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Synthesis and Properties of Oligonucleotides Containing C8-Deoxyguanosine Arylamine Adducts of Borderline Carcinogens†

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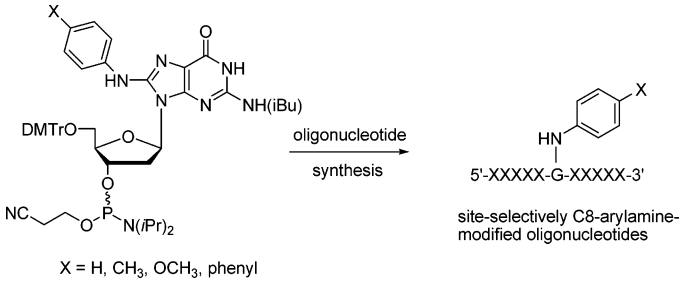
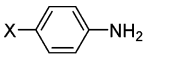


C8-Arylamine−dG adducts of borderline carcinogens and the bladder and breast carcinogen 4-aminobiphenyl were prepared using cross-coupling chemistry. These adducts were converted into the corresponding C8-arylamine−5′-*O*-DMTr-2′-deoxyguanosine phosphoramidites and then used as building blocks for automated synthesis of site-specifically modified oligonucleotides. The oligonucleotides were characterized by UV melting temperature analysis, enzymatic digestion, and circular dichroism.

Introduction

Exposure to chemical carcinogens can occur from environmental or industrial sources, diet, smoking, and endogenous processes. Poly- and monocyclic aromatic amines, e.g., aniline **1**, *p*-toluidine **2**, *p*-anisidine **3**, and 4-aminobiphenyl **4** (Figure 1), belong to the class of chemical carcinogens that are known to form covalent adducts with DNA. If these covalently bonded modifications are not repaired on the DNA level, they might compromise the fidelity of DNA replication, leading to mutations, and may be the reason for the induction of chemical carcinogenesis.[1−3]

Arylamines like **1**−**4** require metabolic activation leading to the ultimate carcinogen, which is an arylnitrenium ion **5** (Scheme 1). The initial step is an oxidation catalyzed by cytochrome P450 of the arylamine to the corresponding *N*-hydroxylamine **6**.[4] The *N*-arylhydroxylamine is then enzymatically esterified into *N*-acetoxyarylamine **7a** by *N*-acetyl transferase (NAT) or to the corresponding sulfate **7b** by sulfotransferase (ST). Such compounds are suspected to act as proximate carcinogens because



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

FIGURE 1. Potential carcinogenic aniline derivatives.

solvolysis generates a highly reactive arylnitrenium ion **5**. The predominant reaction of the arylnitrenium ion is the C8-position of 2′-deoxyguanosine (dG) **8** leading to adduct **9** as the major product. In addition, N^2-hydrazino adducts of 2′-dG **10a** have been identified as minor products. Besides *N*-arylation, C-arylation **10b** was also detected in the case of strong carcinogens such as 2-(acetyl)aminofluorene and the food carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ). In vivo studies this type of adduct showed an even higher persistence as compared to the C8 adduct, particularly in liver cells.[5,6] Other minor products are the C8- (**11**) and N^6-adducts of 2′-deoxyadenosine (Scheme 1).[7,8]

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† Dedicated to Prof. Dr. Joachim Thiem on the occasion of his 65th birthday.

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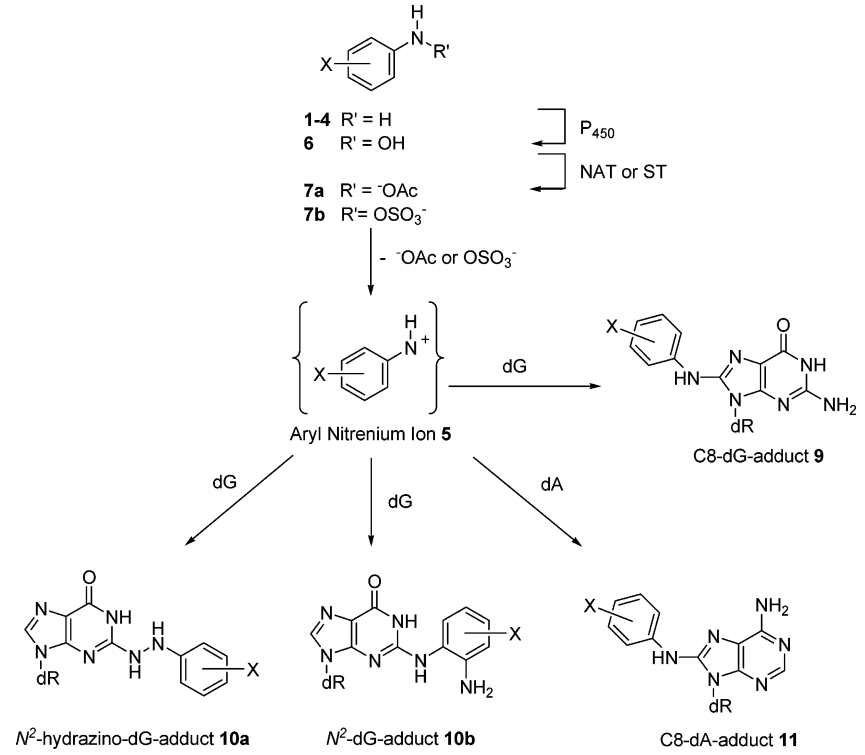
SCHEME 1. Metabolism of Arylamines and the Formed Different Adduct Types[a]



[a] NAT: *N*-acetyl transferase. ST: sulfotransferase.

So far, the most extensively studied arylamine adducts are derived from 2-aminofluorene (AF) and *N*-acetyl-2-aminofluorene (AAF) that were originally used as pesticides.[9] Zhou and Romano reported the synthesis of C8-deoxyguanosine phosphoramidite reagents of 2-aminofluorene and its *N*-acetyl analogue for the site-specific synthesis of oligonucleotide strands containing these C8 adducts.[10,11] However, they started from an adduct formed in an electrophilic amination of dG, and the yield was very poor. Rizzo reported the synthesis of oligonucleotides containing C8 adducts of an heterocyclic amine, the dietary mutagen IQ.[12] However, their approach needs the use of strong bases like LiHMDS or NaO-*tert*-Bu, and/or their protecting group chemistry was not compatible with the condition of the automated oligonucleotide synthesis. In 2002, we published a short report on the first high-yielding protocol for the complete synthesis of phosphoramidites of C8-arylamine−dG adducts using Pd cross-coupling chemistry as the key step.[13] Schärer and Gillet reported the synthesis of C8 adducts of the strong carcinogens 2-aminoflourene and its acetylated adduct using Pd-catalyzed cross-coupling and its incorporation into oligonucleotides.[14,15] Further work was reported by Takamura-Enya related to oligonucleotides damaged by heterocyclic amines.[16] Moreover, the same authors described that adduct formation by Pd-cross-coupling is also possible starting from protected 8-amino-dG with haloarenes.[16]

Our interest is related to DNA adducts of monocyclic aromatic amines like anilines **1-3** that act as so-called borderline carcinogens.[17,18] In contrast to the strong carcinogen 4-aminobiphenyl **4**, these are often used, e.g., as pharmacophores or in azo-dyes. However, nothing is known so far about the reason why the monocyclic anilines are less mutagenic/carcinogenic compared to their polycyclic counterparts like 2-aminofluorene or 1-naphthylamine. Interestingly, the ultimate carcinogens of borderline carcinogens formed the same type of adduct in the same amount as strong carcinogens in biomimetic studies.[17−20] In addition, no biochemical studies related to DNA-replication fidelity or DNA-repair have been conducted in comparison to the strong carcinogens. Thus, the molecular basis for their different mutagenic potential remains unclear. Here, we report on highly efficient syntheses of C8 adducts of borderline carcinogens using palladium-catalyzed cross-coupling chemistries, the synthesis of the 3′-phosphoramidites and their use in DNA solid-phase synthesis to give site-specifically modified oligonucleotides of mixed sequences containing several dG nucleotides.

Results and Discussion

Synthesis of the C8-Deoxyguanosine Adducts of Monocyclic Arylamines. The synthesis of C8-arylamine−dG adducts by electrophilic amination has been reported in very low yields.[19,20] Attempts to optimize the electrophilic amination

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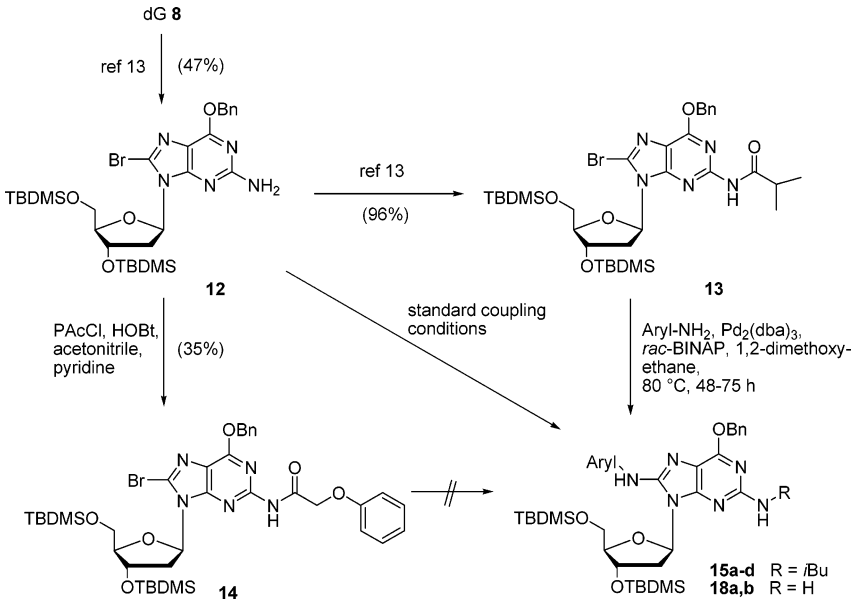
SCHEME 2. Synthesis of the Key Intermediates 12 and 13 and the Synthesis of the Adducts



reaction used in the biomimetic reaction failed (Meier et al., unpublished data), which makes this synthetic approach unsuitable for the synthesis of phosphoramidites of the dG adducts. Moreover, a nucleophilic substitution reaction as reported for protected 8-bromoguanosine was unsuccessful because extensive depurination was observed when protected 8-bromo-2′-*deoxy*-guanosine was treated with arylamines.[21] Recently, C−N bond formation using palladium catalysis (Buchwald−Hartwig reaction)[22] is a suitable method for synthesizing biologically important nucleoside analogs[23] and was introduced by Lakshman[24] and Johnson[25] for the synthesis of N^6-aryl adducts of adenosine and N^2-aryl adducts of guanosine, and Schoffers prepared C8-arylamine adducts of tris-*O*-TBDMS-(ribo)adenosine.[26] However, no suitable process for the corresponding phosphoramidites was reported. While this work was in progress, Rizzo published the synthesis of 2′-deoxyguanosine-phosphoramidites containing a heterocyclic food mutagen (IQ) in the C8 position.[27]

In our approach based also on a palladium cross-coupling as the key step, it became apparent that the O^6-position of guanine as well as the hydroxyl groups of the 2′-deoxyribose should be blocked during synthesis. Thus, the benzyl (Bn) group was introduced to the O^6-position, and *O*-silylation with TBDMS-chloride was done at the hydroxyl groups. Because the exocyclic amino group of guanine needs to be protected for the automated oligonucleotide synthesis and to avoid homo coupling during arylamination, standard N^2-protecting groups like the isobutyryl (*i*-Bu) and the more labile phenoxyacetyl group (PAc) were studied. This fully protected dG-derivatives were synthesized starting from bromination of 2′-dG **8** using NBS to yield 8-bromo-2′-dG in 78% (Scheme 2).[28,14] *O*-Silylation using TBDMS-chloride yielded 8-Br-3′,5′-*O*-TBDMS-2′-dG in 83%. The blocking of the O^6-position was achieved by a Mitsunobu reaction with benzyl alcohol (Bn), DIAD and PPh_3 to give intermediate **12** in 72% yield. Finally, the exocyclic amino group was protected by treatment with isobutyryl chloride or phenoxyacetyl chloride/1-hydroxybenzotriazole to give the key intermediates N^2-isobutyryl-O^6-benzyl-8-bromo-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine **13** and N^2-phenoxyacetyl-O^6-benzyl-8-bromo-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine **14** in 96% and 35% yield.

TABLE 1. Yields of C8-Arylamine−dG Adducts 15a−d and 18a,b

compd	R	arylamine	chem yield (%)
15a	*i*-Bu	aniline	77
15b	*i*-Bu	*p*-toluidine	75
15c	*i*-Bu	*p*-anisidine	76
15d	*i*-Bu	4-aminobiphenyl	80
18a	H	*p*-anisidine	62
18b	H	4-aminobiphenyl	65

The Buchwald−Hartwig reaction was carried out starting from intermediates **13** and **14** using conditions that we have previously published (Scheme 2).[12,27] Efficient cross-coupling was observed for intermediate **13** to give the C8-arylamine adducts **15a**−**d** in 75−80% yields (see Table 1).

To convert C8-arylamine adducts **15a**−**d** into the corresponding phosphoramidites, the O^6-position was deblocked first (Pd/H_2; 99% yield), and then the silyl ethers were cleaved (tetra-*n*-butylammonium fluoride; 96−99% yield) to give N^2-*i*-Bu-protected intermediates **16a**−**d** (Scheme 3). Compounds **16** were 5′-*O*-dimethoxytritylated in 57−82% yield and further converted into 5′-*O*-DMTr-3′-*O*-phosphoramidites **17a**−**d** using 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite and dicyanoimidazole, which in our hands is a stronger activator than tetrazole (58−73% yield; Scheme 3). Neither during the introduction of the DMTr group nor the phosphitylation reaction did a side reaction at the N^8-atom take place.

The overall yields for the preparation of the phosphoramidites **17a**−**d** were 15−19%. Thus, a convincingly efficient synthetic procedure to 3′-phosphoramidites of C8-arylamine−dG adducts using palladium-catalyzed C−N bond formation in the key step has been described.

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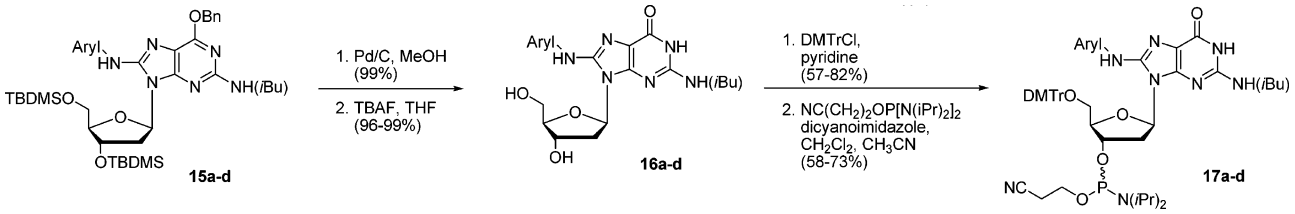
SCHEME 3. Synthesis of the 5′-*O*-DMTr-3′-*O*-phosphoramidites 17a−d



In contrast, the same reaction conditions applied to N^2-PAc compound **14** did not result in the expected products. In fact, when LiHMDS was used as base we found only decomposition of the products; weaker bases like K_3PO_4 or Cs_2CO_3 gave the desired cross-coupling products (65−75% yield), but also considerable N^2-deprotection. With NaOAc no reaction took place. Thus, the PAc group is obviously to labile to resist the reaction condition needed for efficient cross-coupling. However, for oligonucleotide synthesis a more labile protecting group for the N^2-position compared to the *i*-Bu group is desirable. To introduce such a protecting group we tried to achieve the cross-coupling with N^2-unprotected derivative **12** applying the above-mentioned conditions. Surprisingly, comparable yields of the C8-arylamine adducts **18a**,**b** were obtained as in the couplings starting from the N^2-*i*-Bu intermediate **13** (Table 1). However, a subsequent blocking of the exocyclic N^2-amino group of compounds **18a**,**b** with the phenoxyacetyl group according to the protocols of McLaughlin,[29] Ogilvie,[30] Schulhof,[31] or Patil[32] failed. Interestingly, also attempts to protect compound **18a** as its N^2-*i*-Bu derivative failed. Possible alternatives could be groups like formamidine or isopropyl-PAC, which has been used by Schärer and Rizzo.[12,14,15]

Site-Specific Synthesis of Oligonucleotides Containing a C8-Deoxyguanosine Adduct of Different Arylamines. Phosphoramidites **17a**−**d** were successfully used in automated oligonucleotide synthesis applying a modified coupling protocol. Two sequences were prepared: (i) a sequence of the mouse c-H-*ras* proto-oncogene (**19** and **20a**−**d**) and (ii) a self-complementary sequence (**21** and **22a**,**b**) shown in Figure 2.

The c-H-*ras*-sequence **19** was chosen because in earlier studies Marques and Beland identified this sequence as a hotspot for mutations of amines and amides (Figure 3).[33] The same approach has already been successfully used for the synthesis of various site-specifically modified 34-mer oligonucleotides bearing multiple dG in the sequence. These oligonucleotides were used as templates for primer extension assays with DNA-polymerases (data not shown).[34]

For the incorporation of these adducts, 50 mg of phosphoramidites **17a**−**d** were dissolved in acetonitrile (0.1 M solution). The coupling step of the adduct phosphoramidite was doubled compared to regular building blocks with an efficiency of about 60% (based on the commonly used trityl assay). It has been observed, that C8-arylamine adducts undergo an oxidative rearrangement in the presence of strong bases and air analogous to that observed for 8-oxo-2′-deoxyguanosine.[35,36] This reaction that occurs during the deprotection of the oligonucleotides can be avoided by addition of mercaptoethanol to degassed ammonium hydroxide. The deprotection was accomplished at 55 °C overnight followed by purification by reversed-phase HPLC. The oligonucleotides were characterized by LC−MS or MALDI-TOF mass spectrometry and, in the case of the self-complementary oligonucleotides, by enzymatic digestion (see the Supporting Information). The enzymatic digestion was necessary because we observed a fragmentation in the MALDI-TOF mass spectrometry of the self-complementary oligonucleotides. HPLC and LC−MS mass spectrometry indicate that the purity of all oligonucleotides was at least 95%.

Melting Temperature (T_m) and Circular Dichroism Studies. All oligonucleotides were hybridized with the complementary strands and the effect of the incorporated C8-arylamine adduct on the thermal stability of the DNA duplex was measured by UV melting curve analysis (T_m). The results of these measurements are summarized in Table 2. In general, modification of DNA destabilizes the duplex because fewer hydrogen bonds cause a lowering in the T_m value. Indeed, in the case of the c-H-*ras*-sequences **20a**−**d** a decrease in the T_m of the modified oligonucleotides of 5−7 °C compared to the unmodified oligonucleotide **19** was observed. However, no significant difference was observed in the destabilization effect of the oligonucleotides modified with borderline carcinogens (**20a**−**c**) and the oligonucleotide modified with the strong carcinogen 4-aminobiphenyl (**20d**). The T_m values of all modified oligonucleotides were found within a range of 1.5 °C which is within the experimental error.

Comparable observations were made with the self-complementary sequence **21**. However, the difference of the T_m value of unmodified oligonucleotide **21** and modified oligonucleotides **22a** (anisidine-modified) and **22b** (4-aminobiphenyl-modified) is higher ($\Delta = 14$ °C) due to modification in each strand. Again, there is no difference in T_m between the *p*-anisidine- and the 4-aminobiphenyl-modified oligonucleotide.

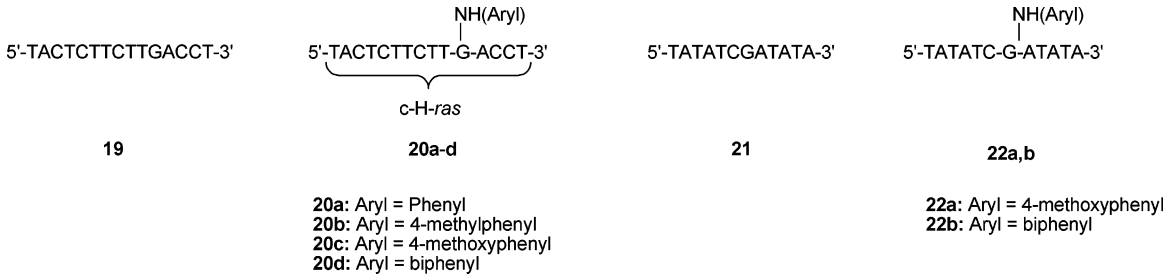


FIGURE 2. Sequences for C8-arylamine-adducted oligonucleotides.

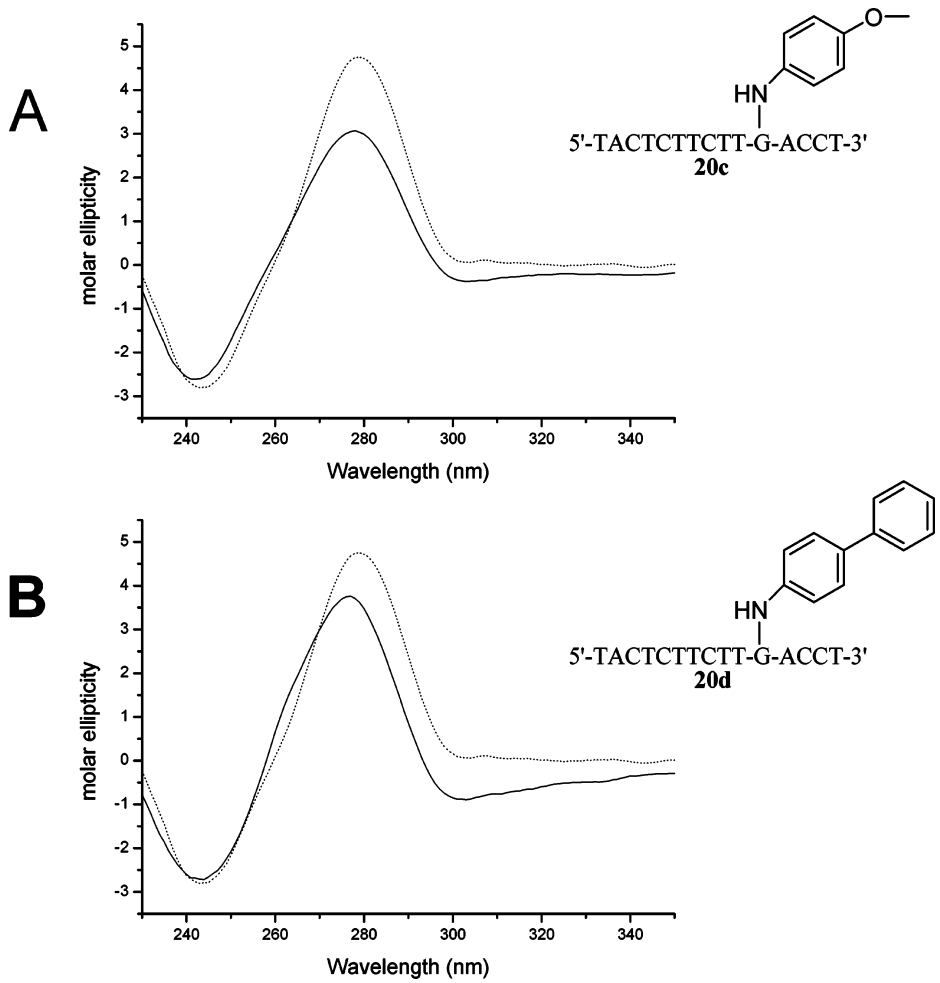


FIGURE 3. CD spectra of the unmodified c-H-*ras* oligonucleotide **19** (—) in comparison to the C8-*p*-anisidine-modified oligonucleotide **20c** (panel A) and the C8-4-aminobiphenyl-modified oligonucleotide **20d** (panel B).

TABLE 2. T_m Studies of Adducted Oligonucleotides (van't Hoff'sche Calculation for ΔH and ΔS)

oligonucleotide	T_m (°C)	ΔH (kcal/mol)	ΔS (cal/mol/K)
19	51.6	−82.0	−280.8
20a	46.3	−163.9	−541.4
20b	46.4	−144.4	−480.8
20c	46.0	−161.5	−534.5
20d	45.0	−175.7	−580.2
21	33.0	−245.5	−773.8
22a	19.1/69.0	−323.1/−186.3	−1075/−523.7
22b	20.2/65.6	−288.2/−121.7	−855.3/−384.4

TABLE 3. T_m Studies of Adducted Oligonucleotides 25 and 26

	T_m value (°C) against dA_5XdA_5 **24a**−**d**			
oligonucleotide	X = C	X = G	X = T	X = A
TTTTT(dG)TTTTT **23**	40	28	30	27
TTTTT(Tol-dG) TTTTT **25**	35	27	28	26
TTTTT(ABP-dG) TTTTT **26**	33	26	26	26

Interestingly, in the cases of the two modified self-complementary oligonucleotides **22a**,**b** a second T_m was detected. This may be due to a second stable solution conformation in addition to the DNA duplex, most probably a hairpin structure. Kukreti, for example, reported a palindromic sequence (12mer) for which a second T_m value of the hairpin was found to be 45 °C above the T_m of the duplex.[37] Our results with oligonucleotides **22a**,**b** are comparable to this observation because the second T_m is 50 °C higher for oligonucleotide **22a** and 46 °C for oligonucleotide **22b**. In both cases, the lower melting transition is sensitive to oligo concentration while the higher was not, which is a strong indication for the proposed hairpin formation. The unmodified oligonucleotide **21** did not show a second T_m within the temperature range of 10−85 °C. Two explanations are possible: (i) the second T_m is higher than 85 °C or (ii) there is no hairpin structure for this oligonucleotide. If the latter is true, the arylamine modification would stabilize the hairpin formation.

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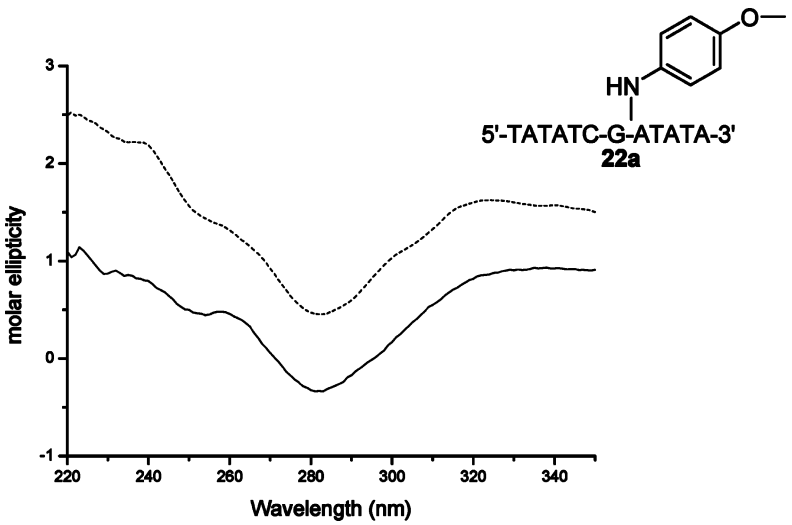

FIGURE 4. CD spectra of the unmodified self-complementary sequence **21** (—) and the C8-*p*-anisidine-modified oligonucleotide **22a**.

Recently, we have been incorporated dG adducts in the middle of a homo(T)$_{14}$ sequence and hybridization experiments were done.[34] For comparison, the unmodified (T)$_7$(dG)(T)$_7$ **23** was hybridized to (dA)$_7$(X)(dA)$_7$ **24a**−**d** (X = dC, T, dA, dG). In the full-matched situation the T_m value was found to be 39 °C (see Table 3). A mismatch within the hybrid caused a decrease of the T_m value of about 10 °C (T_m ~28.5 °C). Incorporation of a adducted dG* (modified with *p*-toluidine **25** and 4-aminobiphenyl **26**) into the homo(T) sequence led to a reduction of 5 °C in the case of the matched (dA)$_7$(dC)(dA)$_7$-strand independently 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 the mismatched duplex (T_m ~27.5 °C).[34] Thus, obviously the incorporated adducted-dG's have a significant less reduction of the thermal stability of the duplex as compared to a mismatch in the sequence. Further studies will be conducted to investigate the structure of the C8-arylamine adducted DNA strands.

In summary, the conclusion that can be taken from the T_m measurements is that there is no major conformational difference between the oligonucleotides containing a C8-modification of a borderline or a strong carcinogen, which has already been postulated by Cho et al. for the C8-aminobiphenyl adduct from NMR studies.[38] To verify this conclusion, the circular dichroism spectra of the oligonucleotides were recorded. CD spectra of modified oligonucleotides **20c** and **20d** and the corresponding unmodified oligonucleotide **19** are shown in Figure 3. All c-H-*ras*-sequence oligonucleotide duplexes **19** showed the same spectral characteristics, a maximum at 280−290 nm and a minimum at 240−250 nm with no change in intensity. Both indicate that all oligonucleotides of the c-H-*ras*-sequence adopt a B-type-DNA-conformation. The shifting of the maxima of the modified oligonucleotides **20c**,**d** to higher wavelength (5 and 8 nm, respectively) is caused by the (partly) conjugated aromatic systems to the guanine heterocycle, which can also be observed in the UV spectra of the adducted oligonucleotides as well as on the nucleoside level (data not shown). In this context it is reasonable that the strongest shift was observed in the case of C8-(4-aminobiphenyl)-modified oligonucleotide **20d**.

(38) Cho, B. P.; Beland, F. A.; Marques, M. M. *Biochemistry* **1992**, *31*, 9587.

Analogous properties were studied using the self-complementary sequence. The CD spectra of the unmodified **21** and the modified oligonucleotide **22a** are shown in Figure 4. Again, there is no conformational difference observable between the modified and the unmodified oligonucleotide. Comparing these spectra with those of the c-H-*ras*-sequence two remarkable changes can be seen. First, the self-complementary sequence did not exist in only one conformation. In addition to the B-type-DNA-conformation (minimum at 240 nm) there is a second minimum at 280 nm (from the Z-DNA-form). This goes along with our interpretation of the T_m-measurements that a hairpin formation is responsible for the second T_m value.

In fact, in all structural studies performed so far point to very similar conformations of oligonucleotides that are modified by borderline carcinogens **1**−**3** or by the strong carcinogen 4-aminobiphenyl **4**. However, further studies are needed to confirm this.

Recently, we ran primer-template extension studies using standing start and standing start +1 conditions. In these studies, it became apparent that DNA pol β showed a considerable loss in fidelity and that replication errors did not take place opposite to the adduct, but one step downstream. But again no clues were found, that could explain the different carcinogenic potential of a borderline carcinogen and a strong carcinogen.[34]

To add more data to the reported results and to gain more insights into the reason for the different carcinogenic potential C8-modified phosphoramidites will be incorporated in further different DNA sequences. Also, including strong, polycyclic carcinogens as 2-aminofluorene in these studies would be interesting for comparison. Structural analysis of modified oligonucleotides like **21** and **22a**,**b** by means of NMR and further biochemical studies, e.g., repair studies, may give hints to explain the difference in carcinogenic potential.

Summary

In summary, we have reported on an efficient strategy for the synthesis of C8-arylamine adducts of 2′-deoxyguanosine involving the Buchwald−Hartwig catalyzed cross-coupling starting from protected 8-bromo-2′-deoxyguanosine derivative **13**. The C8-arylamine adducts were site-specifically incorporated into oligonucleotides using N^2-*i*-Bu-protected phosphoramidites in solid-phase synthesis. We have examined the properties of

two sequences containing different arylamines by combination of UV melting studies and CD spectroscopy. Such C8-arylamine adducts formed by borderline carcinogens and strong carcinogens play an important role in their mutagenicity due to alteration of the structure of the DNA double helix. However, no important differences have been observed so far in the structure of DNA-oligonucleotides modified by borderline or strong carcinogens. Further biochemical studies with the modified oligonucleotides with regard to replication or repair are currently underway in our laboratories.

Experimental Section

***O*^{6}-Benzyl-8-bromo-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12).** 8-Br-dG was prepared according to refs 28 and 34. The spectral data were in accordance with those reported in ref 14. Compound **12** was prepared from 8-Br-dG according to refs 14 and 34. The spectral data were in accordance with those reported in ref 14.

***N*^{2}-Isoutyryl-*O*^{6}-benzyl-8-bromo-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (13).** In a flask, 1.15 g (1.73 mmol) of *O*^{6}-Bn-8-Br-3′,5′-TBDMS-2′-dG (**12**) was twice coevaporated with 4 mL of pyridine and then dissolved in 10 mL of dry pyridine. Then, 0.37 mL of isobutyryl chloride was added slowly via syringe, and the solution was stirred for 1 h at room temperature. After addition of 1 mL of methanol and stirring for 5 min, the solvent was removed in vacuo followed by coevaporation with toluene.

The residue was redissolved in 20 mL of water and 20 mL of CH_2Cl_2, and the layers were separated. The aqueous layer was extracted twice with 20 mL of CH_2Cl_2, and the combined organic layers were washed with 20 mL of water and then dried over sodium sulfate. The solvent was removed in vacuo, and purification of the residue by chromatography on silica gel (5% ethyl acetate/hexanes) gave 1.22 g (96%) of (**13**) as a colorless foam.^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 10.41 (s, 1H), 7.53−7.33 (m, 5H), 6.26 (dd, J = 7.4, 6.1 Hz, 1H), 5.60 (d, J = 12.1 Hz, 1H), 5.57 (d, J = 12.1 Hz, 1H), 4.99−4.95 (m, 1H), 3.84−3.77 (m, 2H), 3.69−3.62 (m, 1H), 3.51 (ddd, J = 13.1, 6.1, 6.1 Hz, 1H), 2.80 (sept, J = 6.8 Hz, 1H), 2.26 (ddd, J = 13.1, 7.4, 4.8 Hz, 1H), 1.10 (d, J = 6.8 Hz, 3H), 1.09 (d, J = 6.8 Hz, 3H), 0.88 (s, 9H), 0.75 (s, 9H), 0.12, (d, J = 8.5 Hz, 6H), −0.11 (d, J = 9.4 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 174.5, 158.8, 153.2, 151.8, 136.1, 129.2, 128.7, 128.6, 128.5, 118.1, 88.1, 85.9, 72.2, 67.9, 63.4, 36.8, 34.8, 25.9, 19.5, 19.4, 18.1, 17.8, −4.7, −4.8, −5.3, −5.4. HRFABMS: calcd (M + H)$^+$ 734.2769, obsd 734.2794.

***N*^{2}-Phenoxyacetyl-*O*^{6}-benzyl-8-bromo-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (14).** To 69 mg (0.51 mmol) of 1-hydroxybenzotriazole, suspended in 2 mL of dry CH_3CN, was added 188 mg (0.48 mmol) of phenoxyacetyl chloride. As soon as the 1-hydroxybenzotriazole had dissolved, dry pyridine (3 mL) was added to dissolve any precipitated salt. This slightly red solution was added dropwise to 200 mg (0.3 mmol) of *O*^{6}-Bn-8-Br-3′,5′-TBDMS-2′-dG (**12**) at 0 °C, which was dissolved in 2 mL of dry pyridine. The mixture was then stirred overnight at ambient temperature. After TLC analysis indicated complete reaction, the mixture was cooled again to 0 °C and the reaction stopped by addition of 1 mL of water. After 5 min of stirring at 0 °C, 4 mL of saturated sodium bicarbonate solution was added and stirred for another 15 min. After separation of the layers, the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried over sodium sulfate, and the solvent was removed in vacuo. Purification of the residue by chromatography on silica gel (10−20% ethyl acetate/hexanes) gave 447 mg (35%) of (**14**) as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 10.48 (s, 1H), 7.52−7,50 (m, 2H), 7.38−7.33 (m, 3H), 7.28−7.26 (m, 2H), 6.94−6.90 (m, 3H), 6.10 (dd, J = 7.1, 7.1 Hz, 1H), 5.53 (s, 2H), 4.89 (d, J = 16.0 Hz, 2H), 4.62 (ddd, J = 2.8, 5.6, 3.1 Hz, 1H), 3.74 (ddd, J = 5.6, 6.0, 3.1 Hz, 1H), 3.58 (dd, J = 9.9, 3.1 Hz, 1H), 3.42 (dd, J = 9.9, 6.0 Hz, 1H), 3.25 (ddd, J = 13.4, 6.8, 6.2 Hz, 1H), 2.01 (ddd, J = 13.4, 6.8, 3.0 Hz, 1H), 0.86 (s, 9H), 0.81 (s, 9H), 0.07 (s, 6H), −0.03 (s, 3H), −0.04 (s, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 171.4, 165.2, 159.6, 159.1, 155.0, 138.3, 129.6, 128.6, 128.4, 128.3, 121.1, 114.6, 87.0, 83.4, 81.2, 72.9, 72.8, 67.8, 35.1, 25.9, 25.8, 18.0, 17.8, −4.5, −4.7, −5.3, −5.3. FABMS: calcd (M) 797.4308, obsd (M + H)$^+$ 798.5021.

General Procedure (I) for the Amination of *O*^{6}-Benzyl-8-bromo-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine Derivatives. A dried flask was purged with nitrogen and charged with bromide (**12, 13** or **14**), 1.5 equiv of K_3PO_4, 10 mol % of tris-(dibenzylideneacetone)dipalladium(0) (Pd_2dba_3), 30 mol % of racemic 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP), and 2 equiv of amine. Dry 1,2-DME (15 mL) was added, and the reaction vessel was stirred at 80 °C until the reaction was complete (TLC analysis). The reaction mixture was allowed to cool to room temperature, and then 1 mL of saturated sodium bicarbonate solution was added. After addition of 10 mL of brine, the layers were separated and the aqueous layer was extracted three times with 10 mL of ethyl acetate. The combined organic layers were washed twice with 10 mL of brine and once with a mixture of 10 mL of brine and 2 mL of water. Then the organic layer was 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.

***N*^{2}-Isobutyryl-*O*^{6}-benzyl-8*N*-phenylamino-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (15a).** The general procedure (I) was conducted with 350 mg (0.48 mmol) of bromide **13** (reaction time 49 h) and afforded 273 mg (77%) of a slightly pink foam.

^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 10.13 (s, 1H), 9.14 (s, 1H), 7.69−6.93 (m, 10H), 6.41 (dd, J = 7.3, 6.3 Hz, 1H), 5.60 (d, J = 12.2 Hz, 1H), 5.56 (d, J = 12.2 Hz, 1H), 4.94 (ddd, J = 4.3, 6.6, 4.3 Hz, 1H), 3.86 (dd, J = 10.6, 4.7 Hz, 1H), 3.80 (ddd, J = 4.7, 4.7, 6.0 Hz, 1H), 3.71 (dd, J = 10.6, 6.0 Hz, 1H), 3.50 (ddd, J = 13.1, 6.3, 6.3 Hz, 1H), 2.81 (sept, J = 6.8 Hz, 1H), 2.17 (ddd, J = 13.1, 7.3, 4.3 Hz, 1H), 1.10 (d, J = 6.8 Hz, 3H), 1.09 (d, J = 6.8 Hz, 3H), 0.89 (s, 9H), 0.77 (s, 9H), 0.13 (d, J = 8.1 Hz, 6H), −0.09 (d, J = 3.2 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 174.4, 156.8, 152.7, 149.4, 148.7, 140.4, 136.8, 128.8, 128.7, 128.6, 128.3, 121.8, 118.6, 115.7, 88.0, 83.0, 72.5, 67.3, 63.7, 37.3, 34.5, 25.9, 19.6, 19.4, 18.1, 17.8, −4.6, −4.7, −5.3, −5.4. HRFABMS: calcd (M + H)$^+$ 747.4086, obsd 747.4066.

***N*^{2}-Isobutyryl-*O*^{6}-benzyl-8*N*-(4-methylphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (15b).** The general procedure (I) was conducted with 450 mg (0.61 mmol) of bromide **13** (reaction time 50 h) and afforded 350 mg (75%) of a light yellow foam. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.13 (s, 1H), 9.05 (s, 1H), 7.58−7.49 (m, 4H), 7.40−7.31 (m, 3H), 7.12−7.08 (m, 2H), 6.39 (dd, J = 7.3, 6.2 Hz, 1H), 5.59 (d, J = 12.1 Hz, 1H), 5.55 (d, J = 12.1 Hz, 1H), 4.93 (ddd, J = 4.3, 6.6, 4.0 Hz, 1H), 3.86 (dd, J = 10.6, 5.1 Hz, 1H), 3.79 (ddd, J = 5.1, 4.0, 5.8 Hz, 1H), 3.70 (dd, J = 10.6, 5.8 Hz, 1H), 3.50 (ddd, J = 13.1, 6.2, 6.6 Hz, 1H), 2.80 (sept, J = 6.8 Hz, 1H), 2.23 (s, 3H), 2.15 (ddd, J = 13.1, 7.3, 4.3 Hz, 1H), 1.09 (d, J = 6.8 Hz, 3H), 1.09 (d, J = 6.8 Hz, 3H), 0.89 (s, 9H), 0.77 (s, 9H), 0.13 (d, J = 8.1 Hz, 6H), −0.09 (d, J = 3.1 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 174.3, 156.5, 152.6, 149.1, 148.8, 137.6, 136.7, 130.5, 129.0, 128.5, 128.4, 128.1, 118.7, 115.6, 87.7, 82.8, 72.3, 67.1, 63.5, 37.0, 34.4, 25.7, 20.4, 19.4, 19.3, 18.0, 17.7, −4.8, −4.9, −5.5, −5.5. HRFABMS: calcd (M + H)$^+$ 761.4242, obsd 761.4216.

***N*^{2}-Isobutyryl-*O*^{6}-benzyl-8*N*-(4-methoxyphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (15c).** The general procedure (I) was conducted with 250 mg (0.34 mmol) of bromide **13** (reaction time 51 h) and afforded 200 mg (76%) of a slightly violet foam: ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 10.20 (s, 1H), 9.06 (s, 1H), 7.69−6.97 (m, 9H), 6.48 (dd, J = 6.6, 7.3 Hz, 1H), 5.67 (d, J = 12.1 Hz, 1H), 5.63 (d, J = 12.1 Hz, 1H), 5.02 (ddd, J = 6.6, 4.2, 4.2 Hz, 1H), 3.96 (dd, J = 5.2, 11.0 Hz, 1H), 3.89 (ddd, J = 5.2, 4.2, 5.9 Hz, 1H), 3.80 (dd, J = 5.9, 11.0 Hz,

1H), 3.61 (ddd, J = 13.0, 6.6, 6.6 Hz, 1H), 2.90 (sept, J = 6.7 Hz, 1H), 2.25 (ddd, J = 13.0, 4.2, 7.3 Hz, 1H), 1.20 (d, J = 6.7 Hz, 3H), 1.19 (d, J = 6.7 Hz, 3H), 1.00 (s, 9H), 0.87 (s, 9H), 0.23 (d, J = 7.6 Hz, 6H), 0.01 (d, J = 2.7 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 174.4, 156.5, 154.7, 152.9, 149.5, 149.1, 136.9, 133.4, 128.7, 128.6, 128.2, 120.8, 115.8, 114.1, 87.9, 82.9, 72.6, 67.3, 63.7, 55.3, 37.2, 34.5, 25.9, 19.6, 19.4, 18.1, 17.9, −4.6, −4.7, −5.3, −5.4. HRFABMS: calcd (M + H)$^+$ 777.4191, obsd 777.4204.

***O*^{6}-Benzyl-8*N*-(4-methoxyphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (18a).** The general procedure (I) was conducted with 3.02 g (4.54 mmol) of bromide **12** (reaction time 70 h) and afforded 2.00 g (62%) of a yellow foam. ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 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 (m, J = 13.2, 9.6, 5.5 Hz, 2 H), 3.70 (s, 3 H), 3.68 (dd, J = 13.2, J = 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 (s, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 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. FABMS: calcd (M) 706.3704, obsd (M + H)$^+$ 707.3211.

***N*^{2}-Isobutyryl-*O*^{6}-benzyl-8*N*-(4-biphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (15d).** The general procedure (I) was conducted with 300 mg (0.41 mmol) of bromide **13** (reaction time 51 h) and afforded 269 mg (80%) of a light-yellow foam. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 10.15 (s, 1H), 9.30 (s, 1H), 7.81−7.27 (m, 14H), 6.44 (dd, J = 6.2, 7.4 Hz, 1H), 5.61 (d, J = 12.1 Hz, 1H), 5.57 (d, J = 12.1 Hz, 1H), 4.96 (ddd, J = 6.7, 4.3, 4.3 Hz, 1H), 3.87 (dd, J = 5.0, 10.6 Hz, 1H), 3.81 (ddd, J = 5.0, 4.3, 6.0 Hz, 1H), 3.72 (dd, J = 6.0, 10.6 Hz, 1H), 3.53 (ddd, J = 13.0, 6.2, 6.7 Hz, 1H), 2.81 (sept, J = 6.7 Hz, 1H), 2.19 (ddd, J = 13.0, 4.3, 7.4 Hz, 1H), 1.11 (d, J = 6.7 Hz, 3H), 1.10 (d, J = 6.7 Hz, 3H), 0.90 (s, 9H), 0.77 (s, 9H), 0.14 (d, J = 8.0 Hz, 6H), −0.09 (d, J = 3.3 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 156.9, 152.7, 149.5, 148.6, 140.0, 139.9, 136.8, 133.4, 129.0, 128.7, 128.6, 128.3, 127.0, 126.9, 126.2, 118.9, 115.7, 88.0, 83.0, 72.5, 67.3, 63.7, 37.2, 34.6, 25.9, 19.6, 19.4, 18.1, 17.9, −4.6, −4.7, −5.3, −5.4. HRFABMS: calcd (M + H)$^+$ 823.4399, obsd 823.4482.

***O*^{6}-Benzyl-8*N*-(4-biphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (18b).** The general procedure (I) was conducted with 1.40 g (1.56 mmol) of bromide **12** (reaction time 55 h) and afforded 1.03 g (65%) of a light-yellow foam. ^{1}H NMR (DMSO-d_6, 400 MHz): 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 (s, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 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. HRFABMS: calcd (M) 752.3967, obsd (M + H)$^+$ 753.4244.

General Procedure (II) for the Debenzylation of *N*^{2}-Isobutyryl-*O*^{6}-benzyl-8*N*-arylamino-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine Derivatives. A dried flask was purged with nitrogen and charged with the N^2-isobutyryl-O^6-benzyl-8N-arylamino-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine derivative and Pd/C. Dry CH_3OH was added, and the reaction was stirred under hydrogen atmosphere, at room temperature, for 1 h. The reaction mixture was centrifuged several times with CH_3OH, filtered, and concentrated in vacuo to give the pure product.

General Procedure (III) for the Desilylation of *N*^{2}-Isobutyryl-8*N*-arylamino-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine Derivatives. In a dried flask, a solution of the N^2-isobutyryl-8N-arylamino-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine derivative in THF was treated with 2.5 equiv of a 1.0 M solution of tetrabutylammonium fluoride in THF at room temperature. The reaction was stirred until the reaction was complete (TLC analysis), concentrated in vacuo, and purified by chromatography on silica gel with 20% CH_3OH in CH_2Cl_2.

***N*^{2}-Isobutyryl-8*N*-phenylamino-2′-deoxyguanosine (16a).** The general procedure (II) was conducted with 800 mg (1.07 mmol) of compound **15a** and 50 mg of Pd/C in 20 mL of dry CH_3OH and afforded 696 mg (99%) of a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 11.99 (s, 1H), 11.21 (s, 1H), 8.66 (s, 1H), 7.57−7.54 (m, 2H), 7.29−7.24 (m, 2H), 6.95−6.91 (m, 1H), 6.32 (dd, J = 8.7, 6.0 Hz, 1H), 4.52−4.48 (m, 1H), 3.88−3.83 (m, 2H), 3.68 (dd, J = 13.5, 7.9 Hz, 1H), 3.40 (ddd, J = 13.2, 8.7, 5.9 Hz, 1H), 2.75 (sept, J = 6.8 Hz, 1H), 2.13 (ddd, J = 13.2, 6.0, 1.8 Hz, 1H), 1.12 (d, J = 6.8 Hz, 3H), 0.89 (s, 9H), 0.81 (s, 9H), 0.11 (s, 6H), −0.01 (d, J = 4.6 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.5, 153.7, 147.3, 146.1, 145.8, 140.8, 128.6, 121.2, 117.8, 117.8, 87.6, 83.8, 73.2, 63.1, 36.5, 34.7, 25.7, 19.0, 18.8, 18.0, 17.8, −4.7, −4.8, −5.4, −5.5. HRFABMS: calcd (M + H)$^+$ 657.3616, obsd 657.3577.

The general procedure (III) was conducted with 330 mg (0.50 mmol) of N^2-isobutyryl-8N-phenylamino-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine in 6 mL of THF (reaction time 2 h). Purification afforded 212 mg (99%) of a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 11.98 (s, 1H), 11.56 (s, 1H), 8.87 (s, 1H), 7.78−7.76 (m, 2H), 7.30−7.26 (m, 2H), 6.96−6.92 (m, 1H), 6.42 (dd, J = 9.9, 5.6 Hz, 1H), 5.99, (s, 1H), 5.35 (d, J = 3.2 Hz, 1H), 4.44−4.43 (m, 1H), 3.97 (ddd, J = 2.0, 2.0, 2.0 Hz, 1H), 3.77, (s, 2H), 2.73 (sept, J = 6.9 Hz, 1H), 2.54 (ddd, J = 12.9, 9.9, 5.9 Hz, 1H), 2.04 (ddd, J = 12.9, 5.6, 1.3 Hz, 1H), 1.11 (d, J = 6.9 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.8, 153.7, 146.9, 146.6, 145.2, 140.2, 128.6, 121.2, 117.9, 116.3, 87.4, 83.2, 71.3, 61.3, 38.4, 34.8, 18.9. HRFABMS: calcd (M + H)$^+$ 429.1886, obsd 429.1934.

***N*^{2}-Isobutyryl-8*N*-(4-methylphenylamino)-2′-deoxyguanosine (16b).** The general procedure (II) was conducted with 500 mg (0.66 mmol) of compound **15b** and 50 mg of Pd/C in 15 mL of dry CH_3OH and afforded 437 mg (99%) of a colorless solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm) 12.02 (s, 1H), 11.26 (s, 1H), 8.53 (s, 1H), 7.47−7.45 (m, 2H), 7.09−7.07 (m, 2H), 6.30 (dd, J = 8.5, 6.1 Hz, 1H), 4.52 (d, J = 5.8 Hz, 1H), 3.87−3.83 (m, 2H), 3.67 (dd, J = 13.3, 7.6 Hz, 1H), 3.38 (ddd, J = 13.3, 8.5, 5.8 Hz, 1H), 2.75 (sept, J = 6.9 Hz, 1H), 2.24 (s, 3H), 2.11 (ddd, J = 13.3, 6.1, 1.8 Hz, 1H), 1.12 (d, J = 6.9 Hz, 3H), 1.12 (d, J = 6.9 Hz, 3H), 0.89 (s, 9H), 0.82 (s, 9H), 0.11 (s, 6H), −0.01 (d, J = 5.4 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 147.4, 146.3, 138.2, 129.9, 129.0, 118.0, 117.7, 87.6, 83.7, 73.2, 63.1, 36.5, 34.8, 25.8, 25.7, 20.3, 19.0, 18.8, 18.0, 17.8, −4.7, −4.8, −5.4, −5.5. HRFABMS: calcd (M + H)$^+$ 671.3773, obsd 671.3769.

The general procedure (III) was conducted with 876 mg (1.31 mmol) of N^2-isobutyryl-8N-(4-methylphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine in 10 mL of THF (reaction time 2 h). Purification afforded 845 mg (99%) of a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 11.97 (s, 1H), 11.55 (s, 1H), 8.78 (s, 1H), 7.67−7.65 (m, 2H), 7.10−7.07 (m, 2H), 6.41 (dd, J = 9.9, 5.5 Hz, 1H), 5.96, (dd, J = 4.6, 4.6 Hz, 1H), 5.34 (d, J = 3.4 Hz, 1H), 3.96 (ddd, J = 2.8, 1.9, 2.0 Hz, 1H), 3.78 (ddd, J = 11.8, 4.6, 1.9 Hz, 1H), 3.74 (ddd, J = 11.8, 4.6, 2.0 Hz, 1H), 2.73 (sept, J = 6.8 Hz, 1H), 2.53 (ddd, J = 13.0, 9.9, 5.8 Hz, 1H), 2.03 (ddd, J = 13.0, 5.5, 1.2 Hz, 1H), 1.11 (d, J = 6.8 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.9, 153.8, 147.0, 146.6, 145.6, 137.9, 130.2, 129.1, 118.1, 116.6, 87.5, 83.4, 71.5, 61.4, 38.5, 34.9, 20.5, 19.0. HRFABMS: calcd (M + H)$^+$ 443.2043, obsd 443.2070.

***N*^{2}-Isobutyryl-8*N*-(4-methoxyphenylamino)-2′-deoxyguanosine (16c).** The general procedure (II) was conducted with 626 mg (0.81 mmol) of compound **15c** and 50 mg of Pd/C in 15 mL of dry

CH_3OH and afforded 548 mg (99%) of a colorless solid. ^1H NMR (DMSO-d_6, 500 MHz): δ (ppm) 11.96 (s, 1H), 11.18 (s, 1H), 8.44 (s, 1H), 7.50−7.48 (m, 2H), 6.88−6.86 (m, 2H), 6.30 (dd, J = 8.7, 6.0 Hz, 1H), 4.50 (d, J = 5.8 Hz, 1H), 3.88−3.84 (m, 2H), 3.71 (s, 3H), 3.68 (dd, J = 13.6, 7.7 Hz, 1H), 3.40 (ddd, J = 13.2, 8.7, 5.8 Hz, 1H), 2.75 (sept, J = 6.9 Hz, 1H), 2.11 (ddd, J = 13.2, 6.0, 1.6 Hz, 1H), 1.12 (d, J = 6.9 Hz, 6H), 0.90 (s, 9H), 0 .81 (s, 9H), 0.11 (d, J = 1.4 Hz, 6H), −0.01 (d, J = 6.9 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.4, 154.3, 153.6, 147.3, 147.0, 145.5, 133.7, 120.2, 117.7, 113.8, 87.6, 83.7, 73.2, 63.1, 55.2, 36.5, 34.7, 25.8, 19.0, 18.8, 18.1, 17.8, −4.7, −4.8, −5.4, −5.5. HRFABMS: calcd $(M + H)^+$ 687.3722, obsd 687.3723.

The general procedure (III) was conducted with 550 mg (0.80 mmol) of N^2-isobutyryl-8*N*-(4-methoxyphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine in 8 mL of THF (reaction time 3 h). Purification afforded 352 mg (96%) of a colorless solid. ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) 11.96 (s, 1H), 11.55 (s, 1H), 8.74 (s, 1H), 7.68−7.65 (m, 2H), 6.89−6.85 (m, 2H), 6.40 (dd, J = 9.9, 5.5 Hz, 1H), 5.95 (dd, J = 4.7, 4.7 Hz, 1H), 5.34 (d, J = 3.3 Hz, 1H), 4.43 (ddd, J = 3.3, 4.5, 1.4 Hz, 1H), 3.96 (ddd, J = 2.0, 2.0, 2.0 Hz, 1H), 3.77−3.75 (m, 2H), 3.72 (s, 3H), 2.73 (sept, J = 6.9 Hz, 1H), 2.55 (ddd, J = 13.0, 9.9, 4.5 Hz, 1H), 2.03 (ddd, J = 13.0, 5.5, 1.4 Hz, 1H), 1.11 (d, J = 6.9 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.7, 154.1, 153.6, 146.9, 146.3, 145.8, 133.5, 119.6, 116.4, 113.8, 87.3, 83.2, 71.4, 61.3, 55.2, 38.3, 34.7, 18.9. HRFABMS: calcd $(M + H)^+$ 459.1992, obsd 459.2007.

N^2-Isobutyryl-8*N*-(4-biphenylamino)-2′-deoxyguanosine (16d). The general procedure (II) was conducted with 695 mg (0.84 mmol) of compound **15d** and 50 mg of Pd/C in 20 mL of dry CH_3OH and afforded 613 mg (99%) of a colorless solid. ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) 12.01 (s, 1H), 11.21 (s, 1H), 8.85 (s, 1H), 7.69−7.58 (m, 6H), 7.44−7.40 (m, 2H), 7.31−7.27 (m, 1H), 6.35 (dd, J = 8.6, 6.0 Hz, 1H), 4.53−4.50 (m, 1H), 3.89−3.83 (m, 2H), 3.68 (dd, J = 14.0, 8.4 Hz, 1H) 3.46 (ddd, J = 13.3, 8.6, 5.9 Hz, 1H), 2.76 (sept, J = 6.7 Hz, 1H), 2.15 (ddd, J = 13.3, 6.0, 1.6 Hz, 1H), 1.13 (d, J = 6.7 Hz, 6H), 0.89 (s, 9H), 0.82 (s, 9H), 0.11 (s, 6H), −0.00 (d, J = 4.2 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.5, 153.8, 147.4, 146.0, 145.8, 140.4, 139.9, 132.8, 128.8, 126.8, 126.7, 126.0, 118.1, 117.9, 87.7, 83.8, 73.3, 63.1, 36.4, 34.7, 25.8, 19.0, 18.8, 18.1, 17.9, −4.7, −4.8, −5.4, −5.4. HRFABMS: calcd $(M + H)^+$ 733.3929, obsd 733.3916.

The general procedure (III) was conducted with 421 mg (0.57 mmol) of N^2-isobutyryl-8*N*-(4-biphenylamino)-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine in 8 mL of dry THF (reaction time 2 h). Purification afforded 288 mg (99%) of a slightly yellow solid. ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) 12.00 (s, 1H), 11.59 (s, 1H), 9.00 (s, 1H), 7.89−7.87 (m, 2H), 7.64−7.60 (m, 4H), 7.45−7.41 (m, 2H), 7.32−7.29 (m, 1H), 6.44 (dd, J = 9.9, 5.6 Hz, 1H), 6.05 (dd, J = 4.6, 4.6 Hz, 1H), 5.36 (d, J = 3.2 Hz, 1H), 4.48−4.43 (m, 1H), 4.00−3.97 (m, 1H), 3.83−3.75 (m, 2H), 2.74 (sept, J = 6.8 Hz, 1H), 2.56 (ddd, J = 13.0, 9.9, 5.8 Hz, 1H), 2.07 (dd, J = 13.0, 5.6 Hz, 1H), 1.12 (d, J = 6.8 Hz, 6H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.7, 153.6, 146.9, 146.6, 145.0, 139.9, 139.7, 132.9, 128.8, 126.8, 126.7, 126.1, 118.2, 116.4, 87.4, 83.3, 71.3, 61.3, 52.0, 38.5, 34.7, 18.9, 7.1. HRFABMS: calcd $(M + H)^+$ 505.2199, obsd 505.2183.

General Procedure (IV) for the Dimethoxytritylation of N^2-Isobutyryl-8*N*-arylamino-2′-deoxyguanosine Derivatives. The N^2-isobutyryl-8*N*-arylamino-2′-deoxyguanosine derivative was twice coevaporated with pyridine and then dissolved in dry pyridine. After that, 2 equiv dimethoxytrityl chloride were added and the solution was stirred at room temperature until the reaction was complete (TLC analysis). The reaction was stopped by adding a saturated $NaHCO_3$ solution. After addition of CH_2Cl_2 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 silica gel with CH_3OH (0−10%) in CH_2Cl_2 gave the desired product.

General Procedure (V) for the Phosphitylation of N^2-Isobutyryl-8*N*-arylamino-*O*5′-dimethoxytrityl-2′-deoxyguanosine Derivatives. The N^2-isobutyryl-8*N*-arylamino-2′-deoxyguanosine derivative was twice coevaporated with dry acetonitrile, dissolved in a mixture of dry CH_3CN and dry CH_2Cl_2 (1:1), and 1 equiv of dicyanoimidazole solution (0.25 M in CH_3CN) and 1.5 equiv of bis-*N*,*N*′-diisopropylamino(2-cyanoethyl) phosphite were added. The solution was stirred at room temperature until no more starting material was detected (TLC analysis). Then, the reaction was stopped by adding saturated $NaHCO_3$ 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 aluminum oxide delivered the desired product.

N^2-Isobutyryl-8*N*-phenylamino-*O*3′-[(2-cyanoethoxy)-(*N*,*N*-diisopropylamino)phosphinyl]-*O*5′-dimethoxytrityl-2′-deoxyguanosine (17a). The general procedure (IV) was conducted with 290 mg (0.68 mmol) of compound **16a** in 15 mL of dry pyridine (reaction time 3 h). Purification afforded 406 mg (82%) of a slightly yellow solid. ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) 12.00 (s, 1H), 11.06 (s, 1H), 8.87 (s, 1H), 7.68−7.65 (m, 2H), 7.30−7.25 (m, 4H), 7.17−7.10 (m, 7H), 6.95−6.91 (m, 4H), 6.73−6.64 (m, 1H), 6.43 (dd, J = 7.6, 5.8 Hz, 1H), 5.16 (d, J = 5.0 Hz, 1H), 4.53 (dddd, J = 5.0, 7.2, 4.9, 4.8 Hz, 1H), 3.98 (ddd, J = 7.9, 4.8, 2.7 Hz, 1H), 3.67 (s, 3H), 3.66 (s, 3H), 3.47 (dd, J = 10.2, 7.9, Hz, 1H), 3.18 (ddd, J = 13.2, 7.2, 5.8 Hz, 1H), 3.08 (dd, J = 10.2, 2.7 Hz, 1H), 2.70 (sept, J = 6.8 Hz, 1H), 2.23 (ddd, J = 13.2, 7.6, 4.9 Hz, 1H), 1.10 (d, J = 6.8 Hz, 3H), 1.08 (d, J = 6.8 Hz, 3H).^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.6, 157.9, 157.8, 153.7, 146.9, 145.9, 145.6, 144.9, 140.9, 135.7, 135.6, 129.7, 129.6, 128.6, 127.8, 127.4, 126.4, 121.0, 117.9, 117.5, 112.8, 112.7, 86.5, 85.2, 83.1, 70.8, 64.8, 54.9, 54.8, 37.4, 34.6, 19.0, 18.8. HRFABMS: calcd $(M + H)^+$ 731.3115, obsd 731.3118.

The general procedure (V) was conducted with 390 mg (0.53 mmol) of N^2-isobutyryl-8*N*-phenylamino-*O*5′-dimethoxytrityl-2′-deoxyguanosine in 20 mL of dry CH_3CN/CH_2Cl_2, 2.12 mL (0.53 mmol) of dicyanoimidazole solution in CH_3CN, and 241 mg (0.80 mmol) of (2-cyanoethoxy)bis(diisopropylamino)phosphine (reaction time 1 h). Purification with CH_2Cl_2/CH_3OH and lyophilization with benzene afforded 332 mg (67%) of a colorless solid. ^1H NMR (500 MHz, C_6D_6): δ (ppm) 11.11 (s, 2H), 9.61 (s, 2H), 8.07 (s, 2H), 7.85−6.70 (m, 36H), 6.42 (dd, J = 6.6, 6.6 Hz, 1H), 6.36 (dd, J = 6.3, 6.3 Hz, 1H), 4.88−4.80 (m, 2H), 4.49−4.45 (m, 2H), 3.67−3.26 (m, 26H), 2.92−2.86 (m, 1H), 2.65−2.59 (m, 1H), 2.50−2.39 (m, 3H), 2.17 (ddd, J = 17.2, 5.8, 1H), 2.05 (ddd, J = 16.7, 5.6, 5.6 Hz, 1H), 1.93 (ddd, J = 16.7, 6.9, 5.3 Hz, 1H), 1.17−1.01 (m, 36H). ^{13}C NMR (126 MHz, C_6D_6): δ (ppm) 178.7, 159.3, 159.2, 147.0, 146.8, 145.4, 145.3, 140.7, 140.6, 136.1, 136.1, 136.0, 136.0, 130.7, 130.6, 130.6, 130.5, 129.3, 128.8, 128.7, 128.6, 128.3, 128.2, 128.0, 127.2, 127.1, 121.9, 121.9, 118.9, 118.7, 118.4, 118.2, 113.6, 87.1, 86.7, 86.6, 86.4, 86.4, 85.9, 75.1, 74.9, 74.1, 73.9, 64.1, 58.8, 58.6, 58.5, 58.4, 54.9, 54.9, 43.7, 43.6, 43.5, 43.5, 38.7, 38.5, 36.1, 24.8, 24.7, 24.6, 24.6, 20.6, 20.5, 20.3, 20.3, 19.3, 19.2, 19.1, 19.1, 1.4. ^{31}P NMR (202 MHz, C_6D_6): δ (ppm) 149.76, 147.90. MALDI-MS: calcd 930.4282, obsd 953.4237 $(M + Na^+)$

N^2-Isobutyryl-8*N*-(4-methylphenylamino)-*O*3′-[(2-cyanoethoxy)-(*N*,*N*′-diisopropylamino)phosphinyl]-*O*5′-dimethoxytrityl-2′-deoxyguanosine (17b). The general procedure (IV) was conducted with 500 mg (1.13 mmol) of compound **16b** in 25 mL of dry pyridine (reaction time 3 h). Purification afforded 605 mg (72%) of a slightly yellow foam. ^1H NMR (DMSO-d_6, 500 MHz): δ (ppm) 11.99 (s, 1H), 11.04 (s, 1H), 8.73 (s, 1H), 7.57−7.56 (m, 2H), 7.28−7.26 (m, 2H), 7.16−7.08 (m, 9H), 6.72−6.65 (m, 4H), 6.41 (dd, J = 7.4, 6.1 Hz, 1H), 5.15 (d, J = 5.2 Hz, 1H), 4.52 (dddd, J = 5.2, 7.0, 4.9, 4.7 Hz, 1H), 3.98 (ddd, J = 8.0, 4.7, 2.7 Hz, 1H), 3.68 (s,

3H), 3.67 (s, 3H), 3.46 (dd, J = 10.1, 8.0, Hz, 1H), 3.17 (ddd, J = 13.1, 7.0, 6.1 Hz, 1H), 3.08 (dd, J = 10.1, 2.7 Hz, 1H), 2.70 (sept, J = 6.8 Hz, 1H), 2.25 (s, 3H), 2.22 (ddd, J = 13.1, 7.4, 4.9 Hz, 1H), 1.10 (d, J = 6.8 Hz, 3H), 1.08 (d, J = 6.8 Hz, 3H).^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.8, 158.1, 158.0, 153.8, 147.1, 146.3, 145.7, 145.1, 138.4, 135.8, 135.7, 129.9, 129.9, 129.8, 129.2, 127.9, 127.6, 126.6, 118.0, 117.9, 112.9, 112.9, 86.6, 85.3, 83.2, 71.0, 64.9, 55.0, 55.0, 37.6, 34.7, 20.5, 19.2, 18.9. HRFABMS: calcd (M + H)$^+$ 745.3350, obsd 745.3363.

The general procedure (V) was conducted with 500 mg (0.69 mmol) of N^2-isobutyryl-8*N*-(4-methylphenylamino)-*O*5′-dimethoxytrityl-2′-deoxyguanosine in 25 mL of dry CH_3CN/CH_2Cl_2, 2.76 mL (0.69 mmol) of dicyanoimidazole solution in CH_3CN, and 304 mg (1.01 mmol) of (2-cyanoethoxy)bis(diisopropylamino)phosphine (reaction time 1 h). Purification with CH_2Cl_2/CH_3OH and lyophilization with benzene afforded 463 mg (73%) of a slightly yellow foam. ^{1}H NMR (500 MHz, C_6D_6): δ (ppm) 12.01 (s, 2H), 9.19 (s, 2H), 8.92 (s, 2H), 7.74−6.69 (m, 34H), 6.18 (dd, J = 6.4, 6.0 Hz, 1H), 6.13 (dd, J = 6.3, 6.0 Hz, 1H), 4.85−4.77 (m, 2H), 4.50−4.45 (m, 2H), 3.59−3.23 (m, 25H), 3.21−3.15 (m, 1H), 2.80 (ddd, J = 14.0, 6.0, 3.0 Hz, 1H), 2.48 (ddd, J = 13.3, 6.0, 2.9 Hz), 2.38 (sept, J = 6.9 Hz, 1H), 2.31−2.21 (m, 2H), 2.15 (s, 6H), 2.04−1.92 (m, 2H), 1.87 (ddd, J = 16.9, 7.0, 5.6 Hz, 1H), 1.14−0.95 (m, 36H). ^{13}C NMR (126 MHz, C_6D_6): δ (ppm) 178.8, 159.3, 159.2, 155.3, 155.2, 147.6, 147.3, 147.1, 146.4, 146.2, 145.4, 145.4, 138.3, 138.1, 136.1, 136.1, 136.0, 136.0, 131.1, 131.0, 130.7, 130.6, 130.6, 130.5, 129.8, 128.8, 128.7, 128.6, 128.3, 128.2, 128.0, 127.2, 127.1, 118.9, 118.9, 118.8, 118.6, 118.6, 118.3, 113.6, 113.6, 87.1, 86.7, 86.6, 86.4, 86.4, 85.8, 75.1, 74.9, 74.0, 73.9, 64.1, 58.8, 58.6, 58.5, 58.4, 54.9, 54.9, 43.6, 43.6, 43.5, 43.5, 38.7, 38.4, 36.2, 36.1, 24.8, 24.7, 24.6, 24.6, 20.9, 20.6, 20.6, 20.4, 20.3, 19.2, 19.2, 19.1, 1.4. ^{31}P NMR (202 MHz, C_6D_6): δ (ppm) 149.97, 147.79 MALDI-MS: calcd 944.4365, obsd. 967.3248 (M + Na$^+$).

N^2-Isobutyryl-8*N*-(4-methoxyphenylamino)-*O*3′-[(2-cyanoethoxy)-(*N*,*N*-diisopropylamino)phosphinyl]-*O*5′-dimethoxytrityl-2′-deoxyguanosine (17c). The general procedure (IV) was conducted with 340 mg (0.74 mmol) of compound **16c** in 20 mL of dry pyridine (reaction time 2 h). Purification afforded 320 mg (57%) of a colorless foam. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 11.97 (s, 1H), 11.04 (s, 1H), 8.65 (s, 1H), 7.61−7.58 (m, 2H), 7.28−7.26 (m, 2H), 7.17−7.11 (m, 7H), 6.89−6.86 (m, 2H), 6.73−6.70 (m, 2H), 6.68−6.65 (m, 2H), 6.40 (dd, J = 7.4, 6.3 Hz, 1H), 5.15 (d, J = 4.9 Hz, 1H), 4.52 (dddd, J = 4.9, 7.0, 4.8, 4.6 Hz, 1H), 3.98 (ddd, J = 7.9, 4.6, 2.8 Hz, 1H), 3.72 (s, 3H), 3.68 (s, 3H), 3.67 (s, 3H), 3.45 (dd, J = 10.1, 7.9 Hz, 1H), 3.18 (ddd, J = 13.0, 7.0, 6.3 Hz, 1H), 3.08 (dd, J = 10.1, 2.8 Hz, 1H), 2.70 (sept, J = 6.8 Hz, 1H), 2.22 (ddd, J = 13.0, 7.4, 4.8 Hz, 1H), 1.10 (d, J = 6.8 Hz, 3H), 1.08 (d, J = 6.8 Hz, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.7, 158.1, 158.0, 154.2, 153.7, 147.1, 146.8, 145.6, 145.1, 135.8, 135.7, 134.1, 129.9, 129.8, 127.9, 127.6, 126.6, 119.7, 118.0, 114.0, 112.9, 112.9, 86.6, 85.3, 83.2, 71.0, 64.9, 55.4, 55.1, 55.0, 52.2, 37.5, 34.7, 19.2, 18.9. HRFABMS: calcd (M + H)$^+$ 761.3299, obsd 761.3324.

The general procedure (V) was conducted with 395 mg (0.52 mmol) of N^2-isobutyryl-8*N*-(4-methoxyphenylamino)-*O*5′-dimethoxytrityl-2′-deoxyguanosine in 20 mL of dry CH_3OH/CH_2Cl_2, 2.08 mL (0.52 mmol) of dicyanoimidazole solution in acetonitrile, and 235 mg (0.78 mmol) of bis-*N*,*N*′-diisopropylamino(2-cyanoethyl) phosphite (reaction time 1 h). Purification with CH_2Cl_2/CH_3OH and lyophilisation with benzene afforded 278 mg (56%) of a slightly yellow foam. ^{1}H NMR (500 MHz, C_6D_6): δ (ppm) 10.99 (s, 2H), 9.63 (s, 2H), 7.70 (s, 2H), 7.67−6.70 (m, 34H), 6.34 (dd, J = 6.6, 6.6 Hz), 6.27 (dd, J = 6.5, 6.5 Hz, 1H), 4.86−4.78 (m, 2H), 3.64−3.16 (m, 32H), 2.79−2.72 (m, 1H), 2.54−2.35 (m, 4H), 2.18 (ddd, J = 17.0, 5.4, 5.4 Hz, 1H), 2.01 (ddd, J = 16.8, 5.8, 5.8 Hz, 1H), 1.94 (ddd, J = 16.8, 6.1, 6.1 Hz, 1H), 1.18−1.02 (m, 36H). ^{13}C NMR (126 MHz, C_6D_6): δ (ppm) 178.7, 159.3, 159.2, 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, 36.1, 30.2, 24.8, 24.7, 24.6, 20.6, 20.5, 20.3, 20.3, 19.1, 1.4. ^{31}P NMR (202 MHz, C_6D_6): δ (ppm) 149.36, 148.08. ESI-MS: calcd 960.4310, obsd. 983.4671 (M + Na$^+$).

N^2-Isobutyryl-8*N*-(4-biphenylamino)-*O*3′-[(2-cyanoethoxy)-(*N*,*N*-diisopropyl-amino)phosphinyl]-*O*5′-dimethoxytrityl-2′-deoxyguanosine (17d). The general procedure (IV) was conducted with 695 mg (1.38 mmol) of compound **16d** in 30 mL of dry pyridine (reaction time 3 h). Purification afforded 800 mg (72%) of a colorless solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 12.02 (s, 1H), 11.07 (s, 1H), 9.00 (s, 1H), 7.78−7.76 (m, 2H), 7.65−7.60 (m, 4H), 7.45−7.42 (m, 2H), 7.31−7.26 (m, 3H), 7.16−7.11 (m, 7H), 6.72−6.65 (m, 4H), 6.45 (dd, J = 6.4, 6.0 Hz, 1H), 5.17 (d, J = 5.0 Hz, 1H), 4.56−4.52 (m, 1H), 3.99 (ddd, J = 8.0, 4.7, 2.7 Hz, 1H), 3.67 (s, 3H), 3.66 (s, 3H), 3.47 (dd, J = 9.9, 8.0 Hz, 1H), 3.20 (ddd, J = 13.1, 7.1, 6.4 Hz, 1H), 3.09 (dd, J = 9.9, 2.7 Hz, 1H), 2.71 (sept, J = 6.7 Hz, 1H), 2.25 (ddd, J = 13.1, 7.6, 6.0 Hz, 1H), 1.11 (d, J = 6.7 Hz, 3H), 1.09 (d, J = 6.7 Hz, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ (ppm) 179.6, 157.9, 157.8, 153.7, 146.9, 145.7, 144.9, 140.4, 139.9, 135.7, 135.6, 132.6, 129.7, 129.6, 128.9, 127.8, 127.4, 126.8, 126.6, 126.4, 126.0, 117.9, 117.9, 112.8, 112.7, 86.5, 85.2, 83.2, 70.8, 64.8, 54.9, 54.8, 37.4, 34.6, 19.0, 18.8. HRFABMS: calcd (M + H)$^+$ 807.3506, obsd 807.3600.

The general procedure (V) was conducted with 400 mg (0.50 mmol) of N^2-isobutyryl-8*N*-(4-biphenylamino)-*O*5′-dimethoxytrityl-2′-deoxyguanosine in 20 mL of dry CH_3CN/CH_2Cl_2, 2.0 mL (0.50 mmol) of dicyanoimidazole solution in acetonitrile, and 224 mg (0.74 mmol) of (2-cyanoethoxy)bis(diisopropylamino)phosphine (reaction time 1 h). Purification with CH_2Cl_2/CH_3OH and lyophilization with benzene afforded 291 mg (58%) of a slightly yellow solid. ^{1}H NMR (500 MHz, C_6D_6): δ (ppm) 11.18 (s, 2H), 9.58 (s, 2H), 7.83 (s, 2H), 7.76−6.68 (m, 44H), 6.27 (dd, J = 6.4, 6.0 Hz, 1H), 6.21 (dd, J = 6.3, 6.3 Hz, 1H), 4.85−4.75 (m, 2H), 4.50−4.42 (m, 2H), 3.60−3.28 (m, 25H), 3.25−3.17 (m, 1H), 2.88−2.80 (m, 1H), 2.55 (ddd, J = 13.6, 6.0, 3.3 Hz, 1H), 2.51−2.42 (m, 2H), 2.37 (ddd, J = 16.8, 7.6, 4.7 Hz, 1H), 2.12 (ddd, J = 16.8, 6.2, 4.5 Hz, 1H), 2.00 (ddd, J = 16.8, 5.7, 5.7 Hz, 1H), 1.91 (ddd, J = 16.8, 6.9, 5.1 Hz, 1H), 1.19−1.02 (m, 36H). ^{13}C NMR (126 MHz, C_6D_6): δ (ppm) 178.8, 159.3, 159.2, 146.8, 146.6, 145.4, 145.4, 141.4, 140.1, 140.0, 136.1, 136.1, 136.0, 136.0, 134.6, 134.6, 130.7, 130.7, 130.6, 130.6, 129.1, 128.8, 128.7, 128.6, 128.3, 128.2, 128.0, 127.5, 127.2, 127.2, 127.0, 126.9, 119.0, 118.8, 118.8, 118.3, 113.6, 113.6, 87.1, 86.7, 86.7, 86.4, 86.4, 85.9, 75.0, 74.8, 74.0, 73.9, 64.1, 58.8, 58.7, 58.6, 58.5, 54.9, 54.9, 45.7, 43.7, 43.6, 43.5, 38.8, 38.6, 36.1, 24.8, 24.7, 24.6, 20.6, 20.6, 20.4, 20.3, 19.1, 19.1, 1.4. ^{31}P NMR (202 MHz, C_6D_6): δ (ppm) 149.71, 147.95. ESI-MS: calcd 1006.4650, obsd 1029.4918 (M + Na$^+$).

Synthesis of the Oligonucleotides. The oligonucleotides were synthesized using isobutyryl-protected dA, isobutyryl-protected dG, benzyl-protected dC, and T phosphoramidites on a 300+ DNA synthesizer (Eppendorf) on a 1 μmol scale using phosphoramidites and solid supports from ChemGenes. The manufacturer's standard synthesis protocol was followed except at the incorporation of the modified phosphoramidites (**17a**−**d**), which were coupled twice and for a longer time.

The oligonucleotide was purified by HPLC using triethylammoniumacetate 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 100% solvent 2; 10 min 100% of solvent 1.

LC−MS for **19**: *m*/*z* calcd for (M + H$^+$) 4469.2, found 4469.0

LC−MS for **20a**: *m*/*z* calcd for (M − H$^+$) 4562.5, found 4562.3

LC−MS for **20b**: *m*/*z* calcd for (M − H$^+$) 4571.8, found 4572.0

LC−MS for **20c**: *m*/*z* calcd for (M) 4590.1, found 4590.5

LC−MS for **20d**: *m*/*z* calcd for (M − H$^+$) 4634.2, found 4634.0

MALDI-TOF for **21**: *m*/*z* calcd 3643.465, found 1183.1, 1147.59, 1486.17, 1833.28

MALDI-TOF for **22a**: *m*/*z* calcd 3766.625, found 1485.64, 1565.67, 1765.7, 1845.63

MALDI-TOF for **22b**: *m*/*z* calcd 3810.531, found 1173.2, 1486.2, 1563.9

Enzymatic Digestion of Oligonucleotides 21 and 22a,b. Enzymatic digestion of the oligonucleotides was performed by treatment of purified oligonucleotides (2 nmol) in a reaction buffer (pH 5.3, 100 μL, 0.03 M sodium acetate) with 5 μL of $ZnSO_4$ solution (20 mM) and 5 units of Nuclease P1 stirred at 37 °C overnight. This was followed by the addition of 20 μL of a buffer (pH 9, 50 mM, Tris/HCl) and 5 units of alkaline phosphatase, and the reaction was stirred at 37 °C overnight. The reaction mixture was concentrated, solved in methanol and filtered. After concentration of the sample, the pellet was solved in 50 μL water and injected directly on HPLC using water (solvent 1) and CH_3CN (solvent 2). The gradient was as follows: initially 100% solvent 1, then a 20 min linear gradient to 25% solvent 2; 5 min 100% solvent 2; 10 min 100% of solvent 1.

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 from 5 to 80 °C.

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

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

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