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Synthesis of Site-Specific Damaged DNA Strands by 8-(Acetylarylamino)-2′-deoxyguanosine Adducts and Effects on Various DNA Polymerases

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Beside the predominately found 8-(arylamino)-2′-dG, 8-(acetylarylamino) damages within DNA-strands may also play an important role in the induction of chemical carcinogenesis. A synthesis pathway leading to these 8-(acetylarylamino)-dG adducts using different aromatic amines has been optimized. The 8-modified dGs were converted into the corresponding phosphoramidites and site-specifically incorporated into different oligonucleotides leading to DNA strands. Lesion-bearing hybrids of these damaged DNA-strands with complementary oligonucleotides were used to study their melting properties and their circular dichroism spectra. It was shown that no *EcoRI* restriction took place with the damage inside the cleavage site. Finally, three different DNA polymerases were used for primer extension studies.

Introduction

Polycyclic aromatic amines, such as 2-aminofluorene, 2-(acetylamino)fluorene (2-AAF), 4-aminobiphenyl and 4-(acetylamino)biphenyl are well-known chemical carcinogens.[1–6] These chemicals are metabolically activated in cells to give highly-reactive arylnitrenium-ions that react with cellular DNA to form covalent adducts predominantly at the 8-position of 2′-deoxyguanosine (dG) residues by means of an electrophilic amination reaction. The aromatic amines form two major DNA adducts, the 8-(arylamino)-2′-dG (**1**) and the 8-(acetylarylamino)-2′-dG (**2**) (Figure 1). In contrast to the polycyclic aromatic amines, their monocyclic counterparts are less toxic and thus they were classified as borderline carcinogens. However, aniline derivatives are ubiquitously present in the environment originating from many sources.[7–10] Interestingly, monocyclic aromatic amines are metabolized through the same enzymatic pathways and give the same type of DNA damage relative to the polycyclic aromatic amines, particularly in the case of donor-substituted derivatives. Recently, owing to the ubiquitous presence of anilines, the potential human carcinogenicity of these compounds has regained interest.[11] Although these two adducts differ only by an *N*-acetyl group, they have very different physicochemical properties and biological effects.

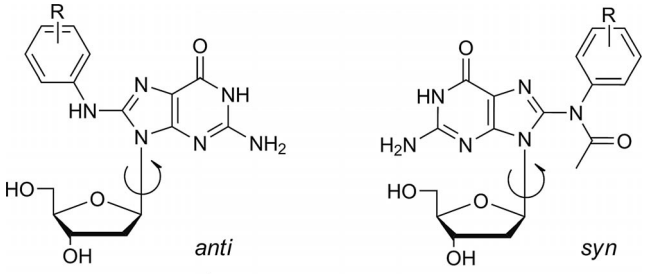

Figure 1. Chemical structure of 8-(arylamino)-2′-dG (**1**) and 8-(acetylarylamino)-2′-dG (**2**).

The acetylated form causes a much more pronounced local distortion within the DNA double-helix than the non-acetylated form.[5,12] This was correlated with a conformational difference of these two adducts. Whereas 8-*N*H-2′-dG adducts (**1**) preferred the *anti*-conformation of the glycosidic bond placing the aromatic moiety in the major groove of the double helix and thus not influencing the hydrogen-bridge interaction within the DNA double helix, 8-(acetylarylamino)-2′-dG adducts (**2**) preferred the *syn*-conformation that places the aromatic system within the double helix and displaces the heterocyclic nucleobase from the helix into the major groove resulting in loss of hydrogen-bond stabilization.[13,14] It has been reported that the 2-AAF adduct is a more potent blocker of replication and transcription; however, it is also a better substrate for DNA-repair enzymes.[15,16]

Zhou and Romano reported on the synthesis of 8-(arylamino)-2′-deoxyguanosine phosphoramidite reagents of 2-aminofluorene and its *N*-acetyl counterpart for the site-specific synthesis of oligonucleotide strands containing these adducts. As a protecting group for the exocyclic amino

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function of dG, the fluorenylmethyloxycarbonyl (Fmoc) group was used.[17,18]

A strategy to prepare the 8-(acetylarylamino)-dG adducts was introduced by Gillet and Schärer in 2005.[19,20] They reported the successful conversion of *N*-acetylated adducts into corresponding phosphoramidites (12 steps overall) and their site-specific incorporation into DNA-oligonucleotides. It was proven that the *N*-acetyl group was not cleaved during the final deprotection.

In 2006, we published the synthesis and the site-specific incorporation of 8-(arylamino)-modified 2′-dG-phosphoramidites into oligonucleotides.[21] Also, we reported on the first synthesis of 8-arylamino-2′-dA adducts and their successful conversion into the corresponding phosphoramidites as well as their site-specific incorporation into an oligonucleotide.[22] This work was recently extended to a comparative study between 8-(arylamino)- and 8-(acetylarylamino)-2′-deoxyadenosine-bearing oligonucleotides.[23] Later, we reported a simple route for the non-optimized synthesis of the 8-(acetylarylamino)-2′-dG adducts and their phosphoramidites.[24] At that time the prepared amidites were not used for the synthesis of oligonucleotides.[24] Here, we report an efficient synthesis of 8-(acetylarylamino)-2′-dG adducts, an improved synthesis of 3′-phosphoramidites, and their use in automated solid-phase DNA synthesis to give site-specific-modified oligonucleotides of several mixed sequences containing multiple dGs. In addition to CD- and T_m measurements, the influence of these adducts on the cleavage of a lesion-bearing DNA duplex by the endonuclease *EcoRI* relative to the 8-(arylamino)-dG adducts was investigated. Moreover, template 20-mer DNA-oligonucleotides bearing *N*-8-(acetylarylamino)-dG lesions were prepared and studied with regard to the primer extension by three different DNA polymerases.

Results and Discussion

Synthesis of 8-(Acetylarylamino)-2′-deoxyguanosine [8-(Ac-aa)-2′-dG] Adducts

As summarized in Scheme 1, the hydroxyl groups of 2′-dG **3** were protected using *tert*-butyldimethylsilyl (TBDMS) chloride. The O^6-position was activated with (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) giving intermediate **4**.[24,25] The key step of the sequence is the reaction between O^6-(1*H*-benzotriazol-1-yl)-2′-dG **4** and *N*-acetyl-hydroxamic acids **6a**–**f** that were prepared by partial reduction of nitrobenzenes **5a**–**f** to give *N*-arylhydroxylamines followed by *N*-acetylation with acetyl chloride.[24,26,27] The target compounds 8-(acetylarylamino) adducts **7a**–**f** were isolated in 70–95% yield. For the conversion of **7a**–**f** into the corresponding phosphoramidites **11a**–**f**, the hydroxy groups were first deprotected. Owing to the introduced *N*-acetyl group, the removal of the TBDMS groups had to be done under mild acid conditions. In contrast to our previous report in which NEt_3–3HF and a different chromatographic purification procedure was used,[24] tetra-*n*-butylammonium fluoride buffered with acetic acid led to an efficient cleavage of the TBDMS groups without loss of the *N*-acetyl group and gave compounds **8a**–**f** in very good yields.

At this step, the *syn*- or *anti*-preference of the glycosidic bond for the acetylated and 8-(arylamino)-dG adducts was studied by NOESY-NMR spectroscopy. As described earlier for the corresponding 8-(acetylarylamino)deoxyadenosine adducts (*syn*),[23] the conformational preferences for the *N*-acetylated dG adducts was also found to be the *syn*-conformation. In contrast, both the 8-*N*H-arylamine-2′-deoxyguanosine and -2′-deoxyadenosine adducts showed a preferred *anti*-conformation.

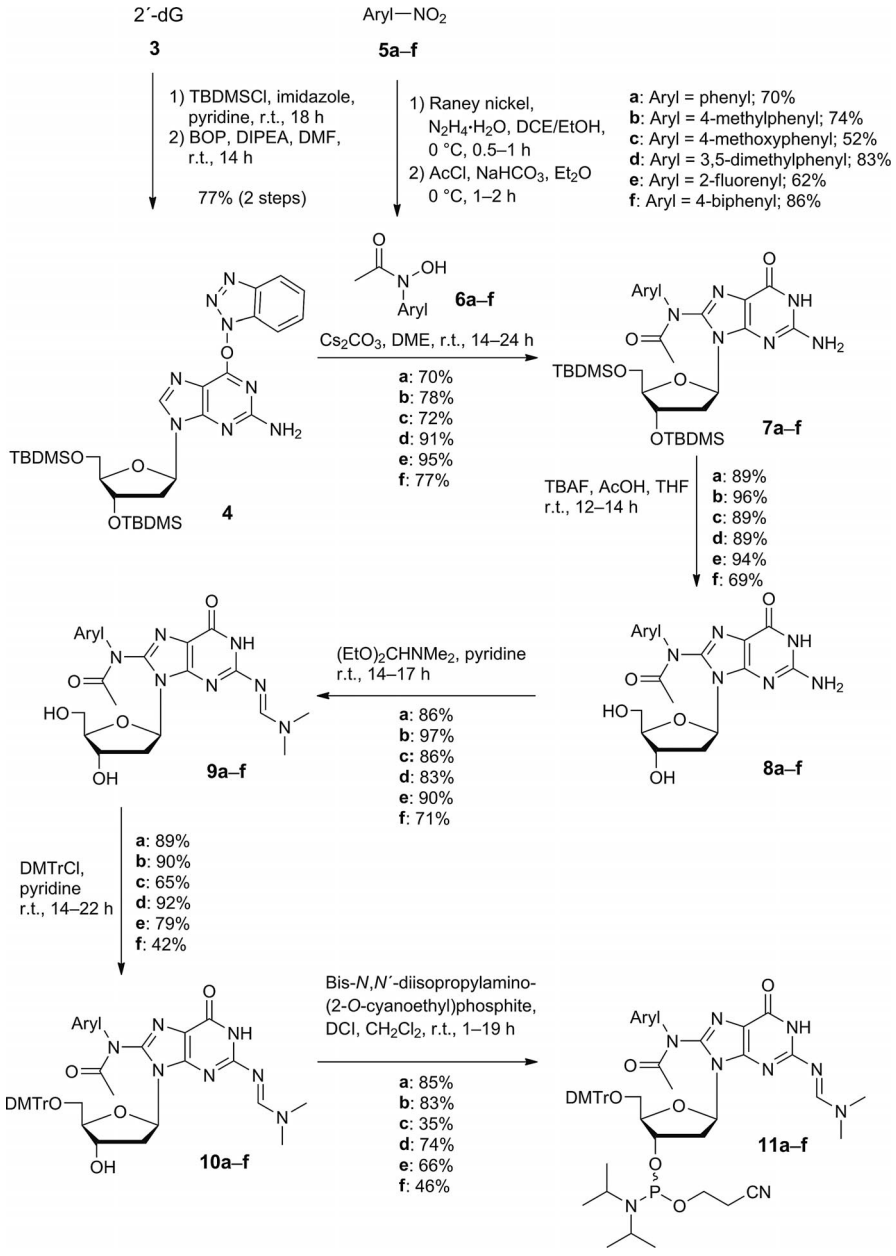
For the oligonucleotide synthesis, the N^2-position was selectively blocked using the formamidine group (mild deprotection strategy). The formamidine group has been used previously and we have recently used this group successfully for 8-*N*H-arylamine-2′-dG oligonucleotides as well.[21,28] After N^2-protection, the 5′-hydroxy function was blocked with a 4,4′-dimethoxytrityl group (DMTr) and corresponding phosphoramidites **11a**–**f** were synthesized using previously described conditions.[22,29] It is worth mentioning that after conversion of compounds **9** to give DMTr-protected nucleosides **10** the purification was also optimized leading to markedly higher chemical yields.[24] For all *N*-acetylated compounds, line broadening in the ^{1}H NMR spectra at room temperature was observed owing to the partial double-bond character of the *N*-acetyl group at the 8-position (see also Supporting Information).[22]

Site-Specific Synthesis of Oligonucleotides Containing 8-(*N*-Ac-aa)-2′-dG Adducts of Different Arylamines

To incorporate the 8-(acetylarylamino)-dG lesions into the oligonucleotides, phosphoramidites **11a**–**f** were dissolved in acetonitrile (0.1 M solutions) and the coupling step of the lesion-bearing-2′-dG phosphoramidite building blocks were repeated three times (every 500 s) with a total coupling efficiency of 70–90%. For the non-modified nucleosides commercially available phosphoramidites were used.

Three different site-specifically modified oligonucleotide-sequences were prepared: *Nar*I oligonucleotide [5′-d(CTC GGC **G**CC ATC)-3′], a palindromic 12mer-*EcoRI* oligonucleotide [5′-d(**G**TA **G**AA TTC TAC)-3′] as well as a 20mer-oligonucleotide [5′-d(ACA T**G**A GCA TCT ACG ACG CG)-3′] needed for DNA polymerase catalyzed primer extension assays.

The final basic deprotection step with concentrated ammonia was completed within 2 h at 45 °C. To avoid oxidative side reactions during the deprotection as observed before in the case of oligonucleotides bearing 8-aminofluorene-dG adduct, β-mercaptoethanol was always added to the deprotection solution.[30–32] The obtained oligonucleotides were purified by reversed-phase HPLC and characterized by ESI-mass spectrometry (neg. mode).



Scheme 1. Synthesis of the 8-(acetylarylamino)-2′-deoxyadenosine adducts and their phosphoramidites.

Melting Temperature (T_m) and Circular Dichroism Studies

Hybrids of the oligonucleotides bearing the 8-(acetylarylamino)-dG adducts as opposed to the 8-(arylamino)-dG adducts at two different positions with the complementary strands were formed and the effect on the thermal stability of these hybrid strands was measured by UV melting-temperature analysis. The data for *Nar*I-oligonucleotides **12a**–**i** is given in Table 1.

For 8-(arylamino)-damaged 2′-dG oligonucleotides **12b**, **12d**, **12f** and **12h** a decrease of approximately 7–9 °C was observed relative to the T_m value of reference *Nar*I-oligonucleotide **12a** (T_m = 58.2 °C). However, for the correspond-

Table 1. T_m values of *Nar*I-oligonucleotides **12a**–**i**.[a]

Oligonucleotide	T_m [°C]
5′-d(CTC GGC GCC ATC) –3′ **12a**	58.2
5′-d[CTC **G(phenyl)**GC GCC ATC] –3′ **12b**	50.3
5′-d[CTC **G(Ac-phenyl)**GC GCC ATC] –3′ **12c**	41.9
5′-d[CTC **G(2-fluorenyl)**GC GCC ATC] –3′ **12d**	49.8
5′-d[CTC **G(Ac-2-fluorenyl)**GC GCC ATC] –3′ **12e**	51.5
5′-d[CTC GGC **G(phenyl)**CC ATC] –3′ **12f**	49.2
5′-d[CTC GGC **G(Ac-phenyl)**CC ATC] –3′ **12g**	44.1
5′-d[CTC GGC **G(2-fluorenyl)**CC ATC] –3′ **12h**	48.8
5′-d[CTC GGC **G(Ac-2-fluorenyl)**CC ATC] –3′ **12i**	44.7

[a] Conditions: 1 nmol *Nar*I-oligonucleotide, 1 nmol complementary oligonucleotide strand, 10 mM phosphate-buffer pH 7.2, 140 mM NaCl, 1.0 mM ethylenediaminetetraacetic (EDTA).

ing 8-(acetylarylamino)-lesions, a decrease in thermal stability of approximately 13–16 °C relative to unmodified *Nar*I-oligonucleotide **12a** was determined. The decrease of thermal stability was nearly independent on the position of the lesion within the oligonucleotide. Surprisingly, for the oligonucleotide bearing 8-[acetyl(2-fluorenyl)amino]-2′-dG lesion **12e** a decrease of only 7 °C in thermal stability was observed. Maybe the distortion within the double helix is partly compensated for by the large, polynuclear aromatic system of the fluorenyl residue.

For the palindromic *EcoRI*-oligonucleotides, the effect on the T_m values was expected to be more pronounced because, after duplex formation, one lesion is present in each strand. Again, the lesions were introduced at two different positions within the oligonucleotide. The first lesion was at the site of cleavage of the restriction endonuclease (4^{th} base from the 5′ end) and the second was at the 5′ terminus, thus outside the restriction region. The T_m values are summarized in Table 2.

Table 2. T_m values of *EcoRI*-oligonucleotides **13a**–**s**.[a]

Oligonucleotide	T_m [°C]
5′-d(GTA GAA TTC TAC) –3′ **13a**	42.1
5′-d[GTA **G(phenyl)**AA TTC TAC] –3′ **13b**	29.5[28]
5′-d[GTA **G(Ac-phenyl)**AA TTC TAC] –3′ **13c**	11.9
5′-d[GTA **G(4-methylphenyl)**AA TTC TAC] –3′ **13d**	23.2[33]
5′-d[GTA **G(Ac-4-methylphenyl)**AA TTC TAC] –3′ **13e**	13.4
5′-d[GTA **G(4-methoxyphenyl)**AA TTC TAC] –3′ **13f**	25.5[33]
5′-d[GTA **G(Ac-4-methoxyphenyl)**AA TTC TAC] –3′ **13g**	11.0
5′-d[GTA **G(3,5-dimethylphenyl)**AA TTC TAC] –3′ **13h**	24.0[33]
5′-d[GTA **G(Ac-3,5-dimethylphenyl)**AA TTC TAC] –3′ **13i**	8.2
5′-d[GTA **G(2-fluorenyl)**AA TTC TAC] –3′ **13j**	23.3[33]
5′-d[GTA **G(Ac-2-fluorenyl)**AA TTC TAC] –3′ **13k**	11.4
5′-d[GTA **G(4-biphenyl)**AA TTC TAC] –3′ **13l**	23.9[33]
5′-d[GTA **G(Ac-4-biphenyl)**AA TTC TAC] –3′ **13m**	7.9
5′-d[**G(phenyl)**TA GAA TTC TAC] –3′ **13n**	34.5
5′-d[**G(Ac-phenyl)**TA GAA TTC TAC] –3′ **13o**	23.8
5′-d[**G(2-fluorenyl)**TA GAA TTC TAC] –3′ **13p**	32.5
5′-d[**G(Ac-2-fluorenyl)**TA GAA TTC TAC] –3′ **13q**	27.0
5′-d[**G(biphenyl)**TA GAA TTC TAC] –3′ **13r**	30.9[33]
5′-d[**G(Ac-biphenyl)**TA GAA TTC TAC] –3′ **13s**	26.2

[a] Conditions: 2 nmol *EcoRI*-oligonucleotide, 10 mM phosphate-buffer pH 7.2, 140 mM NaCl, 1.0 mM EDTA.

A significant decrease in the thermal stabilities of dG-lesion-bearing oligonucleotides **13b**–**s** (T_m values decreased by 8–34 °C) relative to reference oligonucleotides **13a** (T_m = 42.1 °C) was observed. Regarding the 8-(arylamino)-dG lesions, there was a marked thermal destabilization of > 28 °C if 8-(acetylarylamino)-lesions **13c**, **13e**, **13g**, **13i**, **13k**, and **13m** were present within the restriction site relative to non-modified reference oligonucleotide **13a**. However, no significant difference between the oligonucleotide damaged by monocyclic aromatic amines and those damaged by polycyclic aromatic amines was detected. On the other hand, a smaller decrease in thermal stability was observed when the lesions were introduced at the 5′ end of the oligonucleotide. Here, acetylated monocyclic DNA-damaged **13o** led to a decrease of 18 °C (T_m = 23.8 °C), whereas the DNA damage caused by the polycyclic aromatic residue in **13s** has a smaller influence (T_m = 26.2 °C). The T_m values of the *N*H adducts showed the opposite effect. The highest influence in thermal stability was observed with the strong carcinogen biphenylamine (in **13r**), whereas the borderline carcinogen aniline (in **13n**) showed a less significant decrease in thermal stability. The hybridization data for 20mer-oligonucleotides **14a**–**m** is summarized in Table 3.

The double strand of unmodified reference 20mer-oligonucleotide **14a** showed a thermal stability of 68.5 °C. A decrease in the thermal stabilities of dG-adduct-bearing oligonucleotides **14b**–**m** (T_m values decreased by 2–13 °C) relative to the reference oligonucleotide was observed. For 8-(arylamino)- or 8-(acetylarylamino)-dG oligonucleotides **14h**–**m**, modified with strong carcinogens, only a small decrease of the T_m value was observed relative to reference oligonucleotide **14a**. In contrast, the oligonucleotides damaged by acetylated borderline carcinogens **14c** and **14e** showed higher destabilization of the duplex stability, which suggests a larger local distortion caused by the *N*-acetylated dG-lesions.

In addition, the circular dichroism (CD) spectra were measured for all oligonucleotides in their hybridized form. CD spectra were recorded to prove the overall *B*-type-DNA conformation of the lesion-containing DNA hybrids and the unmodified reference duplex. For *EcoRI*-sequence

Table 3. T_m values of 20mer-oligonucleotides **14a**–**m**.[a]

Oligonucleotide	T_m [°C]
5′-d(ACA TGA GCA TCT ACG ACG CG) –3′ **14a**	68.5
5′-d[ACA T**G(phenyl)**A GCA TCT ACG ACG CG] –3′ **14b**	63.1
5′-d[ACA T**G(Ac-phenyl)**A GCA TCT ACG ACG CG] –3′ **14c**	56.3
5′-d[ACA T**G(4-methylphenyl)**A GCA TCT ACG ACG CG] –3′ **14d**	61.0
5′-d[ACA T**G(Ac-4-methylphenyl)**A GCA TCT ACG ACG CG] –3′ **14e**	55.4
5′-d[ACA T**G(4-methoxyphenyl)**A GCA TCT ACG ACG CG] –3′ **14f**	61.1
5′-d[ACA T**G(Ac-4-methoxyphenyl)**A GCA TCT ACG ACG CG] –3′ **14g**	66.6
5′-d[ACA T**G(3,5-dimethylphenyl)**A GCA TCT ACG ACG CG] –3′ **14h**	62.3
5′-d[ACA T**G(Ac-3,5-dimethylphenyl)**A GCA TCT ACG ACG CG] –3′ **14i**	64.7
5′-d[ACA T**G(2-fluorenyl)**A GCA TCT ACG ACG CG] –3′ **14j**	63.4
5′-d[ACA T**G(Ac-2-fluorenyl)**A GCA TCT ACG ACG CG] –3′ **14k**	62.7
5′-d[ACA T**G(biphenyl)**A GCA TCT ACG ACG CG] –3′ **14l**	64.0
5′-d[ACA T**G(Ac-biphenyl)**A GCA TCT ACG ACG CG] –3′ **14m**	63.5

[a] Conditions: 1 nmol 20mer-oligonucleotide, 1 nmol complementary oligonucleotide strand, 10 mM phosphate-buffer pH 7.2, 140 mM NaCl, 1.0 mM EDTA.

13n–q no conformational difference between the unmodified oligonucleotide, the acetylated and non-acetylated modified oligonucleotides was observed and in all cases a positive Cotton-effect was observed at 278 nm and a negative one, which is less pronounced, at 250 nm typically found for *B*-type-DNA structures (Figure 2).

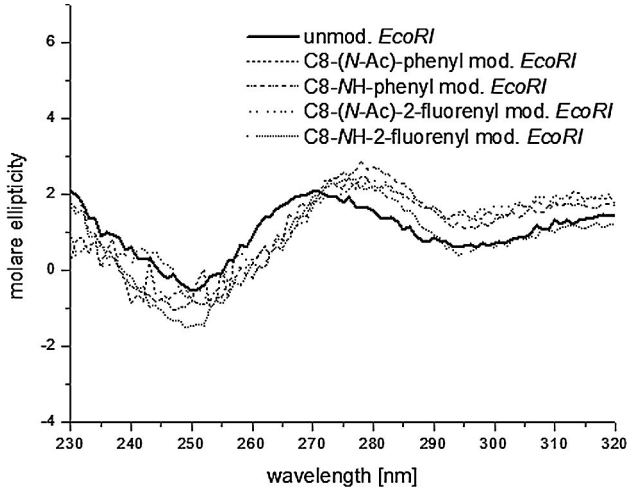


Figure 2. CD spectra of *EcoRI*-oligonucleotides **13a**,**n**–**q**.

The shifts of the maxima in the CD spectra of modified oligonucleotides **13n**–**q** to higher wavelength are caused by the (partly-) conjugated aromatic systems. In addition, no overall conformational difference was observed for the *Nar*I-oligonucleotides and the 20*mer* duplexes.

EcoRI Restriction Assay

To study the effect on *EcoRI* endonuclease restriction of 8-(acetylarylamino)- or 8-(arylamino)-dG modified oligonucleotides a previously used restriction assay was performed.[33] As a control we have shown that the enzyme *EcoRI* cleaved self-complementary, reference 12mer-oligonucleotide **13a** [5′-d(GTA GAA TTC TAC)-3′] into a 4*mer*-(GTAG) and an 8*mer*-strand (AATTCTAC) using identical assay conditions. For oligonucleotide **13a**, a half-life of 2.5 h at 23 °C was determined. The cleavage rates were calculated as described previously.[34]

By using oligonucleotides **13b**–**m**, no restriction of any of these lesion-bearing oligonucleotides was detected. As an example, incubation of **13m** [modification within the *EcoRI*-cleavage site: (acetylamino)biphenyl] for 24 h, as shown in Figure 3, clearly proves that no conversion of the original oligonucleotide occurred. Thus, the 8-arylamino- and 8-(acetylarylamino)-dG lesions within the cleavage site caused a conformational distortion to such an extent that the enzyme is unable to bind and/or cleave the DNA double-strand. A modification introduced by a mono- or polycyclic aromatic amine outside the cleavage site generally led to cleavage of the hybrid although usually an increase in the half-life for the restriction assay was detected. For polycyclic 8-arylamino-dG damage, a half-life of 3.4 h was found whereas damage caused by a monocyclic arylamine led surprisingly to a higher half-life (6.5 h) and lower cleavage rates. In contrast, the cleavage of *N*-acetylated aniline lesion **13o** showed the same half-life as the reference hybrid whereas *N*-acetylated dG-lesions **13p** and **13s** showed opposite and markedly higher stability against restriction although the damage was not placed within the cleavage site. In the latter cases, it seems that the lesions introduced by polycyclic arylamines **13p** and **13s** caused more significant structural changes, which may result in weaker binding of

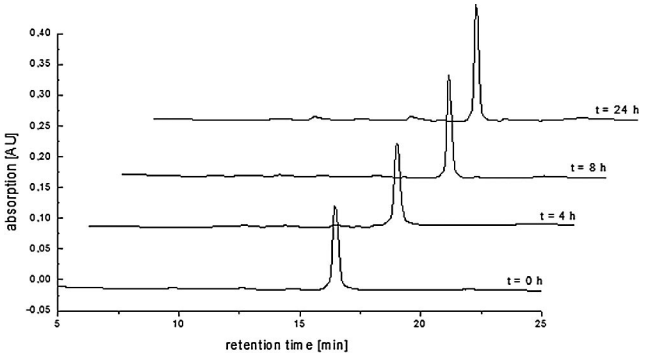


Figure 3. HPLC-chromatogram of the *EcoRI* assay with **13m** (see Table 4; details are given in the Exp. Section).

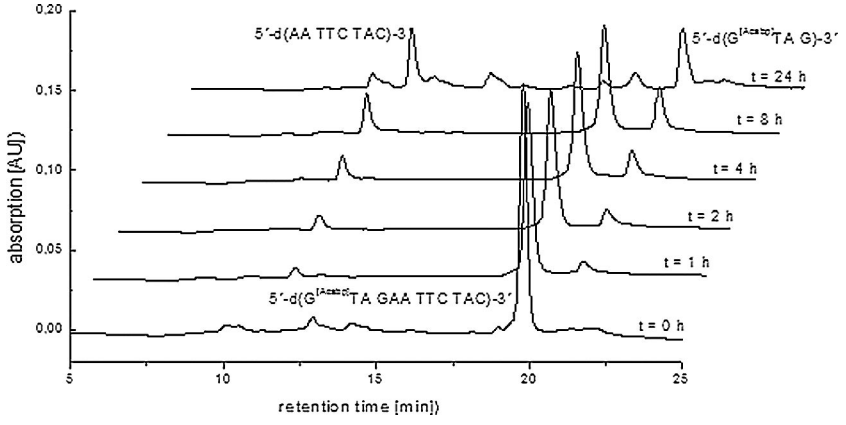


Figure 4. HPLC-chromatogram of the *EcoRI* restriction assay with **13s** (details are given in the Exp. Section).

the enzyme or less efficient recognition of the palindromic hexamer (see **13r** and **13s**; Figure 4). In Table 4 the calculated half-lives are summarized. All values were calculated according to a first-order reaction ($t_{1/2} = \ln 2/k$).

Table 4. Cleavage of damaged oligonucleotides **13** by nuclease *EcoRI*.

Oligonucleotides	*t*1/2
5′-GTAGAATTCTAC-3′ **13a**	2.5
5′-d[GTA **G(phenyl)**AA TTC TAC] –3′ **13b**	no restriction
5′-d[GTA **G(Ac-phenyl)**AA TTC TAC] –3′ **13c**	no restriction
5′-d[GTA **G(2-fluorenyl)**AA TTC TAC] –3′ **13j**	no restriction
5′-d[GTA **G(Ac-2-fluorenyl)**AA TTC TAC] –3′ **13k**	no restriction
5′-d[GTA **G(4-biphenyl)**AA TTC TAC] –3′ **13l**	no restriction
5′-d[GTA **G(Ac-4-biphenyl)**AA TTC TAC] –3′ **13m**	no restriction
5′-d[**G(phenyl)**TA GAA TTC TAC] –3′ **13n**	6.5
5′-d[**G(Ac-phenyl)**TA GAA TTC TAC] –3′ **13o**	2.5
5′-d[**G(Ac-2-fluorenyl)**TA GAA TTC TAC] –3′ **13p**	4.4
5′-d[**G(biphenyl)**TA GAA TTC TAC] –3′ **13r**	3.4
5′-d[**G(Ac-biphenyl)**TA GAA TTC TAC] –3′ **13s**	5.6

Primer Extension Assay

As investigations of other DNA adducts have shown, covalent DNA modifications significantly hamper the selectivity and efficiency of DNA synthesis by replicative DNA polymerases, whereas other DNA polymerases are effective in performing lesion bypass beyond the site of damage.[35] Thus, three DNA polymerases from different DNA polymerase families were used and studied for their effectiveness in bypassing the 8-(acetylarylamino)-dG lesions. The experiments were performed using standing-start conditions with a ^{32}P-labeled primer/template complex (Figure 5, a and b). Single incorporations were examined to gain insight into the impact of the lesion on selectivity, whereas additional experiments employing all four dNTPs were used to study the capability of a lesion bypass.

First, Pyrococcus furiosus (Pfu) DNA polymerase was studied, which is a replicative DNA polymerase belonging to sequence family B. In all cases of the 8-*N*Ac-or the *N*H-arylamine-dG lesions, no incorporation of the canonical or the non-canonical nucleotides was observed proving the high fidelity of DNA polymerase Pfu. This result confirmed well the general opinion that non-acetylated and acetylated 8-arylamine-dG adducts often lead to a slowing or stopping of the replication process.[36,37]

Next, the highly processive, thermostable DNA polymerase *FIREPol*®, which is a member of the family A DNA polymerases and often serves for a wide range of PCR assays was studied. The incorporation of a nucleotide opposite the DNA-lesion was successful and the canonical dCTP was preferably chosen in all cases. However, in the case of the *N*H-phenyl-dG lesion also misinsertion of the non-canonical dATP was observed. Even more striking was that for the *N*Ac-phenyl-dG lesion only the misinsertion of the non-canonical dATP was observed.

If all 4 dNTPs were present, complete elongation to the full-length product (20 nt) was detected in the reference experiment. In contrast, only in the case of *N*Ac-phenyl- **14c** the full-length oligonucleotide was formed, whereas for *N*H-phenyl-dG lesion **14b** the full-length oligonucleotide as well as a one or two nucleotide shorter product was observed in addition to a strong kinetic hold after the nucleotide incorporation opposite the lesion. For both bulky aminobiphenyl lesions **14l** and **14m**, dCTP incorporation was observed exclusively but no elongation took place.

As a member of the X family DNA polymerase involved in DNA repair, we investigated human DNA polymerase β.[38] For the 8-*N*Ac-4-biphenyl- and the 8-*N*H-4-biphenyl lesions the canonical nucleotide dCMP was incorporated predominantly opposite to the modified dG, whereas in cases of the two aniline-lesions again incorporation of the non-canonical dAMP was observed to some extent. In the

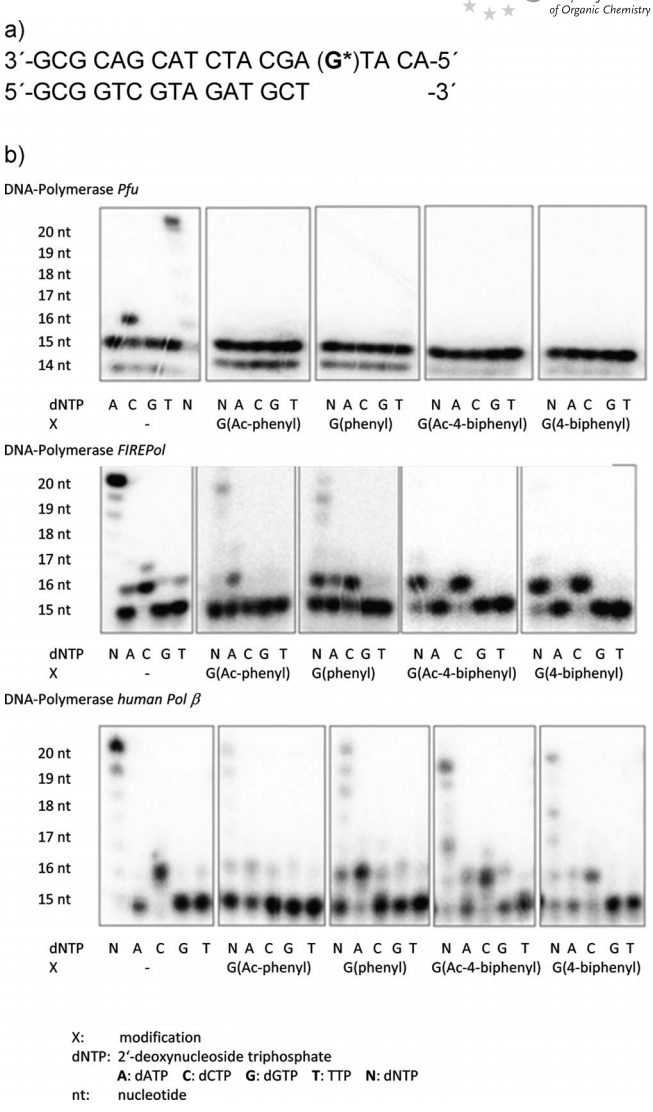


Figure 5. Effect of 8-*N*H- and 8-acetylarylamine-2′-dG lesions on the primer extension by different DNA polymerases. (a) Used duplex for the primer extension assays in which G* points to the site of modification. In the reference strand at this position an unmodified dG is present. (b) Gels obtained from the different primer extension assays using three different DNA polymerases (details are given in the experimental part).

presence of all four dNTPs, complete elongation was observed as expected. Interestingly, in the case of the lesion-containing oligonucleotides beside the full-length product a product that was one, two or more nucleotides shorter was observed. The DNA synthesis proceeded with less efficiency relative to the unmodified reference.

The ability to misincorporate a nucleotide significantly depended on the modification used as well as the modified nucleobase. The use of a 20mer sequence with damaged dGs and three polymerases showed, with the *Pfu* DNA polymerase, predominantly no incorporation of any nucleotide owing to the high-fidelity of this polymerase. In these experiments human DNA polymerase β was able to bypass the dG lesions and predominantly insert the canonical and also the non-canonical nucleotide opposite the lesion. The *FIREPol*® polymerase mainly showed the incorporation of the canonical nucleotide opposite the dG lesions. Surprisingly, in the case of the *N*Ac-phenyl modification, a selective insertion of the non-canonical dA was observed. In our study the strong differences observed for family Y polymerases (e.g. yeast DNA polymerase η) for the acetylated and the non-acetylated DNA lesions could not be observed. In those studies the bulky 2-(acetylamino)fluorene lesion led to a complete stop in elongation, whereas in the case of the 2-aminofluorene lesion a slowing of the replication process but a full elongation was observed.[39] In the case of the *N*-acetylaniline lesion a full elongation was observed without significant slowing of the replication.[40] This might be related to the family of the polymerase that was used in those cases.

Conclusions

In this report, a modified experimental procedure for the synthesis of 8-(acetylarylamino)-dG phosphoramidites is described. These phosphoramidites were site-specifically incorporated into three different oligonucleotides. For the *Nar*I- and the self-complementary *EcoRI* sequence both the thermal stabilities and CD-spectra were determined. In all cases a marked decrease in the T_m values was detected for the lesion-bearing DNA strands suggesting significant distortion of the double helix and resulting in lower hybrid stability. In addition, the decrease was even stronger for the 8-(acetylarylamino) damage relative to the 8-(arylamino) lesions. If this observation can be correlated to the detected difference in *syn*/*anti*-preference of the glycosidic bond of the dG adducts has to be proven in the future. Nevertheless, no difference in the CD-spectra between the compounds modified by an acetylated aromatic amine and those modified by a non-acetylated aromatic amine were measured. The lesion-bearing oligonucleotides were found to be resistant to digestion by *EcoRI* if the modification was present at the cleavage site of the *EcoRI* enzyme. This indicates a marked local distortion of the DNA-hybrid at the modification site that either blocks the cleavage of the double strand or prevents a binding of the restriction enzyme. This effect was identical for all studied 8-(acetylarylamino)-dG damages. Previously, also 8-(arylamino) adducts of dG or dA showed the same behavior. However, irrespective of whether monocyclic or polycyclic arylamine DNA damages were investigated, incorporation of the lesion outside to the *EcoRI* cleavage site only led to slower enzymatic cleavage. Surprisingly, polycyclic acetylated DNA damages were found to have a stronger influence than the corresponding monocyclic acetylated DNA damages. Finally, it was shown that the ability to bypass a lesion within the DNA strand depended strongly on the investigated DNA polymerase. In most cases, the fidelity of the nucleotide incorporation opposite the dG-lesion was not hampered. Surprisingly, only the borderline carcinogen aniline showed a different effect on the replication. With two polymerases, a marked incorporation of dATP beside the canonical GTP was detected leading to a point mutation. The reason for this dC→dA-misincorporation is still unknown. Further work to gain insights into the caused structural changes within the DNA-duplex by the 8-(arylamino)- and 8-(acetylarylamino) adducts is currently underway in our laboratories.

Experimental Section

General Methods: All reactions were performed in glassware dried with a heat gun and under a nitrogen atmosphere, except the syntheses of the hydroxylamines and hydroxamic acids. Commercial solvents and reagents were used without further purification with the following exceptions: pyridine, dichloromethane, and acetonitrile were distilled from calcium hydride; Dimethyl ether (DME) and diethyl ether were dried with potassium and distilled under nitrogen. Water was purified on a Milli-Q water system. ^{1}H NMR and ^{13}C NMR spectra were recorded on AMX 400 MHz or DMX 500 MHz Bruker spectrometer. NMR spectra were reported relative to the NMR solvent peaks [^{1}H NMR: δ = 2.50 (dimethyl sulfoxide; [D_6]DMSO) and 7.16 (C_6D_6). ^{13}C NMR: δ = 39.52 ([D_6]-DMSO) and 128.06 (C_6D_6)]. HRMS analysis was performed with an analytical VG/70-250 F using FAB or Agilent Technologies, 6224 TOF LC/MS1200 spectrometer using ESI. IR spectra were recorded on a Bruker Alpha P spectrophotometer operating in the attenuated total reflection mode. Melting points were measured with an apotec capillary melting point apparatus. Optical rotations were measured on a Perkin–Elmer polarimeter by using a sodium lamp operating at 598 nm and are reported as specific rotation. Thin-layer chromatography was performed on aluminum sheets coated with silica gel 60 F_{254} from Merck.

The synthesis of 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine and O^6-(1*H*-benzotriazolyl)-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine were performed as described previously.[21,33]

General Procedure I. Arylhydroxylamines 5a–f: In a flask nitroaryl compound (1 equiv.) was dissolved in ethanol/dichloroethane (1:1). Raney nickel was added and the mixture was cooled to 0 °C. After the addition of hydrazine hydrate (6.5 equiv.), the solvent was stirred for 30–60 min and filtered. The solvent was removed in vacuo and the product was recrystallized (toluene). Compounds **5** were isolated in 75–94% yields. Analytical data were identical to those reported before.

General Procedure II. *N*-Aryl-*N*-acetylhydroxamic Acids 6a–f: In a flask, *N*-arylhydroxylamine (1 equiv.) and saturated $NaHCO_3$ solution were suspended in Et_2O and stirred at 0 °C. After the addition

of AcCl (1.1 equiv.), the mixture was stirred at r.t. for 1 h. The layers were separated and the organic layer was extracted with 1 M NaOH (3×). Then the aqueous layer was extracted with Et_2O (3×). 3 M HCl was added until the aqueous layer was acidic and extracted with Et_2O (3×). The combined organic layers were dried (Na_2SO_4) and the solvent removed in vacuo. Compounds **6** were isolated in 55–98% yields. Analytical data were identical to those reported before.[7,24]

General Procedure III. 8-(Acetylarylamine) Adducts 7a–f: In a dried flask compound **4** (1 equiv.), Cs_2CO_3 (2 equiv.) and *N*-arylacetylhydroxamic acid **6a–f** (2 equiv.) were suspended in anhydrous DME and stirred under a N_2 atmosphere at room temperature. When the reaction was complete the solvent was removed in vacuo. The product was isolated by column chromatography (5% CH_3OH/CH_2Cl_2).

8-(Acetylphenylamino)-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (7a): Following general procedure III, **4** (5.00 g, 8.17 mmol) and **6a** (10.2 g, 16.2 mmol) gave product **7a** as a slightly brown solid (3.58 g, 5.69 mmol, 70%). Analytical data were identical to those reported.[24]

8-[Acetyl(4-methylphenyl)amino]-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (7b): Following general procedure III, **4** (4.01 g, 6.55 mmol) and **6b** (2.16 g, 13.1 mmol) gave product **7b** as a slightly brown solid (3.29 g, 5.12 mmol, 78%), m.p. 173–175 °C. $[a]_D^{20}$ = +84 (*c* = 0.64, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3151, 2928, 2856, 1703, 1631, 1602, 1542, 1509, 1463, 1434, 1365, 1317, 1285, 1251, 1184, 1080, 1029, 956, 833, 812, 775, 729, 681, 601, 542, 506, 401 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 10.82 (br. s, 1 H), 7.29–7.11 (m, 4 H), 6.39 (s, 2 H), 5.99–5.97 (m, 1 H), 4.53–4.46 (m, 1 H), 3.84–3.61 (m, 3 H), 3.17–3.16 (m, 1 H), 2.29 (s, 3 H), 2.10–2.08 (m, 1 H), 1.99 (s, 3 H), 0.86 (s, 9 H), 0.81 (s, 9 H), 0.08 (s, 3 H), 0.06 (s, 3 H), –0.04 (s, 6 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]-DMSO): δ = 169.2, 156.2, 152.3, 138.3, 129.1, 128.9, 117.2, 113.1, 87.3, 87.0, 72.3, 63.1, 37.9, 25.7, 25.6, 20.5, 17.5, –4.8, –4.9, –5.4, –5.5 ppm. HRMS (FAB): calcd. for $C_{31}H_{50}N_6O_5Si_2$ [M + H] 643.3381; found 643.3454.

8-[Acetyl(4-methoxyphenyl)amino]-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (7c): Following general procedure III, **4** (845 mg, 1.38 mmol) and **6c** (500 mg, 2.76 mmol) gave product **7c** as a slightly brown solid (649 mg, 0.99 mmol, 72%), m.p. 155–157 °C. $[a]_D^{20}$ = +52 (*c* = 0.22, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3145, 2952, 2928, 2855, 1702, 1632, 1606, 1569, 1541, 1508, 1462, 1435, 1365, 1316, 1287, 1246, 1181, 1105, 1081, 1060, 1029, 1006, 947, 830, 775, 731, 712, 682, 665, 626, 600, 552, 528, 402 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 10.82 (br. s, 1 H), 7.55–7.10 (m, 2 H), 6.94 (br. s, 2 H), 6.35 (s, 2 H), 6.01 (s, 1 H), 4.54 (s, 1 H), 3.88–3.60 (m, 8 H), 1.97 (s, 3 H), 0.87 (s, 9 H), 0.81 (s, 9 H), 0.09 (br. s, 6 H), 0.04 (s, 6 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 176.2, 156.2, 153.1, 140.7, 131.0, 120.8, 115.0, 114.2, 87.3, 83.4, 72.3, 63.2, 55.3, 31.4, 25.7, 25.6, 23.7, 17.9, 17.6, –5.4, –5.5 ppm. HRMS (FAB): calcd. for $C_{31}H_{50}N_6O_6Si_2$ [M + H] 659.3330; found 659.3402.

8-[Acetyl(3,5-dimethylphenyl)amino]-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (7d): Following general procedure III, **4** (5.01 g, 8.18 mmol) and **6d** (2.96 g, 16.5 mmol) gave product **7d** as a slightly orange solid (4.88 g, 7.44 mmol, 91%). Analytical data were identical to those reported.[24]

8-[Acetyl(2-fluorenyl)amino]-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (7e): Following general procedure III, **4** (5.01 g, 8.18 mmol) and **6e** (3.89 g, 16.2 mmol) gave product **7e** as a slightly brown solid (4.88 g, 7.77 mmol, 95%). Analytical data were identical to those reported.[24]

8-[Acetyl(4-biphenylyl)amino]-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (7f): Following general procedure III, **4** (2.43 g, 3.97 mmol) and **6f** (1.80 g, 7.93 mmol) gave product **7f** as a slightly brown solid (2.14 g, 3.04 mmol, 77%), m.p. 232–236 °C. $[a]_D^{20}$ = +79 (*c* = 0.53, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3668, 3312, 3150, 2954, 2928, 2857, 1987, 1928, 1701, 1682, 1636, 1594, 1573, 1511, 1471, 1448, 1339, 1284, 1253, 1190, 1103, 1078, 1027, 1007, 970, 945, 881, 693, 632, 576, 555, 532, 502, 475, 464, 430, 403 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 10.84 (s, 1 H), 7.71–7.67 (m, 4 H), 7.67–7.61 (m, 2 H), 7.51–7.42 (m, 2 H), 7.41–7.32 (m, 1 H), 6.40 (s, 2 H), 5.91–6.13 (m, 1 H), 4.69–4.42 (m, 1 H), 3.87–3.56 (m, 3 H), 3.16 (m, 2 H), 2.05 (s, 3 H), 0.85 (s, 9 H), 0.79 (s, 9 H), 0.08 (s, 6 H), –0.05 (s, 6 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 169.5, 156.2, 139.5, 129.0, 127.6, 126.6, 126.4, 124.9, 87.3, 83.3, 72.2, 63.3, 36.0, 25.7, 25.6, 22.8, 5.4, 5.5, –4.8, –5.3 ppm. HRMS (FAB): calcd. for $C_{36}H_{52}N_6O_5Si_2$ [M + H] 705.3538; found 705.3612.

General Procedure IV. Desilylation of C8-*N*-acetylarylamine Adducts 8a–f: Compound **7a–f** was dissolved in tetrahydrofuran (THF), then TBAF in THF (6 equiv.) and AcOH (12 equiv.) were added simultaneously. When the reaction was complete the solvent was removed in vacuo. The product was isolated by column chromatography (10% CH_3OH/CH_2Cl_2).

8-(Acetylphenylamino)-2′-deoxyguanosine (8a): Following general procedure IV, **7a** (1.00 g, 1.50 mmol) gave product **8a** as a yellow solid (568 mg, 1.42 mmol, 89%). Analytical data were identical to those reported.[24]

8-[Acetyl(4-methylphenyl)amino]-2′-deoxyguanosine (8b): Following general procedure IV, **7b** (3.28 g, 5.11 mmol) gave product **8b** as a yellow solid (2.04 g, 4.93 mmol, 96%), m.p. 174–175 °C. $[a]_D^{20}$ = +84 (*c* = 0.64, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3340, 3231, 1684, 1647, 1601, 1568, 1540, 1510, 1474, 1396, 1369, 1320, 1286, 1176, 1102, 1038, 786, 712, 645, 581, 538, 474 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]-DMSO): δ = 11.51 (br. s, 1 H), 7.44–7.23 (m, 4 H), 6.73 (br. s, 2 H), 6.06–6.01 (m, 1 H), 5.31 (s, 1 H), 4.43–4.33 (m, 1 H), 3.92 (s, 1 H), 3.82–3.80 (m, 1 H), 3.66–3.48 (m, 3 H), 2.37–2.34 (m, 1 H), 2.30 (s, 3 H), 1.99 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 176.4, 156.3, 153.9, 149.1, 128.8, 125.1, 120.0, 88.1, 82.5, 71.2, 62.2, 48.6, 20.5, 20.2 ppm. HRMS (FAB): calcd. for $C_{19}H_{22}N_6O_5$ [M + H] 415.4652; found 415.1722.

8-[Acetyl(4-methoxyphenyl)amino]-2′-deoxyguanosine (8c): Following general procedure IV, **7c** (2.25 g, 3.41 mmol) gave product **8c** as a white solid (1.31 g, 3.04 mmol, 89%), m.p. 155–156 °C. $[a]_D^{20}$ = +65 (*c* = 0.45, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3317, 3155, 2931, 1676, 1630, 1596, 1540, 1507, 1438, 1399, 1366, 1320, 1285, 1246, 1171, 1099, 1084, 1053, 1028, 987, 965, 946, 835, 814, 782, 728, 686, 600, 583, 542, 452 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 10.85 (br. s, 1 H), 7.54–7.16 (m, 2 H), 6.98 (br. s, 2 H), 6.47 (br. s, 2 H), 6.07 (s, 1 H), 5.21 (br. s, 1 H), 4.94 (br. s, 1 H), 4.37 (s, 1 H), 3.83 (br. s, 1 H), 3.76 (s, 3 H), 3.70–3.58 (m, 1 H), 3.57–3.46 (m, 1 H), 3.19–3.13 (m, 2 H), 2.00 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]-DMSO): δ = 172.7, 156.2, 153.5, 138.5, 128.0, 114.9, 125.0, 88.2, 85.2, 71.2, 62.1, 57.5, 23.1, 21.2 ppm. HRMS (FAB): calcd. for $C_{19}H_{22}N_6O_6$ [M + H] 431.1601; found 431.1664.

8-[Acetyl(3,5-dimethylphenyl)amino]-2′-deoxyguanosine (8d): Following general procedure IV, **7d** (3.06 g, 4.66 mmol) gave product **8d** as a white solid (1.77 g, 4.13 mmol, 89%), m.p. 193–196 °C. $[a]_D^{20}$ = +5 (*c* = 0.5, CH_2Cl_2/CH_3OH, 1:1; v/v). IR (neat): $\tilde{\nu}$ = 3325, 3148, 2923, 2874, 1677, 1632, 1592, 1539, 1434, 1398, 1366, 1303, 1280, 1260, 1100, 1084, 1053, 989, 966, 852, 835, 781, 715, 691, 674, 600, 561, 542, 450, 434, 403 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]-DMSO): δ = 10.82 (br. s, 1 H), 6.96 (br. s, 3 H), 6.49 (br. s, 2 H),

6.10–5.98 (m, 1 H), 5.03 (br. s, 2 H), 4.38 (s, 1 H), 3.83 (s, 1 H), 3.65 (dd, J_{HH} = 11.5, J_{HH} = 4.8 Hz, 1 H), 3.65 (dd, J_{HH} = 11.7, J_{HH} = 5.1 Hz, 1 H), 3.20–3.11 (m, 2 H), 2.27 (s, 6 H), 2.00 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 156.2, 153.5, 142.0, 88.1, 84.0, 83.9, 71.1, 62.1, 48.6, 22.5, 22.4, 20.8 ppm. HRMS (FAB): calcd. for $C_{20}H_{24}N_6O_5$ [M + H] 429.1808; found 429.1477.

8-[Acetyl)(2-fluorenyl)amino]-2′-deoxyguanosine (8e): Following general procedure IV, **7e** (1.92 g, 2.69 mmol) gave product **8e** as a slightly brown solid (1.25 g, 2.54 mmol, 94%), m.p. 198–19 °C. $[\alpha]_D^{20}$ = +4.3 (c = 0.5, CH_2Cl_2/CH_3OH; 1:1; v/v). IR (neat): $\tilde{\nu}$ = 3431, 3070, 2976, 2939, 2739, 2679, 2492, 1691, 1605, 1475, 1434, 1399, 1170, 1038, 739, 482 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.86 (s, 1 H), 7.94–7.89 (m, 2 H), 7.59–7.58 (m, 2 H), 7.40–7.31 (m, 3 H), 6.44 (s, 2 H), 6.13 (s, 1 H), 5.23 (s, 1 H), 4.97 (s, 1 H), 4.40 (s, 1 H), 3.95 (s, 2 H), 3.85 (s, 1 H), 3.65–3.53 (m, 2 H), 3.17 (d, J_{HH} = 5.1 Hz, 2 H), 2.06 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 161.8, 156.2, 153.4, 150.3, 143.3, 140.1, 127.0, 126.8, 125.1, 120.5, 120.2, 115.1, 88.1, 84.0, 71.1, 62.1, 40.6, 36.4, 22.7 ppm. HRMS (FAB): calcd. for $C_{25}H_{24}N_6O_5$ [M + H] 489.1808; found 489.1873.

8-[Acetyl(4-biphenylyl)amino]-2′-deoxyguanosine (8f): Following general procedure IV, **7f** (1.40 g, 1.99 mmol) gave product **8f** as a yellow solid (1.31 g, 1.37 mmol, 69%), m.p. 90–92 °C. $[\alpha]_D^{20}$ = +48 (c = 1.05, CH_2Cl_2/CH_3OH; 1:1; v/v). IR (neat): $\tilde{\nu}$ = 3675, 3304, 2962, 2927, 2001, 1897, 1685, 1635, 1594, 1539, 1513, 1486, 1448, 1367, 1322, 1281, 1101, 1080, 1054, 1007, 965, 850, 765, 732, 693, 601, 442, 387 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.73 (br. s, 1 H), 7.75–7.70 (m, 4 H), 7.69–7.65 (m, 2 H), 7.49–7.44 (m, 2 H), 7.40–7.34 (m, 1 H), 6.84 (s, 2 H), 6.10 (m, 1 H), 5.19 (br. s, 1 H), 4.80 (br. s, 1 H), 4.38 (s, 1 H), 3.83 (s, 1 H), 3.64 (dd, J_{HH} = 10.9, J_{HH} = 4.0 Hz, 1 H), 3.55–3.45 (m, 1 H), 2.97 (s, 1 H), 2.06 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 173.2, 156.6, 154.1, 150.4, 139.2, 129.0, 127.7, 127.5, 126.7, 125.5, 88.2, 84.0, 71.2, 62.1, 36.9, 22.3 ppm. HRMS (FAB): calcd. for $C_{24}H_{24}N_6O_5$ [M + H] 477.1808; found 477.1886.

General Procedure V. N^2-Formamidation of 8-(Acetylarylamine) Adducts 9a–f: In a dried flask, **8a**–**f** (1 equiv.) and *N*,*N*-dimethylformamide diethyl acetal (2 equiv.) were suspended in anhydrous pyridine and stirred under a N_2 atmosphere at room temp. When the reaction was complete the solvent was removed in vacuo. The product was isolated by column chromatography (20% CH_3OH/CH_2Cl_2).

8-(Acetylphenylamino)-N^2-[(dimethylamino)methylene]-2′-deoxyguanosine (9a): Following general procedure V, **8a** (2.04 g, 5.09 mmol) gave product **9a** as a yellow solid (1.99 g, 4.37 mmol, 86%). Analytical data were identical to those reported.[24]

8-[Acetyl(4-methylphenyl)amino]-N^2-[(dimethylamino)methylene]-2′-deoxyguanosine (9b): Following general procedure V, **8b** (2.58 g, 6.23 mmol) gave product **9b** as a yellow solid (2.83 g, 6.04 mmol, 97%), m.p. 191–193 °C. $[\alpha]_D^{20}$ = +81 (c = 0.48, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3231, 2921, 1680, 1629, 1524, 1507, 1422, 1353, 1320, 1283, 1180, 1112, 1052, 987, 968, 786, 727, 515 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.53 (br. s, 1 H), 8.48 (s, 1 H), 7.40–7.26 (m, 4 H), 6.13–6.12 (m, 1 H), 5.31–5.30 (m, 1 H), 4.88 (s, 1 H), 4.48–4.43 (m, 1 H), 3.87–3.83 (m, 1 H), 3.68–3.66 (m, 1 H), 3.57–3.56 (m, 1 H), 3.14 (s, 3 H), 3.04–3.01 (m, 4 H), 2.31 (s, 3 H), 2.15–2.13 (m, 1 H), 2.03 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]-DMSO): δ = 174.2, 158.3, 157.1, 149.1, 129.3, 128.6, 127.6, 113.2, 87.9, 83.9, 70.9, 61.9, 40.8, 34.6, 20.5, 18.8 ppm. HRMS (FAB): calcd. for $C_{22}H_{27}N_7O_5$ [M + H] 470.2074; found 470.2152.

8-[Acetyl(4-methoxyphenyl)amino]-N^2-[(dimethylamino)methylene]-2′-deoxyguanosine (9c): Following general procedure V, **8c** (1.30 g, 3.02 mmol) gave product **9c** as a yellow solid (1.26 g, 2.60 mmol, 86%), m.p. 150–152 °C. $[\alpha]_D^{20}$ = +87 (c = 0.5, CH_2Cl_2/CH_3OH; 1:1; v/v). IR (neat): $\tilde{\nu}$ = 3234, 2930, 1676, 1628, 1524, 1505, 1421, 1353, 1320, 1283, 1243, 1170, 1108, 1052, 1026, 986, 967, 835, 785, 731, 580, 527, 480 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.48 (br. s, 1 H), 8.47 (s, 1 H), 7.45 (s, 1 H), 7.27 (s, 1 H), 6.98 (s, 2 H), 6.13 (s, 1 H), 5.29 (s, 1 H), 4.90 (br. s, 1 H), 4.56–4.49 (m, 1 H), 3.85 (br. s, 1 H), 3.76 (s, 3 H), 3.70–3.61 (m, 1 H), 3.60–3.50 (m, 1 H), 3.18–3.14 (m, 2 H), 3.13 (s, 3 H), 3.03 (s, 3 H), 2.01 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 168.3, 158.3, 157.1, 140.5, 138.6, 129.2, 128.0, 118.2, 99.5, 87.9, 70.9, 55.4, 48.4, 34.5, 22.3, 19.2 ppm. HRMS (FAB): calcd. for $C_{22}H_{27}N_7O_6$ [M + H] 486.2023; found 486.2089.

8-[Acetyl(3,5-dimethylphenyl)amino]-N^2-[(dimethylamino)methylene]-2′-deoxyguanosine (9d): Following general procedure V, **8d** (1.77 g, 4.13 mmol) gave product **9d** as a yellow solid (1.66 g, 3.43 mmol, 83%), m.p. 176–177 °C. $[\alpha]_D^{20}$ = +44 (c = 0.31, CH_2Cl_2/CH_3OH; 1:1; v/v). IR (neat): $\tilde{\nu}$ = 3307, 2926, 1679, 1629, 1524, 1422, 1353, 1306, 1278, 1180, 1113, 1052, 988, 968, 853, 786, 720, 690, 663, 600, 574, 553, 534, 507, 465 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.52 (s, 1 H), 8.47 (s, 1 H), 7.07–6.98 (m, 3 H), 6.10 (t, J_{HH} = 3.7, J_{HH} = 9.9 Hz, 1 H), 5.29 (d, J_{HH} = 4.7 Hz, 1 H), 4.89–4.86 (m, 1 H), 4.45 (s, 1 H), 3.85–3.84 (m, 1 H), 3.69–3.64 (m, 1 H), 3.58–3.52 (m, 1 H), 3.14 (s, 3 H), 3.03 (s, 3 H), 2.98–2.93 (m, 1 H), 2.27 (s, 6 H), 2.01 (s, 3 H), 1.82–1.81 (m, 1 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 175.6, 158.2, 149.5, 123.8, 123.1, 108.8, 87.8, 87.6, 71.6, 55.2, 40.7, 34.5, 22.4, 20.7, 18.7 ppm. HRMS (FAB): calcd. for $C_{23}H_{29}N_7O_5$ [M + H] 484.2230; found 484.2303.

8-[Acetyl(2-fluorenyl)amino]-N^2-[(dimethylamino)methylene]-2′-deoxyguanosine (9e): Following general procedure V, **8e** (2.05 g, 4.19 mmol) gave product **9e** as a brown solid (2.05 g, 3.77 mmol, 90%). $[\alpha]_D^{20}$ = +59 (c = 0.22, CH_2Cl_2/CH_3OH; 1:1; v/v). IR (neat): $\tilde{\nu}$ = 3425, 2981, 1684, 1631, 1529, 1456, 1425, 1397, 1286, 1174, 1115, 1037, 714, 474 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.53 (s, 1 H), 8.49 (br. s, 1 H), 7.92–7.90 (m, 1 H), 7.80–7.76 (m, 1 H), 7.60–7.58 (m, 1 H), 7.41–7.31 (m, 4 H), 6.19 (br. s, 1 H), 5.39 (br. s, 1 H), 4.92 (br. s, 1 H), 4.51–4.44 (m, 1 H), 3.95 (s, 2 H), 3.87 (s, 1 H), 3.67 (s, 1 H), 3.56 (s, 1 H), 3.17–3.14 (m, 4 H), 3.03 (s, 6 H), 2.23 (s, 1 H), 2.07 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]-DMSO): δ = 187.4, 172.3, 158.3, 157.1, 149.6, 143.4, 140.1, 136.1, 127.1, 126.8, 125.1, 123.9, 120.3, 87.9, 70.9, 61.9, 40.8, 37.5, 36.4, 26.5, 23.0 ppm. HRMS (FAB): calcd. for $C_{28}H_{29}N_7O_5$ [M + H] 544.2230; found 544.2295.

8-[Acetyl(4-biphenylyl)amino]-N^2-[(dimethylamino)methylene]-2′-deoxyguanosine (9f): Following general procedure V, **8f** (1.26 g, 1.31 mmol) gave product **9f** as a brown solid (0.93 g, 0.93 mmol, 71%), m.p. 67–68 °C. $[\alpha]_D^{20}$ = +54 (c = 0.85, CH_2Cl_2/CH_3OH; 1:1; v/v). IR (neat): $\tilde{\nu}$ = 3307, 3055, 3033, 2926, 2871, 2179, 1680, 1629, 1523, 1485, 1422, 1354, 1323, 1283, 1178, 1113, 1054, 1031, 989, 846, 787, 766, 748, 732, 700, 575, 508, 447, 406 cm^{-1}. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.55 (s, 1 H), 8.49 (s, 1 H), 7.79–7.71 (m, 2 H), 7.71–7.64 (m, 2 H), 7.52–7.44 (m, 4 H), 7.41–7.33 (m, 1 H), 6.16 (s, 1 H), 5.30 (s, 1 H), 4.87 (s, 1 H), 4.47 (s, 1 H), 3.85 (s, 1 H), 3.72–3.60 (m, 1 H), 3.61–3.47 (m, 1 H), 3.14 (m, 2 H), 3.04 (s, 6 H), 2.07 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 173.1, 173.0, 158.3, 157.3, 149.6, 128.9, 127.6, 127.5, 126.7, 88.0, 87.9, 70.9, 61.9, 45.7, 40.7, 34.5 ppm. HRMS (FAB): calcd. for $C_{27}H_{29}N_7O_5$ [M + H] 532.2230; found 532.2020.

General Procedure VI. 5′-*O*-Dimethoxytritylation of the 8-(Acetylarylamine) Adducts 10a–f: In a dried flask, **9a**–**f** (1 equiv.) was dis-

solved in anhydrous pyridine under nitrogen atmosphere and 4,4′-dimethoxytrityl chloride (1 equiv.) was added. The mixture was stirred at room temperature until the reaction was complete. The solvent was removed in vacuo. The product was isolated by flash chromatography on Alox (act. III; 5% CH_3OH/CH_2Cl_2).

8-(Acetylphenylamino)-5′-*O*-dimethoxytrityl-*N*²-[(dimethylamino)-methylene]-2′-deoxyguanosine (10a): Following general procedure VI, **9a** (200 mg, 0.44 mmol) gave product **10a** as a slightly brown solid (298 mg, 0.39 mmol, 89%), m.p. 111–113 °C. $[\alpha]_D^{20}$ = +21 (*c* = 0.1, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 2931, 1734, 1653, 1594, 1569, 1491, 1436, 1357, 1286, 1055, 844 cm^{-1}. ^1H NMR (400 MHz, [D₆]DMSO): δ = 11.48 (s, 1 H), 8.19 (s, 1 H), 7.41–7.11 (m, 14 H), 6.74–6.67 (m, 5 H), 6.23–6.17 (m, 1 H), 5.39–5.31 (m, 1 H), 4.62–4.58 (m, 1 H), 3.94–3.89 (m, 1 H), 3.70–3.68 (m, 8 H), 3.29–3.23 (m, 1 H), 3.08–3.01 (m, 1 H), 3.00 (s, 3 H), 2.94 (s, 3 H), 2.04 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D₆]DMSO): δ = 157.8, 157.4, 144.8, 129.4, 129.2, 127.5, 126.4, 112.7, 85.7, 83.0, 70.7, 54.8, 48.5, 34.5, 22.5 ppm. HRMS (FAB): calcd. for $C_{42}H_{43}N_7O_7$ [M + H] 758.3224; found 758.3336.

8-[Acetyl(4-methylphenyl)amino]-5′-*O*-dimethoxytrityl-*N*²-[(dimethylamino)methylene]-2′-deoxyguanosine (10b): Following general procedure VI, **9b** (2.10 g, 4.64 mmol) gave product **10b** as a slightly brown solid (3.22 g, 4.17 mmol, 90%). Analytical data were identical to those reported.[24]

8-[Acetyl(4-methoxyphenyl)amino]-5′-*O*-dimethoxytrityl-*N*²-[(dimethylamino)methylene]-2′-deoxyguanosine (10c): Following general procedure VI, **9c** (1.24 g, 2.55 mmol) gave product **10c** as a slightly brown solid (1.31 g, 1.66 mmol, 65%), m.p. 159–160 °C. $[\alpha]_D^{20}$ = +24 (*c* = 0.1, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3234, 2930, 2836, 1681, 1627, 1607, 1526, 1505, 1463, 1422, 1344, 1322, 1288, 1244, 1173, 1111, 1078, 1027, 986, 827, 786, 754, 726, 701, 581, 527 cm^{-1}. ^1H NMR (400 MHz, [D₆]DMSO): δ = 11.36 (br. s, 1 H), 8.19 (s, 1 H), 7.34–7.22 (m, 9 H), 7.01–6.95 (m, 4 H), 6.78–6.69 (m, 4 H), 6.22–6.16 (m, 1 H), 5.32 (br. s, 1 H), 4.15–4.05 (m, 1 H), 3.97–3.91 (m, 1 H), 3.77 (s, 3 H), 3.74–3.66 (m, 8 H), 3.17 (br. s, 2 H), 3.01 (s, 3 H), 3.00 (s, 3 H), 1.99 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D₆]DMSO): δ = 157.9, 157.7, 157.2, 149.6, 135.6, 129.5, 127.5, 123.9, 112.9, 87.3, 85.2, 55.3, 55.4, 54.9, 40.0, 34.6, 15.3 ppm. HRMS (FAB): calcd. for $C_{43}H_{45}N_7O_8$ [M + H] 788.3330; found 788.3393.

8-[Acetyl(3,5-dimethylphenyl)amino]-5′-*O*-dimethoxytrityl-*N*²-[(dimethylamino)methylene]-2′-deoxyguanosine (10d): Following general procedure VI, **9d** (680 mg, 1.40 mmol) gave product **10d** as a yellow solid (1.01 g, 1.29 mmol, 92%), m.p. 110–112 °C. $[\alpha]_D^{20}$ = +37 (*c* = 0.5, CH_2Cl_2/CH_3OH, 1:1 v/v). IR (neat): $\tilde{\nu}$ = 2915, 1680, 1629, 1609, 1527, 1507, 1423, 1302, 1247, 1175, 1112, 1076, 962, 827, 787, 754, 725, 582, 552 cm^{-1}. ^1H NMR (400 MHz, [D₆]DMSO): δ = 11.39 (br. s, 1 H), 8.18 (s, 1 H), 7.27–6.95 (m, 12 H), 6.70–6.59 (m, 4 H), 6.20 (s,1 H), 5.33 (s, 1 H), 3.97 (s, 1 H), 3.69–3.67 (m, 6 H), 3.33 (s, 1 H), 3.07 (s, 1 H), 3.01 (s, 3 H), 2.94 (s, 4 H), 2.25–2.22 (m, 7 H), 2.04 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D₆]DMSO): δ = 157.8, 157.3, 157.1, 144.9, 135.6, 129.6, 129.5, 129.2, 127.6, 127.5, 127.4, 126.4, 118.1, 112.8, 112.7, 85.1, 83.1, 54.9, 54.8, 48.5, 40.7, 34.5, 20.7 ppm. HRMS (FAB): calcd. for $C_{44}H_{47}N_7O_7$ [M + H] 786.3537; found 786.3606.

8-[Acetyl(2-fluorenyl)amino]-5′-*O*-dimethoxytrityl-*N*²-[(dimethylamino)methylene]-2′-deoxyguanosine (10e): Following general procedure VI, **9e** (2.00 g, 3.77 mmol) gave product **10e** as a slightly brown solid (2.50 g, 2.96 mmol, 79%), m.p. 180–181 °C. $[\alpha]_D^{20}$ = +195 (*c* = 0.3, CH_2Cl_2/CH_3OH, 1:1 v/v). IR (neat): $\tilde{\nu}$ = 2982, 1681, 1628, 1525, 1505, 1454, 1422, 1397, 1285, 1244, 1174, 1111, 1076, 1028, 826, 786, 768, 735, 700, 580 cm^{-1}. ^1H NMR (400 MHz, [D₆]DMSO): δ = 11.48 (s, 1 H), 8.20 (s, 1 H), 7.91–7.87 (m, 2 H), 7.63–7.52 (m, 2 H), 7.37–7.24 (m, 5 H), 7.13–7.12 (m, 7 H), 6.69–6.61 (m, 4 H), 6.28 (br. s, 1 H), 5.74 (s, 1 H), 5.35 (s, 1 H), 4.63 (s, 1 H), 3.96–3.90 (m, 3 H), 3.68 (br. s, 7 H), 3.29–3.27 (m, 1 H), 3.13–3.06 (m, 1 H), 3.01 (br. s, 3 H), 2.95 (s, 3 H), 2.09 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D₆]DMSO): δ = 157.9, 157.8, 157.4, 157.2, 144.8, 144.0, 143.3, 140.1, 135.6, 127.5, 126.8, 126.4, 125.1, 120.2, 112.9, 112.8, 83.2, 54.9, 40.8, 36.4, 34.6 ppm. HRMS (FAB): calcd. for $C_{49}H_{47}N_7O_7$ [M + H] 846.3537; found 846.3593.

8-[Acetyl(4-biphenylyl)amino]-5′-*O*-dimethoxytrityl-*N*²-[(dimethylamino)methylene]-2′-deoxyguanosine (10f): Following general procedure VI, **9f** (0.93 g, 0.93 mmol) gave product **10f** as a yellow solid (600 mg, 0.39 mmol, 42%), m.p. 150–151 °C. $[\alpha]_D^{20}$ = +43 (*c* = 1.1, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3033, 2978, 2945, 2739, 2600, 2530, 2496, 2179, 1682, 1629, 1526, 1507, 1481, 1443, 1423, 1397, 1286, 1247, 1172, 1113, 1072, 1033, 829, 807, 787, 766, 729, 699, 582, 551, 512, 462 cm^{-1}. ^1H NMR (400 MHz, [D₆]DMSO): δ = 11.22 (s, 1 H), 8.10 (s, 1 H), 7.58–7.49 (m, 6 H), 7.36–7.32 (m, 3 H), 7.27–7.24 (m, 3 H), 7.14–7.12 (m, 3 H), 7.06–7.01 (m, 7 H), 6.14–6.10 (m, 1 H), 5.23 (s, 1 H), 4.50 (s, 1 H), 3.82 (s, 1 H), 3.62–3.60 (s, 1 H), 3.56 (s, 6 H), 3.07–3.03 (m, 1 H), 3.02–2.98 (m, 1 H), 2.90 (s, 3 H), 2.84 (s, 3 H), 1.97 (s, 3 H) ppm. ^{13}C NMR (101 MHz, [D₆]DMSO): δ = 180.4, 169.0, 157.9, 157.8, 157.5, 157.2, 144.9, 139.2, 135.6, 129.5, 129.4, 129.0, 127.6, 126.7, 126.5, 125.5, 112.9, 85.8, 85.2, 55.0, 54.9, 48.6, 45.3, 40.8, 34.7 ppm. HRMS (FAB): calcd. for $C_{48}H_{47}N_7O_7$ [M + H] 834.3537; found 834.3607.

General Procedure VII. 3′-*O*-Phosphitylation of the 8-(Acetylarylamine) Adducts 11a–f: In a dried flask, **10a**–**f** (1 equiv.) was dissolved in anhydrous CH_2Cl_2 and acetonitrile (1:1 v/v) under an atmosphere of nitrogen and 4,5-dicyanoimidazole (DCI; 1 equiv.) were added. The reaction mixture was treated with 2-cyanoethyl bis(diisopropylamino)phosphate (1.5 equiv.). The mixture was stirred at room temperature until the reaction was complete. The solvent was removed in vacuo. The product was isolated by flash chromatography on Alox (act. III); 1% CH_3OH/CH_2Cl_2). The product was dissolved in benzene and gave the desired product as a colorless solid after freeze-drying.

2-Cyanoethyl *N*,*N*-Diisopropyl-8-(acetylphenylamino)-5′-*O*-dimethoxytrityl-*N*²-[(dimethylamino)methylene]-2′-deoxyguanosine-3′-phosphoramidite (11a): Following general procedure VII, **10a** (250 mg, 0.33 mmol) gave product **11a** as a white solid (269 mg, 0.28 mmol, 85%). Analytical data were identical to those reported.[24]

Phosphoramidite 11b: Following general procedure VII, **10b** (500 mg, 0.65 mmol) gave product **11b** as a white solid (521 mg, 0.54 mmol, 83%), m.p. 124–126 °C. $[\alpha]_D^{20}$ = +36 (*c* = 0.1, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3056, 2998, 2932, 2835, 1685, 1629, 1606, 1580, 1528, 1506, 1462, 1440, 1423, 1345, 1294, 1244, 1173, 1112, 1078, 1029, 901, 826, 789, 753, 726, 700, 636, 582, 523 cm^{-1}. ^1H NMR (400 MHz, [D₆]benzene): δ = 11.35 (br. s, 1 H), 8.50–8.47 (m, 1 H), 7.78–7.76 (m, 4 H), 7.61–7.59 (m, 4 H), 7.32–7.30 (m, 2 H), 7.25–7.22 (m, 1 H), 7.13–7.11 (m, 2 H), 6.89–6.88 (m, 4 H), 6.55 (br. s, 1 H), 5.43 (br. s, 1 H), 4.56 (br. s, 1 H), 3.83–3.78 (m, 2 H), 3.68 (br. s, 2 H), 3.61–3.59 (m, 7 H), 3.44–3.43 (m, 1 H), 3.05–3.01 (m, 3 H), 2.90–2.88 (m, 3 H), 2.74 (br. s, 3 H), 2.18 (s, 3 H), 1.53–1.49 (m, 4 H), 1.32–1.30 (m, 6 H), 1.25–1.24 (m, 6 H) ppm. ^{13}C NMR (101 MHz, [D₆]benzene): δ = 159.1, 159.0, 158.0, 145.8, 136.4, 136.3, 130.6, 130.4, 129.5, 127.0, 118.9, 116.8, 113.4, 86.7, 58.6, 57.7, 55.0, 54.9, 54.8, 46.4, 43.8, 43.7, 43.5, 35.0, 24.6, 23.9, 21.2, 20.4, 19.9, 13.8 ppm. ^{31}P NMR (162 MHz, [D₆]benzene): δ = 162.8, 162.4 ppm. HRMS (FAB): calcd. for $C_{52}H_{62}N_9O_8P$ [M] 971.4459; found 971.4546.

Phosphoramidite 11c: Following general procedure VII, **10c** (1.29 g, 1.64 mmol) gave product **11c** as a white solid (568 mg, 0.58 mmol, 35%), m.p. 142–143 °C. $[a]_D^{20}$ = +74 (*c* = 0.06, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 2963, 2930, 2836, 1683, 1628, 1607, 1527, 1506, 1462, 1442, 1423, 1345, 1325, 1288, 1246, 1200, 1175, 1112, 1075, 1029, 976, 898, 828, 787, 755, 726, 702, 683, 638, 582, 525 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]benzene): δ = 10.83 (s, 1 H), 8.25 (s, 1 H), 7.44–7.34 (m, 4 H), 7.13–6.55 (m, 13 H), 6.39 (s, 1 H), 5.32–5.15 (m, 1 H), 4.50–4.30 (m, 1 H), 3.68–3.13 (m, 16 H), 1.89 (s, 3 H), 1.16–0.99 (m, 12 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]benzene): δ = 159.1, 146.1, 146.0, 136.5, 136.2, 130.6, 130.4, 129.4, 127.0, 113.5, 113.6, 58.0, 55.0, 54.9, 46.6, 45.3, 43.9, 43.7, 43.6, 34.9, 24.6, 22.8, 19.5, 19.4, 19.1 ppm. ^{31}P NMR (162 MHz, [D$_6$]benzene): δ = 162.6, 162.4 ppm. HRMS (FAB): calcd. for $C_{52}H_{62}N_9O_9P$ [M + Na] 1010.4408; found 1010.4294.

Phosphoramidite 11d: Following general procedure VII, **10d** (370 mg, 0.47 mmol) gave product **11d** as a white solid (346 mg, 0.35 mmol, 74%), m.p. 127–129 °C. $[a]_D^{20}$ = +91 (*c* = 0.1, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3236, 2964, 2930, 2837, 2248, 1682, 1629, 1608, 1527, 1506, 1463, 1443, 1423, 1345, 1303, 1247, 1200, 1175, 1155, 1114, 1066, 1029, 976, 898, 878, 854, 827, 788, 754, 725, 701, 639, 581, 881, 519, 462, 396, 385 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]benzene): δ = 10.77 (s, 1 H), 8.26–8.18 (m, 1 H), 7.13–6.52 (m, 16 H), 6.40 (s, 1 H), 5.32–5.18 (m, 1 H), 4.42 (br. s, 1 H), 3.74–2.91 (m, 16 H), 2.71–2.58 (m, 4 H), 2.52 (s, 3 H), 2.03 (s, 3 H), 1.85–1.68 (m, 4 H), 1.12–0.96 (m, 12 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]benzene): δ = 159.1, 157.8, 145.8, 143.4, 136.5, 136.4, 136.2, 130.7, 130.6, 130.3, 128.6, 127.0, 126.9, 117.6, 113.5, 113.4, 86.7, 86.6, 59.0, 58.9, 57.9, 55.0, 54.9, 43.8, 43.7, 43.6, 40.8, 34.9, 34.8, 24.7, 24.6, 24.5, 24.4, 21.2, 20.1, 20.0 ppm. ^{31}P NMR (162 MHz, [D$_6$]benzene): δ = 162.8, 162.7 ppm. HRMS (FAB): calcd. for $C_{53}H_{64}N_9O_8P$ [M + H] 986.4615; found 986.4681.

Phosphoramidite 11e: Following general procedure VII, **10e** (0.39 mg, 0.46 mmol) gave product **11e** as a white solid (317 mg, 0.30 mmol, 66%), m.p. 124–125 °C. $[a]_D^{20}$ = +19 (*c* = 0.42, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 2964, 1686, 1630, 1526, 1508, 1456, 1424, 1344, 1291, 1249, 1177, 1114, 1076, 1031, 977, 899, 828, 788, 768, 735, 703, 643, 583, 519, 469, 424 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]benzene): δ = 11.33 (s, 1 H), 8.31 (s, 1 H), 7.86–6.66 (m, 20 H), 6.44 (br. s, 1 H), 5.29–5.19 (m, 1 H), 4.41 (br. s, 1 H), 3.64–3.23 (m, 16 H), 2.87–2.81 (m, 1 H), 2.73–2.72 (m, 6 H), 2.55–2.53 (m, 3 H), 1.10–1.01 (m, 12 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]benzene): δ = 159.1, 159.0, 157.9, 157.8, 145.7, 145.6, 144.8, 144.0, 143.5, 141.2, 136.3, 136.2, 130.6, 130.4, 128.6, 128.4, 127.0, 125.3, 120.7, 120.3, 118.5, 117.7, 113.4, 59.0, 58.8, 57.8, 54.9, 46.0, 43.7, 43.5, 40.8, 37.0, 34.9, 24.6, 24.4, 21.2, 20.2, 20.0 ppm. ^{31}P NMR (162 MHz, [D$_6$]-benzene): δ = 147.4, 147.3 ppm. HRMS (FAB): calcd. for $C_{58}H_{64}N_9O_8P$ [M + H] 1046.4615; found 1046.4684.

Phosphoramidite 11f: Following general procedure VII, **10f** (0.56 mg, 0.36 mmol) gave product **11f** as a white solid (186 mg, 0.18 mmol, 46%), m.p. 78–80 °C. $[a]_D^{20}$ = +30 (*c* = 0.11, CH_2Cl_2/CH_3OH). IR (neat): $\tilde{\nu}$ = 2965, 2929, 1685, 1628, 1608, 1526, 1508, 1487, 1462, 1424, 1345, 1326, 1289, 1249, 1177, 1114, 1075, 1031, 978, 898, 828, 788, 764, 728, 700, 584, 551, 516 cm^{-1}. ^1H NMR (400 MHz, [D$_6$]benzene): δ = 11.24 (br. s, 1 H), 8.24 (s, 1 H), 7.73–6.64 (m, 22 H), 6.38 (s, 1 H), 4.38 (s, 1 H), 3.64–3.54 (m, 1 H), 3.37 (s, 3 H), 3.35 (s, 3 H), 3.33–3.14 (m, 2 H), 2.89–2.70 (m, 2 H), 2.58 (dd, J_{HH} = 21.5, J_{HH} = 6.6 Hz, 1 H), 2.47–2.44 (m, 2 H), 2.38 (d, J_{HH} = 7.2 Hz, 1 H), 1.73 (dd, J_{HH} = 13.4, J_{HH} = 5.9 Hz, 1 H), 1.46 (t, J_{HH} = 6.2 Hz, 1 H), 1.15–0.85 (m, 18 H) ppm. ^{13}C NMR (101 MHz, [D$_6$]benzene): δ = 159.0, 145.5, 143.9, 143.2, 136.1, 130.5, 130.3, 125.1, 113.5, 113.3, 83.4, 58.9, 57.7, 54.8, 43.6, 43.4, 40.5, 34.6, 24.5, 24.3, 19.9 ppm. ^{31}P NMR (162 MHz, [D$_6$]-benzene): δ = 162.7, 162.4 ppm. HRMS (FAB): calcd. for $C_{57}H_{64}N_9O_8P$ [M + H] 1034.4615; found 1034.4658.

Synthesis of the Oligonucleotides: Oligonucleotides were synthesized on a 1 μmol scale using bz-protected dA, form-protected dG, bz-protected dC and T phosphoramidites on a 394 DNA synthesizer (Applied Biosystems) using phosphoramidites and solid supports purchased from ChemGenes. The manufacturer's standard synthesis protocol was used, except that at the incorporation position of the modified phosphoramidites the coupling was repeated three times, each for 500 s. The oligonucleotides were purified by HPLC using triethylammonium acetate buffer (pH 6.9; solvent 1) and acetonitrile (solvent 2) on a C-18 reversed-phase column with UV-detection. The solvent gradient was as follows: initially 99% solvent 1, then a 50 min linear gradient to 23% solvent 2; 10 min with 100% solvent 2; 10 min with 100% solvent 1.

HPLC-ESI-MS for **12a**: *m*/*z* calcd. for 3582.4; found 1189.3 (–3)

HPLC-ESI-MS for **12b**: *m*/*z* calcd. for 3673.5; found 1835.7 (–2)

HPLC-ESI-MS for **12c**: *m*/*z* calcd. for 3715.5; found 1856.3 (–2)

HPLC-ESI-MS for **12d**: *m*/*z* calcd. for 3761.5; found 1879.7 (–2)

HPLC-ESI-MS for **12e**: *m*/*z* calcd. for 3803.5; found 1900.4 (–2)

HPLC-ESI-MS for **12f**: *m*/*z* calcd. for 3673.5; found 1835.8 (–2)

HPLC-ESI-MS for **12g**: *m*/*z* calcd. for 3715.5; found 1856.8 (–2)

HPLC-ESI-MS for **12h**: *m*/*z* calcd. for 3761.5; found 1879.8 (–2)

HPLC-ESI-MS for **12i**: *m*/*z* calcd. for 3803.5; found 1900.4 (–2)

HPLC-ESI-MS for **13a**: *m*/*z* calcd. for 3644.5; found 1214.8 (–3)

HPLC-ESI-MS for **13c**: *m*/*z* calcd. for 3777.6; found 1887.4 (–2)

HPLC-ESI-MS for **13e**: *m*/*z* calcd. for 3791.6; found 1894.4 (–2)

HPLC-ESI-MS for **13g**: *m*/*z* calcd. for 3807.6; found 1902.2 (–2)

HPLC-ESI-MS for **13i**: *m*/*z* calcd. for 3805.6; found 1904.4 (–2)

HPLC-ESI-MS for **13k**: *m*/*z* calcd. for 3865.6; found 1931.3 (–2)

HPLC-ESI-MS for **13m**: *m*/*z* calcd. for 3853.6; found 1925.4 (–2)

HPLC-ESI-MS for **13n**: *m*/*z* calcd. for 3735.6; found 1866.8 (–2)

HPLC-ESI-MS for **13o**: *m*/*z* calcd. for 3777.6; found 1887.8 (–2)

HPLC-ESI-MS for **13p**: *m*/*z* calcd. for 3823.6; found 1910.3 (–2)

HPLC-ESI-MS for **13q**: *m*/*z* calcd. for 3865.6; found 1913.3 (–2)

HPLC-ESI-MS for **13s**: *m*/*z* calcd. for 3853.6; found 1925.9 (–2)

HPLC-ESI-MS for **14b**: *m*/*z* calcd. for 6202.1; found 2066.4 (–3)

HPLC-ESI-MS for **14c**: *m*/*z* calcd. for 6244.1; found 2080.4 (–3)

HPLC-ESI-MS for **14d**: *m*/*z* calcd. for 6216.1; found 2071.1 (–3)

HPLC-ESI-MS for **14e**: *m*/*z* calcd. for 6258.1; found 2085.1 (–3)

HPLC-ESI-MS for **14f**: *m*/*z* calcd. for 6232.1; found 2076.4 (–3)

HPLC-ESI-MS for **14g**: *m*/*z* calcd. for 6274.1; found 2090.4 (–3)

HPLC-ESI-MS for **14h**: *m*/*z* calcd. for 6230.1; found 2075.7 (–3)

HPLC-ESI-MS for **14i**: *m*/*z* calcd. for 6272.1; found 2089.4 (–3)

HPLC-ESI-MS for **14j**: *m*/*z* calcd. for 6290.1; found 2095.4 (–3)

HPLC-ESI-MS for **14k**: *m*/*z* calcd. for 6332.1; found 2109.7 (–3)

HPLC-ESI-MS for **14l**: *m*/*z* calcd. for 6278.1; found 2091.44 (–3)

HPLC-ESI-MS for **14m**: *m*/*z* calcd. for 6320.1; found 2105.7 (–3)

Thermal Melting Studies: Equal amounts of the two complementary strands (2 nmol) were dissolved in 1 mL of buffer (10 mM phosphate buffer, 140 mM NaCl, 1 mM EDTA, pH 6.6). The UV absorption at 260 nm was monitored as a function of temperature. The temperature was increased at a rate of 0.5 °C/min over the range 5–80 °C.

Circular Dichroism Measurements: CD measurements were carried out at 10 °C with the same solution as that used for the T_m studies. Samples were scanned from 320 to 220 nm at 0.5 nm intervals averaged over 1 s.

EcoRI Restriction Assay: An amount of 0.4 OD oligonucleotide was dissolved in 100 μL DTT buffer [pH 7.5; $MgCl_2$ (190.4 mg), NaCl (1.17 g), Tris (1.21 g) and DTT (15.4 mg) in water (200 mL)]. The solution was heated to 70 °C for 2 min and then cooled slowly to room temp. After the addition of 270 units of *EcoRI*, the solution was incubated at 23 °C. Aliquots (20 μL) were withdrawn and were analyzed by HPLC using triethylammonium acetate buffer (pH 8.0) containing 5% acetonitrile (solvent 1) and acetonitrile (solvent 2) on a C-18 reversed-phase column with UV detection. The solvent gradient was as follows: initially 99% solvent 1, then a 20 min linear gradient to 25% solvent 2, 5 min with 100% solvent 2, 5 min with 100% solvent 1.

Primer Extension Reactions: 10 μL of the reaction mixture contained 100 nM ^{32}P-labeled primer F15 (5′-CGC GTC GTA GAT GCT-3′), 150 nM of the different templates, 100 μM dNTPs in 1x reaction buffer [for *Pfu* DNA polymerase: 20 mM TrisHCl (pH 8.8), 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% (v/v) Triton X-100, 0.2% (w/v) BSA; for human polymerase β: 5 mM TrisHCl (pH 8.8), 1 mM $MgCl_2$, 0.1 mM DTT, 1 mM KCl, 0.1% (v/v) glycerol; for *Klenow* DNA polymerase: 50 mM TrisHCl (pH 8.0), 1 mM DTT, 5 mM $MgCl_2$]. The final enzyme concentration in the reaction mixture was 10 nM for *Pfu* DNA polymerase, 10 nM for human DNA polymerase β and 10 nM for *Klenow* DNA polymerase. The template sequences used are depicted in Figure 5 were labeled using [γ^{32}P]-ATP according to standard techniques. Annealing of the primer to the template strand was conducted in 20 mM TrisHCl (pH 7.6) and 50 mM NaCl from 95 °C to 25 °C for at least 2 h. Mixtures were incubated for 5–15 min at different temperatures (72 °C, 15 min for *Pfu* DNA polymerase; 37 °C, 15 min for human DNA polymerase β and 37 °C, 5 min for *Klenow* DNA polymerase) and the reactions were stopped by the addition of 45 μL stop solution [80% (v/v) formamide, 20 mM EDTA, 0.025% (w/v) bromophenol blue, 0.025% (w/v) xylene cyanol]. The mixtures were separated using a 12.5% denaturating PAGE following visualization by phosphorimaging.

Supporting Information (see footnote on the first page of this article): ^{1}H NMR spectra of compounds **4**, **7d**–**10d** (as examples); ^{13}C NMR spectra of compounds **8d**–**10d** (as examples); ^{1}H, ^{13}C and ^{31}P NMR spectra of compounds **11a**–**f**; examples of HPLC profiles of the oligonucleotides **12b**,**c**, **13c**,**e**,**g**,**i**,**k**,**m**,**o**,**q**,**s**, **14b**,**d**,**e**,**f**,**j**,**k**,**l**,**m**; examples of ESI mass spectra of the oligonucleotides **12c**,**e**, **13i**,**m**, **14b**,**h**,**l**.

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