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Synthesis and Analysis of Oligonucleotides Containing Abasic Site Analogues

Haidong Huang and Marc M. Greenberg*

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

mgreenberg@jhu.edu

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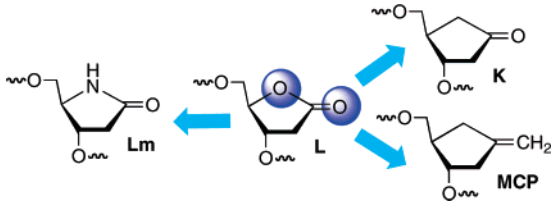
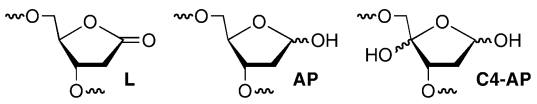


DNA damage results in the formation of abasic sites from the formal hydrolysis of the glycosidic bond (AP) and several oxidized abasic lesions. Previous studies on AP sites revealed that DNA polymerases preferentially incorporated dA opposite them in ~80% of the replication events in *Escherichia coli*. These results were consistent with the hypothesis that the AP sites are noninstructive lesions due to the absence of a Watson−Crick base whose bypass adheres to the "A-rule." Recent replication studies of the oxidized abasic lesion, 2-deoxyribonolactone (L), revealed that DNA polymerase(s) does not apply the A-rule when bypassing it and incorporates large amounts of dG opposite L. These studies suggested that abasic sites such as L do direct polymerases to selectively incorporate nucleotides opposite them. However, it was not possible to determine the structural basis for this molecular recognition from these experiments. A group of oligonucleotides containing analogues of the AP and L lesions were synthesized and characterized as probes to gain insight into the structural basis for the distinct effect of 2-deoxyribonolactone on replication. These molecules will be useful tools for studying replication in cells and in vitro.

Introduction

Abasic sites are a family of DNA lesions that lack the heterocycles involved in Watson−Crick base pair formation in duplex DNA. The unoxidized abasic site, AP, results from spontaneous cleavage of the glycosidic bond but is also formed when DNA is exposed to oxidative stress.[1] A variety of oxidized abasic sites (e.g., L, C4-AP) are produced when DNA is exposed to oxidative stress. 2-Deoxyribonolactone (L) is produced by a



variety of anti-tumor agents that bind in the minor groove of DNA and is the major abasic lesion produced when DNA is exposed to γ-radiolysis or Fe·EDTA, both of which produce hydroxyl radical.[2−5] Despite the inaccessibility of the C1′-hydrogen atom to diffusible species such as hydroxyl radical, L is formed in 3-times greater yield than AP sites.[6,7] Furthermore, C4-AP results from abstraction of the C4′-hydrogen atom that rests on the outer edge of the minor groove and forms a carbon hydrogen bond that is only marginally stronger than that between the C1′-carbon and hydrogen.[6] Nonetheless, the γ-radiolysis-induced yield of C4-AP is only ~1/10 that of 2-deoxyribonolactone (L).[8] One explanation for the higher than expected yield of L involves its formation as the 5′-component of tandem lesions that result from initial radical addition to the pyrimidine base (Scheme 1).[9,10]

(1) Lindahl, T. *Nature* **1993**, *362*, 709−715.
(2) Xi, Z.; Goldberg, I. H. In *Comprehensive Natural Products Chemistry*; Kool, E. T., Ed.; Elsevier: Amsterdam, 1999; Vol. 7, pp 553−592.
(3) Xue, L.; Greenberg, M. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 561−564.
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(6) Miaskiewicz, K.; Osman, R. *J. Am. Chem. Soc.* **1994**, *116*, 232−238.
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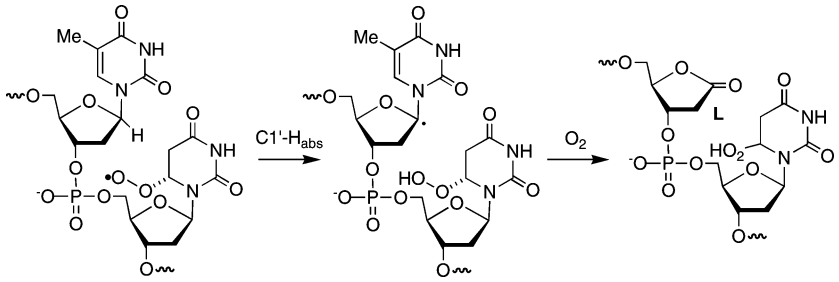
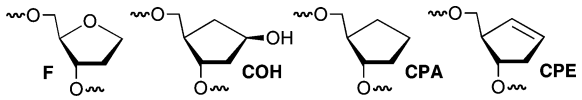
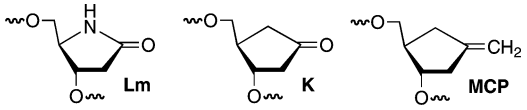
SCHEME 1. 2-Deoxyribonolactone Formed via Internucleotidyl Hydrogen Atom Abstraction



Nucleobase radicals are believed to account for as much as 90% of the reactive intermediates resulting from hydroxyl radical addition to the π-bonds of the Watson−Crick bases, and tandem lesions containing L account for more than 10% of the chemistry attributable to at least one of these.[9,11] The respective nucleobase peroxyl radicals overcome the inaccessibility of the C1′-hydrogen atom faced by a highly reactive diffusible species (e.g., hydroxyl radical) by taking advantage of the helical twist of the duplex, which brings the C1′-hydrogen atom of the 5′-adjacent nucleotide into close proximity. The biochemical significance of 2-deoxyribonolactone's formation is exemplified by its ability to form cross-links with DNA repair enzymes, as well as its effects on replication.[12,13] The distinct effect that L has on replication in *Escherichia coli* provided the impetus for the synthesis and characterization of the oligonucleotide probes presented in this study.[14]



As members of the family of abasic lesions, L was expected to adhere to the "A-rule" and not instruct a DNA polymerase to incorporate a particular nucleotide opposite it.[15,16] 2′-Deoxyadenosine is typically incorporated opposite "noninstructive" lesions, such as AP and its tetrahydrofuran analogue (F) in ∼80% of replication events.[17,18] Surprisingly, transfection of *E. coli* with a single stranded genome containing L resulted in levels of dG incorporation opposite the lesion that were comparable to those of dA.[14] This led to the proposal that L instructed the bypass polymerase, DNA polymerase V to incorporate higher levels of dG than expected for a noninstructive lesion. The most obvious structural source for the differences in AP and L bypass was the presence of a hydrogen bond acceptor in the latter compared to a hydroxyl group at the C1-position of AP. The presence of the carbonyl group in L also affects the conformation of the five-membered ring (Figure 1). In order to probe the structural source for the differences in polymerase bypass of AP and L, we synthesized a group of oligonucleotides containing analogues of these lesions.



Results and Discussion

Probe Design. When evaluating the structural differences between the AP and L lesions we focused primarily on the hydrogen-bonding groups at C1, which are the most obvious. The effect of the cyclic oxygen that is in conjugation with the carbonyl in L on the conformation of the ring was also considered. The structures of the hydroxylated monomeric components were calculated at the B3LYP/6-31G(d.p) level (Figures 1 and 2). These calculations were used as a guide despite the possibility that incorporation into oligonucleotides could alter their structures. Three molecules were considered as candidates to probe whether the carbonyl group is directly responsible for the differences in replication of AP and L. The lactam (Lm) and ketone (K) retain the hydrogen bond acceptor. These molecules also allow us to probe the importance of the cyclic oxygen and the conformation of the 5-membered ring. The five membered rings of the lactam, 2-deoxyribonolactone (L), and cyclopentanone (K) adopt different conformations. The ketone (K) also does not require the cyclic atom at position five to maintain coplanarity with the carbonyl group. In addition, by retaining the C1-carbonyl group but varying the nature of the ring the ketone (K) and Lm provide tests for whether the cyclic oxygen plays a specific role in the bypass of 2-deoxyribonolactone (L) as a hydrogen bond acceptor. In contrast, the methylene cyclopentane (MCP) maintains the planarity of the carbonyl group in L while eliminating the contribution of hydrogen bonding.

Three other analogues were conceived to test the importance of the hydrogen bond acceptor at the C1 position of an abasic site (F, COH), as well as the importance of the conformation of the 5-membered ring (CPE, CPA). The configurationally stable, cyclonucleoside analogue of an AP site (COH) has been studied previously in DNA.[19] This molecule probes whether the cyclic oxygen has any role in polymerase bypass. The tetrahydrofuran analogue (F) is commonly used to mimic AP sites because of its chemical stability.[18] The hydrocarbon analogues (CPA, CPE) probe whether ring conformation significantly influences polymerase activity. The cyclopentane analogue

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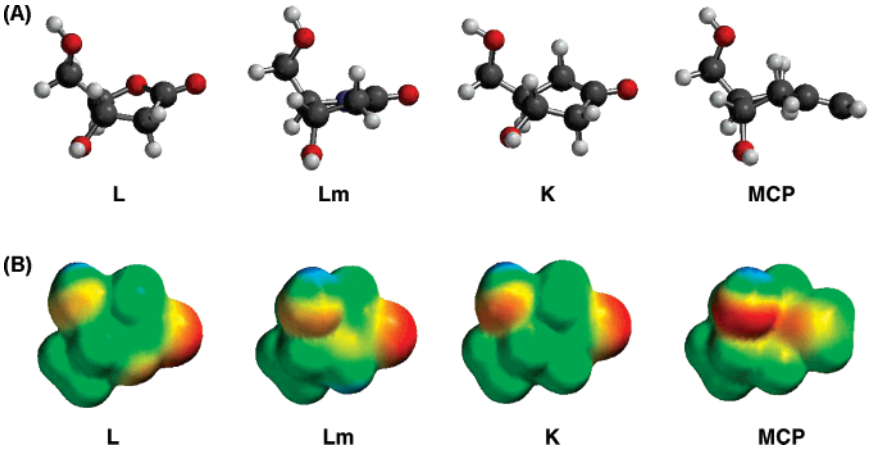


FIGURE 1. Computational analysis of monomeric 2-deoxyribonolactone (L) and analogues. (A) Energy minimized structures. (B) Electron density maps viewed from the bottom faces of the molecules. Electron density correlates with color. Blue−red, minimum−maximum.

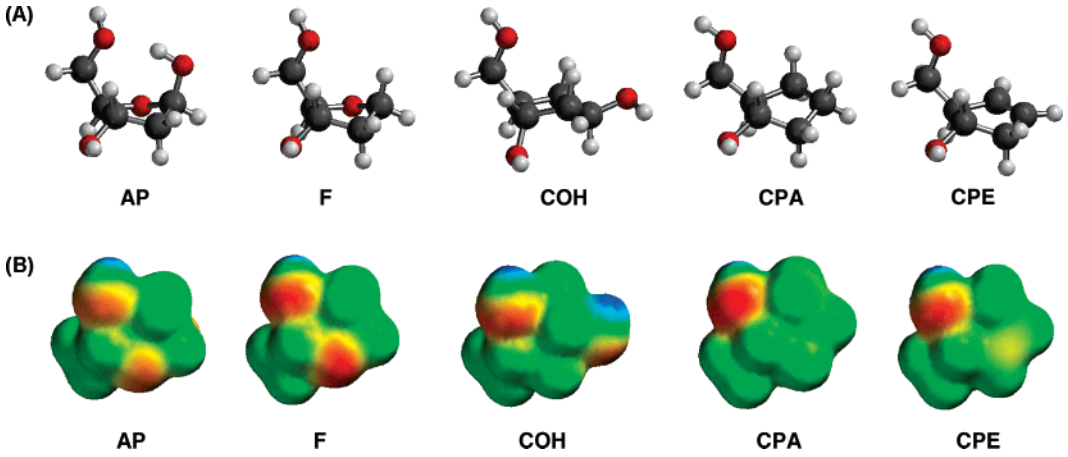


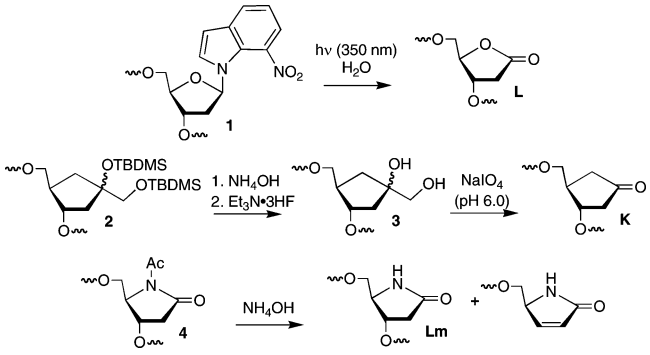
FIGURE 2. Computational analysis of monomeric abasic site (AP) and analogues. (A) Energy minimized structures. (B) Electron density maps viewed from the bottom faces of the molecules. Electron density correlates with color. Blue−red, minimum−maximum.

(CPA) has been used previously as a probe for methylase enzyme activity.[20]

General Synthetic Strategy. Automated solid-phase synthesis was the desired method for preparing oligonucleotides containing the analogues. Syntheses of oligonucleotides containing AP, L, F, COH, and CPA have already been reported. We anticipated that solid-phase oligonucleotide synthesis would be routine for incorporating CPE and MCP following preparation of the respective phosphoramidites because each of these molecules was expected to be stable to the synthesis and alkaline deprotection conditions. The only modification introduced was to substitute *tert*-butyl hydroperoxide for I_2 during the solid-phase synthesis of oligonucleotides containing MCP and CPE to guard against possible complications due to addition−elimination reactions.

Incorporation of Lm and K in oligonucleotides is potentially complicated by their alkali-lability, just as 2-deoxyribonolactone (L) is. The instability of L is circumvented by incorporating it in a more stable disguised form (e.g., **1**, Scheme 2), which is photochemically transformed into the lesion following oligonucleotide purification on an as-needed basis.[21−25] Photochemistry has proven useful for preparing oligonucleotides containing

SCHEME 2. Generation of Alkali-Labile Molecules in Oligonucleotides



other alkali-labile lesions (e.g., C4-AP).[26−28] In contrast, we anticipated using mild periodate oxidation of a vicinal diol (**1**) to generate K in oligonucleotides (Scheme 2). This method has also been used for generating labile molecules in oligonucleotides.[27,29,30] Although we expected that the lactam (Lm) would

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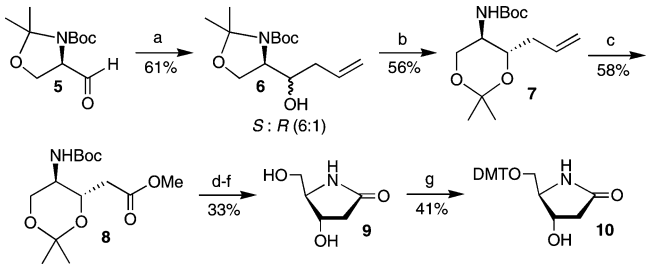
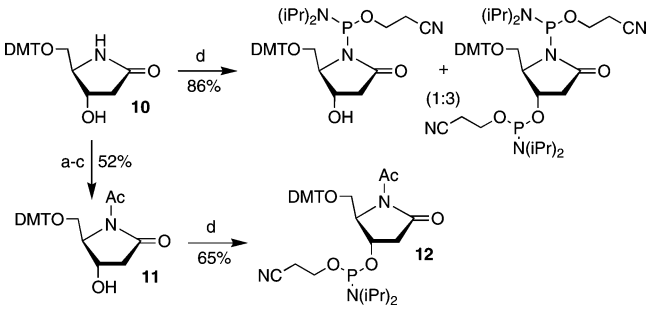
SCHEME 3. Lactam (Lm) Phosphoramidite Precursor Synthesis[a]



[a] Reagents and conditions: (a) allyltributyltin, $BF_3{\cdot}Et_2O$; (b) camphorsulfonic acid, acetone; (c) O_3, NaOH, MeOH/CH_2Cl_2; (d) 1:1 TFA/CH_2Cl_2; (e) 3% TFA, MeOH; (f) K_2CO_3, MeOH; (g) DMTCl, pyridine.

SCHEME 4. Lactam (Lm) Phosphoramidite Synthesis[a]

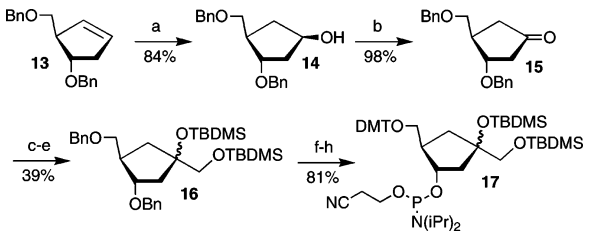


[a] Reagents and conditions: (a) TMSCl, Et_3N, THF; (b) AcBr, Et_3N, THF; (c) $Et_3N{\cdot}3HF$, MeOH; (d) *N*,*N*′-diisopropyl 2-cyanoethyl phosphoramidic chloride, *N*,*N*′-diisopropylethylamine, CH_2Cl_2.

be somewhat labile to alkaline conditions, we predicted that the higher pK_a of a lactam compared to the lactone would enable us to deprotect oligonucleotides containing Lm under mild alkaline conditions.[31]

Synthesis of Oligonucleotides Containing the Lactam (Lm) Analogue. The stereoselective preparation of an appropriate phosphoramidite (**12**, Scheme 4) started with **5**, which is derived from the methyl ester of serine (Scheme 3).[32] The aldehyde (**5**) was allylated by allyltributyltin to yield an inseparable 6:1 mixture of diastereomers of **6**. Comparison to the literature indicated that the major allyl alcohol contained the desired *S*-configuration.[33] Separation of the diastereomers was achieved following rearrangement of **6** to the acetonide (**7**) and only the desired *S* isomer was carried forward.[34] The ultimate C1-carbon was introduced as the methyl ester (**8**) via ozonolysis under

SCHEME 5. Ketone (K) Phosphoramidite Synthesis[a]



[a] Reagents and conditions: (a) 9-BBN, THF; (b) Dess−Martin periodinane, CH_2Cl_2; (c) Nysted's reagent, THF; (d) OsO_4, NMO, *t*-BuOH/H_2O; (e) TBSOTf, 2,6-lutidine, CH_2Cl_2; (f) Pd/C, H_2, MeOH/CH_2Cl_2; (g) DMTCl, Et_3N, DMAP, CH_2Cl_2; (h) *N*,*N*′-diisopropyl 2-cyanoethyl phosphoramidic chloride, *N*,*N*′-diisopropylethylamine, CH_2Cl_2.

alkaline conditions.[35] In order to avoid competitive cyclization by the primary alcohol the Boc and acetonide were deprotected sequentially and the crude trifluoracetate salt was then deprotonated, resulting in spontaneous cyclization to **9**.

Initially, phosphitylation was attempted directly on the dimethoxytritylated lactam (**10**, Scheme 4). However, this led to a mixture of *N*-phosphitylated product and material in which the nitrogen and oxygen were functionalized. Consequently, the lactam nitrogen was selectively acetylated (**11**) by transiently protecting the hydroxyl group prior to preparing the phosphoramidite (**12**). The oligonucleotides were synthesized using fast deprotecting phosphoramidites. The oxidized abasic site was introduced using a 15 min coupling time, while the native nucleotides were coupled using standard coupling times. Although some cleavage occurred at the site of the modification, modest yields of **25a**,**b** were obtained by deprotecting the oligonucleotides in concentrated aqueous ammonia hydroxide at room temperature for 3 h and purifying them by denaturing polyacrylamide gel electrophoresis in a cold room.

Synthesis of Oligonucleotides Containing the Cyclopentanone (K). The preparation of the requisite phosphoramidite (**17**) proceeded through the dibenzyl protected cyclopentene (**13**, Scheme 5).[36] Benzyl protection of the alcohols was desirable because of the ether's orthogonality to the silyl protecting group in **17**. Cyclopentene **13** was obtained via either of two methods, one of which was previously reported starting from cyclopentadienide.[36,37] The other proceeded through the diol (**18**, Scheme 6). We opted to transform **13** into the alcohol (**14**) via regioselective hydroboration instead of the reported multistep phenylselenylation, followed by hydrolysis and radical mediated deselenylation.[37,38] The desired regioisomer was observed as a mixture of stereoisomers (85:15) to the exclusion of that resulting from borane addition to the more hindered carbon. A variety of oxidation methods were explored due to the propensity for **15** to undergo β-elimination.[39] Although PCC and Swern oxidation conditions provided modest yields of the ketone, **15** was obtained in almost quantitative yield using the Dess−Martin

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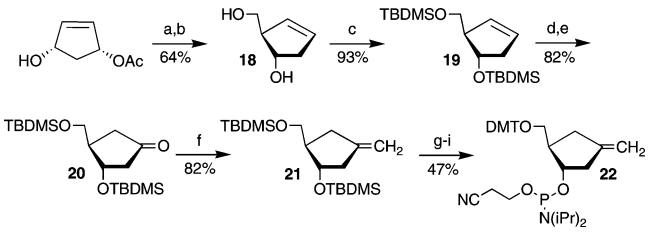
SCHEME 6. Methylenecyclopentane (MCP) Phosphoramidite Synthesis[a]

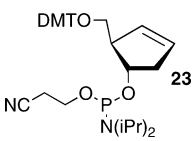


[a] Reagents and conditions: (a) *i*PrO-Si(CH_3)$_2$$CH_2$MgCl, CuCN, THF; (b) KF, K_2CO_3, H_2O_2, MeOH/THF; (c) TBSOTf, 2,6-lutidine, THF; (d) 9-BBN, THF; (e) Dess-Martin periodinane; (f) Nysted's reagent, THF; (g) TBAF, THF; (h) DMTCl, Et_3N, DMAP, CH_2Cl_2; (i) *N*,*N*′-diisopropyl 2-cyanoethyl phosphoramidic chloride, *N*,*N*′-diisopropylethylamine, CH_2Cl_2.

periodinane reagent. The tendency for **15** to undergo elimination also presented a challenge for carrying out the subsequent methylenation reaction. Although conventional Wittig olefination was unsuccessful, the more acidic Nysted's reagent provided a 55% yield of the desired alkene.[39] Osmylation produced an inseparable 60:40 mixture of diastereomers, which were carried on together as their bis-TBDMS ethers (**16**). After cleaving the benzyl protecting groups in **16** by hydrogenolysis. The new diol was carried on to **17** via standard phosphoramidite preparative procedures.

Oligonucleotides (**33a**,**b**) containing the vicinal diol precursor (**3**) were prepared using the same procedures described above for introducing the lactam. The vicinal diol (**3**) was revealed using $Et_3N{\cdot}3HF$, following aqueous ammonia deprotection of the other protecting groups and cleavage of the oligonucleotides from the solid-phase support. The vicinal diol containing oligonucleotides (**33a**,**b**) were purified by denaturing PAGE, and due to its lability (see below) the ketone (**26a**,**b**) was generated on an as needed basis using $NaIO_4$ (5 mM) in sodium acetate buffer (pH 6.0) (Scheme 2). ESI-MS analysis of **26a**,**b** following desalting indicated that the oxidation was complete after 60 min.

Synthesis of Oligonucleotides Containing the Methylenecyclopentane (MCP). Postsynthetic modification was unnecessary for preparing oligonucleotides containing MCP or CPE because the molecules are stable to the alkaline deprotection conditions. Successful synthesis of **17** suggested that the preparation of the necessary phosphoramidite for MCP incorporation (**22**) could proceed via a similar route. However, selective debenzylation proved difficult in the presence of the exocyclic alkene. Consequently, the bis-TBDMS cyclopentene (**19**) was prepared from 1*R*,4*S*-1-acetoxy-4-hydroxycyclopent-2-ene (Scheme 6).[40] The silyl protected cyclopentene (**19**) was hydroborated, oxidized (**20**), and methylenated (**21**) using the same reagents as the dibenzyl derivative (**13**) described above in 67% overall yield. Following desilylation of **21**, the diol was carried on to the phosphoramidite (**22**).



The synthesis of the phosphoramidite (**23**) needed for preparing oligonucleotides containing these abasic site analogues was straightforward and started from the diol (**18**) obtained from 1*R*,4*S*-1-acetoxy-4-hydroxycyclopent-2-ene (Scheme 6). Oligonucleotides containing MCP or CPE were prepared using standard solid-phase synthesis conditions, with the exceptions that oxidation was carried out using *t*-BuOOH and that the coupling time for **22** or **23** was extended to 15 min. Oligonucleotides were purified by denaturing PAGE and characterized by ESI-MS, following deprotection using concentrated aqueous ammonia hydroxide.

5′-d(GAA GAC C**YX** GGC GTC C)

24a,**b** X = L
25a,**b** X = Lm
26a,**b** X = K
27a,**b** X = MCP
28a,**b** X = F
29a,**b** X = COH
30a,**b** X = CPA
31a,**b** X = CPE
32a,**b** X =**1**
33a,**b** X = **3**
a Y = T; **b Y** = C

Comparison of the Stability of Oligonucleotides Containing 2-Deoxyribonolactone (L) and Alkali-Labile Analogues (K, Lm). The sensitivity of the oligonucleotides to alkali conditions is important for determining whether they will be amenable to conditions required for their incorporation into larger nucleic acid constructs (e.g., plasmid DNA) and form determining the thermal stability of duplexes containing the abasic sites. The sensitivity of the ^{32}P-labeled oligonucleotides to various buffer conditions was determined by measuring their fragmentation by gel electrophoresis. Previous studies have documented the susceptibility of L to alkaline treatment.[23,41,42] Hence, it was not surprising that more than 90% of **24a** was cleaved upon mild NaOH treatment (0.1 M, 37 °C, 20 min) (Table 1). The lactam (Lm, **25b**) was more stable to these conditions, which is consistent with our ability to deprotect oligonucleotides containing Lm with concentrated aqueous ammonia. The ketone (K, **26a**) was considerably more susceptible to NaOH treatment and was almost completely cleaved even in formamide loading buffer without any alkaline treatment (Table 1). Treatment of **26a** with hydroxylamine to produce the oxime stabilized the oligonucleotide toward cleavage, supporting the proposal that the loading buffer induced cleavage.

The ketone also proved to be unstable to some conditions commonly used for carrying out enzymatic transformations (e.g., Tris pH 7.5) required for incorporating the respective oligonucleotides in single stranded genomes[43] or UV-melting experiments. For instance, more than 80% of **26a** was cleaved in PIPES (10 mM, pH 7.0) at 75 °C in 1 h. In contrast, no cleavage of the Lm (**25a**) was observed and <15% of an oligonucleotide containing 2-deoxyribonolactone (**24a**) was cleaved under these conditions. Furthermore, although K (**26b**) underwent significant cleavage in Tris (pH 7.5, 100 mM), less than 20% of the oligonucleotide was cleaved at pH 7.0 after 3 h at 25 °C (Table 1).

UV-Melting Measurements of Duplexes Containing Abasic Sites. Abasic sites typically significantly depress the thermal

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TABLE 1. Chemical Stability of Oligonucleotides Containing L, Lm, and K

molecule	conditions	temp (°C)	time (min)	% cleavage
1 (32a)	NaOH (0.1 M)′	37	20	0
L **(24a)**	NaOH (0.1 M)	37	20	93
Lm **(25a)**	NaOH (0.1 M)′	37	20	61
3 (33a)	NaOH (0.1 M)′	37	20	0
K **(26a)**	NaOH (0.1 M)′	37	20	>99
K **(26a)**	formamide loading buffer	25	5	>95
L **(24a)**	PIPES (10 mM, pH 7.5)	75	60	14
L **(24a)**	PIPES (10 mM, pH 7.5)	75	180	92
Lm **(25a)**	PIPES (10 mM, pH 7.5)	75	60	0
Lm **(25a)**	PIPES (10 mM, pH 7.5)	75	180	0
K **(26a)**	PIPES (10 mM, pH 7.5)	75	60	80
K **(26a)**	PIPES (10 mM, pH 7.5)	75	180	96
K **(26a)**	Tris (100 mM, pH 7.5)	0	60	50
K **(26a)**	Tris (100 mM, pH 7.5) then	0	60	
	Tris (100 mM, pH 7.5)	16	180	92
K **(26a)**	Bis-Tris-propane·HCl (10 mM, pH 7.0)	0	60	~1
K **(26a)**	Bis-Tris-propane·HCl (10 mM, pH 7.0) then	0	60	
	Bis-Tris-propane·HCl (10 mM, pH 7.0)	16	180	18

TABLE 2. UV-Melting Temperatures (°C) of Duplexes Containing Abasic Sites[a]

5′-d(GAA GAC C**N X** GGC GTC C)
3′-d(CTT CTG G**N′P** CCG CAG G)

	N:N′ = T:A		N:N′ = C:G	
X	P = A	P = G	P = A	P = G
T	68.0 ± 1.2[b]		70.2 ± 0.6[b]	
F	58.1 ± 0.2	57.1 ± 0.5	59.9 ± 0.3	60.3 ± 0.6
COH	57.7 ± 0.3	56.6 ± 0.2	59.1 ± 0.5	60.4 ± 0.3
CPA	58.0 ± 0.6	57.0 ± 0.7	59.6 ± 0.2	60.5 ± 0.5
CPE	57.0 ± 0.5	56.6 ± 0.5	58.9 ± 0.3	60.1 ± 0.5
L	56.2 ± 0.2	56.7 ± 0.3	59.4 ± 0.4	58.3 ± 0.2
Lm	57.8 ± 0.6	57.4 ± 0.3	58.9 ± 0.3	59.6 ± 0.6
MCP	58.1 ± 0.5	59.1 ± 0.3	60.1 ± 0.4	61.5 ± 0.5

[a] T_m's are the average of 3 measurements ± SD. [b] Data taken from ref 39.

stability of duplex DNA. UV-melting temperatures of duplexes containing the various abasic sites were measured, with the exception of K, which was too unstable to heating. Melting studies were carried out in two sequence contexts, with either dA or dG opposite the abasic sites (Table 2). Purines were chosen as the opposing nucleotides because they are most frequently incorporated opposite L, AP, and F by polymerases in *E. coli*.[14,18] All of the abasic site analogues decreased the T_m's of duplexes containing the native nucleotides by ~10 °C.[44] The T_m's of the duplexes containing dA opposite F were within experimental error of those previously reported, supporting the verity of the data in Table 2.[44] Overall, duplexes containing a flanking dC melted slightly higher than those in which the abasic site was flanked by a 5′-dT. This was to be expected based upon the greater stability of a G·C base pair compared with an A·T base pair. In addition, with the exception of the duplex containing L, the melting temperature was greater when the abasic site was opposed by dG than dA in the same sequence context. However, overall there were no significant differences in thermal stability of duplexes containing the various abasic sites.

Conclusions

The abasic site analogues described above are useful for determining the structural features of abasic sites (AP) and 2-deoxyribonolactone (L) that are responsible for their distinct biochemical effects. Under the appropriate buffer conditions, all of these molecules are sufficiently stable, even the cyclopentanone (K), so as to permit their utilization as probes of replication in cells.[43] These molecules should prove useful for elucidating why L induces high levels of dG incorporation opposite it in *E. coli*, whereas AP sites adhere to the A-rule.[14,18]

Experimental Methods

Compound 7. Compound **5**[29] (3.5 g, 15.3 mmol), allyltributyltin (6.1 mL, 19.9 mmol), and boron trifluoride etherate (2.91 mL, 23 mmol) were stirred at −78 °C in methylene chloride (50 mL) for 3 h. Saturated sodium bicarbonate (30 mL) was added, the solution was warmed to room temperature, and stirring was continued for 30 min. The mixture was extracted with ether (3 × 50 mL). The organic layers were combined and washed with brine. The organic layer was dried over magnesium sulfate and filtered through Celite. The filtrate was concentrated to 30 mL and filtered through Celite again. The solvent was evaporated and the crude product was purified by column chromatography (2:1 hexanes/ethyl acetate) to yield **6** as a mixture of diastereomers (2.6 g, 9.6 mmol, 63%).

A solution of compound **6** (2.6 g, 9.6 mmol) and 10-camphorsulfonic acid (222 mg, 0.96 mmol) was stirred at room temperature in acetone (45 mL) for 48 h, until equilibrium was reached. Acetone was evaporated, and the residue was dissolved in ether (50 mL) and washed successively with saturated sodium bicarbonate solution and brine. The organic layer was dried over sodium sulfate, and the solvent was evaporated. The crude product was purified by column chromatography (5:1 hexanes/ethyl acetate) to yield **7** (1.8 g, 6.6 mmol, 69%). ^{1}H NMR ($CDCl_3$) δ 5.79 (m, 1H), 5.02 (m, 2H), 4.60 (s, br, 1H), 3.82 (br, 1H), 3.49 (br, 3H), 2.48 (br, 1H), 2.20 (m, 1H), 1.35 (m, 15H). ^{13}C NMR ($CDCl_3$) δ 155.1, 134.2, 116.8, 98.7, 79.5, 63.2, 49.0, 36.9, 28.2, 19.8. IR (KBr) 3334, 2987, 1722, 1533, 1388, 1369, 1241, 1201, 1044 cm^{-1}. FAB-HRMS (M + H$^+$) calcd for $C_{14}H_{26}O_4N$ 272.1862, found 272.1848.

Compound 8. A methanolic NaOH solution (2.5 M, 5 mL) was added to a solution of **7** (780 mg, 2.9 mmol) in methylene chloride (20 mL). Ozone was bubbled through at −78 °C while a yellow precipitate formed. The solution turned deep blue after 45 min. The reaction was extracted with ether (3 × 20 mL). The organic layers were combined and dried over magnesium sulfate. The solvent was checked for the presence of peroxides (KI) prior to concentration in vacuo, and the crude product was purified by column chromatography (2:1 hexanes/ethyl acetate) to yield **8** (475 mg, 1.6 mmol, 54%). ^{1}H NMR ($CDCl_3$) δ 4.42 (br, 1H), 4.06 (m, 1H), 3.68 (s, 3H), 3.52 (m, 2H), 3.64 (m, 1H), 2.50 (m, 1H), 1.45

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(m, 15H). ^{13}C NMR ($CDCl_3$) δ 155.1, 134.1, 116.8, 98.7, 79.5, 72.3, 63.2, 49.0, 36.9, 28.2, 28.0, 19.8. IR (KBr) 3369, 2994, 2947, 2872, 1747, 1719, 1688, 1528, 1459, 1438, 1390, 1367, 1299, 1201, 1167, 1080, 1043, 833 cm^{-1}. FAB-HRMS (M + H$^+$) calcd for $C_{14}H_{26}O_6N$ 304.1760, found 304.1743.

Compound 9. Compound **8** (400 mg, 1.3 mmol) was treated with TFA/CH_2Cl_2 (1:1, 10 mL) at room temperature for 30 min. The volatile fraction was removed in vacuo. A 3% solution of TFA in methanol (10 mL) was added, and the reaction was stirred at room temperature overnight. The solvent was removed in vacuo. The residue was dissolved in methanol (10 mL). Potassium carbonate (538 mg, 3.9 mmol) was added, and the reaction was stirred at room temperature for 3 h. The reaction was diluted with methanol (10 mL) and centrifuged. The supernatant was saved, and the solid was washed with CH_2Cl_2/MeOH (1:1, 2 × 5 mL). The combined supernatant was concentrated and purified by column chromatography (1:3 methanol/CH_2Cl_2) to yield **9** (167 mg, 1.27 mmol, 97%). ^{1}H NMR (d_4-MeOH) δ 4.32 (m, 1H), 3.59 (m, 2H), 3.51 (m, 1H), 2.74 (dd, 2H, J = 17, 7 Hz), 2.18 (dd, 2H, J= 17, 3 Hz). ^{13}C NMR (d_4-MeOH) δ 177.6, 68.6, 65.3, 62.1, 39.6. IR (KBr) 3300 (br), 2928, 1696, 1445, 1270, 1084, 713 cm^{-1}. FAB-HRMS (M + H$^+$) calcd for $C_5H_{10}O_3N$ 132.0661, found 132.0661.

Compound 10. Pyridine (5 mL) was added to a mixture of **9** (150 mg, 1.15 mmol) and dimethoxytrityl chloride (507 mg, 1.50 mmol). The reaction was stirred at room temperature for 12 h before quenching with methanol (1 mL). The solvent was removed and the crude product was purified by column chromatography (1:20 methanol/ethyl acetate) to yield **10** (500 mg, 1.15 mmol, 100%). ^{1}H NMR ($CDCl_3$) δ 7.42 (d, 2H, J = 8 Hz), 7.19−7.31 (m, 7H), 6.80 (m, 5H), 4.19 (s, br 1H), 3.94 (b, 1H), 3.75 (m, 6H), 3.69 (b, 1H), 3.24 (m, 1H), 3.09 (m, 1H), 2.70 (dd, 1H, J = 17, 7 Hz), 2.28 (dd, J = 17, 3 Hz). ^{13}C NMR ($CDCl_3$) δ 176.68, 158.57, 158.54, 144.49, 135.70, 135.54, 130.06, 129.95, 128.06, 127.96, 126.95, 113.27, 86.50, 69.85, 64.44, 63.53, 55.20, 40.10. IR (KBr) 3398 (br), 2957, 2834, 1696, 1607, 1577, 1508, 1449, 1301, 1250, 1178, 1089, 1034, 910, 831, 731, 700 cm^{-1}. ESI-HRMS (M + Na$^+$) calcd for $C_{26}H_{27}NO_5Na$ 456.1787, found 456.1789.

Compound 11. TMSCl (1.32 mmol, 145 mg, 169 μL) was added to a solution of **10** (1 mmol, 440 mg) and triethylamine (2.64 mmol, 167 mg, 154 μL) in THF (10 mL) at 0 °C. The reaction was warmed to room temperature and stirred for 2 h. Triethylamine (20 mmol, 2.02 g, 2.8 mL) was added, followed by acetyl bromide (10 mmol, 12.30 g, 0.75 mL). The reaction was continued at room temperature for 2 h. Triethylamine trihydrofluoride (1 mmol, 161 mg, 16 μL) and methanol (5 mL) were added, and the reaction was stirred at room temperature for 15 min. The mixture was diluted with methylene chloride (100 mL) and washed with saturated sodium bicarbonate solution, followed by brine. The organic layer was dried over magnesium sulfate. The solvent was removed, and the crude product was purified by column chromatography (1:2 hexanes/ethyl acetate) to give **11** (255 mg, 0.52 mmol, 52%). ^{1}H NMR ($CDCl_3$) δ 7.22 (m, 10H), 6.82 (m, 4H), 4.30 (m, 2H), 3.78 (s, 6H), 3.49 (dd, 1H, J= 10, 4 Hz), 3.32 (dd, 1H, J = 10, 2 Hz), 3.17 (dd, 1H, J = 18, 6 Hz), 2.52 (m, 3H), 2.49 (m, 1H), 1.81 (br, 1H). ^{13}C NMR ($CDCl_3$) δ 174.3, 171.0, 158.6, 144.3, 135.6, 135.4, 129.9-(1), 129.8(7), 127.9(6), 127.9(3), 127.0, 113.3, 86.8, 77.2, 67.3, 65.9, 61.9, 55.2, 42.9, 29.7, 25.2. IR (KBr), 3503(br), 2959, 2929, 2837, 1745, 1700, 1608, 1509, 1465, 1444, 1370, 1302, 1251, 1208, 1078, 1036, 834 cm^{-1}. ESI-HRMS (M + Na$^+$) calcd for $C_{28}H_{29}NO_6Na$ 498.1893, found 498.1895.

Compound 15. A solution of **14**[37] (2.4 g, 7.7 mmol) and Dess−Martin periodane (3.27 g, 7.7 mmol) was stirred at room temperature in methylene chloride (70 mL) for 1 h. The reaction was diluted with diethyl ether (150 mL) and washed with a mixture of 10% sodium thiosulfate (50 mL), followed by saturated sodium bicarbonate (50 mL). The aqueous layer was back-washed with diethyl ether (100 mL). The organic layers were combined, washed with brine, and dried over magnesium sulfate. The solvent was evaporated, and the crude was purified with column chromatography (3:1 hexanes/ethyl acetate) to yield **15** (2.33 g, 7.53 mmol, 98%). ^{1}H NMR ($CDCl_3$) δ 7.38 (m, 10H), 4.58 (m, 4H), 4.20 (m, 1H), 3.61 (dd, 1H, J= 9, 5 Hz), 3.53 (dd, 1H, J = 9, 3 Hz), 2.63 (m, 3H), 2.35 (dd, 1H, J = 5, 1 Hz), 2.20 (dd, 1H, J = 5, 1 Hz). ^{13}C NMR ($CDCl_3$) δ 215.4 137.7(9), 137.7(5), 128.1(0), 128.0(8), 127.3(9), 127.3(4), 127.3(0), 127.2, 77.7, 72.8, 70.9, 70.1, 44.0, 42.0, 39.6. IR (KBr) 3064, 3030, 2857, 1740, 1497, 1454, 1365, 1199, 1168, 1146, 1121, 1028 cm^{-1}. FAB-HRMS (M + H$^+$) calcd for $C_{20}H_{23}O_3$ 311.1647, found 311.1636.

Compound 16. $TiCl_4$ (7.53 mmol, 7.53 mL, 1 M in methylene chloride) was added dropwise to a suspension of Nysted's reagent in THF (17.17 g, 7.53 mmol, 20 wt % in THF) at 0 °C. Additional THF (20 mL) was added to facilitate stirring. The suspension was kept at 0 °C for 10 min before neat **15** (2.33 g, 7.53 mmol) was added. The reaction was warmed to room temperature and stirred overnight. Saturated sodium bicarbonate solution (5 mL) was added to quench the reaction. The suspension was diluted with ethyl acetate (50 mL) and filtered. Water (20 mL) was added to the filtrate. The organic layer was separated, washed with brine, and dried over magnesium sulfate. The solvent was removed in vacuo and the crude product was purified by column chromatography (10:1 hexanes/ethyl acetate) to yield the olefination product of **15** (1.28 g, 4.15 mmol, 55%). ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 10H), 4.92 (m, 2H), 4.54 (m, 4H), 3.90 (dd, 1H, J = 12, 6 Hz), 3.35 (dd, 1H, J = 10, 1.5 Hz), 3.43 (dd, J = 10, 1.5 Hz), 2.68 (m, 2H), 2.45 (m, 2H), 2.20 (dd, 1H, J = 16, 6 Hz). ^{13}C NMR ($CDCl_3$) δ 148.2, 138.7, 138.5, 128.2(8), 128.2(6), 127.5(9), 127.4(6), 127.4(3), 127.4(0), 107.1, 80.9, 73.0, 71.4, 71.1, 45.2, 38.6, 34.0. IR (KBr) 3064, 3028, 2983, 2940, 2854, 1658, 1496, 1454, 1364, 1205, 1099, 1028, 878, 751, 697 cm^{-1}. FAB-HRMS (MH$^+$) calcd for $C_{25}H_{21}O_2$ 309.1855, found 309.1851.

A solution of *N*-methylmorphine *N*-oxide (305 mg, 2.6 mmol) and osmium tetraoxide (17 mg, 0.065 mmol) in a mixture of *t*-BuOH/H_2O (1:1, 12 mL) was added to the above olefination product (400 mg, 1.3 mmol) at 0 °C. The reaction was warmed to room temperature and stirred for 24 h. Sodium bisulfite (1.5 g) was added, and the suspension was stirred for 30 min. The mixture was diluted with methylene chloride (30 mL) and water (10 mL). The aqueous layer was extracted with methylene chloride (3 × 30 mL). The organic layers were combined and dried over magnesium sulfate. The solvent was evaporated, and the crude product was purified by column chromatography (1:4 hexanes/ethyl acetate) to give the dihydroxylation product (380 mg, 1.11 mmol, 85%) as a mixture of stereoisomers. ^{1}H NMR ($CDCl_3$) δ 7.32 (m, 10H), 4.52 (m, 4H), 4.12 and 3.98 (1H), 3.52−3.31 (m, 5H), 2.70 and 2.36 (2H), 2.19−2.02 (m, 2H), 1.83 and 1.75 (1H), 1.61 and 1.46 (1H). ^{13}C NMR ($CDCl_3$) δ 138.4, 138.2, 138.0, 137.6, 128.4(0), 128.2-(9), 128.2(6), 127.7(5), 127.6(7), 127.5(8), 127.5(6), 127.5(3), 127.4(9), 127.4(7), 127.4(0), 82.7, 82.3, 81.6, 80.3, 73.3, 72.9, 72.1, 71.7, 71.3, 70.7, 69.3, 68.7, 44.6, 44.4, 43.4, 41.4, 38.7, 38.3 cm^{-1}. IR (KBr) 3400 (br), 3005, 2932, 2852, 1474, 1397, 1113, 1098, 909, 734, 697 cm^{-1}. FAB-HRMS (M + H$^+$) calcd for $C_{21}H_{27}O_4$ 343.1909, found 343.1900.

TBSOTf (1.53 mmol, 404 mg, 351 μL) was added dropwise to a solution of the above dihydroxylation product (0.51 mmol, 175 mg) and 2,6-lutidine (2.04 mmol, 219 mg, 238 μL) in methylene chloride (10 mL) at 0 °C. The reaction was warmed to room temperature, and stirring was continued for 6 h. The mixture was diluted with ethyl acetate (100 mL) and washed with ammonium chloride (1 M), followed successively with saturated sodium bicarbonate and brine. The organic layer was dried over magnesium sulfate. The solvent was removed in vacuo, and the crude product was purified by column chromatography (20:1 hexanes/ethyl acetate) to yield **16** (0.44 mmol, 249 mg, 81%) as a mixture of diastereomers. ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 10H), 4.58 (m, 4H), 3.98 and 3.72 (1H), 3.62−3.41 (m, 4H), 2.59−2.24 (m, 2H), 2.00 to 1.66 (m, 3H), 0.95 (m, 18H), 0.11 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 138.9(2), 138.8(4), 138.7(6), 138.6(8), 128.2(6), 128.2(4), 128.2-(2), 128.2(0), 127.6(3), 127.6(0), 127.5(3), 127.4(6), 127.3(8),

127.3(2), 83.0, 81.9, 81.4, 80.8, 72.9, 72.8, 71.6(0), 71.5(8), 71.4, 69.3, 69.2, 45.0, 44.9, 43.4, 42.9, 38.9, 38.8, 26.0, 25.9, 25.8(4), 25.8(0), 18.5, 18.3, 18.0(7), 18.0(6), −2.4(0), −2.4(3), −2.5, −2.6, −5.4, −5.5. IR (KBr) 3030, 2958, 2927, 2883, 2855, 1497, 1463, 1388, 1259, 1112, 832, 773, 732, 697 cm^{-1}. ESI-HRMS ($M + Na^+$) calcd for $C_{33}H_{54}O_4NaSi_2$ 593.3458, found 593.3464.

Hydrogenolysis of 16. The preparation of **16** was repeated to obtain enough material for the next reaction. Pd/C (84 mg) was added to a solution of **16** (420 mg, 0.74 mmol) in a mixture of $MeOH/CH_2Cl_2$ (1:2, 10 mL). The suspension was stirred under H_2 pressure (50 psi) for 2 h. The solution was filtered through Celite and washed with ethyl acetate (50 mL). The filtrate was concentrated in vacuo, and the crude was purified by column chromatography (1:10 hexanes/ethyl acetate) to give the diol (263 mg, 0.67 mmol, 91%). ^1H NMR ($CDCl_3$) δ 4.19 and 3.94 (m, 1H), 3.72 (m, 1H), 3.61−3.39 (m, 3H), 3.20 (br, 2H), 2.30−2.02 (m, 4H), 1.46 and 1.30 (1H), 0.85 (m, 18H), 0.08 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 83.5, 82.4, 76.8, 76.1, 69.5, 68.7, 66.0, 65.6, 49.2, 48.9, 46.1(4), 46.0(7), 38.6, 37.9, 25.9(2), 25.8(8), 25.7(5), 25.7(2), 13.4, 18.3, 17.9(6), 17.9(5), −2.4(2), −2.4(6), −2.4(9), −5.51. IR (KBr) 3405 (br), 2958, 2937, 2839, 2854, 1465, 1429, 1390, 1348, 1248, 1158, 1099, 1056, 999, 830, 767 cm^{-1}. ESI-HRMS ($M + NH_4^+$) calcd for $C_7H_{16}NO_2$ 146.1181, found 146.1178.

Compound 18. A suspension of the dimethylisopropoxysilyl ether of **18**[37] (1.4 g, 6.3 mmol), potassium fluoride (1.86 g, 32 mmol), potassium bicarbonate (1.9 g, 19 mmol) and hydrogen peroxide (6 mL, 30% in H_2O) in methanol/THF (1:1, 40 mL) was heated at reflux for 15 h. Sodium thiosulfate (500 mg) was added. The solution was diluted with ethyl acetate (400 mL) and magnesium sulfate (2 g) was added. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The crude product was purified by column chromatography (ethyl acetate) to yield **18** (578 mg, 5 mmol, 79%). ^1H NMR ($CDCl_3$) δ 5.78 (m, 1H), 5.54 (m, 1H), 4.34 (m, 1H), 3.73 (dd, $J = 10$, 5 Hz), 2.75 (m, 2H), 2.50 (br, 1H), 2.30 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 130.3, 129.0, 74.8, 63.9, 57.5, 41.4. IR (KBr) 3296 (br), 2959, 2922, 2853, 1467, 1351, 1054, 1017 cm^{-1}. ESI-HRMS ($M + NH_4^+$) calcd for $C_6H_{14}NO_2$ 132.1025, found 132.1019.

Compound 19. The reaction was performed at a 4.79 mmol scale (546 mg of **18**, 14.37 mmol TBSOTf, 19.16 mmol 2,6-lutidine) using the same silylation procedure employed for the preparation of **16**. The crude product was purified by column chromatography (50:1 hexanes/ethyl acetate) to yield **19** (1.53 g, 4.47 mmol, 93%). ^1H NMR ($CDCl_3$) δ 5.67 (m, 1H), 5.61 (m, 1H), 4.24 (m, 1H), 3.49 (m, 1H), 2.70−2.56 (m, 2H), 2.21 (m, 1H), 0.87 (s, 18H), 0.51 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 130.5, 129.3, 74.4, 57.9, 42.1, 25.9(4), 25.9(1), 18.4, 18.1, −4.5(6), −4.6(5), −5.3(8), −5.4(0). IR (KBr), 3058, 2959, 2894, 2934, 2894, 2856, 1468, 1364, 1254, 1175, 1103, 1060, 1001, 840, 775 cm^{-1}. ESI-HRMS ($M + H^+$) calcd for $C_{18}H_{39}O_2Si_2$ 343.2489, found 343.2501.

Compound 20. 9-BBN (0.5 M solution in THF, 17 mL, 8.5 mmol) was added to a solution of **18** (2.85 g, 4.27 mmol) in THF (10 mL) at 0 °C. The reaction was warmed to room temperature and continued for 24 h. The reaction was cooled to 0 °C before methanol (4 mL), NaOH (3 N, 10 mL) and hydrogen peroxide (30%, 10 mL) were added successively. The mixture was stirred at room temperature for 12 h and extracted with diethyl ether (4 × 50 mL). The organic layers were combined and dried over magnesium sulfate. The solvent was removed, and the crude product was partially purified by column chromatography (1:1 hexanes/ethyl acetate). The Dess−Martin oxidation (3.38 mmol Dess−Martin periodinane) of the crude material was performed using the same procedure employed for the preparation of **15**. The crude product was purified by column chromatography (hexanes/ethyl acetate, 10:1) to yield **20** (1.03 g, 2.87 mmol, 67% from **18**). ^1H NMR ($CDCl_3$) δ 4.38 (dd, 1H, $J = 12$, 5 Hz), 3.75 (dd, 1H, $J = 10$, 4 Hz), 3.60 (dd, $J = 12$, 5 Hz), 2.59 (dd, 1H, $J = 6$, 1 Hz), 2.54 (dd, $J = 6$, 1 Hz), 2.31 (m, 1H), 2.18 (m, 2H), 0.90 (m, 18H), 0.08 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 216.5, 71.0, 62.1, 47.7, 47.4, 39.3, 25.8, 25.7, 18.2, 18.0, −4.7, −4.9, −5.5, −5.7, IR (KBr), 2961, 2936, 2894, 2855, 1750, 1469, 1400, 1361, 1254, 1195, 1107, 1002, 912, 839, 776, 670 cm^{-1}. ESI-HRMS ($M + H^+$) calcd for $C_{18}H_{39}O_3Si_2$ 359.2438, found 359.2438.

Compound 21. The Nysted olefination of **20** was performed at a 2.60 mmol scale (930 mg of **20**, 5.91 g Nysted's reagent (20 wt % in THF)) using the same procedure for the preparation of **16**. The crude product was purified by column chromatography (50:1 hexanes/ethyl acetate) to yield **21** (0.76 g, 2.12 mmol, 82%). ^1H NMR ($CDCl_3$) δ 4.86 (m, 2H), 4.08 (dd, $J = 10$, 6 Hz), 3.60 (m, 2H), 2.63 (dd, 1H, $J = 16$, 6 Hz), 2.50 (dd, 1H, $J = 16$, 8 Hz), 2.27 (dd, $J = 16$, 6 Hz), 2.17 (m, 1H), 2.06 (m, 1H), 0.87 (m, 18H), 0.08 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 148.6, 106.5, 73.5, 62.9, 50.1, 42.1, 33.0, 26.0, 25.9, 18.3, 18.1, −4.5, −4.8, −5.4, −5.5. IR (KBr) 3074, 2957, 2934, 2894, 2855, 1659, 1469, 1390, 1365, 1115, 1057, 1010, 878, 840, 775, 671 cm^{-1}. ESI-HRMS ($M + H^+$) calcd for $C_{19}H_{41}O_2Si_2$ 357.2645, found 357.2657.

Desilylation of 21. A solution of compound **21** (0.76 g, 2.1 mmol) and tetrabutylammonium fluoride monohydrate (2.35 g, 8.4 mmol) in THF (15 mL) was stirred at room temperature for 18 h. The solvent was evaporated, and the crude was purified by column chromatography (ethyl acetate) to give the desilylation product of **21** (296 mg, 1.62 mmol, 77%). ^1H NMR ($CDCl_3$) δ 4.87 (m, 2H), 4.05 (dd, 1H, $J = 14$, 7 Hz), 3.72 (dd, 1H, $J = 10$, 5 Hz), 3.56 (dd, 1H, $J = 10$, 7 Hz), 3.70−3.30 (br, 2H), 2.71 (dd, 1H, $J = 7$, 1 Hz), 2.67 (m, 1H), 2.33 (m, 1H), 2.12 (m, 1H), 0.95 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 146.9, 107.5, 75.8, 65.1, 48.8, 41.4, 33.1. IR (KBr) 3284 (br), 2945, 1658, 1430, 1346, 1156, 1076, 1021, 881 cm^{-1}. ESI-HRMS ($M + NH_4^+$) calcd for $C_7H_{16}NO_2$ 146.1181, found 146.1178.

Oligonucleotide Synthesis. The 16-nucleotide long oligonucleotides were synthesized on an Applied Biosystems Incorporated 394 DNA synthesizer using standard reagents (Glen Research). Bz-dA-CE, iBu-dG-CE, Ac-dC-CE and T were used as the phosphoramidites for oligonucleotides containing F, L, CPA, CPE and MCP. Fast deprotecting phosphoramidites (Pac-dA-CE, iPac-dG-CE, and Ac-dC-CE) and T were used for synthesizing oligonucleotides containing Lm, COH and K. Pivaloyl anhydride/2,6-Lutidine/THF (1:1:8) was used as the capping reagent when fast deprotecting phosphoramidites were used.[45] *t*-BuOOH in toluene (1 M) was used as oxidation reagent for CPA, CPE, and MCP. Deprotection was carried out in concentrated aqueous ammonia at room temperature for COH (12 h), K (12 h), and Lm (3 h), and at 55 °C for all other oligonucleotides (12 h). The oligonucleotides were purified by 20% denaturing PAGE and desalted by C-18 Sep-Pak cartridge. The oligonucleotides were characterized by ESI-MS or MALDI-MS (Lm) after ammonium acetate precipitation.

UV Melting Temperatures. Oligonucleotides and their complementary stands (2.2 μM for each strand) were hybridized in 10 mM PIPES (pH 7.0), 10 mM $MgCl_2$ and 100 mM NaCl in a total volume of 200 μL. The DNA was denatured at 90 °C (70 °C for Lm), slowly cooled to room temperature, and kept at 0 °C overnight. During the melting experiment the temperature was increased from 25 to 75 °C at 0.5 °C/min. Readings were taken every 0.2 °C. The melting temperatures were derived from the first derivative of the melting curves.

Base Treatment of Oligonucleotides Containing L (24a), Lm (25a), and K (26a). The 5′-OH of oligonucleotides **24a**, **25a**, and **26a** were radiolabeled using a standard protocol.[46] An aliquot (∼ 0.25 pmol, 5 μL) of the 7-nitroindole precursor of **24a** was irradiated at 350 nm for 1 h using a Rayonet Photochemical Reactor equipped with 16 $\lambda_{max} = 350$ nm lamps. An aliquot (∼ 0.40 pmol, 8 μL) of the vicinal diol precursor of **26a** was treated with $NaIO_4$ (5 mM) in NaOAc buffer (10 mM, pH 6.0) at room temperature for 1 h.

(45) Zhu, Q.; Delaney, M. O.; Greenberg, M. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1105−1108.

(46) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1982.

An aliquot of the precursor of **24a** (1.8 μL) was treated with NaOH (0.2 μL, 1 M) and incubated at 37 °C before being quenched with HCl (1 equiv). DNA **24a**, **25a**, and **26a** were treated with NaOH by the same method. An aliquot of **26a** (1.8 μL) was treated with hydroxylamine (0.2 μL, 1 M, pH 4.0) and incubated at room temperature for 30 min. The samples were mixed with 2 μL loading buffer (95% formamide, 10 mM EDTA) and subjected to 20% denaturing PAGE.

Thermal Stability of Oligonucleotides Containing L, K and Lm. The radiolabeled oligonucleotides (~0.25 pmol, 25 μL) **24a**, **25a** and **26a** were heated to 75 °C in PIPES buffer (10 mM, pH 7.0). Ketone **26a** was precipitated in 3 volumes of EtOH (−78 °C, 20 min) and resuspended in cold water prior to heating. An aliquot (4.5 μL) was transferred to a new tube in an ice bath after 1, 2, and 3 h. Ketone **26a** was treated with hydroxylamine (0.5 μL, 1 M) and incubated at room temperature for 30 min. The samples were mixed with 2 μL loading buffer (95% formamide, 10 mM EDTA) and subjected to 20% denaturing PAGE.

Stability of 26a in Various Buffer Conditions. Ketone **26a** (1.8 μL) was incubated at 0 °C for 1 h and then 16 °C for 3 h in TRIS buffer (100 mM, pH 7.5) or buffer 1 (NEB, 20 mM bis-tris-propane, 10 mM $MgCl_2$, pH 7.0). The samples were treated with hydroxylamine (0.2 μL, 1 M), incubated at room temperature for 30 min, mixed with 2 μL loading buffer (95% formamide, 10 mM EDTA), and subjected to 20% denaturing PAGE.

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Supporting Information Available: General experimental methods and procedures for the synthesis of small molecules and oligonucleotides. NMR spectral data. ESI-MS and MALDI-TOF−MS of oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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