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Fluorinated Alcohol Mediated Control over Cis vs Trans Opening of Benzo[*a*]pyrene-7,8-diol 9,10-Epoxides at C-10 by the Exocyclic Amino Groups of O^6-Allyl Protected Deoxyguanosine and of Deoxyadenosine

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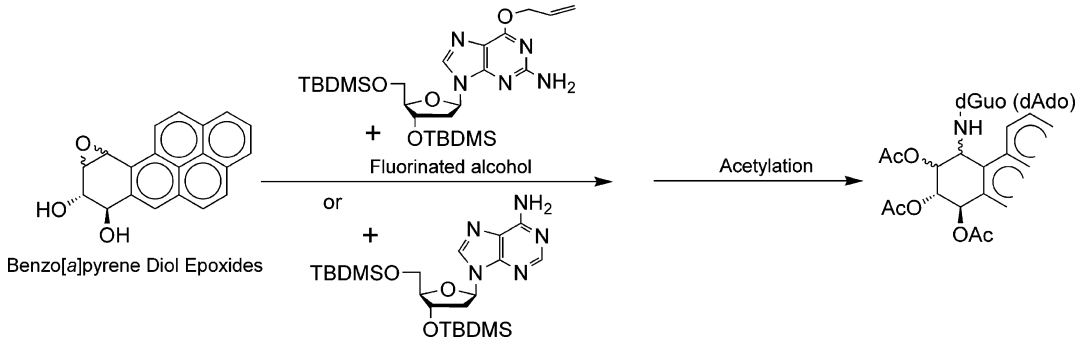

A detailed study was carried out on the stereoselective control of cis- vs trans-opening of (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene {B[*a*]P DE-1 (**1**)} and (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene {B[*a*]P DE-2 (**2**)} at C-10 by the exocyclic amino groups of protected purine nucleosides in the fluorinated alcohols trifluoroethanol (TFE), hexafluoropropan-2-ol (HFP), and perfluoro-*tert*-butanol (PFTB). Addition of the 2-amino group of O^6-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**3**) and of the 6-amino group of 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**4**) occurs at C-10 of the epoxides. The observed cis:trans ratio for the reaction of DE-1 (**1**) in the presence of 5 equiv of **3** over the range of 10−250 equiv of fluorinated alcohol varied from 53:47 to 87:13 for TFE, 60:40 to 92:8 for HFP, and 52:48 to 73:27 for PFTB. The corresponding ratios for DE-2 (**2**) varied from 22:78 to 72:28 for HFP under the same set of conditions. In contrast, the corresponding ratios for DE-2 (**2**) remained unchanged (∼40:60) for TFE and for PFTB over the range of 25−250 molar equiv. Unlike the addition of the dGuo reactant **3**, the corresponding addition of the dAdo reactant (**4**) to the DEs (**1** or **2**) in over 25 molar equiv of TFE occurred highly stereoselectively to afford only cis adducts for both DEs. A highly efficient HPLC separation of dGuo adduct diastereomers derived from DE-2 (**2**) was developed using acetone as a modifier in CH_2Cl_2 or in *n*-hexane. Through the use of varying molar ratios of the different fluorinated alcohols described above and the newly developed HPLC separation method, the four possible phosphoramidites (cis/trans, *R*/*S*) of the B[*a*]P DE-2 N^2-dGuo adducts can be prepared in an efficient fashion on gram scale for use in oligonucleotide synthesis.

Introduction

The potent carcinogenic polycyclic aromatic hydrocarbon benzo[*a*]pyrene (B[*a*]P) is a widespread environmental pollutant that is metabolically activated to bay-region diol epoxides (DEs).[1] These DEs are formed from *trans*-7,8-dihydrodiols as diastereomers in which the benzylic hydroxyl group is either cis (DE-1, **1**) or trans (DE-2, **2**) to the epoxide oxygen (Figure 1); each diastereomer exists as a pair of enantiomers (not shown).[2] Among these four possible stereoisomeric DEs, (+)-(7*R*,8*S*,9*S*,10*R*)-DE-2 is the major DE metabolite formed from

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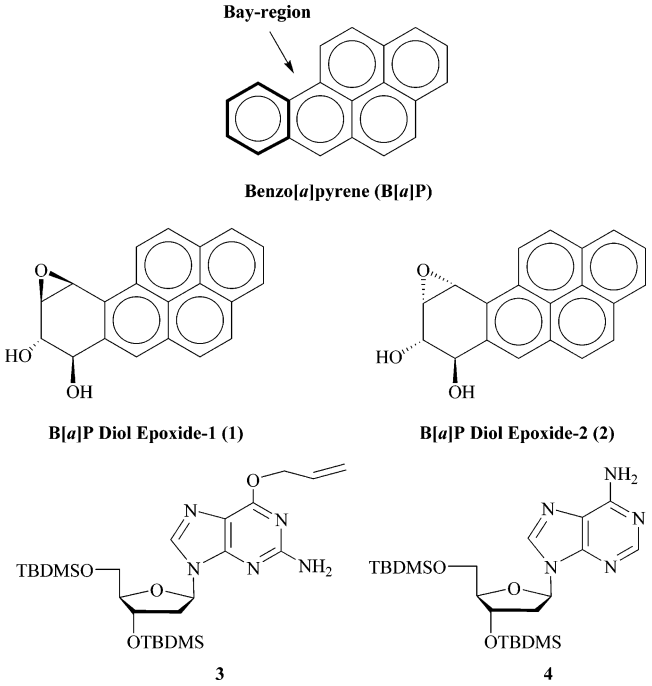

FIGURE 1. Structure of benzo[*a*]pyrene (B[*a*]P) with the bay region and key terminal benzo-ring indicated, structures of B[*a*]P 7,8-diol 9,-10-epoxide DE-1 (**1**) and DE-2 (**2**) diol epoxide diastereomers, the dGuo reactant O^6-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**3**), and the dAdo reactant 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**4**). Only one of the two possible enantiomers of each DE is shown.

B[*a*]P in mammals[2] and is by far the most carcinogenic in several animal models.[3] The major binding site of this ultimate carcinogen to DNA occurs at the exocyclic amino group of deoxyguanosine (dGuo) and to a lesser extend at the exocyclic amino group of deoxyadenosine (dAdo).[4] Adduct formation is thought to be a key step in tumor initiation. Since even minor adducts may have significant biological effects, there has been a continued need for small oligonucleotides containing minor as well as major adducts of B[*a*]P DEs at specific sites in defined sequence contexts. These DE adducted oligonucleotides have been used in structural studies by two-dimensional NMR[5] and X-ray crystallographic analysis.[6] Use of such modified oligonucleotides as probes for the study of the mechanism of DNA processing enzymes such as human DNA polymerases,[7] topoisomerases I[8] and II,[9] vaccinia topoisomerase I,[10] Werner helicase,[11] and HIV-1 integrase[12] is a rapidly developing area of research.

Direct reaction of the exocyclic amino groups of appropriately protected purine nucleosides with the DEs provides a convenient route to the synthesis of adducts. Such modified nucleosides are converted to the corresponding phosphoramidites, which can be site- and stereospecifically incorporated into the target oligonucleotides. We have established highly efficient conditions for the key adduct-forming reaction by utilizing O^6-allyl-3′,5′-di-TBDMS-protected dGuo (**3**)[13] and 3′,5′-diTBDMS-protected dAdo (**4**).[14] Blocking of the purine hydroxyl groups as the very nonpolar TBDMS ethers greatly increases the solubility of the nucleosides in organic solvents. Blocking of the O^6 carbonyl group of dGuo with the allyl group not only prevents adduct formation at this position but also enhances the nucleophilic reactivity of the N^2-amino group. The reaction of B[*a*]P DE-1 (**1**) and DE-2 (**2**) with **3** in dimethylacetamide (DMA) at 100 °C for 2 h formed a mixture of cis and trans adducts (∼50% yield) with cis:trans ratio of ∼1:1.[13] No reaction occurred between **1** or **2** and the TBDMS-protected dAdo (**4**) in DMA,[13] possibly because the N^6-amino group in **4** is less nucleophilic than the N^2-amino group in **3**.

Fluorinated alcohols such as trifluoroethanol (TFE), hexafluoropropan-2-ol (HFP), and perfluoro-*tert*-butyl alcohol (PFTB) have a dramatic catalytic effect on the addition reactions between blocked purines and DEs. Their combination of low nucleophilicity, high hydrogen bonding donor strength, high polarity, and high ionizing power make them ideal solvents for addition reactions that proceed through carbocation intermediates.[15] Thus, reactions of B[*a*]P DE-1 (**1**) and DE-2 (**2**) with **3** in a large excess (1040 molar equiv) of TFE[16] proceeded at room temperature to give mixtures of cis and trans adducted products with cis:trans ratios of 85:15 in an overall yield of 65% (DE-1) and 40:60 in an overall yield of 43% (DE-2). Unlike the corresponding reaction in DMA, which yielded no adducts, addition of **4** to **1** or **2** afforded cis-opened adducts, albeit in

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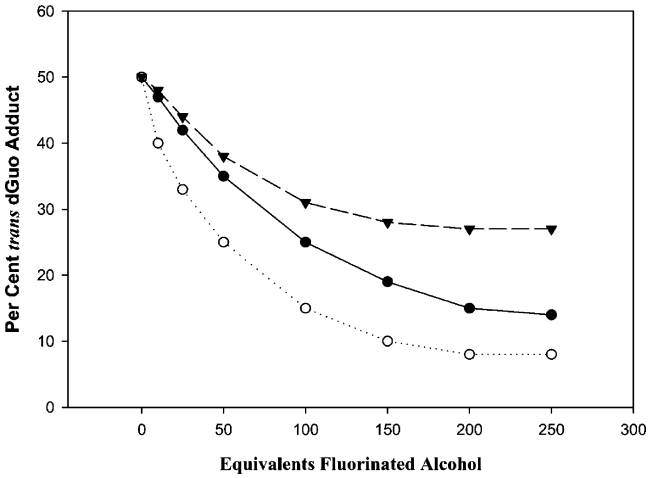

FIGURE 2. Stereoselectivity of fluorinated alcohol-mediated cis- and trans-opening of B[*a*]P DE-1 (1) by the dGuo reactant O^6-allyl-3′,5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (3) in a molar ratio of 1:5: ●, TFE; ○, HFP; ▼, PFTB.

low yield (22−33%). The major side products were from solvent addition and accounted for as much as 50% of the starting material.

A detailed study of the addition reaction of **3** with the less reactive DEs of benzo[*c*]phenanthrene (B[*c*]Ph) revealed that the cis:trans adduct ratios were highly dependent on the amount of solvent HFP used, when less than ∼200 molar equiv of TFE and 5 molar equiv of **3** were used.[17] In the present report, we describe optimization of this addition reaction in fluorinated alcohols for B[*a*]P DEs (**1** and **2**) with **3** and **4** to reduce undesired solvent adduct formation and to control the cis vs trans stereochemistry of addition over a wide range of cis/trans product ratios. We also report a new and highly efficient HPLC separation of the 10*R* and 10*S* diastereomeric adducts that are produced on reaction of racemic B[*a*]P DEs with **3**.

Results and Discussion

Direct opening of B[a]P DEs (**1** and **2**) was investigated using TFE (pK_a = 12.4),[18] HFP (pK_a = 9.3),[18] and PFTB (pK_a = 5.2)[18b] as solvents. In all cases, the DEs were added to a clear solution of the protected dGuo **3** in the solvent. The addition reaction was complete as soon as the DEs dissolved completely, and a clear solution was obtained. This order of addition of the substrate DEs to the reactant **3** is very important to reduce competitive formation of solvent adducts.[19] Unnecessarily prolonged (overnight) treatment in TFE as previously reported[16] also decreases the yield considerably due to decomposition of the desired products.

B[*a*]P DE-1−dGuo Reactions. The observed cis:trans adduct ratio for DE-1 (**1**) varied, as shown in Figure 2 and Table 1 over the range of 10−250 molar equiv of each of the three alcohols. The greatest ratio change was observed for HFP (60:40 to 92:8) followed by TFE (53:47 to 87:13) and PFTB (52:48 to 73:27). Interestingly, the previously observed cis/trans ratio for the solvent-free reaction for **1** was 50:50.[20] This strongly contrasts with the corresponding solvent-free reaction of DEs of B[*c*]Ph with **3**, which produced the corresponding trans adduct almost exclusively.[17] In all cases, the reaction was complete at room temperature within 10−30 min at high molar ratios of fluorinated alcohol to DE-1 (**1**) (>50 molar equiv of TFE or HFP or >150 molar equiv for PFTB). When less than the above amount of solvent was used, the reaction required heating at 70 °C for 30 min in a sealed glass tube to obtain a clear solution, presumably due to the reduced solubility of the reactants. Under these conditions, the yields of the desired adducts were as high as 75%, and the side product of solvent addition was reduced to less than 12% for the reaction in TFE and less than 20% in HFP. No solvent adducts were formed in PFTB (Table 1).

TABLE 1. Fluorinated Alcohol Mediated Synthesis of Cis- and Trans-Opened N^2-dGuo Adducts from B[*a*]P DE-1 (1)[a]

solvent	molar equiv	temp (°C)	time (min)	cis:trans adduct ratio[b]	yield dGuo adducts[c]	yield solvent adducts[c]
TFE	250	rt	10	87:13	68	4.0
	200	rt	10	83:17	64	2.5
	150	rt	15	79:21	62	1.5
	100	rt	15	71:29	63	1.7
	50	rt	30	65:35	54	2.0
	25	70	30	58:42	50	10
	10	70	30	53:47	45	12
HFP	250	rt	10	92:8	70	16
	200	rt	15	92:8	68	18
	150	rt	15	90:10	75	15
	100	rt	30	85:15	68	16
	50	rt	30	75:25	62	20
	25	70	30	68:32	60	15
	10	70	30	60:40	60	15
PFTB	250	rt	10	73:27	70	nd[d]
	200	rt	10	73:27	72	nd
	150	rt	30	72:28	68	nd
	100	70	30	69:31	68	nd
	50	70	30	62:38	65	nd
	25	70	30	56:44	63	nd
	10	70	30	52:48	60	nd

[a] All reactions were run with a molar ratio of the B[*a*]P DE-1 (**1**) to dGuo nucleoside (**3**) of 1:5. [b] The ratio was determined by HPLC (280 nm) as disilyl trihydroxy derivatives. [c] Actual isolated yield of combined cis and trans adducts (10−50 mg scale). [d] Not detectable.

B[*a*]P DE-2−dGuo Reactions. Quite intriguingly, unlike the reaction of DE-1 (**1**), no cis/trans ratio change was observed for the corresponding addition reaction of **3** with DE-2 (**2**) in TFE (cis/trans ratio ∼40/60) and in PFTB (cis:trans ratio ∼40:60) over the range of 25−250 molar equiv (Figure 3 and Table 2). However, a distinct dependency was observed for the reaction of **3** with **2** in HFP over a range of 10−250 molar equiv: the cis:trans ratio varied from 22:78 to 72:28 with increasing HFP. The cis/trans ratio previously observed for the solvent-free reaction[20] was 15:85, which is compatible with the curve obtained in this study, as shown in Figure 3.

The reactivity of B[*a*]P DE-2 (**2**) with **3** in fluorinated alcohols under comparable conditions is appreciably lower than that of DE-1 (**1**). Thus, reaction of DE-2 (**2**) with **3** was complete at room temperature within 6 h in the presence of >150 molar equiv of TFE, within 1 h for >50 molar equiv of HFP, or within 2 h for > 100 molar equiv of PFTB. When less solvent was used, the reaction required heating at 45−70 °C for 1 to 21 h

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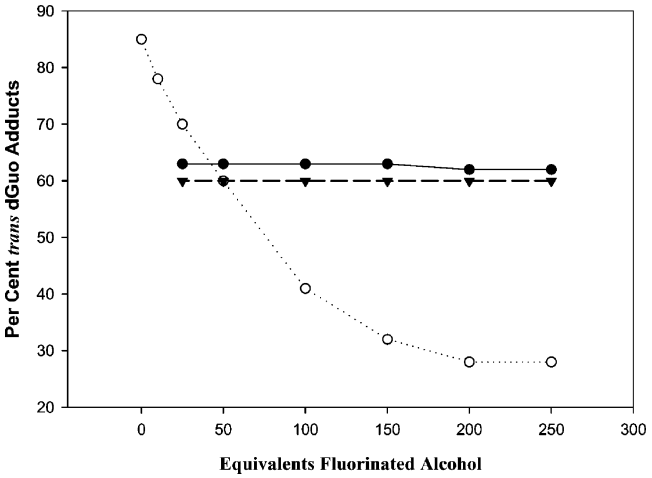

FIGURE 3. Stereoselectivity of fluorinated alcohol-mediated cis- and trans-opening of B[*a*]P DE-2 (**2**) by the dGuo reactant O^6-allyl-3′,5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**3**) in a molar ratio of 1:5: ●, TFE; ○, HFP; ▼, PFTB.

TABLE 2. Fluorinated Alcohol Mediated Synthesis of Cis- and Trans-Opened N^2-dGuo Adducts from B[*a*]P DE-2 (2)[a]

solvent	molar equiv	temp (°C)	time (h)	cis:trans adduct ratio[b]	yield dGuo adducts[c]	yield solvent adducts[c]
TFE						
	250	Rt	2	38:62	59	10
	200	Rt	4	38:62	60	9
	150	Rt	6	37:63	58	7
	100	70	1	36:64	50	5
	50	70	5	38:62	48	7
	25	70	7	37:63	43	3
HFP	250	Rt	1	72:28	78	nd[d]
	200	Rt	1	72:28	75	nd
	150	Rt	1	70:30	78	nd
	100	Rt	1	59:41	80	nd
	50	Rt	1.5	43:57	80	nd
	25	45	5	30:70	65	nd
	10	70	12	22:78	50	nd
PFTB	250	Rt	1	40:60	80	nd
	200	Rt	1	40:60	80	nd
	150	Rt	1	39:61	85	nd
	100	Rt	2	40:60	87	nd
	50	45	4	38:62	90	nd
	25	70	21	40:60	45	nd

[a] All reactions were run with a molar ratio of the B[*a*]P DE-2 (**2**) to dGuo nucleoside (**3**) of 1:5. [b] The ratio was determined by HPLC (280 nm) as disilyl tracetoxy derivatives for the reaction in TFE and as disilyl trihydroxy derivatives for the reaction in HFP and in PFTB. [c] Actual isolated yield of combined cis and trans adducts (10−50 mg scale). [d] Not detectable.

in a sealed glass tube. Under these conditions, yields of desired adducts were 43∼90% and formation of the solvent adduct **11** was observed only for the reaction in TFE (Table 2). For the preparative scale reactions (1.2 g of **2**; see Experimental Section), the best yield (90%) was obtained when a 1:8 ratio of **2** to **3** was heated at 45 °C in PFTB (50 molar equiv) for 4−6 h in a sealed glass tube. The cis and trans mixed diastereomers and byproduct solvent adducts (**9**−**11**) were separated by a combination of column chromatography and HPLC, and the individual acetylated diastereomers (**5a**, **5b**, **6a**, **6b**, and **7a**, **7b**, **8a**, **8b**) were separated by HPLC (Scheme 1).

Mechanism of Addition Reaction. At solvent ratios where the cis:trans adduct ratio remains constant, the reaction is presumed to proceed largely by an S_N1 mechanism, which is consistent with the formation of substantial amounts of cis-opened adducts. As the amount of solvent is decreased relative to the other reactants, the medium becomes less polar, and the portion of the reaction that proceeds through an S_N1 mechanism must compete with that through an S_N2 mechanism, which increases the amount of trans-opened adducts. Unlike the solvent-free reaction of the less reactive B[*c*]Ph DEs, which produced almost exclusively the trans-opened adduct through an S_N2 mechanism,[17] the corresponding solvent-free reaction of B[*a*]P DEs proceeds substantially through an S_N1 mechanism. This is consistent with formation of a much more stable carbocation at the C-10 position of B[*a*]P DEs compared with a much less stable carbocation at the C-1 position of the B[*c*]-Ph DEs.[21] A plausible mechanism for the observed dependency of adduct ratio on the amount of solvent observed for B[*a*]P DEs in this study is similar to that given for the corresponding reaction of B[*c*]Ph DEs.[17] Since exclusive formation of cis-opened adducts was observed for the reaction of **4** with B[*a*]P DEs, as will be discussed later, the rate of capture of the two possible carbocation conformations might also be dependent on the nature of the nucleophile used.

Separation and Identification of B[*a*]P DEs−dGuo Reaction Products. The solvent adducts (**9a**, **10a**, and **11**) were also acetylated. Relative cis stereochemistry of the 9,10-substituents in the acetylated adducts **9b**, **10b**, and **12** was assigned by comparison of their ^{1}H NMR spectra with those of a known, related compound.[22]

We have developed a highly efficient separation of the diastereomeric adducts by HPLC on a silica column (16 × 250 mm). As shown in Figure 4, using 3% acetone in methylene chloride as solvent, a baseline separation of a mixture of **7a** and **7b** (50 mg/injection) was easily achieved. An even better separation was achieved for **8a** and **8b**. Thus, a complete baseline separation of as much as 200 mg/injection of **8a** and **8b** was achieved using 12% acetone in *n*-hexane as solvent. A previous separation of these diastereomers using CH_2Cl_2−MeOH (98:2) on an Axxiom silica column (9.5 × 250 mm) allowed a maximum of 3 mg of sample per injection.[13] Acetone has rarely been used as an HPLC solvent, since it has rather strong UV absorption below 300 nm. Fortunately, all of the present adducts have a strong UV absorption at ∼340−370 nm due to the pyrene chromophore, which can be used to monitor the HPLC effluent in the presence of acetone. The high solubility of these adducts in acetone is another advantage in that it minimizes the volume of injections.

A comparison of benzo-ring ^{1}H NMR data (600 MHz) for the triacetates of the O^6-allyl protected cis- and trans-opened DE-2 N^2-dGuo adducts (**7a,b** and **8a,b**) is given in Table 3. Previously unassigned $J_{9,10}$ for **8a,b**[13] is now clearly assignable to be 5.5 Hz, the value of which is consistent with the previously observed coupling constant for the structurally related acetylated trans-opened DE-2 N^2-dGuo adducts.[22]

dAdo Adducts. Unlike the addition reaction of **1** and **2** with the O^6-allyl dGuo di-TBDMS ether (**3**), the corresponding reaction of these DEs (**1** and **2**) with the dAdo di-TBDMS ether (**4**) in TFE proceeded highly stereoselectively to give only cis dAdo adducts **13a,b** and **14a,b**, respectively (>25 molar equiv of TFE as solvent, Scheme 2). Despite the relatively low yields

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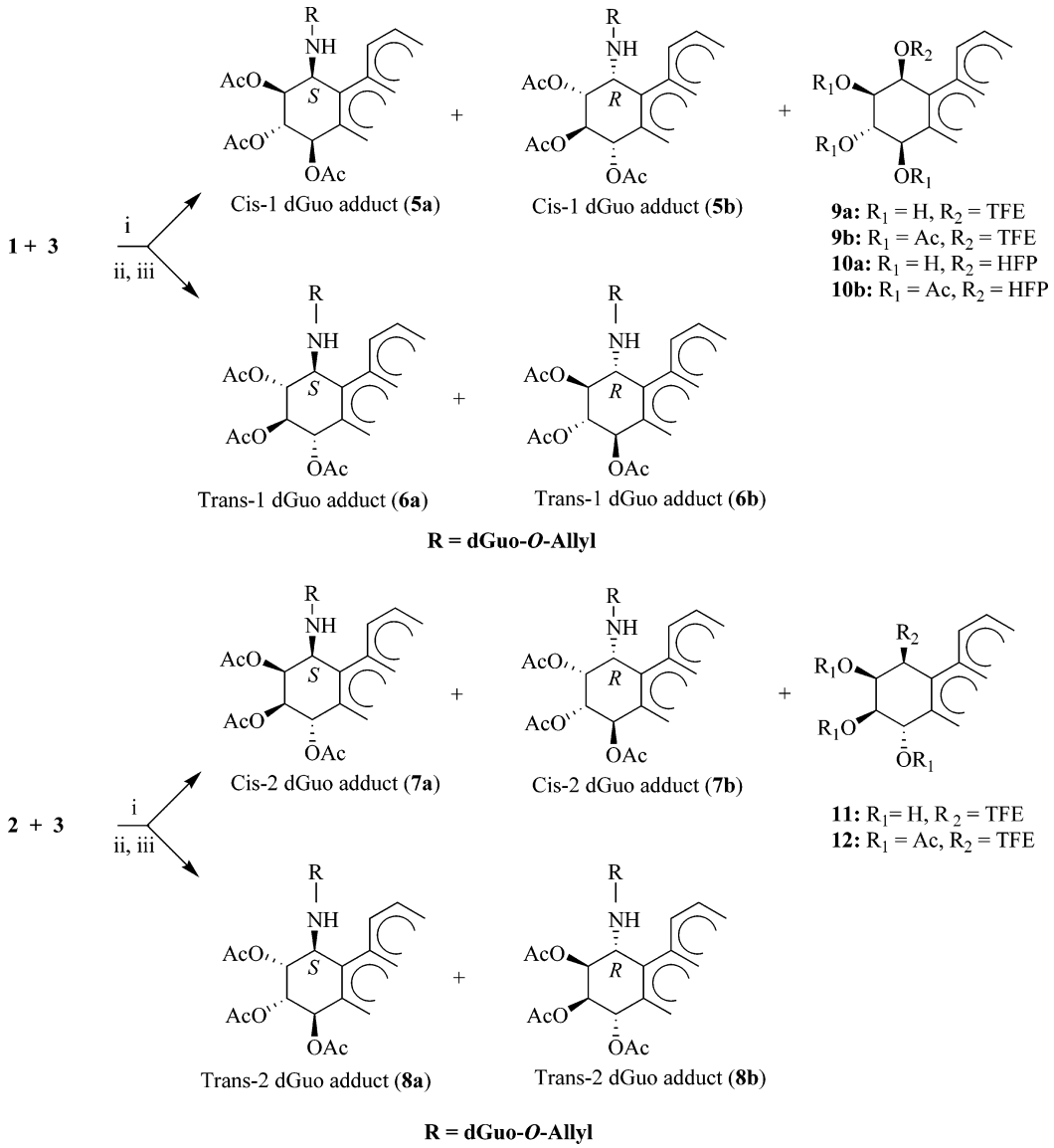
SCHEME 1[a]



[a] Key: (i) fluorinated alcohols; (ii) Ac_2O−DMAP−pyridine; (iii) HPLC. Only one of the two enanthiomers is shown for **9a**, **9b**, **10a**, **10b**, **11**, and **12**.

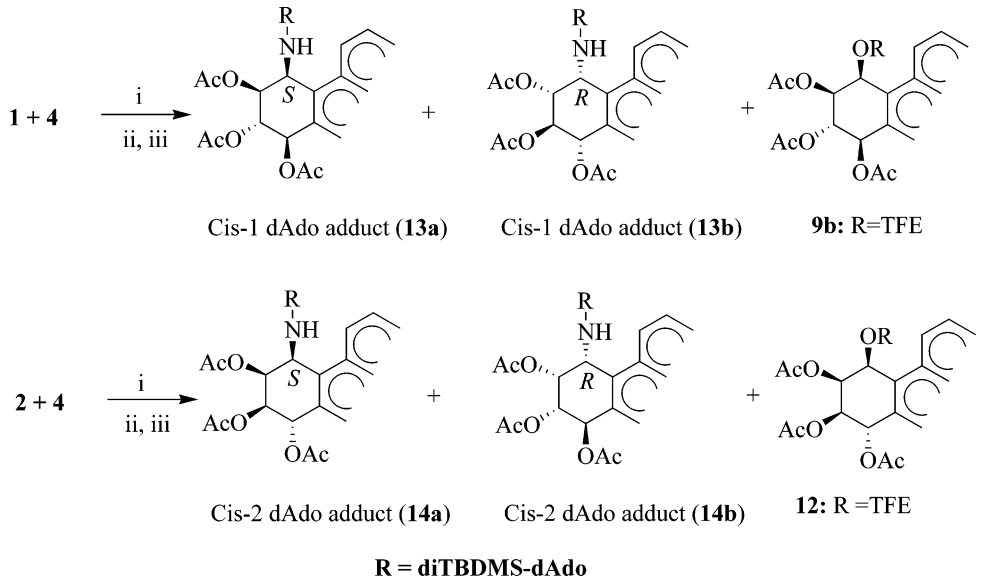
previously reported (22% for **1** and 33% for **2**, respectively),[16] this method provides an attractive source for the *cis*-dAdo adducts, which are often difficult to synthesize. Surprisingly, no reaction occurred between the DEs and **4** in HFP or in PFTB.[16]

In the present study, we attempted to modify previous reaction conditions to see if it is possible to increase the yield of the objective dAdo adducts. Thus, the reaction was carried out by portionwise addition of DEs (**1** or **2**) to a solution of **4** (6 molar equiv) in a much smaller amount of TFE (∼1/7 of the amount previously used) at 45−60 °C. The reaction mixture was acetylated and the products (Scheme 2, **13a,b** and **9b** from **1**, **14a,b** and **12** from **2**, respectively) were separated by HPLC. Under these conditions, we could successfully reduce the formation of the unwanted solvent adducts (**9b** and **12**) to less than half (25%) and double the yield of the objective dAdo adducts (40% for **1** and 60% for **2**, respectively). The direct addition reaction between DE-1 (**1**) and **4** is by far the simplest method to obtain the cis-opened dAdo adduct. An earlier method required the use of the cis-opened aminotriol derived from DE-1 (**1**) via a multiple step synthesis.[14] Although the yield for **2** in the present study is comparable with that previously obtained through an aminohydroxylation approach,[14] the procedure and workup for the present addition reaction are much simpler, and the reaction is more robust.

Phosphoramidite Synthesis. In a previous study[23] we reported the synthesis of 5′-dimethoxytrityl (DMT)-protected 3′-phosphoramidite building blocks of N^2-dGuo adducts derived from cis- and trans-opening of B[*a*]P DE-1 (**1**) and DE-2 (**2**) at C-10. Since the starting material for these syntheses is a racemic DE, each of the *cis*- and *trans*-phosphoramidites consists of a mixture of diastereomers. Their use in modified oligonucleotide synthesis results in a mixture of 10*S*/10*R* constructs. Although separation of the two diastereomeric adducted oligonucleotides has generally been achieved, use of single phosphoramidites diastereomers of known configuration greatly simplifies the HPLC purification and characterization of the modified oligo-

(23) Kroth, H.; Yagi, H.; Sayer, J. M.; Kummer, S.: Jerina, D. M. *Chem. Res. Toxicol.* **2001**, *14*, 708−719.

SCHEME 2[a]



[a] Key: (i) CF_3CH_2OH; (ii) Ac_2O−DMAP−pyridine; (iii) HPLC. Only one of the two enanthiomers is shown for **9b** and **12**.

nucleotides. In addition, if only one isomer is desired, the other diastereomer reduces the effective yield and essentially wastes half of the starting material. In general, separation of *R*/*S* pairs of adducted oligonucleotides tends to become increasingly more difficult as the length of the sequence increases. Thus, use of diastereomerically pure phosphoramidites as building blocks for oligonucleotide synthesis is desirable for synthesis of longer oligonucleotides.

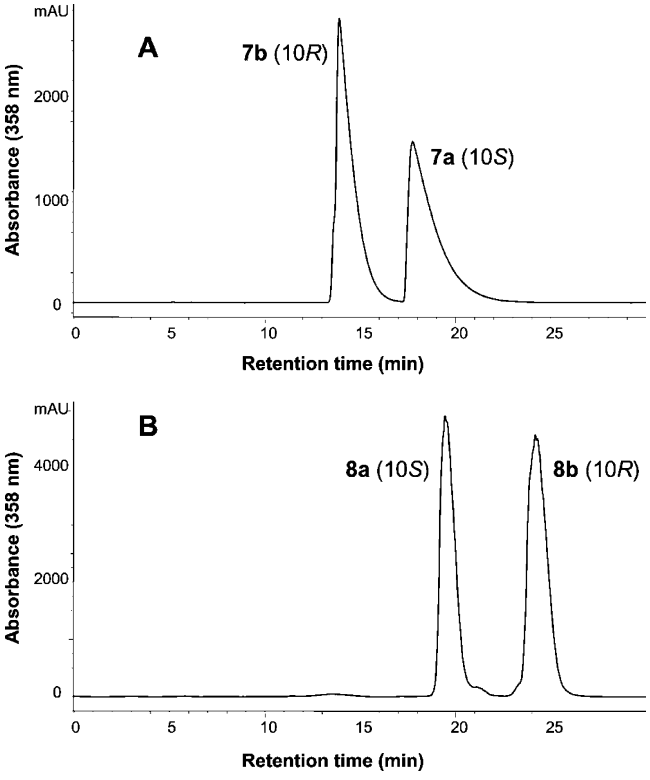


FIGURE 4. HPLC separation of the diastereomeric O^6-allyl-protected dGuo adduct disilyl triacetates derived from B[*a*]P DE-2 (**2**) on a LiChrosorb-60 Si (16 × 250 mm) column. (A) Separation of the early eluting (−)-*cis*-**7b** (10*R*) and the late eluting (+)-*cis*-**7a** (10*S*). (B) Separation of the early eluting (−)-*trans*-**8a** (10*S*) and the late eluting (+)-*trans*-**8b** (10*R*). See Supporting Information for details.

TABLE 3. Comparison of Benzo-Ring ^{1}H NMR Data for the O^6-Allyl Protected Cis- and Trans-Opened N^2-dGuo Adducts as Their Disylyl Triacetates (600 MHz, Acetone-d_6)[a]

compound	H_7 (ppm)	$J_{7,8}$ (Hz)	H_8 (ppm)	$J_{8,9}$ (Hz)	H_9 (ppm)	$J_{9,10}$ (Hz)	H_{10} (ppm)
(+)-**7a** (10*S*)	6.64		5.74		5.94		6.85
cis-dGuo O^6-all-DE-2		3.4		2.3		5.4	
(−)-**7b** (10*R*)	6.64		5.72		5.96		6.85
		3.4		2.3		5.4	
(−)-**8a** (10*S*)	6.81		5.97		6.12		6.32[b]
trans-dGuo O^6-all-DE-2		9.4		2.3		2.6	
(+)-**8b** (10*R*)	6.81		5.97		6.12		6.32[b]
		8.9		2.3		2.6	

[a] Assignments were confirmed by decoupling and COSY experiments. [b] Previously reported[13] chemical shifts for the H_{10} proton for (+)-**8a** (7.10 ppm) and for (+)-**8b** (7.23 ppm) were in error.

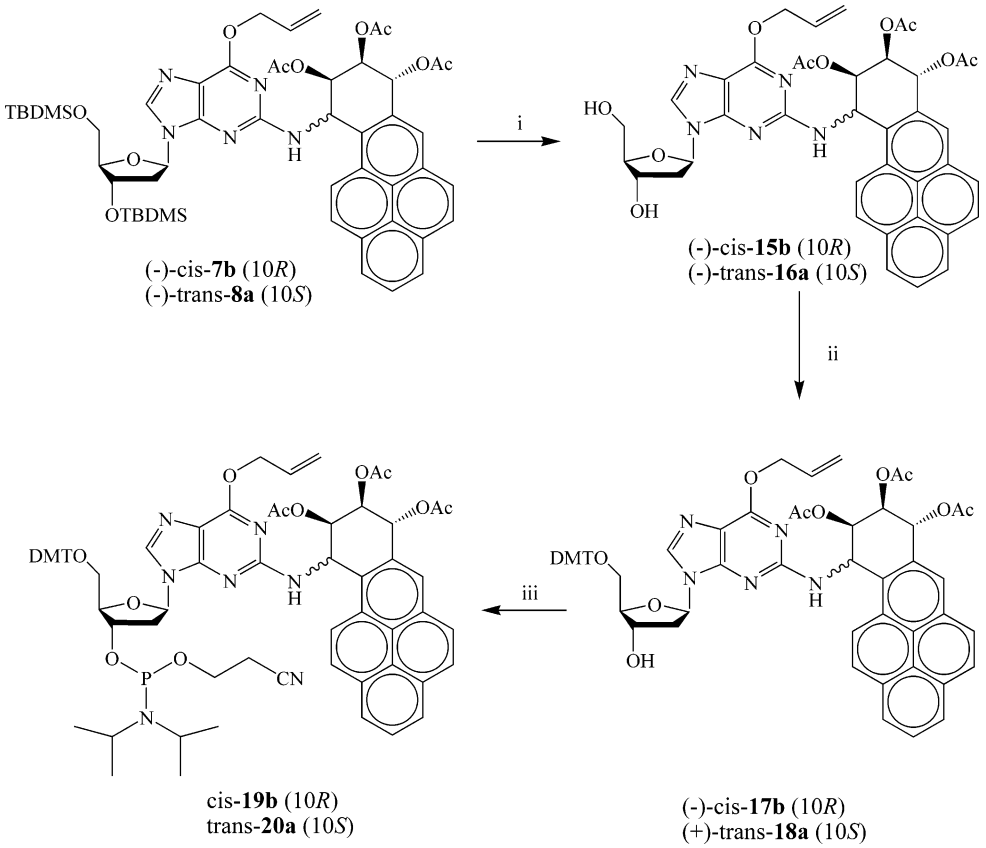
Major improvements have been achieved for the desilylation of the sugar hydroxyl groups in **7a**, **7b**, **8a**, and **8b** and the successive introduction of the 5′-DMT protecting group on the sugar (Scheme 3). Thus, a complete and nearly quantitative desilylation (>97%) was achieved using 7% HF in pyridine for 12 h at room temperature to give **15a,b** and **16a,b** instead of using 2% HF in a mixture of acetonitrile and pyridine (4:1).[23] The assignment of the relative stereochemistry and the absolute configuration of (+)-*trans*-**16b** were recently confirmed by X-ray crystallographic analysis.[24] The newly developed method for the DMT protection of the 5′ sugar hydroxyl group involved replacing THF with CH_2Cl_2 in the presence of DMT fluoroborate.[25] As before, formation of any side products was prevented.[17] Thus, **17a,b** and **18a,b** were obtained from **15a,b** and **16a,b**, respectively, in nearly quantitative yield (>95%).

Finally, introduction of the phosphoramidite function at the 3′-sugar hydroxyl group in the 5′-DMT derivatives (**17a,b** and **18a,b**) was achieved with 2-cyanoethyltetraisopropyl phos-

(24) Karle, I. L.; Yagi, H.; Sayer, J. M.; Jerina, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 1433−1438.

(25) Bleasdale, C.; Ellwood, S. B.; Golding, B. T. *J. Chem. Soc. Perkin Trans.* **1990**, *1*, 803−805.

SCHEME 3[a]



[a] Key: (i) 7% HF/pyridine; (ii) DMT+ BF_4−CH_2Cl_2; (iii) 2-cyanoethyltetraisopropyl phosphoramidite.

phoramidite and *N*,*N*-diisopropylammonium tetrazolide in CH_2Cl_2 to give **19a,b** and **20a,b**, respectively, in 80−90% yield. Each of the diastereomeric phosphoramidites thus obtained consisted of a mixture of two peaks due to the asymmetry of the phosphorus. Although these phosphorus diastereomers could be separated by HPLC (see Experimental Section in the Supporting Information), such separation is not necessary prior to their use in oligonucleotide synthesis, as the resulting phosphates are no longer chiral at phosphorus.

Conclusions

The present observations on the addition reactions of the B[*a*]P DEs (**1** and **2**) are mechanistically similar to results obtained for the hydrolysis of these DEs in water.[26] The predominant *cis*-N^2-dGuo adduct formation from DE-1 (**1**) (87% for TFE, 92% for HFP, 73% for PFTB, respectively, Table 1) in the presence of high amounts of solvent (250 molar equiv) is consistent with the predominant formation of *cis*-tetraol (85%) on acid-catalyzed hydrolysis of DE-1 (**1**).[26b] This result suggests that the same conformation of the carbocation intermediate from DE-1 (**1**) is trapped by solvent water and by the O^6-allyl dGuo di-TBDMS ether (**3**) in the fluorinated alcohols used in the present study. The cis:trans ratio of the N^2-dGuo adducts obtained from DE-2 (**2**) in TFE and PFTB (∼40:60, Table 2) is also very similar to the cis:trans tetraol ratio (35:65) formed from its acid-catalyzed hydrolysis at high chloride ion concentrations,[26a] where the two carbocation conformers are in rapid equilibrium. This observation is consistent with the speculation that the carbocation for DE-2 (**2**) undergoes conformational equilibration in these two fluorinated alcohols faster than it is captured by the weakly nucleophilic O^6-allyl dGuo di-TBDMS ether (**3**). The only exception to this explanation is found for the cis:trans N^2-dGuo adduct ratio observed for the reaction of DE-2 (**2**) (72:28, Table 2) in the presence of high concentration of HFP (>200 molar equiv). The mechanism for this unusually high cis adduct formation is presently unknown and may involve solvent effects on the equilibrium between the two carbocation conformations and/or the relative rates of their capture by **3**. It is also interesting to point out that exclusive cis adduct formation was observed for the solvent adducts and for the reaction of both DEs **1** and **2** with the dAdo di-TBDMS ether (**4**) in TFE.

Experimental Section

Caution: Benzo[*a*]pyrene-7,8-dihydrodiol and diol epoxides DE-1 and DE-2 are mutagenic and carcinogenic and must be handled carefully in accordance with NIH guidelines.[27]

Reaction of B[*a*]P DE-1 (1) with O^6-Allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (3) in TFE. To a solution of the O^6-allyl dGuo di-TBDMS ether (**3**) (5 molar equiv) in TFE (10−250 molar equiv) was added B[*a*]P DE-1 (**1**) (1 molar equiv), and the reaction mixture was stirred at room temperature or at

(26) (a) Doan, L.; Yagi, H.; Jerina, D. M.; Whalen, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 14382−14387. (b) Doan, L.; Yagi, H.; Jerina, D. M.; Whalen, D. L. *J. Org. Chem.* **2004**, *69*, 8012−8017.

(27) *NIH Guidelines for the Laboratory Use of Chemical Carcinogens*; NIH Publication No. 81−2385; U. S. Government Printing Office: Washington, DC, 1981.

70 °C (bath temperature) in a sealed glass tube until a clear solution was obtained (for the conditions, see Table 1). After the reaction was complete, the mixture was evaporated in vacuo to remove TFE, and the residue was subjected to HPLC on two coupled Axxiom silica columns (9.5 × 250 mm) using 25% *n*-hexane in EtOAc (containing 0.4% THF) at a flow rate of 6 mL/min (detected at 280 nm). Evaporation of the fraction corresponding to the peak at t_R = 5.8 min gave recovered **3** (~75% recovery). Evaporation of the fraction corresponding to the peak at t_R = 8.8 min gave **(±)-10*β*-trifluoroethyl-7*β*,8α,9*β*-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (9a)** as a colorless solid. ^{1}H NMR and HRMS of this solvent adduct and its triacetate **(±)-10*β*-trifluoroethyl-7*β*,8α,9*β*-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (9b)** are given in the Supporting Information. 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- and trans-opening of (±)-DE-1 (**1**) had t_R = 13.4 and 14.3 min, respectively. The cis/trans ratios obtained are shown in Table 1 and Figure 2. Each of the diasteromeric mixtures of cis- and trans-adducts was separately acetylated and separated by HPLC into diastereomerically pure *cis*- and *trans*-triacetates (**5a,b**), and (**6a,b**), respectively. Their structures were established by comparison of ^{1}H NMR and CD spectra with those of the authentic samples.[13]

Reaction of B[*a*]P DE-1 (1) with *O*^{6}-Allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (3) in HFP. Reaction of **1** with **3** in HFP was carried out essentially the same way as above for the corresponding reaction in TFE (for the conditions see Table 1). Separation of the unreacted **3** and products by HPLC was carried out as above. Evaporation of the fraction corresponding to the peak at t_R = 5.8 min gave unreacted **3** (~75% recovery). Evaporation of the fraction corresponding to the solvent adduct peak at t_R = 8.8 min gave **(±)-10*β*-hexafluoroisopropyl-7*β*,8α,9*β*-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (10a)** as a colorless solid. ^{1}H NMR and HRMS of this solvent adduct and its triacetate **(±)-10*β*-hexafluoroisopropyl-7*β*,8α,9*β*-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (10b)** are given in the Supporting Information. 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- and trans-opening of (±)-DE-1 (**1**) had t_R = 13.4 and 14.3 min, respectively, and were characterized as above.

Reaction of B[*a*]P DE-1 (1) with *O*^{6}-Allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (3) in PFTB. The reaction of **1** with **3** in PFTB was carried out essentially as above for the corresponding reaction in TFE (for the conditions, see Table 1). Separation of unreacted **3** (~75% recovery) and the cis- and the trans-adducts by HPLC was also carried out as above.

Reaction of B[*a*]P DE-2 (2) with *O*^{6}-Allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (3) in Fluorinated Alcohols. To a solution of the *O*^{6}-allyl dGuo di-TBDMS ether **(3)** (5 molar equiv) in TFE, HFP, or PFTB (10−250 molar equiv) was added B[*a*]P DE-2 (**2**) (1 molar equiv), and the reaction mixture was stirred at room temperature or heated at 45−70 °C (bath temperature) in a sealed glass tube until a clear solution was obtained (for the conditions, see Table 2). After reaction, the mixture was evaporated in vacuo to remove solvent, and the residue was subjected to HPLC on two coupled Axxiom silica columns (9.5 × 250 mm) using 25% *n*-hexane in EtOAc (containing 0.4% THF) at a flow rate of 6 mL/min (detected at 280 nm). Evaporation of the fraction corresponding to the peak t_R = 5.8 min gave unreacted **3** (~75% recovery). Diastereomeric mixtures of the *trans*-dGuo and the *cis*-dGuo adducts eluted, respectively, as single peaks at t_R = 12.3 and 16.1 min. In the case of the reaction in TFE, the solvent adduct (**11**) cochromatographed with the *cis*-dGuo adducts. Therefore, the combined fractions corresponding to the peak at t_R = 12.3 and 16.1 min were evaporated, the resulting mixture was acetylated and further separated by HPLC on two coupled Axxiom silica columns (9.5 × 250 mm) using 14% acetone in *n*-hexane at a flow rate of 6 mL/min (detected at 345 and 358 nm). The cis TFE solvent adduct triacetate **12 [(±)-10α-Trifluoroethyl-7*β*,8α,9α-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene**, mp 233−234 °C (EtOAc−*n*-hexane)] had t_R = 14.4 min. See the Supporting Information for ^{1}H NMR and HRMS. The *cis*-(10*S*)-(**7a**) and *cis*-(10*R*)-dGuo adduct triacetate (**7b**) had t_R = 19.2 and 20.1 min, respectively. The *trans*-(10*S*)-(**8a**) and *trans*-(10*R*)-dGuo adduct triacetate (**8b**) had t_R = 24.4 and 29.9 min, respectively. The structures of these dGuo adduct triacetates were identified by comparison of HPLC retention times, CD, ^{1}H NMR, and mass spectra with those of the authentic samples.[13]

(+)-*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 (7a). $[\alpha]_D$ = +145° (*c* = 4.9, CH_2Cl_2). **(−)-*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 (7b).** $[\alpha]_D$ = −111° (*c* = 5.5, CH_2Cl_2). **(−)-*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 (8a).** $[\alpha]_D$ = −10° (*c* = 6.4, CH_2Cl_2). **(+)-*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 (8b).** $[\alpha]_D$ = + 16° (*c* = 6.0, CH_2Cl_2). For ^{1}H NMR (600 MHz, acetone-d_6) spectra of compounds **7a**, **7b**, **8a**, and **8b**, see the listings of tetrahydrobenzo-ring protons.

An Optimized Large-Scale Reaction of B[*a*]P DE-2 (2) with 3 in PFTB. To a solution of **3** (17.1 g, 32 mmol) in PFTB (28 mL, 200 mmol) was added B[*a*]P DE-2 (**2**) (1.21 g, 4 mmol), and the reaction mixture was stirred in a sealed tube under Ar gas at 45 °C (bath temperature) until a clear solution was obtained (4−6 h). Evaporation of the solvent in vacuo gave a hard oil that was subjected to column chromatography on silica gel (4 × 20 in.) eluted with of 30% EtOAc in *n*-hexane to give unreacted **3** (14.5 g, 84.7% recovery). The column was washed with 40% EtOAc (2000 mL) and 50% EtOAc in *n*-hexane (200 mL) and then eluted with 65% EtOAc in *n*-hexane. Three fractions were collected. The first 2000 mL of eluent contained the diasteromeric mixture of the *trans*-dGuo adducts (1.5 g, >98% pure). The second 3000 mL afforded a mixture of *trans*/*cis*-dGuo adduct diastereomers (635 mg), and the third 200 mL of eluent gave a diasteromeric mixture of *cis*-dGuo adducts (900 mg, >98% pure). The mixture obtained from the second fraction above was further separated by HPLC on two coupled Axxiom silica columns (9.5 × 250 mm) as described above using 25% *n*-hexane in EtOAc (containing 0.4% THF) into the *trans*-dGuo adduct diastereomers (250 mg) and the *cis*-dGuo adduct diastereomers (360 mg). Thus, the combined yield for the *trans*- and the *cis*-dGuo diastereomers was 52.2% (1.75 g) and 37.6% (1.26 g), respectively. Thus, the combined yield for the total dGuo adducts was 89.8%.

Reaction of B[*a*]P DE-1 (1) with 3′,5′-Di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (4) in TFE. To a solution of 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**4**) (2.95 g, 6 mmol) in TFE (10.9 mL, 150 mmol) was added B[*a*]P DE-1 (**1**) (302 mg, 1 mmol) portionwise (~20 mg at a time over a period of 10 h with sonication at 45 °C bath temperature. When the reaction mixture became a clear solution, an additional ~20 mg of **1** was added. After the addition of **1** was complete, the reaction mixture was further sonicated at 45 °C for 4 h to ensure complete reaction. The solvent was removed in vacuo, and the residue was acetylated by the standard procedures to give a hard oil that was subjected to column chromatography on a silica gel column (2.5 × 40 cm) developed with 20% EtOAc in *n*-hexane to give a solid (520 mg) that was further separated by HPLC on an Axxiom silica column (2.5 × 250 mm) eluted with 24% EtOAc in *n*-hexane at a flow rate of 10 mL/min (detected at 295 nm). Evaporation of the combined fractions corresponding to the peak at t_R = 3.26 min

(28) Steinbrecher, T.; Wameling, C.; Oesch, F.; Seidel, A. In *Polycyclic Aromatic Comouunds: Synthesis, Analytical Measurements, Occurrence and Biological Effects*; Garrigues, P., Lamotte, M., Eds.; Gordon and Breach: Amsterdam, 1993; pp 223−230.

gave **(±)-10*β*-Trifluoroethyl-7*β*,8α,9*β*-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (9b)** (132 mg, 25%). Evaporation of the combined fractions corresponding to the peak at t_R = 10.4 min and the peak at t_R = 12.3 min afforded ***N*⁶-{10*S*-(7*S*,8*R*,9*S*-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl}-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (13a)** (180 mg, 20%) and ***N*⁶-{10*R*-(7*R*,8*S*,9*R*-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)}-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (13b)** (182 mg, 20%), respectively. The structures of these adducts were established by comparison of the ^{1}H NMR, mass, and CD spectra with those of the authentic samples.[14]

Reaction of B[*a*]P DE-2 (2) with 3′,5′-Di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (4) in TFE. To a solution of 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**4**) (12.7 g, 26.5 mmol) in TFE (52.2 mL) was added B[*a*]P DE-2 (**2**) (1.34 g, 4.42 mmol) portionwise, ∼200 mg at a time over a period of 10 h under sonication at 60 °C. As soon as the reaction mixture became a clear solution, another 200 mg was added. After completion of the addition of **2**, the reaction mixture was further sonicated at 60 °C for 10 h to ensure complete reaction. The reaction mixture was evaporated in vacuo to remove solvent, and the residue was acetylated by the standard procedures to give a hard oil that was subjected to column chromatography on 500 mL of silica gel eluted with 20% EtOAc in *n*-hexane (2400 mL) to give a solid (3.2 g) that was further separated by HPLC on a Vertex LiChrosorb Si-60 column eluted with 24% EtOAc in *n*-hexane at a flow rate of 25 mL/min (detected at 295 nm). Evaporation of the combined fractions corresponding to the peak at t_R = 8.0 min gave **(±)-10α-trifluoroethyl-7*β*,8α,9α-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]-pyrene (12)** (592 mg, 24.5%). Evaporation of the combined fractions corresponding to the peak at t_R = 12.8 min) and the peak at t_R = 15.3 min afforded ***N*⁶-{10*S*-(7*S*,8*R*,9*S*-triacetoxy-7,8,9,-10-tetrahydrobenzo[*a*]pyrenyl)}-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (14a)** (1.17 g, 30.1%) and ***N*⁶-{10*R*-(7*R*,8*S*,9R-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)}-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (14b)** (1.17 g, 30.1%), respectively. The structures of these adducts were identified by comparison of ^{1}H NMR, mass, and CD spectra with those of authentic samples.[14]

Synthesis of Diastereomerically Pure *O*⁶-Allyl-dGuo Building Blocks for Phosphoramidite Derived from B[*a*]P DE-2 (2). The diastereomerically pure *O*⁶-allyl-protected (+)-*cis*-(10*S*)-**7a** or (-)-*cis*-(10*R*)-**7b** and (-)-*trans*-(10*S*)-**8a** or (+)-*trans*-(10*R*)-**8b**, derived from B[*a*]P DE-2 (**2**) (each on a 200 mg scale) were separately dissolved in 7% HF−pyridine (15 mL) under cooling at 5 °C in a polyethylene vial. The reaction mixture was stirred at room temperature for 18 h. To the reaction mixture was added 5% $NaHCO_3$ solution under cooling (ice bath) until evolution of CO_2 gas subsided. The mixture was evaporated in vacuo to remove pyridine, and the residue was diluted with water (100 mL) and extracted with EtOAc (250 mL). The extract was washed with water (50 mL), dried (Na_2SO_4), and evaporated to give a colorless solid (>95% yield).

(+)-*N*²-{10*S*-(7*S*,8*R*,9*S*-Triacetoxy-7,8,9,10-tetrahydrobenzo-[*a*]pyrenyl)}-*O*⁶-allyl-2′-deoxyguanosine (15a). $[\alpha]_D$ = +105° (*c* = 6.6, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 1.94, 2.11, 2.24 (each s, each 3H, 3 × CH_3CO), 2.16 (m, 1H, $H_{2''}$), 2.83 (m, 1H, $H_{2'}$), 3.54 (d, 1H, $H_{5'}$, *J* = 12.6), 3.84 (d, 1H, $H_{5''}$, *J* = 12.6), 4.12 (m, 1H, $H_{4'}$), 4.50 (d, 1H, $H_{3'}$, *J* = 4.7), 5.13 (m, 2H, $CH_{2\ allyl}$), 5.40 (d, 1H, $H_{c\ allyl}$, *J* = 10.5), 5.44 (d, 1H, NH, *J* = 10.4), 5.53 (d, 1H, $H_{t\ allyl}$, *J* = 17.6), 5.63 (dd, 1H, H_8, *J* = 3.1, 2.2), 6.00 (dd, 1H, H_9, *J* = 5.6, 2.2), 6.19 (m, 1H, $H_{1'}$), 6.27 (m, 1H, $H_{v\ allyl}$), 6.64 (d, 1H, H_7, *J* = 3.1), 6.97 (dd, 1H, H_{10}, *J* = 10.4, 5.6), 7.69 (s, 1H, H_{G8}), 7.98−8.26 (m, 8H, pyrene aromatic protons). HRMS (FAB+): calcd for $C_{39}H_{37}O_{10}N_5Cs$ (M^+ + Cs) 868.1595, found 868.1590.

(−)-*N*²-{10*R*-(7*R*,8*S*,9*R*-Triacetoxy-7,8,9,10-tetrahydrobenzo-[*a*]pyrenyl)}-*O*⁶-allyl-2′-deoxyguanosine (15b). $[\alpha]_D$ = −63° (*c* = 6.6, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 1.90, 2.08, 2.20 (each s, each 3H, 3 × CH_3CO), 2.12 (m, 1H, $H_{2''}$), 2.83 (m, 1H, $H_{2'}$), 3.59 (d, 1H, $H_{5'}$, *J* = 11.3), 3.84 (d, 1H, $H_{5''}$, *J* = 11.3), 4.14 (m, 1H, $H_{4'}$), 4.52 (d, 1H, $H_{3'}$, *J* = 4.7), 5.17 (m, 2H, CH_2 allyl), 5.40 (d, 1H, $H_{c\ allyl}$, *J* = 12.3), 5.51 (d, 1H, NH, *J* = 10.4), 5.58 (d, 1H, $H_{t\ allyl}$, *J* = 12.3), 5.61 (dd, 1H, H_8, *J* = 3.1, 2.2), 6.00 (dd, 1H, H_9, *J* = 5.6, 2.2), 6.24 (m, 1H, $H_{1'}$), 6.29 (m, 1H, $H_{v\ allyl}$), 6.64 (d, 1H, H_7, *J* = 3.1), 6.95 (dd, 1H, H_{10}, *J* = 10.4, 5.6), 7.95−8.30 (m, 9H, H_{G8} and 8 pyrene aromatic protons). HRMS (FAB+): calcd for $C_{39}H_{37}O_{10}N_5Cs$ (M^+ + Cs) 868.1595, found 868.1588.

(−)-*N*²-{10*S*-(7*R*,8*S*,9*R*-Triacetoxy-7,8,9,10-tetrahydrobenzo-[*a*]pyrenyl)}-*O*⁶-allyl-2′-deoxyguanosine (16a). Colorless needles, mp 184−185 °C (EtOH); $[\alpha]_D$ = −64.5° (*c* = 6.6, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 2.05, 2.11, 2.31 (each s, each 3H, 3 × CH_3CO), 2.30 (m, 1H, $H_{2''}$), 2.94 (m, 1H, $H_{2'}$), 3.59 (m, 1H, $H_{5'}$,), 3.72 (m, 1H, $H_{5''}$), 4.07 (m, 1H, $H_{4'}$), 4.66 (m, 1H, $H_{3'}$), 5.07 (m, 2H, CH_2 allyl), 5.28 (m, 1H, $H_{c\ allyl}$), 5.44 (d, 1H, $H_{t\ allyl}$, *J* = 17.5), 5.45 (d, 1H, NH, *J* = 6.3), 5.91 (dd, 1H, H_8, *J* = 9.0, 2.1), 6.10 (dd, 1H, H_{10}, *J* = 6.3, 3.5), 6.15 (m, 1H, $H_{v\ allyl}$), 6.16 (app t, 1H, H_9), 6.31 (m, 1H, $H_{1'}$), 6.78 (d, 1H, H_7, *J*= 9.0), 7.95−8.37 (m, 9H, H_{G8} and 8 pyrene aromatic protons). HRMS (FAB+): calcd for $C_{39}H_{37}O_{10}N_5Cs$ (M^+ + Cs) 868.1595, found 868.1592.

(+)-*N*²-{10*R*-(7*S*,8*R*,9*S*-Triacetoxy-7,8,9,10-tetrahydrobenzo-[*a*]pyrenyl)}-*O*⁶-allyl-2′-deoxyguanosine (16b). Colorless prisms, mp 185−187 °C (EtOH); $[\alpha]_D$ = +31°(*c* = 0.9, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 2.03, 2.09, 2.29 (each s, each 3H, 3 × CH_3-CO), 2.20 (m, 1H, $H_{2''}$), 3.01(m, 1H, $H_{2'}$), 3.63 (m, 1H, $H_{5''}$), 3.90 (m, 1H, $H_{5''}$), 4.07 (m, 1H, $H_{4'}$), 4.63 (m, 1H, $H_{3'}$), 5.00 (m, 2H, CH_2 allyl), 5.24 (m, 1H, $H_{c\ allyl}$), 5.35 (d, 1H, $H_{t\ allyl}$, *J* = 17.5), 5.40 (d, 1H, NH, *J* = 6.3), 5.91 (dd, 1H, H_8, *J* = 9.0, 2.1), 6.10 (app. t, 1H, H_9), 6.15(m, 1H, $H_{v\ allyl}$), 6.18 (dd, 1H, H_{10}, *J* = 6.7, 3.5), 6.20 (m, 1H, $H_{1'}$), 6.74 (d, 1H, H_7, *J* = 9.0), 7.69 (s, 1H, H_{G8}), 7.90−8.64 (m, 8H, pyrene aromatic protons). HRMS (FAB+): calcd for $C_{39}H_{37}O_{10}N_5Cs$ (M^+ + Cs) 868.1595, found 868.1616.

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Supporting Information Available: Synthetic procedures for the preparation of dimethoxytrityl and phosphoramidite derivatives, characterization of solvent adducts, a highly improved HPLC separation of acetylated cis and trans dG adducts, as well as proton NMR spectra of the new compounds **9a,b**, **10a,b**, **12**, **15a,b**, and **16a,b** are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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