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Synthesis of DNA Strands Site-Specifically Damaged by C8-Arylamine Purine Adducts and Effects on Various DNA Polymerases

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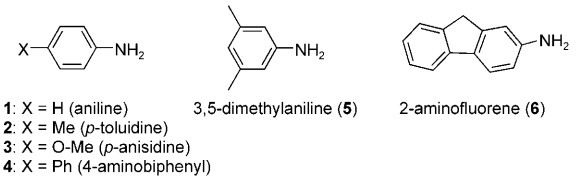
Abstract: C8-Arylamine-dG and C8-arylamine-dA adducts have been prepared using palladium cross-coupling chemistry. These adducts were subsequently converted into the corresponding 5′-*O*-DMTr-C8-arylamine-3′-*O*-phosphoramidites and then used for the automated synthesis of different site-specifically modified oligonucleotides. These "damaged" oligonucleotides have been characterized by ESI-MS, UV thermal stability assays, and circular dichroism, and they have been used in EcoRI assays as well as in primer extension studies using various DNA polymerases.

Keywords: aromatic amines · cross-coupling · DNA adducts · DNA damage · palladium

Introduction

Exposure to carcinogens can occur through environmental or work conditions, diet, smoking, and/or endogenous processes. Poly- and monocyclic aromatic amines, such as aniline (**1**), *p*-toluidine (**2**), 4-aminobiphenyl (**4**), and 2-aminofluorene (**6**) (Figure 1), belong to the class of chemical carcinogens that form covalently bonded adducts with the DNA double helix. Covalent damage of DNA (by electrophiles) may be the reason for the induction of chemical carcinogenesis.[1] If such DNA damage is not repaired, it might compromise the fidelity of DNA replication, eventually leading to mutations and possibly cancer.[2,3]

Arylamines belong to the group of indirect carcinogens because they require metabolic activation in order to generate the so-called ultimate carcinogen (Scheme 1). The initial step is a cytochrome P450-catalyzed oxidation of the arylamine to the corresponding *N*-hydroxylamine.[4] The *N*-hydroxylamine is then esterified to an *N*-acetoxy derivative by *N*-acetyltransferase (NAT) or to a sulfate by a sulfotransferase (ST) to give the ultimate carcinogens. Solvolysis of these compounds generates the highly reactive arylnitrenium ion **8**.



1: X = H (aniline)
2: X = Me (*p*-toluidine)
3: X = O-Me (*p*-anisidine)
4: X = Ph (4-aminobiphenyl)

3,5-dimethylaniline (**5**)

2-aminofluorene (**6**)

Figure 1. Carcinogenic arylamines.

The predominant reaction of the arylnitrenium ion occurs at the C8-position of 2′-deoxyguanosine (dG) and 2′-deoxyadenosine (dA), leading to the corresponding adducts **9a** and **9b** as major products. Moreover, N^2-adducts of dG (**10**) and N^6-*ortho*-arylamine adducts of dA have been identified as minor products.[5,6]

So far, the most extensively studied arylamine adducts have been those derived from 2-aminofluorene (AF) and *N*-acetyl-2-aminofluorene (AAF).[7] Zhou and Romano reported on the synthesis of C8-deoxyguanosine phosphoramidite reagents of 2-aminofluorene and its *N*-acetyl counterpart for the site-specific synthesis of oligonucleotide strands containing these C8 adducts. As protecting group for the exocyclic amino function of dG, the Fmoc group was used.[8,9]

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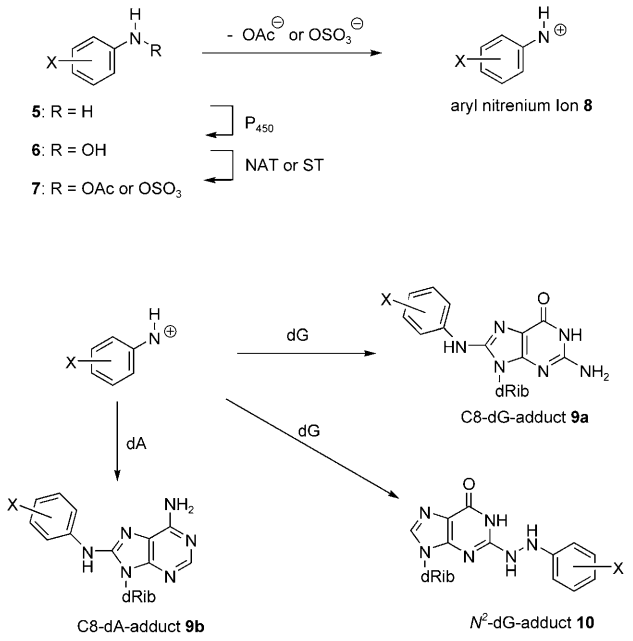


Scheme 1. Metabolism of arylamines and the adducts formed.

A strategy for obtaining the *N*-acetyl adducts was introduced by Schärer and Gillet,[10] which involves transient dimethoxytrityl (DMTr) protection of the N^2-position of an 8-Br-dG derivative. After a palladium-catalyzed cross-coupling reaction of 8-Br-dG with the arylamine, the products are subsequently acetylated at the N^8-position. In 2005, these authors reported the successful conversion of the *N*-acetylated adducts into the corresponding phosphoramidites (12 steps overall) and their site-specific incorporation into oligonucleotides. It was proven that the N^8-acetyl group was not cleaved during the final deprotection.[11]

Recently, Rizzo reported the synthesis of oligonucleotides containing C8 adducts of a heterocyclic amine, the dietary mutagen 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ).[12]

In contrast, our interest is related to DNA adducts of monocyclic aromatic amines that act as so-called borderline carcinogens, such as toluidine, dimethylaniline, and anisidine.[13,14] In contrast to arylamines such as 4-aminobiphenyl (**4**), these are often used, for example, as pharmacophores. In 2002, we reported on an efficient synthesis of a phosphoramidite building block for the C8-dG adduct of toluidine.[15] In 2006, we published the synthesis and the site-specific incorporation of these C8-arylamine-modified dG-phosphoramidites into oligonucleotides.[16] Also in 2006, we reported on the first synthesis of C8-dA adducts and their successful conversion into the corresponding phosphoramidites as well as their site-specific incorporation into an oligonucleotide.[17] In 2007, we published a simple and efficient route for the synthesis of the C8-*N*-Ac-dG adducts and their phosphoramidites, and recently we have developed a synthetic route for the N^2-hydrazinoaryl- and N^2-azoaryl-dG adducts.[18,19] Here, we report on a highly efficient synthesis of these C8 adducts using palladium-catalyzed cross-coupling chemistry, an improved synthesis of the 3′-phosphoramidites, and their use in solid-phase DNA synthesis to give site-specifically modified oligonucleotides of mixed sequences containing several dGs or dAs. The effect of these modifications in relation to the restriction of a damaged DNA duplex by EcoRI is investigated. Moreover, the site-specific incorporation of C8-arylamine-damaged phosphoramidites into 30 mer oligonucleotides for DNA polymerase studies is reported for the first time.

Results and Discussion

Synthesis of the C8 adducts of monocyclic arylamines: The synthesis of C8-arylamine-dG adducts by simple electrophilic amination has been reported to give only low yields.[20,21] Thus, this approach is not suitable as the key reaction for the synthesis of phosphoramidites.[8,9] Attempts to optimize the electrophilic amination used in these biomimetic reactions failed in our hands (unpublished data). Also, the use of nucleophilic substitution as reported for C8-Br-(ribo)guanosine was unsuccessful because extensive depurination was observed when fully protected 8-Br-2′-deoxyguanosine was treated with arylamines.[22] C8–N bond formation was first reported by Lakshman[23] and Johnson[24] in the synthesis of N^6-aryl adducts of adenosine and N^2-aryl-dG adducts of guanosine using palladium catalysts (Buchwald–Hartwig reaction).[25] Schoffers prepared C8-arylamine adducts of tris-*O*-TBDMS-(ribo)adenosine using the same reaction.[26] However, these compounds were never converted into their phosphoramidites. In 2004, Rizzo et al. published the synthesis of 2′-dG-phosphoramidites containing a heterocyclic food mutagen (IQ) in the C8-position.[12] Their approach required the use of strong bases such as LiHMDS or NaO*t*Bu and/or their protecting group chemistry was incompatible with the conditions of automated oligonucleotide synthesis. In 2006, we published the synthesis and site-specific incorporation of C8-arylamine-modified 2′-deoxyguanosine phosphoramidites into oligonucleotides.[16] In that work, the exocyclic amino function of dG was protected using the isobutyryl group, which is a standard protecting group in oligonucleotide synthesis. A drawback of this group is the long reaction time needed for the deprotection after the synthesis using ammonia solution (8 h at 55 °C). Nevertheless, oligonucleotides incorporating the adducts were successfully isolated. Also, the first primer template extensions using standing start as well as standing start +1 conditions were accomplished. However, in these studies, no difference in primer extension was observed between borderline and strong carcinogens.[27]

Due to the fact that C8-arylamine-damaged oligonucleotides are base-labile, there was a pressing need to reduce the time required for N^2-deprotection. In this context, we reported on the synthesis and incorporation of C8-arylamine-modified 2′-dG phosphoramidites using formamidine as the protecting group for the exocyclic amino function.[28] Using this strategy, which allows for milder deprotection, the deprotection was completed after 4 h at 40 °C instead of 8 h at

55 °C, and the yield of modified oligonucleotides was four- to fivefold higher as compared to that with the isobutyryl strategy.

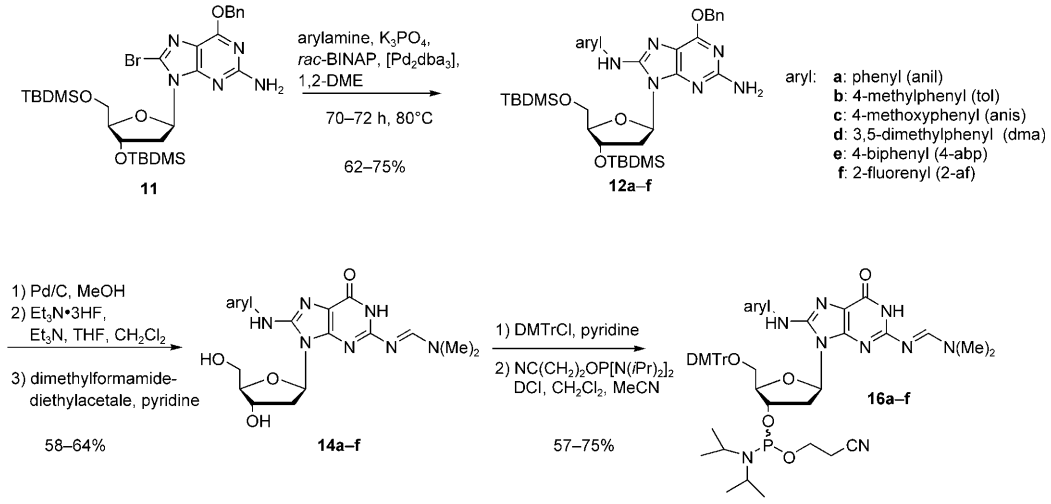
For the synthesis of the formamidine-protected C8-arylamine-2′-dG phosphoramidites **16**, palladium-catalyzed cross-coupling was again used as the key step. The O^6-position of guanine as well as the hydroxyl groups of the glycon need to be blocked during the reaction. The fully protected dG derivative was synthesized as described previously.[16] However, protection of the exocyclic amino group of dG was not necessary for the Buchwald–Hartwig reaction, which was carried out starting from intermediate **11** under previously published conditions (Scheme 2).[15] The coupling proceeded smoothly to give the C8-arylamine adducts **12a–f** in yields of 65–92 %. The C8-arylamine adducts **12a–f** were converted into the corresponding phosphoramidites as summarized in Scheme 2. The O^6-position was deblocked using Pd/H_2 and the hydroxyl groups were desilylated using triethylamine trihydrofluoride to give the unprotected intermediates. The formamidine group was introduced using dimethylformamide diethyl acetal to give **14a–f**. These compounds were 5′-*O*-dimethoxytritylated in yields of 78–84 % and further converted into the 5′-*O*-DMTr-3′-*O*-phosphoramidites **16a–f** (57–88 % yields). No side reaction at the N^8-atom took place, neither during the introduction of the DMTr group nor in the course of the phosphoramidite reaction.

In the case of 2′-deoxyadenosine, nothing was known about the synthesis of C8 adducts with aromatic amines prior to our recent report on the synthesis of C8-arylamine-modified 2′-dA adducts. Moreover, we proved that the adducts could be converted into the phosphoramidites and they were successfully site-specifically incorporated into a DNA sequence.[17] In contrast to the preparation of the dG-C8 adducts, we selected the Markiewicz (TIPDS) protecting group instead of the *tert*-butyldimethylsilyl group for the protection of the 3′- and 5′-hydroxyl moieties of 2′-dA. This was necessary because of incomplete cleavage of the *tert*-butyldimethylsilyl ethers from the N^6-benzoyl-protected C8-substituted 2′-dA adducts. Neither with tetrabutylammonium fluoride nor with triethylamine trihydrofluoride as deprotecting reagent could a satisfactory deprotection be realized. Interestingly, the desilylation proceeded in nearly quantitative yield after the Buchwald–Hartwig coupling when the exocyclic N^6-amino function was still unprotected. Since this first report in 2006, improvements have been achieved using morpholine for the selective debenzoylation of the N^6-amino position instead of a mixture of aqueous ammonia, water, and pyridine.[29] The commonly used technique with sodium methanolate failed in our hands. A modification of the reaction conditions was also necessary for the 5′-dimethoxytritylation because the originally used method showed only poor regioselectivity and required long reaction times (Scheme 3).

As a consequence, a considerable amount of the 3′,5′-bis-dimethoxytritylated product was detected while there was still starting material present. This problem was reduced by the addition of one equivalent each of silver nitrate and *sym*-collidine to accelerate the reaction, which led to a considerable improvement in the yield of the 5′-DMTr-protected compound.[30]

To improve the yield of the cross-coupling, several commonly used ligands were studied. Thus, Buchwald's ligand,[31] PEPPSI-*i*Pr,[32] benzyldi-1-adamantylphosphine,[33] and Xantphos[34] were used, as well as *rac*-BINAP (Figure 2).

The Buchwald ligand generated the desired product only in moderate yield and with low purity. Use of the PEPPSI-*i*Pr ligand resulted in an undefined product, while the benzyldi-1-adamantylphosphine showed no reaction at all. Xantphos and *rac*-BINAP proved to be the most appropriate ligands for the cross-coupling with aromatic amines. To obtain very good to excellent yields with these two ligands, it is necessary to pre-react the catalyst and ligand for 1 h prior to addition of the nucleoside, amine, and base. This pre-reaction procedure increased the yield from about 70 % to over 90 % (Table 1).



Scheme 2. Synthesis of C8-arylamine-modified 2′-dG phosphoramidites.

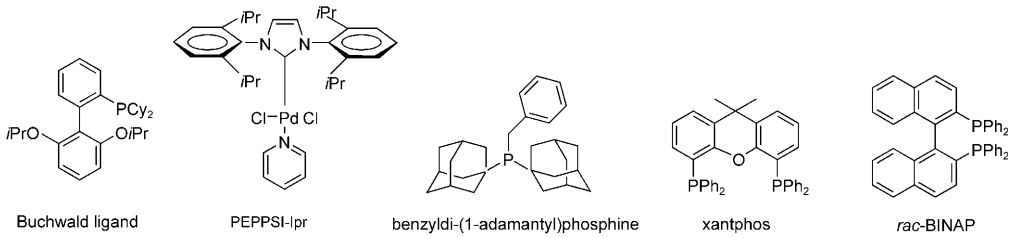
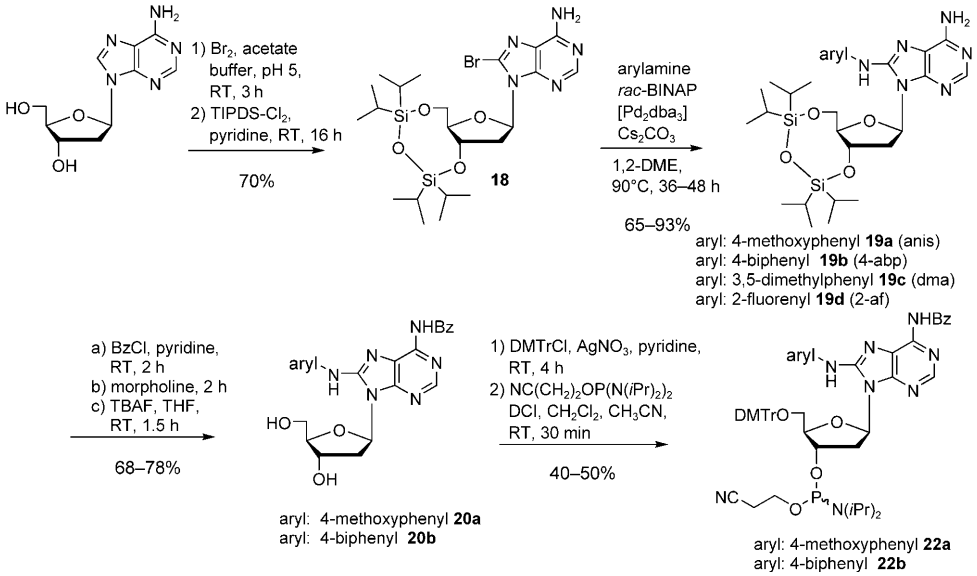


Figure 2. Ligands tested for the Buchwald–Hartwig reaction.



Scheme 3. Synthesis of C8-arylamine-modified 2′-dA phosphoramidites.

Table 1. Optimization of Buchwald–Hartwig cross-coupling for dA.

Ligand	Base/ Solvent	Aryl- amine	*t* [h]	Yield [%]	Pre-reaction of catalyst and ligand	Product
rac-BINAP	Cs_2CO_3/1,2-DME	4-anisidine	48	75	no	**19a**
rac-BINAP	Cs_2CO_3/1,2-DME	4-aminobiphenyl	58	65	no	**19b**
rac-BINAP	Cs_2CO_3/1,2-DME	3,5-dimethylaniline	48	73	no	**19c**
rac-BINAP	Cs_2CO_3/1,2-DME	2-aminofluorene	48	93	yes	**19d**
Xantphos	Cs_2CO_3/1,2-DME	3,5-dimethylaniline	18	92	yes	**19c**

Site-specific synthesis of oligonucleotides containing C8-adducts of different arylamines: Compounds **16a–f** and **22a,b**, which are readily soluble in acetonitrile, were employed in oligonucleotide synthesis using a modified coupling protocol with three coupling steps for the C8 adducts. In this way, we synthesized a site-specifically modified 12mer oligonucleotide including the *Nar*I sequence **23a–f** (Table 2). Additionally, we prepared 30 mer oligonucleotides **24a–j** (Table 3) needed for DNA polymerase assays and 12 mer oligonucleotides **25a–q** for an EcoRI assay (Table 4).

In the automated DNA synthesis, we used commercially available protected phosphoramidites for the regular nucleosides. For the incorporation of the adducts, the C8-modified dG and dA phosphoramidites were dissolved in acetonitrile (0.1 M solution) and the coupling step was repeated three times with a total coupling efficiency of 60–70% for the modified 2′-deoxyguanosine and a coupling efficiency of >98% for the modified 2′-deoxyadenosine phosphoramidites. The basic deprotection step was completed within 4 h at 45°C according to Johnson et al. by adding ethanethiol.[35,36] They observed that the C8-aminofluorene adduct undergoes an oxidative rearrangement in the presence of strong bases and oxygen, analogous to that observed for 8-oxo-2′-dG. The addition of ethanethiol to the degassed ammonium hydroxide prevents the oxidative side reaction during the final deprotection. The deprotected oligonucleotides were purified by reversed-phase HPLC and characterized by ESI mass spectrometry.

Melting temperature (T_m) and circular dichroism studies: In the past, dG adducts were incorporated in the middle of a homo(T)$_{14}$ sequence and hybridization experiments were conducted.[16] For comparison, the unmodified (T)$_7$(dG)(T)$_7$ was hybridized to (dA)$_7$(X)(dA)$_7$ (X=dC, T, dA, dG). A mismatch within the hybrid caused a decrease of the T_m value of about 10°C ($T_m \approx 28.5$°C). Incorporation of a dG* adduct into the homo(T) sequence led to a reduction of 5°C in the case of the matched (dA)$_7$(dC)(dA)$_7$ strand, irrespective of the arylamine modification. Hybridization of the mismatched (dA)$_7$(X)(dA)$_7$ strand with the modified strand led to the same thermal stability as in the case of the mismatched duplex and no stabilization from a possible Hoogsteen base pairing was observed ($T_m \approx 27.5$°C).[16]

For the present investigations, all oligonucleotides were hybridized to complementary strands and the effect of the C8-arylamine adduct on the thermal stability of the DNA

duplex was measured through UV melting temperature analysis (T_m values). The data for the 12 mer oligonucleotides **23a–f** are given in Table 2.

Table 2. Synthesized oligonucleotides **23a–f** and T_m values.

Oligonucleotide	T_m [°C]
5′-CTC GGC **G**CC ATC-3′ **23a**	58
5′-CTC GGC **G(anis)**CC ATC-3′ **23b**	51
5′-CTC GGC **G(4-abp)**CC ATC-3′ **23-c**	40
5′-CTC GGC **A**CC ATC-3′ **23d**	56
5′-CTC GGC **A(anis)**CC ATC-3′ **23e**	47
5′-CTC GGC **A(4-abp)**CC ATC-3′ **23f**	49

For C8-anisidine-damaged dG oligonucleotide **23b**, a decrease of 7 °C was observed compared to the T_m value of the unmodified *Nar*I oligonucleotide **23a** (T_m = 58 °C). Interestingly, for the oligonucleotide bearing the abp lesion **23c**, a dramatic decrease in thermal stability was observed (T_m = 40 °C). Thus, the second aromatic ring of the abp lesion has a significant effect on the duplex stability. However, the situation was different in the case of the mutated *Nar*I sequences **23e** and **23f** bearing a damaged dA nucleoside. As compared with the unmodified reference oligonucleotide **23d**, both lesions caused a similar destabilization of about 8 °C (Table 2). Here, the strong carcinogen abp clearly did not cause a further decrease in stability as in the case of the dG adducts.

The data collected for the 30 mer oligonucleotides **24a–j** are summarized in Table 3. For the C8-arylamine-2′-dG oligonucleotides modified with borderline carcinogens **24b–d**, no effect on the T_m value was observed with respect to the unmodified oligonucleotide **24a** (62 °C). In contrast, the oligonucleotides damaged by strong arylamine carcinogens (**24e–g**) showed a decrease in thermal stability (59 °C). This decrease is certainly higher than the experimental error of ±0.5 °C. Astonishingly, the oligonucleotides modified with different monocyclic aromatic amines always showed the same thermal stability as compared to the non-damaged reference strands **24a** and **24h**.

Similar trends were observed for C8-arylamine-2′-dA oligonucleotides **24i,j**. For the *p*-anisidine-modified oligonucleotide **24i**, an identical T_m value was measured as for the unmodified oligonucleotide **24h** (59 °C), whereas the oligonucleotide modified with 4-aminobiphenyl **24j** showed a decrease of the T_m value (57 °C).

Thus, oligonucleotides modified by strong carcinogens consistently showed a 2–3 °C reduction in T_m compared to the reference. The conformational distortion as a result of C8-dG or C8-dA damage of the DNA double-helix caused by a borderline or a strong carcinogen is therefore significantly different and is in the range of the hybridization contribution of one G–C or one A–T base pair, respectively.

For the self-complementary 12 mer oligonucleotides of the EcoRI sequence, the effect on the T_m values was much more pronounced (Table 4). This was expected because in the duplexes formed in this case one adduct occurs in each strand.

Table 4. Synthesized oligonucleotides **25a–q** and corresponding T_m values.

Oligonucleotide	T_m [°C]
5′-GTAGAATTCTAC-3′ **25a**	42
5′-GTA**G(anil)**AATTCTAC-3′ **25b**	29
5′-GTA**G(tol)**AATTCTAC-3′ **25c**	24
5′-GTA**G(anis)**AATTCTAC-3′ **25d**	25
5′-GTA**G(dma)**AATTCTAC-3′ **25e**	24
5′-GTA**G(4-abp)**AATTCTAC-3′ **25f**	24
5′-GTA**G(2-af)**AATTCTAC-3′ **25g**	24
5′-**G(anil)**TA GAATTCTAC-3′ **25h**	35
5′-**G(4-abp)**TAGAATTCTAC-3′ **25i**	30
5′-GT**A(anis)**GAATTCTAC-3′ **25j**	29
5-GT**A(4-abp)**GAATTCTAC-3′ **25k**	27
5′-GTAG**A(anis)**ATTCTAC-3′ **25l**	35
5′GTAG**A(4-abp)**GAATTCTAC-3′ **25m**	42
5′-GTAGA**A(anis)**TTCTAC-3′ **25n**	37
5′-GTAGA**A(4-abp)**TTCTAC-3′ **25o**	39
5′-GTAGAATTCT**A(anis)**C-3′ **25p**	28
5′-GTAGAATTCT**A(4-abp)**C-3′ **25q**	33

A decrease in the thermal stabilities of the dG-adduct-bearing oligonucleotides **25b–g** (T_m values decreased by 13–18 °C) compared to reference oligonucleotide **25a** (T_m = 42 °C) was observed. Here, no significant difference between the oligonucleotides damaged by monocyclic aromatic amines and those damaged by a polycyclic aromatic amine was measured. In contrast, a significant difference in the influences on thermal stability can be observed for the oligonucleotides **25h,i**. Here, the monocyclic DNA damage leads to a decrease of 7 °C to a T_m of 35 °C, whereas the polycyclic DNA damage has a bigger influence leading to a T_m of 30 °C.

In the case of the dA-modified oligonucleotides, there is no such strong influence on the T_m value. The oligonucleotides modified with *p*-anisidine **25j,l,n,p** showed only a 5–14 °C decrease in the T_m value compared to that of the unmodi-

Table 3. Synthesized oligonucleotides **24a–j** and T_m values.

Oligonucleotide	T_m [°C]
5′-AAA T**G**A ACC TAT CCT CCT TCA GGA CCA ACG-3′ **24a**	62
5′-AAA T**G(anil)**A ACC TAT CCT CCT TCA GGA CCA ACG-3′ **24b**	62
5′-AAA T**G(tol)**A ACC TAT CCT CCT TCA GGA CCA ACG-3′ **24c**	62
5′-AAA T**G(anis)**A ACC TAT CCT CCT TCA CGA CCA ACG-3′ **24d**	62
5′-AAA T**G(dma)**A ACC TAT CCT CCT TCA GGA CCA ACG-3′ **24e**	59
5′-AAA T**G(4-abp)**A ACC TAT CCT CCT TCA GGA CCA ACG-3 **24f**	59
5′-AAA T**G(2-af)**A ACC TAT CCT CCT TCA GGA CCA ACG-3′ **24g**	59
5′-AAA T**A**A ACC TAT CCT CCT TCA GGA CCA ACG-3′ **24h**	59
5′-AAA T**A(anis)**A ACC TAT CCT CCT TCA GGA CCA ACG-3′ **24i**	59
5′-AAA T**A(4-abp)**A ACC TAT CCT CCT TCA GGA CCA ACG-3′ **24j**	57

fied oligonucleotide **25a**. Surprisingly, the influence on the thermal stabilities of the oligonucleotides modified with the strong carcinogen 4-aminobiphenyl **25k**,**m**,**o**,**q** was lower than that for the monocyclic aromatic amines. Here, a decrease of 3–12 °C could be observed. For the oligonucleotide **25m**, the T_m value was found to be identical to that of the non-damaged oligonucleotide **25a**.

In addition, the circular dichroism (CD) spectra of all of the synthesized oligonucleotides as hybrids with the complementary strand were measured. CD spectra were recorded to prove the overall conformation of the adduct-modified DNA hybrids. For the *Nar*I sequence **23a–f**, no difference between the unmodified oligonucleotide and those bearing lesions of dG and dA could be observed and all showed typical spectra of a B-type DNA conformation (see Supporting Information).

The same result was obtained for the 2′-dA-damaged oligonucleotides **24i** and **24j** and the unmodified strand **24h** (Figure 3). Again, the three oligonucleotides are predominantly in a B-type DNA conformation. Thus, no conformational difference was observed between oligonucleotides modified with a borderline or a strong carcinogen.

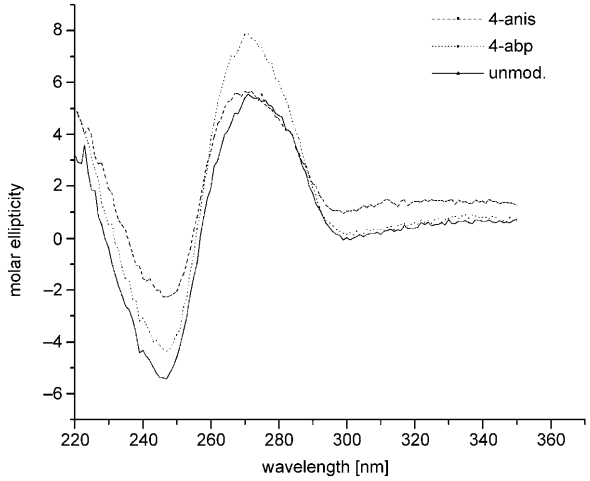


Figure 3. CD spectra of dA-modified oligonucleotides **24h–j**.

Moreover, all of the 30 mer oligonucleotide duplexes modified at 2′-dG show the same characteristics, a maximum at 280–290 nm and a minimum at 240–250 nm without significant changes in intensity (see the Supporting Information). These features indicate that all of the 30 mer oligonucleotides are in a B-type DNA conformation. The shifts of the maxima in the CD spectra of the modified oligonucleotides **24e**,**f** to higher wavelength (6 and 9 nm, respectively) are caused by the (partly) conjugated aromatic systems.

An analogous study was performed with the EcoRI sequences. Again, all of the modified 12 mer oligonucleotides **25b–q** exhibited the same overall conformation (see Supporting Information).

EcoRI restriction assay: To investigate the effect on enzymatic cleavage of arylamine-modified oligonucleotides **25** by an endonuclease, the EcoRI restriction assay was chosen. EcoRI cleaved the self-complementary, undamaged 12 mer oligonucleotide **25a** into a 4 mer (GTAG) and an 8 mer strand (AATTCTAC). It is known that EcoRI cleaves both strands of a DNA double-helix between dG[4] and dA[5]. For the reference oligonucleotide **25a**, a half-life of 2.5 h using 270 units of enzyme in a DTT buffer at 20 °C was determined (Figure 4). The half-life was calculated as described previously.[37]

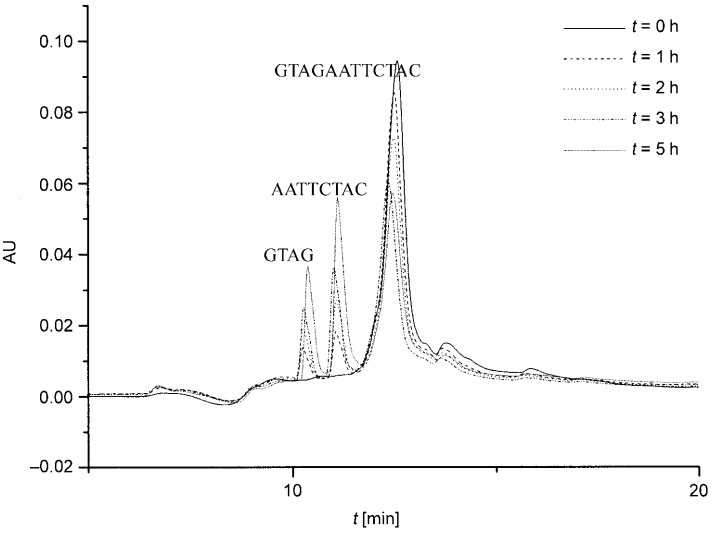


Figure 4. HPLC chromatograms of the EcoRI assay with the unmodified oligonucleotide **25a**.

Performing the cleavage assay using the arylamine-modified oligonucleotides **25b–g** and **25l–o** under the same experimental conditions, no cleavage of any of the oligonucleotides could be detected. Even after incubation for 76 h, the modified double-strands were not digested (Figure 5). Thus, the arylamine damage of either dA or dG within the cleavage site evidently causes a conformational distortion in such a way that the enzyme is unable to bind and/or to cleave the DNA double-strand.

A modification by a mono- or polycyclic aromatic amine away from the recognition site generally leads to an increase in the half-life for the restriction assay. For polycyclic DNA damage of dG[1], a half-life of 4.5 h was calculated. Surprisingly, a monocyclic modification has a more pronounced effect, leading to a longer half-life (6.3 h). A similar effect can be observed for modification at dA[3] (see Table 5). Here again, the monocyclic arylamine modification clearly results in a greater structural change, which could be a reason for a weaker binding of the enzyme or inferior recognition of the acquired palindromic hexamer (see the example in Figure 6).

Primer extension assay: As investigations of other DNA adducts have shown, covalent DNA modifications significantly

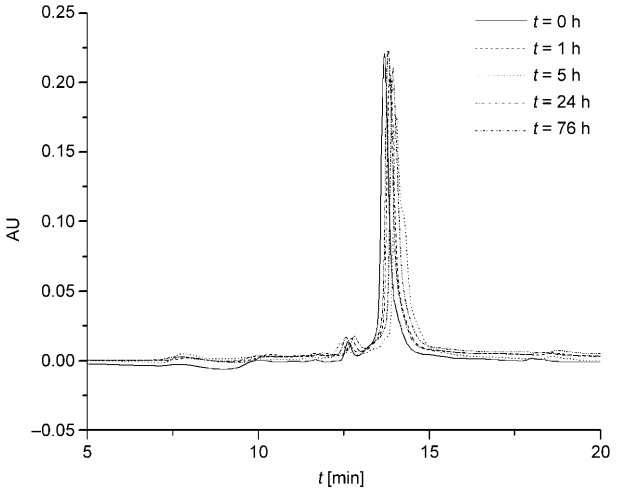


Figure 5. HPLC chromatograms of the EcoRI assay with the modified oligonucleotide **25b**.

Table 5. Calculated half-lives for the EcoR1 restriction assay for oligonucleotides **25a–q**.

Oligonucleotides	$t_{1/2}$ [h]
5′-GTAGAATTCTAC-3′ **25a**	2.5
5′-GTA**G(anil)**AATTCTAC-3′ **25b**	n.r.
5′-GTA**G(tol)**AATTCTAC-3′ **25c**	n.r.
5′-GTA**G(anis)**AATTCTAC-3′ **25d**	n.r.
5′-GTA**G(dma)**AATTCTAC-3′ **25e**	n.r.
5′-GTA**G(4-abp)**AATTCTAC-3′ **25f**	n.r.
5′-GTA**G(2-af)**AATTCTAC-3′ **25g**	n.r.
5′-**G(anil)**TA GAATTCTAC-3′ **25h**	6.3
5′-**G(4-abp)**TAGAATTCTAC-3′ **25i**	4.5
5′-GT**A(anis)**GAATTCTAC-3′ **25j**	>24
5′-GT**A(4-abp)**GAATTCTAC-3′ **25k**	3.1
5′-GTAG**A(anis)**ATTCTAC-3′ **25l**	n.r.
5′-GTAG**A(4-abp)**ATTCTAC-3′′ **25m**	n.r.
5′-GTAGA**A(anis)**TTCTAC-3′ **25n**	n.r.
5′-GTAGA**A(4-abp)**TTCTAC-3′ **25o**	n.r.

n.r. = no restriction.

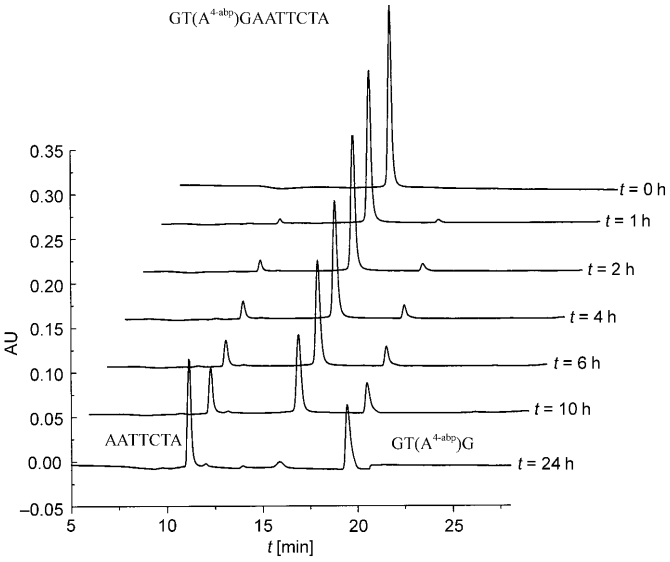


Figure 6. HPLC chromatograms of the EcoRI assay with the modified oligonucleotide **25k**.

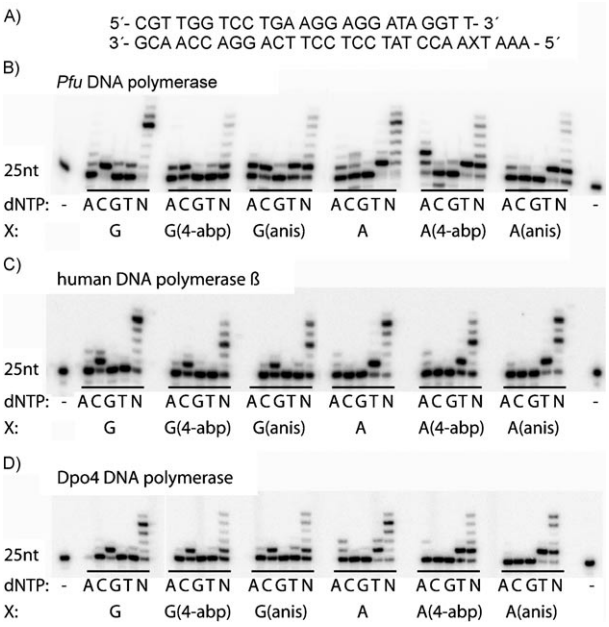


Figure 7. Effect of C8-arylamine adducts on DNA polymerases (A): DNA sequences employed. X = modified dG or dA residues. (B–D): reactions catalyzed by the indicated DNA polymerase in the presence of one dNTP or all four dNTPs (indicated by N).

hamper the selectivity and efficiency of lesion bypass synthesis by replicative DNA polymerases, while other DNA polymerases are effective in performing DNA synthesis beyond the site of damage.[38] Thus, to gain some initial insights, we investigated several DNA polymerases from different DNA polymerase families with regard to their effectiveness in bypassing the C8-arylamine adducts studied here (Figure 7).

We conducted experiments using a radioactively labeled primer–template complex, which was designed in such a way that the modified nucleotide in the template strand codes for the first nucleotide after primer extension (Figure 7). Single incorporations were examined in order to gain first insights into the impact of the modification on selectivity, in addition to experiments employing all four dNTPs to study lesion bypass. First, we investigated the high-fidelity *Pyrococcus furiosus* (*Pfu*) DNA polymerase (3′→5′ exonuclease-deficient mutant),[39,40] a replicative DNA polymerase belonging to sequence family B, the same as the human replicative DNA polymerases.

The investigated lesions significantly block the progress of DNA synthesis by this enzyme, as indicated by a strong pausing band after incorporation of only one nucleotide, even when all four dNTPs are present (Figure 7B). Interestingly, the ability to misincorporate a nucleotide significantly depends on the chemical composition of the modification as well as the modified nucleobase. While predominately the canonical dC is incorporated opposite G(4-abp) and the

primer strand is extended by 60%, G(anis) promotes misincorporation of dT and dA more efficiently, extending the primer by 60% and 54%, respectively (see the Supporting Information). On the other hand, incorporation opposite A(anis) is more selective and the most significant primer extension by 90% was observed when the canonical dTTP was present. However, A(4-abp) promotes misinsertion of dA more significantly (78%). Comparing A(anis) with G(anis), the latter seems to promote mismatch formation to a greater extent. In addition, we investigated the ability of the 3′→5′ exonuclease-proficient *Pfu* DNA polymerase to bypass the studied lesions (see the Supporting Information). Interestingly, we found that this enzyme was only able to bypass the lesions in the case of G(anis), indicating the proofreading activity involved in the bypass process.

Next, we investigated human DNA polymerase β (Figure 7C). Interestingly, this enzyme, a member of the DNA polymerase X family involved in DNA repair,[41] is able to bypass the lesions and predominantly inserts the canonical nucleotide opposite the lesion (see the Supporting Information). When investigating single nucleotide insertion, it was apparent that both modifications render primer extension less efficient in most cases (47–63%), as compared to reactions employing the unmodified templates (80–83%). However, the effects of nucleobase and composition of the modification have a lower impact on error formation when compared to the family B *Pfu* DNA polymerase. Next, we studied *Sulfolobus solfataricus* P2 DNA polymerase IV (*Dpo4*), which often serves as a functional and structural model for Y-family DNA polymerases (Figure 7D).[42–45] This enzyme is effective in bypassing these lesions, albeit with reduced efficiency, as evidenced by a strong pausing band after nucleotide insertion opposite the lesions when all four nucleotides are employed. Single nucleotide incorporation assays show that the canonical nucleotide is incorporated predominantly opposite the lesion, as has also been shown for other lesions.[44,45] Only in the cases of the G(4-abp) and G(anis) modifications insertion of the non-canonical dT was observed to some extent (7–11%).

Conclusion

We have presented detailed experimental procedures for the successful synthesis of C8-arylamine-dG phosphoramidites as well as for their hitherto unknown dA counterparts. The amidites have been successfully site-specifically incorporated into two different oligonucleotides. For both 30 mer oligonucleotides and the self-complementary 12 mers, the thermal stabilities of the damaged DNA strands showed a difference between the compounds modified by a monocyclic aromatic amine and those modified by 4-aminobiphenyl. Moreover, the adduct-bearing oligonucleotides were found to be resistant to digestion by EcoRI when these modifications were present within the recognition sequence of the EcoRI enzyme. An influence on the half-life of this enzymatic digestion was also found. Surprisingly, monocyclic DNA damages were found to have a stronger influence, leading to higher half-lives in this restriction assay. We have shown that the effect of the damaged oligonucleotides on DNA polymerases very much depends on the respective DNA polymerase, the nucleobase, as well as the chemical nature of the adduct. Interestingly, the most significant potential for incorporating a non-Watson–Crick nucleotide was found when a high-fidelity DNA polymerase promoted nucleotide insertion opposite the lesion. Investigations concerning the effect on repair enzymes of the different modified oligonucleotides are currently in progress in our laboratories.

Experimental Section

General methods: All air- or water-sensitive reactions were performed in flame-dried glassware under a nitrogen atmosphere. Commercial solvents and reagents were used without further purification with the following exceptions: 1,4-dioxane and 1,2-DME were distilled from potassium under nitrogen; pyridine, dichloromethane, and acetonitrile were distilled from calcium hydride under nitrogen. Water was purified on a Milli-Q water system. NMR spectra are reported relative to the respective solvent peaks (^{1}H NMR: 2.50 ppm ([D$_6$]DMSO), 7.26 ppm ($CDCl_3$), 3.31 ppm (CD_3OD), and 7.16 ppm (C_6D_6); ^{13}C NMR: 39.52 ppm ([D$_6$]DMSO), 77.16 ppm ($CDCl_3$), 49.0 ppm (CD_3OD), and 128.06 ppm (C_6D_6)). Thin-layer chromatography (TLC) was performed on aluminium sheets coated with silica gel 60 F_{254} from Merck.

***O*^{6}-Benzyl-8-bromo-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (11)** was prepared as described previously.[16]

General procedure I for the C–N bond formation of *O*^{6}-benzyl-8-bromo-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine derivatives by Pd-catalyzed cross-coupling chemistry: A dried flask was purged with nitrogen and charged with bromide **11**, K_3PO_4 (1.5 equiv), tris(dibenzylideneacetone)dipalladium(0) ([Pd_2dba_3]; 10 mol%), racemic 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (*rac*-BINAP; 30 mol%), and the appropriate amine (2 equiv). Dry 1,2-DME (15 mL) was added and the mixture was stirred at 80°C until the reaction was complete (TLC analysis). The mixture was allowed to cool to room temperature, whereupon saturated sodium hydrogencarbonate solution (1 mL) was added. After the addition of brine (10 mL), the layers were separated and the aqueous layer was extracted with ethyl acetate (3×10 mL). The combined organic layers were washed with brine (2×10 mL) and finally with a mixture of brine (10 mL) and water (2 mL). The organic phase was then dried over sodium sulfate and the solvent was removed in vacuo. Purification by chromatography on silica gel, eluting with 10→30% ethyl acetate in hexanes, gave the desired product.

***O*^{6}-Benzyl-8*N*-(phenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12a)**: GP I was conducted with 3.00 g (4.51 mmol) of bromide **11** (reaction time 72 h), which afforded a yellow foam (1.97 g, 64%). M.p. 161°C; $[\alpha]_{546}^{20}=+13.8°$ ($c=1.64$, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.69$ (s, 1H), 7.59 (dd, $J=7.6$ Hz, 2H), 7.49 (dd, $J=6.7$ Hz, 2H), 7.40–7.38 (m, 1H, H_ε), 7.37–7.33 (m, 2H, H_γ), 7.26 (ddd, $J=7.5$, 7.5 Hz, 2H), 6.91 (ddd, $J=7.3$, 7.3 Hz, 1H), 6.31 (dd, $J=6.9$, 6.9 Hz, 1H), 6.05 (s, 2H), 5.48 (s, 2H), 4.64 (ddd, $J=3.1$, 3.1, 6.2 Hz, 1H), 3.88–3.80 (m, 2H), 3.68 (dd, $J=4.4$, 10.0 Hz, 1H), 3.47–3.40 (m, 1H), 2.15–2.10 (m, 1H), 0.90 (s, 9H), 0.81 (s, 9H), 0.12 (s, 6H), −0.02 (s, 3H), −0.03 ppm (s, 3H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=157.1$, 156.9, 153.3, 145.5, 140.2, 136.4, 128.2, 128.0, 127.8, 127.8, 127.3, 120.4, 117.3, 86.6, 82.4, 72.1, 65.8, 62.4, 35.8, 25.2, 17.4, 17.2, −5.2, −5.4, −6.0 ppm; IR (KBr): $\tilde{\nu}=3227$, 3034, 1180, 1005, 917, 895, 725, 669, 560, 505 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 676.3589, found: 677.3616 [*M*+H$^+$].

***O*^{6}-Benzyl-8*N*-(4-methylphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12b)**: GP I was conducted with bromide **11** (2.50 g, 3.76 mmol; reaction time 70 h), which afforded a yellow foam (1.96 g, 75%). M.p. 86°C; $[\alpha]_{546}^{20}=+16.9°$ ($c=0.9$, $CHCl_3$); ^{1}H NMR (400 MHz,

[D$_6$]DMSO): δ=8.56 (s, 1H), 7.49–7.47 (m, 2H), 7.40–7.30 (m, 3H), 7.07 (d, J=8.4 Hz, 2H), 6.80 (d, J=8.0 Hz, 2H), 6.30 (dd, J=6.9 Hz, 1H), 6.01 (s, 2H), 5.47 (s, 2H), 4.62 (ddd, J=6.1, 3.0, 3.0 Hz, 1H), 3.82–3.78 (m, 2H), 3.68 (dd, J=10.2, 4.5 Hz, 1H), 3.42–3.38 (m, 1H), 2.22 (s, 3H), 2.11–2.09 (m, 1H), 0.89 (s, 9H), 0.80 (s, 9H), 0.11 (s, 6H), −0.02 (s, 3H), −0.03 ppm (s, 3H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ=157.6, 157.4, 146.4, 138.1, 137.0, 129.9, 129.2, 128.4, 128.3, 127.9, 123.9, 117.4, 111.0, 87.1, 83.0, 72.7, 66.4, 63.0, 36.5, 25.7, 20.3, 18.0, −4.6, −5.2 ppm; IR (KBr): $\tilde{\nu}$=3465, 3348, 2953, 1600, 1409, 1257, 1105, 1060, 835, 698 cm^{-1}; MS (HRFAB): m/z: calcd for: 690.3745, found 691.3809 [M+H$^+$].

O^6-Benzyl-8*N*-(4-methoxyphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12c): GP I was conducted with 3.02 g (4.54 mmol) of bromide **11** (reaction time 70 h), which afforded a yellow foam (2.00 g, 62%). M.p. 117–121 °C; $[\alpha]_{546}^{20}$=+15° (c=0.1, CHCl$_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.47 (s, 1H), 7.51 (d, J=9.0 Hz, 2H), 7.47 (d, J=7.2 Hz, 2H), 7.38 (dd, J=7.2 Hz, 3H), 6.88 (d, J=9.0 Hz, 2H), 6.29 (dd, J=6.9 Hz, 1H), 5.97 (s, 2H), 5.46 (s, 2H), 4.63 (ddd, J=6.2, 3.1, 3.1 Hz, 1H), 3.84 (ddd, J=13.2, 9.6, 5.5 Hz, 2H), 3.70 (s, 3H), 3.68 (dd, J=13.2, 5.5 Hz, 1H), 3.43 (ddd, J=13.3, 6.7 Hz, 1H), 2.10 (ddd, J=13.3, 6.7, 3.2 Hz, 1H), 0.90 (s, 9H), 0.81 (s, 9H), 0.12 (s, 6H), −0.02 (s, 3H), −0.03 ppm (s, 3H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ=157.6, 157.4, 147.2, 139.1, 137.3, 133.9, 128.6, 128.5, 128.1, 120.0, 114.0, 87.3, 83.0, 72.9, 66.6, 63.2, 55.3, 36.6, 25.9, 18.0, −5.2 ppm; IR (KBr): $\tilde{\nu}$=3332, 2952, 2929, 1633, 1605, 1565, 1414, 1256, 835 cm^{-1}; MS (HRFAB): m/z: calcd for: 706.3718, found 707.3622 [M+H$^+$].

O^6-Benzyl-8*N*-(3,5-dimethylphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12d): GP I was conducted with 2.50 g (3.76 mmol) of bromide **11** (reaction time 72 h), which afforded an orange solid (1.74 g, 66%). M.p. 135 °C; $[\alpha]_{546}^{20}$=+15° (c=1.64, CHCl$_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.38 (s, 1H), 7.46 (d, J=7.2 Hz, 2H), 7.36 (t, J=7.5 Hz, 2H), 7.32–7.29 (dd, J=7.2, 8.2 Hz, 2H), 7.25 (d, J=7.1 Hz, 1H), 6.55 (s, 1H), 6.26 (t, J=6.9 Hz, 1H), 6.02 (s, 2H), 5.49 (s, 2H), 4.61–4.59 (m, 1H), 3.85–3.79 (m, 2H), 3.67 (dd, J=4.6, 5.8 Hz, 1H), 2.20 (s, 6H), 2.11–2.08 (m, 2H), 0.88 (s, 9H), 0.80 (s, 9H), 0.10 (s, 6H), 0.00 ppm (s, 6H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ=157.8, 154.9, 154.1, 149.3, 141.0, 137.7, 128.7, 128.5, 128.4, 128.3, 128.0, 120.8, 117.9, 87.2, 84.0, 72.8, 66.6, 63.2, 36.7, 25.9, 21.4, 17.4, 16.7, −5.2, −5.4, −6.0 ppm; IR (KBr): $\tilde{\nu}$=3377, 3333, 2950, 2857, 1616, 1565, 1464, 1413, 1253, 1108, 833, 783 cm^{-1}; MS (HRFAB): m/z: calcd for: 704.3902, found 705.3993 [M+H$^+$].

O^6-Benzyl-8*N*-(4-biphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12e): GP I was conducted with bromide **11** (1.40 g, 1.56 mmol, reaction time 55 h), which afforded a light-yellow foam (1.03 g, 65%). M.p. 146 °C; $[\alpha]_{546}^{20}$=+16.3° (c=0.32, CHCl$_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.05 (s, 1H), 7.52–7.20 (m, 14H), 6.50 (s, 2H), 6.20 (dd, J=6.8, 7.4 Hz, 1H), 5.48 (s, 2H), 4.51 (ddd, J=6.7, 4.3, 4.3 Hz, 1H), 3.81 (dd, J=10.6, 5.0 Hz, 1H), 3.73 (ddd, J=5.0, 4.3, 6.0 Hz, 1H), 3.69 (dd, J=10.6, 6.0 Hz, 1H), 3.63 (ddd, J=13.0, 6.2, 6.8 Hz, 1H), 2.26 (ddd, J=13.0, 4.3, 7.4 Hz, 1H), 0.89 (s, 9H), 0.85 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H), −0.04 (s, 3H), −0.05 ppm (s, 3H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ=160.2, 159.9, 154.3, 148.5, 140.9, 137.8, 136.8, 128.9, 128.6, 128.4, 128.2, 127.5, 127.4, 127.2, 125.8, 125.5, 114.4, 82.5, 72.4, 72.3, 67.0, 62.9, 37.2, 26.0, 25.9, 18.1, 17.9, −4.5, −4.7, −5.2, −5.3 ppm; IR (KBr): $\tilde{\nu}$=2940, 2908, 2877, 1607, 1559, 1423, 1159, 1005, 938, 785 cm^{-1}; MS (HRFAB): m/z: calcd for: 752.3902, found 753.3992 [M+H$^+$].

O^6-Benzyl-8*N*-(2-aminofluorenyl)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12e): GP I was conducted with bromide **11** (1.95 g, 2.91 mmol; reaction time 78 h), which afforded a yellow solid (2.05 g, 91%). M.p. 85 °C; $[\alpha]_{546}^{20}$=−5.2° (c=0.5, CHCl$_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.77 (s, 1H), 7.91–7.88 (m, 1H), 7.76 (dt, J=7.8, 5.9 Hz, 1H), 7.59 (dd, J=8.3, 1.9 Hz, 1H), 7.52–7.46 (m, 3H), 7.42–7.20 (m, 5H), 7.11–7.07 (m, 1H), 6.34 (dd, J=6.9 Hz, 1H), 6.07 (s, 2H), 5.50 (s, 2H), 4.63 (ddd, J=6.1, 3.1 Hz, 1H), 3.92 (s, 2H), 3.89–3.86 (m, 2H), 3.71–3.69 (m, 1H), 3.43 (ddd, J=10.1, 6.5 Hz, 1H), 2.13 (ddd, J=10.1, 7.2, 3.5 Hz, 1H), 0.90 (s, 9H), 0.81 (s, 9H), 0.12 (s, 6H), −0.00 (s, 3H), −0.01 ppm (s, 3H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ=157.8, 157.6, 153.9, 146.1, 143.8, 142.5, 141.3, 140.1, 137.0, 134.5, 128.4, 128.4, 127.9, 126.7, 124.9, 124.3, 120.1, 119.0, 116.8, 114.4, 111.3, 87.2, 83.0, 72.7, 66.5, 63.0, 57.9, 36.5, 25.7, 17.9, −4.7, −5.4 ppm; IR (KBr): $\tilde{\nu}$=3495, 3357, 2952, 2927, 1597, 1560, 1456, 1416, 1254, 835, 778 cm^{-1}; MS (HRFAB): m/z: calcd for: 764.3902, found 765.3974 [M+H$^+$].

General procedure II for the debenzylation of O^6-benzyl-8*N*-arylamino-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine derivatives: A dried flask was purged with nitrogen and then charged with the O^6-benzyl-8*N*-arylamino-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine derivative and Pd/C. Dry methanol was added and the reaction mixture was stirred under a hydrogen atmosphere at room temperature for 1–48 h. The reaction mixture was centrifuged several times with methanol, filtered, and concentrated in vacuo to give the pure product.

8*N*-(Phenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (13a): GP II was conducted with **12a** (1.90 g, 2.80 mmol; reaction time 24 h), which afforded a white solid (1.48 g, 90%). M.p. 155 °C; $[\alpha]_{546}^{20}$=−10.8° (c=0.8, CHCl$_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=10.52 (s, 1H), 8.35 (s, 1H), 7.45 (dd, J=7.6 Hz, 2H), 7.23 (dd, J=8.5 Hz, 2H), 6.87 (dd, J=7.3, 7.3 Hz, 1H), 6.22 (dd, J=7.1 Hz, 1H), 6.19 (s, 2H), 4.53 (ddd, J=3.1, 3.1, 6.2 Hz, 1H), 3.84–3.78 (m, 2H), 3.71 (ddd, J=8.2, 8.2, 8.1 Hz, 1H), 3.23–3.16 (m, 1H), 2.18–2.14 (m, 1H), 0.88 (s, 9H), 0.83 (s, 9H), 0.10 (s, 6H), 0.00 ppm (s, 6H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ=155.3, 152.0, 149.5, 143.2, 141.3, 128.2, 120.0, 116.5, 113.0, 86.8, 82.5, 72.3, 62.7, 36.5, 25.4, 25.3, 17.7, 17.4, −5.1, −5.3 ppm; IR (KBr): $\tilde{\nu}$=3353, 1179, 1006, 953, 692, 667, 576, 501 cm^{-1}; MS (HRFAB): m/z: calcd for: 586.8736, found 587.8705 [M+H$^+$].

8*N*-(4-Methylphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (13b): GP II was conducted with **12b** (1.90 g, 2.74 mmol; reaction time 24 h), which afforded a yellow solid (1.69 g, 85%). M.p. 119 °C; $[\alpha]_{546}^{20}$=−7.9° (c=0.47, CHCl$_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=10.55 (s, 1H), 8.22 (s, 1H), 7.37 (d, J=8.4 Hz, 2H), 7.04 (d, J=8.4 Hz, 2H), 6.20 (dd, J=7.0 Hz, 1H), 6.17 (s, 2H), 4.55–4.52 (m, 1H), 3.84–3.81 (ddd, J=8.3, 9.3 Hz, 2H), 3.73–3.70 (m, 1H), 3.18 (ddd, J=13.2, 7.0, 6.7 Hz, 1H), 2.23 (s, 3H), 2.06 (ddd, J=13.2, 6.7, 3.0 Hz, 1H), 0.88 (s, 9H), 0.83 (s, 9H), 0.09 (s, 6H), 0.02 (s, 3H), 0.00 ppm (s, 3H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ=155.7, 152.3, 146.0, 143.9, 139.0, 128.3, 123.9, 114.5, 111.0, 87.1, 82.9, 72.7, 63.1, 36.9, 25.7, 20.1, 18.1, −4.6, −5.3 ppm; IR (KBr): $\tilde{\nu}$=3312, 2918, 1693, 1603, 1517, 1369, 1252, 1083, 837, 776 cm^{-1}; MS (HRFAB): m/z: calcd for: 600.3276, found 601.3344 [M+H$^+$].

8*N*-(4-Methoxyphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (13c): GP II was conducted with **12c** (1.92 g, 2.69 mmol; reaction time 4 h), which afforded a white solid (1.58 g, 96%). M.p. 112 °C; $[\alpha]_{546}^{20}$=−9.9° (c=0.8, CHCl$_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=10.53 (s, 1H), 8.14 (s, 1H), 7.42 (d, J=9.0 Hz, 2H), 6.84 (d, J=9.0 Hz, 2H), 6.20 (dd, J=7.1 Hz, 1H), 6.15 (s, 2H), 4.52 (ddd, J=6.2, 3.1, 3.1 Hz, 1H), 3.85–3.83 (m, J=13.2, 9.6, 5.5 Hz, 2H), 3.70 (s, 3H), 3.68 (dd, J=13.2, 5.5 Hz, 1H), 3.20 (ddd, J=13.8, 6.6 Hz, 1H), 2.10 (ddd, J=13.8, 6.6, 3.2 Hz, 1H), 0.88 (s, 9H), 0.83 (s, 9H), 0.10 (s, 6H), 0.00 (s, 3H), −0.03 ppm (s, 3H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ=155.8, 153.9, 152.2, 150.0, 144.7, 134.8, 128.4, 119.3, 114.0, 87.3, 83.0, 72.9, 63.3, 55.3, 37.0, 26.0, 25.9, 18.0, 17.9, −4.6, −5.2 ppm; IR (KBr): $\tilde{\nu}$=2929, 2856, 1698, 1613, 1513, 1248, 1108, 834, 777 cm^{-1}; MS (HRFAB): m/z: calcd 616.3222, found 617.3312 [M+H$^+$].

8*N*-(3,5-Dimethylphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (13d): GP II was conducted with of **12d** (1.17 g, 1.54 mmol; reaction time 12 h), which afforded a white solid (739 mg, 78%). M.p. 185 °C; $[\alpha]_{546}^{20}$=−43° (c=0.8, CHCl$_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=10.57 (s, 1H), 8.04 (s, 1H), 7.31–7.28 (m, 2H), 6.97 (s, 1H), 6.51 (s, 2H), 6.20–6.11 (m, 1H), 4.49 (t, J=2.8 Hz, 1H), 4.02 (q, J=7.1 Hz, 1H), 3.82–3.57 (m, 2H), 3.08 (q, J=6.8 Hz, 1H), 2.23–2.19 (m, 1H), 0.86 (s, 9H), 0.83 (s, 9H), 0.7 (s, 6H), 0.00 ppm (s, 6H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ=155.9, 152.7, 150.2, 143.6, 142.0, 137.7, 119.7, 117.8, 112.2, 93.7, 87.2, 73.0, 63.2, 37.2, 30.5, 26.0, 26.0, 21.4, 18.3, 17.3, −4.7, −6.1 ppm; IR (KBr): $\tilde{\nu}$=3489, 2928, 2857, 1696, 1596, 1497, 1406, 1285, 1091, 836, 775 cm^{-1}; MS (HRFAB): m/z: calcd for: 614.3439, found 615.3510 [M+H$^+$].

8*N*-(4-Biphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (13e): GP II was conducted with **12e** (1.00 g, 1.32 mmol; reaction time

16 h), which afforded a white solid (820 mg, 93%). M.p. decomposition at 200°C; $[\alpha]_{546}^{20}=-8°$ ($c=0.3$, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=10.59$ (s, 1H), 7.87 (s, 1H), 7.83 (d, $J=8.3$ Hz, 2H), 7.60 (dd, $J=8.3$ Hz, 4H), 7.42 (dd, $J=7.6$ Hz, 2H), 7.29 (dd, $J=7.6$ Hz, 1H), 6.47 (s, 2H), 6.10 (dd, $J=6.1$, 7.6 Hz, 1H), 4.48 (ddd, $J=6.3$, 5.3, 4.3 Hz, 1H), 3.81 (dd, $J=10.2$, 4.7 Hz, 1H), 3.73 (ddd, $J=6.3$, 4.7, 4.3 Hz, 1H), 3.69 (dd, $J=10.2$, 6.3 Hz, 1H), 3.63 (ddd, $J=13.0$, 6.1, 6.8 Hz, 1H), 2.22 (ddd, $J=13.0$, 4.3, 7.6 Hz, 1H), 0.88 (s, 9H), 0.86 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H), −0.03 (s, 3H), −0.04 ppm (s, 3H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=160.4$, 159.9, 154.5, 148.6, 140.9, 139.8, 128.9, 127.6, 127.3, 127.2, 125.8, 125.5, 114.4, 82.3, 72.4, 72.3, 62.9, 37.2, 26.0, 25.9, 18.1, 17.9, −4.5, −4.7, −5.2, −5.3 ppm; IR (KBr): $\tilde{\nu}=3346$, 3173, 2954, 2928, 1647, 1603, 1537, 1486, 1383, 1169, 1075, 776 cm^{-1}; MS (HRFAB): m/z: calcd for: 662.3432, found 663.3496 [M+H$^+$].

8*N*-(2-Aminofluorenyl)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (13f): GP II was conducted with **12f** (1.95 g, 2.55 mmol; reaction time 48 h), which afforded a white solid (1.45 g, 84%). M.p. 169°C; $[\alpha]_{546}^{20}=-21°$ ($c=0.34$, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=$ 10.61 (s, 1H), 8.50 (s, 1H), 7.80 (m, 1H), 7.75 (dt, $J=7.9$, 6.0 Hz, 1H), 7.63 (dd, $J=8.3$, 1.9 Hz, 1H), 7.52–7.50 (m, 1H), 7.41 (dd, $J=8.3$, 2.0 Hz, 1H), 7.32 (dt, $J=7.6$ Hz, 1H), 7.08–7.06 (m, 1H), 6.55 (dd, $J=6.6$ Hz, 1H), 6.21 (s, 2H), 4.55–4.53 (m, 1H), 3.99 (s, 2H), 3.92–3.65 (m, 3H), 3.21 (dd, $J=6.8$ Hz, 1H), 2.11–2.09 (m, 1H), 0.87 (s, 9H), 0.84 (s, 9H), 0.09 (s, 6H), 0.08 (s, 3H), 0.01 ppm (s, 3H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=158.3$, 157.3, 152.3, 149.8, 143.9, 143.5, 140.9, 140.1, 137.0, 126.9, 125.4, 124.8, 120.1, 118.9, 115.9, 113.4, 110.3, 87.2, 83.0, 72.6, 63.1, 57.9, 36.9, 25.8, 17.9, −4.7, −5.4 ppm; IR: (KBr): $\tilde{\nu}=3357$, 2952, 2928, 1684, 1591, 1562, 1456, 1359, 1256, 836 cm^{-1}; MS (HRFAB): m/z: calcd 674.3432, found 675.3483 [M+H$^+$].

General procedure III for the desilylation and N^2-formamidine protection of 8*N*-arylamino-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine derivatives: The 8*N*-arylamino-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine derivative was dissolved in dichloromethane/tetrahydrofuran 1:1 and triethylamine (10 equiv) and triethylamine trihydrofluoride (12.5 equiv) were added. The resulting mixture was stirred at room temperature until the reaction was complete (TLC analysis). After the solvent had been removed in vacuo, the residue was purified by chromatography. The crude product, containing the deprotected adduct and triethylammonium salts, was subjected to co-evaporation of the volatiles with pyridine and was then dissolved in dry pyridine. Dimethylformamide diethyl acetal (2 equiv) was then added and the mixture was stirred at room temperature for 16 h. Thereafter, the solvent was removed in vacuo and the residue was purified by chromatography eluting with methanol (0→30%) in dichloromethane.

N^2-Formamidino-8*N*-(phenylamino)-2′-deoxyguanosine (14a): GP III was conducted with **13a** (1.41 g, 2.40 mmol; reaction time 5 h for the first step), which afforded a slightly yellow solid (800 mg, 81%). M.p. 161°C; $[\alpha]_{546}^{20}=+18.8°$ ($c=1.2$, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.26$ (s, 1H), 8.72 (s, 1H), 8.53 (s, 1H), 7.74 (dd, $J=$ 7.7 Hz, 2H), 7.26–7.21 (m, 2H), 6.92 (dd, $J=6.8$, 7.5 Hz, 1H), 6.44 (dd, $J=5.6$, 9.3 Hz, 1H), 5.87 (dd, $J=4.8$, 4.8 Hz, 1H), 5.39 (d, $J=3.9$ Hz, 1H), 4.46–4.44 (m, 1H), 3.92 (d, $J=2.2$ Hz, 2H), 3.76 (dd, $J=12.0$, 4.6 Hz, 1H), 3.17 (d, $J=5.6$ Hz, 1H), 3.15 (s, 3H), 3.02 (s, 3H), 2.09–2.04 ppm (m, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=157.1$, 156.2, 155.5, 147.7, 143.9, 140.3, 128.1, 120.4, 117.1, 115.2, 86.8, 82.3, 70.7, 60.8, 38.0, 34.2 ppm; IR (KBr): $\tilde{\nu}=3265$, 1115, 991, 960, 915, 859, 504 cm^{-1}; MS (HRFAB): m/z: calcd for: 414.1812, found 415.2543 [M+H$^+$].

N^2-Formamidino-8*N*-(4-methylphenylamino)-2′-deoxyguanosine (14b): GP III was conducted with **13b** (1.30 g, 2.16 mmol; reaction time 4 h for the first step), which afforded a slightly yellow solid (590 mg, 72%). M.p. 182°C; $[\alpha]_{546}^{20}=+28.5°$ ($c=0.33$, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.24$ (s, 1H), 8.64 (s, 1H), 8.52 (s, 1H), 7.63 (d, $J=$ 8.5 Hz, 2H), 7.06 (d, $J=8.4$ Hz, 2H), 6.42 (dd, $J=9.4$, 5.9 Hz, 1H), 5.87 (dd, $J=4.7$ Hz, 1H), 5.39 (d, $J=3.8$ Hz, 1H), 4.47–4.45 (m, 1H), 3.94–3.91 (m, 1H), 3.79–3.75 (m, 2H), 3.01 (s, 3H), 3.14 (s, 3H), 2.57 (ddd, $J=$ 13.1, 9.5, 6.4 Hz, 1H), 2.24 (s, 3H), 1.99–2.01 ppm (m, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=157.5$, 156.5, 155.8, 148.1, 144.5, 138.2, 129.5, 128.9, 117.6, 115.6, 87.2, 82.7, 71.1, 61.2, 38.3, 34.6, 20.3 ppm; IR (KBr): $\tilde{\nu}=3307$, 1667, 1632, 1533, 1344, 1114, 1061, 960, 819 cm^{-1}; MS (HRFAB): m/z: calcd for: 427.1968, found 428.2046 [M+H$^+$].

N^2-Formamidino-8*N*-(4-methoxyphenylamino)-2′-deoxyguanosine (14c): GP III was conducted with **13c** (1.14 g, 1.86 mmol; reaction time 2 h for the first step), which afforded a white solid (680 mg, 76%). M.p. 124°C; $[\alpha]_{546}^{20}=+8.4°$ ($c=0.8$, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.21$ (s, 1H), 8.57 (s, 1H), 8.51 (s, 1H), 7.64 (d, $J=9.0$ Hz, 2H), 6.85 (d, $J=9.0$ Hz, 2H), 6.42 (dd, $J=9.4$, 5.9 Hz, 1H), 5.86 (dd, $J=4.8$ Hz, 1H), 5.37 (d, $J=3.8$ Hz, 1H), 4.44 (ddd, $J=5.7$ Hz, 1H), 3.91 (ddd, $J=$ 5.7 Hz, 2H), 3.73 (dd, $J=13.2$, 5.5 Hz, 1H), 3.71 (s, 3H), 3.40 (ddd, $J=$ 13.8, 6.6 Hz, 1H), 3.14 (s, 3H), 3.01 (s, 3H), 2.04 ppm (ddd, $J=12.6$, 5.9 Hz, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=158.3$, 156.1, 153.8, 153.3, 149.9, 144.1, 120.8, 119.3, 113.9, 112.0, 87.5, 83.0, 71.7, 61.7, 55.4, 38.5, 34.7 ppm; IR (KBr): $\tilde{\nu}=3338$, 3161, 2944, 2910, 1651, 1599, 1531, 1475, 1361, 1139, 1038, 776 cm^{-1}; MS (HRFAB): m/z: calcd for: 443.4699, found 444.4802 [M+H$^+$].

N^2-Formamidino-8*N*-(3,5-dimethylphenylamino)-2′-deoxyguanosine (14d): GP III was conducted with **13d** (1.17 g, 1.75 mmol; reaction time 5 h for the first step), which afforded a yellow solid (280 mg, 46%). M.p. 151°C; $[\alpha]_{546}^{20}=-10°$ ($c=1.0$, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.23$ (s, 1H), 8.50 (s, 1H), 8.49 (s, 1H), 7.30 (s, 2H), 6.53 (s, 1H), 6.39 (dd, $J=5.9$, 6.7 Hz, 1H), 5.81 (dd, $J=4.6$, 9.2 Hz, 1H), 5.34 (d, $J=$ 3.9 Hz, 1H), 4.44–4.42 (m, 1H), 3.88 (d, $J=2.3$ Hz, 1H), 3.73–3.68 (m, 2H), 3.12 (s, 3H), 2.99 (s, 3H), 2.52–2.39 (m, 1H), 2.20 (s, 6H), 2.04–1.99 ppm (m, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=158.6$, 157.8, 156.8, 156.6, 148.5, 144.5, 140.8, 137.6, 122.3, 115.6, 87.3, 82.1, 71.2, 61.3, 38.5, 34.8, 21.5 ppm; IR (KBr): $\tilde{\nu}=3302$, 2921, 1675, 1630, 1560, 1345, 1113 cm^{-1}; MS (HRFAB): m/z: calcd for: 441.2120, found 442.2221 [M+H$^+$].

N^2-Formamidino-8*N*-(4-biphenylamino)-2′-deoxyguanosine (14e): GP III was conducted with **13e** (600 mg, 0.90 mmol; reaction time 3 h for the first step), which afforded a white solid (332 mg, 64%). M.p. 245°C; $[\alpha]_{546}^{20}=+28.9°$ ($c=0.5$, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.28$ (s, 1H), 8.68 (s, 1H), 8.57 (s, 1H), 7.85 (d, $J=$ 8.3 Hz, 2H), 7.63 (dd, $J=8.3$ Hz, 4H), 7.42 (dd, $J=7.6$ Hz, 2H), 7.29 (dd, $J=7.6$ Hz, 1H), 6.46 (dd, $J=9.3$, 5.9 Hz, 1H), 5.93 (s, 1H), 5.40 (s, 1H), 4.49–4.47 (m, 1H), 3.94 (dd, $J=12.1$ Hz, 1H), 3.79–3.77 (m, 3H), 3.07 (s, 3H), 3.06 (s, 3H), 2.08 ppm (ddd, $J=13.0$, 5.9 Hz, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=157.7$, 156.7, 156.0, 149.7, 148.3, 140.4, 140.1, 132.7, 129.0, 126.9, 126.8, 126.2, 115.8, 87.4, 83.0, 71.3, 61.4, 38.6, 34.8 ppm; IR (KBr): $\tilde{\nu}=3346$, 3173, 2954, 2928, 1647, 1603, 1537, 1486, 1383, 1169, 1075, 776 cm^{-1}; MS (HRFAB): m/z: calcd for: 489.2125, found 490.2229 [M+H$^+$].

N^2-Formamidino-8*N*-(2-aminofluorenyl)-2′-deoxyguanosine (14f): GP III was conducted with **13f** (1.35 g, 2.00 mmol; reaction time 5 h for the first step), which afforded a slightly red solid (237 mg, 31%). M.p. 209°C; $[\alpha]_{546}^{20}=+5°$ ($c=0.1$, CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.30$ (s, 1H), 8.83 (s, 1H), 8.53 (s, 1H), 8.11 (d, $J=1.3$ Hz, 1H), 7.79–7.77 (m, 2H), 7.68 (dd, $J=8.4$, 1.8 Hz, 1H), 7.53 (d, $J=7.4$ Hz, 1H), 7.33 (t, $J=7.4$ Hz, 1H), 7.22 (dt, $J=7.4$, 7.4, 1.0 Hz, 1H), 6.34 (dd, $J=6.0$, 9.3 Hz, 1H), 5.96 (t, $J=4.7$ Hz, 1H), 5.41 (d, $J=3.7$ Hz, 1H), 4.50–4.48 (m, 1H), 3.99–3.94 (m, 1H), 3.90 (s, 2H), 3.83–3.79 (m, 2H), 3.15 (s, 3H), 3.02 (s, 3H), 2.65–2.61 (m, 1H), 2.15–2.08 ppm (m, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=157.5$, 156.6, 155.9, 152.8, 148.1, 144.2, 143.8, 142.4, 141.4, 139.9, 134.1, 126.7, 125.4, 124.9, 120.0, 119.0, 116.5, 115.6, 114.0, 87.2, 82.8, 71.1, 61.3, 48.6, 36.5, 34.6 ppm; IR (KBr): $\tilde{\nu}=3283$, 2923, 1673, 1630, 1343, 1113, 1058, 946, 731 cm^{-1}; MS (HRFAB): m/z: calcd 501.2203, found 502.2237 [M+H$^+$].

General procedure IV for the 5′-*O*-dimethoxytritylation of N^2-formamidino-8*N*-arylamino-2′-deoxyguanosine derivatives: The N^2-formamidino-8*N*-arylamino-2′-deoxyguanosine derivative was twice subjected to co-evaporation of the volatiles with pyridine and then dissolved in dry pyridine. Dimethoxytrityl chloride (2 equiv) was then added and the resulting mixture was stirred at room temperature until the reaction was complete (TLC analysis). The reaction was stopped by adding saturated aqueous $NaHCO_3$ solution. The layers were separated and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were dried over sodium sulfate and the solvent was removed in vacuo. Purifi-

cation of the residue by chromatography on silica gel eluting with methanol (0→10%) in dichloromethane furnished the desired product.

N^2-Formamidino-8*N*-(phenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyguanosine (15a): GP IV was conducted with **14a** (542 mg, 1.31 mmol; reaction time 3.5 h), which afforded a slightly yellow solid (737 mg, 79%). M.p. 168°C; $[\alpha]_{546}^{20}=-2.9°$ ($c=1.0$, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.30$ (s, 1H), 8.69 (s, 1H), 8.23 (s, 1H), 7.66 (dd, $J=7.7$ Hz, 2H), 7.60 (dd, $J=7.7$ Hz, 1H), 7.33–7.30 (m, 2H), 7.20–7.13 (m, 13H), 6.37 (dd, $J=5.0$, 7.7 Hz, 1H), 5.36–5.35 (m, 1H), 4.59 (ddd, $J=5.5$ Hz, 1H), 3.90 (ddd, $J=3.1$, 6.8, 6.9 Hz, 2H), 3.70–3.68 (m, 7H), 3.17 (d, $J=4.6$ Hz, 1H), 2.99 (s, 3H), 2.97 (s, 3H), 2.24–2.20 ppm (m, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=157.2$, 156.7, 156.5, 156.4, 155.6, 155.2, 155.0, 154.7, 153.7, 153.4, 146.7, 146.3, 143.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.1, 126.5, 126.4, 126.2, 126.1, 125.1, 119.1, 115.7, 115.4, 111.8, 111.5, 84.0, 83.9, 81.0, 62.6, 36.1, 33.1 ppm; IR (KBr): $\tilde{\nu}=2931$, 2835, 914, 790, 777, 727, 583, 555, 503 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 715.3118, found 716.3202 [M+H$^+$].

N^2-Formamidino-8*N*-(4-methylphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyguanosine (15b): GP IV was conducted with **14b** (500 mg, 1.16 mmol; reaction time 5 h), which afforded a white solid (675 mg, 81%). M.p. 165°C; $[\alpha]_{546}^{20}=-9.7°$ ($c=0.69$, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.27$ (s, 1H), 8.56 (s, 1H), 8.23 (s, 1H), 7.56 (d, $J=8.5$ Hz, 2H), 7.28–7.13 (m, 8H), 7.06 (d, $J=8.4$ Hz, 2H), 6.77–6.68 (m, 5H), 6.42 (dd, $J=7.7$, 5.1 Hz, 1H), 5.34 (d, $J=3.8$ Hz, 1H), 4.50–4.48 (m, 1H), 3.93–3.90 (m, 1H), 3.77–3.72 (m, 2H), 3.67 (s, 6H), 3.21–3.19 (m, 1H), 2.99 (s, 3H), 2.96 (s, 3H), 2.24 (s, 3H), 2.19 ppm (ddd, $J=12.3$, 6.7 Hz, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=157.9$, 157.8, 157.0, 156.6, 155.1, 148.1, 144.9, 138.7, 135.5, 129.6, 129.5, 129.3, 128.9, 127.6, 126.5, 117.4, 116.8, 112.9, 85.4, 82.3, 70.6, 64.0, 54.9, 37.5, 34.5, 20.3 ppm; IR (KBr): $\tilde{\nu}=3361$, 2927, 1674, 1628, 1527, 1342, 1247, 827 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 729.3275, found 730.3353 [M+H$^+$].

N^2-Formamidino-8*N*-(4-methoxyphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyguanosine (15c): GP IV was conducted with **14c** (650 mg, 1.46 mmol; reaction time 3.5 h), which afforded a white solid (850 mg, 79%). M.p. 131°C; $[\alpha]_{546}^{20}=-10.6°$ ($c=0.76$, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.24$ (s, 1H), 8.47 (s, 1H), 8.22 (s, 1H), 7.60 (d, $J=9.0$ Hz, 2H), 7.26–7.13 (m, 10H), 6.86 (d, $J=9.0$ Hz, 2H), 6.75–6.70 (m, 5H), 6.42 (dd, $J=7.5$, 5.1 Hz, 1H), 5.34 (d, $J=5.4$ Hz, 1H), 4.58 (ddd, $J=5.4$ Hz, 1H), 4.09 (ddd, $J=5.2$, 5.4 Hz, 2H), 3.73 (dd, $J=13.2$, 5.5 Hz, 1H), 3.71 (s, 3H), 3.68 (s, 6H), 3.40 (ddd, $J=13.8$, 6.6 Hz, 1H), 3.17 (s, 3H), 3.16 (s, 3H), 2.32 ppm (ddd, $J=12.6$, 5.4 Hz, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=158.3$, 156.1, 153.8, 153.3, 149.9, 144.1, 140.6, 139.9, 135.8, 135.7, 132.6, 129.7, 129.6, 128.9, 127.8, 127.4, 126.8, 126.6, 126.4, 126.0, 120.8, 119.2, 113.9, 113.1, 87.5, 83.0, 71.7, 61.7, 55.4, 38.5, 34.7 ppm; IR (KBr): $\tilde{\nu}=3423$, 3041, 2982, 1638, 1476, 1037 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 745.3224, found 746.3215 [M+H$^+$].

N^2-Formamidino-8*N*-(3,5-dimethylphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyguanosine (15d): GP IV was conducted with **14d** (280 mg, 0.64 mmol; reaction time 12 h), which afforded a white solid (383 mg, 82%). M.p. 164°C; $[\alpha]_{546}^{20}=+10°$ ($c=1.0$, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.25$ (s, 1H), 8.41 (s, 1H), 8.20 (s, 1H), 8.05 (s, 1H), 7.24–7.10 (m, 11H), 6.68 (dd, $J=8.9$, 4.6 Hz, 4H), 6.29 (dd, $J=5.1$, 2.6 Hz, 1H), 5.28 (d, $J=4.8$ Hz, 1H), 4.52 (dd, $J=5.7$, 6.2 Hz, 1H), 3.86–3.82 (m, 2H), 3.64 (d, $J=1.1$ Hz, 6H), 3.12 (d, $J=5.2$ Hz, 1H), 2.94 (d, $J=8.9$ Hz, 6H), 2.18 (s, 7H), 1.99–1.86 (m, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=159.3$, 159.2, 159.1, 158.6, 158.4, 156.3, 152.3, 149.5, 148.0, 146.6, 146.3, 136.1, 130.6, 129.8, 128.8, 118.9, 117.8, 113.7, 113.6, 85.9, 85.3, 80.0, 64.4, 54.9, 34.9, 24.7, 20.0 ppm; IR (KBr): $\tilde{\nu}=3366$, 2921, 1675, 1629, 1560, 1342, 1249, 1113, 1032, 830 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 743.3430, found 744.3407 [M+H$^+$].

N^2-Formamidino-8*N*-(4-biphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyguanosine (15e): GP IV was conducted with **14e** (182 mg, 0.37 mmol; reaction time 3.5 h), which afforded a white solid (247 mg, 84%). M.p. 120°C; $[\alpha]_{546}^{20}=-15.4°$ ($c=0.5$, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.29$ (s, 1H), 8.81 (s, 1H), 8.24 (s, 1H), 7.65–6.70 (m, 22H), 6.39 (dd, $J=7.6$, 5.1 Hz, 1H), 5.37–5.34 (m, 1H), 4.60 (ddd, 1H), 3.91 (dd, $J=2.1$ Hz, 1H), 3.75–3.71 (m, 3H), 3.67 (s, 6H), 3.00 (s, 3H), 2.98 (s, 4H), 2.22 ppm (ddd, $J=5.1$ Hz, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=157.7$, 156.7, 156.0, 149.7, 148.3, 140.4, 140.1, 139.9, 135.8, 135.7, 132.7, 132.6, 129.7, 129.6, 129.0, 128.9, 127.8, 127.4, 126.9, 126.8, 126.2, 115.8, 87.4, 83.0, 71.3, 61.4, 55.4, 38.6, 34.8 ppm; IR (KBr): $\tilde{\nu}=3380$, 2931, 1672, 1628, 1527, 1343, 1249 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 791.3431, found 792.3535 [M+H$^+$].

N^2-Formamidino-8*N*-(2-aminofluorenyl)-5′-*O*-dimethoxytrityl-2′-deoxyguanosine (15f): GP IV was conducted with **14f** (200 mg, 0.39 mmol; reaction time 3.5 h), which afforded a slightly reddish solid (247 mg, 78%). M.p. 180°C; $[\alpha]_{546}^{20}=-11°$ ($c=0.3$, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.32$ (s, 1H), 8.79 (s, 1H), 8.25 (s, 1H), 8.03 (d, $J=1.3$ Hz, 1H), 7.78–7.76 (m, 2H), 7.56 (dd, $J=8.4$, 2.0 Hz, 1H), 7.53–6.65 (m, 16H), 6.40 (dd, $J=5.1$, 7.7 Hz, 1H), 5.35 (d, $J=3.6$ Hz, 1H), 4.61–4.59 (m, 1H), 3.96–3.91 (m, 1H), 3.88 (s, 2H), 3.69–3.67 (m, 8H), 3.22–3.18 (m, 1H), 3.00 (s, 3H), 2.98 (s, 3H), 2.27–2.22 ppm (m, 1H); ^{13}C NMR (101 MHz, [D$_6$]DMSO): $\delta=157.9$, 157.8, 157.0, 156.7, 155.9, 152.8, 148.2, 145.0, 143.8, 142.4, 141.4, 140.0, 135.6, 134.9, 133.9, 129.6, 129.5, 126.7, 126.5, 125.5, 124.9, 120.1, 119.0, 116.9, 116.2, 113.6, 112.9, 85.5, 82.5, 70.6, 64.1, 54.9, 36.5, 34.5 ppm; IR (KBr): $\tilde{\nu}=3354$, 2928, 1674, 1628, 1455, 1424, 1342, 1175, 1031, 827, 765, 701 cm^{-1}; MS (HRFAB): *m*/*z*: calcd 803.3431, found 804.3483 [M+H$^+$].

General procedure V for the phosphitylation of N^2-formamidino-8*N*-arylamino-5′-*O*-dimethoxytrityl-2′-deoxyguanosine derivatives: The N^2-formamidino-8*N*-arylamino-2′-deoxyguanosine derivative was twice subjected to co-evaporation of the volatiles with dry acetonitrile and then dissolved in a mixture of anhydrous acetonitrile and dry dichloromethane (1:1). A 0.25 M solution of dicyanoimidazole in anhydrous acetonitrile (1 equiv) and bis-*N*,*N*′-diisopropylamino-(2-cyanoethyl)phosphite (1.5 equiv) were added. The resulting mixture was stirred at room temperature until the starting material could no longer be detected (TLC analysis). The reaction was then stopped by adding saturated aqueous $NaHCO_3$ solution. The layers were separated and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were dried over sodium sulfate and the solvent was removed in vacuo. Purification of the residue by chromatography on alumina furnished the desired product, which was obtained as a fine powder after lyophilization from benzene.

N^2-Formamidino-8*N*-(phenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyguanosin-3′-yl-β-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite (16a): GP V was conducted with **15a** (200 mg, 0.21 mmol; reaction time 1 h), which afforded a white solid (140 mg, 61%). M.p. 136°C; $[\alpha]_{546}^{20}=+7°$ ($c=0.1$, $CHCl_3$); ^{1}H NMR: (400 MHz, C_6D_6): $\delta=11.29$ (s, 2H), 8.36 (s, 1H), 8.23 (s, 1H), 7.62–6.62 (m, 38H), 6.41 (dd, $J=6.1$, 6.1 Hz, 1H), 6.31 (dd, $J=6.3$, 6.3 Hz, 1H), 4.89–4.86 (m, 1H), 4.85–4.81 (m, 1H), 4.41–4.37 (m, 2H), 3.56–3.30 (m, 38H), 2.81–2.77 (m, 1H), 2.61–2.58 (m, 2H), 2.23 (ddd, 1H), 1.85 (dd, $J=6.1$, 6.1 Hz, 2H), 1.15–0.96 ppm (m, 24H); ^{13}C NMR (101 MHz, C_6D_6): $\delta=178.7$, 159.3, 159.2, 158.2, 147.6, 147.5, 145.3, 145.3, 136.3, 133.2, 130.9, 130.8, 130.7, 130.6, 130.5, 129.3, 128.8, 128.7, 128.6, 128.4, 118.4, 118.2, 114.6, 113.7, 113.6, 87.1, 86.6, 85.6, 75.0, 74.8, 74.0, 73.8, 64.1, 64.0, 58.7, 58.6, 58.5, 58.5, 55.1, 54.9, 54.8, 43.6, 43.6, 43.5, 43.5, 38.6, 38.4, 37.7, 30.2, 24.8, 24.7, 24.6, 20.6, 20.5, 20.3 ppm; ^{31}P NMR (161 MHz, C_6D_6): $\delta=148.03$, 148.21 ppm; IR (KBr): $\tilde{\nu}=3385$, 2964, 2930, 1628, 1527, 1509, 1344, 1250, 1032, 978 cm^{-1}; UV (MeCN): $\lambda_{max}=345$, 256 nm; MS (ESI): *m*/*z*: calcd for: 915.4197, found 938.4091 [M+Na$^+$].

N^2-Formamidino-8*N*-(4-methylphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyguanosin-3′-yl-β-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite (16b): GP V was conducted with **15b** (50 mg, 0.05 mmol; reaction time 1 h), which afforded a white solid (43 mg, 68%). M.p. 111°C; $[\alpha]_{546}^{20}=-21°$ ($c=0.07$, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): $\delta=12.04$ (s, 2H), 8.47 (s, 1H), 8.42 (s, 1H), 8.00–6.66 (m, 36H), 6.43 (dd, $J=6.1$, 6.1 Hz, 1H), 6.39 (dd, $J=6.3$, 6.3 Hz, 1H), 5.04–5.00 (m, 1H), 4.94–4.91 (m, 1H), 4.37–4.33 (m, 2H), 3.51–3.32 (m, 38H), 2.89–2.85 (m, 1H), 2.62–2.54 (m, 8H), 2.05 (ddd, 1H), 1.81 (dd, $J=6.2$, 6.2 Hz, 1H), 1.76–1.72 (m, 1H), 1.15–1.10 ppm (m, 24H); ^{13}C NMR (101 MHz, C_6D_6): $\delta=159.2$, 158.3, 155.5, 155.4, 148.1, 147.1, 145.3, 145.3, 136.1, 135.9, 133.9, 133.8, 130.6, 130.5, 129.7, 128.8, 127.2, 127.2, 120.4, 120.3, 118.9, 118.8, 117.3, 116.1, 114.6, 113.6, 113.6, 87.1, 86.9, 85.6, 75.0, 74.8, 74.0, 73.8, 64.1, 64.0, 54.9, 43.6, 43.6, 43.5, 43.5, 40.7, 38.6, 38.4, 36.3, 30.2, 24.6, 20.8, 19.6 ppm; ^{31}P NMR (161 MHz, C_6D_6): $\delta=148.93$, 148.87 ppm; IR (KBr): $\tilde{\nu}=3853$, 3744, 3675,

2927, 2219, 1669, 1628, 1528, 1249, 1115 cm^{-1}; UV (MeCN): λ_{max} = 330, 297 nm; MS (ESI): *m/z*: calcd for: 929.4353, found 952.4256 [*M*+Na^+].

N^2-Formamidino-8*N*-(4-methoxyphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyguanosin-3′-yl-β-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite (16c): GP V was conducted with **15c** (200 mg, 0.21 mmol; reaction time 1 h), which afforded a white solid (140 mg, 61 %). M.p. 122 °C; $[\alpha]_{546}^{20}$ = +15° (*c* = 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ = 10.99 (s, 2H), 8.35 (s, 1H), 8.31 (s, 1H), 7.68 (s, 1H), 7.66 (s, 1H), 7.59–6.71 (m, 34H), 6.44 (dd, *J* = 6.3, 6.3 Hz, 1H), 6.37 (dd, *J* = 6.6, 6.6 Hz, 1H), 4.95–4.92 (m, 1H), 4.87–4.85 (m, 1H), 4.41–4.36 (m, 2H), 3.64–3.16 (m, 44H), 2.83–2.80 (m, 1H), 2.60–2.54 (m, 2H), 2.20 (ddd, 1H), 1.89 (dd, *J* = 6.2, 6.2 Hz, 1H), 1.86–1.83 (m, 1H), 1.24–1.10 ppm (m, 24H); ^{13}C NMR (101 MHz, C_6D_6): δ = 178.7, 159.3, 159.2, 158.3, 155.5, 155.4, 147.6, 147.5, 145.3, 145.3, 136.1, 136.0, 136.0, 135.9, 133.9, 133.8, 130.7, 130.6, 130.6, 130.5, 128.8, 128.7, 128.6, 128.3, 128.2, 128.0, 127.2, 127.2, 120.4, 120.3, 118.9, 118.8, 118.2, 114.6, 113.6, 113.6, 87.1, 86.6, 86.5, 86.4, 86.3, 86.2, 85.6, 75.0, 74.8, 74.0, 73.8, 64.1, 64.0, 58.8, 58.6, 58.6, 58.5, 55.2, 54.9, 54.9, 43.6, 43.6, 43.5, 43.5, 38.6, 38.4, 37.7, 30.2, 24.8, 24.7, 24.6, 20.6, 20.5, 20.3 ppm; ^{31}P NMR (161 MHz, C_6D_6): δ = 147.34, 148.63 ppm; IR (KBr): $\tilde{\nu}$ = 3411, 2965, 2360, 1628, 1527, 1510, 1344, 1247, 1114 cm^{-1}; UV (MeCN): λ_{max} = 331, 258, 224 nm; MS (ESI): *m/z*: calcd for: 945.4302, found 968.4191 [*M*+Na^+].

N^2-Formamidino-8*N*-(3,5-dimethylphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyguanosin-3′-yl-β-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite (16d): GP V was conducted **15d** with (100 mg, 0.22 mmol; reaction time 1 h), which afforded a white solid (122 mg, 57 %). M.p. 124 °C; $[\alpha]_{546}^{20}$ = −28° (*c* = 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ = 11.61 (s, 1H), 11.43 (s, 1H), 8.51 (s, 1H), 8.43 (s, 1H), 7.65–6.54 (m, 36H), 6.46 (dd, *J* = 5.5, 5.5 Hz, 1H), 6.36 (dd, *J* = 6.2, 6.2 Hz, 1H), 5.02–4.96 (m, 1H), 4.85–4.79 (m, 1H), 4.37–4.33 (m, 2H), 3.74–3.33 (m, 26H), 2.79–2.59 (m, 13H), 2.46–2.29 (m, 3H), 2.16 (s, 6H), 2.18 (s, 6H), 1.94–1.89 (m, 2H), 1.16–0.91 ppm (m, 24H); ^{13}C NMR (101 MHz, C_6D_6): δ = 159.3, 159.2, 158.4, 158.0, 156.2, 146.1, 145.4, 145.3, 140.9, 140.7, 138.7, 138.6, 136.1, 116.5, 116.4, 113.7, 87.1, 86.8, 85.0, 84.8, 78.6, 78.7, 73.9, 73.1, 63.6, 62.9, 59.1, 57.2, 56.8, 54.9, 43.7, 43.6, 43.5, 41.1, 39.8, 34.8, 34.7, 24.7, 24.6, 24.5, 21.6, 21.5, 21.4, 20.6, 20.2, 20.1, 16.0 ppm; ^{31}P NMR (161 MHz, C_6D_6): δ = 149.3, 148.7 ppm; IR (KBr): $\tilde{\nu}$ = 3411, 2965, 1686, 1629, 1528, 1341, 1250, 1103 cm^{-1}; MS (ESI): *m/z*: calcd for: 943.4510, found 965.7158 [*M*+Na^+].

N^2-Formamidino-8*N*-(4-biphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyguanosin-3′-yl-β-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite (16e): GP V was conducted with **15e** (45 mg, 0.05 mmol; reaction time 1 h), which afforded a yellow solid (30 mg, 60 %). M.p. 64 °C; $[\alpha]_{546}^{20}$ = +13° (*c* = 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ = 12.23 (s, 2H), 8.36 (s, 1H), 8.34 (s, 1H), 7.96 (s, 2H), 7.52–6.67 (m, 44H), 6.34 (dd, *J* = 6.6, 6.6 Hz, 1H), 6.29 (dd, *J* = 6.3, 6.3 Hz, 1H), 5.04–5.00 (m, 2H), 4.35–4.29 (m, 2H), 3.64–3.29 (m, 38H), 2.84–2.80 (m, 1H), 2.37–2.31 (m, 3H), 1.85–1.81 (m, 2H), 0.99–0.96 ppm (m, 24H); ^{13}C NMR (101 MHz, C_6D_6): δ = 177.3, 158.2, 158.1, 157.3, 154.6, 154.2, 146.6, 146.5, 144.3, 144.2, 136.1, 136.0, 136.0, 135.9, 133.9, 133.8, 130.7, 130.6, 130.6, 130.5, 128.8, 128.7, 128.6, 128.3, 128.2, 128.0, 127.2, 127.2, 120.4, 120.3, 118.9, 118.8, 118.2, 114.6, 113.6, 113.6, 87.1, 86.6, 86.5, 86.4, 86.3, 86.2, 85.6, 75.0, 74.8, 74.0, 73.8, 64.1, 64.0, 58.8, 58.6, 58.6, 58.5, 55.2, 46.6, 46.6, 46.5, 46.5, 38.6, 38.4, 37.7, 30.2, 24.8, 24.7, 24.6, 19.6, 19.5, 19.3 ppm; ^{31}P NMR (161 MHz, C_6D_6): δ = 149.04, 149.01 ppm; IR (KBr): $\tilde{\nu}$ = 3422, 2926, 1629, 1529, 1384, 1249, 1103 cm^{-1}; UV (MeCN): λ_{max} = 330, 296, 251, 226 nm; MS (ESI): *m/z*: calcd for: 991.4510, found 1014.4410 [*M*+Na^+].

N^2-Formamidino-8*N*-(2-aminofluorenyl)-5′-*O*-dimethoxytrityl-2′-deoxyguanosin-3′-yl-β-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite (16f): GP V was conducted **15f** with (50 mg, 0.06 mmol; reaction time 1 h), which afforded a white solid (60 mg, 88 %). M.p. 112 °C; $[\alpha]_{578}^{20}$ = +3° (*c* = 0.21, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ = 11.05 (s, 2H), 8.39 (s, 2H), 8.10 (s, 1H), 8.08 (s, 1H), 8.05–8.02 (m, 2H), 7.81–7.76 (m, 4H), 7.62–7.36 (m, 26H), 7.28 (d, *J* = 7.3 Hz, 2H), 7.23–7.21 (m, 2H), 7.09–7.03 (m, 4H), 6.59 (dd, *J* = 6.7 Hz, 1H), 6.53 (dd, *J* = 6.1 Hz, 1H), 5.05–5.01 (m, 1H), 4.94–4.91 (m, 1H), 4.44–4.39 (m, 2H), 3.74–3.70 (m, 10H), 3.39–3.33 (m, 12H), 2.69–2.63 (m, 14H), 1.89 (ddd, *J* = 5.6, 11.5 Hz, 2H), 1.12 (dd, *J* = 6.1 Hz, 12H), 1.06 (d, *J* = 6.7 Hz, 6H), 0.93 ppm (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (101 MHz, C_6D_6): δ = 160.7, 159.2, 158.2, 157.9, 156.1, 155.7, 149.3, 143.4, 136.1, 135.8, 130.6, 128.8, 128.6, 128.5, 128.3, 128.0, 127.8, 113.6, 54.9, 43.6, 34.7, 24.7, 24.6, 21.2, 20.5, 20.1, 8.4 ppm; ^{31}P NMR (202 MHz, C_6D_6): δ = 162.0, 161.78 ppm; IR (KBr): $\tilde{\nu}$ = 3377, 2966, 2930, 2836, 2760, 2722, 1681, 1607, 1575, 1457, 1427, 1344, 1301, 1251, 1178, 1154, 1115, 1076, 1033, 978, 829, 767, 732, 703 cm^{-1}; UV (MeCN): λ_{max} = 336 nm; MS (ESI): *m/z*: calcd for: 1003.4823, found 1026.4440 [*M*+Na^+].

8-Bromo-2′-deoxyadenosine (17) was prepared as described previously.[46]

8-Bromo-3′,5′-*O*-tetrakisisopropyldisiloxanediyl-2′-deoxyadenosine (18): 8-Bromo-2′-deoxyadenosine (**17**; 515 mg, 1.56 mmol) was subjected to co-evaporation of the volatiles with anhydrous pyridine three times, and then suspended in anhydrous pyridine (10 mL) under a nitrogen atmosphere. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2) (530 μL, 526 mg, 1.67 mmol) was then added and the reaction mixture was stirred for 16 h at room temperature. The reaction was stopped by the addition of methanol (1 mL). The mixture was diluted with dichloromethane and washed once with water and once with brine. The solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel (5 % MeOH in CH_2Cl_2) and recrystallized from hexane to give **18** as a colourless solid (880 mg, 1.54 mmol, 98 %). M.p. 158 °C; $[\alpha]_{546}^{20}$ = −53.9° (*c* = 0.750, $CHCl_3$); ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.22 (s, 1H), 6.29 (dd, *J* = 9.0, 2.9 Hz, 1H), 5.76 (s, 2H), 5.47 (dd, *J* = 14.9, 8.0 Hz, 1H), 4.01–3.93 (m, 2H), 3.89 (ddd, *J* = 6.4, 4.4 Hz, 1H), 3.22 (ddd, *J* = 13.5, 8.9, 2.9 Hz, 1H), 2.60 (ddd, *J* = 13.4, 8.5, 4.2 Hz, 1H), 1.26–1.05 ppm (m, 28H); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 159.8, 152.5, 150.4, 122.2, 85.6, 84.5, 72.2, 63.1, 38.6, 17.7–17.1, 13.4–12.7 ppm; IR (KBr): $\tilde{\nu}$ = 3329, 3174, 2945, 2893, 1662, 1602, 1574, 1461, 1388, 1351, 1321, 1299, 1280, 1252, 1178, 1139, 1097, 1035, 885, 693 cm^{-1}; MS (HRFAB): *m/z*: calcd for: 571.1646, found 572.1723 [*M*+H^+].

General procedure VIa for the amination of 3′,5′-*O*-silyl-protected 8-Br-dA derivatives (19a,b): Racemic 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (*rac*-BINAP; 30 mol %), tris(benzylideneacetone)dipalladium(0) ([Pd_2dba_3]; 10 mol %), **18**, the amine (2 equiv), and Cs_2CO_3 (1.5 equiv) were dissolved in anhydrous 1,2-dimethoxyethane (50 mL) and the mixture was stirred under reflux until the reaction was complete (30–48 h). The reaction mixture was allowed to cool to room temperature, whereupon saturated sodium hydrogencarbonate solution (1 mL) was added. After the addition of brine (10 mL), the layers were separated and the aqueous layer was extracted with ethyl acetate (3×10 mL). The combined organic layers were washed with brine (2×10 mL) and with a mixture of brine (10 mL) and water (2 mL). The organic layer was dried over sodium sulfate and the solvent was removed in vacuo. Purification of the residue by flash chromatography on silica gel, eluting with 0→50 % ethyl acetate in hexane, gave the desired product.

8*N*-(4-Methoxyphenylamino)-3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-2′-deoxyadenosine (19a): GP VIa was conducted with **18** (2.34 g, 4.09 mmol), which afforded the desired product as a pale-yellow foam (1.85 g, 3.01 mmol, 74 %). M.p. 59–61 °C; $[\alpha]_{546}^{20}$ = −29.0° (*c* = 0.400, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.16 (s, 1H), 7.48 (m, 2H), 7.41 (br s, 1H), 6.88 (m, 2H), 6.31 (dd, *J* = 7.8, 3.8 Hz, 1H), 5.34 (br s, 2H), 4.90 (dd, *J* = 15.2, 7.9 Hz, 1H), 4.16 (dd, *J* = 9.2, 3.4 Hz, 1H), 3.98 (dd, *J* = 12.5, 5.0 Hz, 1H), 3.90 (ddd, *J* = 7.0, 4.9, 3.4 Hz, 1H), 3.79 (s, 3H), 3.06 (ddd, *J* = 13.4, 8.1, 3.6 Hz, 1H), 2.59 (ddd, *J* = 13.4, 7.9 Hz, 1H), 1.13–0.89 ppm (m, 28H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.8, 152.5, 149.4, 132.5, 121.5, 117.4, 114.5, 85.3, 83.8, 70.5, 61.9, 55.7, 38.8, 17.6–17.0, 13.5–12.6 ppm; IR (KBr): $\tilde{\nu}$ = 3378, 2945, 2867, 1638, 1607, 1561, 1511, 1465, 1420, 1342, 1286, 1246, 1180, 1114, 1037, 886, 827, 780, 697, 598, 460 cm^{-1}; MS (HRFAB): *m/z*: calcd for: 614.3068, found 615.3149 [*M*+H^+].

8*N*-(4-Aminobiphenyl)-3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-2′-deoxyadenosine (19b): GP VIa was conducted with **18** (2.35 g, 4.10 mmol), which afforded the desired product as a pale-yellow foam (1.76 g, 2.67 mmol, 65 %). M.p. 102 °C; $[\alpha]_{546}^{20}$ = −58.4° (*c* = 0.125, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19 (s, 1H), 7.78 (br s, 1H), 7.69 (m, 2H), 7.58 (m, 4H), 7.44 (m, 2H), 7.33 (m, 1H), 6.35 (dd, *J* = 7.5, 3.8 Hz, 1H), 5.59 (s, 2H), 4.88 (dd, *J* = 15.2, 7.9 Hz, 1H), 4.16 (dd, *J* = 12.6, 3.6 Hz, 1H), 4.04 (dd, *J* = 12.5, 4.8 Hz, 1H), 3.95 (ddd, *J* = 7.3, 4.4, 3.0 Hz, 1H), 3.07 (ddd, *J* = 13.4, 8.0, 3.6 Hz, 1H), 2.59 (ddd, *J* = 13.4, 7.8 Hz, 1H), 1.12–0.91 ppm (m, 28H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.7, 152.4, 149.7, 148.3, 140.9, 132.5, 121.5, 117.4, 114.5, 85.3, 83.8, 70.5, 61.9, 55.7,

38.8, 17.6–17.0, 13.5–12.6 ppm; IR (KBr): $\tilde{\nu}$ = 3367, 2364, 1972, 1954, 1908, 1892, 1811, 1659, 1624, 1577, 1495, 1474, 1449, 1179, 1123, 1073, 1001, 918, 847, 771, 703, 685, 650, 635, 614, 530, 414 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 660.3276, found 661.3377 [*M*+H$^+$].

General procedure VIb for the amination of 3′,5′-*O*-silyl-protected 8-Br-dA derivatives (19c,d): This procedure was the same as **VIa**, but with a pre-incubation of Pd_2dba_3 and *rac*-BINAP for 1 h.

8*N*-(3,5-Dimethylphenylamino)-3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-2′-deoxyadenosine (19c): GP VIb was conducted with **18** (829 mg, 1.45 mmol), which afforded the desired product as a pale-yellow foam (818 mg, 1.33 mmol, 92 %). M.p. 184 °C; $[\alpha]_{546}^{20} = -37°$ (*c* = 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.18 (s, 1H), 7.99 (s, 1H), 7.41 (s, 1H), 7.18 (s, 2H), 6.29 (dd, *J* = 7.5, 4.0 Hz, 1H), 5.30 (s, 2H), 4.88 (dd, *J* = 14.3, 7.5 Hz, 1H), 3.93–3.91 (m, 2H), 3.89–3.84 (m, 1H), 3.11 (ddd, *J* = 13.4, 8.0, 3.9 Hz, 1H), 2.60–2.52 (m, 1H), 2.31 (s, 6H), 1.11–1.03 (m, 24H), 0.93 ppm (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.5, 150.4, 146.9, 142.8, 139.1, 138.9, 124.8, 119.4, 116.8, 85.3, 84.0, 70.9, 62.3, 38.9, 21.6, 17.9, 17.8, 17.7, 17.6, 17.5, 17.5, 17.4, 17.4, 17.3, 13.9, 13.5, 13.1, 12.9 ppm; IR (KBr): $\tilde{\nu}$ = 3370, 3178, 3055, 2944, 2866, 1637, 1567, 1465, 1437, 1387, 1336, 1289, 1251, 1186, 1116, 1035, 947, 920, 885, 836, 776, 746, 699, 560, 575, 541, 524, 458 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 612.3276, found 613.3339 [*M*+H$^+$].

8*N*-(2-Aminofluorenyl)-3′,5′-*O*-(1,1,3,3-tetrakis(isopropyl)-1,3-disiloxanediyl)-2′-deoxyadenosine (19d): GP VIb was conducted with **18** (331 mg, 0.579 mmol), which afforded the desired product as a pale-yellow foam (362 mg, 0.538 mmol, 93 %). M.p. 99 °C; $[\alpha]_{546}^{20} = -27°$ (*c* = 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.21 (s, 1H), 7.91 (br s, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 7.8 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 1H), 7.31 (s, 1H), 7.18 (dd, *J* = 7.4, 7.4 Hz, 2H), 6.35 (dd, *J* = 7.5, 3.8 Hz, 1H), 5.22 (s, 2H), 4.89 (dd, *J* = 15.0, 7.9 Hz, 1H), 4.12 (dd, *J* = 14.3, 7.0 Hz, 2H), 4.07–4.02 (m, 1H), 3.97–3.94 (m, 2H), 3.13–3.07 (m, 1H), 2.61–2.54 (m, 1H), 1.11–1.04 (m, 18H), 0.96–0.91 ppm (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.3, 149.7, 149.3, 148.8, 144.8, 143.3, 141.9, 138.3, 137.2, 128.5, 127.2, 126.4, 125.3, 120.6, 119.7, 118.6, 117.6, 85.6, 84.4, 70.4, 62.0, 45.8, 39.8, 17.9, 17.8, 17.7, 17.6, 17.5, 17.5, 17.4, 17.4, 17.3, 13.9, 13.5, 13.1, 12.9 ppm; IR (KBr): $\tilde{\nu}$ = 3365, 3189, 3060, 2945, 2866, 1708, 1637, 1600, 1560, 1493, 1459, 1430, 1404, 1331, 1288, 1251, 1184, 1117, 1038, 946, 919, 885, 826, 765, 731, 698, 580, 422 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 672.3276, found 673.3370 [*M*+H$^+$].

General procedure VII for the N^6-benzoylation and 3′,5′-desilylation of C8-arylamine-dA adducts (20a,b): The 3′,5′-*O*-silyl-protected C8-arylamine-modified dA derivative **19a,b** was dissolved in anhydrous pyridine (30 mL) under an atmosphere of nitrogen and distilled benzoyl chloride (1.5 equiv) was added. The reaction mixture was stirred for 2 h at room temperature. It was then diluted with dichloromethane (200 mL) and washed with saturated sodium hydrogencarbonate solution, and the aqueous layer was extracted twice with dichloromethane. After removal of the dichloromethane in vacuo, morpholine (2.5 equiv) was added and the resulting mixture was stirred at room temperature for a further 2 h. The reaction mixture was again diluted with dichloromethane (200 mL) and then washed twice with 0.5 M sodium dihydrogenphosphate solution. The aqueous layer was extracted three times with dichloromethane. After complete removal of the dichloromethane in vacuo, the residue was dissolved in tetrahydrofuran and tetrabutylammonium fluoride (2.5 equiv) was added. After complete desilylation, the solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel, eluting with dichloromethane containing 10 % methanol, to yield the product.

N^6-Benzoyl-8*N*-(4-aminobiphenyl)-2′-deoxyadenosine (20a): GP VII was conducted with **19a** (2.30 g, 3.74 mmol), which gave the desired product as a pale-yellow solid (1.28 g, 2.69 mmol, 72 %). M.p. 161 °C; $[\alpha]_{546}^{20} = -27°$ (*c* = 1, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ = 8.44 (s, 1H), 7.97 (m, 2H), 7.59 (tt, *J* = 7.4, 1.3 Hz, 1H), 7.49–7.43 (m, 4H), 6.84 (d, *J* = 8.9 Hz, 2H), 6.73 (dd, *J* = 9.0, 5.7 Hz, 1H), 4.64–4.58 (m, 1H), 4.14–4.10 (m, 1H), 3.94 (dd, *J* = 11.7, 2.5 Hz, 1H), 3.88 (dd, *J* = 11.7, 1.7 Hz, 1H), 3.73 (s, 3H; OMe), 2.81 (ddd, *J* = 13.5, 9.4, 6.1 Hz, 1H), 2.27 ppm (ddd, *J* = 13.4, 5.9, 1.7 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 156.2, 152.2, 150.1, 135.0, 132.6, 131.8, 129.5, 129.0, 128.8, 128.0, 123.1, 114.4, 87.9, 84.5, 72.4, 63.3, 55.7, 39.5 ppm; IR (KBr): $\tilde{\nu}$ = 3353, 3060, 2932, 2835, 2550, 2348, 2254, 2125, 2048, 1911, 1692, 1654, 1611, 1565, 1444, 1248, 1176, 1028, 828, 703, 582 cm^{-1}; MS (HRESI): *m*/*z*: calcd for: 476.1808, found 499.1706 [*M*+Na$^+$].

N^6-Benzoyl-8*N*-(4-aminobiphenyl)-2′-deoxyadenosine (20b): GP VII was conducted with **19b** (1.60 g, 2.42 mmol), which gave the desired product as a pale-yellow solid (906 mg, 1.89 mmol, 78 %). M.p. 151 °C; $[\alpha]_{546}^{20} = +4.3°$ (*c* = 0.5, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): δ = 8.41 (s, 1H), 7.91–7.85 (m, 2H), 7.69–7.64 (m, 2H), 7.49 (tt, *J* = 7.4, 1.2 Hz, 1H), 7.47–7.43 (m, 4H), 7.42–7.39 (m, 2H), 7.36–7.30 (m, 2H), 7.24 (tt, *J* = 7.4, 1.2 Hz, 1H), 6.71 (dd, *J* = 9.4, 5.8 Hz, 1H), 4.62–4.55 (m, 1H), 4.13–4.08 (m, 1H), 3.94 (dd, *J* = 11.6, 2.4 Hz, 1H), 3.87 (dd, *J* = 11.7, 2.5 Hz, 1H), 2.74 (ddd, *J* = 13.5, 9.4, 6.2 Hz, 1H), 2.24 ppm (ddd, *J* = 13.4, 5.9, *J* = 1.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 168.3, 154.0, 150.0, 141.7, 138.0, 135.4, 133.5, 129.8, 129.6, 129.2, 128.5, 128.1, 127.6, 122.7, 120.1, 89.3, 85.4, 73.4, 62.8, 39.9 ppm; IR (KBr): $\tilde{\nu}$ = 3447, 3277, 3050, 3031, 2927, 2874, 2362, 2344, 1719, 1709, 1696, 1685, 1624, 1555, 1486, 1448, 1271, 1174, 1088, 836, 763, 697 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 522.2016, found 523.2094 [*M*+H$^+$].

General procedure VIII for the 5′-*O*-dimethoxytritylation of N^6-benzoylated C8-arylamine-dA adducts (21a,b): The N^6-benzoylated C8-arylamine-dA adduct (**20a,b**) was dissolved in anhydrous pyridine (30 mL) under a nitrogen atmosphere and 4,4′-dimethoxytrityl chloride (1.2 equiv) and silver nitrate (1.2 equiv) were added. The mixture was stirred at room temperature until the reaction was complete (≈3 h). It was then diluted with dichloromethane (200 mL) and washed with saturated sodium hydrogencarbonate solution and brine. The aqueous layer was extracted twice with dichloromethane. The organic layers were combined, dried over sodium sulfate, and filtered, and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel eluting with 0→10 % methanol in dichloromethane to afford the desired product.

N^6-Benzoyl-8*N*-(4-methoxyphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyadenosine (21a): GP VIII was conducted with **20a** (1.10 g, 2.31 mmol), which gave the desired product as a pale-yellow foam (1.32 g, 1.69 mmol, 73 %). M.p. 93 °C; $[\alpha]_{546}^{20} = -37.2°$ (*c* = 0.5, CH_3OH); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.78 (s, 1H), 9.32 (s, 1H), 8.27 (s, 1H), 8.04–7.99 (m, 2H), 7.78 (d, *J* = 9.2 Hz, 2H), 7.64–7.58 (m, 1H), 7.56–7.50 (m, 2H), 7.33–7.27 (m, 2H), 7.22–7.14 (m, 7H), 6.86 (d, *J* = 9.2 Hz, 2H), 6.81–6.74 (m, 4H), 6.53 (dd, *J* = 9.0, 2.9 Hz, 1H), 5.41 (d, *J* = 4.3 Hz, 1H), 4.74–4.67 (m, 1H), 4.01 (dd, *J* = 9.8, 4.7 Hz, 1H), 3.70 (s, 3H), 3.69 (s, 6H), 3.59–3.49 (m, 1H), 3.25 (dd, *J* = 10.0, 4.2 Hz, 1H), 3.15 (dd, *J* = 10.0, 6.0 Hz, 1H), 2.31–2.22 ppm (m, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.3, 158.0, 154.7, 152.8, 150.4, 147.9, 145.0, 144.6, 135.8, 135.6, 134.0, 132.8, 129.7, 129.6, 128.4, 128.3, 127.6, 126.5, 126.2, 120.8, 113.8, 113.0, 85.6, 85.3, 82.9, 70.8, 63.7, 55.2, 55.0, 36.4 ppm; IR (KBr): $\tilde{\nu}$ = 3356, 3057, 2999, 2932, 2835, 2361, 1735, 1670, 1609, 1563, 1462, 1444, 1372, 1301, 1177, 1034, 910, 828, 791, 754, 726, 617, 584 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 778.3115, found 779.3193 [*M*+H$^+$].

N^6-Benzoyl-8*N*-(4-aminobiphenyl)-5′-*O*-dimethoxytrityl-2′-deoxyadenosine (21b): GP VIII was conducted with **20b** (880 mg, 1.684 mmol), which gave the desired product as a pale-yellow foam (1.056 g, 1.280 mmol, 76 %). M.p. decomposition at 132 °C; $[\alpha]_{546}^{20} = +14°$ (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.86 (br s, 1H), 9.60 (br s, 1H), 8.32 (s, 1H), 8.07–7.93 (m, 4H), 7.67–7.59 (m, 5H), 7.57–7.51 (m, 2H), 7.45–7.38 (m, 2H), 7.33–7.27 (m, 3H), 7.22–7.14 (m, 7H), 6.81–6.74 (m, 4H), 6.62–6.56 (m, 1H), 5.43 (dd, *J* = 4.3 Hz), 4.76–4.69 (m, 1H), 4.06–4.00 (m, 1H), 3.69 (s, 3H), 3.68 (s, 3H), 3.60–3.51 (m, 1H), 3.29–3.23 (m, 1H), 3.20–3.13 (m, 1H), 2.34–2.25 ppm (m, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 165.3, 158.0, 149.7, 148.3, 145.2, 145.0, 139.8, 139.3, 135.8, 135.6, 133.9, 133.8, 132.1, 129.7, 129.6, 128.9, 128.5, 128.3, 127.7, 126.8, 126.2, 125.8, 119.3, 113.1, 85.7, 83.1, 70.7, 63.7, 55.0, 36.4 ppm; IR (KBr): $\tilde{\nu}$ = 3416, 3056, 2931, 2834, 2348, 2282, 1701, 1607, 1577, 1557, 1508, 1487, 1445, 1339, 1299, 1250, 1176, 1047, 1033, 828, 700, 583 cm^{-1}; MS (HRFAB): *m*/*z*: calcd 824.3322, found 825.3424 [*M*+H$^+$].

General procedure IX for phosphitylation of N^6-benzoyl-(8*N*-arylamino)-5′-*O*-dimethoxytrityl-2′-deoxyadenosine (22a,b): The N^6-Bz-8-*N*-(arylamino)-5′-*O*-DMTr-2′-dA (**21a,b**) was dissolved in anhydrous CH_2Cl_2

(4 mL) and anhydrous CH_3CN (3 mL) under an atmosphere of nitrogen and treated with a 0.25 M solution of 4,5-dicyanoimidazole in CH_3CN (2 equiv) and 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (1.5 equiv). After stirring the mixture for 30 min at room temperature, the reaction was stopped by adding CH_3OH (0.5 mL). The solution was diluted with CH_2Cl_2 (50 mL) and washed sequentially with 5% aqueous $NaHCO_3$ solution and brine. The organic layer was dried and concentrated to dryness. The residue was purified by chromatography on silica gel, eluting with CH_2Cl_2 containing 2% NEt_3. The product was redissolved in CH_2Cl_2 and further washed with H_2O to give the product as a solid after freeze-drying from benzene.

N^6-Benzoyl-8*N*-(4-methoxyphenylamino)-5′-*O*-dimethoxytrityl-2′-deoxyadenosin-3′-yl-β-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite (22a): GP IX was conducted with **21a** (200 mg, 0.257 mmol), which gave the desired product as a pale-yellow solid (189 mg, 0.193 mmol, 75%). M.p. decomposition at 82 °C; ^{1}H NMR (400 MHz, C_6D_6): 2 diastereomers + 2 rotamers: δ = 9.55 (m, 2H), 9.18 (s, 1H), 9.16 (s, 1H), 8.90 (m, 2H), 8.67 (s, 1H), 8.66 (s, 1H), 8.29 (s, 1H), 8.27 (s, 1H), 7.91 (m, 2H), 7.82 (m, 5H), 7.75 (m, 3H), 7.70–7.64 (m, 4H), 7.62–7.54 (m, 6H), 7.53–7.43 (m, 8H), 7.32 (m, 12H), 7.32 (m, 5H), 7.16–7.08 (m, 8H), 7.06–6.87 (m, 22H), 6.80–6.64 (m, 24H), 6.13–5.99 (m, 2H), 5.91 (m, 2H), 5.25 (m, 2H), 5.07 (m, 4H), 4.64–4.50 (m, 2H), 4.41 (m, 2H), 3.93–3.81 (m, 4H), 3.75–3.61 (m, 4H), 3.62–3.44 (m, 16H), 3.43–3.22 (m, 36H), 2.53–2.28 (m, 4H), 2.00–1.68 (m, 12H), 1.23–1.11 ppm (m, 48H); ^{13}C NMR (100 MHz, C_6D_6): δ = 165.9, 165.1, 159.2, 159.2, 159.9, 154.1, 152.6, 151.1, 150.1, 149.4, 146.1, 145.9, 145.3, 144.2, 142.0, 136.9, 136.8, 136.0, 135.8, 135.0, 132.7, 132.6, 130.9, 130.8, 130.5, 128.6, 127.1, 127.0, 124.0, 123.4, 121.6, 121.5, 117.8, 117.6, 115.4, 114.6, 113.6, 113.5, 111.7, 86.9, 86.8, 86.0, 85.4, 82.5, 82.3, 64.9, 63.4, 63.2, 59.2, 59.1, 59.0, 58.8, 58.6, 55.0, 54.9, 54.9, 43.6, 43.6, 37.5, 37.2, 30.5, 30.2, 24.8, 24.8, 24.7, 20.2, 20.1, 20.1 ppm; ^{31}P NMR (161 MHz, C_6D_6): δ = 149.20, 148.95, 148.84, 148.20 ppm; IR (KBr): $\tilde{\nu}$ = 3426, 3061, 2964, 2930, 2362, 2344, 2253, 1701, 1686, 1676, 1670, 1664, 1655, 1618, 1612, 1569, 1511, 1491, 1483, 1250, 1178, 1034, 829, 704, 582 cm^{-1}; MS (HRESI): *m*/*z*: calcd for: 978.4193, found 1001.4120 [*M*+Na$^+$].

N^6-Benzoyl-8*N*-(4-aminobiphenyl)-5′-*O*-dimethoxytrityl-2′-deoxyadenosin-3′-yl-β-cyanoethyl-*N*,*N*′-diisopropylphosphoramidite (22b): GP IX was conducted with **21b** (200 mg, 0.242 mmol), which gave the desired product as a pale-yellow solid (171 g, 0.167 mmol, 69%). M.p. 77–79 °C; ^{1}H NMR (400 MHz, C_6D_6): 2 diastereomers + 2 rotamers: δ = 9.87 (m, 2H), 8.94 (s, 1H), 8.92 (s, 1H), 8.85 (s, 1H), 8.84 (s, 1H), 8.60 (m, 2H), 8.44 (s, 1H), 8.42 (s, 1H), 8.24 (s, 1H), 8.21 (s, 1H), 8.03 (m, 4H), 7.92 (m, 4H), 7.86 (m, 4H), 7.69 (m, 12H), 7.63–7.49 (m, 20H), 7.40 (m, 12H), 7.32 (m, 12H), 7.22 (m, 10H), 7.18–7.04 (m, 12H), 6.97 (m, 2H), 6.85 (m, 8H), 6.77 (m, 10H), 6.03–5.87 (m, 2H), 5.34–5.17 (m, 2H), 5.15–4.99 (m, 2H), 4.66–4.54 (m, 2H), 4.41 (m, 2H), 3.88 (m, 4H), 3.74 (m, 2H), 3.66–3.57 (m, 10H), 3.31 (m, 12H), 3.30 (m, 12H), 3.16–3.00 (m, 8H), 2.62–2.28 (m, 4H), 1.97–1.70 (m, 10H), 1.10–1.01 ppm (m, 48H); ^{13}C NMR (100 MHz, C_6D_6): δ = 159.2, 149.7, 141.1, 136.0, 135.9, 134.9, 132.6, 131.9, 130.8, 130.6, 129.0, 129.0, 128.7, 128.6, 127.9, 127.1, 127.0, 119.7, 117.6, 117.0, 116.3, 113.6, 113.5, 87.0, 86.8, 85.8, 59.2, 59.0, 58.0, 58.0, 54.8, 45.4, 45.3, 43.7, 43.6, 24.8, 24.8, 24.7, 24.6, 23.7, 22.9, 22.8, 22.8, 22.8, 20.6, 20.1, 20.1, 19.5, 19.4 ppm; ^{31}P NMR (161 MHz, C_6D_6): δ = 149.37, 149.11, 148.69, 148.38 ppm; IR (KBr): $\tilde{\nu}$ = 3377, 3057, 3032, 2966, 2931, 2872, 2836, 2362, 2344, 2252, 1734, 1700, 1607, 1508, 1250, 1179, 1034, 978, 829, 700, 526, 419, 406 cm^{-1}; MS (HRFAB): *m*/*z*: calcd for: 1024.4401, found 1025.4433 [*M*+H$^+$].

Synthesis of the oligonucleotides: Oligonucleotides were synthesized on a 1 μmol scale using benzoyl-protected dA, dmf-protected dG, acetyl-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 a duration of 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.

ESI-MS for **23a**: *m*/*z*: calcd for [*M*−H$^+$]: 3582.4, found 3581.2
ESI-MS for **23b**: *m*/*z*: calcd for [*M*−H$^+$]: 3703.4, found 3702.3
ESI-MS for **23c**: *m*/*z*: calcd for [*M*−H$^+$]: 3750.4, found 3751.8
ESI-MS for **23d**: *m*/*z*: calcd for [*M*−H$^+$]: 3566.4, found 3565.2
ESI-MS for **23e**: *m*/*z*: calcd for [*M*−H$^+$]: 3688.6, found 3687.3
ESI-MS for **23f**: *m*/*z*: calcd for [*M*−H$^+$]: 3734.6, found 3733.2
ESI-MS for **24a**: *m*/*z*: calcd for [*M*−H$^+$]: 9099.5, found 9101.2
ESI-MS for **24b**: *m*/*z*: calcd for [*M*−H$^+$]: 9191.6, found 9192.5
ESI-MS for **24c**: *m*/*z*: calcd for [*M*−H$^+$]: 9205.6, found 9206.5
ESI-MS for **24d**: *m*/*z*: calcd for [*M*−H$^+$]: 9221.6, found 9220.0
ESI-MS for **24e**: *m*/*z*: calcd for [*M*−H$^+$]: 9219.7, found 9220.1
ESI-MS for **24f**: *m*/*z*: calcd for [*M*−H$^+$]: 9271.2, found 9273.2
ESI-MS for **24g**: *m*/*z*: calcd for [*M*−H$^+$]: 9283.2, found 9284.5
ESI-MS for **24h**: *m*/*z*: calcd for [*M*−H$^+$]: 9087.0, found 9086.5
ESI-MS for **24i**: *m*/*z*: calcd for [*M*−H$^+$]: 9209.2, found 9208.6
ESI-MS for **24j**: *m*/*z*: calcd for [*M*−H$^+$]: 9255.2, found 9253.9
ESI-MS for **25a**: *m*/*z*: calcd for [*M*−H$^+$]: 3643.4, found 3644
ESI-MS for **25b**: *m*/*z*: calcd for [*M*−H$^+$]: 3736.6, found 3737
ESI-MS for **25c**: *m*/*z*: calcd for [*M*−H$^+$]: 3749.6, found 3746
ESI-MS for **25d**: *m*/*z*: calcd for [*M*−H$^+$]: 3765.6, found 3766
ESI-MS for **25e**: *m*/*z*: calcd for [*M*−H$^+$]: 3764.7, found 3764
ESI-MS for **25f**: *m*/*z*: calcd for [*M*−H$^+$]: 3812.7, found 3810.5
ESI-MS for **25g**: *m*/*z*: calcd for [*M*−H$^+$]: 3822.7, found 3820.5
ESI-MS for **25h**: *m*/*z*: calcd for [*M*−H$^+$]: 3736.6, found 3737
ESI-MS for **25i**: *m*/*z*: calcd for [*M*−H$^+$]: 3812.7, found 3809
ESI-MS for **25j**: *m*/*z*: calcd for [*M*−H$^+$]: 3765.6, found 3765
ESI-MS for **25k**: *m*/*z*: calcd for [*M*−H$^+$]: 3812.7, found 3811
ESI-MS for **25l**: *m*/*z*: calcd for [*M*−H$^+$]: 3765.6, found 3764
ESI-MS for **25m**: *m*/*z*: calcd for [*M*−H$^+$]: 3812.7, found 3812
ESI-MS for **25n**: *m*/*z*: calcd for [*M*−H$^+$]: 3765.6, found 3760
ESI-MS for **25o**: *m*/*z*: calcd for [*M*−H$^+$]: 3812.7, found 3811
ESI-MS for **25p**: *m*/*z*: calcd for [*M*−H$^+$]: 3765.6, found 3766
ESI-MS for **25q**: *m*/*z*: calcd for [*M*−H$^+$]: 3812.7, found 3811.

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 6.8). 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^{-1} over the range 5–80 °C.

Circular dichroism measurements: CD measurements were carried out at 25 °C with the same solutions as used for the T_m studies. Samples were scanned from 350 to 220 nm at 0.5 nm intervals averaged over 1 s.

EcoRI cleavage 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 at 70 °C for 2 min and then cooled in an ice-bath. After the addition of 270 units of EcoRI, the solution was incubated at 23 °C. Aliquots of 20 μL were withdrawn, which 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: The recombinant enzymes were purified as described in the literature (*Pfu* DNA polymerase, see ref. [40]; human DNA polymerase β: adapted from ref. [47]; Dpo4 DNA polymerase: adapted from ref. [48]). 20 μL of the reaction mixture contained 150 nM ^{32}P-labeled primer F25 (5′-(CGT TGG TCC TGA AGG AGG ATA GGT)-3′, 225 nM of the different templates, 200 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 DNA polymerase β: 50 mM TrisHCl (pH 8.0), 10 mM $MgCl_2$, 2 mM DTT, 20 mM NaCl, 20 mM KCl, 1% (v/v) glycerol; for Dpo4 DNA polymerase: 50 mM TrisHCl (pH 7.8), 5 mM DTT, 50 mM NaCl, 5 mM $MgCl_2$, 5% (v/v) glycerol]. The final enzyme concentration in the reaction mixture was 10 nM for *Pfu* DNA polymerase, 300 nM for human DNA polymerase β, and 50 nM for Dpo4 DNA polymerase. The template sequences used are depicted in Figure 7. F25 was 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 7.5) and 50 mM NaCl from 95 °C to 25 °C over at least 1 h (. Mixtures were incubated for 30 min at different temperatures (68 °C for *Pfu* DNA polymerase; 37 °C for human DNA polymerase β and Dpo4 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% denaturing PAGE with visualization by phosphoimaging.

Efficiencies of primer extension reactions: The primer extension reactions were quantified by phosphoimaging (BioRad FX) of the dried polyacrylamide gels. The ratio of primer extension was determined by subtracting the intensity of the band corresponding to the remaining primer from the total intensity in the lane. The results presented below are averages from repeated experiments.

Primer extension reactions employing *Pfu* (exo+) DNA polymerase: The reaction mixtures (20 μL) contained 150 nM of ^{32}P-labeled primer F25 (5′-(CGT TGG TCC TGA AGG AGG ATA GGT)-3′, 225 nM of the different templates, 200 μM each of dATP, dGTP, dTTP, and dCTP in 1x reaction buffer [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]. The final enzyme concentration in the mixture was 10 nM. The template sequences used are depicted in Figure 7. F25 was 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 7.5) and 50 mM NaCl from 95 °C to 25 °C over at least 1 h (see Supporting Information). Mixtures were incubated for 30 min at different temperatures (68 °C for *Pfu* 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% denaturing PAGE with visualization by phosphoimaging.

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