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Synthesis of Oligonucleotides Containing Fapy·dG (N^6-(2-Deoxy-α,β-D-erythropentofuranosyl)-2,6-diamino-4-hydroxy-5-formamidopyrimidine) Using a 5′-Dimethoxytrityl Dinucleotide Phosphoramidite

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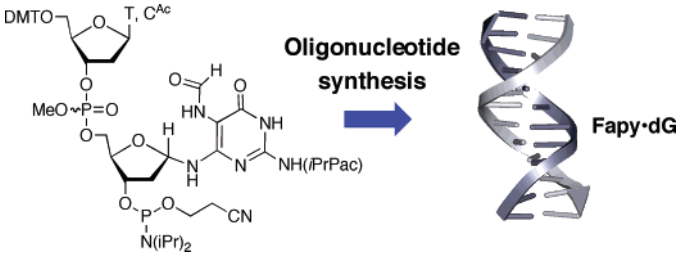

Fapy·dG (N^6-(2-deoxy-α,β-D-erythropentofuranosyl)-2,6-diamino-4-hydroxy-5-formamidopyrimidine) is a modified purine lesion produced by a variety of DNA-damaging agents, which shows interesting biochemical properties. The previous method for synthesizing oligonucleotides containing Fapy·dG utilized a reverse dinucleotide phosphoramidite, which also required the synthesis of the appropriate reverse phosphoramidites. An improved method for synthesizing oligonucleotides containing Fapy·dG, which does not require reverse phosphoramidites, is described. Fapy·dG containing dinucleotide phosphoramidites containing 5′-thymidine (**11a**) or 5′-deoxycytidine (**15**) are prepared and employed in oligonucleotide synthesis. Oligonucleotide purity is assayed using the DNA repair enzyme formamidopyrimidine DNA glycosylase and by ESI-MS.

DNA damage manifests itself in the form of strand breaks, abasic lesions, and modified nucleotides. The transformations of DNA lesions into premutagenic base pairs have important ramifications in aging and a variety of diseases, including cancer.[1−4] Determining the effects of individual lesions on polymerases and repair enzymes is an important endeavor. Organic chemistry plays a vital role in such studies through the synthesis of oligonucleotides containing specific lesions at defined sites.[5−7] Although solid-phase oligonucleotide synthesis is automated, the chemical instability of many DNA lesions often presents challenges that require the involvement of organic chemists.[8−12] One such family of lesions is the formamidopyrimidines (Fapy·dA, Fapy·dG), which are derived from the formal addition of hydroxyl radical to the C8-position of the respective purines (Scheme 1).[13] We now report an improved method for the synthesis of oligonucleotides containing Fapy·dG (*N*-(2-deoxy-α,β-D-erythropentofuranosyl)-*N*-(2,6-diamino-4-hydroxy-5-formamidopyrimidine)).

The formamidopyrimidines and 8-oxopurines (e.g., OxodG) are believed to arise from a common intermediate (Scheme 1).[13] The formamidopyrimidines are produced in comparable yield in vitro under anoxic conditions and

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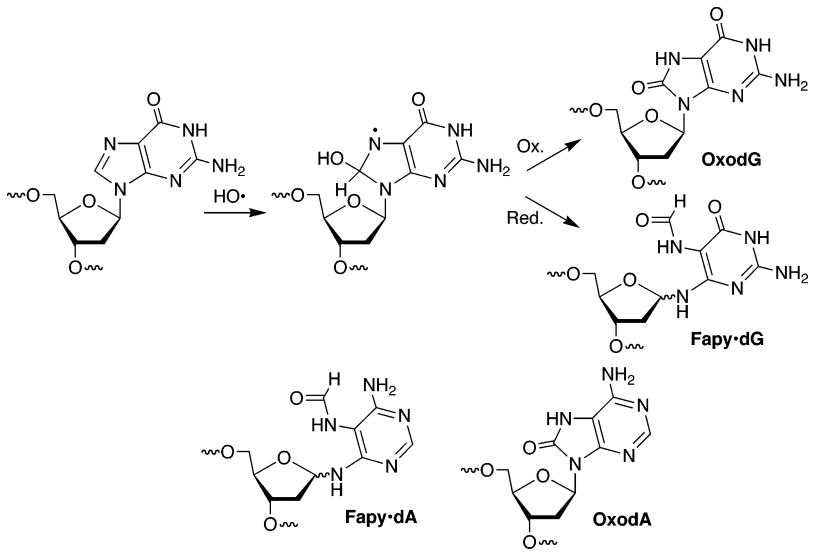
SCHEME 1



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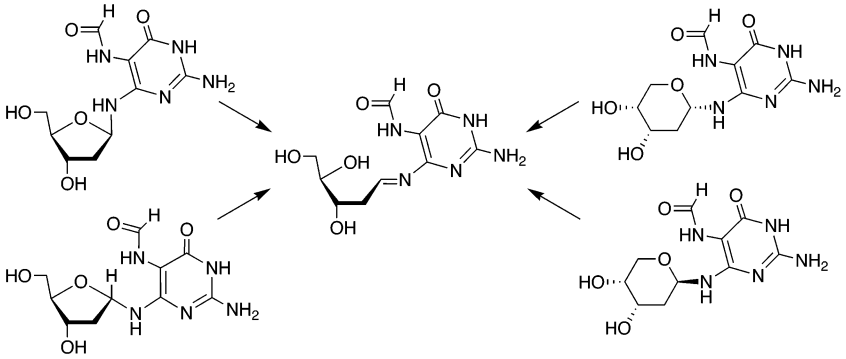

in larger amounts in some cells than the 8-oxopurines.[14−16] However, little was known about the effects of the formamidopyrimidines on DNA until recently when Haraguchi reported the first syntheses of oligonucleotides containing Fapy·dA or Fapy·dG.[17−19] In vitro studies revealed that Fapy·dA and Fapy·dG are avidly excised by formamidopyrimidine DNA glycosylase (Fpg), a base excision repair enzyme that recognizes damaged purines.[20,21] Repair of the latter is highly selective for duplexes containing dC versus dA opposite the lesion, suggesting that Fpg evolved to protect against the premutagenic Fapy·dG:dA base pair. Experiments that demonstrate misincorporation of dA opposite Fapy·dG and dA incision from Fapy·dG:dA base pairs by the deoxyadenosine mismatch repair enzyme, MutY, also point to the biological relevance of the lesion.[22]

The aforementioned biochemical studies, the prevalence of formamidopyrimidine formation, and the recognized importance of DNA damage in general have engendered significant interest in these molecules. Two properties of the formamidopyrimidine lesions warranted particular attention when designing chemical syntheses.[17,18] First, the relatively labile formamide required modifying the capping procedure used in solid-phase synthesis to prevent the accumulation of single nucleotide deletions. However, utilization of a more hindered anhydride and elimination of the imidazole activating agent prevented deformylation at the expense of capping efficiency. The alkaline lability of the formamide was overcome by deprotecting the oligonucleotides with anhydrous K_2CO_3 in methanol. In addition, the thermodynamic preference of the readily epimerizable formamidopyrimidine nucleosides for their respective pyranose isomers (Scheme 2) required that they be incorporated as dinucleotide units in order to avoid exposing the 5′-hydroxyl group in the presence of the formamide.[23,24] The requisite Fapy·dA containing phosphoramidite (**1**) was

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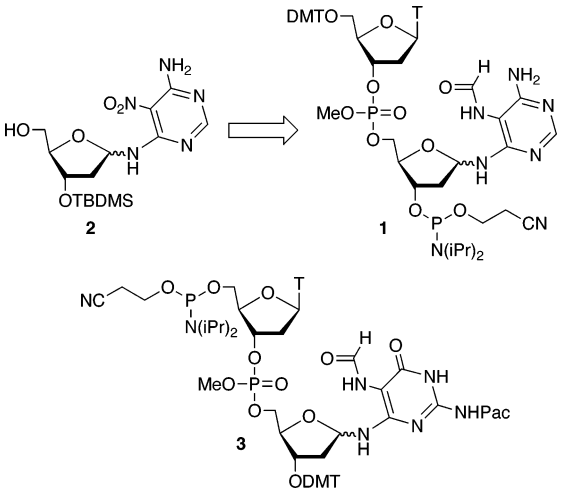
prepared via key intermediate **2** in which the electron-withdrawing nitro substituent sufficiently increased the barrier to rearrangement. Unfortunately, conditions

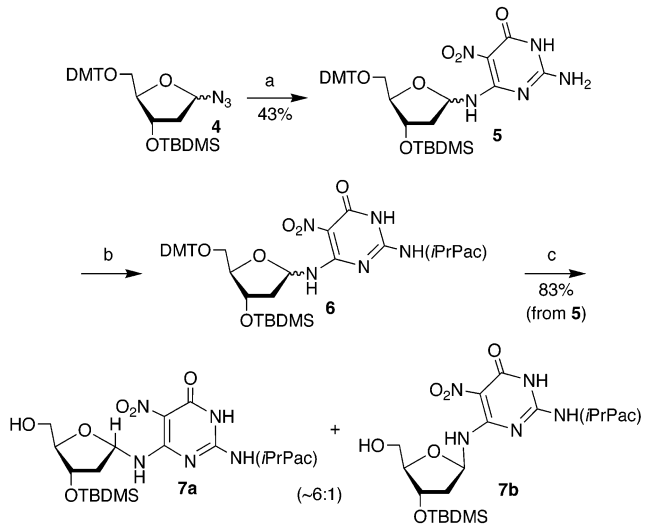


could not be found at that time to prevent rearrangement of the analogous Fapy·dG precursor. This limitation was overcome by using a reverse dinucleotide phosphoramidite (**3**) and assembling the biopolymers in the 5′ → 3′ direction instead of the more common 3′ → 5′ direction. In addition to the synthesis of **3** being difficult, this approach required the preparation of the appropriately protected reverse phosphoramidites for dA, dC, and dG, which are not commercially available. The oligonucleotide synthesis method described herein proceeds in the more preferable 3′ → 5′ direction. Furthermore, overall improvements in the synthesis of Fapy·dG containing dinucleotide phosphoramidites have made the synthesis of oligonucleotides containing a 5′-deoxycytidine practical.

Results and Discussion

Synthesis of a 5′-*O*-Dimethoxytrityl Fapy·dG Dinucleotide Phosphoramidite. The first and perhaps most important improvement to be made in the syntheses of oligonucleotides containing Fapy·dG was to eliminate the need for carrying out the solid-phase synthesis in the 5′ → 3′ direction. This required that we unmask the primary hydroxyl group in the nitropyrimidine intermediate (**6**) without any concomitant rearrangement to the pyranose (Scheme 3). Prior to investigating appropriate reaction conditions for selective 5′-detritylation of the nitropyrimidine intermediate, the exocyclic amine protecting group was changed from phenoxyacetyl (Pac) used in previous studies to 4-isopropylphenoxyacetyl (*i*PrPac, **6**). Amide **6** was most readily obtained on a large scale using PyBOP and the respective carboxylic acid. The desired product could be purified for spectroscopic characterization. However, large-scale preparations were contaminated with byproducts from the PyBOP reagent, and **6** was typically carried forward with these impurities present. The ^{i}PrPac group was advantageous during the latter stages of the phosphoramidite synthesis. Its lower polarity compared to that of a Pac group facilitated chromatographic purification of the dinucleotides. The ^{i}PrPac group was also more resistant to adventitious cleavage. Having previously tried a variety of Bronsted

SCHEME 3[a]



[a] Reagents and conditions: (a) (i) 5% Pd/C (Lindlar's), H_2 (45 psi), EtOH, (ii) 2-amino-6-chloro-4-hydroxy5-nitropyrimidine, diisopropylethylamine, EtOH, 70 °C; (b) (4′-isopropyl)-phenoxyacetic acid, PyBOP, diisopropylethylamine, CH_2Cl_2, 0−25 °C; (c) $FeCl_3 \cdot 6H_2O$, CH_3CN, 25 °C.

acid conditions for carrying out the detritylation without success, we chose to investigate mild Lewis acids. A recent report using ceric ammonium nitrate adsorbed on silica gel provided promising results, but in our hands the yields of this reaction were low upon scale-up.[25] Encouraged by this we investigated the use of $FeCl_3 \cdot 6H_2O$ and found that high yields of **7** could be obtained even on a large scale.[26,27] The anomers (∼6:1 ratio) of **7** were separable by column chromatography, and no pyranose rearrangement product could be detected. In large-scale reactions, the minor anomer was often contaminated with ∼2−5% of the major isomer. In previous syntheses of formamidopyrimidine phosphoramidites, interpretation of differences in the ^{1}H NMR spectra of diastereomers led to the suggestion that the α-anomer was the major product.[18] The differences in the chemical shifts of the C2′-protons was much greater in the major isomer than in the minor isomer. This is consistent with assignment of the α-anomer (**7a**) as the major isomer.[18] Any uncertainty in this assignment was dispelled by X-ray analysis of a crystal of **7a** obtained from acetonitrile. Although the *tert*-butyldimethylsilyloxy group was disordered in the structure, the assignment of α-stereochemistry was certain.

The major isomer (**7a**) was carried forward (Scheme 4). Coupling to the *O*-methyl phosphoramidite of thymidine was carried out as previously described for the synthesis of the dinucleotide phosphoramidite of Fapy·dA using tetrazole as activator, followed by in situ oxidation with *tert*-butyl hydroperoxide.[18] The coupling product (**8a**) and all subsequent reaction products were obtained as a mixture of diastereomers that were isomeric at the phosphate triester. Introduction of the

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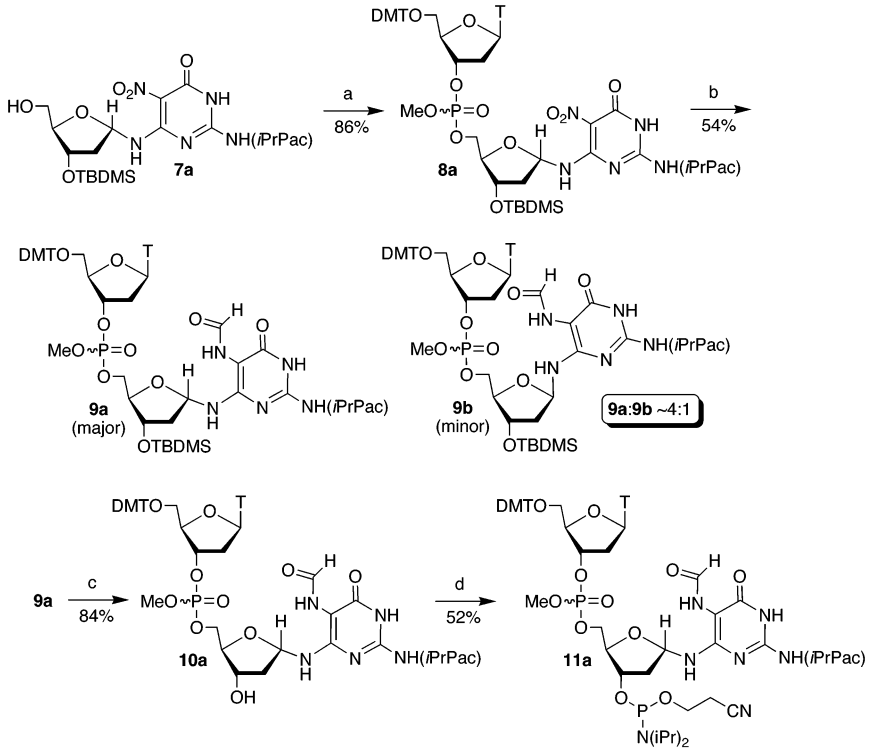
SCHEME 4[a]



[a] Reagents and conditions: (a) (i) 5′-DMT-thymidine phosphoramidite, tetrazole, CH_3CN, 0−25 °C, (ii) *t*BuOOH, 0 °C; (b) (i) 10% Pd/C, diisopropylethylamine, H_2 (80 psi) THF, 25 °C, (ii) acetic formic anhydride, pyridine, 0 °C; (c) $Bu_4N^+ F^-$, AcOH, THF, 0 °C; (d) 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite, diisopropylethylamine, THF, −78 to 25 °C.

formamide group via reduction of the nitro moiety and formylation of the resulting amine was a capricious reaction in the original Fapy·dG phosphoramidite (**3**) synthesis. The hydrogenation of the Fapy·dG precursor was considerably slower than the respective reaction in Fapy·dA phosphoramidite (**2**) synthesis. A more active form of Pd/C and a greater quantity of catalyst were required. Furthermore, the intermediate amine decomposed to products that complicated purification of the formamide. Methanol was used as cosolvent in order to accelerate the rate of hydrogenation. However, this prohibited circumventing the amine's instability by carrying out formylation in situ. The overall transformation of **8** into **9** was improved by carrying out the hydrogenation (80 psi) using 10% Pd/C (75 wt %) in the presence of 8 equiv of diisopropylethylamine. Under these conditions the reduction was complete in 2 h despite using the less polar solvent tetrahydrofuran. (One note of caution is that the activity of commercially available catalyst varied from lot to lot. Hydrogenation conditions needed to be examined whenever a new bottle of catalyst was used.) Formylation was then carried out in situ by adding 3 equiv of acetic formic anhydride and pyridine (in slight excess of the anhydride) to a 0 °C solution of amine. Purification of **9** revealed that the addition of diisopropylethylamine not only accelerated the hydrogenation but also significantly reduced the isomerization at the anomeric center of the penultimate formamidopyrimidine component (see below). The formamidopyrimidine dinucleotide phosphate triester (**9**) was isolated as a separable ∼4:1 (**9a**:**9b**) mixture of diastereomers in ∼50% yield.

Previously, isomerization at the formamidopyrimidine component's anomeric center was also a complication during desilylation. Epimerization of the formamidopyrimidine component was minimized upon desilylation of **9a** by using 6 equiv of acetic acid buffered TBAF at 0 °C. Although either anomer of **10** is useful, the β-anomer (**10b**) undergoes phosphitylation significantly more poorly than **10a** (data not shown). Hence, preventing epimerization decreased the reaction time and in effect increased the yield of phosphoramidite (**11a**). A crucial detail for improving the yield of the phosphitylation reaction was to add the phosphitylating reagent at −78 °C, followed by removal of the cold bath after dissolution of the reagent. If necessary, additional reagent could be added in order to push the conversion of starting material provided the reaction was cooled to −78 °C. Under these conditions >50% isolated yields of **11a** were routinely achieved. Addition of the phoshitylating reagent at higher temperatures led to an inseparable product, which was distinguishable by ^{31}P NMR. On the basis of its observed chemical shift (δ 132), we believe the impurity was an *O*-aryl phosphoramidite resulting from reaction of the phosphitylating reagent at O4 of the formamidopyrimidine ring.[28]

Epimerization at the Anomeric Center of Fapy·dG Is the Source of Isomerization. The modifications described above enabled us to reproducibly prepare **11a** ca. 100 mg at a time. This is enough phosphoramidite for several 1-μmol scale oligonucleotide syntheses. As

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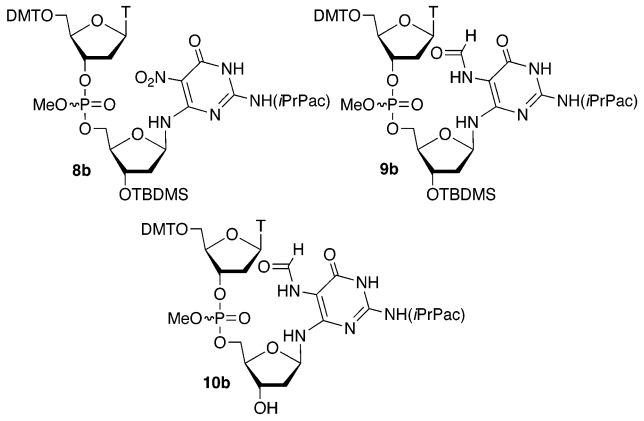
noted above, we believed that the minor products isolated from the reduction/formylation and desilylation reactions resulted from epimerization to the β-Fapy·dG isomer. Although carrying through a mixture of anomers is not detrimental to oligonucleotide preparation, it makes determining purity difficult. Given the complexity of the ^{1}H NMR spectra for the dinucleotides (**9**−**11**), we wanted to be sure that the minor products formed did not result from more drastic rearrangements. The stereochemical relationships between the major and minor products discussed above was firmly established by carrying the β-isomer of the detritylated nitropyrimidine (**7b**), containing a small amount of **7a**, through the synthetic procedures described above (Scheme 4). The coupling of **7b** to the thymidine phosphoramidite proceeded in poorer yield (**8b** is obtained in 48% yield) than when using the α-isomer (**7a**). Importantly a diastereomeric mixture of phosphate triester dinucleotides in which the nitropyrimidine anomer was readily distinguishable from its α-isomer (**8a**) was obtained. Moreover, hydrogenation/formylation of **8b** produced a mixture of the same formamidopyrimidine products obtained from **8a**. However, the major product (**9b**) isolated from reaction of **8b** corresponded to the minor one obtained from **8a**. Likewise, the minor product (**9a**) was identical to the major isomer produced from reduction/formylation of the α-anomer (**8a**). Confirmation that the minor product (**10b**) isolated from desilylation of **9a** also corresponded to the isomer containing the β-formamidopyrimidine was obtained by subjecting **9b** to buffered TBAF. Indeed, the major product (**10b**) isolated was identical to the minor product obtained from desilylation of **9a**.



The ease with which epimerization occurs is illustrated by the isomerization of **9a** upon standing overnight in wet acetone. The facile epimerization of the Fapy·dG components in **9** and **10** under these various reaction conditions is not surprising given what is known about the behavior of such molecules in aqueous solution.[23,24,29,30] However, it underscores the fact that chemical synthesis of oligonucleotides containing single anomers of Fapy·dG is probably not a practical objective. Moreover, a recent study on the configuration of formamidopyrimidine lesions in duplex DNA suggests that the duplex structure dictates the equilibrium distribution of anomers.[31]

Synthesis of a Fapy·dG Dinucleotide Phosphoramidite Containing 5′-Adjacent Deoxycytidine (15). Having developed a practical synthesis of **11a** we sought to prepare the corresponding phosphoramidite (**15**) containing a 5′-adjacent *N*-acetyl-protected deoxycytidine. Deoxycytidine was chosen as the 5′-adjacent nucleotide because a number of mutational hotspots in genes implicated in cancer (e.g. p53) involve deoxyguanosine in 5′-d(CG) sequences.[32] The dinucleotide was prepared from **7a** (Scheme 5), and only the α-isomer (major) obtained following reduction/formylation and desilylation was purified and carried forward. The yields of individual steps were comparable to the corresponding ones obtained in the synthesis of **11a**.

Oligonucleotide Synthesis, Deprotection, and Characterization. Solid-phase synthesis conditions were adjusted for the preparation of oligonucleotides using phosphoramidites **11a** and **15** as previously described for the incorporation of Fapy·dA. Modification of standard oligonucleotide synthesis conditions included using trimethylacetic anhydride as capping agent throughout the synthesis and eliminating the use of *N*-methyl imidazole to activate the anhydride after Fapy·dG was introduced into the biopolymer.[17,18] 4,5-Dicyanoimidazole was used as activating agent for the entire synthesis, as was *tert*-butyl hydroperoxide in place of I_2 as oxidant.[17,18,33] This activator is less acidic than either tetrazole or *S*-ethyl tetrazole.[34] One important deviation from previous syntheses of oligonucleotides containing Fapy·dA and Fapy·dG concerned the coupling of **11a** and **15**.

Dimethoxytrityl cation response indicated that **11a** and **15** coupled in ∼50% yield when reacted for 15 min. Coupling **11a** twice ("double-coupling") prior to progressing to capping and oxidation did not double the yield as indicated by dimethoxytrityl cation response. Consequently, to preserve the valuable phosphoramidites (**11a** and **15**) oligonucleotides (**16**−**21**) were prepared using a

5′-d(TGC AGT **Fapy·dG**TC AGC)
16
5′-d(GAA GAC CT**Fapy·dG** AGC GTC C)
17
5′-d(GAA GAC CT**Fapy·dG** CGC GTC C)
18
5′-d(GAA GAC CT**Fapy·dG** GGC GTC C)
19
5′-d(GAA GAC CT**Fapy·dG** TGC GTC C)
20
5′-d(CCA GGT GC**Fapy·dG** TGT TGG T)
21

single coupling. Modifications in the previously reported deprotection procedure were attempted.[17,18] For instance, demethylation was carried out in methanol, followed by potassium carbonate addition after the prescribed period. However, the advantage of this sequential one-pot reaction was negated by the necessity to desalt the crude

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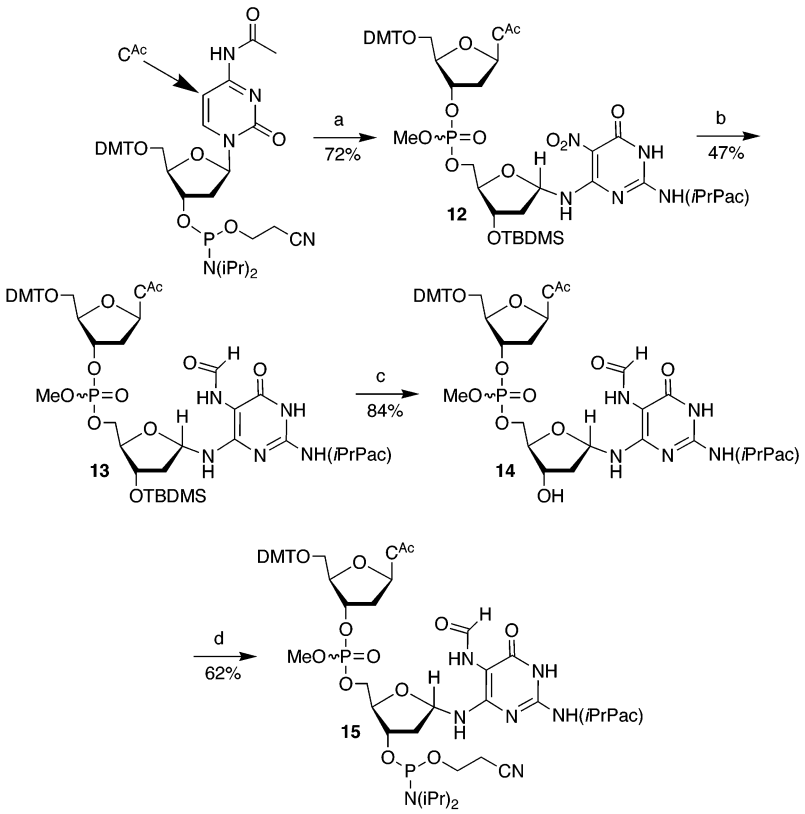
SCHEME 5[a]



[a] Reagents and conditions: (a) (i) **7a**, tetrazole, CH_3CN, 0−25 °C, (ii) *t*BuOOH, 0 °C; (b) (i) 10% Pd/C, diisopropylethylamine, H_2 (80 psi) THF, 25 °C, (ii) acetic formic anhydride, pyridine, 0 °C; (c) Bu_4N^+ F^-, AcOH, THF, 0 °C; (d) 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite, diisopropylethylamine, THF, −78 to 25 °C.

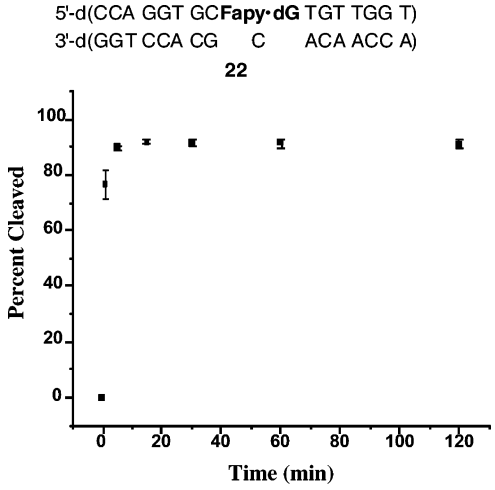


FIGURE 1. Fpg (100 nM) digestion of 5′-^{32}P-**22** (10 nM).

material prior to PAGE purification. Hence, the oligonucleotides were deprotected as previously described and purified via denaturing PAGE.[17,18] The isolated oligonucleotides were characterized by ESI-MS and by formamidopyrimidine DNA glycosylase digestion of the oligonucleotide hybridized to its complement (**22**, Figure 1).

Experimental Section

Preparation of 5. Dimethoxytrityl azide **4** (13.2 g, 23 mmol) was reduced by hydrogen (45 psi) in the presence of Lindlar's catalyst (5% Pd, 2.2 g) in ethanol (160 mL) to afford the crude amine in 75 min. The resulting mixture was passed through Celite, concentrated, and dried under high vacuum. The residue was dissolved in ethanol (150 mL) and reacted with freshly made 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine (17.2 g, 90 mmol) in the presence of diisopropylethylamine (18.6 g, 25 mL, 144 mmol) at 70 °C for 14 h. The cooled mixture was filtered through a thick layer of Celite, concentrated, dried under reduced pressure, and purified by column chromatography (3 times; 0.5−2% methanol in CH_2Cl_2) to afford **5** (7.0 g, 43% yield) as a mixture of anomers: ^{1}H NMR ($CDCl_3$) δ 10.89 (s, 1 H), 10.80 (s, 1H), 10.32 (d, 1 H, J = 9.0 Hz), 9.87 (d, 1 H, J = 8.1 Hz), 8.45 (s, 1H), 8.36 (s, 1H), 6.85−6.90, 7.20−7.49 (m, 18 H), 6.40 (t, 1H, J = 7.8 Hz), 6.24 (dd, 1H, J = 6.9, 13.4 Hz), 5.57 (s, 1H), 5.51 (s, 1H), 4.45−4.47 (m, 1H), 4.39−4.40 (m, 1H), 4.28−4.31 (m, 1H), 4.04−4.05 (m, 1H), 3.08−3.16 (m, 1H), 3.22−3.28 (m, 1H), 2.37−2.42 (m, 1H), 2.21−2.27 (m, 1H), 1.88−2.07 (m, 2H), 0.95 (s, 9H), 0.93 (s, 9H), 0.16 (s, 6H), 0.05 (s, 6H), 0.08 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 158.7, 158.6, 154.2, 144.8, 136.9, 136.0, 130.5, 130.2, 128.2, 128.0, 126.9, 113.3, 87.6, 86.6, 82.2, 63.9, 55.4, 54.2, 42.4, 40.4, 29.7, 26.2, 26.0, 18.9, 18.7, 17.7, 12.3, −4.3, −4.4; IR (thin film) 3309, 2929, 2856, 1682, 1652, 1568, 1558, 1510, 1446, 1372, 1250, 1178, 1118, 1069, 1033 cm^{-1}; HRMS (M + H$^+$) calcd for $C_{36}H_{46}N_5O_8Si$ 704.3116, found 704.3094.

Preparation of 6. Diisopropylethylamine (3.86 g, 5.2 mL, 30 mmol), PyBOP (7.6 g, 14.6 mmol), and (4′-isopropyl)-phenoxyacetic acid (2.85 g, 14.7 mmol) were added to a solution of **5** (7.0 g, 9.9 mmol) in CH_2Cl_2 (60 mL) at 0 °C. The reaction mixture was stirred for 1 h at 25 °C, at which time it was concentrated to dryness and purified by column chromatography (0.5−0.6% methanol and gradually changing diisopropylethylamine 0.25−0.05% in CH_2Cl_2) to afford compound **6** contaminated with PyBOP byproducts (10.1 g). Analytical samples were obtained and spectroscopically characterized: ^{1}H

NMR ($CDCl_3$) δ 10.31 (d, 0.7 H, J = 8.8 Hz), 9.78 (d, 0.3 H, J = 8.4 Hz), 7.17−7.41 (m, 13 H), 6.79−6.91 (m, 4 H), 6.32 (t, 0.7 H, J = 8.0 Hz), 6.17 (dd, 0.3 H, J = 8.0, 13.2 Hz), 4.67 (s, 0.6 H), 4.63 (s, 1.4 H), 4.40 (d, 0.7 H, J = 4.8 Hz), 4.30 (m, 0.3 H), 4.24 (dd, 0.7 H, J = 3.6, 5.2 Hz), 4.00 (m, 0.3 H), 3.76 (s, 6 H), 3.13 (m, 2 H), 2.89 (septet, 1 H, J = 6.8 Hz), 2.35 (m, 1 H), 2.00 (d, 1 H, J = 13.6 Hz), 1.22 (m, 6 H), 0.89 (m, 6.3 H), 0.86 (m, 2.7 H), 0.09 (s, 4.2 H), 0.01 (d, 1.8 H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$), δ 170.5, 158.5, 157.8, 154.3, 148.9, 144.6, 143.9, 135.8, 135.7, 130.0, 127.9, 127.8, 126.8, 114.7, 113.1, 87.6, 86.6, 86.4, 82.3, 74.4, 67.2, 63.5, 55.2, 53.8, 40.2, 33.3, 29.3, 26.0, 25.8, 24.1, 18.4, −4.6, −4.7; IR (KBr film) 3248, 2958, 1686, 1623, 1584, 1510, 1420, 1251, 1179, 1031, 840, 789, 558 cm^{-1}; HRMS (M + Na) calcd for $C_{47}H_{57}N_5O_{10}NaSi$ 902.3767, found 902.3774.

Preparation of 7. A solution of $FeCl_3$·$6H_2O$ (615 mg, 2.28 mmol) in CH_3CN (2 mL) was added to **6** in CH_3CN (100 mL) and stirred for 60 min at room temperature. Additional $FeCl_3$·$6H_2O$ (307 mg, 1.14 mmol) in CH_3CN (2 mL) was added, and the reaction was stirred 30 min longer. The reaction was quenched by diisopropylethylamine (0.165 g, 0.222 mL, 1.3 mmol) and methanol (0.162 mL). After concentration, the mixture was purified by column chromatography (1−4% methanol in CH_2Cl_2) to afford fast isomer **7a** (4.1 g, 72%) and slow isomer **7b** (0.69 g, 12%). The fast isomer was recrystallized from CH_3CN (12 mL) to provide long, white needlelike crystals (2.3 g, mp 145−147 °C). **7a**: ^{1}H NMR ($CDCl_3$) δ 10.26 (d, 1 H, J = 8.4 Hz), 9.57 (bd s, 1 H), 7.19 (d, 2 H, J = 8.8 Hz), 6.89 (d, 2 H, J = 8.8 Hz), 6.25 (t, 1 H, J = 7.6 Hz), 4.64 (s, 2 H), 4.44 (d, 1 H, J = 5.6 Hz), 4.15 (dd, 1 H, J = 1.2, 3.6 Hz), 3.67 (dd, 1 H, J = 4.4, 12 Hz), 3.58 (dd, 1 H, J = 3.6, 12 Hz), 2.86 (septet, 1 H, J = 6.8 Hz), 2.34 (m, 1 H), 2.02 (d, 1 H, J = 13·6 Hz), 1.21 (d, 6 H, J = 6.8 Hz), 0.89 (s, 9 H), 0.12 (d, 6 H, J = 3.6 Hz); ^{13}C NMR ($CDCl_3$) δ 170.9, 158.1, 154.5, 149.2, 143.8, 127.8, 114.9, 113.7, 88.4, 82.5, 73.9, 67.5, 62.8, 41.0, 33.3, 30.9, 25.9, 24.1, 18.3, −4.7, −4.8; IR (KBr film) 3462, 3242, 2957, 1680, 1628, 1582, 1511, 1421, 1244, 1027, 839, 788 cm^{-1}; HRMS (M + Na) calcd for $C_{26}H_{39}N_5O_8NaSi$ 600.2460, found 600.2447. **7b**: ^{1}H NMR ($CDCl_3$), slow isomer, δ 9.98 (d, 1 H, J = 8.4 Hz), 9.20 (bs, 1 H), 7.21 (d, 2 H, J = 8.8 Hz), 6.91 (d, 2 H, J = 8.8 Hz), 6.25 (dd, 1 H, J = 6.4, 14.4 Hz), 4.65 (s, 2 H), 4.48 (m, 1 H), 3.96 (dd, 1 H, J = 2.8, 5.6 Hz), 3.67−3.82 (m, 2 H), 2.88 (septet, 1 H, J = 6.8 Hz), 2.17−2.30 (m, 2 H), 1.22 (d, 6 H, J = 6.8 Hz), 0.88 (s, 9 H), 0.08 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 170.7, 158.2, 154.3, 149.2, 143.9, 127.9, 114.8, 113.8, 87.5, 82.3, 72.7, 67.2, 55.7, 43.6, 42.3, 33.3, 25.7, 24.1, 18.6, 18.0, 17.2, 12.4, −4.7, −4.8; IR (KBr film) 3401, 3236, 2958, 1686, 1624, 1511, 1426, 1250, 1098, 842, 788 cm^{-1}; HRMS (M + Na) calcd for $C_{26}H_{39}N_5O_8NaSi$ 600.2460, found 600.2487.

Preparation of 8a. A mixture of **7a** (600 mg, 1.04 mmol) and 5′-DMT-dT-*O*-methyl phosphoramidite (870 mg, 1.24 mmol) was coevaporated with CH_3CN (2 × 4 mL). Tetrazole (128.6 mg, 4.08 mL of 0.45 M in acetonitrile, 1.84 mmol) was added to the dried material that was dissolved in CH_3CN (16 mL) at 0 °C. After 1 h at 25 °C, the solution was cooled to 0 °C, *t*-BuOOH (0.724 mL of 5−6 M solution in decane, 3.62 mmol) was added, and stirring was continued for 30 min. The reaction mixture was concentrated in vacuo and chromatographed (2−2.5% methanol in CH_2Cl_2) to give product **8a** (1.045 g, 84%): ^{1}H NMR ($CDCl_3$) δ 10.27 (d, 0.5 H, J = 8.4 Hz), 10.23 (d, 0.5 H, J = 8.4 Hz), 9.58 (s, 0.5 H), 9.44 (s, 0.5 H), 7.60 (d, 0.5 H, J = 1.2 Hz), 7.57 (d, 0.5 H, J = 1.2 Hz), 7.11−7.36 (m, 12 H), 6.81−6.84 (m, 6 H), 6.31 (m, 2 H), 5.09−5.15 (m, 1 H), 4.70 (d, 1 H, J = 1.6 Hz), 4.66 (d, 1 H, J = 4.0 Hz), 4.44 (t, 1 H, J = 5.6 Hz), 4.18−4.32 (m, 2 H), 3.99−4.07 (m, 2 H), 3.78 (s, 6 H), 3.71 (dd, 3 H, J = 11.6, 23.2 Hz), 3.51−3.55 (m, 1 H), 3.40 (dd, 1 H, J = 10.8, 16.0 Hz), 2.84 (septet, 1 H, J = 6.8 Hz), 2.63 (m, 1 H), 2.48 (m, 1 H), 2.31 (m, 1 H), 1.9 (dd, 1 H, J = 2.8, 13.2 Hz), 1.35 (d, 1.5 H, J = 0.4 Hz), 1.33 (d, 1.5 H, J = 0.8 Hz), 1.20 (d, 6 H, J = 7.2 Hz), 0.92 (d, 9 H, J = 2.0 Hz), 0.15 (s, 3 H), 0.14 (d, 3 H, J = 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 171.7, 171.3, 163.74, 163.66, 158.8, 158.0, 154.7, 154.6, 154.5, 150.6, 149.6, 149.4, 144.0, 143.9, 143.4, 143.3, 135.0, 134.95, 134.90, 130.0, 128.11, 128.05, 128.0, 127.7, 127.3, 114.6, 113.63, 113.55, 113.3, 111.74, 111.70, 87.21, 87.16, 86.0, 85.93, 85.86, 84.5, 84.4, 84.3, 84.23, 84.15, 82.6, 82.5, 78.1, 77.8, 73.7, 67.6, 67.4, 67.2, 62.8, 62.5, 55.2, 54.7, 54.61, 54.58, 54.5, 44.5, 44.4, 40.6, 39.1, 33.2, 25.9 24.1, 23.6, 22.9, 22.6, 18.3, 11.7, 11.6, −4.7, −4.8; ^{31}P NMR ($CDCl_3$) δ −0.06, −0.042; IR (KBr film) 3248, 2958, 1686, 1624, 1584, 1510, 1421, 1251, 1179, 1031, 840, 789, 558 cm^{-1}; HRMS (M + Na) calcd for $C_{58}H_{72}N_7O_{17}NaSiP$ 1220.4384, found 1220.4328.

Preparation of 8b. The reaction between **7b** (252 mg, 0.44 mmol) and 5′-DMT-dT-*O*-methyl phosphoramidite (368 mg, 0.525 mmol) was carried out as described above for **7a** using tetrazole (54 mg, 1.71 mL of 0.45 M, 0.77 mmol), followed by *t*-BuOOH (0.30 mL of 5−6 M solution in decane, 1.50 mmol). The reaction mixture was concentrated and chromatographed (2% methanol in CH_2Cl_2) to give product **8b** (250 mg, 47%): ^{1}H NMR (CD_3CN) δ 9.54 (d, 1 H, J = 8.0 Hz), 9.42 (bd s, 1 H), 7.15−7.44 (m, 14 H), 6.80−6.91 (m, 6 H), 6.17−6.28 (m, 2 H), 5.08−5.18 (m, 1 H), 4.72 (t, 2 H, J = 4.0 Hz), 4.42 (m, 1 H), 4.15−4.21 (m, 1 H), 4.03−4.10 (m 1 H), 3.93 (m, 1 H), 3.79 (m, 1 H), 3.73−3.74 (m, 6 H), 3.63−3.70 (m, 3 H), 3.29−3.34 (m, 2 H), 2.84 (septet, 1 H, J = 6.8 Hz), 2.40−2.55 (m, 2 H), 2.16−2.23 (m, 2 H), 1.47 (t, 1.5 H, J = 1.2 Hz), 1.47 (dd, 1.5 H, J = 1.2, 4.0 Hz), 1.18 (m, 6 H), 0.88 (d, 9 H, J = 1.2 Hz), 0.08 (m, 6 H); ^{13}C NMR (CD_3CN) δ 173.0, 164.7, 159.8, 156.2, 151.4, 151.2, 145.73, 145.68, 143.6, 136.6, 136.4, 131.0, 129.0, 128.4, 128.3, 128.0, 115.6, 115.2, 114.6, 114.1, 111.7, 111.6, 87.7, 85.8, 85.1, 85.0, 83.2, 79.1, 76.7, 73.2, 68.2, 68.0, 65.8, 64.1, 63.92, 63.85, 55.9, 55.5, 55.41, 55.35, 52.6, 42.0, 39.2, 34.0, 30.9, 26.1, 24.4, 18.6, 12.2, −4.5, −4.7; ^{31}P NMR (CD_3CN) δ 8.45, −1.64; IR (KBr film) 3224, 2954, 1690, 1608, 1584, 1508, 1472, 1249, 1179, 1032, 840, 832, 785 cm^{-1}; HRMS (M + Na) calcd for $C_{58}H_{72}N_7O_{17}NaSiP$ 1220.4384, found 1220.4418.

Preparation of 9 from 8a. Compound **8a** (1.0 g, 0.84 mmol) was reduced by H_2 (80 psi) in the presence of palladium on carbon (10%, 728 mg) and diisopropylethylamine (0.89 g, 1.2 mL, 6.8 mmol) in THF (40 mL) for 2 h at 25 °C. The reaction mixture was cooled to 0 °C, whereupon acetic formic anhydride (0.220 mL, 2.51 mmol) and pyridine (268 mg, 0.274 mL, 3.39 mmol) were added. After 10 min at 0 °C, the reaction mixture was filtered through Celite. After concentration, the residue was chromatographed (3−5% methanol in CH_2Cl_2) to afford **9a** (less polar, 431 mg, 43%) and **9b** (110 mg, 11%). **9a**: ^{1}H NMR (CD_3OD) δ 8.20 (s, 0.3 H), 8.19 (s, 0.3 H), 7.87 (s, 0.1 H), 7.76 (s, 0.1 H), 7.60 (s, 1 H), 7.08−7.39 (m, 12 H), 6.82−6.91 (m, 6 H), 6.33 (m, 1 H), 6.10−6.14 (m, 1 H), 5.13 (m, 1 H), 4.65−4.68 (m, 2 H), 4.34 (m, 2 H), 3.99−4.08 (m, 3 H), 3.70−3.79 (m, 9 H), 3.44 (dd, 2 H, J = 3.2, 11.6 Hz), 2.83 (septet, 1 H, J = 6.8 Hz), 2.51−2.64 (m, 2 H), 2.38 (m, 1 H), 1.92−1.97 (m, 1 H), 1.39 (d, 1.5 H, J = 1.2 Hz), 1.38 (d, 1.5 H, J = 1.2 Hz), 1.19 (d, 6 H, J = 6.8 Hz), 0.91−0.93 (m, 9 H), 0.12−0.15 (m, 6 H); ^{13}C NMR (CD_3OD) δ 210.0, 173.0, 169.1, 166.0, 163.9, 163.8, 160.3, 159.2, 159.0, 157.0, 156.9, 152.2, 149.5, 145.8, 143.7, 137.3, 137.2, 136.6, 136.5, 131.4, 129.4, 129.0, 128.4, 128.2, 115.7, 114.3, 112.1, 94.5, 88.4, 86.0, 85.9, 85.8, 85.1, 83.3, 83.2, 80.0, 74.4, 74.3, 69.1, 68.7, 68.2, 64.3, 55.8, 55.6, 55.5, 55.4, 41.0, 39.8, 34.6, 30.7, 29.5, 26.4, 24.6, 19.0, 12.2, −4.6; ^{31}P NMR (CD3OD) δ −1.85, −2.20; IR (KBr film) 3401, 3246, 2956, 1696, 1655, 1583, 1510, 1460, 1252, 1179, 1032, 831, 778, 584 cm^{-1}; HRMS (M + Na) calcd for $C_{59}H_{74}N_7O_{16}NaSiP$ 1218.4591, found 1218.4638. **9b**: ^{1}H NMR (CD_3OD) δ 8.17 (s, 0.4 H), 8.18 (s, 0.4 H), 7.93 (s, 0.1 H), 7.92 (s, 0.1 H), 7.61 (d, 0.5 H, J = 1.2 Hz), 7.57 (d, 0.5 H, J = 1.2 Hz), 7.11−7.40 (m, 12 H), 6.83−6.92 (m, 6 H), 6.28 (m, 1 H), 6.09 (m, 1 H), 5.11 (m, 1 H), 4.68 (s, 2 H), 4.38 (m, 1 H), 4.27 (m, 1 H), 4.08 (m, 1 H), 4.02 (m, 1 H), 3.84−3.88 (m, 1 H), 3.75 (s, 6 H), 3.72 (dd, 3 H, J = 11.2, 15.2 Hz), 3.40 (m, 2 H), 2.83 (septat, 1 H, J = 6.8 Hz), 2.47−2.59 (m, 2 H), 2.20 (m, 1 H), 2.08 (m, 1 H), 1.41 (d, 1.5 H, 1.2 Hz), 1.40 (d, 1.5 H, 1.2 Hz), 1.20 (d, 6 H, J = 6.8 Hz), 0.89 (d, 9 H, J = 2.0 Hz), 0.08−0.10 (m, 6 H); ^{13}C NMR (CD_3OD) δ 210.0, 173.04, 172.99,

166.02, 165.98, 163.9, 160.3, 159.4, 159.2, 156.9, 151.2, 149.53, 149.46, 145.8, 143.7, 137.2, 136.6, 136.5, 131.3, 129.3, 129.0, 128.4, 128.2, 115.8, 115.7, 114.3, 112.1, 95.7, 88.32, 88.29, 85.9, 85.8, 85.7, 85.6, 83.6, 80.3, 73.7, 73.6, 69.3, 68.2, 64.4, 55.8, 55.7, 55.52, 55.46, 40.9, 39.7, 39.5, 34.5, 30.7, 26.4, 26.3, 24.6, 18.9, 18.8, 12.2, −4.5, −4.56, −4.64; ^{31}P NMR (CD_3OD) δ −1.96, −2.03, −2.21, −2.28; IR (KBr film) 3260, 2956, 1691, 1655, 1586, 1510, 1466, 1252, 1179, 1033, 831, 778, 586 cm^{-1}; HRMS (M + Na) calcd for $C_{59}H_{74}N_7O_{16}NaSiP$ 1218.4591, found 1218.4614.

Preparation of 9 from 8b. The reaction of **8b** (0.25 g, 0.21 mmol) was carried out as described above for **8a** in the presence of palladium on carbon (10%, 182 mg) and diisopropylethylamine (0.222 g, 0.3 mL, 1.7 mmol) in THF (10 mL) for 2 h at 25 °C. The crude amine was formylated using acetic formic anhydride (0.055 mL, 0.63 mmol) and pyridine (67 mg, 0.068 mL, 0.85 mmol). The residue was chromatographed (3−5% methanol in CH_2Cl_2) to afford **9a** (15 mg, 6.0%) and more polar **9b** (118 mg, 47.2%).

Preparation of 10a from 9a. Acetic acid (69.2 mg, 0.066 mL, 1.15 mmol) and $Bu_4NF{\cdot}3H_2O$ (0.78 g, 2.48 mmol) were added to a solution of **9a** (570 mg, 0.48 mmol) in THF (20 mL) at 0 °C. The reaction mixture was stirred for 5 h at this temperature, evaporated to dryness, and chromatographed (5−7% methanol in CH_2Cl_2) to give compound **10a** (433 mg, 83%): ^{1}H NMR (CD_3OD) δ 8.21 (s, 0.4 H), 8.19 (s, 0.4 H), 7.88 (s, 0.1 H), 7.78 (s, 0.1 H), 7.60 (s, 1 H), 7.06−7.38 (m, 11 H), 6.81−6.91 (m, 6 H), 6.32 (m, 1 H), 6.06 (m, 1 H), 5.13 (m, 1 H), 4.67 (s, 1 H), 4.66 (s, 1 H), 4.22−4.38 (m, 2 H), 3.90−4.11 (m, 3 H), 3.66−3.77 (m, 9 H), 3.40 (d, 2 H, J = 12.8 Hz), 2.83 (m, 1 H), 2.49−2.63 (m, 2 H), 2.32 (m, 1 H), 1.94 (m, 1 H), 1.38 (s, 1.5 H), 1.37 (s, 1.5 H), 1.17 (d, 6 H, J = 6.8 Hz); ^{13}C NMR (CD_3OD) δ 173.0, 166.1, 163.7, 163.6, 160.3, 156.9, 152.2, 149.6, 145.8, 143.7, 137.3, 137.2, 136.6, 131.4, 129.3, 129.0, 128.4, 128.2, 115.7, 115.6, 114.3, 112.1, 95.3, 88.4, 86.00, 85.95, 85.90, 85.85, 85.2, 84.8, 83.3, 80.0, 72.7, 68.2, 64.3, 55.8, 54.0, 40.4, 39.7, 34.6, 30.7, 26.9, 24.6, 20.9, 13.9, 12.1; ^{31}P NMR (CD_3OD) δ −1.92, −1.95, −2.11, −2.19, −2.22, −2.29; IR (KBr film) 3389, 3248, 2959, 1696, 1654, 1577, 1509, 1446, 1252, 1179, 1031, 827, 770, 584 cm^{-1}; HRMS (M + Na) calcd for $C_{53}H_{60}N_7O_{16}NaP$ 1104.3726, found 1104.3674.

Preparation of 10b from 9b. Acetic acid (36 mg, 0.035 mL, 0.61 mmol) and $Bu_4NF{\cdot}3H_2O$ (0.41 g, 1.30 mmol) were reacted with a solution of **9b** (300 mg, 0.25 mmol) in THF (6 mL) at 0 °C as described above. The reaction mixture was stirred for 40 h at this temperature, evaporated to dryness, and chromatographed (5−9% methanol in CH_2Cl_2) to give compound **10b** (250 mg, 93%): ^{1}H NMR (CD_3CN) δ 9.74 (bd s, 1 H), 8.18 (s, 0.4 H), 8.17 (s, 0.4 H), 8.14 (s, 0.1 H), 8.13 (s, 0.1 H), 7.82−7.88 (m, 1 H), 7.10−7.42 (m, 14 H), 6.81−6.89 (m, 4 H), 6.23 (dd, 1 H, J = 2.8, 7.2 Hz), 5.96−6.04 (m, 1 H), 5.06 (m, 1 H), 4.66 (s, 2 H), 4.18−4.25 (m, 2 H), 3.99−4.05 (m, 2 H), 3.76 (m, 1 H), 3.62−3.72 (m, 9 H), 3.31−3.34 (m, 2 H), 2.97 (m, 1 H), 2.83 (septet, 1 H, J = 6.4 Hz), 2.42−2.54 (m, 2 H), 2.25 (m, 1 H), 2.08 (m, 1 H), 1.43 (s, 3 H), 1.14−1.17 (m, 6 H); ^{13}C NMR (CD_3CN) δ 173.1, 172.3, 164.8, 159.7, 159.1, 157.6, 156.4, 151.6, 148.8, 145.7, 143.4, 136.5, 136.4, 131.0, 128.9, 128.4, 128.0, 115.5, 114.1, 111.7, 87.6, 85.5, 85.3, 85.0, 84.4, 83.0, 82.9, 78.7, 72.7, 72.3, 68.3, 67.8, 64.1, 63.9, 55.9, 55.4, 55.3, 53.1, 39.2, 34.0, 30.9, 26.2, 26.0, 24.4, 20.8, 20.6, 13.8, 12.3; ^{31}P NMR (CD_3CN) δ −1.70, −1.73, −1.80, −1.87, −1.93; IR (KBr film) 3389, 3226, 2954, 1684, 1654, 1578, 1502, 1420, 1249, 1173, 1032, 826 cm^{-1}; HRMS (M + Na) calcd for $C_{53}H_{60}N_7O_{16}NaP$ 1104.3726, found 1104.3716.

Preparation of 11a from 10a. Alcohol substrate **10a** (141 mg, 0.13 mmol) was azeotroped using THF (2 × 0.5 mL) and dried under high vacuum. 2-Cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite (37.8 mg, 0356 mL, 0.160 mmol) was added to a solution of azeotropically dried **10a** and diisopropylethylamine (46.7 mg, 0.063 mL, 0.36 mmol) in THF (0.8 mL) at −78 °C. The reaction mixture was allowed to warm to room temperature by removing the cold bath, stirred for 10 min, and checked by TLC. The solution was recooled to −78 °C, and more 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite (25.5 mg, 0.024 mL, 0.11 mmol) was added. Thereafter, the addition of 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite was repeated two additional times, 12.7 mg (0.012 mL, 0.054 mmol) and 8.0 mg (0.0075 mL, 0.034 mmol), along with 2 equiv of diisopropylethylamine, to achieve more than 80% conversion of **10a**. The reaction was quenched by adding brine (1 mL), and the resulting mixture was extracted with THF and dried over $MgSO_4$. After removing the THF under high vacuum, the residue was chromatographed (2−5% methanol in CH_2Cl_2) to afford **11a** (87 mg, 52%): ^{1}H NMR (CD_3CN) δ 9.62 (bd s, 1 H), 8.20 (d, 0.7 H, J = 3.2 Hz), 7.86 (m, 0.3 H), 7.63 (t, 0.7 H, J = 5.6 Hz), 7.11−7.31 (m, 9 H), 6.82−6.86 (m, 6 H), 6.46 (m, 1 H), 6.25 (dd, 1 H, J = 7.6, 14 Hz), 6.04 (m, 1 H), 5.05 (m, 1 H), 4.67 (s, 1 H), 4.64 (s, 1 H), 4.41 (m, 1 H), 4.16−4.28 (m, 2 H), 3.98−4.07 (m, 2 H), 3.53−3.82 (m, 11 H), 3.34 (m, 2 H), 2.81 (m, 2 H), 2.35−2.69 (m, 7 H), 1.44 (s, 3 H), 1.14−1.17 (m, 18 H); ^{31}P NMR (CD_3CN) δ 147.71, 147.30, 147.25, 147.07, −2.17, −2.21, −2.23, −2.34; HRMS (M + Na) calcd for $C_{62}H_{77}N_9O_{17}NaP_2$ 1304.4805, found 1304.4734.

Preparation of 12. The mixture of compound **7a** (600 mg, 1.04 mmol) and 5′-DMT-*N*-Ac-dC-*O*-methyl phosphoramidite (920 mg, 1.26 mmol) was coevaporated with CH_3CN (2 × 4 mL). Tetrazole (128.6 mg, 4.08 mL of 0.45 M, 1.84 mmol) was added to the dried material that was dissolved in CH_3CN (16 mL) at 0 °C. After 1 h at 25 °C, the solution was cooled to 0 °C, *t*-BuOOH (0.724 mL of 5−6 M solution in decane, 3.6 mmol) was added, and stirring was continued for 30 min. The reaction mixture was concentrated in vacuo and chromatographed (2−2.5% methanol in CH_2Cl_2) to give product **12** (0.919 g, 72%): ^{1}H NMR (CD_3CN) δ 12.79 (bd s, 0.4 H), 11.32 (bd s, 0.6 H), 10.19, (d, 0.4 H, J = 8.4 Hz), 10.10, (d, 0.6 H, J = 8.4 Hz), 9.06 (s, 0.8 H), 8.78 (s, 0.2 H), 8.30 (d, 0.4 H, J = 7.6 Hz), 8.26 (d, 0.6 H, J = 7.6 Hz), 7.08−7.46 (m, 12 H), 6.79−6.88 (m, 6 H), 6.29 (dd, 0.4 H, J = 6.8, 8.8 Hz), 6.18 (dd, 0.6 H, J = 6.8, 8.4 Hz), 6.04 (m, 1 H), 5.13−5.23 (m, 1 H), 4.80−4.89 (m, 2 H), 4.50 (t, 1 H, J = 4.0 Hz), 4.34 (dd, 0.6 H, J = 3.4, 4.0 Hz), 3.99−4.34 (m, 3.4 H), 3.80 (s, 3 H), 3.74 (s, 3 H), 3.65−3.72 (m, 3 H), 3.36−3.47 (m, 2 H), 2.75−2.93 (m, 2 H), 2.52−2.67 (m, 1 H), 2.19−2.32 (m, 2 H), 2.08 (s, 3 H), 1.14−1.17 (m, 6 H), 0.93 (d, 9 H, J = 20 Hz), 0.14−0.16 (m, 6 H); ^{13}C NMR (CD_3CN) δ 174.1, 174.0, 171.4, 163.4, 159.7, 159.3, 159.2, 157.6, 157.4, 156.61, 156.63, 155.5, 151.7, 151.6, 145.4, 142.9, 136.5, 136.29, 136.26, 131.0, 130.9, 128.9, 128.2, 128.0, 115.1, 114.1, 97.3, 97.2, 88.8, 88.0, 87.8, 87.7, 87.1, 86.9, 86.8, 86.1, 86.0, 85.4, 85.3, 83.4, 83.3, 77.43, 77.35, 75.0, 68.9, 68.3, 67.8, 62.7, 62.0, 55.8, 55.1, 55.02, 54.96, 41.8, 40.2, 40.0, 33.9, 30.8, 26.2, 26.1, 24.82, 24.79, 24.4, 24.3, 18.9, 18.8, −4.6, −4.7; ^{31}P NMR (CD_3CN) δ −0.04, −0.69; IR (KBr film), 3436, 2943, 1684, 1625, 1578, 1502, 1484, 1349, 1249, 1179, 1032, 832, 791 cm^{-1}; HRMS (M + Na) calcd for $C_{59}H_{73}N_8O_{17}NaSiP$ 1247.4493, found 1247.4490.

Preparation of 13. Compound **12** (0.919 g, 0.75 mmol) was reduced by hydrogen (80 psi) in the presence of palladium on carbon (10%, 827 mg) and diisopropylethylamine (0.749, 1.01 mL, 5.8 mmol) in THF (35 mL) for 2 h at 25 °C. The reaction mixture was cooled to 0 °C, whereupon acetic formic anhydride (0.194 mL, 2.21 mmol) and pyridine (0.235 g, 0.241 mL, 3.0 mmol) were added. After 10 min at 0 °C, the reaction mixture was filtered through Celite. After concentration, the residue was chromatographed (3−5% methanol in CH_2Cl_2) to afford **13** (431 mg, 47%): ^{1}H NMR (CD_3OD) δ 8.15−8.20 (m, 1.8 H), 7.88 (s, 0.1 H), 7.85 (s, 0.1 H), 7.04−7.35 (m, 12 H), 6.83 (m, 6 H), 6.7 (m, 0.2 H), 6.07−6.19 (m, 1.8 H), 5.06 (m, 0.8 H), 4.71 (s, 2 H), 4.33−4.41 (m, 2 H), 3.99−4.10 (m, 3 H), 3.68−3.74 (m, 9 H), 3.42−3.50 (m, 2 H), 2.85−2.88 (m, 1 H), 2.76−2.80 (m, 1 H), 2.45−2.48 (m, 1 H), 2.31−2.36 (m, 1 H), 1.97 (s, 3 H), 1.93 (m, 1 H), 1.16 (d, 3 H, J = 2.0 Hz), 1.14 (d, 3 H, J = 2.0 Hz), 0.91 (d, 9 H, J = 3.6 Hz), 0.11−0.12 (m, 6 H); ^{13}C NMR (CD_3OD) δ 210.1, 173.1, 172.9, 164.3, 163.9, 160.4, 160.3, 159.2, 157.6, 156.99, 157.00, 149.6, 145.74, 145.67, 145.6,

143.6, 136.6, 136.5, 136.4, 131.4, 131.3, 129.3, 129.0, 128.4, 128.2, 115.8, 115.7, 114.3, 98.0, 95.3, 88.8, 88.7, 88.5, 86.54, 86.47, 85.23, 85.16, 85.0, 83.3, 83.1, 78.8, 78.6, 74.4, 74.2, 69.11, 68.7, 68.4, 68.3, 63.5, 55.8, 55.6, 55.5, 41.2, 41.1, 34.5, 30.7, 26.38, 26.35, 26.3, 24.61, 24.56, 20.8, 18.9, −4.59, −4.62; ^{31}P NMR (CD_3OD) δ −2.04, −2.15, −2.26, −2.33; IR (KBr film), 3417, 2957, 1653, 1613, 1579, 1505, 1347, 1249, 1178, 1032, 829 cm^{-1}; HRMS (M + Na) calcd for $C_{60}H_{75}N_8O_{16}NaSiP$ 1245.4700, found 1245.4758.

Preparation of 14. Acetic acid (29 mg, 0.028 mL, 0.48 mmol) and $Bu_4NF{\cdot}3H_2O$ (0.325 g, 1.03 mmol) were added to a solution of **13** (244 mg, 0.20 mmol) in THF (9 mL) at 0 °C. The reaction mixture was stirred for 4.5 h at this temperature, evaporated to dryness, and chromatographed (5−9% methanol in CH_2Cl_2) to give **14** (155 mg, 70%): ^{1}H NMR (CD_3OD) δ 8.16−8.22 (m, 1.8 H), 7.91 (s, 0.1 H), 7.85 (s, 0.1 H), 7.03−7.34 (m, 13 H), 6.83 (m, 5 H), 6.14−6.18 (m, 1 H), 6.08 (m, 0.4 H), 6.02 (m, 0.6 H), 5.03 (m, 1 H), 4.70 (m, 2 H), 4.36−4.40 (m, 1 H), 4.25 (m, 1 H), 4.01−4.12 (m, 3 H), 3.70−3.74 (m, 6 H), 3.68−3.70 (m, 3 H), 3.47 (m, 2 H), 2.84−2.90 (m, 1 H), 2.74−2.79 (m, 1 H), 2.45−2.46 (m, 1 H), 2.12 (m, 1 H), 1.97 (s, 3 H), 1.95 (m, 1 H), 1.16 (dd, 6 H, J = 2.0, 6.8 Hz); ^{13}C NMR (CD_3OD) δ 175.9, 173.1, 172.9, 169.3, 164.2, 163.8, 160.4, 160.3, 159.3, 159.2, 157.6, 156.97, 156.99, 149.6, 145.8, 145.7, 143.6, 138.9, 136.5, 136.4, 131.4, 131.3, 129.9, 129.3, 129.2, 129.0, 128.5, 128.4, 128.2, 126.3, 115.8, 115.7, 114.3, 98.0, 95.2, 88.9, 88.7, 88.45, 86.48, 85.1, 84.9, 84.8, 84.7, 83.3, 83.2, 78.9, 78.7, 72.8, 72.7, 69.5, 69.2, 68.3, 63.6, 55.8, 55.6, 55.5, 55.4, 54.0, 41.1, 40.6, 40.3, 34.5, 30.7, 26.8, 24.6, 21.5, 21.2, 20.9, 13.9; ^{31}P NMR (CD_3OD) δ −2.14; IR (KBr film), 3389, 3224, 2954, 1654, 1584, 1501, 1349, 1243, 1173, 1031, 832 cm^{-1}; HRMS (M + Na) calcd for $C_{54}H_{61}N_8O_{16}NaP$ 1131.3825, found 1131.3813.

Preparation of 15. Alcohol substrate **14** (100 mg, 0.090 mmol) was azeotroped by using THF (2 × 0.5 mL) and dried under high vacuum. 2-Cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite (26.5 mg, 0.025 mL, 0.11 mmol) was added to a solution of azeotropically dried **14** and diisopropylethylamine (30 mg, 0.040 mL, 0.23 mmol) in THF (0.9 mL) at −78 °C. The reaction mixture was allowed to warm to room temperature by removing the cold bath, stirred for 10 min, and checked by TLC. The solution was recooled to −78 °C, and more 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite (26.5 mg, 0.025 mL, 0.111 mmol) was added. Thereafter, the addition of diisopropylethylamine (9.6 mg, 0.013 mL, 0.074 mmol) and 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite (8.8 mg, 0.0083 mL, 0.037 mmol) was repeated one more time in order to achieve more than 95% conversion of **14**. The reaction was quenched by adding brine (1 mL), and the resulting mixture was extracted with THF and dried over $MgSO_4$. After removing the THF under high vacuum, the residue was chromatographed (2−5% methanol in CH_2Cl_2) to afford **15** (72 mg, 61%): ^{1}H NMR (CD_3CN) δ 11.43 (bd s, 1 H), 9.58 (bd s, 1 H), 8.13−8.21 (m, 1.6 H), 7.89−7.94 (m, 0.3 H), 7.81 (s, 0.1 H), 7.75 (s, 0.2 H), 7.70 (s, 0.2 H), 7.07−7.44 (m, 13 H), 6.86 (m, 6.4 H) 6.42 (m, 1 H), 6.01−6.07 (m, 2 H), 4.77−5.12 (m, 3 H), 4.04−4.43 (m, 5 H), 3.69−3.74 (m, 11 H), 3.34−3.41 (m, 2 H), 2.31−2.84 (m, 7 H), 1.92−2.12 (m, 2 H), 2.08 (s, 3 H), 1.14−1.16 (m, 18 H); ^{31}P NMR (CD_3CN) δ 147.71, 147.53, 147.43, 147.38, 147.18, 147.04, −2.41, −2.50, −2.57, −2.61, −2.88, −2.94, −2.99; HRMS (M + Na) calcd for $C_{64}H_{78}N_8O_{18}NaP_2$ 1331.4802, found 1331.4874.

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Supporting Information Available: Experimental procedures for the synthesis of 2-amino-6-chloro-4-hydroxy-5-nitropyrimidine, oligonucleotide synthesis, preparation of samples for ESI-MS, and Fpg cleavage of **22**; NMR spectra of **5**−**15**; ESI-MS of **16**−**21**; X-ray crystal structure of **7a** in CIf format; and phosphorimage autoradiogram for Fpg cleavage of **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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