JOC Article

Independent Generation and Characterization of a C2′-Oxidized Abasic Site in Chemically Synthesized Oligonucleotides

Jaeseung Kim, Yvonne N. Weledji, and Marc M. Greenberg*

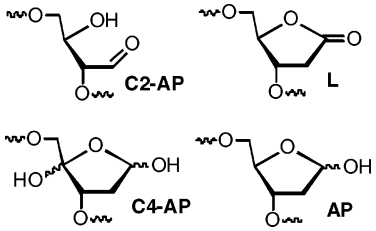
Department of Chemistry, Johns Hopkins University, 3400 N. Charles St., Baltimore, Maryland 21218

mgreenberg@jhu.edu

Received June 8, 2004

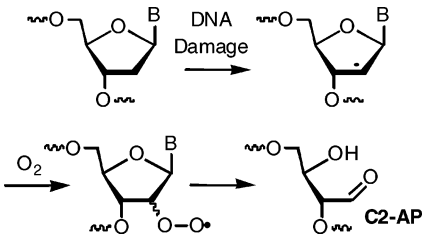
Abasic lesions, which are formed endogenously and as a consequence of exogenous agents, are lethal and mutagenic. Hydrogen atom abstraction from C2′ in DNA under aerobic conditions produces an oxidized abasic lesion (C2-AP), along with other forms of DNA damage. The effects of C2-AP on DNA structure and function are not well understood. A method for the solid-phase synthesis of oligonucleotides containing C2-AP lesions is reported. The lesion is released via periodate oxidation of a triol containing a vicinal diol. The triol is introduced via a phosphoramidite that is compatible with standard oligonucleotide synthesis and deprotection conditions. UV-melting studies indicate that the C2-AP lesion has a comparable effect on the thermal stability of duplex DNA as other abasic lesions. The C2-AP lesion is rapidly cleaved by piperidine at 90 °C. However, cleavage by NaOH (0.1 M, 37 °C) shows that C2-AP is considerably less labile ($t_{1/2}$ = 3.3 ± 0.2 h) than other abasic lesions.

DNA damage is involved in aging and a variety of diseases, including cancer.[1−4] Chemistry plays an important role in identifying and characterizing endogenously and/or exogenously produced lesions, which induce significant biological effects. Abasic sites, which as their name suggests are missing their nucleobases, are an important family of lesions. It is estimated that ~10,000 AP sites are produced per cell each day.[5,6] Oxidized AP sites (e.g., C4-AP, L) are also produced in varying amounts depending upon the damaging agent.[7−10]



Recent in vitro and in vivo studies reveal that the effects of oxidized abasic lesions on DNA replication and repair are distinct from those of AP lesions and each other.[11−13] The oxidized abasic site resulting from formal hydrogen atom abstraction from the C2′-nucleotide position (C2-AP) is observed during γ-irradiation of DNA and photolysis of DNA containing 5-halopyrimidines (Scheme 1).[10,14−18] Very little is known about the effects of this lesion on DNA structure and function. We describe herein the synthesis and characterization of oligonucleotides containing C2-AP, which will facilitate evaluation of the lesion's biochemical and biological effects.

SCHEME 1



Abasic lesions have often been referred to as noninstructive lesions because they cannot form Watson−Crick

* To whom correspondence should be addressed. Phone: 410-516-8095. Fax: 410-516-7044.

(1) DePinho, R. A. *Nature* **2000**, *408*, 248−254.
(2) Friedberg, E. C.; McDaniel, L. D.; Schultz, R. A. *Curr. Opin. Genet. Dev.* **2004**, *14*, 5−10.
(3) Finkel, T.; Holbrook, N. J. *Nature* **2000**, *408*, 239−247.
(4) Cooke, M. S.; Evans, M. D.; Dizdaroglu, M.; Lunec, J. *FASEB* **2003**, *17*, 1195−1214.
(5) Lindahl, T. *Nature* **1993**, *362*, 709−715.
(6) Lindahl, T.; Wood, R. D. *Science* **1999**, *286*, 1897−1905.
(7) Carter, K. N.; Greenberg, M. M. *J. Am. Chem. Soc.* **2003**, *125*, 13376−13378.
(8) Rabow, L. E.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 3196−203.
(9) Xi, Z.; Goldberg, I. H. In *Comprehensive Natural Products Chemistry*; Kool, E. T., Ed.; Elsevier: Amsterdam, 1999; Vol. 7, pp 553−592.
(10) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor & Francis: London, 1987.
(11) Kroeger, K. M.; Jiang, Y. L.; Kow, Y. W.; Goodman, M. F.; Greenberg, M. M. *Biochemistry* **2004**, *43*, 6723−6733.
(12) Berthet, N.; Roupioz, Y.; Constant, J. F.; Kotera, M.; Lhomme, J. *Nucleic Acids Res.* **2001**, *29*, 2725−2732.
(13) Faure, V.; Constant, J. F.; Dumy, P.; Saparbaev, M. *Nucleic Acids Res.* **2004**, *32*, 2937−2946.
(14) Dizdaroglu, M.; Schulte-Frohlinde, D.; Von Sonntag, C. *Z. Naturforsch.* **1977**, *32c*, 1021−1022.
(15) Hissung, A.; Isildar, M.; von Sonntag, C. *Int. J. Radiat. Biol* **1981**, *39*, 185−193.
(16) Sugiyama, H.; Tsutsumi, Y.; Fujimoto, K.; Saito, I. *J. Am. Chem. Soc.* **1993**, *115*, 4443−4448.
(17) Sugiyama, H.; Fujimoto, K.; Saito, I. *J. Am. Chem. Soc.* **1995**, *117*, 2945−2946.
(18) Sugiyama, H.; Fujimoto, K.; Saito, I. *Tetrahedron Lett.* **1996**, *37*, 1805−1808.

10.1021/jo049033d CCC: $27.50
Published on Web 08/12/2004

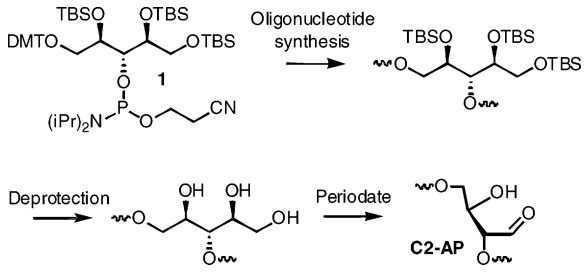
hydrogen bonds with nucleotides. However, studies on 2-deoxyribonolactone (L) have shown that the oxidized abasic site and AP interact differently with individual polymerases involved in replication in *E. coli*.[11] Replication of L does not follow the "A-rule", an empirical rule developed in response to studies on AP lesions in *E. coli*.[19] Furthermore, the lactone lesion (L) exhibits distinct behavior in its interactions with some base excision repair enzymes, which is manifested in the formation of DNA−protein cross-links.[20−22] These experiments suggest that the term noninstructive should not be used when describing the biochemistry of abasic lesions. Moreover, it suggests that detailed studies on other abasic lesions, such as C4-AP and C2-AP, could provide important information concerning the biological effects of these lesions.[23]

Studies on DNA lesions are greatly facilitated by synthesis of oligonucleotides containing them at defined sites.[24−26] Synthesis of oligonucleotides containing abasic sites is more complex because the lesions are alkali-labile. Successful strategies for the synthesis of L- or C4-AP-containing oligonucleotides unmask the lesions in a final photochemical step.[27−30] The latter has also been incorporated chemoenzymatically in oligonucleotides.[31] AP lesions have been incorporated into oligonucleotides chemoenzymatically by exploiting the deglycosylation of 2′-deoxyuridine by uracil DNA glycosylase.[32] More recently, oligonucleotides containing AP lesions were prepared via mild periodate oxidation in the final step.[33]

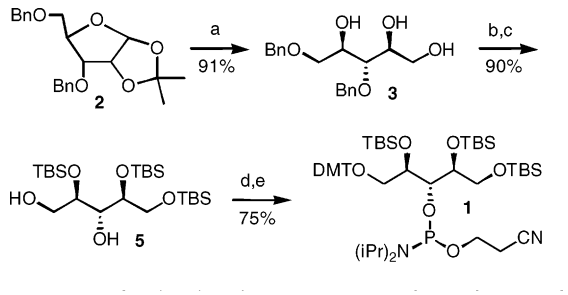
Results and Discussion

The C2-AP has been shown to undergo a retroaldol reaction under mild alkaline conditions.[16] Consequently, chemical synthesis of oligonucleotides containing this molecule using standard 5′-dimethoxytrityl, β-cyanoethyl phosphoramidites required disguising the lesion until after the protecting groups are removed and the biopolymer is cleaved from its solid-phase synthesis support (Scheme 2). We chose to disguise C2-AP by introducing the latent aldehyde as a vicinal diol. This strategy is analogous to that employed by Johnson in his recent chemical synthesis of oligonucleotides containing AP sites.[33] The aldehyde is unmasked by mild sodium periodate oxidation, a common method for introducing aldehydes in chemically synthesized RNA and DNA.[34−37] The diol and hydroxyl group derived from the C4′-position of the nucleotide are in turn protected as their silyl ethers, which is a common protecting group for the 2′-hydroxyl during the chemical synthesis of RNA.[38] This is desirable because the hydroxyl groups remain protected during the alkaline ammonia treatment, which prevents cleavage and/or phosphoryl migration due to intramolecular attack by the hydroxyl group(s) on the phosphate diesters.

SCHEME 2



SCHEME 3[a]

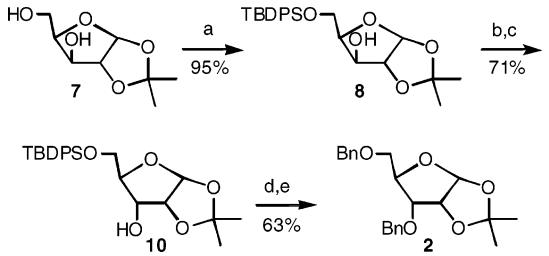


[a] (a) (i) aq AcOH (30%), reflux, (ii) $NaBH_4$, MeOH, 0 → 25 °C; (b) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C; (c) H_2 (50 psi), $Pd(OH)_2$/C, EtOAc, 25 °C; (d) DMTCl, pyridine, 25 °C; (e) 2-cyanoethyl diisopropylchlorophosphoramidite (5 equiv), diisopropylethylamine (8 equiv), CH_2Cl_2, reflux.

Implementation of this synthetic approach was effected by the synthesis of phosphoramidite **1** (Scheme 3). This was accomplished from triol (**3**), which was obtained in high yield from 3,5-dibenzyl-1,2-isopropylidene ribofuranose (**2**) using a previously reported procedure for the C3-epimer of **2**.[39] The ribofuranose isopropylidene (**2**) was previously prepared from the 1,2-isopropylidene of xylose (**7**).[40] In this project **2** was prepared from commercially available **7** via the silyl ether (Scheme 4).[41] Stereochemical inversion of the C3-hydroxyl was accomplished

(19) Strauss, B. S. *BioEssays* **1991**, *13*, 79−84.
(20) Hashimoto, M.; Greenberg, M. M.; Kow, Y. W.; Hwang, J.-T.; Cunningham, R. P. *J. Am. Chem. Soc.* **2001**, *123*, 3161−3162.
(21) Kroeger, K. M.; Hashimoto, M.; Kow, Y. W.; Greenberg, M. M. *Biochemistry* **2003**, *42*, 2449−2455.
(22) DeMott, M. S.; Beyret, E.; Wong, D.; Bales, B. C.; Hwang, J.-T.; Greenberg, M. M.; Demple, B. *J. Biol. Chem.* **2002**, *277*, 7637−7640.
(23) Greenberg, M. M.; Weledji, Y. N.; Kroeger, K. M.; Kim, J.; Goodman, M. F. *Biochemistry* **2004**, *43*, 2656−2663.
(24) Wang, D.; Kreutzer, D. A.; Essigmann, J. M. *Mutat. Res.* **1998**, *400*, 99−115.
(25) Kamiya, H. *Nucleic Acids Res.* **2003**, *31*, 517−531.
(26) Butenandt, J.; Burgdorf, L. T.; Carell, T. *Synthesis* **1999**, 1085−1105.
(27) Zheng, Y.; Sheppard, T. L. *Chem. Res. Toxicol.* **2004**, *17*, 197−207.
(28) Kotera, M.; Bourdat, A.-G.; Defrancq, E.; Lhomme, J. *J. Am. Chem. Soc.* **1998**, *120*, 11810−11811.
(29) Kim, J.; Gil, J. M.; Greenberg, M. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 5882−5885.
(30) Goodman, B. K.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 2−3.
(31) Chen, J.; Stubbe, J. *Biochemistry* **2004**, *43*, 5278−5286.
(32) Shibutani, S.; Takeshita, M.; Grollman, A. P. *J. Biol. Chem.* **1997**, *272*, 13916−13922.
(33) Shishkina, I. G.; Johnson, F. *Chem. Res. Toxicol.* **2000**, *13*, 907−912.
(34) Bellon, L.; Workman, C.; Scherrer, J.; Usman, N.; Wincott, F. *J. Am. Chem. Soc.* **1996**, *118*, 3771−2.
(35) Tilquin, J. M.; Dechamps, M.; Sonveaux, E. *Bioconjugate Chem.* **2001**, *12*, 451−457.
(36) Forget, D.; Renaudet, O.; Boturyn, D.; Defrancq, E.; Dumy, P. *Tetrahedron Lett.* **2001**, *42*, 9171−9174.
(37) Forget, D.; Boturyn, D.; Defrancq, E.; Lhomme, J.; Dumy, P. *Chem. Eur. J.* **2001**, *7*, 3976−3984.
(38) Wincott, F.; DiRenzo, A.; Shaffer, C.; Grimm, S.; Tracz, D.; Workman, C.; Sweedler, D.; Gonzalez, C.; Scaringe, S.; Usman, N. *Nucleic Acids Res.* **1995**, *23*, 2677−84.
(39) Kakefuda, A.; Shuto, S.; Nagahata, T.; Seki, J.-i.; Sasaki, T.; Matsuda, A. *Tetrahedron* **1994**, *50*, 10167−10182.
(40) Chen, T.; Greenberg, M. M. *Tetrahedron Lett.* **1998**, *39*, 1103−1106.
(41) van Straten, N. C. R.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1997**, *53*, 6509−6522.

SCHEME 4[a]

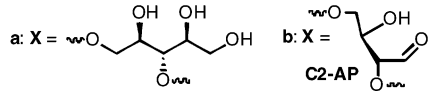


[a] (a) TBDPSCl, imidazole, DMF, 25 °C; (b) DMSO, Ac_2O, 25 °C; (c) $NaBH_4$, $EtOH/H_2O$, 25 °C; (d) TBAF, THF, 25 °C; (e) NaH, BnBr, THF, reflux.

via oxidation to the ketone and selective reduction from the less hindered β-face. Transformation of **3** into the desired phosphoramidite (**1**) was accomplished by a series of hydroxyl group deprotection and functionalization steps. The diastereomers of **1** were chromatographically separable. However, there was no advantage to this (vide infra), and the phosphoramidite (**1**) was routinely employed as a mixture of diastereomers in solid-phase synthesis.

Oligonucleotides containing up to 30 nucleotides (**12**−**15**) were prepared using **1** and commercially available

5'-d(CGC ATG **X**GT AGT)
12a,**b**
5'-d(GAA GAC CC**X** GGC GTC C)
13a,**b**
5'-d(GAA GAC CT**X** GGC GTC C)
14a,**b**
5'-d(GTC ACG TGC TGC A**X**A CGA CGT GCT GAG CCT)
15a,**b**



"fast deprotecting" phosphoramidites (*N*-acetyl-2′-deoxycytidine, *N*-phenoxyacetyl-2′-deoxyadenosine, *N*-isopropylphenoxyacetyl-2′-deoxyguanosine). Standard coupling cycles provided by the manufacturer were employed except when coupling **1**, which required additional experimentation. Extending the coupling time of **1** to 5 min resulted in a 50% yield according to trityl cation measurement. Surprisingly, carrying out a double-coupling of **1** did not improve the yield. Furthermore, solid-phase synthesis coupling yields were not any higher using individual diastereomers of **1** epimeric at the phosphorus center. Despite the lower than typical coupling yield for phosphoramidite **1**, oligonucleotides **12a**−**15a** were readily purified by denaturing polyacrylamide gel electrophoresis following a two-step deprotection. Desilylation was carried out using $Et_3N{\cdot}3HF$ for 3 h following cleavage from the solid-phase support and deprotection of the phosphate and exocyclic amine groups using concentrated ammonium hydroxide.[38] The four triol-containing oligonucleotides exhibited the expected *m*/*z* ratio for the fully protonated and single sodium adduct molecules (see Supporting Information). Periodate oxidation was carried out on 2−6 nmol of oligonucleotides using buffered (0.1 M NaOAc, pH 6.0) sodium periodate (5 M). The reactions were quenched by desalting using C-18 Sep pak cartridges after 30 min. No further purification was necessary, as evidenced by ESI-MS (see Supporting Information). MS analysis of these oligonucleotides showed no evidence for the triol starting materials, indicating that the reactions had proceeded to completion.

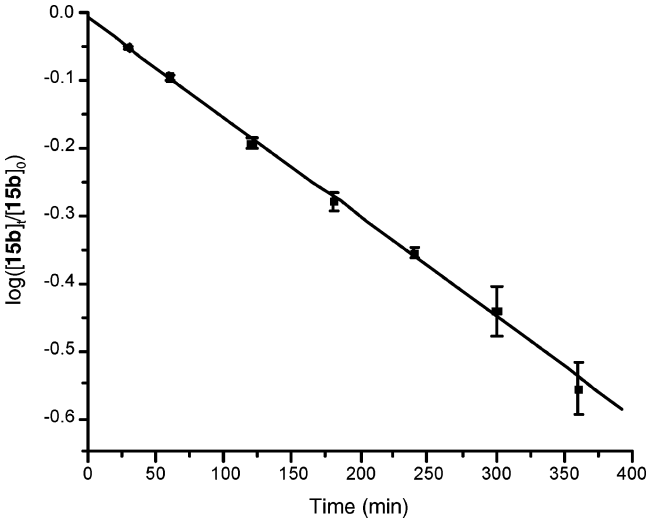


FIGURE 1. Cleavage of C2-AP (**15b**) as a function of time upon treatment with NaOH (0.1 M, 37 °C).

The C2-AP lesion had previously been shown to be alkaline-labile.[16] The lesion contains β-hydroxy phosphate groups as are present in RNA and is also capable of undergoing a retroaldol reaction. As is the case with many alkali-labile lesions, 5′-^{32}P-**15b** is completely cleaved by 1 M piperidine (90 °C, 20 min). However, C2-AP is significantly more stable to NaOH than other abasic lesions. AP, L, or C4-AP are completely cleaved upon treatment with NaOH (0.1 M, 37 °C, 20 min).[7,29,42] C2-AP is cleaved with first-order kinetics (Figure 1), but the half-life is considerable (3.3 ± 0.2 h, $5.9 \pm 0.2 \times 10^{-5}$ s^{-1}).

The effect of C2-AP on the thermal stability of duplex DNA was analyzed by UV-melting. Despite the relative robust behavior of this lesion toward alkali treatment, care was taken to prevent adventitious cleavage during hybridization. Duplex **16** was formed by hybridizing **12b** with its complement at 37 °C for 30 min, followed by cooling at 25 °C for 2 h.[29] The T_m was determined to be 38.6 °C. The T_m for **16** was within 1.5 °C of those measured for C4-AP and the tetrahydrofuran analogue of AP in otherwise identical duplexes.[29]

5'-d(CGC ATG **X**GT AGT)
3'-d(GCG TAC ACA TCA)
16

Conclusion

We have developed a method for the chemical synthesis of oligonucleotides containing C2-AP, an oxidized abasic lesion. The method is compatible with standard solid-phase synthesis reagents and as such is not restricted in terms of the sequences that can be prepared. Preliminary studies suggest that the C2-AP lesion reduces the thermal stability of duplex DNA by an amount comparable to reduction by other abasic lesions. DNA containing C2-AP is considerably more resistant to strand

(42) Roupioz, Y.; Lhomme, J.; Kotera, M. *J. Am. Chem. Soc.* **2002**, *124*, 9129−9135.

scission than other abasic lesions, suggesting that it will be necessary for repair enzymes to excise it.

Experimental Section

(2*S*,3*R*,4*R*)-3,5-*O*-Dibenzyloxy-1,2,4-*O*-tri-*tert*-butyldimethylsilyloxypentane (4). TBSOTf (1.95 g, 7.36 mmol) was added to (2*S*,3*S*,4*R*)-3,5-*O*-dibenzyloxy-1,2,4-pentanetriol[39] (**3**, 546 mg, 1.64 mmol) and 2,6-lutidine (1.06 g, 9.87 mmol) in CH_2Cl_2 (17 mL) at 0 °C. After 1 h at 0 °C, the solution was poured into cold saturated NH_4Cl solution, extracted with EtOAc, and then washed with brine. The organic layer was dried with Na_2SO_4. The solution was filtered and concentrated on a rotary evaporator. The crude material was purified by column chromatography (2−5% EtOAc in hexanes) to give **3** (1.08 g, 97.6%): R_f 0.62 (1:9 EtOAc/hexanes); IR (neat) 3032, 2929, 2857, 1472, 1361, 1254, 1095, 836 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36−7.33 (m, 10H), 4.80 (dd, J = 11.2, 19.2 Hz, 2H), 4.54 (d, J = 1.2 Hz, 2H), 4.22−4.18 (m, 1H), 3.96−3.93 (m, 1H), 3.80−3.69 (m, 4H), 3.60 (dd, J = 6.4, 10.0 Hz, 1H), 0.96 (s, 9H), 0.95 (s, 9H), 0.94 (s, 9H), 0.14 (s, 3H), 0.12 (s, 6H), 0.11 (s, 3H), 0.08 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 139.1, 138.7, 128.2, 128.1, 127.8, 127.6, 127.4, 127.2, 82.8, 74.5, 74.1, 73.3, 72.7, 72.3, 64.8, 26.0, 25.9, 18.3, 18.2, 18.1, −4.2, −4.3, −4.8, −5.3, −5.4; HRMS (MALDI-TOF) M + Na^+ calcd for $C_{37}H_{66}O_5NaSi_3$ 697.4110, found 697.4094.

(2*S*,3*R*,4*R*)-3,5-Dihydroxy-1,2,4-*O*-tri-*tert*-butyldimethylsilyloxypentane (5). 2*S*,3*R*,4*R*-3,5-*O*-Dibenzyl-1,2,4-*O*-tri-*tert*-butyldimethylsilyloxypentane **4** (741 mg, 1.10 mmol) and 20% $Pd(OH)_2$/C (240 mg) were stirred in EtOAc (25 mL) under H_2 (50 psi) overnight. After the reaction mixture was filtered through a Celite pad, which was then washed with EtOAc, the filtrate was concentrated on a rotary evaporator. The crude material was purified by column chromatography (1−2% MeOH in CH_2Cl_2) to give (2*S*,3*R*,4*R*)-3,5-dihydroxy-1,2,4-*O*-tri-*tert*-butyldimethylsilyloxypentane **5** (507 mg, 92.7%): R_f 0.50 (1:50 MeOH/ CH_2Cl_2); IR (neat) 3452, 2930, 2858, 1463, 1362, 1256, 1083, 836 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.92−3.81 (m, 3H), 3.76−3.62 (m, 4H), 2.98 (brs, 2H), 0.89 (s, 27H), 0.11 (s, 3H), 0.10 (s, 6H), 0.09 (s, 3H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 76.1, 73.1, 72.1, 64.7, 63.4, 25.9, 25.8, 18.3, 18.1, 18.0, −4.4, −4.5, −4.8, −4.9, −5.4, −5.5; HRMS (FAB) M + H^+ calcd for $C_{23}H_{55}O_5Si_3$ 495.3357, found 495.3361.

(2*S*,3*R*,4*R*)-5-*O*-DMT-1,2,4-*O*-tri-*tert*-butyldimethylsilyloxypentan-3-ol (6). 4,4′-Dimethoxytrityl chloride (360 mg, 1.06 mmol) was added to a solution of **5** (438 mg, 0.88 mmol) in pyridine (4.4 mL) at room temperature. After 6 h, the reaction mixture was concentrated on a rotary evaporator. The crude material was dissolved in EtOAc (100 mL) and then washed sequentially with saturated $NaHCO_3$, H_2O, and saturated NaCl. The organic layer was dried over Na_2SO_4, filtered, and concentrated on a rotary evaporator. The crude material was purified by column chromatography (oven-dried silica gel, 5% EtOAc in hexanes) to afford **6** (678 mg, 96.6%): R_f 0.33 (1:9 EtOAc/hexanes); IR (film) 3508, 2929, 2856, 1608, 1509, 1471, 1252, 1177, 1081, 833 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.51−7.22 (m, 9H), 6.84 (d, J = 8.8 Hz, 4H), 4.14 (dd, J = 4.4, 8.8 Hz, 1H), 3.80 (s, 6H), 3.76−3.74 (m, 1H), 3.66 (dd, J = 5.2, 10.4 Hz, 1H), 3.56−3.52 (m, 1H), 3.29 (d, J = 4.0 Hz, 2H), 3.14 (d, J = 4.0 Hz, 1H), 0.97 (s, 9H), 0.92 (s, 9H), 0.84 (s, 9H), 0.16 (s, 3H), 0.12 (s, 3H), 0.07 (s, 6H), 0.03 (s, 3H), −0.14 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.4, 144.7, 136.0, 135.9, 130.2, 130.1, 128.3, 127.7, 126.6, 113.0, 86.3, 75.7, 73.9, 71.8, 65.4, 65.2, 55.1, 25.9, 25.8, 18.3, 18.1, 18.0, −4.1, −4.4, −4.8, −5.3, −5.4; HRMS (MALDI-TOF) M + Na^+ calcd for $C_{44}H_{72}O_7NaSi_3$ 819.4478, found 819.4463.

Phosphoramidite 1. 2-Cyanoethyl diisopropylchlorophosphoramidite (753 mg, 3.18 mmol) was added to a solution of **6** (500 mg, 0.63 mmol) and DIPEA (649 mg, 5.02 mmol) in CH_2Cl_2 (10 mL). After refluxing for 5 h, the solution was cooled to room temperature, diluted with CH_2Cl_2 (90 mL), and then washed sequentially with saturated $NaHCO_3$, H_2O, and saturated NaCl. The organic layer was dried over Na_2SO_4, filtered, and concentrated on a rotary evaporator. The crude material was purified by column chromatography (oven-dried silica gel, 20% Et_2O in hexanes) to afford a diastereomeric mixture of phosphoramidite **1** (485 mg, 77.8%). A small amount of sample was rechromatographed to separate the phosphoramidite diastereomers for analytical characterization. Lower R_f 0.18, major isomer, 1:9 EtOAc/hexanes: IR (film) 2930, 2856, 1608, 1510, 1464, 1363, 1252, 1077, 834 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46−7.20 (m, 9H), 6.82 (dd, J = 1.2, 9.2 Hz, 4H), 4.23−4.19 (m, 1H), 4.02−3.98 (m, 1H), 3.79 (s, 6H), 3.77−3.69 (m, 2H), 3.68−3.49 (m, 5H), 3.28 (dd, J = 4.4, 10.0 Hz, 1H), 3.16 (dd, J = 7.2, 10.4 Hz, 1H), 2.88−2.24 (m, 2H), 1.15 (d, J = 7.2 Hz, 12H), 0.90 (s, 9H), 0.88 (s, 9H), 0.86 (s, 9H), 0.16 (s, 3H), 0.09 (s, 3H), 0.02 (s, 6H), 0.00 (s, 3H), −0.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.3, 145.1, 136.4, 130.3, 130.2, 128.4, 127.6, 126.6, 117.6, 112.8, 86.3, 79.8 (d, J = 12.9 Hz), 73.9 (d, J = 4.5 Hz), 73.6, 66.2, 65.1, 57.9 (d, J = 21.2 Hz), 55.1, 43.1 (d, J = 12.9 Hz), 26.1, 24.8, 24.7, 24.5, 19.9, 18.4, 18.2, 18.1, −4.0, −4.5, −4.7, −5.1, −5.3; ^{31}P NMR ($CDCl_3$) δ 152.3; HRMS (MALDI-TOF) M + Na^+ calcd for $C_{53}H_{89}N_2O_8NaSi_3P$ 1019.5557, found 1019.5608. Higher R_f 0.23, minor isomer, 1:9 EtOAc/hexanes: ^1H NMR ($CDCl_3$) δ 7.47−7.17 (m, 9H), 6.77 (d, J = 8.8 Hz, 4H), 4.42 (dd, J = 1.2, 7.2 Hz, 1H), 4.00 (dd, J = 1.6, 10.8 Hz, 1H), 3.78 (s, 6H), 3.74−3.60 (m, 3H), 3.47−3.24 (m, 5H), 2.98 (dd, J = 2.0, 10.0 Hz, 1H), 2.54 (t, J = 6.4 Hz, 2H), 1.08 (d, J = 6.0 Hz, 6H), 0.97 (s, 9H), 0.93 (d, J = 6.8 Hz, 6H), 0.87 (s, 9H), 0.86 (s, 9H), 0.20 (s, 3H), 0.17 (s, 3H), 0.01 (s, 9H), −0.13 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.2, 145.0, 136.6, 136.3, 130.3, 130.2, 128.4, 127.5, 126.4, 117.4, 112.8, 85.9, 79.3 (d, J = 9.1 Hz), 75.3, 72.7, 66.4, 65.4, 57.6 (d, J = 22.8 Hz), 55.1, 42.8 (d, J = 12.9 Hz), 26.1, 24.9, 24.8, 24.4, 20.3, 20.2, 18.5, 18.2, −3.9, −4.1, −4.3, −5.1, −5.3; ^{31}P NMR ($CDCl_3$) δ 151.8.

Oligonucleotide Synthesis, Deprotection, and Purification. Standard cycles (ABI 394) were used for incorporating native nucleotides, which were introduced using commercially available fast deprotecting phosphoramidites. The coupling time allowed for phosphoramidite **1** was increased to 5 min. Deprotection of the exocyclic amine protecting groups and β-cyanoethyl groups and cleavage from the solid-phase synthesis support was carried out using concentrated aqueous NH_4OH for 2 h at 55 °C. The TBDMS groups were removed under conditions reported by Wincott for deprotecting the 2′-hydroxyl group in chemically synthesized RNA (250 μL of a solution containing 1.5 mL *N*-methylpyrrolidinone, 750 μL of Et_3N, and 1.0 mL of $Et_3N{\cdot}3HF$; 3 h at 65 °C). Fully deprotected oligonucleotides containing the triol were purified by 20% denaturing PAGE.[38]

Oxidative Cleavage of Oligonucleotides using $NaIO_4$. The oligonucleotides (**12**−**15a**, 2−6 nmol, 35−100 μM) were treated with 50−60 μL of 5 mM $NaIO_4$ in 0.1 M sodium acetate buffer (pH 6.0) for 30 min at room temperature and then desalted on a Sep-Pak C18 cartridge (Waters).

Treatment of Single Strand Oligonucleotide Containing C2-AP (15b) with NaOH and Piperidine. A solution (25 μL) containing 0.1 μM **15b** was added to 25 μL of NaOH (0.2 M), and the reaction incubated at 37 °C for 360 min. Aliquots (5 μL) were removed at 30, 60, 120, 180, 240, and 360 min. The aliquots were neutralized immediately with 5 μL of HCl (0.1 M), and diluted in 95% formamide loading buffer containing 10 mM EDTA (20 μL). The kinetic analysis was carried out using three reactions. The rate constant reported is the average of three such experiments. The result of one experiment (three replicates) is shown in Figure 1).

For treatment with piperidine, 25 μL of **15b** (0.1 μM) was added to 25 μL of piperidine (2 M), and the reaction incubated at 90 °C for 20 min, at which time the samples were placed in a dry ice/ethanol cold bath. The samples were then placed in a Savant speed vacuum at medium heat for 10 min to remove the piperidine before resuspending in 95% formamide loading buffer containing 10 mM EDTA (20 μL).

All samples were separated on a 20% denaturing PAGE.

Acknowledgment. We are grateful to the National Institute of General Medical Sciences (NIH GM-063028) for financial support.

Supporting Information Available: Experimental procedures for the synthesis of **3**, preparation of samples for ESI-MS, UV-melting experiment (**16**), and NaOH cleavage of **15b**; NMR spectra of **1**−**6** and **8**−**11**; ESI-MS of **12**−**15a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO049033D