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Site-Specific Synthesis and Properties of Oligonucleotides Containing C8-Deoxyguanosine Adducts of the Dietary Mutagen IQ

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Abstract: The site-specific synthesis of oligonucleotides containing the C8-deoxyguanosine adduct of the highly mutagenic heterocyclic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) has been achieved, and the oligonucleotides were characterized by UV melting temperature analysis, circular dichroism, and UV absorption spectroscopy. Examination of these data indicated that the IQ-adduct is accommodated in dramatically different environments. This sequence-dependent conformational preference is likely to play a key role in the mutagenicity and repair of IQ-modified oligonucleotides.

Introduction

Exposure to carcinogens can occur from environmental or work conditions, diet, smoking, and endogenous processes. In the mid-1970s, it was discovered that the processes of cooking beef, pork, fish, and poultry produced a variety of mutagenic compounds in the parts per billion to parts per million quantities.[1−7] Since then, over 20 such mutagens have been structurally characterized; these compounds are collectively known as the heterocyclic amines (HCAs), and representative structures (**1**−**11**) are shown in Figure 1. The systematic names of these compounds are cumbersome, and they are more commonly referred to by their simpler acronyms. The distribution and quantity formed is dependent upon the type of meat, cooking method, temperature, and time. In general, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP, **8**) is the most abundantly produced, while 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ, **2**) is the most mutagenic. PhIP and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ, **1**) have also been shown to be a constituent of cigarette smoke.[8]

The HCAs are highly mutagenic in Ames *Salmonella* reversion assays. A listing of their mutagenicities in an Ames testers strain designed for frameshift mutations is shown in Table 1.[3] The most mutagenic of these compounds, MeIQ and IQ, are on the order of 24 and 20 times more mutagenic than aflatoxin B_1 in these assays. In bacteria, the HCAs are potent inducers of frameshift mutations in CG repeat sequences; however, in mammalian cells, point mutations are largely observed.[1,2,4]

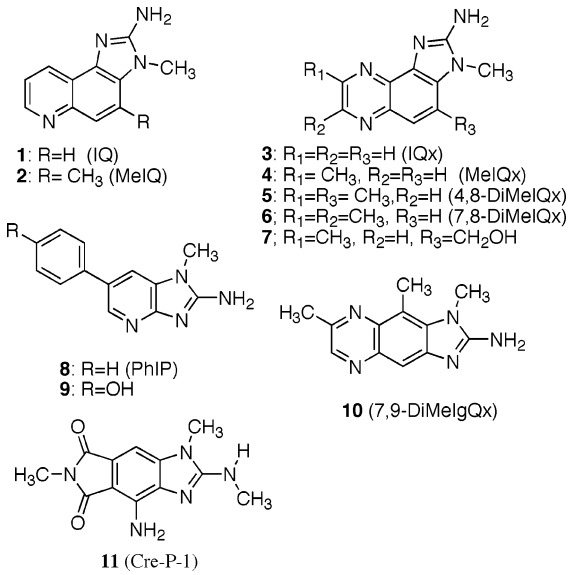

Figure 1. Structures of representative heterocyclic amines (HCAs) found in cooked meats.

The HCAs, as with other arylamines, require metabolic activation to covalently modify DNA (Scheme 1). The initial step is the cytochrome P450 oxidation of the arylamine to the corresponding *N*-hydroxylamine.[9] For IQ, P450 1A2 has been identified as the major metabolizing enzyme in humans. The *N*-hydroxylamine is then esterified to an acetyl by *N*-acetyl transferase enzyme (NAT) or related derivative. Solvolysis of the *N*-acetyl group generates a highly reactive arylnitrenium ion, which is the DNA modifying species. The aryl nitrenium ion

(1) Turesky, R. J. *Drug Metab. Rev.* **2002**, *34*, 625−650.
(2) Schut, H. A.; Snyderwine, E. G. *Carcinogenesis* **1999**, *20*, 353−368.
(3) Sugimura, T. *Mutat. Res.* **1997**, *376*, 211−219.
(4) Felton, J. S.; Knize, M. G.; Dolbeare, F. A.; Wu, R. W. *Environ. Health Perspect.* **1994**, *102* (suppl. 6), 201−204.
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(6) Jagerstad, M.; Skog, K.; Arvidsson, P.; Solyakov, A. *Z. Lebensm.-Unters.-Forsch. A* **1998**, *207*, 419−427.
(7) Skog, K. I.; Johansson, M. A.; Jagerstad, M. I. *Food Chem. Toxicol.* **1998**, *36*, 879−896.
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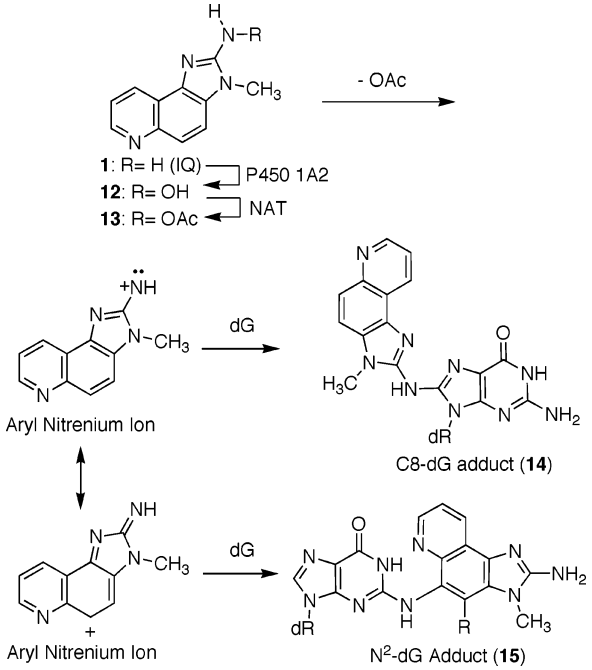
(9) Guengerich, F. P. *Drug Metab. Rev.* **2002**, *34*, 607−623.

Table 1. Relative Mutagenicity of HCA in *S. typhimurium* Tester Strains TA 104[a]

2 (MeIQ)	1000
1 (IQ)	656
5 (4,8-DiMeIQx)	277
6 (7,8-DiMeIQx)	247
4 (MeIQx)	220
7	150
3 (IQx)	114
aflatoxin B_1	42
11 (Cre-P-1)	29
8 (PhIP)	3
10 (7,9-DiMeIgQx)	1
benzo[*a*]pyrene	1
9	0.003

[a] Reference 3.

Scheme 1



reacts with DNA to give the C8-deoxyguanosine adducts (**14**) as the major product, and, in some cases, modification of the N^2-position of deoxyguanosine (**15**) has been unambiguously identified as minor products.[10,11] Some simple arylamines also give C8-deoxyadenosine adducts; however, most of the evidence indicates that deoxyadenosine adducts do not play a significant role in HCA mutagenicity, and no HCA adducts of deoxyadenosine have been characterized.[2,12]

Perhaps the most extensively studied of the arylamine adducts are 2-aminofluorene (AF) and *N*-acetyl-2-aminofluorene (AAF).[13] These compounds were of commercial interest as pesticides, but were found to be highly mutagenic and never used as intended. Zhou and Romano reported the synthesis of phosphoramidite reagents of C8-deoxyguanosine adducts of 2-aminofluorene and its *N*-acetyl analogue, thereby allowing for these lesions to be site-specifically incorporated into oligonucleotides.[14,15] Because of the base-sensitive nature of the *N*-acetyl group, alternative protecting group chemistry was developed for its solid-phase DNA synthesis. Although structurally very similar, the biological response to these lesions was dramatically different. In bacterial mutagenesis assays, AAF gave predominantly −1 and −2 frameshift mutations, while base-pair substitution were largely observed for AF.[1,2]

Results and Discussion

Synthesis of the C8-Deoxyguanosine Adducts of IQ and Simple Arylamine. The Buchwald−Hartwig[16−20] palladium-catalyzed *N*-arylation reaction has been used recently for the preparation of nucleoside-carcinogen adducts by Lakshman and later Johnson, for the preparation of N^6-2′-deoxyadenosine derivatives.[21,22] Hopkins and Sigurdsson, and Johnson synthesized N^2-deoxyguanosine-N^2-deoxyguanosine and N^2-deoxyguanosine-N^6-deoxyadenosine nitrous acid cross-links as well as other N^2-aryl derivatives of deoxyguanosine via the Buchwald−Hartwig cross-coupling reaction.[23−27] It is worth noting that Johnson's *N*-arylation approach involved coupling of the exocyclic amino group of deoxyadenosine or deoxyguanosine with bromoarenes, while Lakshman and Hopkins and Sigurdsson employed the corresponding bromopurine with arylamines. This reaction has become an important method for the preparation of deoxyadenosine and deoxyguanosine derivatives that are modified at the exocyclic amino groups.[28−31] At the time of our initial report, we were the first to employ this strategy for the synthesis of C8-deoxyguanosine adducts of arylamines.[32] Since then, the Buchwald−Hartwig reaction has been utilized by Meier and Scharer for the synthesis of C8-deoxyguanosine adducts of simple arylamines and by Schoffers for the synthesis of C8-arylamines adducts of adenosine.[33−35] The coupling of a suitably protected 8-amino-2′-deoxyguanosine derivative with bromoarene has also been demonstrated as a feasible strategy.[36]

Buchwald and others have reported the *N*-arylation of amides.[37] Thus, conventional amide protecting groups for N^2 of deoxyguanosine were judged as unsatisfactory. We found that the tetramethyldisilylazacyclopentane (STABASE) group developed by Magnus was a compatible N^2-protecting group

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(11) Turesky, R. J.; Markovic, J. *Chem. Res. Toxicol.* **1994**, *7*, 752−761.
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(15) Zhou, Y.; Chladek, S.; Romano, L. J. *J. Org. Chem.* **1994**, *59*, 556−563.
(16) Yang, B. H.; Buchwald, S. L. *J. Organomet. Chem.* **1999**, *576*, 125−146.
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(22) De Riccardis, F.; Bonala, R. R.; Johnson, F. *J. Am. Chem. Soc.* **1999**, *121*, 10453−10460.
(23) Harwood, E. A.; Sigurdsson, S. T.; Edfeldt, N. B. F.; Reid, B. R.; Hopkins, P. B. *J. Am. Chem. Soc.* **1999**, *121*, 5081−5082.
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(25) De Riccardis, F.; Johnson, F. *Org. Lett.* **2000**, *2*, 293−295.
(26) Bonala, R. R.; Yu, P. L.; Johnson, F. *Tetrahedron Lett.* **1999**, *40*, 597−598.
(27) Bonala, R. R.; Shishkina, I. G.; Johnson, F. *Tetrahedron Lett.* **2000**, *41*, 7281−7284.
(28) Lakshman, M. K. *J. Organomet. Chem.* **2002**, *653*, 234−251.
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(31) Chakraborti, D.; Colis, L.; Schneider, R.; Basu, A. K. *Org. Lett.* **2003**, *5*, 2861−2864.
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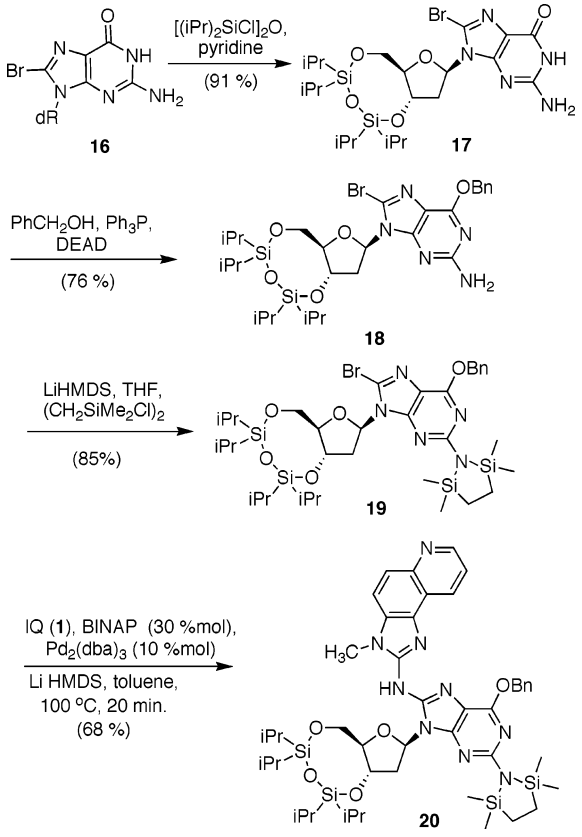
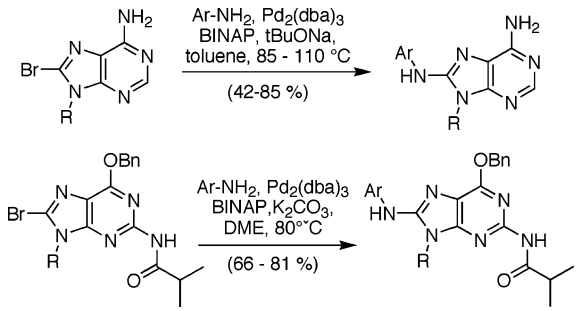
(37) Yin, J.; Buchwald, S. L. *Org. Lett.* **2000**, *2*, 1101−1104.

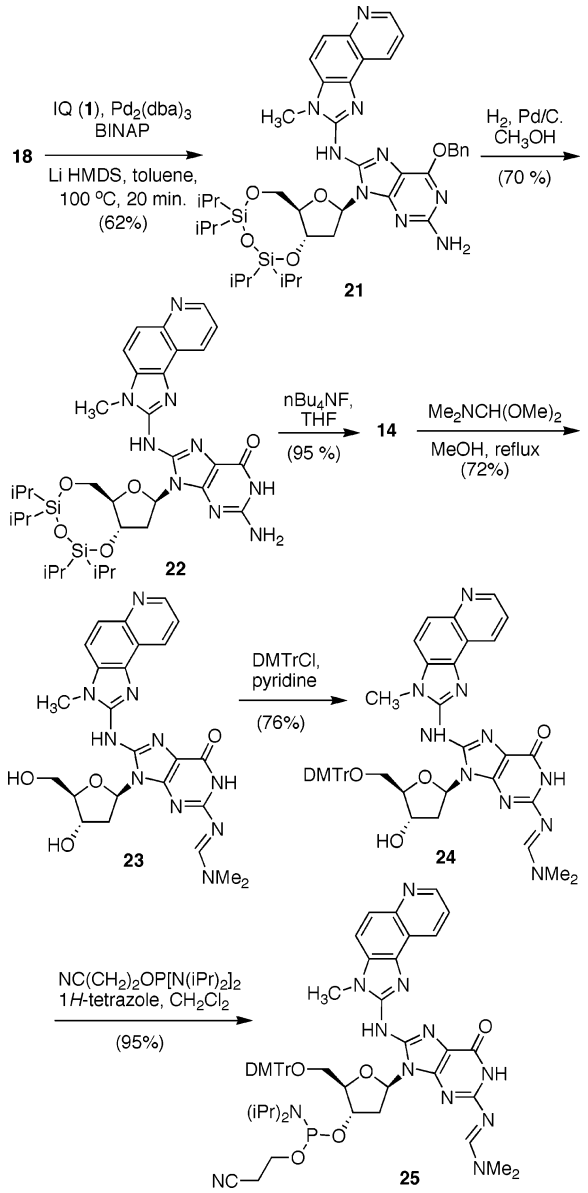
Scheme 2



Scheme 3



Scheme 4



for the *N*-arylation reaction.[38] The desired substrate for the *N*-arylation reaction (**19**) was prepared from 8-bromo-2′-deoxyguanosine in three steps according to Scheme 2. The optimal conditions for the cross-coupling reaction of **19** with IQ (**1**) were achieved using 10 mol % catalyst and lithium hexamethyldisilazide as the base to give **20** in 68% yield.[39,40] Sequential deprotection of **20** gave the C8-IQ adduct of deoxyguanosine **14**.[32]

After our initial communication on the synthesis of the C8-IQ adduct of deoxyguanosine nucleoside (**14**), Schoffers reported the synthesis of C8-arylamine adducts of adenosine involving a Buchwald−Hartwig *N*-arylation reaction (Scheme 3). Of significance, it was demonstrated that the N^6-amino group did not require protection. Subsequently, Meier reported that N^2-isobutyryl-8-bromo-2′-deoxyguanosine also undergoes Buchwald−Hartwig reaction with arylamines. These reports indicated that our initial assumption that the N^2-amino group required special protection to affect the Buchwald−Hartwig reaction was incorrect. In light of these reports, we reexamined our synthetic scheme. For the synthesis of the C8-IQ-adducted nucleoside, the silyl protecting groups for the N^2 and ribose hydroxyls could be removed simultaneously with fluoride ion. However, for the preparation of a phosphoramidite reagent of the C8-modified nucleoside, it would be advantageous to have the N^2-amino group differentially protected to avoid a deprotection−reprotection sequence.

We attempted the Buchwald−Hartwig reaction of N^2-unprotected 8-bromo-2′-deoxyguanosine derivative **18** under conditions we previously worked out (Scheme 4). The coupling proceeded smoothly to give **21** in comparable yield to that reported for the fully deprotected series. However, attempts to protect the N^2-position of **21** or the O^6-deprotected analogue **22** as the corresponding acetyl or phenoxyacetyl derivative proceeded in poor yield in our hands. Previous studies of the conformation of the C8-IQ-adducted nucleoside (**14**) indicate that the *syn* conformation about the glycosidic bond is preferred

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(39) Louie, J.; Hartwig, J. F.; Fry, A. J. *J. Am. Chem. Soc.* **1997**, *119*, 11695−11696.
(40) Louie, J.; Hartwig, J. F. *Tetrahedron Lett.* **1995**, *36*, 3609−3612.

Scheme 5

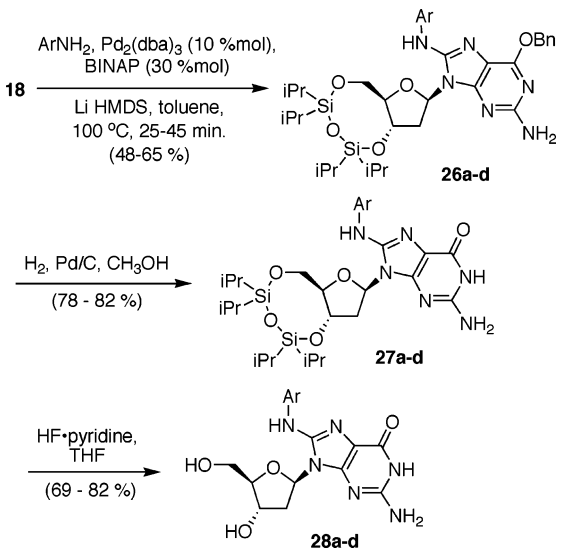
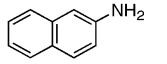
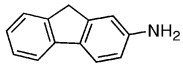
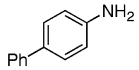
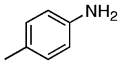


Table 2. Synthesis of C8-Deoxyguanosine Adducts of Simple Arylamine

entry	Ar-NH_2	% yield 26	27	28
a		65	86	82
b		54	80	77
c		62	82	69
d		48	78	70
e	IQ (**1**)	62		

due to the bulk of the C8-substituent.[10] This may place the N^2-amino group in an unfavorable steric environment and hinder its reactivity toward acetylization. We were able to protect this position as a dimethylformamidine, which was selective for N^2 even in the presence of free ribose hydroxyl groups. We found it more convenient for purification reasons to first remove the silyl groups of **22** with fluoride to give the adducted nucleoside **14** and subsequently treat with the dimethylformamide dimethyl acetal to give the N^2-formamidine **23**, which precipitated from the reaction in 72% yield. Conversion to the desired phosphoramidite reagent **25** was accomplished by selective protection of the primary 5′-hydroxy group to give the dimethoxytrityl ether followed by phosphitylation of the 3′-hydroxyl group in 72% yield for two steps. The synthesis of the C8-IQ-adducted phosphoramidite required eight steps from commercially available 8-bromo-2′-deoxyguanosine and proceeded in 17% overall yield. The Buchwald−Hartwig *N*-arylation reaction of IQ (**1**) and N^2-unprotected 8-bromo-2′-deoxyguanosine derivative **18** shortens the synthesis of the adducted nucleoside **14** to five steps and improves the overall yield to 33% from 8-bromo-2′-deoxyguanosine.

One potential drawback to phosphoramidite **25** is that it is only sparingly soluble in acetonitrile, the standard solvent used in automated DNA synthesizers. A phenoxyacetyl (PAC) or related protecting group for N^2 may impart more desirable solubility properties. We attempted the Buchwald−Hartwig *N*-arylation reaction with IQ (**1**) and the N^2-phenoxyacetyl protected 8-bromoguanosine prepared by the reaction of phenoxyacetyl chloride with **18**. However, the phenoxyacetyl group did not survive the strongly basic conditions used previously to couple IQ with **18** and **19**. During the course of our work, Gillet and Scharer reported the cross-coupling of 2-aminofluorene with a N^2-(4-isopropylphenoxy)acetyl (^{i}PrPAC) protected 8-bromo-2′-deoxyguanosine derivative.[34] In this work, strong bases such sodium *tert*-butoxide lead to rapid loss of the N^2-^{i}PrPAC group analogous to our observations. It was also reported that K_2CO_3 was effective in promoting the Buchwald−Hartwig reaction to give the C8-aminofluorene adduct with an N^2-^{i}PrPAC protecting group. Unfortunately, in our hands, these conditions did not give satisfactory yields when IQ was the amine.

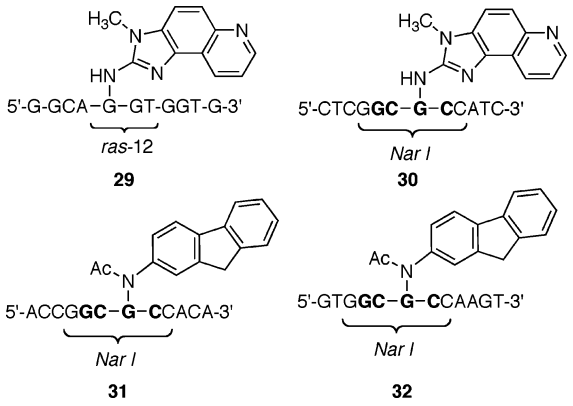


Figure 2. Sequences for C8-IQ- and AFF-adducted oligonucleotides.

To examine the generality of the Buchwald−Hartwig reaction of N^2-unprotected derivative **18**, we examined its reactivity with simple arylamines. We found that simple arylamines undergo rapid cross-coupling with **18** in 48−65% yield (Scheme 5), which is comparable to that obtained for the coupling with IQ. Two-step deprotection provided the adducted nucleosides **28a**−**d** in good overall yield (Table 2).

Site-Specific Synthesis of Oligonucleotides Containing a C8-Deoxyguanosine Adduct of IQ. Phosphoramidite **25** is readily soluble in methylene chloride, and we proceeded with the oligonucleotide synthesis using a manual coupling protocol to incorporate the IQ-modified nucleotide. For our initial synthesis, we prepare the *N-ras*-12 sequence (**29**) shown in Figure 2. This particular sequence was chosen as an initial target because there is substantial experience in our department in preparing N^2-deoxyguanosine-PAH-adducted oligonucleotides using this sequence. For the synthesis of the adducted *N-ras*-12 11-mer **29**, automated DNA synthesis was used for the first six unmodified nucleotides (from the 3′-end) using standard commercially available PAC-protected phosphoramidites. The cassette containing the solid support was then removed from the DNA synthesizer, and each end was fitted with a syringe, one contained a methylene chloride solution of the IQ-adducted phosphoramidite **25**, while the other syringe contained a solution of 1*H*-tetrazole. The solutions of **25** and 1*H*-tetrazole were periodically drawn through the cassette over 30 min. After a manual wash with acetonitrile, the cassette was placed back on the DNA synthesizer and capped with phenoxyacetyl chloride. The remainder of the synthesis was completed as usual. Johnson had observed that the C8-aminofluorene adduct undergoes

oxidative rearrangement in the presence of strong base and air, analogous to that observed for 8-oxo-2′-deoxyguanosine.[41,42] It was found that this reaction could be suppressed by the addition of mercaptoethanol during the deprotection of the oligonucleotide. To prevent complications due to oxidation of the C8-IQ-adducted deoxyguanosine, the deprotection of oligonucleotide **25** was accomplished with degassed ammonium hydroxide under an argon atmosphere at room temperature overnight followed by purification by reversed-phase HPLC. The oligonucleotide was characterized by MALDI-TOF mass spectrometry and enzymatic digestion, and the purity was assessed by capillary zone electrophoresis (CZE) analysis.

We also prepared an oligonucleotide containing the *Nar*I restriction sequence ($G_1G_2CG_3CC$) site-specifically adducted with IQ (**30**, Figure 2) at the G_3-position. The *Nar*I sequence contains a CG-repeat (shown in bold in **30**−**32**, Figure 2) and is considered a "hotspot" for arylamine modification and is prone to −2 frameshift mutations. Site-specific mutagenesis studies of the C8-AF and C8-AAF adducts have been reported at all three guanine positions of this sequence, and the NMR structural analyses of the C8-AF adducts have also been disclosed. [43]

As with oligonucleotide **29**, a manual coupling protocol was utilized to incorporate the IQ-modified base of **30**, and the adducted oligonucleotide was purified and analyzed as described above. Enzyme digests of oligonucleotides **29** and **30** are shown in Figure 3 along the UV spectra of the adducted base as obtained by a diode array detector from the HPLC analysis of the enzymatic digestion. The modified base (**14**) for the enzyme digest integrated low, which we believe is due to precipitation from its low water solubility. HPLC, CZE, and MALDI-TOF mass spectrometric analysis indicate that the adducted oligonucleotide is greater than 92% pure.

Melting Temperature (T_m), Light Absorption, and Circular Dichroism Studies. Oligonucleotides **29** and **30** were hybridized to complementary strands, and the effect of the C8-IQ adduct on the thermal stability of the DNA duplex was measured through UV melting temperature analysis (T_m) by monitoring the absorbance at 260 nm versus temperature. These data are summarized in Table 3 along with related melting temperatures for C8-AAF-adducted oligonucleotides at the G_3-position in the *Nar*I sequence, **31** and **30** (Figure 2), previously reported by Fuchs and Romano, respectively.[14,44,45] In general, modification of DNA destabilizes the duplex, resulting in a lower T_m. In the case of the *ras*-12-adducted oligonucleotides **29**, we observed a 9 °C decrease in the T_m versus that of the corresponding unmodified oligonucleotides (entry a). A modest 4 °C destabilization for the IQ-adducted *Nar*I oligonucleotide **30** was observed when compared to the unmodified duplex (entry b, T_m = 61 vs 65 °C). These results should be compared with the independent observations of Fuchs and Romano who examined oligonucleotides **31** and **32** containing a C8-AAF adduct at the G3-position of the *Nar*I restriction sequences.[14,44] The AAF-adducted oligonucleotides **31** and **32** (Table 3, entries

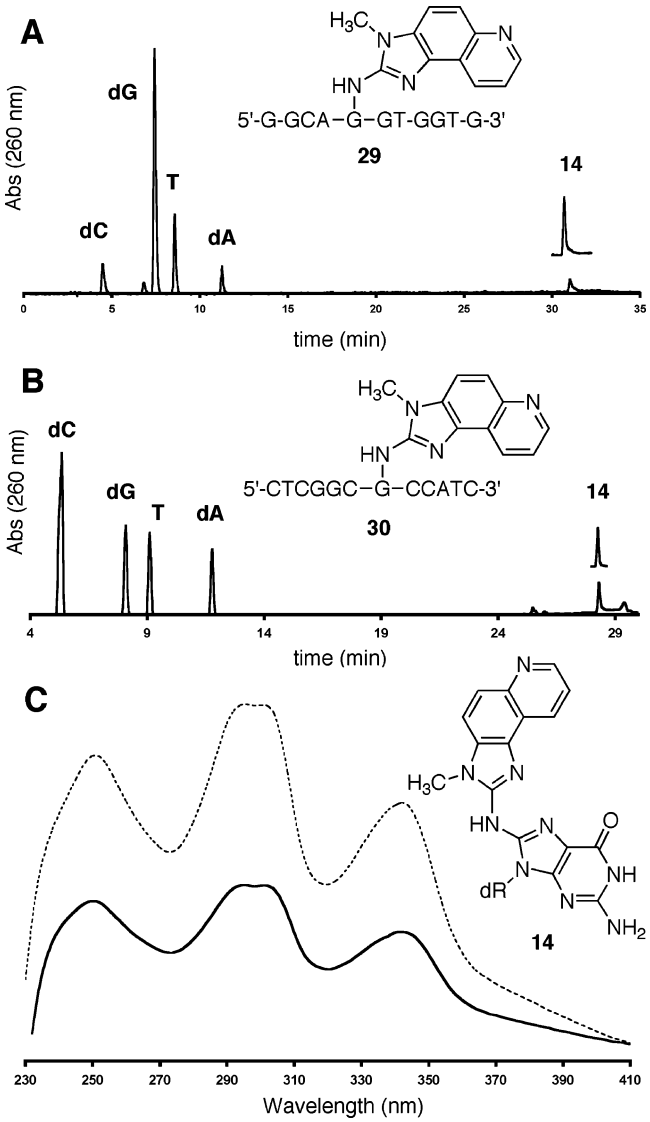


Figure 3. Enzyme digests of oligonucleotides **29** (panel A) and **30** (panel B) containing the C8-deoxyguanosine adduct of IQ. Panel C shows the UV spectrum of the IQ-adducted base (**14**) from the enzyme digest taken via diode array detector (solid line) versus that of an authentic standard (dashed line).

c and d) were significantly more destabilized (ΔT_m = 13 and 17 °C) versus the unadducted duplex than that for the IQ-adducted oligonucleotide **30** (entry b). We note that the sequence of 12-mers **31** and **32** flanking the *Nar*I restriction sequence and the buffer conditions used for the T_m measurements by Fuchs and Romano were slightly different than ours; however, in all cases we are comparing the difference in T_m for the adducted versus the unadducted oligonucleotides. Thus, we believe the observed thermal destabilizations are due largely to the modification, and the comparisons are qualitatively useful.

As mentioned, the G_3-position of the *Nar*I restriction sequence is part of a CG-repeat and is a hotspot for −2 frameshift mutations by arylamines such as AAF and IQ. One interesting difference is that AAF blocks replication and its mutagenesis is SOS-dependent, while IQ is by-passed and has been shown to be SOS-independent.[46] The accepted model for frameshift mutagenesis within a repetitive sequence involves strand slip-

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Table 3. T_m Studies of Adducted Oligonucleotides (Temperature Was Raised 1 °C min^{-1})

	oligonucleotide	Tm		
		modified	unmodified	Δ Tm
a.	IQ 5'-GGCA−G−GTGGTG-3' **(28)** 3'-CCGT−C−CACCAC-5'	51° C [a,d]	60° C	-9° C
b.	IQ 5'-CTCGGC−G−CCATC-3' **(30)** 3'-GAGCCG−C−GGTAG-5'	61° C [a,d]	65° C	-4° C
c.	AAF 5'-ACCGGC−G−CCACA-3' **(31)** 3'-TGGCCG−C−GGTAG-5'	48° C [a,e]	61° C	-13 °C
d.	AAF 5'-GTGGC−G−CCAAGT-3' **(32)** 3'-CACCG−C−GGTTCA-5'	41° C [c,g]	58° C	-17° C
e.	IQ 5'-CTCGGC−G−CCATC-3' **(30)** 3'-GAGCC———GGTAG-5'	48° C [a,d]	38° C	+10° C
f.	AAF 5'-ACCGGC−G−CCACA-3' **(31)** 3'-TGGCC———GGTAG-5'	49° C [b,f]	34 °C	+15° C
g.	AAF 5'-GTGGC−G−CCAAGT-3' **(32)** 3'-CACC———GGTTCA-5'	41° C [c,g]		
h.	IQ 5'-CTCGGC−G−CCATC-3' **(30)** 3'-GAGCCG−T−GGTAG-5'	60° C [a,d]	53° C	+7° C

[a] Conditions: 100 mM NaCl, 10 mM pH 7 phosphate, 0.05 mM EDTA, 0.5 A_{260}/mL of each oligonucleotide. [b] Conditions: 50 mM NaCl, 1 mM EDTA, 10 mM Tris. [c] Conditions: 100 mM NaCl, 10 mM NaH_2PO_4, pH 7, 0.5 mM EDTA. [d] This work. [e] Reference 44. [f] Reference 45. [g] Reference 14.

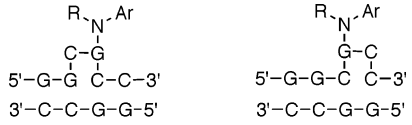

Figure 4. Possible structures for the slipped mutagenic intermediate of C8-arylamine adducts in a CG-repeat sequence.

page during replication.[47] In the present case, two bases are extruded, leading to the −2 frameshift deletion. To simulate the slipped-mutagenic intermediate for the observed −2 frameshifts, Fuchs and Romano independently examined the stability of **31** and **32** when hybridized to a complementary 10-mer in which two bases opposite the lesions were deleted (Table 3, entries f and g), thereby creating a two-base bulge involving the adducted base.[14,45] In the case of the *Nar*I CG-repeat sequence, this two-base bulge can be accommodated in two ways when hybridized to a complementary strand as shown in Figure 4. NMR studies showed that structure **34** existed predominantly or exclusively in the 12•10 duplex involving **31**; however, the NMR analysis of an aminofluorene-adducted oligonucleotide in a related *Nar*I sequence reached the opposite conclusion.[43,45,48] Melting temperature studies showed that the AAF-adducted 12•10 duplex is significantly stabilized when compared to the unadducted 12•10 hybrid (Table 3, entry f). When the IQ-adducted oligonucleotide **30** was hybridized with a complementary 10-mer in which the GC bases opposite the adduct were deleted, a similar stabilization was observed versus the unmodified 12•10 duplex (Table 3, entry e). However, the stabilization of the IQ-adducted 12•10 duplex was less pronounced than that for AAF ($\Delta T_m = 10$ vs 15 °C). Interestingly, Fuchs and Romano reported that the T_m value of the AAF-adducted 12•10 duplex was essentially identical to that of the corresponding AAF-adducted 12•12 duplex (Table 3, entries c vs f and entries d vs g, respectively). This led Romano to speculate that the AAF-adducted 12•12 and 12•10 duplexes containing oligonucleotide **32** had similar structures.[14] In the case of the IQ-adducted oligonucleotide **30**, we find that the T_m of the full length 12•12 duplex is 13 °C higher than the 12•10 duplex with the two-base deletion opposite the adducted base (Table 3, entries b vs e), in contrast to the results reported by Fuchs and Romano for the AAF-adducted oligonucleotide.

Fuchs examined the circular dichroism spectra of the AAF-adducted oligonucleotides at all three deoxyguanosine positions of the *Nar*I restriction sequence (i.e., **31**).[44] It was observed that the long wavelength absorptions from 290 to 305 nm, assigned to the *N*-acetylaminofluorene group, had a negative optical activity. This was interpreted as a local left-handed helical structure around the adducted nucleotide of **31**, which may play a significant role in the mutagenic mechanism and polymerase arresting properties of AAF. This local left-handed or Z-DNA conformation is due to the AAF group inducing an *anti* to *syn* rotation of the glycosidic bond accompanied by the AAF moiety intercalating into the DNA helix with the adducted guanine base displaced into the minor groove. This results in a local denaturation of the DNA near the adducted base and an increased susceptibility of AAF-adducted oligonucleotide to nuclease S_1, which is a single-strand-specific endonuclease. This model has been termed base displacement or insertion-denaturation.[49,50] In addition, the positive band at 268 nm for the unmodified duplex was slightly blue-shifted to 263 nm and increased in amplitude by 100% upon modification by AAF at the G_3-position.

The CD spectra of the IQ-adducted oligonucleotides **29** and **30** as well as their corresponding modified and unmodified duplexes are shown in Figure 5. The duplex of the IQ-adducted oligonucleotide at the G_3-position in the *Nar*I restriction sequence (**30**) showed few of the characteristics of the AAF spectra reported by Fuchs. The induced CD signal assigned to the IQ group from ∼300−360 is broad, weak, and has a positive optical activity, indicating that there is no pronounced left-handed helical structure around the adducted base. The positive band at 272 nm for the unadducted duplex is slightly red-shifted upon adduction at the G_3-position by IQ to 274, and its intensity is relatively unchanged. Overall, the spectrum of the adducted single-strand and duplex oligonucleotides strongly resembles that of the unmodified. We contrast this spectrum to that of the 12•12 duplex involving the *ras*-12 sequence (**29**). The intensity of the band at 270 nm is shifted to approximately 278 nm and is significantly decreased upon adduction by IQ at the G_3-position. In the adducted single-stranded oligonucleotides, a strong negative band is observed at 260 nm. The significant reduction in the intensity for the duplex at 278 nm versus that of the unmodified oligonucleotide may be due to specific conformations of the adducted base responsible for the negative band observed in the single-stranded adducted oligonucleotide (**29**), and this conformation is largely preserved in both single- and double-stranded DNA. We also note that the induced CD signals assigned to the IQ moiety in **29** from ∼300 to 360 nm are well defined and reasonably intense.

We believe that the dramatic differences in the IQ absorptions between duplexes of **29** and **30** are an indication that the adduct

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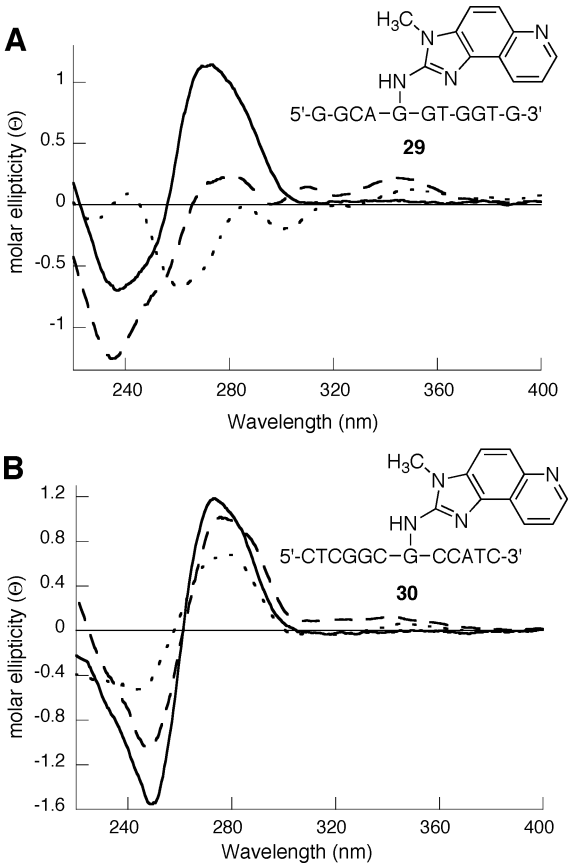

Figure 5. CD spectra of unmodified duplex (−), adducted duplex (− − −), and adducted single-strand (- - -) DNA in the *ras*-12 sequence (panel A) and *Nar*I restriction sequence (panel B).

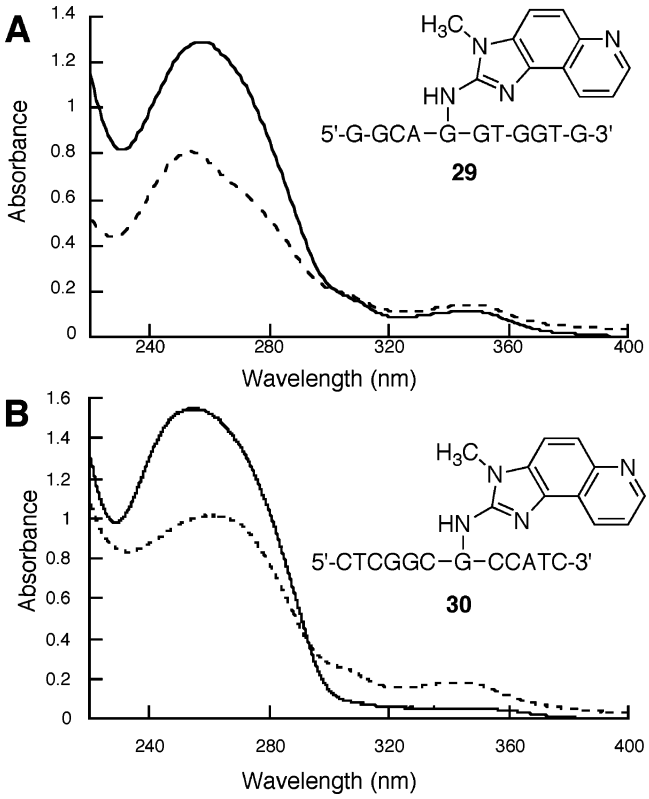

Figure 6. UV absorption spectra of adducted single-stranded (- - -) and double-stranded (−) DNA in the r*as*-12 sequence (panel A) and *Nar*I restriction sequence (panel B).

is accommodated in dramatically different environments, the preference of which is highly sequence-dependent. Absorbance and fluorescence emission spectroscopy has been used to characterize the environment of the lesion of PAH-adducted oligonucleotides.[51] For N^2-PAH adducts of deoxyguanosine, it was observed that base-displaced intercalated conformations (termed site I) resulted in a ∼10 nm red shift in the absorbance and a significantly quenched fluorescence emission. For groove-bound conformations (site II) where base stacking is expected to have less influence on the adduct chromophore, there is a ∼2 nm red-shift in the absorbance maximum and only a modest quenching of the fluorescence emission. This analysis had been applied to a C8-aminopyrene (AP)-adducted oligonucleotide in the sequence 5′-d(CTCATG^{AP}ATTCC) and it was concluded that a mixture of groove-bound and base-displaced intercalated conformers were present.[52]

Absorption spectra of oligonucleotides **29** and **30** and their duplexes are shown in Figure 6. Our interpretation of the spectra is that the IQ-adduct of **30**, which is in the CG-repeat portion of the *Nar*I restriction sequence, adopts a base-displaced intercalated conformation and is groove-bound in **29**. The intensity of the IQ absorption for **30** is dramatically reduced when the oligonucleotide is hybridized to its complementary strand as compared to the absorbance of the adducted single-stranded oligonucleotide. We believe this behavior is consistent with a base-displaced intercalated conformation where $\pi-\pi$ stacking interactions with neighboring DNA bases can significantly perturb the UV absorbance of the IQ moiety. For groove-bound conformations, the UV absorbance of the adduct is minimally perturbed by the DNA bases. This observation is consistent for the C8-IQ adduct in the *ras*-12 sequence **30** in which we believe the IQ is accommodated in a groove-bound conformation. As with previous studies with PAHs, we would also expect the fluorescence of the base-displaced intercalated adduct to be quenched and red-shifted, while the groove-bound conformation would be less perturbed. Unfortunately, the C8-IQ adduct of deoxyguanosine was not fluorescent.

For N^2-deoxyguanosine adducts of PAHs, it was observed that the intensity of the adduct absorbance was reduced for the intercalated conformation. In the case of the duplex of **30**, the intensity of the IQ absorbance is barely detectable. We speculated that this dramatic reduction in the absorbance is due to a hydrogen-bonding interaction between the quinoline nitrogen of IQ and the N^4-amino group of the cytosine base in the complementary strand of the duplex (Figure 7). Such a conformation would result in an unfavorable local distortion of the DNA duplex near the adduct site; however, this strain could be offset by more favorable hydrophobic stacking interactions and an additional hydrogen bond. To test this hypothesis further, we examined the properties of adducted oligonucleotide **30** when hybridized to a complementary strand in which a thymidine was placed opposite the adducted base. In this case, the proposed hydrogen-bonding interaction would be disrupted or eliminated. The melting temperature of the thymidine mismatch duplex of **30** was not significantly lower than that with a normal complement (entries h vs b in Table 3). G−T-mismatches can

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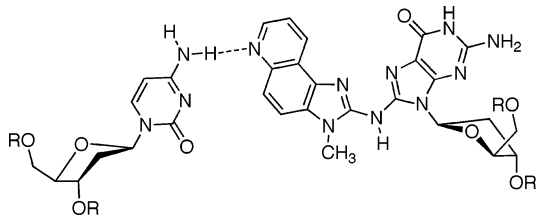

Figure 7. Proposed hydrogen bonding of the base-displaced intercalated conformation of the adducted C8-deoxyguanosine with the complementary deoxycytidine in the CG repeat of the *Nar*I restriction sequence.

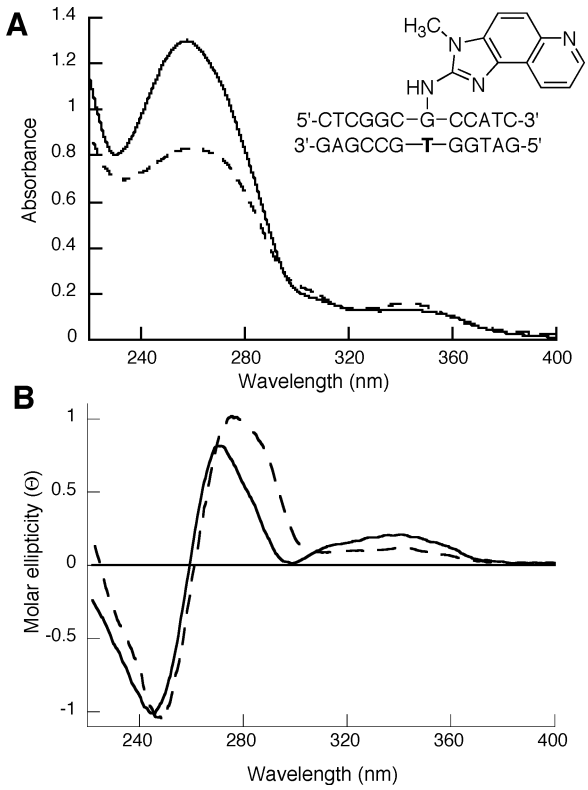

Figure 8. UV (panel A) and CD (panel B) spectra of the IQ-adducted *Nar*I restriction sequence (**30**) hybridized to a complementary strand with a thymidine opposite the adducted base (−). The UV spectrum is compared to the adducted single-strand oligonucleotide (− − −), and the CD spectrum is compared to the duplex with a normal complementary strand (− − −).

severely destabilize DNA duplexes, and in fact the IQ-adducted duplex with the T-mismatch was stabilized versus the unadducted G−T-mismatch.[53,54] From these studies, it is difficult to assess the degree of stabilization provided by the proposed hydrogen bond. The UV absorption and CD spectra of this duplex are shown in Figure 8. The CD spectrum of the duplex of **30** with the thymidine mismatch opposite the adducted base is similar to that of **30** with a normal complementary strand (Figure 5, panel B) except that the IQ absorbance is clearly visible and its intensity is only modestly reduced versus the adducted single-stranded oligonucleotide. With the T-mismatch, the induced CD of the IQ moiety is positive and noticeably more intense; the band at 270 nm is blue-shifted, and its intensity is slightly reduced as compared to that with the normal complement (274 nm) and the unmodified duplex (272 nm). Unfortunately, the IQ absorbance when in a duplex is broad and rather featureless. Consequently, it is difficult to determine

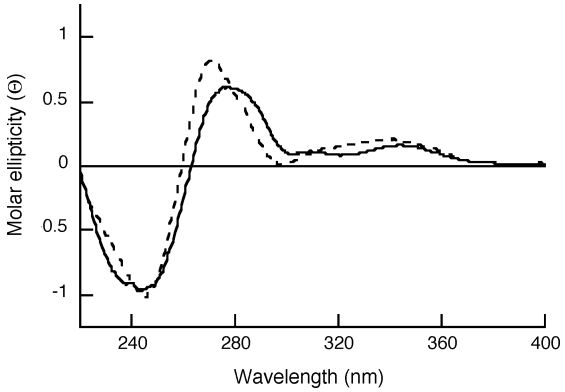

Figure 9. Averaged CD spectra of **29** and **30** with the normal complement (−) and **30** with a T-mismatch (- - -).

small shifts in the absorbance maximum, although there appears to be little change from the single stand, which would be more consistent with a groove-bound adduct.

NMR solution structures of oligonucleotides containing C8-deoxyguanosine adducts of simple arylamines have shown that the adducted base may be in conformational exchange between the groove bound and base-displaced intercalated conformations. For example, solution structures of AF-adducted oligonucleotides at all three positions of the *Nar*I restriction sequence have been determined by NMR, and the equilibrium between the groove-bound and base-displaced intercalated conformations was highly dependent upon the sequences. At the G_1 and G_2 positions, the groove-bound conformation was strongly favored in a ratio of 70:30 and 90:10, respectively, while at the G_3-position an equal mixture of the groove-bound and base-displaced intercalated conformations was reported.[43,55] Solution structures of C8-AAF and aminobiphenyl have been determined in other sequences and were reported to exist in equilibrium between the groove-bound and base-displaced intercalated conformations as well.[56,57] A solution structure of C8-aminopyrene adducts was found to exist exclusively in a base-displaced intercalated conformation in a sequence in which the corresponding AF-adduct was found to be largely groove-bound.[58] On the basis of their CD and UV spectra, we believe that the IQ moiety of oligonucleotides **29** and **30** exists predominantly in the groove-bound and base-displaced intercalated conformations, respectively. However, the CD spectra of **30** with a thymidine opposite the adducted base possess features observed in both the groove-bound and the base-displaced intercalated spectra, which may indicate that this duplex exists in conformational exchange. The CD spectra of oligonucleotides **29** and **30** (with the normal complement strand) were averaged in an attempt to model a spectrum of an oligonucleotide in conformational exchange with equal populations of base-displaced intercalated and groove bound. This analysis assumes that the spectra for **29** and **30** are predominantly one conformation. The result of this analysis is shown in Figure 9. The averaged spectrum (−) closely resembles that of **30** with the T-mismatch (- - -) and leads us to believe that this duplex is in conformational exchange.

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Our interpretation of the CD and UV data for oligonucleotide **30** is in contrast to modeling studies which predict that the C8-IQ adduct at the G_3-position of the *Nar*I restriction sequence would adopt a groove-bound conformation.[59,60] In this work, a nonbiased conformational search was performed on a duplex DNA sequence 5′-$G_1G_2CG_3CCA$-3′ in which G_3 (designated G_4 in their manuscript) was adducted with IQ at the C8-position. Families of structures were identified in which the IQ moiety was bound in either the minor groove or the major groove. The lowest energy base-displaced intercalated conformation was approximately 10 kcal/mol higher in energy than the lowest energy minor groove-bound conformation. It was noted that no attempt was made to correct for non-Coulombic (hydrophobic) forces, and it was estimated that such interaction may account for up to 2 kcal/mol. It is possible that the hydrophobic contribution of C8-IQ adduct may have been underestimated; IQ itself has been shown to intercalate into CG-rich sequences of double-stranded DNA.[61] It was also noted that the lack of a lower energy base-displaced intercalated conformation might be due to a "multiple minimum problem," in which the observed groove-bound conformations are kinetically trapped structures. Similar results were found for the C8-adduct of MeIQx.[60]

Recently, Cosman reported the solution structure of an oligonucleotide containing a C8-PhIP adduct in the sequence 5′-CCATCGCTACC; this work represents the first and to date only structural analysis of an HCA-adducted oligonucleotide.[62,63] The overall structure was described as similar to the C8-AF-adducted oligonucleotide with some key differences. The major conformation of the C8-PhIP-adducted oligonucleotides was a base-displaced intercalated structure in which the adducted guanine is situated in the major groove in a *syn* conformation. The C6-phenyl and N3-methyl groups of PhIP protrude into the minor groove, causing a widening of the minor groove and a compression of the major groove. This causes an overall bending of the DNA structure. A slow conformational exchange with a minor conformation was reported. Few details of this minor conformation were disclosed; however, it was described as a minimally perturbed B-DNA duplex with the PhIP adduct located in the major groove. Previous ^{1}H and ^{31}P NMR, UV, and fluorescence titration studies indicated that the major noncovalent interaction of free PhIP with DNA was as a groove binder and not an intercalator.[64] The synthesis of the PhIP-adducted oligonucleotide was accomplished via a biomimetic approach in which the single-stranded DNA was reacted with the PhIP nitrenium ion. The low yield of this process may have limited the sequence to a single deoxyguanosine. Although the full CG repeat portion of the frameshift-prone *Nar*I restriction sequence was not examined, deoxycytidines were flanking the adducted bases. Our conclusion that the C8-IQ-adducted deoxyguanosine of **30** adopts a base-displaced intercalated conformation is based on light absorption and CD analysis and is consistent with the NMR analysis of the related C8-PhIP adduct. It was noted that the C6-phenyl group of the PhIP adduct was twisted approximately 22° from the plane of the imidazaopyridine ring. Eclipsing of ortho protons about the biaryl bond represents a significant energy barrier and this biaryl twist may impede complete intercalation of the PhIP group into the DNA helix; extended aromatic structures such as IQ and AF would not be subject to such steric constraints. This may prevent PhIP from hydrogen bonding to the complementary deoxycytidine as proposed above for IQ.

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Summary

In summary, we have developed an efficient and general strategy for the synthesis of C8-deoxyguanosine adducts of arylamines involving the Buchwald−Hartwig palladium-catalyzed *N*-arylation reaction of a suitably protected 8-bromo-2′-deoxyguanosine derivative **16** with various mutagenic arylamines including the dietary mutagen IQ (**1**). The C8-IQ-adducted nucleoside was site-specifically incorporated into oligonucleotides using the phosphoramidite approach. We have examined the properties of two adducted oligonucleotides by a combination of thermal UV melting studies, UV spectroscopy, and circular dichrosim. We believe that the C8-IQ adduct of the *N-ras*-12 sequence (**29**) adopts a groove-bound conformation, while at the G_3-position of the *Nar*I restriction sequence (**30**) it is in a base-displaced intercalated conformation. Furthermore, on the basis of the strong decrease of the IQ absorption, we believe that a hydrogen-bonding interaction between the IQ quinoline nitrogen and the complementary cytosine base stabilizes the base-displaced intercalated form. This differentiates IQ and potentially the rest of the HCAs from simple arylamines and may play an important role in their mutagenicity.

Experimental Section

All commercially obtained chemicals were used as received. Toluene was freshly distilled from sodium and degassed with argon. THF was distilled from a sodium/benzophenone ketyl. Pyridine was freshly distilled from calcium hydride. Anhydrous 1,4-dioxane and 1, 2-dimethoxyethane (DME) were purchased from Aldrich in Sure-seal bottles and were used as received. All reactions were performed in oven-dried glassware and under an argon atmosphere. Melting points are uncorrected. Proton and carbon-13 NMR data were recorded at 300 or 400 and 75 or 100 MHz, respectively, in $CDCl_3$ unless otherwise noted. High-resolution FAB mass spectra were obtained from the University of Notre Dame Mass Spectrometry Center using nitrobenzyl alcohol (NBA) as the matrix. MALDI-TOF mass spectrometry of oligonucleotides was performed at the Vanderbilt University Mass Spectrometry Resource Center using a 3-hydroxypicolinic acid (HPA) matrix containing ammonium hydrogen citrate (7 mg/mL) to suppress sodium and potassium adducts. Electrophoretic analyses were carried out using a Beckman P/ACE Instrument System 5500 Series monitored at 260 nm on a 27 cm × 100 mm column packed with the manufacturer's 100-R gel (for ss DNA) using a Tris-borate buffer system containing 7 M urea.

8-Bromo-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-β-D-2′-deoxyribofuranosyl]guanine (17). A stirred suspension of 8-bromo-2′-deoxyguanosine (**16**, 2.9 g, 8.37 mmol) in dry pyridine (50 mL) was cooled 0 °C, and then 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.9 g, 9.18 mmol) was added. The reaction mixture was warmed to room temperature and stirred overnight. The pyridine was evaporated under reduced pressure, and the residue was poured into water and extracted with chloroform. The combined organic extracts were successively washed with 10% HCl, saturated sodium bicarbonate,

and brine, and then dried over sodium sulfate and evaporated. Purification by flash chromatography on silica, eluting with 1−2% methanol in chloroform, gave **17** (4.5 g, 91% yield) as a white solid, mp 194−196 °C; $[\alpha]^{23}_D$ +5.7° (*c* 2.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 12.00 (br s, 1H), 6.46 (br s, 2H), 6.15 (dd, J = 8.6, 3.0, 1H), 5.17 (m, 1H), 4.03 (dd, J = 11, 2.3, 1H), 3.93−3.84 (m, 2H), 3.28−3.22 (m, 1H), 2.54−2.45 (m, 1H), 1.14−0.93 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 157.8, 153.5, 152.0, 121.6, 117.9, 85.2, 83.8, 72.9, 63.6, 38.0, 17.6, 17.5, 17.7, 17.3, 17.2, 17.0, 13.4, 13.2, 13.0, 12.6; HRMS (FAB, NBA) *m*/*z* calcd for $C_{22}H_{39}O_5N_5BrSi_2$ (M + H) 588.1673, found 588.1657.

***O*^{6}-Benzyl-8-bromo-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]guanine (18).** Triphenylphosphine (285 mg, 1.08 mmol) and **17** (0.58 g, 0.98 mmol) were placed in a dry flask, and the flask was evacuated for 10 min, and then refilled with argon. Anhydrous 1,4-dioxane (10 mL) and benzyl alcohol (117 mg, 1.08 mmol) were added, and the resulting suspension was cooled to 0 °C. Diethylazodicarboxylate (190 mg, 1.08 mmol) was added dropwise via syringe. After the addition was complete, the reaction was warmed to room temperature and stirred for 1 h, after which time it was concentrated in vacuo to give a yellow oil. Purification by flash chromatography on silica, eluting with 10% ethyl acetate in hexanes, gave **18** (0.51 g, 76% yield) as a white solid. mp 127−129 °C; $[\alpha]^{23}_D$ −11° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.47 (d, J = 7.5, 2H), 7.36−7.29 (m, 3H), 6.21 (dd, J = 8.9, 3.4, 1H), 5.51 (s, 2H), 5.23 (dt, J = 7.8, 6.0, 1H), 4.75 (s, 2H), 4.03−3.97 (m, 1H), 3.90−3.83 (m, 2H), 3.31−3.24 (m, 1H), 2.54−2.46 (m, 1H), 1.15−0.97 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 159.79, 158.6, 154.0, 136.0, 128.3, 128.2, 128.0, 125.3, 116.2, 85.0, 83.7, 72.9, 68.0, 63.6, 37.8, 17.5, 17.4, 17.3, 17.3, 17.2, 17.1, 17.0, 16.9, 13.3, 13.1, 12.9, 12.5; HRMS (FAB, NBA) *m*/*z* calcd for $C_{29}H_{45}O_5N_5BrSi_2$ (M + H) 678.2143, found 678.2156.

***O*^{6}-Benzyl-8-bromo-2-(2,2,5,5-tetramethyl-1-aza-2,5-disilylcyclopent-1-yl)-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]inosine (19).** A solution of 18 (220 mg, 0.33 mmol) in anhydrous THF (20 mL) was cooled to −78 °C, and then lithium bis(trimethylsilyl)amide (1.0 M in THF, 0.65 mL, 0.65 mmol) was added over 5 min. The solution was stirred for 15 min, and then 1,2-bis(chlorodimethylsilyl)ethane (72 mg, 0.33 mmol) in THF (1 mL) was added dropwise. The reaction was warmed to room temperature and stirred for an additional 20 min. The reaction was quenched by the addition of saturated sodium bicarbonate (10 mL). The layers were separated, and the aqueous phase was extracted with ether. The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and evaporated. Purification by flash chromatography on silica, eluting with 3% ethyl acetate in hexanes, gave **19** (226 mg, 85% yield) as white solid. mp 94−96 °C; $[\alpha]^{23}_D$ −13° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.43 (d, J = 6.9, 2H), 7.37−7.29 (m, 3H), 6.28 (t, J = 7.6, 1H), 5.52 (s, 2H), 4.77−4.71 (m, 1H), 4.15−4.06 (m, 1H), 3.97−3.89 (m, 2H), 3.75−3.64 (m, 1H), 2.28 (ddd, J = 13.5, 6.9, 3.0, 1H), 1.12−0.98 (m, 28H), 0.83 (s, 4H), 0.33 (s, 6H), 0.31 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 160.9, 159.0, 154.7, 136.1, 128.4, 128.0, 127.7, 124.6, 116.1, 85.5, 83.8, 73.7, 68.0, 64.4, 37.4, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 16.9, 13.4, 13.2, 13.0, 12.6, 8.2, −0.5, −0.7; HRMS (FAB, NBA) *m*/*z* calcd for $C_{35}H_{59}O_5N_5BrSi_4$ (M + H) 820.2777, found 820.2771.

***O*^{6}-Benzyl-8-[(3-methyl-3*H*-imidazo[4,5-*f*]quinolin-2-yl)amino]-2-(2,2,5,5-tetramethyl-1-aza-2,5-disilylcyclopent-1-yl)-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]inosine (20).** To a stirred suspension of **19** (220 mg, 0.268 mmol), 2-amino-3*H*-methylimidazo[4,5-*f*]-quinoline (IQ, 106 mg, 0.536 mmol), $Pd_2(dba)_3$ (25 mg, 0.0268 mmol), and BINAP (50 mg, 0.0804 mmol) in anhydrous, degassed toluene (2 mL) under an argon atmosphere was added lithium bis(trimethylsilyl)amide (1.0 M in THF, 0.54 mL, 0.54 mmol). The reaction was heated to 100 °C and stirred for 25 min, and then cooled to room temperature and quenched with saturated sodium bicarbonate (1 mL). The layers were separated, and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and evaporated. Purification by flash chromatography on silica, eluting with 20% ethyl acetate in hexanes, gave **20** (170 mg, 68%) as a yellow solid. mp 233−235 °C; $[\alpha]^{23}_D$ −12° (*c* 0.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.50−8.00 (br s, 1H), 8.85 (d, J = 4.1, 1H), 8.60 (d, J = 8.3, 1H), 7.92 (d, J = 8.9, 1H), 7.63 (d, J = 8.9, 1H), 7.52 (d, J = 7.4, 2H), 7.46−7.36 (m, 4H), 6.66 (t, J = 7.7, 1H), 5.60 (s, 2H), 4.83−4.80 (m, 1H), 4.15 (dd, J = 10.5, 3.1, 1H), 4.07−4.02 (m, 1H), 3.99−3.88 (m, 2H), 3.83 (s, 3H), 2.32−2.24 (m, 1H), 1.15−1.05 (m, 28H), 0.85 (s, 4H), 0.37 (s, 6H), 0.35 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 159.6, 154.7, 154.0, 152.6, 151.5, 148.1, 145.1, 136.9, 129.8, 129.5, 128.5, 128.4, 127.9, 127.2, 122.8, 120.6, 118.0, 112.3, 105.7, 85.5, 81.4, 74.7, 67.5, 65.1, 36.6, 28.7, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 13.5, 13.3, 13.0, 12.7, 8.7, −0.4, −0.6; HRMS (FAB, NBA) *m*/*z* calcd for $C_{46}H_{68}O_5N_9Si_4$ (M + H) 938.4421, found 938.4456.

***O*^{6}-Benzyl-8-[(3-methyl-3*H*-imidazo[4,5-*f*]quinolin-2-yl)amino]-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]guanine (21).** To a stirred suspension of **18** (182 mg, 0.268 mmol), 2-amino-3-methylimidazo[4,5-*f*]-quinoline (IQ, 106 mg, 0.536 mmol), $Pd_2(dba)_3$ (25 mg, 0.0268 mmol), and BINAP (50 mg, 0.0804 mmol) in anhydrous, degassed toluene (2 mL) under an argon atmosphere was added lithium bis(trimethylsilyl)amide (1.0 M in THF, 0.54 mL, 0.54 mmol). The reaction was heated to 100 °C and stirred for 25 min, and then cooled to room temperature and quenched with saturated sodium bicarbonate (1 mL). The layers were separated, and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and evaporated. Purification by flash chromatography on silica, eluting with 2% methanol in methylene chloride, gave **21** (141 mg, 66% yield) as a yellow solid. mp 203−205 °C; $[\alpha]^{23}_D$ −48° (*c* 0.5, $CHCl_3$); IR (KBr) 3348, 3215, 2943, 2866, 1601, 1465, 1421, 1134, 1081, 1033 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.87 (dd, J = 4.18, 1.66 1H), 8.56 (d, J = 8.20, 1H), 7.93 (d, J = 8.86, 1H), 7.63 (d, J = 8.92, 1H), 7.57 (d, J = 7.16, 2H), 7.46−7.36 (m, 4H), 6.69 (dd, J = 9.02, 3.64, 1H), 5.61 (s, 2H), 5.32 (q, J = 12.66, 7.00, 1H), 4.65 (s, 2H), 4.13−4.05 (m, 1H), 3.99−3.93 (m, 2H), 3.81 (s, 3H), 3.32−3.25 (m, 1H), 2.56−2.49 (m, 1H), 1.18−1.05 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 157.67, 155.5, 153.7, 152.3, 151.7, 147.9, 144.8, 129.7, 129.0, 128.5, 128.3, 128.0, 127.9, 122.8, 120.6, 117.7, 112.4, 106.5, 84.9, 80.6, 73.8, 67.6, 64.4, 37.9, 28.6, 17.5, 17.5, 17.4, 17.3, 17.0, 13.5, 13.3, 13.0, 12.7; HRMS (FAB NBA) *m*/*z* calcd for $C_{40}H_{54}N_9O_5Si_2$ (M + H) 796.3786, found 796.3760.

8-[(3-Methyl-3*H*-imidazo[4,5-*f*]quinolin-2-yl)amino]-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]guanine (22). A stream of hydrogen gas was bubbled through a solution of **21** (55 mg, 0.088 mmol) and 5% Pd/C (42 mg, 0.02 mmol) in methanol (7 mL) for 2 h. The mixture was then stirred at room temperature overnight under 1 atm of hydrogen. The catalyst was removed by suction filtration, and the filtrate evaporated. Purification by flash chromatography on silica, eluting with 10% methanol in methylene chloride, gave **22** (35 mg, 74% yield) as a light yellow solid. mp 280 °C (dec); $[\alpha]^{23}_D$ −18.4° (*c* 0.5, MeOH:CH_2Cl_2 = 1:1); IR (KBr) 3347, 3215, 3021, 2944, 2867, 1679, 1592, 1496, 1382, 1137, 1088, 1032 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.80 (dd, J = 4.08, 1.64, 1H), 8.66 (dd, J = 8.26, 0.68, 1H), 7.87 (d, J = 8.92, 1H), 7.76 (d, J = 8.84, 1H), 7.56 (dd, J = 8.32, 4.16, 1H), 6.51−6.46 (m, 3H), 4.60−4.58 (m, 1H), 3.97−3.92 (m, 1H), 3.87−3.81 (m, 2H), 3.74 (s, 3H), 3.47−3.41 (m, 1H), 2.16−2.10 (m, 1H), 0.94−0.87 (m, 28H); ^{13}C NMR (DMSO-d_6) δ 154.7, 153.7, 153.0, 149.5, 149.1, 148.0, 144.9, 131.5, 129.4, 128.8, 121.9, 121.1, 1183, 113.4, 103.8, 87.2, 82.0, 71.5, 63.7, 35.8, 28.9, 17.5, 17.4, 13.3, 12.7; HRMS (FAB NBA) *m*/*z* calcd for $C_{33}H_{48}N_9O_5Si_2$ (M + H) 706.3317, found 706.3292.

8-[(3-Methyl-3*H*-imidazo[4,5-*f*]quinolin-2-yl)amino]-2′-deoxyguanosine (14). To a stirred solution of **22** (320 mg, 0.45 mmol) in THF (8 mL) was added tetrabutylammonium fluoride (1.06 mL, 1.0 M in

THF, 1.06 mmol). The reaction was stirred at room temperature for 1.5 h, after which time the solvent was removed under reduced pressure. Purification by flash chromatography on silica, eluting with 10% methanol in methylene chloride containing 1% NH_3, gave **14** (190 mg, 90% yield) as a light green solid. mp 250 °C (dec); $[\alpha]^{23}_D$ +16° (*c* 0.55, MeOH:$CHCl_3$ =1:1); ^{1}H NMR (DMSO-d_6) δ 11.05 (br s, 1H), 8.77 (dd, J = 4.2, 1.7, 1H), 8.66 (d, J = 8.3, 1H), 7.88 (d, J = 8.9, 1H), 7.77 (d, J = 8.9, 1H), 7.57 (dd, J = 8.4, 4.2, 1H), 6.53−6.49 (m, 3H), 4.52−4.49 (m, 1H), 3.83−3.79 (m, 1H), 3.75 (s, 3H), 3.69 (dd, J = 11.5, 5.2, 1H), 3.54 (dd, J = 11.5, 5.6, 1H), 3.37−3.30 (m, 1H), 2.10−2.04 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 154.11, 153.38, 152.81, 149.19, 148.60, 147.78, 144.55, 130.68, 129.11, 128.46, 121.75, 120.80, 117.73, 113.17, 104.05, 87.40, 81.94, 71.32, 62.34, 35.86, 28.58; HRMS (FAB, NBA) *m*/*z* calcd for $C_{21}H_{22}O_4N_9$ (M + H) 464.1795, found 464.1806.

***N*^{2}-[(Dimethylamino)methylene]-8-[(3-methyl-3*H*-imidazo[4,5-*f*]-quinolin-2-yl)amino]-*β*-D-2′-deoxyribofuranosyl]guanine (23).** To a stirred solution of **14** (100 mg, 0.22 mmol) in anhydrous methanol (5 mL) under an argon atmosphere was added *N*,*N*-dimethylformamide diethyl acetal (0.3 mL, 1.76 mmol). The reaction was heated to 50 °C and stirred for 2 h. The reaction mixture was allowed to cool to room temperature, upon which time a precipitate formed. The precipitate was collected by suction filtration and washed with cold methanol to give **23** (75 mg, 66% yield) as a light yellow solid. mp 280 °C (dec); $[\alpha]^{23}_D$ +12.4° (*c* 0.5, MeOH:CH_2Cl_2 = 1:1); IR (KBr) 3346, 3220, 2924, 1666, 1629, 1601, 1544, 1493, 1348, 1283, 1112 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.81 (dd, J = 4.16, 1.68, 1H), 8.69 (dd, J = 4.64, 0.92, 1H), 8.43 (s, 1H), 7.86 (d, J = 8.92, 1H), 7.78 (d, J = 8.96, 1H), 7.58 (dd, J = 8.56, 4.16, 1H), 4.57−4.53 (m, 1H), 3.85−3.82 (m, 2H), 3.74 (s, 3H), 3.72−3.68 (m, 2H), 3.59−3.54 (m, 1H), 3.32−3.27 (m, 1H), 3.14 (s, 3H), 3.02 (s, 3H), 2.17−2.14 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 158.18, 156.92, 154.33, 153.91, 150.08, 148.19, 147.59, 144.85, 130.05, 129.53, 128.64, 122.29, 121.20, 117.72, 113.48, 108.84, 87.63, 82.33, 71.55, 62.60, 55.27, 36.86, 34.94, 28.88; HRMS (FAB NBA) *m*/*z* calcd for $C_{24}H_{26}N_{10}O_4$ (M^+) 518.2138, found 518.2162.

***N*^{2}-[(Dimethylamino)methylene]-8-[(3-methyl-3*H*-imidazo[4,5-*f*]-quinolin-2-yl)amino]-*N*9[5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]]-*β*-D-2′-deoxyribofuranosyl]guanine (24).** To a stirred solution of **23** (50 mg, 0.096 mmol) in anhydrous pyridine (1.0 mL) and freshly distilled diisopropylethylamine (0.116 mL, 0.066 mmol) under an argon atmosphere was added 4,4′-dimethoxytrityl chloride (98 mg, 0.29 mmol) at 0 °C. After the addition, the reaction was allowed to warm to room temperature and was stirred for 3 h. The solvent was then removed under reduced pressure, and the residue was purified by flash chromatography on silica eluting with 1.0% methanol in methylene chloride containing 0.5% triethylamine gave **24** (62 mg, 79% yield) as a light yellow solid. mp 157° C (dec); $[\alpha]^{23}_D$ +37.6° (*c* 0.5, CH_2Cl_2); IR (KBr) 3397, 2927, 1626, 1531, 1340; ^{1}H NMR (CD_2Cl_2) δ 9.31 (s, 1H), 8.81 (d, J = 2.81, 2H), 8.77 (d, J = 8.19, 1H), 8.37 (s, 1H), 7.84 (d, J = 8.78, 1H), 7.50 (d, J = 8.87, 1H), 7.46 (dd, J = 8.25, 3.94, 1H), 7.36 (d, J = 7.92, 1.31, 2H), 7.24 (dd, J = 7.73, 2.40, 4H), 7.14 (t, J = 7.80, 2H), 7.07 (t, J = 7.25, 1H), 6.70−6.63 (m, 5H), 5.10 (q, J = 6.02, 3.88, 1H), 4.85 (q, J = 7.02, 2.30, 1H), 3.71−3.65 (m, 2H), 3.62 (s, 3H), 3.58 (s, 3H), 3.51 (dd, J = 0.024, 0.017, 1H), 3.43 (s, 3H), 3.29 (dd, J = 9.62, 4.67, 1H), 3.03 (s, 3H), 2.97 (s, 3H), 2.47−2.41 (m, 1H); ^{13}C NMR (CD_2Cl_2) δ 158.83, 158.78, 157.82, 155.55, 152.92, 151.89, 149.09, 148.47, 145.38, 145.16, 136.32, 136.27, 130.31, 130.17, 129.90, 128.40, 128.14, 128.01, 127.38, 126.98, 123.35, 121.13, 117.13, 113.24, 112.31, 86.41, 85.73, 82.40, 73.53, 65.29, 55.38, 41.51, 37.20, 35.16, 30.05, 28.50; HRMS (FAB NBA) *m*/*z* calcd for $C_{45}H_{44}N_{10}O_6$ (M + H) 821.3524, found 821.3552.

***N*^{2}-[(Dimethylamino)methylene]-8-[(3-methyl-3*H*-imidazo[4,5-*f*]-quinolin-2-yl)amino]-*N*9-[5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-3′-*O*-[[bis(1-methylethyl)amino](2-cyanoethoxy)phosphino]]-*β*-D-2′-deoxyribofuranosyl]guanine (25).** To a stirred suspension of **24** (50 mg, 0.061 mmol) and 1*H*-tetrazole (5 mg, 0.07 mmol) in anhydrous methylene chloride (3 mL) was added 2-cyanoethyl tetraisopropylphosphorodiamidite (0.028 mL, 0.085 mmol). The reaction was stirred for 1.5 h, after which time the solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica, eluting with 4% methanol in methylene chloride containing 0.5% triethylamine, to give **25** (85 mg, 95% yield) as a yellow solid. mp 135 °C; $[\alpha]^{23}_D$ +55.8° (*c* 1.0, CH_2Cl_2); IR (KBr) 3408, 2964, 1625, 1530, 1340; ^{1}H NMR (CD_2Cl_2) δ 9.23 (s, 1H), 8.84 (dd, J = 4.14, 1.66, 1H), 8.76 (d, J = 8.46, 1H), 8.49 (d, J = 8.30, 1H), 7.91 (d, J = 8.87, 1H), 7.61 (dd, J = 8.92, 1.91, 1H), 7.48 (q, J = 8.40, 4.18, 1H), 7.38 (t, J = 7.07, 2H), 7.26 (t, J = 8.47, 4H), 7.19−7.11 (m, 3H), 6.78−6.64 (m, 5H), 5.07−4.93 (m, 1H), 4.24−4.19 (m, 1H), 3.91−3.22 (m, 16H), 3.10 (s, 3H), 3.05 (s, 3H), 2.60−2.57 (m, 4H), 1.34−1.07 (m, 12H); ^{13}C NMR (CD_2Cl_2) δ 159.28, 159.25, 159.22, 159.19, 159.31, 156.22, 155.61, 155.54, 153.89, 153.83, 152.30, 152.26, 149.20, 149.00, 145.86, 145.80, 136.80, 136.66, 130.83, 130.78, 130.67, 130.61, 130.30, 128.93, 128.84, 128.47, 128.43, 128.36, 127.44, 127.36, 123.91, 123.85, 121.54, 118.50, 117.85, 117.79, 113.69, 112.81, 112.61, 112.42, 86.82, 86.78, 85.42, 82.84, 82.60, 75.89, 75.63, 74.92, 74.65, 65.27, 64.82, 59.38, 59.16, 59.01, 58.77, 55.88, 55.84, 55.79, 44.19, 44.02, 41.94, 41.90, 35.65, 29.20, 25.24, 25.15, 25.09, 25.00; HRMS (FAB NBA) *m*/*z* calcd for $C_{54}H_{61}N_{12}O_7P$ (M + H) 1021.4603, found 1021.4647.

General Procedure for the Buchwald−Hartwig Palladium-Catalyzed Cross-Coupling Reaction of 20 with Simple Arylamines. *O*^{6}-Benzyl-8-(2-naphthalenylamino)-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]guanine (26a). 2-Aminonaphthalene (44 mg, 0.303 mmol), $Pd_2(dba)_3$ (14 mg, 0.015 mmol), BINAP (28 mg, 0.0449 mmol), and **18** (100 mg, 0.147 mmol) were placed in an oven-dried flask, and the flask was evacuated for 20 min and then filled with argon. Freshly distilled toluene (3 mL) was degassed by bubbling a stream of argon through the solution for 5 min and then was added to the flask, and the mixture was stirred for 15 min while maintaining an argon atmosphere. Lithium bis(trimethylsilyl)-amide (1.0 M in THF, 0.3 mL, 0.30 mmol) was added, and the reaction was heated to 100 °C for 30 min, and then cooled to room temperature and quenched with saturated sodium bicarbonate (1 mL). The layers were separated, and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with 10% HCl and brine, dried over sodium sulfate, filtered, and evaporated. Purification by flash chromatography on silica, eluting with 18% ethyl acetate in hexanes, gave **26a** (71 mg, 65% yield) as a yellow solid: mp 78−82 °C; $[\alpha]^{23}_D$ −44° (*c* 1.0, $CHCl_3$); IR (KBr) 1680, 1589, 1364, 1038 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.12 (bs, 1H), 7.70−7.22 (m, 14H), 6.15 (dd, J = 7.6, 3.8, 1H), 5.53 (s. 2H), 5.78 (dt, J = 7.5, 7.3, 1H), 4.77 (s, 2H), 4.0−3.8 (m, 3H), 3.10 (m, 1H), 2.44−2.39 (m, 1H), 1.0−0.75 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 158.9, 158.3, 147.5, 183.1, 138.1, 135.0, 130.4, 129.1, 128.8, 127.9, 124.7, 120.6, 114.2, 113.1, 86.0, 84.9, 71.4, 68.6, 62.9, 39.5, 17.8, 17.6, 14.2, 13.5; HRMS (FAB, NBA) *m*/*z* calcd for $C_{39}H_{53}N_6O_5Si_2$ (M + H) 741.3616, found 741.3639.

***O*^{6}-Benzyl-8-(9*H*-fluoren-2-ylamino)-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis-(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]guanine (26b).** Obtained in 54% as a yellow solid: mp 95−97 °C; $[\alpha]^{23}_D$ −24° (*c* 1.0, $CHCl_3$); IR (KBr) 1680, 1591, 1462, 1038 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.9 (bs, 1H), 7.67−7.21 (m, 16H), 6.21 (dd, J = 8.7, 3.2, 1H), 5.58 (s, 2H), 4.79 (dt, J = 7.1, 5.7, 1H), 4.63 (s, 2H), 4.12−3.87 (m, 6H), 3.04 (m, 1H), 2.46 (m, 1H), 1.08−0.69 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 158.9, 158.2, 154.1, 147.7, 145.3, 143.8, 142.4, 139.6, 137.8, 136.7, 129.2, 129.2, 128.8, 127.4, 126.5, 125.6, 120.8, 119.8, 118.1, 115.8, 113.1, 85.9, 84.8, 71.5, 68.5, 62.9, 39.4, 37.86, 18.1, 17.6, 14.2, 13.3; HRMS (FAB, NBA) *m*/*z* calcd for $C_{42}H_{55}N_6O_5Si_2$ (M + H) 779.3772, found 779.3740.

***O*^{6}-Benzyl-8-[(1,1′-biphenyl)-4-ylamino]-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]guanine (26c).** Obtained in 62% yield as a yellow solid: mp 86−91 °C; $[\alpha]^{23}_D$ −28° (*c* 1.0, $CHCl_3$); IR (KBr) 1589, 1560, 1460, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.82−7.24 (m, 18H), 6.21 (dd, J = 6.7, 3.6, 1H),

5.57 (s, 2H), 4.82 (dt, J = 7.3, 6.5, 1H), 4.64 (s, 2H), 4.1 (m, 1H), 4.1−3.9 (m, 2H), 3.0 (m, 1H), 2.47−2.39 (m, 1H), 1.0−0.85 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 157.4, 156.5, 152.3, 145.9, 140.0, 138.0, 136.1, 133.9, 127.8, 126.7, 125.8, 117.7, 111.5, 84.3, 82.9, 70.0, 67.1, 61.4, 37.8, 16.4, 12.3, 11.6; HRMS (FAB, NBA) m/z calcd for $C_{41}H_{55}N_6O_5Si_2$ (M + H) 767.3772, found 767.3776.

***O*^{6}-Benzyl-8-(4-methylphenylamino)-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-d-2′-deoxyribofuranosyl]guanine (26d).** Obtained in a 48% yield as a white solid: mp 82−86 °C; $[\alpha]^{23}_D$ −16° (c 1.0, $CHCl_3$); IR (KBr) 1595, 1560, 1470, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.12 (bs, 1H), 7.49−7.06 (m, 14H), 6.17 (dd, J = 7.5, 4.3, 1H), 5.56 (s, 2H), 4.78 (dt, J = 7.5, 5.8, 1H), 4.62 (s, 2H), 4.1−4.08 (m, 1H), 3.90 (m, 1H), 3.0 (m, 1H), 2.44−2.38 (m, 1H), 1.06−0.88 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 158.6, 157.6, 153.6, 147.6, 137.4, 131.9, 129.8, 128.2, 118.8, 112.8, 85.5, 83.9, 71.6, 68.3, 62.9, 38.9, 21.2, 17.6, 17.3, 13.8, 12.8; HRMS (FAB, NBA) m/z calcd for $C_{36}H_{53}N_6O_5Si_2$ (M + H) 705.3616, found 705.3634.

General Procedure for the Hydrogenolysis of 35. 8-(2-Naphthalenylamino)-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]guanine (27a). A stream of hydrogen gas was bubbled through a solution of **26a** (39 mg, 0.052 mmol) and 10% Pd/C (50 mg, 0.047 mmol) in methanol (10 mL) and tetrahydrofuran (10 mL) for 2 h. The mixture was then stirred at room temperature overnight under 1 atm of hydrogen. The catalyst was removed by suction filtration, and the filtrate evaporated. Purification by flash chromatography on silica, eluting with 5% methanol in methylene chloride, gave **27a** (31 mg, 86% yield) as a yellow solid: mp 178−184 °C; $[\alpha]^{23}_D$ 52° (c 1.0, $CHCl_3$); IR (KBr) 3368, 2945, 2866, 1689, 1360 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.90−7.18 (m, 6H), 6.0 (dd, J = 7.15, 3.8, 1H), 4.66 (m, 3H), 4.0−3.71 (m, 3H), 2.81 (m, 1H), 2.33 (m, 1H), 0.99−0.83 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 158.3, 153.3, 150.9, 145.6, 138.9, 134.6, 129.9, 129.0, 127.9, 127.4, 126.6, 124.1, 120.1, 113.7, 85.3, 83.8, 71.4, 62.7, 39.2, 30.1, 17.4, 13.5, 12.9; HRMS (FAB, NBA) m/z calcd for $C_{32}H_{47}N_6O_5Si_2$ (M + H) 651.3146, found 651.3132.

8-(9*H*-Fluoren-2-ylamino)-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]guanine (27b). Obtained in a 80% yield as a yellow solid: mp 182−188 °C; $[\alpha]^{23}_D$ 15° (c 1.0, $CHCl_3$); IR (KBr) 3371, 2944, 2866, 1684, 1461, 1038 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.67−7.13 (m, 7H), 5.93 (dd, J = 7.8, 3.9, 1H), 4.65 (dt, J = 7.6, 7.4, 1H), 4.0−3.71 (m, 5H), 2.78 (m, 1H), 2.31 (m, 1H), 1.0−0.77 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 153.2, 150.6, 146.0, 143.4, 142.12, 139.7, 136.32, 127.0, 126.1, 125.24, 120.4, 119.5, 118.0, 115.9, 113.3, 85.2, 83.7, 71.1, 62.5, 39.1, 37.4, 30.1, 17.4, 12.9; HRMS (FAB, NBA) m/z calcd for $C_{35}H_{49}N_6O_5Si_2$ (M + H) 689.3303, found 689.3332.

8-[(1,1′-Biphenyl)-4-ylamino]-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]guanine (27c). Obtained in 82% yield as a yellow solid: mp 180−184 °C; $[\alpha]^{23}_D$ 43° (c 1.0, $CHCl_3$); IR (KBr) 3376, 2948, 2868, 1694, 1594, 1038 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.49−7.18 (m, 9H), 6.01 (dd, J = 7.5, 3.7, 1H), 4.67 (m, 3H), 4.01−3.71 (m, 3H), 2.76 (m, 1H), 2.32 (m, 1H), 0.98−0.81 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 158.3, 153.2, 150.8, 145.7, 141.2, 135.0, 129.1, 127.8, 127.0, 119.1, 113.5, 85.2, 83.6, 71.3, 62.5, 39.2, 30.1, 17.7, 17.4, 13.7, 12.8; HRMS (FAB, NBA) m/z calcd for $C_{34}H_{49}N_6O_5Si_2$ (M + H) 677.3303, found 689.3334.

8-(4-Methylphenylamino)-*N*9-[3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-*β*-D-2′-deoxyribofuranosyl]guanine (27d). Obtained in 78% yield as a white solid: mp 179−185 °C; $[\alpha]^{23}_D$ 13° (c 1.0, $CHCl_3$); IR (KBr) 3316, 2944, 2867, 1681, 1564, 1038 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40−7.01 (m, 5H), 6.05 (dd, J = 7.5, 4.6, 1H), 4.70 (dt, J = 7.4, 7.3 1H), 4.03−3.76 (m, 3H), 2.85 (m, 1H), 2.34 (m, 1H), 2.24 (s, 3H), 1.01−0.81 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 158.3, 153.1, 150.9, 146.4, 138.3, 132.2, 129.9, 119.6, 113.2, 85.4, 83.4, 71.6, 62.8, 39.0, 30.1, 21.2, 17.7, 17.4, 13.8, 12.9; HRMS (FAB, NBA) m/z calcd for $C_{29}H_{47}N_6O_5Si_2$ (M + H) 615.3146, found 615.3168.

General Procedure for the Desilylation of 27. 8-(2-Naphthalenylamino)-2′-deoxyguanosine (28a). Using a plastic syringe and needle, pyridinium poly(hydrogen fluoride) (1.0 mL, 70% hydrogen fluoride, ∼35 mmol of fluoride) was added to dry THF (20 mL) containing pyridine (5.0 mL, 62 mmol), giving a stock solution of pyridinium hydrofluoride (∼1.4 N).[65] **27a** (29 mg, 0.045 mmol) was dissolved in the pyridinium hydrofluoride solution (2.0 mL, ∼2.4 mmol of fluoride), and the solution was stirred at room temperature for 24 h. The reaction was then concentrated under reduced pressure and purified by flash chromatography on silica, eluting with 10% methanol in methylene chloride to give **28a** (15 mg, 82% yield) as a light brown solid: mp > 300 °C; $[\alpha]^{23}_D$ −15.7° (c 1.0, $MeOH/CH_2Cl_2$); IR (KBr) 3371, 2961, 1672, 1558, 1384, 1093 cm^{-1}; ^{1}H NMR (DMSO) δ 10.58 (bs, 1H), 8.83 (bs, 1H), 8.32 (d, J = 2.25, 1H), 7.80−7.70 (m, 4H), 7.45 (t, J = 7.9, 1H), 7.30 (t, J = 7.95, 1H), 6.40−6.30 (m, 3H), 6.05 (t, J = 4.7, 1H), 5.32 (d, J = 3.5, 1H), 4.42 (bs, 1H), 3.95 (d, J = 1.5, 1H), 3.78 (bs, 2H), 2.08−2.0 (m, 1H); ^{13}C NMR (DMSO) δ 155.7, 152.8, 149.5, 143.1, 138.5, 133.8, 128.5, 128.1, 127.4, 126.6, 126.3, 123.4, 119.5, 111.7, 87.2, 82.8, 71.3, 61.3; HRMS (FAB, NBA) m/z calcd for $C_{20}H_{21}N_6O_4$ (M + H) 409.1624, found 409.1643.

8-(9*H*-Fluoren-2-ylamino)-2′-deoxyguanosine (28b). Obtained in 77% yield as a light red solid: mp > 300 °C; $[\alpha]^{23}_D$ −14.7° (c 1.0, $MeOH/CH_2Cl_2$); IR (KBr) 3323, 2929, 1679, 1599, 1461, 1085 cm^{-1}; ^{1}H NMR (DMSO) δ 10.6 (bs, 1H), 8.96 (bs, 1H), 8.12 (d, J = 2.25, 1H), 7.86 (m, 1H), 7.65−7.50 (m, 4H), 7.25 (m, 1H), 6.40 (bs, 2H), 6.32 (dd, J = 5.4, 9.5, 1H), 6.05 (s, 1H), 5.32 (d, J = 3.5, 1H), 4.42 (bs, 1H), 4.08 (dd, J = 5.0, 10.1, 1H), 3.93 (d, J = 2.0, 1H), 3.76 (bs, 2H), 2.06−2.0 (m, 1H); ^{13}C NMR (DMSO) δ 193.4, 155.7, 149.5, 144.6, 142.6, 142.0, 135.8, 134.2, 133.4, 128.0, 123.8, 122.5, 121.5, 120.3, 113.1, 112.2, 87.2, 82.8, 71.3, 61.4, 48.6; HRMS (FAB, NBA) m/z calcd for $C_{23}H_{23}N_6O_4$ (M + H) 447.1781, found 447.1784.

8-[(1,1′-Biphenyl)-4-ylamino]-2′-deoxyguanosine (28c). Obtained in 69% yield as a brown solid: mp > 300 °C; $[\alpha]^{23}_D$ 2.7° (c 1.0, $MeOH/CH_2Cl_2$); IR (KBr) 3323, 2929, 1677, 1561, 1486, 1384, 1100 cm^{-1}; ^{1}H NMR (DMSO) δ 10.53 (bs, 1H), 8.75 (bs, 1H), 7.83 (m, 2H), 7.65−7.55 (m, 4H), 7.45−7.40 (t, J = 7.3, 2H), 7.30 (m, 1H), 6.36 (bs, 2H), 6.32 (dd, J = 4.1, 9.8, 1H), 5.96 (t, J = 5.0, 1H), 5.32 (d, J = 3.5, 1H), 4.43 (bs, 1H), 3.93 (d, J = 1.8, 1H), 3.76 (bs, 2H), 2.05−2.0 (m, 1H); ^{13}C NMR (DMSO) δ 156.0, 153.2, 149.8, 143.5, 140.6, 140.4, 132.6, 129.2, 127.1, 126.9, 126.4, 118.1, 112.5, 87.5, 83.2, 71.6, 61.7; HRMS (FAB, NBA) m/z calcd for $C_{22}H_{23}N_6O_4$ (M + H) 435.1781, found 435.1761.

8-[(4-Methylphenyl)amino]-2′-deoxyguanosine (28d). Obtained in 70% yield as a yellow solid: mp > 300 °C; $[\alpha]^{23}_D$ 1.5° (c 1.0, $MeOH/CH_2Cl_2$); IR (KBr) 3323, 2930, 1678, 1581, 1475, 1081 cm^{-1}; ^{1}H NMR (DMSO) δ 10.52 (bs, 1H), 8.59 (bs, 1H), 7.60 (d, J = 8.5, 2H), 7.05 (d, J = 8.5, 1H), 6.38−6.28 (m, 3H), 5.89 (bs, 1H), 5.31 (bs, 1H), 4.42 (d, J = 5.7, 1H), 3.90 (d, J = 1.9, 1H), 3.76 (bs, 2H), 2.05−1.95 (m, 1H); ^{13}C NMR (DMSO) δ 155.9, 153.1, 149.8, 143.9, 138.6, 129.7, 129.3, 117.8, 87.5, 83.2, 71.6, 61.6, 20.7; HRMS (FAB, NBA) m/z calcd for $C_{17}H_{21}N_6O_4$ (M + H) 373.1624, found 373.1626.

Synthesis of 5′-d(GGCA-G^{IQ}-GTGGTG) (29). The IQ-adducted oligonucleotides were synthesized using the phenoxyacetyl-protected dA, 4-isopropyl-phenoxyacetal-protected dG, acetyl-protected dC, and T phosphoramidites on an Expedite 8909 DNA synthesizer (PerSeptive Biosystems) on a 1 μmol scale using the UltraMild line of phosphoramidites and solid supports from Glen Research. The manufacturer's standard synthesis protocol was followed except at the incorporation of the modified phosphoramidite (**25**), which was accomplished manually off-line. At this point, the column was removed from the instrument and sealed with two syringes, one which contained 250 μL of the manufacturer's 1*H*-tetrazole activator solution (1.9−4.0% in acetonitrile) and the other which contained 250 μL of **25** (25 mg, 0.098

(65) Trost, B. M.; Caldwell, C. G.; Murayama, E.; Heissler, D. *J. Org. Chem.* **1983**, *48*, 3252−3265.

M in anhydrous methylene chloride). The 1*H*-tetrazole and **25** were sequentially drawn through the column, and this procedure was repeated periodically over 30−60 min. After this time, the column was washed with anhydrous manufacturer's grade acetonitrile and returned to the instrument to proceed with capping, oxidation, detritylation, and the remainder of the synthesis. The oligonucleotide was purified by HPLC using 0.1 M ammonium formate (solvent 1) and methanol (solvent 2) on a C-18 reversed-phase column with UV detection. The solvent gradient was as follows: initially 99% solvent 1, then a 40 min linear gradient to 50% solvent 1; 5 min isochratic at 50% solvent 1, then a 5 min linear gradient to the initial conditions. The yield of **29** was 9.0 units. MALDI-TOF MS (HPA) *m*/*z* calcd for (M − H) 3647.76, found 3647.22

Synthesis of 5′-d(CTCGGC-G^{IQ}-CCATC) (30). This synthesis followed the same procedure as for **29**, with a difference in amounts of modified phosphoramidite and tetrazole solution, where only 150 μL of the modified phosphoramidite was used (15 mg, 0.098 M in anhydrous methylene chloride) and 200 μL of the manufacturer's 1*H*-tetrazole/acetonitrile activator solution was used. The synthesis and purification proceeded as described above to give 5.5 units of **30**. MALDI-TOF MS (HPA) *m*/*z* calcd for (M − H) 3776.58, found 3776.38

Enzymatic Digestion of Oligonucleotides. Enzymatic digestion of IQ-modified oligonucleotides was performed by treatment of purified oligonucleotides (0.2 units) in a reaction buffer (pH 7, 20 μL, 0.1 M Tris HCl, 10 mM $MgCl_2$) with 4 μL of Nuclease P1 stirred for 4 h at 37 °C. This was followed by the addition of 20 μL of pH 9 buffer solution, 3 μL of alkaline phosphatase, and 5 μL of snake venom phosphodiesterase, and the reaction was stirred for overnight. The reaction mixtures were filtered and injected directly on HPLC: 90% buffer initially, 15 min linear gradient to 70% buffer, 10 min linear gradient to 25% buffer, 10 min isochratic gradient at 25% buffer, then 5 min linear gradient to initial conditions.

Thermal Melting Studies. Equal amounts of the adducted and the complementary strands (0.3 units each) were dissolved in 0.5 mL of buffer (100 mM NaCl, 0.05 mM EDTA, and 10 mM phosphate buffer, pH 7.1). The UV absorption at 260 nm was monitored as a function of temperature. The temperature was increased at a rate of 1 °C/min from 5 to 90 °C. The melting temperatures of the native and modified oligonucleotides were calculated by determining the first derivative of the melting curve.

Circular Dichroism Measurements. CD measurements were carried out with the same solutions as the T_m studies and at 23 °C. Samples were scanned from 410 to 220.0 nm at 0.5 nm intervals averaged over 1 s in a 300 μL strain-free quartz cuvette. The corresponding raw CD spectra were subtracted from a blank, converted to molar ellipticity, and processed using the provided manufacturer's software package.

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Supporting Information Available: C^1H, ^{13}C, and ^{31}P NMR spectra of all new compounds. MALDI-TOF spectra, HPLC traces of enzyme digests, CZE, and T_m spectra of adducted oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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