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Preparation and Analysis of Oligonucleotides Containing the C4′-Oxidized Abasic Site and Related Mechanistic Probes

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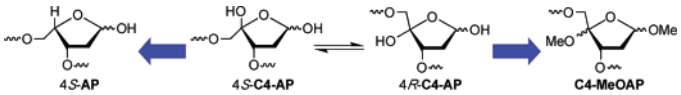
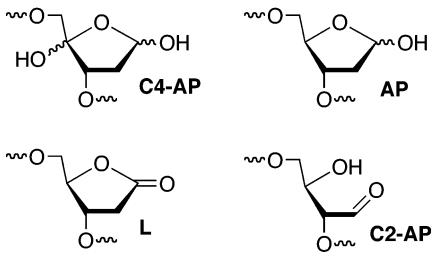


The C4′-oxidized abasic site (C4-AP) is produced by a variety of DNA damaging agents. This alkali labile lesion can exist in up to four diastereomeric cyclic forms, in addition to the acyclic keto-aldehyde. Synthetic oligonucleotides containing the lesion were prepared from a stable photochemical precursor. Chemical integrity of the lesion containing oligonucleotides was probed using phosphodiesterase lability. Analysis of the 3′,5′-phosphate diester of the monomeric lesion released from single diastereomers of photolabile precursors by ^{1}H NMR indicates that isomerization of the hemiacetal and/or hemiketal is rapid. The syntheses and characterization of oligonucleotides containing configurationally stable analogues of C4-AP, which serve as mechanistic probes for deciphering the structural basis of the biochemical and biological effects of the C4′-oxidized abasic lesion, are also described.

Introduction

The C4′-hydrogen atom of a nucleotide forms one of the two weakest carbon−hydrogen bonds in the 2′-deoxyribose component of DNA.[1,2] This relatively labile atom is readily accessible to diffusible species and minor groove binding molecules that oxidize DNA.[3] Consequently, the C4′-oxidized abasic site (C4-AP) is often formed from abstraction of the C4′-hydrogen atom.[4,5] However, the lesion is formed in highest yield (∼40%) when DNA is damaged by the antitumor agent bleomycin.[6−8] It was difficult to examine the effects of the C4-AP lesion on DNA replication and its repair until recently. In the past two years, two methods for preparing oligonucleotides containing C4-AP at a defined site were reported.[9,10] The results of biochemical and biological experiments using these oligonucleotides have provided motivation to prepare related molecules designed to probe the structural basis of C4-AP's effects on DNA repair and replication. The synthesis and physical characterization of oligonucleotides containing the C4-AP lesion and related probes are described herein.



Studies using synthetic oligonucleotides have shown that C4-AP incision by phosphodiesterases present in *Escherichia coli* is as efficient as that of a typical abasic site (AP).[11] Replication of C4′-oxidized abasic site by the Klenow exo$^-$ fragment of DNA polymerase I is also

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(1) Miaskiewicz, K.; Osman, R. *J. Am. Chem. Soc.* **1994**, *116*, 232−238.
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(8) Rabow, L. E.; McGall, G. H.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 3203−3208.
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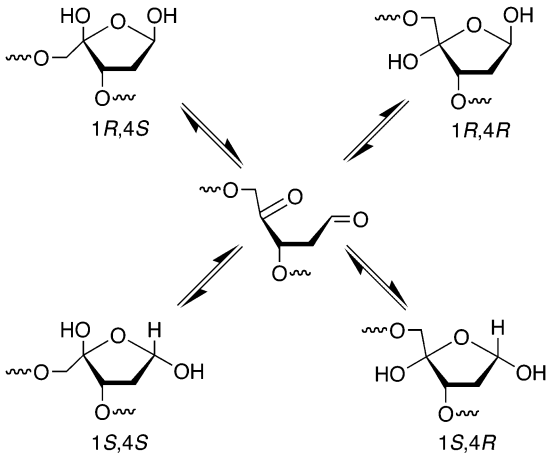
SCHEME 1. Isomerization of the C4′-Oxidized Abasic Site



similar to an AP site.[12] However, single-stranded shuttle vector studies in *E. coli* revealed that replication of the C4′-oxidized abasic template site is unique.[13] Replication of a template containing this lesion, which is smaller than a native nucleotide, produces three nucleotide deletions. The three nucleotide deletion products are formed exclusively when the *E. coli* are exposed to conditions that up regulate the three DNA polymerases typically associated with bypassing DNA damage. Replication of templates containing other abasic lesions (AP, L) does not give rise to the formation of three nucleotide deletion products.[14,15] In general, this is an unusual occurrence, which is seldom observed in the replication of even bulky DNA lesions, and then only in limited sequence contexts.

A structural explanation for the formation of three nucleotide deletions from the replication of plasmids containing C4-AP has not been formulated. However, the C4-AP and AP lesions differ only by the substitution of a hydrogen atom by a hydroxyl group in the former. We suggest that the 4-hydroxyl group must play a role in replication, perhaps via hydrogen bonding with the polymerase(s). The additional hydroxyl group may also affect replication indirectly by introducing configurational lability at the C4-position (Scheme 1). C4-AP can exist as a mixture of four cyclic isomers and the acyclic ketoaldehyde. However, studies on AP suggest that the latter should be present in very small (≤1%) amounts.[16] The ability of C4-AP to epimerize at C4 raises the possibility that the lesion could significantly alter the trajectory of the DNA backbone. We describe the synthesis and characterization of oligonucleotides designed to probe the role of the C4-hydroxyl group in the replication of the C4′-oxidized abasic lesion.

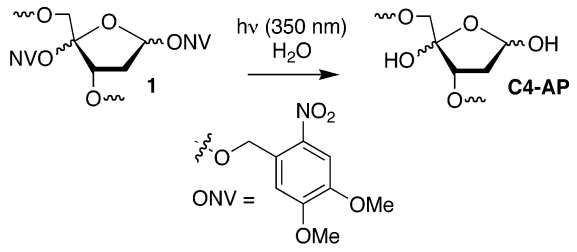
Results and Discussion

Photochemical Generation of the C4′-Oxidized Abasic Lesion (C4-AP). The C4-AP lesion is generated from the thermally stable precursor (**1**) upon UV irradiation (350 nm). The lesion is released using the venerable *ortho*-nitrobenzyl photoredox reaction. This photochemical reaction enjoys wide application ranging from organic synthesis to biomolecule caging and plays a prominent role in the preparation of oligonucleotide microarrays.[17−22] We typically employ a Rayonet Photoreactor equipped with lamps that have maximum emission at 350 nm (2.4 mW/cm^2) and less than 1% relative fluence at 300 nm to carry out these reactions. The photochemical conversion of **1** to a NaOH (0.1 M) labile lesion in a 30-nucleotide duplex (**2a**−**d**) is independent of the opposing nucleotide (Table 1). Greater than 93% conversion is achieved in 30 min. This is not surprising, for although the mechanistic details describing the release of molecules following the photochemical reaction have been discussed in the literature, the initial photochemical process is believed to involve a singlet excited state with a several nanosecond-long lifetime.[23−25] Quenching of short-lived singlet excited states is inefficient, which is consistent with the independence of the reaction on opposing nucleotide.

TABLE 1. Effect of Opposing Nucleotide on the Photochemical Conversion of 1 in 2

	% cleavage upon NaOH treatment[a]	
opposing nucleotide	before hν	after hν[b]
dA	1.1 ± 0.1	94.2 ± 0.6
dG	1.1 ± 0.3	96.2 ± 0.9
dC	1.0 ± 0.1	94.5 ± 1.9
dT	2.1 ± 0.6	93.8 ± 0.9

[a] NaOH (0.1 M) at 37 °C for 30 min. [b] Rayonet photoreactor (λ_{max} = 350 nm), 30 min.



5′-d(GTC ACG TGC TGC A**X**A CGA CGT GCT GAG CCT)
3′-d(CAG TGC ACG ACG T**Y**T GCT GCA CGA CTC GGA)

2 X = **1** **2**,**3a** Y = A
3 X =C4-AP **2**,**3b** Y =G
2,**3c** Y = C
2,**3d** Y =T

(12) Greenberg, M. M.; Weledji, Y. N.; Kroeger, K. M.; Kim, J.; Goodman, M. F. *Biochemistry* **2004**, *43*, 2656−2663.
(13) Kroeger, K. M.; Kim, J.; Goodman, M. F.; Greenberg, M. M. *Biochemistry* **2004**, *43*, 13621−13627.
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(21) Pirrung, M. C. *Angew. Chem., Int. Ed.* **2002**, *41*, 1276−1289.
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(24) Il'ichev, Y. V.; Schwörer, M. A.; Wirz, J. *J. Am. Chem. Soc.* **2004**, *126*, 4581−4595.
(25) Corrie, J. E. T.; Barth, A.; Munasinghe, V. R. N.; Trentham, D. R.; Hutter, M. C. *J. Am. Chem. Soc.* **2003**, *125*, 8546−8554.

TABLE 2. Probing the Integrity of the Photochemical Conversion of 1 (2a) to C4-AP Using Endonuclease IV

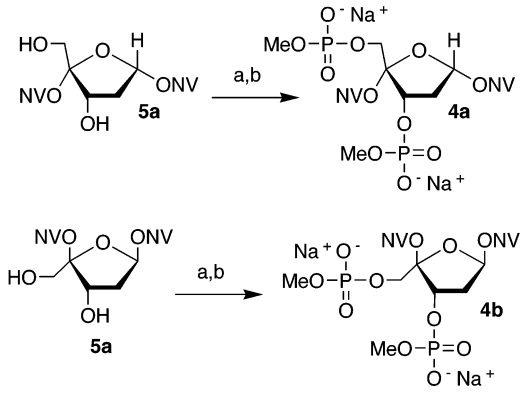
	% cleavage	
treatment	before hν	after hν[b]
background (none)	0.6 ± 0.2	6.2 ± 0.4
NaOH[a]	2.7 ± 2.8	95.6 ± 3.1
endonuclease IV	1.3 ± 0.7	95.0 ± 0.7

[a] NaOH (0.1 M) at 37 °C for 30 min. [b] Rayonet photoreactor (λ_{max} = 350 nm), 30 min.

The integrity of C4-AP containing oligonucleotides prepared in this way has been examined using ESI-MS.[9,13] Two species that differ by 18 atomic mass units are detected in a ratio that is dependent upon the temperature of the inlet source. These ions are attributed to the cyclic and acyclic (keto-aldehyde) forms of C4-AP (Scheme 1). The integrity of a 30-nucleotide-long oligonucleotide duplex containing C4-AP (**3a**) was also probed using the type II *E. coli* base excision repair enzyme endonuclease IV (Endo IV) (Table 2). We previously reported that C4-AP is efficiently incised by endonuclease IV.[11] In the current situation, we used the enzyme as a reagent to probe the integrity of the C4-AP containing DNA. Treatment of 5′-^{32}P-**2a** (10 nM) with Endo IV (50 nM) or NaOH (0.1 M) showed that the C4-AP precursor (**1**) is not incised by the enzyme. In contrast, the amount of cleavage by NaOH or endonuclease IV measured after irradiation (**3a**) was within experimental error of one and other. This suggests that if **2a** is converted into a product(s) other than C4-AP (**3a**), the undesired product is also a substrate for endonuclease IV, whereas the photochemical precursor is not. A simpler explanation, which is consistent with all observations, is that the precursor that is converted to NaOH labile material is transformed into C4-AP exclusively.

Epimerization of the C4′-Oxidized Abasic Lesion. The original report describing the photochemical synthesis of oligonucleotides containing C4-AP advocated using mixtures of stereoisomers. It was assumed that the configurational composition of the lesion in DNA would rapidly equilibrate to the appropriate thermodynamic mixture dictated by the duplex. This assumption was based upon the half-lives for pyranose mutarotation in water, which are approximately 30 min.[26,27] Moreover, the respective half-life for the epimerization of the furanose form of ribose is considerably faster.[28] Despite the indication that C4-AP should readily adopt its thermodynamically preferred distribution of isomers in DNA, we sought evidence to support this supposition. Individual diastereomers of bis-phosphate diester **4a**,**b** were synthesized from the previously reported diols (**5a**,**b**, Scheme 2).[9] The individual phosphate triesters were prepared by phosphitylation and in situ oxidation. The purified phosphate triesters (**6a**,**b**) were deprotected using sodium iodide, and the desired substrates were purified by reverse-phase HPLC as their triethylammonium salts, followed by exchange with sodium using Dowex resin. Diastereomers **4a** and **4b** were readily distinguished by a significant upshield shift of the C1-proton in the latter, which is occluded by the residual water peak.[29] Solutions (D_2O) of individual diastereomers of **4** (0.1 mM) were photolyzed in a Rayonet photoreactor (20 min) and immediately analyzed by ^{1}H NMR (with water suppression). Dilute solutions were irradiated, despite the fact that obtaining NMR data was more difficult, to mimic conditions in which oligonucleotides are photolyzed and to minimize bimolecular interactions. Although the spectra were obtained with differing single-to-noise ratios, analysis of the region where the C1-protons resonate (5.30−5.75 ppm) indicated that each diastereomer of **4** was converted into the same mixture of **7**. This was evident by examining the ratio of peak intensities in the region where the C1-proton of **7** was expected to resonate (Figure 1). This experiment supports the supposition that stereoisomer distribution

SCHEME 2. Synthesis of a Bis-phosphate Diester Model for C4-AP Generation[a]



[a] (a) i. Dimethoxy *N*,*N*-diisopropylphosphoramidite, tetrazole, CH_3CN; ii. *t*-BuOOH, decane. (b) NaI, acetone

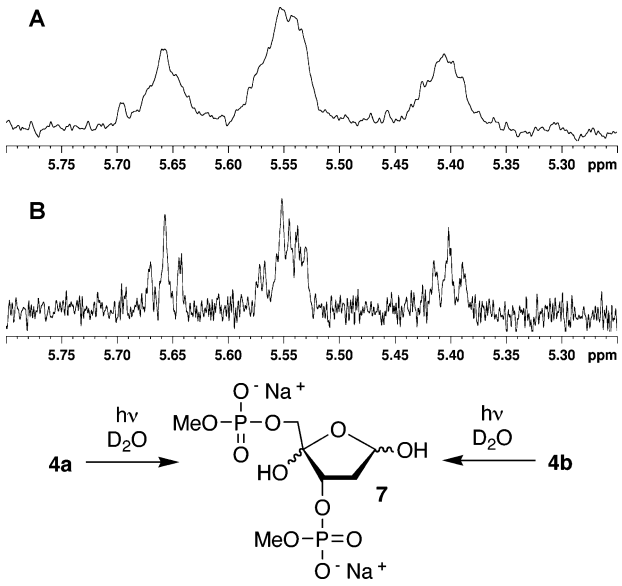
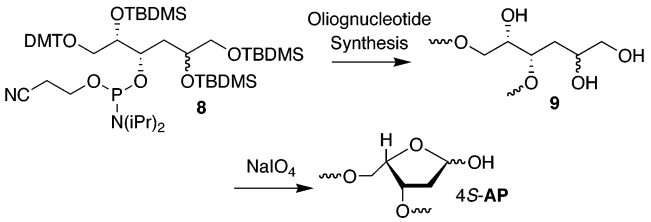


FIGURE 1. ^{1}H NMR analysis following photolysis of individual diastereomers of **4**. (A) **4a**. (B) **4b**.

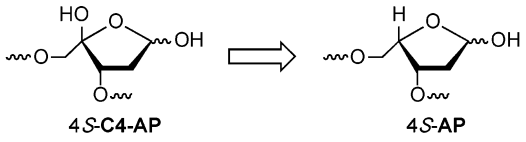
(26) Moriyasu, M.; Kato, A.; Okada, M.; Hashimoto, Y. *Anal. Lett.* **1984**, *17*, 1533−1538.
(27) Panov, M. Y.; Sokolova, O. B. *Russ. J. Gen. Chem.* **2004**, *74*, 1451−1453.
(28) Ryu, K.-S.; Kim, C.; Park, C.; Choi, B.-S. *J. Am. Chem. Soc.* **2004**, *126*, 9180−9181.
(29) See Supporting Information.

SCHEME 3. Strategy for the Synthesis of Oligonucleotides Containing 4*S*-AP



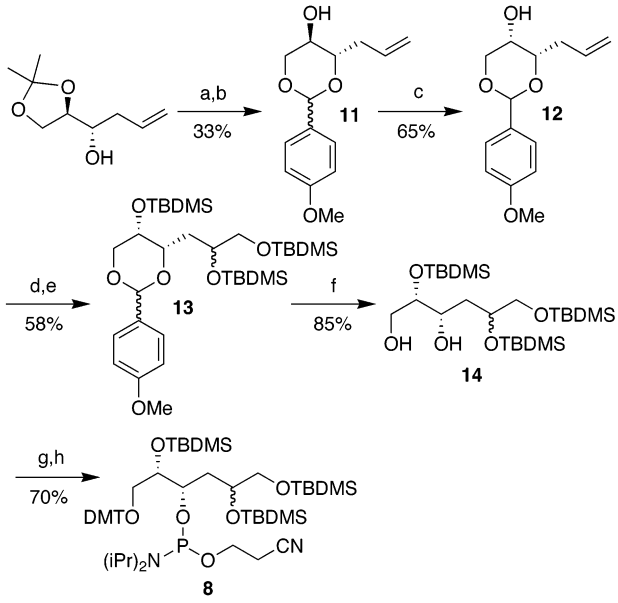
in a C4-AP precursor (e.g., **2**) is unimportant, because the lesion will readily adopt the distribution of isomers dictated by its environment in duplex DNA provided the neighboring nucleotides do not impose a kinetic barrier.

Synthesis of Oligonucleotides Containing Analogues of C4-AP. The configurational lability of the C4′-oxidized abasic site, particularly at the C4-position, raises the question of whether the lesion's extraordinary effect on replication in *E. coli* is attributable to the 4*S*-configuration and/or the presence of a hydroxyl group at this position. We are using configurationally stable analogues of C4-AP to investigate these issues. The 4*S*-epimer of an AP site (4*S*-AP) lesion was designed as a configurationally stable probe to investigate the effect of an altered backbone, which could be introduced into DNA by the C4′-oxidized abasic lesion independent of the hydrogen bonding hydroxyl group. We previously reported that the 4*S*-AP did not give rise to three nucleotide deletions upon replication in *E. coli*, but did not describe the synthesis of oligonucleotides containing this useful probe.[13]



Oligonucleotides containing 4*S*-AP were prepared using the strategy employed by Johnson and Kim in their syntheses of DNA containing AP and C2′-oxidized AP sites, respectively.[30,31] In these syntheses, the alkali labile lesions were introduced following purification of an oligonucleotide containing an appropriate vicinal diol (**9**) using $NaIO_4$. Applying this approach to synthesizing oligonucleotides containing 4*S*-AP required that we prepare **8** (Scheme 3). Phosphoramidite **8** was prepared starting from the product obtained from Lewis acid mediated allyl addition to D-glyceraldehyde (Scheme 4).[32] The acetonide was converted into the 1,3-protected triol (**11**), which was previously prepared by a different method.[33] Preparation of the benzylidene acetal allowed us to invert the stereochemistry at the carbon that ultimately becomes C4. Following Mitsunobu inversion, **12** was osmylated and the crude diastereomeric mixture of triols was persilylated. Phosphoramidite **8** was obtained following hydrogenolysis via dimethoxytritylation of the primary alcohol and phosphitylation.

SCHEME 4. Synthesis of the Phosphoramidite Required for Preparing Oligonucleotides Containing 4*S*-AP[a]

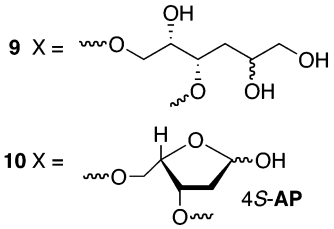


[a] (a) Aqueous acetic acid (75%). (b) *p*-Anisaldehyde dimethyl acetal, camphorsulfonic acid, CH_2Cl_2. (c) i. Diethyl azo dicarboxylate, triphenylphosphine, *p*-nitrobenzoic acid, THF; ii. LiOH, H_2O/THF. (d) OsO_4, NMO, *t*-BuOH, H_2O. (e) *tert*-Butyldimethysilyl triflate, 2,6-lutidine, CH_2Cl_2. (f) $Pd(OH)_2$/C, H_2, EtOH. (g) Dimethoxytriyl chloride, pyridine. (h) *N*,*N*-Diisopropyl 2-cyanoethyl phosphoramidic chloride, diisopropylethylamine, CH_2Cl_2.

5′-d(GAA GAC CC**X** GGC GTC C)
9a, **10a**

5′-d(GAA GAC CT**X** GGC GTC C)
9b, **10b**

5′-d(GTC ACG TGC A**X**A CGA CGT GCT GAG CCT)
9c, **10c**



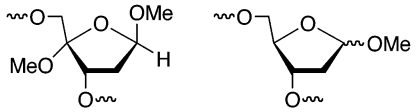
The triol containing DNA (**9**) was obtained using standard solid-phase oligonucleotide synthesis methods and commercially available fast deprotecting phosphoramidites, with the exception of coupling **8**. Extending the reaction time for the protected triol to 5 min provided >75% coupling. Following deprotection and cleavage from the resin using concentrated aqueous ammonia (2 h, 55 °C), the silyl protecting groups were removed using $Et_3N{\cdot}3HF$ in *N*-methylpyrrolidine (3 h, 65 °C).[34] The triol containing oligonucleotides (**9a**−**c**) were purified by denaturing PAGE and characterized by ESI-MS. The 4*S*-AP lesion was introduced on an as needed basis (nanomole scale) by treating a 0.1 M sodium acetate solution (pH 6.0) for 30 min with sodium periodate

(30) Shishkina, I. G.; Johnson, F. *Chem. Res. Toxicol.* **2000**, *13*, 907−912.

(31) Kim, J.; Weledji, Y.; Greenberg, M. M. *J. Org. Chem.* **2004**, *69*, 6100−6104.

(32) Almendros, P.; Thomas, E. J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2561−2568.

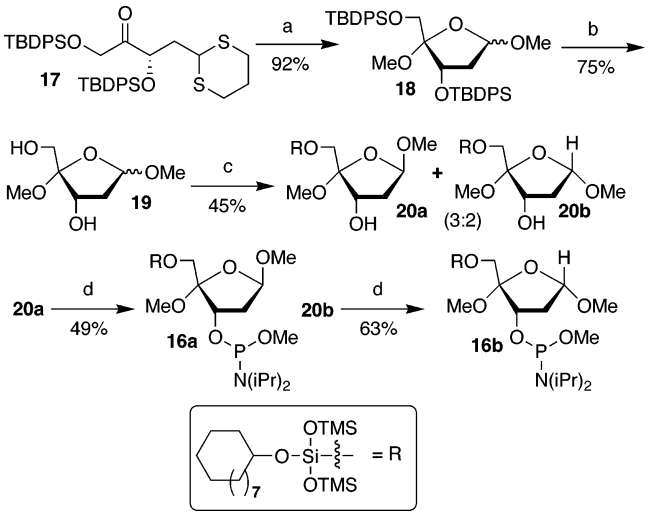
(5 mM). Following desalting, no further purification of **10a**−**c** was necessary, as evidenced by ESI-MS analysis.



C4-MeOAP **MeOAP**

The dimethoxy abasic site (C4-MeOAP) was envisioned to act as a configurationally stable analogue of the C4′-oxidized abasic site, which retains the oxidation state of C4-AP. Oligonucleotides containing the methoxy analogue of an AP site (MeOAP) have been prepared using standard solid-phase oligonucleotide synthesis conditions.[35] However, C4-MeOAP is unstable to the acidic detritylation conditions. Consequently, we employed the strategy used for synthesizing oligonucleotides containing C4-AP, which takes advantage of the 5′-silyloxy protecting groups to prepare the biopolymer without exposing it to acid following incorporation of the labile component.[9,36] Implementation of this approach required that we synthesize phosphoramidites **16a**,**b**, which was accomplished in a manner similar to that used for synthesizing oligonucleotides containing C4-AP (Scheme 5).

SCHEME 5. Synthesis of the Phosphoramidite Required for Preparing Oligonucleotides Containing C4-MeOAP[a]

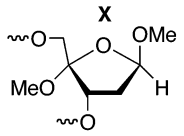
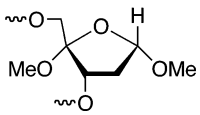


[a] (a) NBS, MeOH, CH_3CN. (b) TBAF·hydrate, THF. (c) Cyclododecyloxy bis-trimethylsilyloxy chloride, pyridine, DMAP, CH_2Cl_2. (d) *N*,*N*-Diisopropyl methyl phosphoramidic chloride, diisopropylethylamine, CH_2Cl_2.

Cyclization of in situ deprotected **17** in the presence of methanol produced silylated C4-MeOAP (**18**) as a mixture of isomers.[9] Separation of the two major stereoisomers (~83% of the mixture) from the minor isomers was achieved upon desilylation. The mixture of the two major isomers of **19** was silylated, and **20a** and **20b** were separated from one another as well as from bis-silylated materials.

TABLE 3. UV Melting Temperatures of Duplexes Containing the Stereoisomers of C4-MeOAP[a]

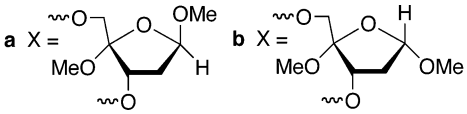
5′-d(GAA GAC CG**X** CGC GTC C)
3′-d(CTT CTG GCA GCG CAG G)

X	T_M (°C)
	59.5 ± 0.6
	59.2 ± 0.5

[a] [Duplex] = 2.2 μM.

Stereochemical assignments were made based upon ROSEY analysis of the mixture of the two major isomers of **19** and comparison of the ^{1}H NMR spectra of **20a**,**b**. The chemical shifts of the C3 and C5 protons in **20a**,**b** are very similar to one another and suggest that the stereochemistry at C3 and C4 is the same. The ^{1}H NMR spectra of **20a** and **20b** are most readily distinguished from one another in the regions where the C1 and C2 protons resonate. The chemical shifts of anomeric protons are often very sensitive to structural variation. In **20a**,**b**, the chemical shifts of the diastereotopic C2 protons are very similar to one another in the major isomer (**20a**) but are separated by ~0.5 ppm in the minor stereoisomer. The large difference in C2-proton chemical shifts in **20b** is ascribed to the cis relationship of the pro-*R* proton to the C1 and C3 oxygen substitutents, whereas each C2-proton in **20a** is cis to one of the oxygen containing substituents and the protons exhibit similar chemical shifts. The individual isomers of **20** were phosphitylated and purified. Care must be taken during chromatography, as the phosphoramidites (**16a**,**b**) are relatively nonpolar and are not detectable using UV light.

5′-d(GAA GAC CG**X** CGC GTC C)
21a,**b**



Oligonucleotide synthesis (**21a**,**b**) was carried out using typical dimethoxytrityl protected phosphoramidites until C4-MeOAP is incorporated, because the coupling efficiency of **16a** and **16b** as well as the corresponding silyloxy protected phosphoramidites of the native nucleotides cannot be monitored. Automated synthesis was paused prior to introduction of **16**, at which time the reagents were switched, and the remainder of **21a**,**b** was synthesized using the method previously described for oligonucleotides containing C4-AP.[9] Deprotection was also carried out as described. The UV melting temperatures of the respective duplexes were measured at 2.2 μM (Table 3). The T_M's of the two diastereomers were within experimental error of one another. Furthermore,

(33) Furstner, A.; Schlede, M. *Adv. Synth. Catal.* **2002**, *344*, 657−665.
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(35) McCullough, A. K.; Sanchez, A.; Dodson, M. L.; Marapaka, P.; Taylor, J.-S.; Lloyd, R. S. *Biochemistry* **2001**, *40*, 561−568.
(36) Scaringe, S. A.; Wincott, F. E.; Caruthers, M. H. *J. Am. Chem. Soc.* **1998**, *120*, 11820−11821.

they were very similar to those reported for C4-AP and the tetrahydrofuran analogue of an abasic site in otherwise identical duplexes, but distinct from the 4*S*-AP containing DNA.[13] The latter indicates that the configuration at C4 of C4-MeOAP was *R* in each instance, as suggested above based upon analysis of the NMR data.

Experimental Methods

Preparation of 4a and 4b. Dimethoxy *N*,*N*-diisopropylphosphoramidite (43 mg, 0.22 mmol) was added to **5a**[9] (22 mg, 41 μmol) in an acetonitrile solution of tetrazole (0.5 mL, 0.45 M). A solution of *tert*-butyl hydroperoxide (5 M) in decane (0.1 mL) was added after being stirred for 30 min. After being stirred for 2 h, the mixture was concentrated under vacuum. The crude material was taken up in CH_2Cl_2 (10 mL) and washed with saturated aqueous $NaHCO_3$, water, and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (2% MeOH in CH_2Cl_2) to provide the bis-phosphate triester, **6a** (20 mg, 64%). R_f 0.31 (1:15 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1H), 7.56 (s, 1H), 7.39 (s, 1H), 6.95 (s, 1H), 5.38 (dd, J = 4.0, 5.6 Hz, 1H), 5.13 (d, J = 7.2 Hz, 2H), 5.00−4.89 (m, 3H), 4.28−4.18 (m, 2H), 3.93 (s, 6H), 3.91 (s, 3H), 3.83 (s, 3H), 3.80 (d, J = 1.6 Hz, 3H), 3.79 (d, J = 2.0 Hz, 3H), 3.78 (d, J = 2 Hz, 3H), 3.76 (d, J = 2.0 Hz, 3H), 2.84−2.77 (m, 1H), 2.45−2.38 (m, 1H); ^{31}P NMR ($CDCl_3$) δ 1.26, 0.77. The bis-phosphate triester was refluxed in acetone (2 mL) in the presence of excess NaI (178 mg, 1.19 mmol) for 6 h. The mixture was cooled to room temperature and evaporated to dryness. The residue was taken up in water and extracted with EtOAc. The water layer was concentrated, and the product (**4a**) was purified by reverse-phase HPLC (Waters μ-Bondpak, 7.8 × 25 cm; solvent A: 100 mM TEAA (pH 7.8); solvent B: 50% CH_3CN in 100 mM TEAA (pH 7.8); 0−100% B linearly over 30 min). The triethylammonium ion was then exchanged by passing over a Dowex-Na^+ column to provide **4a** (6 mg). ^{1}H NMR (D_2O, water suppression) δ 7.45 (s, 1H), 7.44 (s, 1H), 7.08 (s, 1H), 6.75 (s, 1H), 5.66 (dd, J = 4.4, 7.0 Hz, 1H), 4.89−4.56 (m, 5H), 4.22−4.08 (m, 2H), 3.84 (s, 6H), 3.81 (s, 3H), 3.71 (s, 3H), 3.61 (d, J = 10.8 Hz, 3H), 3.56 (d, J = 10.8 Hz, 3H), 2.74−2.68 (m, 1H), 2.51−2.45 (m, 1H); ^{31}P NMR (D_2O) δ 1.25, 0.18. ESI-MS (M − H) calcd for $C_{25}H_{34}N_2O_{19}P_2$, 727.48; found, 727.27.

Diastereomeric **4b** was prepared from **5b** on the same scale in identical manner. **6b**: ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 1H), 7.51 (s, 1H), 7.22 (s, 1H), 6.84 (s, 1H), 5.60 (t, J = 4.8 Hz, 1H), 5.06−4.92 (m, 5H), 4.26 (dd, J = 6.4, 10.8 Hz, 1H), 4.21 (dd, J = 5.6, 11.2 Hz, 1H), 3.90 (s, 6H), 3.70 (d, J = 2.0 Hz, 3H), 3.69 (d, J = 2.0 Hz, 3H), 2.69−2.63 (m, 1H), 2.52−2.46 (m, 1H); ^{31}P NMR ($CDCl_3$) δ 1.2, 0.4. **4b**: ^{1}H NMR (D_2O, water suppression) δ 7.72 (s, 1H), 7.55 (s, 1H), 7.05 (s, 1H), 6.99 (s, 1H), 4.98−4.50 (m, 6H), 4.20−4.05 (m, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.57 (d, J = 10.8 Hz, 3H), 3.54 (d, J = 10.8 Hz, 3H), 2.53−2.48 (m, 1H), 2.23−2.20 (m, 1H); ^{31}P NMR (D_2O) δ 1.23, 0.03. ESI-MS (M − H) calcd for $C_{25}H_{34}N_2O_{19}P_2$, 727.48; found, 727.13.

Preparation of 11. To a −78 °C solution of allyl tributyltin (6.29 g, 19.0 mmol) in CH_2Cl_2 (30 mL) under an Ar atmosphere was added a solution of $SnBr_4$ (8.08 g, 18.4 mmol) in CH_2Cl_2 (20 mL).[31] After 30 min, D-glyceraldehyde acetonide (2.00 g, 15.4 mmol) in CH_2Cl_2 (30 mL) was added via cannular. After 1.5 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, warmed to room temperature, and stirred for an additional 1 h. The reaction mixture was diluted with EtOAc. The organic layer was washed with saturated NH_4Cl, saturated $NaHCO_3$, and brine, and then dried over Na_2SO_4. The solution was filtered and concentrated on a rotary evaporator. The residue was purified by chromatography (10−33% EtOAc in hexanes) to yield the alcohol. The alcohol was hydrolyzed in 75% aqueous acetic acid at 60 °C for 3 h, cooled to room temperature, and concentrated. The crude was purified by chromatography (1−5% MeOH in CH_2Cl_2) to give the triol (1.13 g, 55.5%). ^{1}H NMR (acetone-d_6 with TMS) δ 5.99−5.89 (m, 1H), 5.11−4.99 (m, 2H), 3.90 (d, J = 5.2 Hz, 1H), 3.83−3.47 (m, 6H), 2.48−2.42 (m, 1H), 2.25−2.17 (m, 1H); ^{13}C NMR (acetone-d_6 with TMS) δ 136.7, 116.7, 74.9, 73.0, 64.5, 38.6.

To a solution of the triol (1.10 g, 8.32 mmol) in CH_2Cl_2 (30 mL) was added camphorsulfonic acid (0.20 g, 0.86 mmol) and *p*-anisaldehyde dimethyl acetal (3.10 g, 17.0 mmol). After 18 h, the reaction mixture was neutralized by addition of triethylamine, concentrated, and purified by flash chromatography (10−30% EtOAc in hexanes) to give the benzylidene alcohol **11** (1.23 g, 59.1%). R_f 0.34 (1:2 EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 7.41 (d, J = 8.4 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.04−5.94 (m, 1H), 5.42 (s, 1H), 5.22−5.11 (m, 2H), 4.20 (dd, J = 4.0, 10.4 Hz, 1H), 3.80 (s, 3H), 3.61−3.49 (m, 3H), 2.64−2.58 (m, 1H), 2.46−2.39 (m, 1H), 2.26 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ 159.9, 134.2, 130.2, 127.3, 117.4, 113.5, 100.7, 81.0, 70.9, 65.3, 55.2, 36.4; IR (film) 3446, 3076, 2910, 1614, 1519, 1255, 1081, 827 cm^{-1}; HRMS (EI) M − H^+ calcd, for $C_{14}H_{17}O_4$ 249.1127; found, 249.1137.

Preparation of 12. Diethyl azo dicarboxylate (0.25 g, 1.43 mmol) was added dropwise to a solution of Ph_3P (365 mg, 1.39 mmol), *p*-nitrobenzoic acid (230 mg, 1.38 mmol), and **11** (230 mg, 0.92 mmol) in THF (9 mL) at −45 °C. The mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was partitioned with ether and H_2O, extracted with ether, washed with brine, and dried over Na_2SO_4. The organics were filtered, concentrated, and purified by column chromatography (2−10% EtOAc in hexanes) to afford the inverted *p*-nitrobenzoate derivative of **11** as a white solid (250 mg, 68.0%). R_f 0.68 (1:4 EtOAc/hexanes); mp 115 °C; ^{1}H NMR ($CDCl_3$) δ 8.30 (d, J = 8.8 Hz, 1H), 8.20 (d, J = 8.8 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 8.8 Hz, 1H), 5.96−5.89 (m, 1H), 5.55 (s, 1H), 5.14−5.04 (m, 3H), 4.50 (dd, J = 5.2, 10.4 Hz, 1H), 4.05−4.00 (m, 1H), 3.81 (s, 3H), 3.76 (t, J = 10.4 Hz, 1H), 2.54−2.44 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 163.5, 160.2, 150.7, 134.7, 133.0, 130.8, 129.7, 127.4, 123.6, 117.8, 113.6, 101.2, 78.4, 67.8, 55.3, 36.6; IR (KBr) 2866, 1720, 1616, 1529, 1310, 1274, 1123, 833 cm^{-1}; HRMS (FAB) M^+ calcd for $C_{21}H_{21}NO_6$, 400.1396; found, 400.1372.

The benzoate (250 mg, 0.63 mmol) was stirred with LiOH (20 mg, 0.84 mmol) in THF/H_2O (10:1, 6.6 mL) overnight. The reaction was quenched with aqueous NH_4Cl, extracted with ethyl acetate, washed with brine, and dried over Na_2SO_4. The filtrate was concentrated and purified by column chromatography (10−30% EtOAc in hexanes) to afford **12** (150 mg, 95%). R_f 0.28 (1:2 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.42 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 6.04−5.94 (m, 1H), 5.43 (s, 1H), 5.22−5.11 (m, 2H), 4.22 (dd, J = 4.4, 10.4 Hz, 1H), 4.19−4.10 (m, 1H), 3.80 (s, 3H), 3.64−3.58 (m, 2H), 3.53 (t, J = 10.0 Hz, 1H), 2.65−2.58 (m, 1H), 2.47−2.37 (m, 1H), 2.09 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ 159.9, 134.2, 130.2, 127.3, 117.4, 113.5, 100.8, 81.0, 70.9, 65.5, 55.2, 36.5; IR (film) 3439, 2840, 1614, 1518, 1251, 1079, 827 cm^{-1}; HRMS (EI) M^+ calcd for $C_{14}H_{18}O_4$, 250.1205; found, 250.1207.

Preparation of 13. A solution of OsO_4 (12 mg, 47.2 μmol) and *N*-methyl morpholine-*N*-oxide (141 mg, 1.2 mmol) in *t*-BuOH/H_2O (1:1, 6 mL) was added to **12** (150 mg, 0.60 mmol) at 0 °C. The mixture was allowed to warm to room temperature and was stirred overnight. Sodium bisulfite (1.0 g) was added and stirred for 30 min. The mixture was diluted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (5−10% MeOH in CH_2Cl_2) to afford the triol, which was carried on immediately to **13**. *tert*-Butyldimethylsilyl triflate (544 mg, 2.1 mmol) and 2,6-lutidine (286 mg, 2.7 mmol) were added to the triol in CH_2Cl_2 (10 mL) at 0 °C. After 1 h, the solution was poured into cold aqueous NH_4Cl, extracted with CH_2Cl_2, and washed with $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (2−5% EtOAc in hexanes) to afford a mixture of

two diastereomers of **13** (220 mg, 58.3% over two steps). R_f 0.65 (1:9 EtOAc/Hexanes); ^{1}H NMR ($CDCl_3$) δ 7.43−7.38 (m, 2H), 6.91−6.86 (m, 2H), 5.43 (s, 1H), 4.19−4.14 (m, 1H), 4.02−3.95 (m, 1H), 3.81 (s, 3H), 3.76−3.47 (m, 5H), 2.18−1.90 (m, 1H), 1.70−1.62 (m, 1H), 0.92 (s, 9H), 0.91 (s, 9H), 0.90 (s, 9H), 0.11 (s, 3H), 0.09 (s, 6H), 0.07 (s, 3H), 0.06 (s, 3H), 0.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 159.8, 130.6, 127.3, 113.5, 113.4, 100.6, 100.5, 79.2, 78.6, 71.9, 70.6, 68.9, 68.3, 68.0, 67.2, 66.8, 55.2, 37.1, 36.9, 26.0, 25.9, 25.8, 25.7, 18.4, 18.1, 18.0, 17.9, −3.9, −4.1, −4.3, −4.6, −4.7, −4.9, −5.3; IR (film) 2929, 2857, 1518, 1463, 1251, 1107, 1039, 836 cm^{-1}; HRMS (MALDI-TOF) M + Na^+ calcd for $C_{32}H_{62}O_6NaSi_3$, 649.3747; found, 649.3734.

Preparation of 14. To a solution of benzylidene **13** (160 mg, 0.26 mmol) in EtOH (6 mL) was added $Pd(OH)_2/C$ (20%, 50 mg) under H_2 (50 psi). After 0.5 h, the mixture was filtered through a Celite pad, washed with EtOH, and concentrated. The crude was purified by column chromatography (0−2% MeOH in CH_2Cl_2) to afford a mixture of two diastereomers of **14** (110 mg, 84.7%). R_f 0.31 (1:4 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 3.97−3.45 (m, 8H), 2.62−2.51 (m, 1H), 2.07−1.87 (m, 1H), 1.70−1.59 (m, 1H), 0.89 (s, 18H), 0.88 (s, 9H), 0.11−0.05 (m, 18H); ^{13}C NMR ($CDCl_3$) δ 75.2, 75.0, 73.0, 72.7, 72.1, 71.3, 67.5, 66.6, 65.1, 64.8, 37.9, 37.1, 25.9, 25.8, 25.7, 18.3, 18.0, 17.9, −4.2, −4.4, −4.5, −4.8, −5.0, −5.4; IR (film) 3445, 2956, 2858, 1464, 1256, 1091, 837 cm^{-1}; HRMS (FAB) M + H^+ calcd for $C_{24}H_{57}O_5Si_3$, 509.3514; found, 509.3525.

Preparation of 15. A solution of **14** (135 mg, 0.27 mmol) in dimethoxytrityl chloride (120 mg, 0.35 mmol) was stirred in pyridine (3 mL) overnight. The solution was quenched with MeOH (1 mL), concentrated, and purified by column chromatography (10−20% EtOAc in hexanes) to give **15** (200 mg, 93.2%). R_f 0.52 (1:4 EtOAc/Hexanes); ^{1}H NMR ($CDCl_3$) δ 7.46−7.17 (m, 9H), 6.82−6.80 (m, 4H), 4.00−3.90 (m, 2H), 3.79 (s, 6H), 3.76−3.72 (m, 1H), 3.61−3.40 (m, 2H), 3.21−3.05 (m, 2H), 2.97 (d, J = 3.2 Hz, 0.5H), 2.75 (d, J = 3.6 Hz, 0.5H), 1.75−1.44 (m, 2H), 0.88−0.86 (m, 27H), 0.09−0.03 (m, 18H); ^{13}C NMR ($CDCl_3$) δ 158.4, 144.9, 136.2, 136.1, 130.1, 128.2, 127.7, 126.6, 113.0, 86.3, 75.0, 72.3, 71.1, 70.6, 69.8, 67.5, 65.1, 55.1, 36.4, 35.9, 26.0, 25.9, 18.3, 18.0, −4.3, −4.4, −4.8, −4.9, −5.4; IR (film) 3518, 2954, 2857, 1608, 1510, 1464, 1252, 1082, 835 cm^{-1}; HRMS (MALDI-TOF) M + Na^+ calcd for $C_{45}H_{74}O_7NaSi_3$, 833.4635; found, 833.4625.

Preparation of 8. *N*,*N*-Diisopropyl 2-cyanoethyl phosphoramidic chloride (233 mg, 0.98 mmol) was added to a solution of **15** (160 mg, 0.20 mmol) and diisopropylethylamine (257 mg, 2.0 mmol) in CH_2Cl_2 (1.8 mL). After 7 h at room temperature, the mixture was diluted with EtOAc (80 mL) and washed with saturated aqueous $NaHCO_3$ (2×), H_2O, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude compound was purified by column chromatography (oven dried silica gel, 20% EtOAc in hexanes) to afford a diastereomeric mixture of **8** (150 mg, 75.1%). R_f 0.48 (1:4 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.46−7.17 (m, 9H), 6.85−6.79 (m, 4H), 4.32−4.03 (m, 2H), 3.93−3.51 (m, 6H), 3.79 (s, 6H), 3.42−3.35 (m, 1H), 3.15−3.03 (m, 2H), 2.59−2.39 (m, 2H), 1.89−1.34 (m, 2H), 1.19−1.03 (m, 12H), 0.91−0.84 (m, 27H), 0.13−0.05 (m, 18H); ^{13}C NMR ($CDCl_3$) δ 158.4, 144.0, 136.3, 136.1, 130.1, 130.0, 128.3, 128.2, 127.6, 126.6, 117.6, 117.5, 113.0, 86.4, 75.6, 74.8, 74.0, 70.5, 70.1, 67.9, 65.4, 65.2, 58.2, 58.0, 57.8, 57.6, 55.1, 43.2, 43.1, 43.0, 42.8, 37.1, 36.1, 35.4, 26.0, 25.9, 25.8, 24.8, 24.7, 24.6, 24.5, 20.2, 20.1, 18.4, 18.3, 18.1, 18.0, −3.7, −3.8, −3.9, −4.0, −4.1, −4.3, −4.4, −4.8, −4.9, −5.2, −5.3, −5.4; ^{31}P NMR ($CDCl_3$) δ 151.3, 151.0, 147.5, 147.1; IR (film) 2929, 2857, 1608, 1510, 1463, 1252, 1077, 834 cm^{-1}; HRMS (MALDI-TOF) M + Na^+ calcd for $C_{54}H_{91}N_2O_8NaSi_3P$, 1033.5713; found, 1033.5744.

Preparation of 18. *N*-Bromosuccinimide (1.09 g, 6.15 mmol) was added to a −10 °C solution of **17**[9] (0.86 g, 1.23 mmol) in a mixture of CH_3CN/MeOH (30:1, 15.5 mL). After 1 h, the solution was warmed to room temperature and quenched with saturated aqueous NaS_2O_3 (5 mL). The layers were separated, and the aqueous phase was extracted with ether. The combined organic layers were washed with saturated aqueous $NaHCO_3$, H_2O, and brine. The organics were dried over Na_2SO_4 and concentrated, and the crude compound was purified by column chromatography (2−10% EtOAc in hexanes) to afford **18** (a mixture of 4 isomers, 0.74 g, 91.9%). R_f 0.51 (1:9 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.83−7.30 (m, 20H), 5.16−4.97 (m, 1H), 4.84−4.53 (m, 1H), 4.10−3.62 (m, 2H), 3.48−2.98 (m, 6H), 2.21−1.80 (m, 2H), 1.15−0.93 (m, 18H); ^{13}C NMR ($CDCl_3$) δ 136.1, 135.9, 135.8, 135.5, 133.6, 133.5, 129.7, 129.5, 127.6, 127.5, 110.1, 106.5, 104.8, 103.0, 74.7, 71.4, 63.7, 63.1, 56.0, 55.7, 50.8, 49.1, 40.2, 39.6, 27.0, 26.9, 26.7, 19.4, 19.2, 19.1; IR (neat) 3049, 2932, 2857, 1589, 1472, 1113, 1027, 823 cm^{-1}; HRMS (FAB) M − OCH_3^+ calcd for $C_{38}H_{47}O_4Si_2$, 623.3013; found, 623.3238.

Preparation of 19. TBAF·hydrate (1.49 g, 4.74 mmol) was added to a 0 °C solution of **18** (620 mg, 0.95 mmol) in THF (12 mL). The mixture was allowed to warm to room temperature and was stirred overnight, at which time it was concentrated on a rotary evaporator. The crude compound was purified by column chromatography (0−5% MeOH in CH_2Cl_2) to afford **20** as two sets of mixtures, each containing two diastereomers of **19** (minor set, 20 mg, 11.8%; major set, 97 mg, 57.4%). Only the major set of isomers was characterized and carried on. **19**: R_f 0.34 (10% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 5.25 (dd, J = 4.4, 5.6 Hz, 1H), 4.35−4.33 (m, 1H), 3.85−3.69 (m, 2H), 3.45 (s, 3H), 3.31 (s, 3H), 3.13 (brs, 1H), 2.29−2.12 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 109.4, 106.7, 104.2, 103.8, 75.8, 72.6, 60.6, 58.9, 56.2, 56.0, 49.7, 48.9, 40.0, 39.0; IR (neat): 3430, 2937, 1447, 1266, 1100, 1022, 739 cm^{-1}.

Preparation of 20a and 20b. A solution of cyclododecyloxy bis-trimethylsilyloxy chloride (188 mg, 0.44 mmol) in CH_2Cl_2 (3 mL) was added to a 0 °C solution of **19** (45 mg, 0.25 mmol), pyridine (391 mg, 4.95 mmol), and DMAP (8 mg, 0.07 mmol) in CH_2Cl_2 (3 mL) over 10 min. After 5 h, the solution was warmed to room temperature, quenched with saturated aqueous $NaHCO_3$, and diluted with EtOAc (100 mL). The organic layer was washed with saturated aqueous $NaHCO_3$, H_2O, and brine, and dried over Na_2SO_4. The solution was then concentrated, and the crude compound was purified by column chromatography (0−10% EtOAc in hexanes containing 0.5% triethylamine) to afford **20a** and **20b** (**20a** 40 mg, 28.2%; **20b** 25 mg, 17.6%). **20a**: R_f 0.43 (1:4 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 5.27 (t, J = 5.6 Hz, 1H), 4.25−4.22 (m, 1H), 4.04−4.00 (m, 1H), 3.93 (dd, J = 11.6, 48 Hz, 2H), 3.73−3.71 (dd, J = 1.6, 3.6 Hz, 1H), 3.44 (s, 3H), 3.30 (s, 3H), 2.27−2.13 (m, 2H), 1.69−1.25 (m, 22H), 0.15 (s, 9H), 0.14 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 110.1, 107.1, 75.5, 71.3, 58.4, 56.1, 48.6, 39.4, 31.9, 24.3, 23.8, 23.4, 23.3, 23.2, 20.9, 20.7, 1.5, 1.4; IR (film) 3463, 2932, 2864, 1469, 1252, 1083, 843 cm^{-1}; HRMS (FAB) M − OCH_3^+ calcd for $C_{24}H_{51}O_7Si_3$, 535.2943; found, 535.2924. **20b**: R_f 0.35 (1:4 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 4.97 (dd, J = 4.0, 5.6 Hz, 1H), 4.27 (dd, J = 8.0, 14.8 Hz, 1H), 4.02−3.97 (m, 1H), 3.72 (dd, J = 15.2, 53.2 Hz, 2H), 3.43 (s, 3H), 3.42 (s, 3H), 2.75 (d, J = 7.6 Hz, 1H), 2.48−2.41 (m, 1H), 1.98−1.92 (m, 1H), 1.67−1.25 (m, 22H), 0.13 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 104.5, 104.4, 72.4, 70.8, 61.5, 55.9, 49.9, 39.4, 32.0, 24.4, 24.0, 23.3, 23.2, 20.8, 1.5; IR (film) 3566, 2932, 2852, 1470, 1252, 1083, 847 cm^{-1}; HRMS (FAB) M − OCH_3^+ calcd for $C_{24}H_{51}O_7Si_3$, 535.2943; found, 535.3060.

Preparation of 16a and 16b. *N*,*N*-Diisopropyl methyl phosphoramidic chloride (35 mg, 0.18 mmol) was added to a 0 °C solution of **20a** (40 mg, 70 μmol) and diisopropylethylamine (45 mg, 0.35 mmol) in CH_2Cl_2 (1 mL). The solution was allowed to warm to room temperature and was stirred overnight. The reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$, H_2O, and brine. The layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated, and the crude compound was purified by column chromatography (0−5% EtOAc in hexanes containing 0.5% triethylamine) to afford a mixture of two diastereomers of **16a** (25 mg, 49%). **16a**: R_f 0.67 (1:4 EtOAc/

hexanes); ^{1}H NMR ($CDCl_3$) δ 5.23−5.18 (m, 1H), 4.40−4.30 (m, 1H), 4.01−3.78 (m, 3H), 3.63−3.54 (m, 2H), 3.45−3.32 (m, 9H), 2.43−2.28 (m, 1H), 2.23−2.15 (m, 1H), 1.64−1.25 (m, 22H), 1.19−1.15 (m, 12H), 0.13−1.12 (m, 18H); ^{31}P NMR ($CDCl_3$) δ 149.4, 148.7; IR (film) 2933, 2867, 1470, 1364, 1252, 1185, 1090, 1060, 976, 846 cm^{-1}; HRMS (FAB) M − OCH_3^+ calcd for $C_{31}H_{67}NO_8PSi_3$, 696.3912; found, 696.4069.

Phosphitylation was carried out on **20b** (25 mg, 43.8 μmol) under the same conditions to provide **16b** (20 mg, 63%). **16b**: R_f 0.62 (1:4 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 4.97−4.94 (m, 1H), 4.57−4.43 (m, 1H), 4.00−3.80 (m, 2H), 3.70−3.56 (m, 3H), 3.45−3.36 (m, 9H), 2.57−2.41 (m, 1H), 2.09−2.01 (m, 1H), 1.63−1.28 (m, 22H), 1.20−1.16 (m, 12H), 0.14−1.13 (m, 18H); ^{31}P NMR ($CDCl_3$) δ 149.7, 149.1; IR (film) 2932, 1469, 1379, 1261, 1184, 1086, 847 cm^{-1}; HRMS (FAB) M + H^+ calcd for $C_{32}H_{71}NO_9PSi_3$, 728.4174; found, 728.3860.

Preparation of Duplexes Containing C4-AP. Duplexes containing **1** (**2**) were prepared by hybridizing the ^{32}P-labeled lesion containing oligonucleotide with 1.5 equiv of the appropriate complement in a solution containing 100 mM KCl and 10 mM $MgCl_2$. Hybridization was carried out at 90 °C for 5 min, followed by cooling to room temperature over 5 h. Duplexes **2a**−**d** (120 nM) were photolyzed at 350 nm for 30 min in 50 mM KCl and 10 mM $MgCl_2$ immediately prior to use.

Photoconversion of 1 to C4-AP and Analysis Using Endo IV. Duplexes containing the bases A, T, G, and C (**2a**−**d**) opposite **1** were prepared as described above. The strand containing **1** was 5′-labeled prior to hybridization. Duplexes **2a**−**d** (20 nM) were photolyzed at 350 nm for 30 min in 100 mM KCl and 10 mM $MgCl_2$. Portions of **2a**−**d** (20 nM) in 100 mM KCl and 10 mM $MgCl_2$ were also prepared and stored in the dark. The photoconversion of **1** in each solution was determined by incubating 5-μL aliquots with 0.2 M NaOH (5 μL) for 20 min at 37 °C. The reactions were quenched by adding 1 M HCl (1 μL) and diluting with 9 μL of 95% formamide loading buffer. Lesion integrity was determined by reacting both the precursor (**2a**−**d**) and the lesion (**3a**−**d**) containing duplexes (5 μL) with 5 μL of a 2× enzyme solution containing 50 mM HEPES-KOH (pH 7.5), 50 mM KCl, 10% glycerol, 1 mM dithiothreitol, 100 μg/mL BSA, and 100 nM endonuclease IV. The reactions were incubated at 37 °C for 45 min and then quenched by the addition of 10 μL of formamide. Background cleavage was determined by treating aliquots (5 μL) with the 2× enzyme buffer (5 μL) described above without enzyme and quenching with 10 μL of formamide to determine background cleavage. All samples were analyzed on a 12% denaturing polyacrylamide gel.

UV Melting Experiments. Duplexes (5 μM) were prepared by heating **21a** or **21b** and the appropriate complement in a one-to-one ratio in 10 mM PIPES (pH 7.0), 10 mM $MgCl_2$, and 100 mM NaCl at 90 °C for 5 min. The duplexes were cooled to room temperature over 2.5 h, followed by an additional period of 2.5 h at 4 °C. Solutions of 2.2 μM were prepared by diluting the hybridized duplex in PIPES buffer. Melting experiments were carried out in 1-cm path length quartz cells containing 150 μL of duplex solution covered by 100 μL of mineral oil. UV absorbance was recorded as the temperature was ramped at 0.5 °C/min from 15 to 85 °C.

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Supporting Information Available: General experimental methods. NMR spectral data of **4**, **6**, **8**, **12**−**16**, **18**−**20**. ESI-MS of oligonucleotides (**9**, **10**, **21**). This material is available free of charge via the Internet at http://pubs.acs.org.

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