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Preparation and Analysis of Oligonucleotides Containing Lesions Resulting from C5'-Oxidation

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Abstract

Hydrogen atom abstraction from the C5'-position of nucleotides in DNA results in direct strand scission. The newly formed 5'-termini of the cleaved DNA consists of alkali-labile fragments of the oxidized nucleotide. One terminus contains a 5'-aldehyde as part of an otherwise undamaged nucleotide (**T-al**). A second more structurally distinct product that is produced in lower yields results from cleavage of the C4'–C5' carbon–carbon bond. The 1,4-dioxo-2-phosphorylbutane (**DOB**) is a precursor of the alkylating agent but-2-ene-1,4-dial. To facilitate studies on these lesions, methods for synthesizing oligodeoxynucleotides containing **DOB** or **T-al** at their 5'-termini were developed. The effects of these lesions on the UV-melting temperatures of duplex DNA, and their cleavage labilities were determined. **T-al** cleaves very slowly ($t_{1/2} = 100.7$ h), whereas **DOB** has a half-life at 37 °C (pH 7.2) of less than 11 h. In addition, **DOB** forms a stable adduct very efficiently with Tris, which protects the abasic site against cleavage.

Introduction

DNA strand breaks resulting from oxidative processes require hydrogen atom abstraction from the carbohydrate backbone. Pathways for direct strand scission emanating from C3', C4', or C5'-hydrogen atom abstraction are precedented.^{1,2} The latter two positions are abstracted by several minor groove binding molecules. The positioning of the C4'- and C5'-hydrogen atoms on the edge of the minor groove also makes them accessible to diffusible species.^{3,4} Some strand scission processes produce termini that retain nucleotide fragments, which must be removed as part of the repair process.⁵ Hence, consideration of the interactions of these terminal fragments with biomolecules could have important biological consequences. The products resulting from C4'-hydrogen atom abstraction, the mechanisms of their formation, and their biological effects are well characterized.^{6,10} These types of studies are facilitated by independent synthesis of oligonucleotides containing individual lesions.^{11,12} The products resulting from C5'-oxidation are less well understood, in part because methods for preparing oligonucleotides containing them have not been reported. We describe herein the synthesis and characterization of oligonucleotides containing the fragments produced upon C5'-oxidation.

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Supporting Information Available: General experimental methods and procedures for the synthesis of **15** and **16**, NMR spectral data, and ESI-MS of oligonucleotides. This material is available free of charge via the Internet at <http://pubs.acs.org>.

C5'-Hydrogen atom abstraction gives rise to direct strand breaks containing two major families of products at the newly formed termini (Scheme 1). Some reagents produce fragments containing 3'-phosphate and 5'-termini containing a C5'-oxidized but otherwise intact nucleotide (**T-al**).^{13,15} **T-al** is susceptible to cleavage upon treatment with heat and/or alkali, and it forms adducts with nucleophiles.^{15,16} Alternatively, the initially formed C5'-radical ultimately gives rise to products resulting from C4'-C5' carbon-carbon bond fragmentation. The newly formed 3'-terminus contains a phosphate group, formed via hydrolysis of an activated formate.¹⁷ An unusual oxidized abasic lesion, 1,4-dioxo-2-phosphorylbutane (**DOB**), a 2-deoxytetrodialdose, is formed concomitantly at the 5'-terminus of the other DNA fragment.^{18,19} The elimination product, 1,4-dioxo-but-2-ene alkylate nucleosides, suggest that **DOB** may be toxic.^{20,21}

SCHEME 1.

DNA Fragmentation Products following C5'-Hydrogen Atom Abstraction

Results and Discussion

Synthesis and Characterization of DNA Containing a 5'-Aldehyde Nucleoside (T-al) at Its 5'-Terminus. Oligonucleotides containing 5'-oxidized nucleosides at their 5'-termini are labile to the alkaline conditions typically used to deprotect oligonucleotides synthesized on solid-phase supports and to heat.^{15,16} Consequently, we introduced **T-al** into the oligonucleotide postsynthetically through phosphoramidite **1** (Scheme 2). The aldehyde was produced via NaIO₄ treatment of the deprotected oligonucleotide containing the vicinal diol (**2**), a method used previously to introduce alkali-labile aldehydes in DNA.^{22,23}

SCHEME 2.

General Approach for Synthesizing Oligonucleotides Containing T-al

SCHEME 3.

Synthesis of a Phosphoramidite Precursor (1) to T-al^{aa} Key: (a) AD mix- β , ^tBuOH/H₂O; (b) DMTCl, pyridine; (c) Ac₂O, Et₃N, DMAP, CH₃CN; (d) TBAF, THF; (e) 2-cyanoethyl diisopropylchlorophosphoramidite, DIPEA, CH₂Cl₂.

Although phosphoramidites similar to **1** were previously reported, we synthesized the regioisomer via a slightly different route (Scheme 3).²⁴ Instead of utilizing ring opening of an epoxide, the previously reported alkene (**3**) was converted to the diol (**4**).^{24,25} Ligand-free OsO₄ produced **4** as a 1.5:1 ratio of isomers in good yield (88%). The alkene (**3**) was osmylated using AD mix- β to produce a 4–5:1 mixture of diastereomers of **4** in comparable yield (85%). Interestingly, AD mix- α produced the same diastereoselectivity but the reaction was very slow and only ~50% of **3** was converted after 24 h. Although it was not necessary to control the stereoselectivity of the dihydroxylation reaction, AD mix- β was used in the synthesis of **1** on a preparative scale because it facilitated purification and characterization of the products. The diol was then carried on to the phosphoramidite (**1**) via standard protecting group transformations (Scheme 3).

During solid-phase synthesis, phosphoramidite **1** was activated using tetrazole and coupled in >99% yield to the solid-phase supported oligodeoxynucleotide for 15 min prior to standard I₂ oxidation. Following detritylation, which enabled us to determine the coupling yield of **1**, and concentrated aqueous ammonia deprotection, the vicinal diol (**2**, Scheme 2) containing oligonucleotide (**7**) was purified by denaturing polyacrylamide gel electrophoresis and characterized by ESI-MS.²⁶ The final step in the synthesis of DNA containing T-al (**8**) was carried out on nanomole quantities of **7** using NaIO₄ in sodium acetate buffer (pH 6.0) at 37 °C for 1 h. The crude reactions were desalted, precipitated from ammonium acetate and characterized by ESI-MS (Figure 1A). Two species were observed in addition to the expected ion for **8**. One ion corresponded to the anticipated elimination product of T-al ($m/z = 4320.0$). The third ion possessed m/z that could be unreacted **7** or the methanol adduct of **8** ($m/z =$

4573.7), which is present in the matrix solution used for sample analysis. The latter ion was absent following treatment of the crude oxidized mixture with NaBH₄, suggesting that it was not unreacted **7** (Figure 1B). This indicates that conversion of **7** was complete. In addition, analysis of the reduced material reveals that most of the elimination product is an artifact of the ESI-MS (Figure 1B).

5'-d(XCC GTA ATG CAG TCT)

7 X = **2**

8 X = **T-al**

Further evidence that **T-al** formation was complete and did not undergo elimination to a significant extent was obtained using freshly prepared 3'-³²P-**8**. Formation and reaction of **T-al** was examined using denaturing polyacrylamide gel electrophoresis (PAGE) (Figure 2). Reaction of 3'-³²P-**7** (4 pmol, 80 nM) with NaIO₄ (25 mM) for 1 h quantitatively converted the diol to **T-al** (3'-³²P-**8**). Less than 6% of elimination product was evident on the gel using independently synthesized 5'-phosphate containing fragmentation product as a migration marker. The aldehyde did not react efficiently to form an oxime (3.2%) with aldehyde reactive probe (ARP) at pH 7.2 but was efficiently reduced (≥89%) by NaBH₄ and cleaved using NaOH (0.1 M, 37 °C, 30 min).²⁷ **T-al** was previously shown to form an oxime but at pH 9.¹⁵

Synthesis and Characterization of Oligonucleotides Containing 1,4-Dioxo-2-phosphorylbutane (DOB) at Their 5'-Termini. Abasic sites (e.g. **AP**, **L**, **C4-AP**) are typically very labile to alkaline conditions due to the increased acidity of the α-protons imparted by the carbonyl group. Photochemistry and the *o*-nitrobenzyl photoredox reaction in particular are useful for synthesizing oligonucleotides containing oxidized abasic sites (**L**, **C4-AP**).^{11,28,30} On the basis of our reported preparation of **C4-AP**, we pursued the synthesis of phosphoramidite **9** as a precursor for **DOB** (Scheme 4). The bis(*o*-nitrobenzyl)-protected abasic site would be stable to the alkaline oligonucleotide deprotection conditions and would enable us to unmask **DOB** on an as needed basis using a high yielding, reliable photochemical reaction.

SCHEME 4.

General Approach for Synthesizing Oligonucleotides Containing DOB

SCHEME 5.

Attempted Synthesis of a Phosphoramidite Precursor to DOB^{aa} Key: (a) DMTCl, pyridine; (b) TBDPSCl, imidazole, DMF; (c) DIBAL-H, toluene; (d) MsCl, Et₃N, CH₂Cl₂; (e) I₂, PPh₃ imidazole, toluene; (f) Li salt of dithiane.

SCHEME 6.

Synthesis of a Phosphoramidite Precursor (9) to DOB^{aa} Key: (a) TBDMSCl, imidazole, DMF; (b) AcOH, THF, H₂O (3:2:1); (c) Dess–Martin periodinane, K₂CO₃, CH₂Cl₂; (d) 4,5-dimethoxy-2-nitrobenzyl alcohol, NBS, CH₃CN, CH₂Cl₂; (e) TBAF, THF; (f) 2-cyanoethyl diisopropylchlorophosphoramidite, DIPEA, CH₂Cl₂.

Using the synthesis of the respective **C4-AP** phosphoramidite as a guide, we viewed **11** (Scheme 6) as a key synthetic intermediate.¹¹ Stereocontrolled synthesis of **11** was initially pursued by attempted nucleophilic substitution of the protected glycerol derivatives (**14**, **15**) (Scheme 5). The stereochemistry was set using the asymmetric dihydroxylation of the *p*-methoxybenzoate of allyl alcohol (**12**).³¹ However, dithiane anion displacement of the mesylate (**14**) or iodide (**15**) was unsuccessful. The dithiane protected dialdehyde (**11**) was prepared from **16**, which was synthesized using the previously reported dithiane anion induced ring opening of the triethylsilyl ether of *R*-glycidol (Scheme 6).³² Migration of the triethylsilyl group decreased the overall yield of **17**. Using the dimethoxytrityl ether of *R*-glycidol in place of the triethylsilyl group did not improve the overall yield (data not shown). In situ deprotection of the dithiane and cyclization provided **18** as a mixture of 4 stereoisomers, along with incompletely cyclized material. Complete separation of diastereomers from undesired compounds was achieved by silica gel chromatography of the desilylated products (**19**). Although it was impractical and unnecessary for preparative purposes, it was possible to separate **19** into pairs of diastereomers by flash chromatography. After doing so the 1*S*,4*R*-diastereomer of **19** was crystallized from ethyl acetate and characterized by X-ray diffraction.²⁶ In practice, the 4 isomers of **19** were carried on together to produce a mixture of 8 diastereomers of **9**.

Oligonucleotides containing protected **DOB** (**10**, Scheme 4) were prepared using commercially available fast deprotecting phosphoramidites and deprotected in concentrated aqueous ammonia at 25 °C for 12 h. Phosphoramidite **9** was coupled for an extended period (15 min). It was not possible to determine the coupling efficiency of **9** in real time due to the lack of a dimethoxytrityl (DMT) group. However, examination of the purification gel and comparison of the intensity of the product band with that of the single nucleotide deletion indicated that the phosphoramidite coupled as well as those of the native nucleotides (~98%).

Oligonucleotides containing **10** (**20–22**) were characterized by ESI-MS.²⁶ Oligonucleotides containing **10** were irradiated ($\lambda_{\text{max}} = 350 \text{ nm}$) for 20 min inside the cavity of a Rayonet photoreactor. ESI-MS analysis revealed a similar distribution of types of products detected during examination of **T-al**. No starting material was detected by ESI-MS, indicating that the precursor was completely converted. Analysis of **24** in a MeOH/H₂O (1:1 by volume) mixture indicated the presence of **DOB**, eliminated product, and a third product exhibiting m/z 32 amu greater than that calculated for the **DOB** containing oligonucleotide (Figure 3A). As was the case above when analyzing **T-al** (**8**) by ESI-MS, the higher molecular weight ion was believed to be a MeOH adduct of **DOB** (or mixture of such adducts). Unlike the **C4-AP** lesion, the cyclized form of **DOB** is either not detected in the ESI-MS or present as a minor component.¹¹ The source of the ion ($m/z = 4333.8$) corresponding to eliminated material was of greater concern because of its possible ramifications with respect to the photochemical process. To distinguish between elimination occurring as a result of photolysis or mass spectral analysis, the crude photolyzate was treated with NaBH₄ and then analyzed by ESI-MS (Figure 3B). Neither elimination product nor MeOH adduct was observed under these conditions. The sole products observed corresponded to the diol and its sodium ion adduct.

5'-d(XCC GTA ATG CAG TCT)

20 X = **10**

23 X = **DOB**

5'-d(XTC GTA ATG CAG TCT)

21 X = **10**

24 X = **DOB**

5'-d(XTT TTT TTT T)

22 X = **10**

25 X = **DOB**

Additional evidence that supports the proposal that the phosphate elimination product was an artifact of the ESI-MS analysis was gleaned from denaturing PAGE analysis of 3'-³²P-**20** (Figure 4). Photolysis produced a single product (**DOB**, 3'-³²P-**23**) that migrated more rapidly than the photocaged material. The absence of any photochemical precursor indicated that the photochemical conversion was complete. Mild treatment of the photolyzed material with NaOH (0.1 M, 37 °C, 30 min) converted it completely to an even faster moving product that comigrated with independently synthesized material. Similarly, treatment with NaBH₄

produced a single product that migrated more rapidly than 3'-³²P-**23** but slightly more slowly than independently prepared DNA containing a 5'-hydroxyl group. Overall, denaturing PAGE and ESI-MS reveal that **DOB** is produced cleanly and efficiently from the *o*-nitrobenzyl precursor within DNA (**10**).

These experiments and all others described below were carried out in nonnucleophilic buffer solutions due to reaction of **DOB** with tris(hydroxymethyl)aminomethane (Tris). Reaction of **DOB** with Tris was discerned by ESI-MS on unlabeled material and via denaturing PAGE using 3'-³²P-**23**. Incubation of 3'-³²P-**23** (37 °C, 24 h) in phosphate buffer pH 7.2 (20 mM) results in extensive cleavage of **DOB** to product containing 5'-phosphate (Figure 5). Treatment of the incubated material with NaBH₄ or NaOH results in reduction or elimination of the remaining **DOB** lesion, respectively. In contrast, less than 2% of 3'-³²P-**23** is cleaved following incubation in Tris buffer (20 mM, pH 7.2) for 24 h (37 °C). Furthermore, the oligonucleotide is not modified by treatment with NaBH₄ or NaOH. The change in reactivity suggests that the **DOB** lesion is modified by reaction with Tris. ESI-MS analysis of the reaction between **23** (2.5 μM) and Tris (1 mM, pH 7.2) revealed that the major product is a monoadduct whose molecular weight corresponds to the tricyclic monoadduct (Figure 6). A small amount of a bisadduct is also evident. The efficient trapping of **DOB** is consistent with that of other 1,4-dial compounds and appears to be more efficient than the previously reported reaction between **T-al** and Tris at this pH.^{15,33}

Chemical Stability of T-al and DOB Lesions. The respective rate constants for β-elimination by the lesions as part of ternary complexes (see Table 1) were determined in phosphate buffer (10 mM, pH 7.2) at 37 °C using denaturing PAGE. Comparison of the migratory ability of independently synthesized 5'-phosphate product with that of the product observed from reaction of **T-al** confirmed that decomposition occurred via β-elimination (Figure 2 and data not shown). **T-al** was very stable under these conditions, exhibiting a 100.7 h half-life ($k = 1.84 \times 10^{-6} \text{ s}^{-1}$) for elimination (Figure 7A).

The dioxobutane abasic lesion (**DOB**) was much more labile. Elimination from **DOB** was also monitored by denaturing PAGE. However, the remaining lesion in aliquots removed for analysis was stabilized by reducing with NaBH₄.³⁴ Again, the independently synthesized 5'-phosphate product produced upon elimination confirmed the nature of the product produced from **DOB** (Figure 4 and data not shown). The half-life for the disappearance of **DOB** in the respective ternary complex (Table 1) was 10.7 h ($k = 1.86 \times 10^{-5} \text{ s}^{-1}$). The **DOB** lesion is considerably less stable than **AP**, **L**, or **C4-AP** are in duplex DNA.^{12,30}

UV-Melting Temperatures of DNA Containing T-al and DOB. When **T-al** and **DOB** are formed in DNA, they are adjacent to a 5'-fragment containing a 3'-phosphate. In the case of **T-al**, stabilization due to π-stacking is still possible. Melting temperatures were determined using a 30 nucleotide long template in the presence or absence of the corresponding 5'-fragment. It is not possible to determine whether the melting of the ternary complexes involves an equal and simultaneous dissociation of all three strands. However, comparison of the T_m 's measured for the duplex and ternary systems indicate that there is some interaction between the fragmented strands (Table 1). The T_m 's of the respective ternary complexes are higher than the duplexes when **T-al** or thymidine is present at the 5'-terminus of the fragment. In addition, all of the T_m 's are higher than the duplex containing the 5'-fragment employed in the ternary complex hybridized to the template. It is also interesting to note that DNA containing **T-al** melts at higher temperatures than those containing thymidine. It will be interesting to determine whether there is any correlation between the high T_m of DNA containing **T-al** and its recognition by DNA repair enzymes.

In contrast, ternary complexes containing the tetrahydrofuran analogue of an abasic lesion (**F**), **DOB**, or the phosphate elimination product melted at lower temperatures than the respective duplexes. Furthermore, the T_m 's of duplexes containing thymidine, **DOB**, **F**, or phosphate were very similar to one another. Although one might expect the duplexes that contain the same charge but lack a 5'-terminus containing a nucleobase to melt at similar temperatures (e.g. **DOB**, **F**, phosphate), the similarity to the duplex containing thymidine might seem surprising at first. However, the results are predicted using the HyTher program in which the dA opposite thymidine is treated as a dangling end group in duplexes containing the phosphate or either abasic site (Table 2).³⁵

Conclusions

Methods for the synthesis of oligonucleotides containing the previously observed products resulting from C5'-oxidation of thymidine in DNA have been developed. Both **T-al** and **DOB** are alkali-labile, but the former is significantly less so. **T-al** does not destabilize duplex DNA compared to thymidine. **DOB** affects the thermal stability of a duplex to a similar extent as does the tetrahydrofuran model (**F**) of an abasic site. The chemical properties and physical effects of **T-al** and **DOB** suggest that their interactions with DNA repair enzymes warrant examination. The synthetic method reported herein will facilitate examination of the lesions' biochemistry.

Experimental Methods

Preparation of 4. A mixture of AD-mix β (1.4 g) in t BuOH-H₂O (1:1, 10 mL) was cooled to 0 °C. A solution of compound **3** (350 mg, 1.0 mmol) was added to the suspension, and stirred vigorously at 4 °C for 24 h. Solid sodium bisulfite (1 g) was added to the reaction mixture, followed by extraction with CH₂Cl₂. The organic layers were combined, dried over Na₂SO₄, and concentrated to dryness. The crude mixture was purified by column chromatography (25–80% ethyl acetate in hexanes) to give a diastereomeric mixture (ca. 5:1) of **4** (340 mg, 0.88 mmol, 88%) as a colorless amorphous solid: ¹H NMR (CDCl₃) δ 9.53, 9.48 (each br s, total 1 H), 7.50 (d, 0.15 H, J = 1.2 Hz), 7.40 (d, 0.85 H, J = 1.2 Hz), 6.12 (t, 0.15 H, J = 6.8 Hz) and 6.08 (dd, 0.85 H, J = 7.6, 6.3 Hz), 4.59 (ddd, 0.85 H, J = 5.9, 2.9, 2.9 Hz), 4.55 (ddd, 0.15 H, J = 6.2, 3.1 Hz), 3.98–3.62 (m, 5 H), 3.06 and 2.95 (each br s, total 1 H), 2.40 (m, 1 H), 2.15 (ddd, 1 H, J = 13.3, 6.2, 2.9 Hz), 1.89 and 1.88 (each d, total 3 H, each J = 1.2 Hz), 0.88 and 0.88 (each s, total 9 H), 0.08, 0.08 and 0.08 (each s, total 6 H); ¹³C NMR (CDCl₃) δ 164.10, 164.06, 150.6, 137.5, 111.12, 111.07, 88.0, 87.9, 87.4, 87.3, 72.8, 72.3, 71.4, 71.1, 64.8, 63.6, 40.1, 39.9, 25.70, 25.67, 17.9, 17.8, 12.5, 12.4, -4.5, -4.7, -4.9; IR (film) 3396, 3062, 2930, 2857, 1698, 1472, 1362, 1276, 1253, 1198, 1105, 1065, 843, 778 cm⁻¹; FAB-HRMS (M^+ + H^+) calcd for C₁₇H₃₁N₂O₆Si 387.1951, found 387.1958.

Preparation of 5. A mixture of compound **4** (193 mg, 0.50 mmol) and DMTCl (339 mg, 1.0 mmol) in pyridine (5.0 mL) was stirred at room temperature for 12 h. The resulting mixture was diluted with ethyl acetate, washed with H₂O, saturated NaHCO₃, and brine. After

concentration of the dried (Na_2SO_4) mixture, the crude mixture was purified by column chromatography (25–33% ethyl acetate in hexanes) to give a diastereomeric mixture (ca. 6:1) of **5** (344 mg, 0.50 mmol, 100%) as a foam: ^1H NMR (CDCl_3) δ 8.65 and 8.39 (each br s, total 1 H), 7.71–7.16 (m, 32 H), 6.25 (t, 0.86 H, $J = 7.0$ Hz), 6.18 (dd, 0.14 H, $J = 7.3$ and 6.8 Hz), 4.49 (ddd, 0.15 H, $J = 5.9$, 3.3, 3.0 Hz), 4.39 (m, 0.85 H), 4.09 (m, 1 H), 3.79 and 3.78 (each s, total 6 H), 3.38 (t, 0.14 H, $J = 8.6$ Hz), 3.24 (dd, 1 H, $J = 9.4$, 3.7 Hz), 3.15 (t, 0.86, $J = 9.2$ Hz), 2.85 (br s, 0.86 H), 2.81 (br s, 0.14 H), 2.14 (dd, 1 H, $J = 7.0$, 4.0 Hz), 1.92 and 1.91 (each d, total 3 H, each $J = 1.1$ Hz), 0.89, 0.81 (each s, total 9 H), 0.07, 0.04, -0.04 and -0.12 (each s, total 6 H); ^{13}C NMR (CDCl_3) δ 171.2, 170.0, 169.6, 163.9, 163.8, 158.5, 150.3, 150.2, 144.4, 135.6, 135.5, 135.0, 134.6, 129.91, 129.85, 127.9, 127.8, 126.8, 113.1, 110.9, 110.7, 86.3, 86.2, 85.9, 85.8, 85.3, 84.7, 72.4, 72.3, 72.2, 71.9, 64.3, 62.6, 62.3, 60.3, 55.1, 53.8, 41.0, 40.4, 30.5, 29.2, 25.6, 25.4, 21.1, 21.0, 20.94, 20.91, 19.0, 17.8, 14.1, 13.6, 12.6, 12.5, -4.7 , -4.8 , -5.0 ; IR (film) 3448, 3189, 3061, 2929, 2856, 1694, 1608, 1510, 1470, 1251, 1177, 1058, 1035, 833, 778 cm^{-1} ; FAB-HRMS (M^+) calcd for $\text{C}_{38}\text{H}_{48}\text{N}_2\text{O}_8\text{Si}$ 688.3180, found 688.3120.

Preparation of 6. A mixture of **5** (344 mg, 0.50 mmol), Et_3N (110 μL , 0.79 mmol, 80 mg), acetic anhydride (71 μL , 0.75 mmol, 77 mg), and DMAP (6 mg, 0.05 mmol) in acetonitrile (5.0 mL) was stirred at room temperature for 2 h. The resulting mixture was diluted with ethyl acetate, washed with H_2O , saturated NaHCO_3 , and brine. The dried (Na_2SO_4) mixture was concentrated and purified by column chromatography (33–50% ethyl acetate in hexanes) to give a diastereomeric mixture (ca. 6:1) of **6** (359 mg, 0.49 mmol, 98%) as a yellow foam: ^1H NMR (CDCl_3) δ 8.13 (br s, 1 H), 7.41–7.17 (m, DMTr, CHCl_3), 6.95 (d, 1H, $J = 1.2$ Hz), 6.83–6.80 (m, 4 H), 6.25 (dd, 0.16 H, $J = 7.6$, 5.6 Hz), 6.17 (dd, 0.84 H, $J = 8.8$ and 5.2 Hz), 5.31 (m, 0.16 H), 5.25 (ddd, 0.84 H, $J = 6.0$, 4.4 Hz), 4.33 (ddd, 0.84 H, $J = 5.6$, 2.0, 1.8 Hz), 4.21 (m, 0.16 H), 4.08 (dd, 0.84 H, $J = 6.0$, 2.0 Hz), 3.78 (s, 6 H), 3.40 (dd, 0.16 H, $J = 10.0$, 7.6 Hz), 3.31–3.20 (m, 0.16 H), 3.27 (dd, 0.84 H, $J = 10.2$, 6.2 Hz), 3.25 (dd, 0.84 H, $J = 10.2$, 4.6 Hz), 2.26–2.14 (m, total 4 H), 1.92–1.81 (m, 4 H), 0.87, 0.86 (each s, total 9 H), 0.04, 0.03 and -0.01 (each s, total 6 H); ^{13}C NMR (CDCl_3) δ 171.2, 170.0, 169.6, 163.9, 163.8, 158.5, 150.3, 150.2, 144.4, 135.6, 135.5, 135.0, 134.6, 129.91, 129.85, 127.9, 127.8, 126.8, 113.1, 110.9, 110.8, 86.3, 86.2, 85.9, 85.8, 85.3, 84.7, 72.4, 72.3, 72.2, 71.9, 64.3, 62.6, 62.3, 60.3, 55.1, 53.8, 41.0, 40.4, 30.5, 29.2, 25.6, 25.4, 21.1, 21.0, 20.94, 20.91, 19.0, 17.8, 14.1, 13.6, 12.6, 12.5, -4.7 , -4.8 , -5.0 ; IR (film) 3183, 3056, 2954, 2931, 2857, 1745, 1694, 1608, 1510, 1464, 1371, 1251, 1177, 1059, 1035, 833, 779, 702, 596, 584 cm^{-1} ; FAB-HRMS (M^+) calcd for $\text{C}_{40}\text{H}_{50}\text{N}_2\text{O}_9\text{Si}$ 730.3286, found 730.3301.

Preparation of Desilylated 6. A solution of TBAF (1.0 M in THF, 900 μL) was added to a solution of **6** (435 mg, 0.60 mmol) in THF (5.0 mL) at room temperature, and the mixture was stirred at the same temperature for 12 h. The resulting mixture was concentrated, and the crude mixture was purified by column chromatography (75–100% ethyl acetate in hexanes) to give the desilylated product as a mixture of diastereomers (336 mg, 0.55 mmol, 92%) as a white foam: ^1H NMR (CDCl_3) δ 8.50 (br s, 1 H), 7.42–7.21 (m, 9.12 H), 7.06 (d, 0.88 H, $J = 0.9$ Hz), 6.85–6.83 (m, 4.12 H), 6.20 (t, 1 H, $J = 6.6$ Hz), 5.29 (dd, 1 H, $J = 7.5$ and 3.8 Hz), 4.68 (m, 1 H), 4.24 (m, 0.12), 4.10–3.97 (m, 1 H), 3.79–3.73 (m, 6.48 H), 3.52–3.47 (m, 1.88 H), 3.39 (dd, 0.12 H, $J = 10.2$, 6.1 Hz), 3.31 (dd, 0.12 H, $J = 10.2$, 4.5 Hz), 3.21 (dd, 0.88 H, $J = 10.8$, 4.0 Hz), 2.45–2.35 (m, total 1 H), 2.18 (s, 3 H), 1.91 (s, 3 H); ^{13}C NMR (CDCl_3) δ 170.2, 170.0, 163.9, 163.8, 158.6, 158.54, 158.50, 150.39, 150.35, 144.3, 144.1, 135.5, 135.4, 135.3, 135.1, 134.8, 129.9, 129.8, 127.9, 127.8, 127.0, 126.9, 113.2, 113.2, 111.1, 86.8, 86.5, 85.9, 84.7, 84.4, 72.0, 71.3, 70.3, 62.6, 55.1, 40.0, 21.0, 12.6, 12.5; IR (film) 3417, 3194, 3055, 2933, 2837, 1694, 1608, 1509, 1465, 1371, 1250, 1177, 1035, 829 cm^{-1} ; FAB-HRMS (Na^+) calcd for $\text{C}_{34}\text{H}_{36}\text{N}_2\text{O}_9$ 616.2421, found 616.2426.

Preparation of Phosphoramidite 1. To a solution of the above alcohol (185 mg, 0.3 mmol) and DIPEA (105 μL , 0.60 mmol, 78 mg) in CH_2Cl_2 (3 mL) was added 2-cyanoethyl

diisopropylchlorophosphoramidite (100 μ L, 0.45 mmol, 107 mg) at 0 $^{\circ}$ C, and the resulting solution was stirred at room temperature for 4 h. The reaction mixture was diluted with ethyl acetate and washed with saturated NaHCO_3 , H_2O , and brine. After concentration of the dried (Na_2SO_4) mixture, residue was purified by column chromatography (25–35% ethyl acetate in hexanes with 0.5% Et_3N) to give **1** (195 mg, 0.24 mmol, 80%) as a pale yellow amorphous solid: ^1H NMR (CDCl_3) δ 7.42–7.18 (m, 9.14 H), 6.99–6.96 (m, 0.86 H), 6.84–6.79 (m, 4 H), 6.29 (m, 0.14 H), 6.19 (dd, 0.86 H, J = 8.4, 5.7 Hz), 5.40–5.23 (m, 1 H), 4.54 (m, 0.86 H), 4.44 (m, 0.14 H), 4.22 (m, 1 H), 3.89 (t, 0.4 H, J = 6.2 Hz), 3.85–3.48 (m, 11 H), 3.42–3.23 (m, 2 H), 2.63–2.58 (m, 1.4 H), 2.54–2.40 (m, 2 H), 2.15, 2.14, 2.13, 2.12 (each s, total 3 H), 2.06–1.93 (m, 2.3 H), 1.89 (m, 0.4 H), 1.86 (m, 2.6 H), 1.19–1.13 (m, total 12 H); ^{13}C NMR (CDCl_3) δ 169.9, 163.83, 163.78, 158.4, 158.3, 150.3, 150.2, 144.4, 135.5, 135.4, 134.52, 134.46, 129.8, 127.9, 127.7, 127.6, 126.72, 126.66, 117.5, 117.4, 113.0, 112.9, 111.0, 86.3, 86.2, 84.9, 84.8, 84.6, 84.5, 84.3, 84.2, 73.6, 73.5, 72.8, 72.6, 72.2, 62.2, 62.8, 58.28, 58.27, 58.0, 57.8, 55.0, 43.2, 43.2, 43.1, 43.0, 38.9, 24.5, 24.4, 24.3, 24.2, 21.00, 20.96, 20.2, 20.1, 20.0, 19.9, 12.5, 12.3; ^{31}P NMR (CDCl_3) δ 149.36, 149.11, 149.03, 149.01; IR (film) 3189, 3056, 2967, 2933, 2837, 1743, 1693, 1608, 1583, 1509, 1465, 1366, 1250, 1179, 1036, 979, 830, 736 cm^{-1} ; FAB-HRMS ($[\text{M} + \text{Na}]^+$) calcd for $\text{C}_{43}\text{H}_{53}\text{N}_4\text{O}_{10}\text{NaP}$ 839.3397, found 839.3387.

Preparation of 17. Imidazole (450 mg, 6.6 mmol) and TBDMSCl (500 mg, 3.3 mmol) were added to a solution of **16** (925 mg, 3.0 mmol) in DMF (30 mL) and stirred at room temperature for 1 h. The reaction mixture was diluted with diethyl ether, washed with H_2O and brine, dried (Na_2SO_4), and evaporated to give a yellow oil. A solution of $\text{AcOH-H}_2\text{O}$ (3:1, 24 mL) was added to the crude material in THF (12 mL) at 0 $^{\circ}$ C, and the mixture was stirred at this temperature for 2 h. The resulting mixture was poured into ice-cooled saturated NaHCO_3 , and the contents were extracted with CH_2Cl_2 . The combined organic layers were washed with saturated NaHCO_3 and brine, dried with Na_2SO_4 , and concentrated. The residue was purified by column chromatography (5% acetone in hexanes) to give **17** (400 mg, 1.3 mmol, 43%) as a colorless solid: ^1H NMR (CDCl_3) δ 4.05 (m, 2 H), 3.61 (m, 1 H), 3.49 (ddd, 1 H, J = 12.0, 8.0, 4.0 Hz), 2.92–2.80 (m, 4 H), 2.10 (m, 1 H), 1.94–1.84 (m, 4 H), 0.91 (s, 9 H), 0.14 (s, 3 H), 0.11 (s, 3 H); ^{13}C NMR (CDCl_3) δ 69.3, 66.3, 43.7, 39.4, 30.4, 30.1, 25.9, 25.8, 18.1, –4.57, –4.62; IR (film) 3284, 3186, 2926, 2895, 2853, 1248, 1126, 1110, 1071, 1039, 934, 836, 782, 744 cm^{-1} ; FAB-HRMS ($\text{M}^+ + \text{H}$) calcd for $\text{C}_{13}\text{H}_{29}\text{O}_2\text{S}_2\text{Si}$ 309.1378, found 309.1347.

Preparation of 11. A mixture of **17** (926 mg, 3.0 mmol), Dess–Martin periodinane (1.91 g, 4.5 mmol), and anhydrous K_2CO_3 (1.24 g, 9.0 mmol) in CH_2Cl_2 (90 mL) was stirred at 25 $^{\circ}$ C for 30 min. A mixture of saturated $\text{Na}_2\text{S}_2\text{O}_3$ and saturated NaHCO_3 (1:4, 100 mL) was added to the resulting mixture and stirred vigorously for 5 min. The organic layer was washed with saturated NaHCO_3 and brine and dried over Na_2SO_4 . The mixture was concentrated to give **11** (925 mg, 3.0 mmol, 100%) as an amorphous solid: ^1H NMR (CDCl_3) δ 9.66 (d, 1 H, J = 1.2 Hz), 4.24 (ddd, 1H, J = 6.8, 5.2, 0.8 Hz), 4.07 (t, 1 H, J = 7.4 Hz), 2.89 (ddd, 1 H, J = 14, 6.8, 2.8 Hz), 2.82–2.69 (m, 3 H), 2.20–2.16 (m, 2 H), 1.97–1.88 (m, 1 H), 0.93 (s, 9 H), 0.11 (s, 6 H); ^{13}C NMR (CDCl_3) δ 203.3, 74.7, 41.2, 38.7, 28.7, 28.5, 25.7, 25.6, 18.1, –4.6, –4.9; IR (film) 2953, 2930, 2897, 2856, 1735, 1472, 1424, 1361, 1253, 1120, 938, 838, 779 cm^{-1} ; FAB-HRMS ($\text{M}^+ + \text{H}$) calcd for $\text{C}_{13}\text{H}_{27}\text{O}_2\text{S}_2\text{Si}$ 307.1222, found 307.1247.

Preparation of 19. To a mixture of compound **11** (340 mg, 1.1 mmol) and 4,5-dimethoxy-2-nitrobenzyl alcohol (1.17 g, 5.5 mmol) in $\text{MeCN-CH}_2\text{Cl}_2$ (2:1, 30 mL) was added NBS (1.17 g, 6.6 mmol) at –10 $^{\circ}$ C. The resulting mixture was stirred for 1 h at this temperature and poured into an ice-cooled mixture of saturated $\text{Na}_2\text{S}_2\text{O}_3$ and saturated NaHCO_3 (1:4). The mixture was extracted with CH_2Cl_2 , and the organic layer was washed with saturated NaHCO_3 brine, followed by drying over Na_2SO_4 prior to concentration. The residue was purified by column chromatography (20–25% ethyl acetate in hexanes) to give an amorphous solid (319 mg)

containing **18** and one or more other products that are derivatized with 4,5-dimethoxy-2-nitrobenzyl alcohol. A solution of the yellow amorphous solid (281 mg) in THF (5.0 mL) was added to a solution of TBAF (1.0 M in THF, 500 μ L, 0.5 mmol) at 0 °C and stirred for 2 h at this temperature. The mixture was evaporated, and the residue was purified by column chromatography (a small amount of CH_2Cl_2 was used as a loading solution and the mixture was eluted using 60–70% ethyl acetate in hexanes) to give a diastereomeric mixture of **19** (199 mg, 0.39 mmol, 40% from **11**) as a yellow solid. One diastereomer was crystallized from a mixture of two isomers using ethyl acetate after further purification by column chromatography (65% ethyl acetate in hexanes with 0.5% Et_3N). Data for the crystallized diastereomer (**1S**, **4R-19**): ^1H NMR (CDCl_3) δ 7.65, 7.64 (each s, each 1 H), 7.16, 7.08 (each s, each 1 H), 5.30 (dd, 1 H, $J = 5.6, 4.0$ Hz), 5.15 (d, 1 H, $J = 4.4$ Hz), 5.11 (s, 2 H), 5.03 (d, 1 H, $J = 14.6$ Hz), 4.94 (d, 1 H, $J = 14.6$ Hz), 4.34 (m, 1 H), 3.94, 3.92, 3.89, 3.88 (each s, each 3 H), 2.58 (ddd, 1 H, $J = 13.2, 7.6, 5.6$ Hz), 2.52 (d, 1 H, $J = 14.0$ Hz), 2.09 (ddd, 1 H, $J = 13.2, 7.2, 4.0$ Hz); ^{13}C NMR (CDCl_3) δ 153.4, 147.84, 147.80, 139.63, 139.57, 129.3, 129.1, 110.4, 110.3, 108.0, 103.4, 101.6, 71.4, 67.2, 67.0, 56.31, 56.27, 39.5. Data for a mixture of 4 diastereomers: ^1H NMR (CDCl_3) δ 7.61, 7.60 (each s, each 1 H), 7.09, 7.02 (each s, each 1 H), 5.57 (dd, 1 H, $J = 5.6, 4.0$ Hz), 5.16 (s, 1 H), 5.03–4.91 (m, 4 H), 4.50 (dd, 1 H, $J = 5.6, 2.8$ Hz), 3.91, 3.91, 3.86, 3.83 (each s, each 3 H), 2.70 (br s, 1 H), 2.38 (ddd, 1 H, $J = 14.0, 5.6, 4.0$ Hz), 2.25 (ddd, 1 H, $J = 14.0, 5.6, 2.8$ Hz); ^{13}C NMR (CDCl_3) δ 153.47, 153.45, 153.1, 153.33, 153.30, 153.23, 153.19, 147.9, 147.8, 147.61, 147.59, 147.55, 139.6, 139.5, 139.44, 139.41, 139.3, 139.2, 129.6, 129.40, 129.36, 129.30, 129.21, 129.15, 129.0, 110.6, 110.3, 110.2, 109.9, 109.7, 109.2, 108.8, 108.0, 107.94, 107.92, 107.85, 107.80, 107.7, 105.5, 105.0, 103.3, 102.5, 101.5, 101.3, 75.6, 74.4, 71.3, 70.7, 67.4, 67.2, 67.1, 66.8, 66.5, 66.2, 56.34, 56.25, 56.19, 56.17, 56.12, 56.07, 39.9, 38.8, 38.59, 38.57; IR (film) 1581, 1520, 1325, 1275, 1220, 1066, 986, 873, 796, 756 cm^{-1} ; FAB-HRMS (M^+) calcd for $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_{12}$ 510.1486, found 510.1480.

Preparation of Phosphoramidite 9. To a solution of **19** (255 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) was added DIPEA (175 μ L, 1.00 mmol, 130 mg) and 2-cyanoethyl diisopropylchlorophosphoramidite (170 μ L, 0.76 mmol, 180 mg) at 0 °C. The resulting solution was warmed to room temperature. After being stirred for 1 h, the reaction mixture was diluted with ethyl acetate, washed with saturated $\text{NaHCO}_3/\text{H}_2\text{O}$, and brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (40–50% ethyl acetate in hexanes with 0.5% Et_3N) to give **9** (271 mg, 0.38 mmol, 76%) as a yellow amorphous solid: ^1H NMR (CDCl_3) δ 7.74–7.00 (m, 4 H), 5.55–4.85 (m, 6 H), 4.69–4.50 (m, 0.43 H), 4.40–4.22 (m, 0.57 H), 4.10–3.69 (m, 14 H), 3.67–3.51 (m, 2 H), 2.70–2.20 (m, 4 H), 1.38–1.01 (m, 12 H); ^{13}C NMR (CDCl_3) δ 153.6, 153.54, 153.47, 153.29, 153.27, 153.25, 153.23, 147.70, 147.68, 147.63, 147.59, 147.56, 147.52, 147.48, 139.5, 139.42, 139.39, 139.37, 139.35, 139.33, 139.25, 139.1, 138.8, 130.6, 130.3, 130.2, 129.8, 129.6, 129.50, 129.49, 129.42, 129.39, 129.35, 129.30, 117.55, 117.50, 110.5, 110.4, 110.3, 110.24, 110.21, 110.15, 110.11, 110.08, 110.05, 110.02, 109.99, 109.85, 109.77, 108.11, 108.05, 108.00, 107.94, 107.91, 107.8, 107.7, 107.6, 105.7, 105.2, 105.1, 102.8, 102.6, 102.1, 100.6, 100.4, 67.3, 67.2, 67.14, 67.05, 66.8, 66.7, 66.7, 66.3, 66.1, 65.9, 58.5, 58.42, 58.36, 58.33, 58.24, 58.17, 58.06, 56.42, 56.36, 56.31, 56.26, 56.25, 56.20, 56.18, 56.17, 56.13, 56.11, 43.22, 43.18, 43.09, 43.05, 39.4, 39.3, 39.10, 39.05, 38.2, 37.1, 24.54, 24.47, 24.42, 24.35, 24.29, 24.27, 24.23, 20.34, 20.32, 20.30, 20.27, 20.23; ^{31}P NMR (CDCl_3) δ 148.79, 148.76, 148.70, 148.66, 148.55, 148.32; IR (film) 2967, 1581, 1520, 1413, 1363, 1327, 1276, 1221, 1067, 981, 876, 796 cm^{-1} ; FAB-HRMS (M^+) calcd for $\text{C}_{31}\text{H}_{43}\text{N}_4\text{O}_{13}\text{P}$ 710.2564, found 710.2567.

Preparation of DOB-Containing Oligonucleotides. Following radiolabeling using standard methods (terminal deoxynucleotidyl transferase, α - ^{32}P -ddATP), the oligonucleotide (10 pmol) was separated from unincorporated α - ^{32}P -ddATP using a C_{18} -Sep pak cartridge. Photolysis ($\lambda_{\text{max}} = 350$ nm) was carried out in sodium phosphate (50 μ L, 50 mM, pH 7.2) at room

temperature for 20 min. When **DOB** was to be used in duplex or ternary complexes, hybridization was carried out prior to photolysis to avoid elimination of the lesion. Nanomole scale preparations were carried out in TEAA buffer (10 mM, pH 7.2). Following irradiation (20 min), TEAA was removed using a C₁₈-Sep pak cartridge and washing with NH₄OAc (5 mM).

Preparation of T-al-Containing Oligonucleotides. Following radiolabeling using standard methods (terminal deoxynucleotidyl transferase, α -³²P-ddATP), the oligonucleotide (10 pmol) was separated from unincorporated α -³²P-ddATP using a C₁₈-Bakerbond spe cartridge (100 mg). The 3'-³²P-labeled oligonucleotide was reacted in 100 mM NaOAc (pH 6.0) with 25 mM NaIO₄ at room temperature for 60 min (total volume: 50 μ L). The oligonucleotide was desalted by passing through a G-25 Sephadex column that was equilibrated with H₂O, followed by washing with 50 μ L of H₂O. The pH of the combined fractions was adjusted with 500 mM (5 μ L) sodium phosphate (pH 7.2) and concentrated to 50 μ L. Larger scale preparations of unlabeled **8** (e.g. 4 nmol/1 mL) were carried out for 1 h at 37 °C. Samples were diluted with NH₄OAc (10 mM, 4 mL) and desalted using a C₁₈-Sep pak cartridge, which was washed with NH₄OAc (50 mM).

Reaction of DOB with Tris-HCl. 3'-³²P-**23** (10 pmol, 45 μ L) in 10 mM sodium phosphate (pH 7.2) was mixed with 5 μ L of 200 mM Tris-HCl buffer pH 7.2 and incubated at 37 °C for 24 h. Each solution was divided into 3 tubes and mixed with 1.8 μ L of (I) H₂O, (II) 500 mM NaBH₄ in H₂O, or (III) 1.0 M NaOH. The mixtures were incubated for 30 min at room temperature (I, II) or at 37 °C (III). The NaOH reaction was neutralized with 1.0 M HCl. Control experiments were carried out in 10 mM sodium phosphate buffer (pH 7.2) without the addition of Tris-HCl. After incubation, each solution was mixed with 95% formamide loading buffer and analyzed by 20% denaturing PAGE.

Decomposition Kinetics of DOB and T-al. Ternary complexes of 3'-³²P-**7** (**T-al**) or 3'-³²P-**20** were prepared in 50 mM sodium phosphate (pH 7.2) and 100 mM NaCl. Complex containing **10** was irradiated at room temperature for 20 min, and the DNA was used without further purification. Complex containing **7** was incubated in 25 mM NaIO₄ and 100 mM NaOAc (pH 6.0) at room temperature for 1 h and then passed through a G-25 Sephadex column (50 mM sodium phosphate (pH 7.2), 100 mM NaCl). The ternary complexes were incubated at 37 °C for 24 h (**DOB**) or 96 h (**T-al**). Aliquots (4.5 μ L) were mixed with 0.5 μ L of 0.5 M NaBH₄, incubated at room temperature for 30 min, and stored at -20 °C while awaiting denaturing PAGE separation.

Thermal Denaturation Study of Duplexes. Oligonucleotides **8** and **23** were prepared as described above. Samples were 2 μ M in each oligonucleotide. Melts were carried out in 10 mM sodium phosphate (pH 7.2) and 100 mM NaCl, and the temperature was increased at 0.5 °C/min. Readings were taken every 0.2 °C.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.
ESI-MS analysis of **8** prior to (A) or after (B) treatment with NaBH₄. R = CCG TAA TGC
AGT CT-3'. * = Na⁺ adduct.

FIGURE 2.

Denaturing PAGE analysis of formation of **T-al** in **8** from **2** by treatment with NaIO₄. * = slower migrating product attributable to ARP adduct of **T-al**. R = CCG TAA TGC AGT CT-3'-³²P-ddA.

FIGURE 3.
ESI-MS analysis of **DOB** in **24** (A) in MeOH:H₂O and (B) after NaBH₄ reduction. R = TCG
TAA TGC AGT CT-3'. * = Na⁺ adduct.

FIGURE 4.
Denaturing PAGE analysis of **DOB** (**23**) produced upon photolysis of **20**. R = CCG TAA TGC
AGT CT-3'-³²P-ddA.

FIGURE 5.

Denaturing PAGE analysis of **DOB (23)** with or without incubation with Tris (pH 7.2, 37 °C, 24 h). R = CCG TAA TGC AGT CT-3'-pddA.

FIGURE 6.
ESI-MS analysis of the reaction of **DOB (23)** with Tris. R = CCG TAA TGC AGT CT-3'. *
= Na⁺ adduct.

FIGURE 7. Decomposition within ternary complexes in sodium phosphate (50 mM, pH 7.2) of (A) **T-al** from **8** and (B) **DOB** from **23**. See Table 1 for ternary complex structures.

Table 1UV-Melting Temperatures of Duplexes and Ternary Complexes^{a,b}

	X	T_m(°C)
5'-TAA TGG CTA ACG CAGp	T	53.7 ± 0.2
3'-ATT ACC GAT TGC GTC AGG CAT TAC GTC AGA-5'	T-al	55.2 ± 0.1
Ternary complexes	DOB	51.6 ± 0.1
	F	51.7 ± 0.4
	p	51.6 ± 0.2
	X	T_m(°C)
	T	52.9 ± 0.1
5'-TAA TGG CTA ACG CAGp	T-al	53.5 ± 0.1
3'-ATT ACC GAT TGC GTC AGG CAT TAC GTC AGA-5'	DOB	52.7 ± 0.3
Duplexes	F	52.8 ± 0.3
	p	52.2 ± 0.2
5'-TAA TGG CTA ACG CAGp		50.8 ± 0.4
3'-ATT ACC GAT TGC GT C AGG CAT TAC GTC AGA-5'		
5'-TAA TGG CTA ACG CAG TCC GTA ATG CAG TCT-3'		73.5 ± 0.1
3'-ATT ACC GAT TGC GTC AGG CAT TAC GTC AGA-5'		

^a Oligonucleotides, 2 μM, sodium phosphate (pH 7.2), 10 mM, NaCl, 100 mM.^b p = phosphate terminus.

Table 2Predicted UV-Melting Temperatures of Duplexes^{a,b}

	T_m(°C)
CC GTA ATG CAG TCT-3'	
GG CAT TAC GTC AGA-5'	50.5
TCC GTA ATG CAG TCT-3'	
AGG CAT TAC GTC AGA-5'	52.7
CC GTA ATG CAG TCT-3'	
AGG CAT TAC GTC AGA-5'	52.7

^a Predictions made using HyTher.³⁵ Oligonucleotides, 2 μM, Na⁺, 100 mM.