# New and Highly Efficient Synthesis of *Cis*- and *Trans*-Opened Benzo[*a*]pyrene 7,8-Diol 9,10-Epoxide Adducts at the Exocyclic $N^2$-Amino Group of Deoxyguanosine

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We describe a new and facile method for the synthesis of both *cis*- and *trans*-opened $N^2$-deoxyguanosine (dG) adducts of (±)-7α,8β-dihydoxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and (±)-7α,8β-dihydoxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene at C-10. The key step in our approach is the direct coupling of $O^6$-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine with these epoxides followed by the separation of the mixtures of *cis*- and *trans*-diastereomers produced. Overall coupling yields ranged from 45 to 65%. Stereochemistry of addition of the $N^2$-exocyclic amino group of dG (*cis*−*trans*, ∼1:1) was assigned by NMR, and the absolute configuration of the dG adducts was unequivocally assigned by CD spectroscopy after separation of each individual diastereomer and cleavage of the allyl protecting group. A strong CD band at 279 nm in the $O^6$-protected adduct was found to be diagnostic for configuration at C-10, with a negative band correlating with 10*R* configuration. The synthetic methodology described allows easy access to *cis*- and *trans*-opened $N^2$-dG adducts which are valuable building blocks for the synthesis of adduct-containing oligonucleotides for physical and biochemical studies.

## Introduction

Polycyclic aromatic hydrocarbons (PAHs), formed during incomplete combustion of organic material, are ubiquitous environmental pollutants that require metabolic activation to electrophilic, reactive metabolites in order to exert their mutagenic and tumorigenic activity.[1] The strong carcinogenicity of a number of the PAHs, including benzo[*a*]pyrene (B[*a*]P), has been attributed to the metabolic formation of sterically hindered bay- and fjord-region diol epoxides (DEs) on benzo-rings of the hydrocarbons. Due to their electrophilic character, these DEs (DE-1 with the benzylic hydroxyl group and epoxide oxygen *cis* and DE-2 with these groups *trans*, cf. Scheme 1) react with the exocyclic $N^6$- and $N^2$-amino groups of deoxyadenosine (dA) and deoxyguanosine (dG) residues in DNA by both *cis*- and *trans*-opening of the oxirane ring at the reactive benzylic position.[2] Formation of these stable adducts at specific sites in protooncogenes (e.g., *ras* family members)[3] or tumor suppressor genes such as p53[4] is thought to play an important role in the mutagenic activity of the DE and appears to be the initial step for cancer development caused by PAH. Recently, it was demonstrated that B[*a*]P dG adducts can block normal DNA cleavage by topoisomerase I, trap the enzyme−DNA covalent intermediate, and promote illegitimate recombinations, thus providing a new possible mechanism for the induction of cancer by the PAH.[5]

The only highly carcinogenic DE of the four possible stereoisomeric B[*a*]P DEs formed in mammals,[6] the (+)-DE-2 enantiomer with (7*R*,8*S*,9*S*,10*R*)-absolute configuration, reacts predominantly with dG (95%) in DNA to form predominantly a stable *trans*-opened $N^2$-dG adduct.[7,8] The B[*a*]P DE-1 enantiomers also favor reaction at dG in DNA, but to a slightly lesser extent (∼82−86%). Interestingly, the (−)-DE-2 enantiomer with (7*S*,8*R*,9*R*,10*S*)-absolute configuration reacts at both dG and dA to comparable extents.[8] Despite the importance of dG ad-

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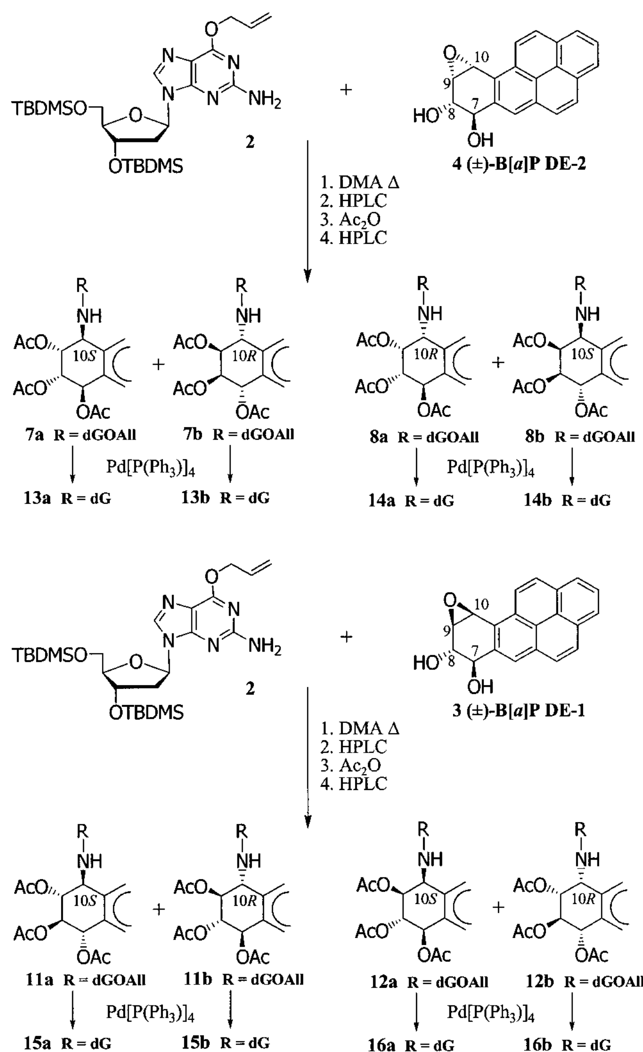
**Scheme 1**




ducts, there has been no convenient method described for their synthesis and their site-specific incorporation into oligonucleotides.

An early approach to the synthesis of oligonucleotides containing DEs attached to the exocyclic amino groups of the purines was the direct alkylation of the oligonucleotide with the DE.[9] This approach has been successfully used to prepare enough oligonucleotide to establish solution conformations of dG adducted duplexes by 2D NMR[10] as well as a number of biochemical studies.[11] In general, this approach has been restricted to sequences containing only one purine residue in a C**G**C sequence context in order to avoid attack of the DE at other purine sites, thus resulting in a complex separation problem.[9c] A postoligomerization modification procedure,[12] in which a fluoropurine analogue incorporated into the oligonucleotide is allowed to react with a PAH aminotriol, has been used for the synthesis of oligonucleotides containing either dA[13] or dG adducts.[14] Prolonged heating (55 °C, 5 d) of the support bound oligonucleotide with the aminotriol leads to partial decomposition of the oligonucleotide. The resulting decomposition products bearing the adduct are difficult to separate from the desired product, and the yields for the coupling step are relatively modest. Finally, selective blocking group strategies have been developed for the synthesis of adducted dA phosphoramidites to be used in partially automated DNA synthesis. In this approach, dA with the sugar blocked by silyl groups and the exocyclic C-6 amino group replaced by either a fluoro-[15] or sulfonate-[16] leaving group is allowed to react with an aminotriol. As with postoligomerization modification, there are no limitations on the choice of sequence. This approach has been used to synthesize oligonucleotides containing dA adducts for 2D NMR[17] and biochemical studies.[18] Similar reactions utilizing dG analogues containing either fluoro-[19] or sulfonate-[20] leaving groups at C-2 resulted in low to moderate yields of adducted dG phosphoramidites for incorporation into oligonucleotides. Although the crude oligonucleotide products are relatively pure, preparation of the requisite phosphoramidites entails several steps. The "*trans*" aminotriols are readily prepared by direct ammonolysis of DEs,[14a,15,21] whereas preparation of the corresponding "*cis*" aminotriols requires multiple steps.[22] Recently, our laboratory described a highly efficient route to *cis*-opened $N^6$-dA adducts suitable for conversion to phosphoramidites based on the Sharpless asymmetric aminohydroxylation (AA) reaction.[23] The procedure stereospecifically produces $N^6$-dA adducts that correspond to *cis* opening

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of DE-2. Thus far, we have been unable to identify AA reaction conditions that allow preparation of either dG adducts or adducts that correspond to *cis*-opening of DE-1.

The present report describes a highly efficient general approach for the synthesis of *cis*- and *trans*-opened $N^2$-dG adducts of B[*a*]P DE-1 and B[*a*]P DE-2. The key step in our synthesis is the direct opening of either racemic DE by the exocyclic $N^2$-amino group of $O^6$-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine. The resulting mixture of *cis*- and *trans*-opened diastereomers is readily separated by HPLC. Simultaneous formation of both *cis*- and *trans*-opened adducts is desirable because both types of adducts are useful in biochemical studies.

## Results and Discussion

The weak nucleophilicity of the exocyclic $N^2$-amino group in dG (**1**), due to the electron-withdrawing character of the carbonyl group,[24] necessitated protection of the $O^6$-position prior to direct reaction with the DEs. We chose allyl as the protecting group for the $O^6$-position because of its stability and because of its small size which decreases possible steric interactions. The allyl group can be selectively cleaved without affecting other protecting groups[25] and can act as an electron donor to the purine system. Failure to block the $O^6$-position or the use of other blocking groups such as the *p*-nitrophenethyl group failed to produce any of the desired coupling product described below.

The desired dG building block for synthesis of the adducts, $O^6$-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine **2**, was prepared from commercially available 2′-deoxyguanosine **1** in 40% overall yield in two steps.[26] The racemic mixtures of both B[*a*]P DE-1 **3** and B[*a*]P DE-2 **4** were prepared from racemic *trans*-7,8-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene by the published method.[27] The coupling reaction of racemic DE-1 or DE-2 with the dG building block **2** was performed in dimethylacetamide (DMA) at 90−100 °C under nitrogen (Scheme 1). For each reaction, four diastereomers were obtained through *cis*- and *trans*-opening of the oxirane ring of either DE-1 (45% yield) or DE-2 (65% yield) at C-10. Interestingly, reaction of **2** with DE-2 resulted in predominant formation of *cis*-opened $N^2$-dG adducts (60:40 based on HPLC). In contrast, reaction of **2** with DE-1 yielded a 40:60 mixture of *cis*- and *trans*-opened $N^2$-dG adducts. The formation of *cis*-opened adducts indicates that a significant portion of the overall reaction occurs by an $S_N1$ like pathway, although some or all of the *trans*-opened adducts may be formed by an $S_N2$ pathway. A potential mechanism for formation of *cis* adducts would consist of axial attack by **2** at C-10 on a carbocation derived from the aligned conformation of the epoxide in which the cleaved carbon−oxygen bond is perpendicular to the plane of the aromatic system.[28] In contrast, axial attack to form *trans*-opened adducts would have to occur on the carbocation derived from the nonaligned conformation of the epoxide.[28]

**Table 1. Comparison of Benzo-Ring $^1$H NMR Data for the $O^6$-Allyl Protected *Cis*- and *Trans*-Opened $N^2$-dG Adducts as Their Disilyl Triacetates (300 MHz, acetone-$d_6$)[a]**

| compound | $H_7$ | $H_8$ | $H_9$ | $H_{10}$ |
|---|---|---|---|---|
| **7a** (10*S*) | 6.90 | 6.04 | 6.20 | 7.10 |
| *trans*-dG OAll-DE-2 | | $J_{7,8} = 9.8$ | $J_{8,9} = 2.8$ | $J_{9,10} = 2.9$ |
| **7b** (10*R*) | 6.87 | 6.01 | 6.15 | 7.23 |
| | | $J_{7,8} = 9.8$ | $J_{8,9} = 1.9$ | $J_{9,10} = 1.9$ |
| **8a** (10*R*) | 6.50 | 5.59 | 6.01−5.84 | 6.75 |
| *cis*-dG OAll-DE-2 | | $J_{7,8} = 3.9$ | $J_{8,9} = 1.9$ | $J_{9,10}$ = NA |
| **8b** (10*S*) | 6.47 | 5.58 | 5.96−5.79 | 6.74 |
| | | $J_{7,8} = 3.9$ | $J_{8,9}$ = NA | $J_{9,10}$ = NA |
| **11a** (10*S*) | 6.75 | 5.54 | 5.95 | 6.69 |
| *trans*-dG OAll-DE-1 | | $J_{7,8} = 6.0$ | $J_{8,9} = 6.0$ | $J_{9,10} = 3.3$ |
| **11b** (10*R*) | 6.76−6.71 | 5.54 | 5.92 | 6.76−6.71 |
| | | $J_{7,8} = 6.0$ | $J_{8,9} = 6.0$ | $J_{9,10} = 3.6$ |
| **12a** (10*S*) | 6.72−6.69 | 6.40−6.26 | 5.76 | 7.16−7.04 |
| *cis*-dG OAll-DE-1 | | $J_{7,8}$ = NA | $J_{8,9} = 11.5$ | $J_{9,10} = 4.9$ |
| **12b** (10*R*) | 6.71 | 6.29 | 5.76 | 7.25−7.07 |
| | | $J_{7,8} = 8.3$ | $J_{8,9} = 11.7$ | $J_{9,10}$ = NA |

[a] NA = not assigned due to broad peaks.

Separation of the mixture of *cis*- and *trans*-opened $N^2$-dG DE-2 adducts from the reaction of **2** with DE-2 was performed in two steps. First, the crude mixture of the 4 diastereomers (Scheme 1) was separated by HPLC (EtOAc in hexane on silica) into a mixture of the *cis*-opened adducts (**5**) and a mixture of the *trans*-opened adducts (**6**). After acetylation of **5** and **6**, the individual *trans* distereomers **7a,b** and *cis* diastereomers **8a,b** were separated by HPLC (**7a,b** with EtOAc in hexane and **8a,b** with MeOH in $CH_2Cl_2$, both on silica). Stereochemistry of the *cis*-(**8a,b**) and *trans*-(**7a,b**) DE-2 $N^2$-dG adducts was assigned by examination of the coupling constants for the hydrogens on their saturated benzo-rings (Table 1). The NMR data showed a large coupling constant $J_{7,8} = 9.8$ Hz for the acetylated *trans*-opened $N^2$-dG DE-2 adducts **7a,b**, which is typical for *trans*-opened DE-2 adducts.[2a,29] In contrast, the value of $J_{7,8}$ was small (3.9 Hz) as expected for the acetylated *cis*-opened dG DE-2 adducts **8a,b**.[2a,29]

Initial separation of the corresponding diasteromeric mixture of adducts from the reaction between **2** and DE-1 was also accomplished by HPLC as above. In contrast to the DE-2 adducts, the pair of *cis*-opened $N^2$-dG DE-1 adducts (**10a,b**) separated from each other as well as from the mixture of *trans*-opened $N^2$-dG DE-1 adducts (**9**). Acetylation of the separated *cis*-opened adducts yielded **12a** and **12b**. The acetylated *trans*-opened $N^2$-dG DE-1 adducts **11a,b** were separated by HPLC (EtOAc in hexane on silica) using a gradient. The NMR data showed a large coupling constant $J_{8,9} = 11.5$ Hz for **12a,b** indicating that these dG adducts were derived from *cis*-opening of DE-1.[2a,29] The other pair of diastereomers (**11a,b**) had a coupling constant of $J_{7,8} = 6.0$ Hz indicating that *trans*-opening of DE-1 had occurred. For both the DE-1 and DE-2 adducts, the unacetylated *trans*-diastereomers eluted prior to the *cis*-isomers on the silica column eluted with EtOAc in hexane.

The presence of the $O^6$-allyl protecting group made it difficult to assign absolute configuration to the $O^6$-allyl

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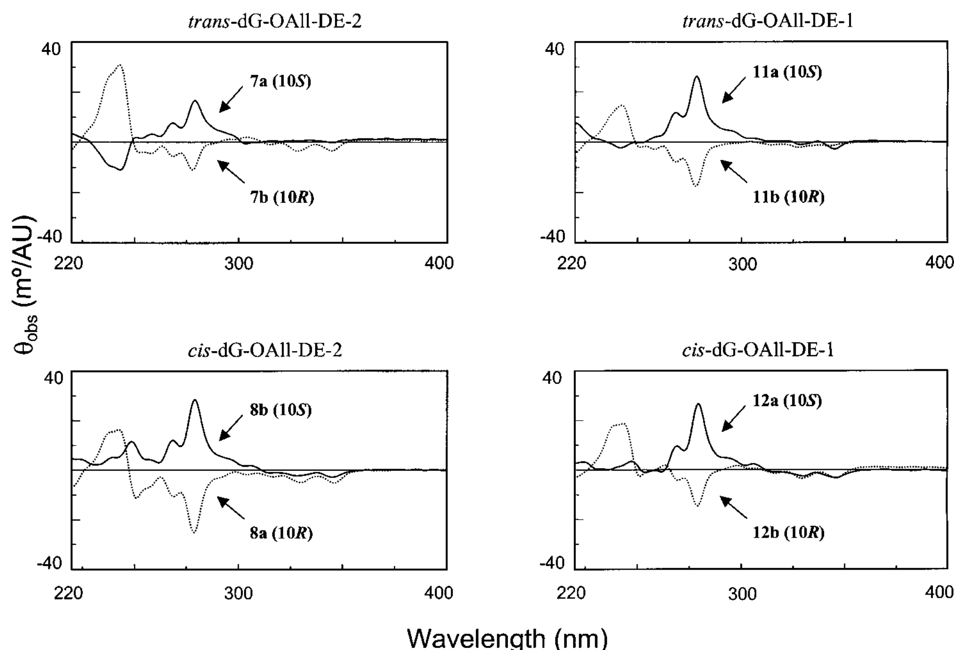


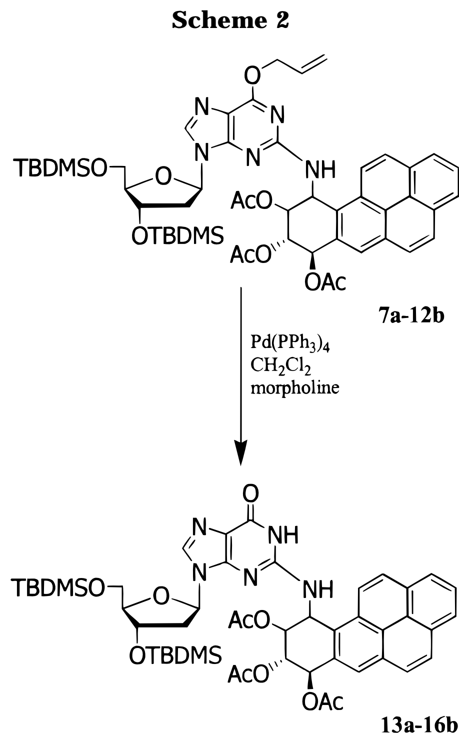

**Figure 1.** CD spectra (normalized to 1 absorbance unit at 279 nm, methanol) of the *O*[6]-allyl protected *cis*- and *trans*-opened *N*[2]-dG adducts **7a**, **7b**, **8a**, **8b**, **11a**, **11b**, **12a**, and **12b** (as their disilyl triacetates) of B[*a*]P DE-1 and B[*a*]P DE-2 (Scheme 1). The positive exciton band at 279 nm correlates with 10*S* absolute configuration. Protection of the *O*[6]-position of dG substantially decreases the exciton interaction between the hydrocarbon moiety and the purine system.

protected dG adducts **7a**, **7b**, **8a**, **8b**, **11a**, **11b**, **12a**, and **12b** (as their disilyl triacetates) by comparison of their CD spectra (Figure 1) with those reported for the completely unprotected *cis*- and *trans*-opened *N*[2]-dG adducts obtained from the reaction of DE-1 and DE-2 with DNA or deoxyguanylic acid.[7b,8,30] The presence of the acetate and silyl protecting groups on the *O*[6]-allyl protected dG adducts is not expected to substantially influence either the shape or intensity of their CD spectra.

In contrast to the reported CD spectra of the unprotected dG adducts which all had their major CD bands at 250 nm, the major CD band at 279 nm for the *O*[6]-allyl protected dG adducts **7a**, **7b**, **8a**, **8b**, **11a**, **11b**, **12a**, and **12b** was relatively weak (Figure 1). In addition, the shapes of the CD spectra for the *O*[6]-allyl protected dG adducts were completely different from those of their *O*[6]-unprotected counterparts. The CD bands at 279 nm were positive in sign for the dG adducts **7a**, **8b**, **11a**, and **12a** and negative for **7b**, **8a**, **11b**, and **12b**. All of the *O*[6]-allyl protected dG adducts had weak, negative bands above 300 nm. In addition, adducts **7b**, **8a**, **11b**, and **12b** showed an additional positive CD band at about 242 nm (Figure 1). Therefore, assignment of absolute configuration to the *O*[6]-allyl protected dG adducts based on comparison of their CD spectra with the spectra of their *O*[6]-unprotected counterparts was not possible.

To unambiguously assign the absolute configuration of the *O*[6]-allyl protected dG adducts, it was necessary to remove the allyl protecting group. Initial attempts to remove the allyl group with the Wilkinson catalyst chlorotris(triphenylphosphine)-rhodium(I)[31] in EtOH/$H_2O$ at 70 °C were unsuccessful. In addition to removing the allyl group, side reactions at the hydrocarbon moiety occurred. Tetrakis(triphenylphosphine)-palladium(0)[32] as catalyst in a transesterfication reaction at room temperature with excess morpholine as a scavenger for the allyl group allowed cleavage of the allyl group in good (75–90%) yield (Scheme 2). CD spectra of the now *O*[6]-deprotected dG adducts, **13a**, **13b**, **14a**, **14b**, **15a**, **15b**, **16a**, and **16b** (Figure 2), showed the expected major CD bands at 250 nm and allowed assignment of the absolute

**Scheme 2**

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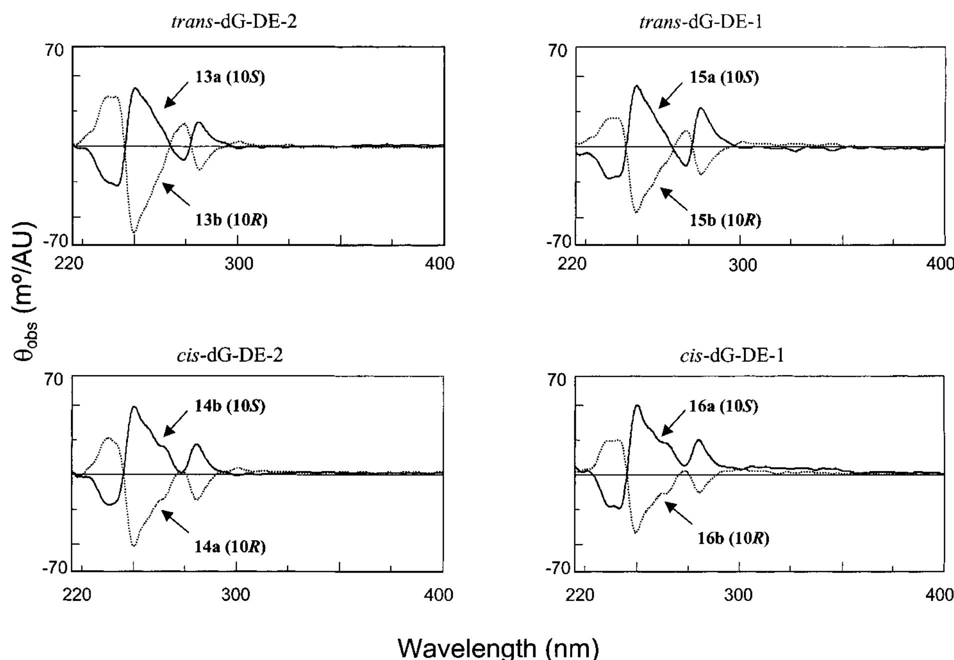


**Figure 2.** CD spectra (normalized to 1 absorbance unit at 250 nm, methanol) of the *cis*- and *trans*-opened $N^2$-dG adducts **13a**, **13b**, **14a**, **14b**, **15a**, **15b**, **16a**, and **16b** (as their disilyl triacetates) of B[*a*]P DE-1 and B[*a*]P DE-2 (Scheme 2). The strong positive exciton band at 250 nm indicates 10*S* absolute configuration.[7b,8,30]

configuration to all of the adducts by comparison with reported literature spectra.[7b,8,30] For example, the chemically related *trans*-opened $N^2$-dG adducts **7a** ($O^6$-protected) and **13a** ($O^6$-deprotected) with positive CD bands at 279 and 250 nm, respectively, have (10*S*)-configuration and were formed from (+)-(7*R*,8*S*,9*S*,10*R*) B[*a*]P DE-2 by inversion at C-10. Thus, the sign of the 279 nm band in these *$O^6$-protected* dG adducts correlates with their absolute configuration: a positive band at 279 nm implies 10*S*-configuration. In addition, the broad positive band around 242 nm for **7b**, **8a**, **11b**, and **12b** correlates with 10*R*-absolute configuration. Notably, an analogous, strong positive (10*R*) or negative (10*S*) band is present in the *$O^6$-unprotected* dG adducts at short wavelength (Figure 2). Once absolute configurations had been established, it became apparent that, in the EtOAc/hexane solvent system on silica, the *$O^6$-deprotected* 10*S*-dG adducts (as the disilyl triacetates) were always early eluting relative to their 10*R*-isomers.

In summary, the present report describes an efficient synthesis for both *cis*- and *trans*-opened $N^2$-dG adducts of B[*a*]P DE from racemic DE-1 and DE-2. Our approach circumvents the multistep syntheses needed to prepare fluoro- or sulfonate-activated purines as well as "*cis*" and "*trans*" aminotriols. The excellent chromatographic separation of the $O^6$-allyl protected *cis* and *trans* diastereomers as their disilyl ethers before acetylation makes this methodology suitable for the synthesis of dG adducts on an adequate scale for demandingly large studies such as solution conformation analysis of adducted oligonucleotides by 2D NMR. Conversion of the present adducts into 5′-DMT-3′-phosphoramidites for use in such oligonucleotide synthesis is straightforward.[15,16,19] We are presently examining whether there is advantage to maintaining the $O^6$-allyl protection throughout oligonucleotide synthesis. In general, there seems to be little need to separate the *R*- and *S*-diastereomers at the adduct stage because we have found that the adducted oligonucleotides (up to 25-mers, unpublished) are readily separable. For example, we have prepared and separated oligonucleotide 22-mers containing *cis*- and *trans*-opened $N^2$-dG adducts of B[*a*]P DE-2 derived from the (+)-(7*R*,8*S*,9*S*,10*R*)- or (−)-(7*S*,8*R*,9*R*,10*S*)-enantiomers.[5] Preliminary results (unpublished) have established that the present approach can be extended to highly hindered fjord-region diol epoxides such as those from benzo[*c*]-phenanthrene.[33]

## Experimental Section

**CAUTION:** *Benzo[a]pyrene-7,8-dihydrodiol and the B[a]P-diol epoxides DE−1 and DE−2 are carcinogenic and mutagenic and must be handled carefully in accordance with NIH guidelines.*[34] $^1$H NMR spectra were measured at 300 MHz in acetone-$d_6$. Chemical shifts ($\delta$) are reported in ppm and coupling constants ($J$) are in Hz. For adducts and related compounds, singly primed numbers are used for the protons on the ribose moiety (1′−5′) and the purine protons are doubly primed (8″). For the vinyl protons of the allyl protecting group, $H_v$ designates the vinyl hydrogen adjacent to the methylene, and $H_c$ and $H_t$ are the terminal vinyl protons *cis* and *trans* to it, respectively. These assignments are not made for *cis* adducts due to their broad spectra. All chemicals used were purchased from Aldrich in highest quality and used without further purification. DMA, morpholine, and $CH_2Cl_2$ used for reactions were dried prior to use over 4 Å molecular sieves. Column chromatography was conducted on silica gel 60 (220−440 mesh) from Fluka.

**Diastereomeric $N^2$-[10-(7,8,9-Trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-$O^6$-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine Adducts Derived from *Cis* (5) and *Trans* (6) Opening of (±)-DE-2.** A mixture of **4** (180 mg, 0.60 mmol), **2** (963 mg, 1.86 mmol), and dry DMA (2 mL) was heated at 90−100 °C for 2 h under nitrogen. The cooled orange solution was concentrated under reduced pressure to an orange oil, which was purified by chromatography

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on silica gel using $CH_2Cl_2$−MeOH (95:5). Two fractions were collected. The first fraction contained unreacted **2** (650 mg) and the second contained the crude mixture of the four *cis*- and *trans*-dG adducts (324 mg, 65%). The crude products were dissolved in EtOAc−hexane (3:1) and the *cis*- and *trans*-dG diastereomers were separated by HPLC using a 5-μm, 10 × 250-mm Axxiom silica-gel column eluted at 5 mL/min with EtOAc−hexane (3:1). The pair of *trans*-dG diastereomers **6** had $T_R$ = 7.1 min and the pair of *cis*-dG diastereomers **5** $T_R$ = 8.6 min (*trans*-dG diastereomers, 99.2 mg; *cis*-dG diastereomers, 135.9 mg; 47% combined yield).

***N*²-[10-(7,8,9-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine Adducts (7a) and (7b) Derived from *Trans* Opening of (±)-DE-2.** A solution of **6** (30 mg, 0.036 mmol), $Ac_2O$ (1.5 mL), and DMAP (2 mg) in pyridine (5 mL) was stirred overnight. The solvents were evaporated under reduced pressure and the remaining residue was purified by chromatography on silica gel using $CH_2Cl_2$−MeOH (98:2). The *trans* diastereomers were separated by HPLC using a 5-μm, 10 × 250-mm Axxiom silica-gel column eluted at 5 mL/min with EtOAc−hexane (1:3). The *trans* adducts had $T_{R(early)}$ = 17.3 min and $T_{R(late)}$ =19.9 min. (***early*** diastereomer **7a**, 16.4 mg; ***late*** diastereomer **7b**, 13.8 mg; 87% combined yield). The $^1$H NMR and mass spectra for **7a** and **7b** matched those reported in the literature.[20]

***N*²-[10*S*-(7*R*,8*S*,9*R*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (7a).** $^1$H NMR δ: 8.40−8.13 (m, 8$H_{aromatic}$, 1$H_{8''}$), 7.10 (br s, 1$H_{10}$), 6.90 (d, 1$H_7$, *J* = 9.8), 6.50 (br s, 1$H_{NH}$), 6.41−6.39 (m, 1$H_{1'}$), 6.30−6.20 (m, 1$H_{v(allyl)}$), 6.20 (d, 1$H_9$, *J* = 2.9), 6.04 (dd, 1$H_8$, *J* = 9.3, 2.8), 5.53 (d, 1$H_{t(allyl)}$, *J* = 17.6), 5.34 (d, 1$H_{c(allyl)}$, *J* = 9.8), 5.11 (br s, 2H, $CH_{2(allyl)}$), 4.76 (br s, 1$H_{3'}$), 4.02 (br s, 1$H_{4'}$), 3.92 (br s, 2$H_{5',5'}$), 2.90−2.85 (m, 1$H_{2'}$), 2.58−2.49 (m, 1$H_{2'}$), 2.37−2.11 (3s, 9H, OAc), 0.96 (*tert*-butyl methyls), 0.19−0.09 (Si-bonded methyls). LRMS (FAB⁺): *m*/*z* 964 ([M + H]⁺). HRMS calcd for ($C_{51}H_{65}O_{10}N_5Si_2$ + Cs): 1096.3324. Found: 1096.3306.

***N*²-[10*R*-(7*S*,8*R*,9*S*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (7b).** $^1$H NMR δ: 8.38−8.11 (m, 8$H_{aromatic}$, 1$H_{8''}$), 7.23 (br s, 1$H_{10}$), 6.87 (d, 1$H_7$, *J* = 9.8), 6.41 (br s, 1$H_{NH}$), 6.35−6.33 (m, 1$H_{1'}$), 6.21−6.19 (m, 1$H_{v(allyl)}$), 6.15 (pt, 1$H_9$, *J* = 1.9), 6.01 (dd, 1$H_8$, *J* = 9.8, 1.9), 5.54 (d, 1$H_{t(allyl)}$, *J* = 17.6), 5.31−5.29 (m, 1$H_{c(allyl)}$), 5.03 (br s, $CH_{2(allyl)}$), 4.69 (br s, 1$H_{3'}$), 3.98−3.89 (m, 1$H_{4',5',5'}$), 2.34−2.09 (3s, 9H, OAc), 0.95−0.78 (*tert*-butyl methyls), 0.19−0.00 (Si-bonded methyls). LRMS (FAB⁺): *m*/*z* 964 ([M + H]⁺). HRMS calcd for ($C_{51}H_{65}O_{10}N_5Si_2$ + Cs): 1096.3324. Found: 1096.3358.

***N*²-[10-(7,8,9-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine Adducts (8a) and (8b) Derived from *Cis* Opening of (±)-DE-2.** Acetylation of **5** (40 mg, 0.048 mmol) was carried out as described above and products were separated by HPLC using a 5-μm, 10 × 250-mm Axxiom silica-gel column eluted at 5 mL/min with $CH_2Cl_2$−MeOH (98:2). The *cis* adducts had $T_{R(early)}$ = 10.3 min and $T_{R(late)}$ = 11.7 min (***early*** diastereomer **8a**, 17.4 mg; ***late*** diastereomer **8b**, 17.5 mg; 76% combined yield).

***N*²-[10*R*-(7*R*,8*S*,9*R*-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (8a).** $^1$H NMR δ: 8.32 (d, 1$H_{11}$, *J* = 8.9), 8.19−7.93 (m, 7$H_{aromatic}$, 1$H_{8''}$), 6.75 (br s, 1$H_{10,}$), 6.50 (d, 1$H_7$, *J* = 3.9), 6.38 (m, 1$H_{1'}$), 6.20−6.12 (m, 1$H_{v(allyl)}$), 6.01−5.84 (m, 1$H_{NH}$, 1$H_9$), 5.59 (d, 1$H_8$, *J* = 1.9), 5.49−4.50 (m, 2$H_{(allyl)}$, $CH_{2(allyl)}$, 1$H_{3'}$), 3.90−3.66 (m, 3$H_{4',5',5'}$), 2.61−2.20 (m, 2$H_{2',2'}$), 2.08−1.64 (3s, 9H, OAc), 0.79−0.61 (*tert*-butyl methyls), −0.3−0.05 (Si-bonded methyls). LRMS (FAB⁺): *m*/*z* 964 ([M + H]⁺). HRMS calcd for ($C_{51}H_{65}O_{10}N_5Si_2$ + Cs): 1096.3324. Found: 1096.3373.

***N*²-[10*S*-(7*S*,8*R*,9*S*-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (8b).** $^1$H NMR δ: 8.31−7.87 (m, 8$H_{aromatic}$, 1$H_{8''}$), 6.74 (m, 1$H_{10}$), 6.47 (d, 1$H_7$, *J* = 3.9), 6.21 (m, 1$H_{1'}$), 5.96−5.79 (m, 1$H_9$, 1$H_{NH}$), 5.58 (s, 1$H_8$), 5.49−4.50 (m, 2$H_{(allyl)}$, $CH_{2(allyl)}$, 1$H_{3'}$), 3.95−3.33 (m, 3$H_{4',5',5'}$), 2.51−2.49 (m, 1$H_{2'}$), 2.00−1.53 (3s, 9H, OAc), 0.77−0.53 (*tert*-butyl methyls), −0.32−0.01 (Si-bonded methyls). LRMS (FAB⁺): *m*/*z* 964 ([M + H]⁺). HRMS calcd for ($C_{51}H_{65}O_{10}N_5Si_2$ + Cs): 1096.3324. Found: 1096.3292.

**Diastereomeric *N*²-[10-(7,8,9-Trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine Adducts from *Cis* (10) and *Trans* (9) Opening of (±)-DE-1.** A mixture of **3** (192 mg, 0.64 mmol), **2** (1.24 g, 1.90 mmol), and dry DMA (2 mL) was treated under the same conditions as described for the synthesis of the *cis*- and *trans*-dG DE-2 adducts to afford a crude mixture of *cis*- and *trans*-dG DE-1 adducts (273 mg, 45%). The crude products were dissolved in EtOAc−hexane (7:3) and the *cis*- and *trans*-dG diastereomers were separated by HPLC using a 5-μm, 10 × 250-mm Axxiom silica-gel column eluted at 5 mL/min with EtOAc−hexane (7:3). The *trans*-dG diastereomers **9** had $T_R$ = 6.0 min and the *cis*-dG diastereomers **10a** $T_{R(early)}$ = 7.6 min and **10b** $T_{R(late)}$ = 8.1 min. (*trans*-dG diastereomers, 102.6 mg; ***early*** *cis*-dG diastereomer **10a**, 38.4 mg; ***late*** *cis*-dG diastereomer **10b**, 32 mg; 32.5% combined yield).

***N*²-[10-(7,8,9-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine Adducts (11a) and (11b) Derived from *Trans* Opening of (±)-DE-1.** Acetylation of **9** (20 mg, 0.024 mmol) was carried out as described and the products were separated by HPLC using a 5-μm, 10 × 250-mm Axxiom silica-gel column eluted at 5 mL/min using a gradient ramped from 22% EtOAc/78% hexane to 28% EtOAc/72% hexane in 30 min. The *trans* adducts had $T_{R(early)}$ = 21.8 min and $T_{R(late)}$ = 22.6 min (***early*** diastereomer **11a**, 11.4 mg; ***late*** diastereomer **11b**, 7.3 mg; 84% combined yield).

***N*²-[10*S*-(7*S*,8*R*,9*R*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (11a).** $^1$H NMR δ: 8.44 (d, 1$H_{11}$, *J* = 9.3), 8.32−8.06 (m, 7$H_{aromatic}$, 1$H_{8''}$), 6.75 (d, 1$H_7$, *J* = 6.0), 6.69−6.64 (m, 1$H_{10}$), 6.42−6.38 (m, 1$H_{1'}$, 1$H_{NH}$), 6.20−6.12 (m, 1$H_{v(allyl)}$), 5.95 (dd, 1$H_9$, *J* = 6.0, 3.3), 5.54 (pt, 1$H_8$, *J* = 6.0), 5.44 (d, 1$H_{t(allyl)}$, *J* = 17.6), 5.24 (d, 1$H_{c(allyl)}$, *J* = 9.8), 5.10−4.96 (m, $CH_{2(allyl)}$), 4.67 (br s, 1$H_{3'}$), 3.95−3.83 (m, 3$H_{4',5',5'}$), 2.79−2.72 (m, 1$H_{2'}$), 2.43−2.38 (m, 1$H_{2'}$), 2.26−2.00 (3s, 9H, OAc), 0.89−0.85 (*tert*-butyl methyls), 0.11−0.04 (Si-bonded methyls). LRMS (FAB⁺): *m*/*z* 964 ([M + H]⁺). HRMS calcd for ($C_{51}H_{65}O_{10}N_5Si_2$ + Cs): 1096.3324. Found: 1096.3306.

***N*²-[10*R*-(7*R*,8*S*,9*S*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl])-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (11b).** $^1$H NMR δ: 8.39 (d, 1$H_{11}$, *J* = 9.3), 8.33−8.04 (m, 7$H_{aromatic}$, 1$H_{8''}$), 6.76−6.71 (m, 1$H_7$, 1$H_{10}$), 6.41 (dd, 1$H_{1'}$, *J* = 8.5, 3.6), 6.18−6.10 (m, 1$H_{v(allyl)}$), 5.92 (dd, 1$H_9$, *J* = 6.0, 3.6), 5.54 (pt, 1$H_8$, *J* = 6.0), 5.42 (d, 1$H_{t(allyl)}$, *J* = 17.6), 5.22 (d, 1$H_{c(allyl)}$, *J* = 9.8), 5.10−4.96 (m, $CH_{2(allyl)}$), 4.67−4.66 (m, 1$H_{3'}$), 3.94−3.82 (m, 3$H_{4',5',5'}$), 2.45−2.38 (m, 2$H_{2',2'}$), 2.25−2.01 (3, 9H, OAc), 0.91−0.81 (*tert*-butyl methyls), −0.03−0.14 (Si-bonded methyls). LRMS (FAB⁺): *m*/*z* 964 ([M + H]⁺). HRMS calcd for ($C_{51}H_{65}O_{10}N_5Si_2$ + Cs): 1096.3324. Found: 1096.3358.

***N*²-[10*S*-(7*R*,8*S*,9*S*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12a) and *N*²-[10*R*-(7*S*,8*R*,9*R*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-*O*⁶-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12b).** Acetylation of **10a** (10 mg, 0.012 mmol) or **10b** (9 mg, 0.011 mmol), the previously separated *cis*-opened adducts, was carried out as described to afford **12a** (8.4 mg, 73%) or **12b** (8.1 mg, 78%).

**12a.** $^1$H NMR δ: 8.41−8.14 (m, 8$H_{aromatic}$, 1$H_{8''}$), 7.16−7.04 (m, 1$H_{10}$, 1$H_{NH}$), 6.72−6.69 (m, 1$H_7$), 6.40−6.26 (m, 1$H_8$, 1$H_{1'}$), 5.76 (dd, 1$H_9$, *J* = 11.5, 4.9), 5.58−4.72 (m, 2$H_{(allyl)}$, $CH_{2(allyl)}$, 1$H_{3'}$), 4.08−3.81 (m, 3$H_{4',5',5'}$), 2.76−270 (m, 1$H_{2'}$), 2.35−1.94 (3s, 9H, OAc), 1.09−0.85 (*tert*-butyl methyls), 0.28−0.00 (Si-bonded methyls). LRMS (FAB⁺): *m*/*z* 964 ([M + H]⁺). HRMS calcd for ($C_{51}H_{65}O_{10}N_5Si_2$ + Cs): 1096.3324. Found: 1096.3423.

**12b.** $^1$H NMR δ: 8.69−8.68 (m, 1$H_{11}$), 8.41−8.14 (m, 6$H_{aromatic}$, 1$H_{8''}$), 7.86 (pt, 1$H_2$, *J* = 7.6), 7.45 (dd, 1$H_{1'}$, *J* = 7.6,

5.6), 7.25−7.07 (m, $1H_{NH}$, $1H_{10}$), 6.71 (d, $1H_7$, $J$ = 8.3), 6.29 (dd, $1H_8$, $J$ = 11.7, 8.3), 6.20−6.14 (m, $1H_{v(allyl)}$), 5.76 (d, $1H_9$, $J$ = 11.7), 5.56−4.78 (m, $2H_{(allyl)}$, $CH_{2(allyl)}$, $1H_{3'}$), 4.04−3.96 (m, $3H_{4',5',5'}$), 2.60−2.54 (m, $1H_{2'}$), 2.36−1.99 (3s, 9H, OAc), 1.04−0.89 (*tert*-butyl methyls), 0.29−0.00 (Si-bonded methyls). LRMS (FAB$^+$): *m*/*z* 964 ([M + H]$^+$). HRMS calcd for ($C_{51}H_{65}O_{10}N_5Si_2$ + Cs): 1096.3324. Found: 1096.3333.

***N*$^2$-[10*S*-(7*R*,8*S*,9*R*-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (13a) and *N*$^2$-[10*R*-(7*S*,8*R*,9*S*-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (13b).** To a solution of **7a** (10 mg, 0.01 mmol) or **7b** (8 mg, 0.008 mmol) in $CH_2Cl_2$ (5 mL) were added morpholine (87 μL, 1.0 mmol) and Pd($PPh_3$)$_4$ (1.2 mg, 0.001 mmol), and the mixture was stirred for 30 min to become a slightly yellow solution. The reaction mixture was diluted with 50 mL EtOAc and extracted with 25 mL brine (pH = 2.0), 25 mL saturated $NaHCO_3$, and 25 mL $H_2O$. The organic layer was separated, dried with $MgSO_4$, and evaporated under reduced pressure. The remaining material was purified by HPLC using a 5-μm, 10 × 250-mm Axxiom silica-gel column eluted at 5 mL/min with EtOAc−hexane (9:1) to yield **13a** (8 mg, 83%, $T_R$ = 8.6 min) or **13b** (6.1 mg, 80%, $T_R$ = 13.0 min). The $^1$H NMR and mass spectra data were identical with those reported in the literature.[14b]

***N*$^2$-[10*R*-(7*R*,8*S*,9*R*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (14a) and *N*$^2$-[10*S*-(7*S*,8*R*,9*S*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (14b).** To a solution of **8a** (10 mg, 0.01 mmol) or **8b** in $CH_2Cl_2$ (5 mL) were added morpholine (87 μL, 1 mmol) and Pd($PPh_3$)$_4$ (1.2 mg, 0.001 mmol), and the mixture was stirred for 30 min to become a slightly yellow solution. Identical workup and HPLC purification yielded **14a** (9 mg, 94%, $T_R$ = 12.9 min) or **14b** (7.2 mg, 75%, $T_R$ = 11.4 min).

**14a.** $^1$H NMR δ: 10.1 (br s, $1H_{NHamide}$), 8.36−8.09 (m, $8H_{aromatic}$), 7.86 (s, $1H_{8''}$), 6.86−6.63 (m, $1H_{10}$), 6.63 (d, $1H_7$, $J$ = 3.3), 6.43−6.30 (m, $1H_{1'}$, $1H_{NH}$), 6.01 (dd, $1H_9$, $J$ = 5.5, 2.2), 5.70 (s, $1H_8$), 4.74 (br s, $1H_{3'}$), 4.06−3.89 (m, $3H_{4',5',5'}$), 3.13−3.11 (m, $1H_{2'}$), 2.47−2.42 (m, $1H_{2'}$), 2.15−1.97 (3s, 9H, OAc), 0.92−0.75 (*tert*-butyl methyls), −0.16−0.17 (Si-bonded methyls). LRMS (FAB$^+$): *m*/*z* 924 ([M + H]$^+$). HRMS calcd for ($C_{48}H_{61}O_{10}N_5Si_2$ + Cs): 1056.3011. Found: 1056.3010.

**14b.** $^1$H NMR δ: 10.2 (br s, $1H_{NHamide}$), 8.36−8.07 (m, $8H_{aromatic}$), 7.94 (s, $1H_{8''}$), 6.83−6.80 (m, $1H_{10}$), 6.61 (d, $1H_7$, $J$ = 3.3), 6.60−6.58 (m, $1H_{NH}$), 6.27−6.24 (m, $1H_{1'}$), 6.01 (dd, $1H_9$, $J$ = 5.8, 2.5), 5.70 (d, $1H_8$, $J$ = 2.2), 4.77 (br s, $1H_{3'}$), 4.09−3.96 (m, $3H_{4',5',5'}$), 2.74−2.65 (m, $2H_{2',2'}$), 2.18−1.91 (3s, 9H, OAc), 0.96−0.93 (*tert*-butyl methyls), 0.20−0.15 (Si-bonded methyls). LRMS (FAB$^+$): *m*/*z* 924 ([M + H]$^+$). HRMS calcd for ($C_{48}H_{61}O_{10}N_5Si_2$ + Cs): 1056.3011. Found: 1056.3052.

***N*$^2$-[10*S*-(7*S*,8*R*,9*R*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (15a) and *N*$^2$-[10*R*-(7*R*,8*S*,9*S*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (15b).** To a solution of **11a** (9 mg, 0.009 mmol) or **11b** (7 mg, 0.007 mmol) in $CH_2Cl_2$ (5 mL) were added morpholine (80 μL, 0.9 mmol) and Pd($PPh_3$)$_4$ (1.1 mg, 0.001 mmol), and the mixture was stirred for 30 min to become a slightly yellow solution. Identical workup and HPLC purification yielded **15a** (7.4 mg, 86%, $T_R$ = 11.8 min) and **15b** (5.9 mg, 88%, $T_R$ = 14.6 min). The $^1$H NMR and mass spectra were identical with those reported in the literature.[14b]

***N*$^2$-[10*S*-(7*R*,8*S*,9*S*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (16a) and *N*$^2$-[10*R*-(7*S*,8*R*,9*R*-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (16b).** To a solution of **12a** or **12b** (6 mg, 0.006 mmol) in $CH_2Cl_2$ (5 mL) were added morpholine (53 μL, 0.6 mmol) and Pd($PPh_3$)$_4$ (0.72 mg, 0.0006 mmol), and the mixture was stirred for 30 min to become a slightly yellow solution. Identical workup and HPLC purification yielded **16a** (5.1 mg, 89%, $T_R$ = 8.9 min) and **16b** (5.1 mg, 89%, $T_R$ = 10.1 min).

**16a.** $^1$H NMR δ: 9.92 (br s, $1H_{NHamide}$), 8.35−8.16 (m, $7H_{aromatic}$), 8.10 (pt, $1H_2$, $J$ = 7.7), 7.96 (s, $1H_{8''}$), 7.03−6.90 (m, $1H_{10}$, $1H_{NH}$), 6.66 (d, $1H_7$, $J$ = 8.2), 6.55 (pt, $1H_{1'}$, $J$ = 6.6), 6.00 (dd, $1H_8$, $J$ = 11.8, 8.2), 5.67 (dd, $1H_9$, $J$ = 11.8, 2.2), 4.77−4.75 (m, $1H_{3'}$), 4.09−4.08 (m, $1H_{4'}$), 3.95 (d, $2H_{5',5'}$, $J$ = 4.4), 2.76−2.59 (m, $1H_{2',2'}$), 2.23−1.88 (3s, 9H, OAc), 0.96−0.93 (*tert*-butyl methyls), 0.20−0.15 (Si-bonded methyls). LRMS (FAB$^+$): *m*/*z* 924 ([M + H]$^+$). HRMS calcd for ($C_{48}H_{61}O_{10}N_5Si_2$ + Cs): 1056.3011. Found: 1056.3053.

**16b.** $^1$H NMR δ: 9.90 (br s, $1H_{NHamide}$), 8.35−8.14 (m, $7H_{aromatic}$), 8.11 (pt, $1H_2$, $J$ = 7.7), 7.93 (s, $1H_{8''}$), 6.91−6.90 (m, $1H_{10}$, $1H_{NH}$), 6.66 (d, $1H_7$, $J$ = 8.2), 6.47 (m, $1H_{1'}$), 6.01 (dd, $1H_8$, $J$ = 11.8, 8.2), 5.67−5.62 (m, $1H_9$), 4.75−4.74 (m, $1H_{3'}$), 4.06−3.92 (m, $3H_{4',5',5'}$), 2.58−2.53 (m, $1H_{2'}$), 2.22−1.95 (3s, 9H, OAc), 0.93−0.84 (*tert*-butyl methyls), −0.18−0.19 (Si-bonded methyls). LRMS (FAB$^+$): *m*/*z* 924 ([M + H]$^+$). HRMS calcd for ($C_{48}H_{61}O_{10}N_5Si_2$ + Cs): 1056.3011. Found: 1056.3060.

**Supporting Information Available:** Two tables containing $\lambda_{max}$ and $\Delta\epsilon$ data for the CD spectra of compounds **7a,b**/**8a,b**, **11a,b**−**16a,b** and $^1$H NMR spectra of compounds **7a,b**/**8a,b**, **11a,b**/**12a,b**/**14a,b** and **16a,b** depicted schematically in Schemes 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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