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Novel Stereoselective Control over Cis vs Trans Opening of Benzo[*c*]phenanthrene 3,4-Diol 1,2-Epoxides by the Exocyclic N^2-Amino Group of Deoxyguanosine in the Presence of Hexafluoropropan-2-ol

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We describe a novel and efficient synthesis (62−84% yields) of the eight possible, diastereomerically pure, cis and trans, *R* and *S* O^6-allyl-protected N^2-dGuo phosphoramidite building blocks derived through cis and trans opening of (±)-3α,4*β*-dihydroxy-1*β*,2*β*-epoxy-1,2,3,4-tetrahydrobenzo[*c*]-phenanthrene [BcPh DE-1 (**1**)] and (±)-3α,4*β*-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenzo[*c*]-phenanthrene [BcPh DE-2 (**2**)] by hexafluoropropan-2-ol (HFP)-mediated addition of O^6-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**3**) at C-1 of the epoxides. Simply changing the relative amount of HFP used in the reaction mixture can achieve a wide ratio of cis/trans addition products. Thus, the observed cis/trans adduct ratio for the reaction of DE-1 (**1**) in the presence of 5 equiv of **3** varied from 17/83 to 91/9 over the range of 5−532 equiv of HFP. The corresponding ratios for DE-2 (**2**) varied from 2/98 to 61/39 under the same set of conditions. When **1** or **2** was fused with a 20-fold excess of **3** at 140 °C in the absence of solvent HFP, almost exclusive trans addition (>95%) was observed for the both DEs. Through the use of varying amounts of HFP in the reaction mixture as described above, each of the eight possible phosphoramidite oligonucleotide building blocks (DE-1/DE-2, cis/trans, *R*/*S*) of the BcPh DE N^2-dGuo adducts can be prepared in an efficient fashion. To rationalize the varying cis-to-trans ratio, we propose that the addition of **3** to **1** or **2** in the absence of solvent or in the presence of small amounts of HFP proceeds primarily via an S_N2 mechanism to produce mainly trans-opened adducts. In contrast, increasing amounts of HFP promote increased participation of an S_N1 mechanism involving a relatively stable carbocation with two possible conformations. One of these conformations reacts with **3** to give mostly trans adduct, while the other conformation reacts with **3** to give mostly cis adduct.

Introduction

Metabolically formed bay-region and fjord-region diol epoxides (DEs) are known ultimate carcinogens of the environmentally prevalent polycyclic aromatic hydrocarbons.[1] These diol epoxides are formed from *trans*-dihydrodiols and exist as pairs of diastereomers in which the epoxide oxygen is either cis (DE-1) or trans (DE-2) to the benzylic hydroxyl group.[2] Typical examples of hydrocarbons with a bay region (benzo[*a*]pyrene, BaP) and a fjord region (benzo[*c*]phenanthrene, BcPh) as well as the DE-1 and DE-2 diastereomers are shown in Figure 1. Interestingly, the highly hindered fjord-region DEs are much more tumorigenic than their bay-region counterparts.[1,3] Each diastereomer exists as a pair of enantiomers. These DEs are known to form stable adducts by cis and trans addition of the exocyclic amino groups of dAdo and dGuo in DNA to the benzylic position of the epoxide.[4] Damage to genes such as the ras family of proto-oncogenes[5] and

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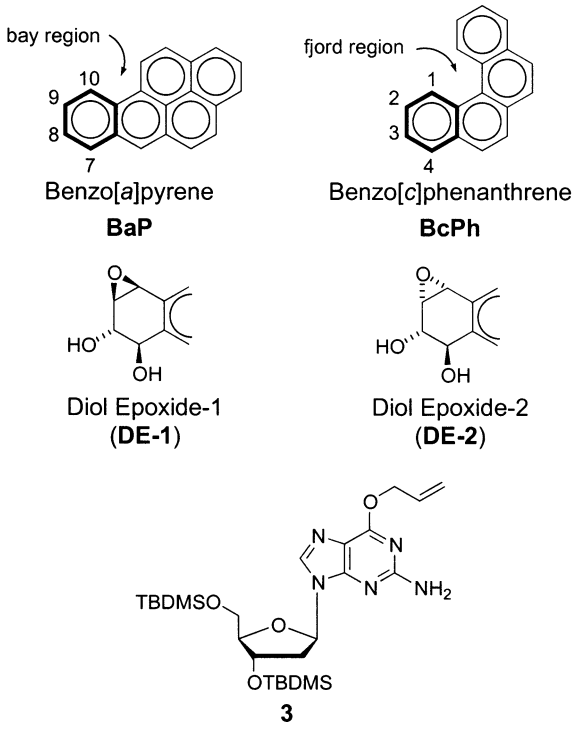

FIGURE 1. Structures of benzo[*a*]pyrene (BaP) and benzo[*c*]phenanthrene (BcPh) with their respective bay region and fjord regions as well as key benzo-rings (bold) indicated. Partial structures of the DE-1 (**1**) and DE-2 (**2**) diol epoxide diastereomers and the dGuo reactant O^6-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**3**) are also shown.

the tumor suppressor genes such as p53[6] and p21[6c] has been implicated in the causation of cancer by DEs.

There exists a great deal of interest in small oligonucleotides containing DE adducts. Determination of the solution conformation of DNA adducts by two-dimensional NMR has identified minor-groove-bound as well as intercalated adducts.[7a,b] Site specific mutagenesis studies have indicated a strong dependence on sequence context and the nature of the adduct.[7c,d] Translesional bypass synthesis with a variety of DNA polymerases has shown that fidelity versus misincorporation is enzyme, sequence, and adduct dependent.[7e−g] Studies of human topoisomerase I have shown that, depending on the adduct, cleavage of the DNA can be blocked or subsequent religation of the cleaved DNA can be inhibited.[7h,i] Rates of DNA repair are highly dependent on the hydrocarbon and the structure of the DE adduct.[7j,k] Thus, synthetic access to oligonucleotides containing a variety of different DE adducts in different sequence contexts is of high significance.

To date, three strategies have been successful in the preparation of oligonucleotides containing N^6-dAdo or N^2-dGuo adducts of the polycyclic aromatic hydrocarbon DEs: direct reaction of DEs with short oligonucleotides, postsynthetic modification in which an oligonucleotide containing an activated nucleotide unit is allowed to react with an amino triol derived from a DE, and synthesis of a DE-adducted phosphoramidite suitably protected for use in standard, solid-phase DNA synthesis. The advantages and disadvantages of each of these approaches have been detailed elsewhere.[8] We have preferred the third approach in that it allows the greatest flexibility in terms of the choice of specific adduct in any sequence context. In addition, the desired oligonucleotides are generally much more easily purified from the crude product mixtures compared to the other two approaches. Initially, the stepwise synthesis of phosphoramidites involved nucleophilic displacement by aminotriols derived from DEs on appropriately protected purine nucleosides with either 6-fluoro-[9a,10a] or 6-sulfonate-[9b,c] leaving groups (dAdo precursors) and 2-fluoro-[7j,10b] or 2-triflate-[10c] deoxyinosine derivatives (dGuo precursors). Although trans-opened aminotriols are readily available by direct aminolysis of DEs, synthesis of the corresponding *cis*-aminotriols requires multiple steps.[11,12a] This difficulty was partially resolved by our discovery that cis-opened N^6-dAdo adducts of BaP DE-2 and BcPh DE-2 (**2**) could be prepared in a single[12] step from the corresponding dihydrodiols and 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine in a highly stereoselective fashion under Sharpless aminohydroxylation conditions. Notably, however, we have been unable to find reaction conditions for preparation of dGuo adducts by this procedure.

Direct reaction of the exocyclic amino groups of the purines with the DEs constitutes an attractive route to adducts. Only recently have we been able to establish

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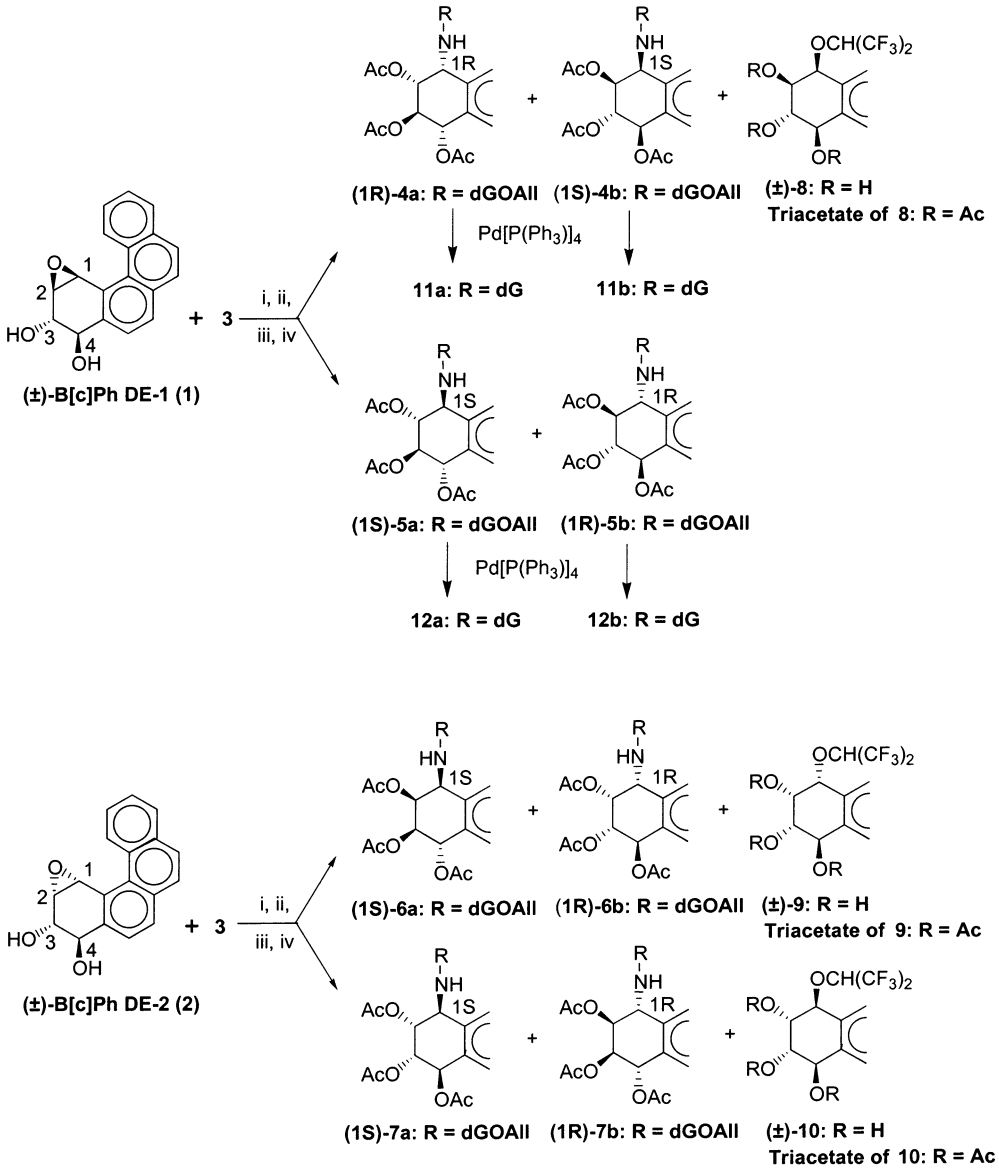
SCHEME 1[a]



[a] Key: (i) $(CF_3)_2CHOH$; (ii) HPLC or column; (iii) Ac_2O, DMAP, pyridine; (iv) HPLC.

satisfactory conditions for this reaction.[8] Blocking the sugar hydroxyl groups of dGuo with the very nonpolar *tert*-butyldimethylsilyl ether group (TBDMS) and O^6-allyl protection (O^6-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**3**), Figure 1) provides an organic soluble form of the purine with enhanced nucleophilicity of the 2-amino group that efficiently reacts with the DEs in dimethylacetamide (DMA) at elevated temperature. For BaP DE-1 and DE-2 (100 °C, 2 h), a mixture of cis and trans adducts was formed (∼50% yield) with a cis/trans ratio of ∼1/1.[8a] The more hindered and less reactive BcPh DE-2 (**2**) (100 °C, 5 h) gave a cis/trans ratio of 27/73 in much lower yield (26%).[8b] Surprisingly, we have been unable to find similar conditions where 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine reacts with DEs to form adducts.

Fluorinated alcohols have a dramatic catalytic effect on the addition reactions between blocked purines and DEs. Thus, reaction of BaP DE-1 and DE-2 with **3** in 2,2,2-trifluoroethanol (TFE) *proceeds at room temperature* to give a mixture of cis and trans adducts in 65 and 43% overall yields, respectively, with cis/trans ratios of 85/15 (DE-1) and 40/60 (DE-2).[13] Unlike the reaction in DMA, reaction of 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine in TFE with BaP DE-1 and DE-2 afforded exclusively cis-opened adducts, albeit in low yield (22−33%).[13] Encouraged by such facile reaction at room temperature, we explored the possibility of solvent-free reaction.[14] Simply grinding BaP DE-1 or DE-2 with **3** with a pestle in a porcelain mortar afforded a mixture of the corresponding cis and trans adducts (cis/trans ratio for DE-1: 50/50, 45% yield; for DE-2: 15/85, 54% yield).[14] As with reaction in DMA, very little dAdo adduct forms in the solvent-free reaction.

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Although there have been a number of studies using site-specifically modified oligonucleotides containing dGuo adducts of BaP DEs, corresponding oligonucleotides containing BcPh DE-dGuo adducts have been less accessible due to the greater difficulty in synthesizing such adducts from the more hindered and less reactive intermediates in the BcPh series. Thus there is a pressing need for new synthetic routes to oligonucleotides containing such BcPh DE-dGuo adducts for physical and biochemical studies. Since reaction of BcPh DE-2 with **3** in DMA proceeded in modest yield,[8b] we have investigated in the present study the potential of fluorinated alcohols as catalysts in the formation of the desired adducts. We have found that reaction in 1,1,1,3,3,3-hexafluoropropan-2-ol (HFP) results in much higher yields (62−84%) and that the cis/trans ratio of adducts is highly dependent on the amount of fluorinated alcohol used. The use of HFP provides ready access to the eight possible diastereomerically pure, fully protected N^2-dGuo adducts of BcPh DE-1 (**1**) and BcPh DE-2 (**2**) for use in oligonucleotide synthesis (Scheme 1).

Results and Discussion

Direct opening of the highly hindered fjord-region BcPh DEs (**1** and **2**) with **3** was investigated using TFE (pK_a = 12.4),[15] HFP (pK_a = 9.3),[15] and perfluoro-*tert*-butyl alcohol (PFTB; pK_a = 5.2)[15b] as solvents. HFP was found to be the solvent of choice. Although the addition reactions occurred in PFTB, the yield was lower than in HFP, and several unidentified products were formed. Increased ionizing power and acidity of PFTB might have caused degradation through N^7-adduct formation and deglycosylation. Unlike BaP DEs,[13] the corresponding BcPh DEs did not undergo this addition reaction in the less acidic TFE at room temperature. The C-1 carbocation corresponding to the BcPh DEs ($\Delta E_{deloc} = 0.600\beta$) is higher in energy and less easily formed than the C-10 carbocation corresponding to the BaP DEs ($\Delta E_{deloc} = 0.794\beta$).[16] Thus, the BcPh DEs are much less reactive[17] than the BaP DEs. Increased steric hindrance of the fjord region of BcPh DEs compared with the bay region of BaP DEs may also retard their reaction rates.

Reaction of a 5-fold molar excess of **3** with BcPh DEs (**1** or **2**) was complete within 3 h at room temperature when a 100-fold molar equivalent or more of HFP was present (Table 1). When a lesser amount of HFP was used (75 molar equiv or less), the reaction required heating (45−140 °C, 3 h) to reach homogeniety, at which time reaction was complete. Yields of the adducts as their triacetates for the DE-1 reaction (62−67%) and the DE-2 reaction (72−84%) were much higher than that for DE-2 in DMA (26%).[8b] Although decomposition products of the DEs were not detected, minor amounts of solvent addition of HFP (∼10%) were observed (Scheme 1). In reactions of DE-1 (**1**), only the *cis*-HFP adduct (**8**) was formed. In reactions of DE-2, the *cis*-HFP adduct (**9**) was accompanied by a lesser amount (1/5) of the trans adduct (**10**). For reactions of DE-1 (**1**), residual **3** was removed by column chromatography on silica gel, and the resultant adduct fraction was acetylated. The triacetate of the *cis*-HFP adduct (**8**) and the diastereomeric triacetates of the *O*-allyl disilyl dGuo adducts *cis*-(1*R*)-**4a**, *cis*-(1*S*)-**4b**, *trans*-(1*S*)-**5a**, and *trans*-(1*R*)-**5b** (Scheme 1) were readily separated by HPLC. For the reactions of DE-2 (**2**), direct HPLC provided four fractions: the *cis*-HFP adduct (**9**), the *trans*-HFP adduct (**10**), the cis-opened diastrereomeric adduct mixture, and the trans-opened diastereomeric adduct mixture. After acetylation, the diastereoisomeric pair of cis isomers (*cis*-(1*S*)-**6a** and *cis*-(1*R*)-**6b**) and the diastereoisomeric pair of trans isomers (*trans*-(1*S*)-**7a** and *trans*-(1*R*)-**7b**) were separated by HPLC. These DE-2 dGuo adducts were identified (^{1}H NMR, CD, and mass spectra) by comparison with authentic samples.[8b]

TABLE 1. HFP-Mediated Synthesis of Cis- and Trans-Opened N^2-dGuo Adducts from BcPh DE-1 (1) and DE-2 (2)[a]

diol epoxide	HFP (molar equiv)	temp (°C)	cis:trans adduct ratio[b]	yield (%)[c]
1	532	rt	91:9	66
2	532	rt	61:39	78
1	266	rt	90:10	67
2	266	rt	53:47	80
1	100	rt	84:16	63
2	100	rt	40:60	84
1	75	45	83:17	62
2	75	60	36:64	83
1	30	50	52:48	69
2	30	70	18:82	81
1	24	75	42:58	68
2	24	75	14:86	72
1	5	110	17:83	62
2	5	110	2:98	74
1	0	140	5:95	74
2	0	140	2:98	60

[a] All reactions were run for 3 h with a molar ratio of the DE to dGuo nucleoside (**3**) of 1 to 5, except for the fusion reaction in the absence of solvent, where the ratio was 1 to 20. [b] Ratio was determined by HPLC (300 nm) as disilyl triacetates for DE-1 and directly as disilyl trihydroxy derivatives for DE-2. [c] Actual isolated yield of combined cis and trans adducts (as triacetates for DE-1, 50−100 mg scale).

The cis/trans ratio of adducts from both DE-1 (**1**) and DE-2 (**2**) in HFP changes with the relative amount of HFP solvent used. As shown in Table 1 and in Figure 2, a maximum cis/trans ratio (91/9) for DE-1 was obtained when 532 molar equiv of HFP was present. A significant increase in the formation of the trans adduct began below ∼150 molar equiv of HFP. At 5 molar equiv of HFP, the ratio reached 17/83. A similar, but less dramatic change was observed for DE-2. The observed cis/trans ratios ranged from 61/39 to 2/98 for this DE.

Unlike the BaP DEs,[14] the less reactive BcPh DEs (**1** or **2**) did not undergo solvent-free reaction with **3** at room temperature. However, when **1** or **2** and a 20-fold molar excess of **3** were fused at 140 °C (Table 1), reaction was complete within 3 h (74 and 60% yields, respectively). Almost exclusive formation of the trans isomers was observed for both DE-1 (**1**) (cis/trans = 5/95) and DE-2 (**2**) (cis/trans = 2/98). This high trans selectivity provides an excellent route to these trans isomers. Much less selectivity was observed with the BaP DEs in the absence of solvent.[14]

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TABLE 2. Comparison of the BcPh DE-1 Benzo-Ring ^{1}H NMR Data (500 MHz, Acetone-d_6) for the O^6-Allyl-Protected Cis- and Trans-Opened N^2-dGuo Adducts as Their Disilyl Triacetates[a]

compd	H_1	H_2	H_3	H_4
4a (1*R*)	6.92	4.90	5.97	6.17
cis-*O*-allyl-dGuo-DE-1	$J_{1,2} = 3.3$	$J_{2,3} = 9.3$	$J_{3,4} = 3.3$	
4b (1*S*)	6.95	4.92	5.98	6.18
	$J_{1,2} = 3.3$	$J_{2,3} = 9.3$	$J_{3,4} = 3.4$	
5a (1*S*)	6.19	5.84	5.12	6.88
trans-*O*-allyl-dGuo-DE-1	$J_{1,2} = 3.3$	$J_{2,3} = 2.4$	$J_{1,3} = 0.9$	$J_{3,4} = 7.8$
5b (1*R*)	6.19	5.84	5.13	6.85
	$J_{1,2} = 3.3$	$J_{2,3} = 2.4$	$J_{1,3} = 0.9$	$J_{3,4} = 8.2$

[a] Assignments and coupling constants were confirmed by decoupling experiments.

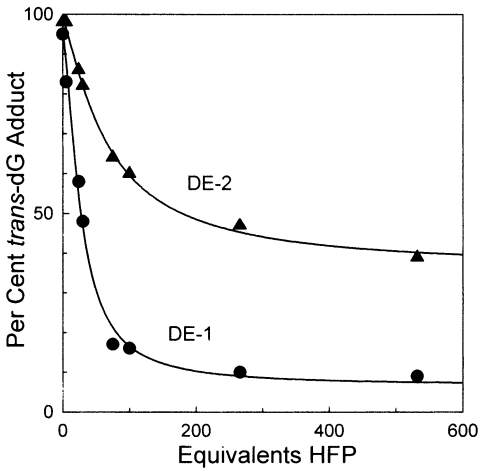

FIGURE 2. Stereoselectivity of the HFP-mediated cis and trans opening of BcPh DE-1 (**1**) and DE-2 (**2**) by the dGuo reactant O^6-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**3**) in a molar ratio of 1:5. See Table 1 for details.

In the presence of very low amounts of HFP, reaction of the DEs (**1** and **2**) with **3** is presumed to proceed largely by an S_N2 mechanism. As the amount of HFP is increased relative to the other reactants, the medium becomes more polar, and an S_N1 reaction mechanism becomes more favorable. This would account for the increase in the amount of cis-opened products. Conformations of the starting DEs and their intermediate carbocations (Scheme 2) provide a plausible explanation for the cis/trans product ratios observed in the presence of high amounts of HFP (Figure 2). On the basis of observed NMR spectra in Me_2SO-d_6,[17] **1** is expected to prefer conformation **1b** (Scheme 2) in polar media such as HFP. This conformation differs from that observed for DE-1 isomers with a bay region[18,19] as a result of steric crowding between H_1 and H_{12} in the fjord region. The conformation of the carbocation formed upon initial epoxide ring opening of **1b** is **1d**. This conformation is also expected to be the preferred one at equilibrium.[20] Energetically favorable axial attack[20,21] by nucleophiles on this conformation will lead to cis products. Thus, under S_N1 conditions, acid-catalyzed hydrolysis of **1** and its reaction with **3** in the presence of solvent HFP give predominantly *cis*-tetrol (~85%)[17] and cis-1 adduct (~90%), respectively. Similarly, only the *cis*-HFP adduct (**8**) was formed as a side product in the reaction of **1**.

In contrast to **1**, the fjord region has less of an effect on the preferred conformation of **2** since relief of steric strain between H_1 and H_{12} in **2a** is offset by an adverse eclipsing interaction between the epoxide C_2−O bond and the nonbenzylic hydroxyl group (C_3−OH) in **2b** (Scheme 2). Consequently, fjord-region DE-2 (**2**) prefers the same conformation[17] (**2a**) as DE-2 isomers with a bay region.[18,19] The corresponding carbocation (**2c**) would be expected to undergo preferential axial attack, as above, to give trans opened products, and indeed only *trans*-tetrol is observed in the acid-catalyzed hydrolysis of **2**.[17] In the present case, a marked increase in cis addition of **3** [~60% cis-2 adduct] is observed. Similarly, *cis*-(**9**) and *trans*-(**10**) HFP adducts were formed from **2** in a ratio of 80 to 20. We speculate that this preference for cis addition in HFP may result from an increased lifetime of the carbocation, which allows the initially formed **2c** to undergo conformational equilibration with **2d** at a rate faster than its capture by solvent or by **3**. Thus, assuming that capture of **2c** and **2d** by axial attack of **3** occurs at similar rates, the product ratio under S_N1 conditions will be largely determined by the position of the equilibrium between these conformers. Although previous studies[20,22] indicate that S_N1 solvolyses of DE-2 isomers in water proceed predominantly via the conformation corresponding to **2c** to give *trans*-tetrols, the present results suggest that for BcPh DE-2 in HFP conformations **2c** and **2d** may be of comparable stability and have sufficiently long lifetimes to equilibrate before they are captured by a nucleophile.

^{1}H NMR spectral data for the saturated benzo-ring protons of the DE-1 O^6-allyl disilyl dGuo triacetates (**4a**,**b** and **5a**,**b**) are given in Table 2. Practically identical chemical shifts and coupling constants were observed for members of the cis pair and of the trans pair of diastereomers. Assignment of relative stereochemistry for these adducts was readily achieved by comparison of the present NMR data with those obtained for the BcPh tetrol tetraacetates[23] and the peracetates of the dGuo adducts[23] derived from DE-1. Notably, only products derived from cis opening of DE-1 (i.e., **4a**,**b**) have a large

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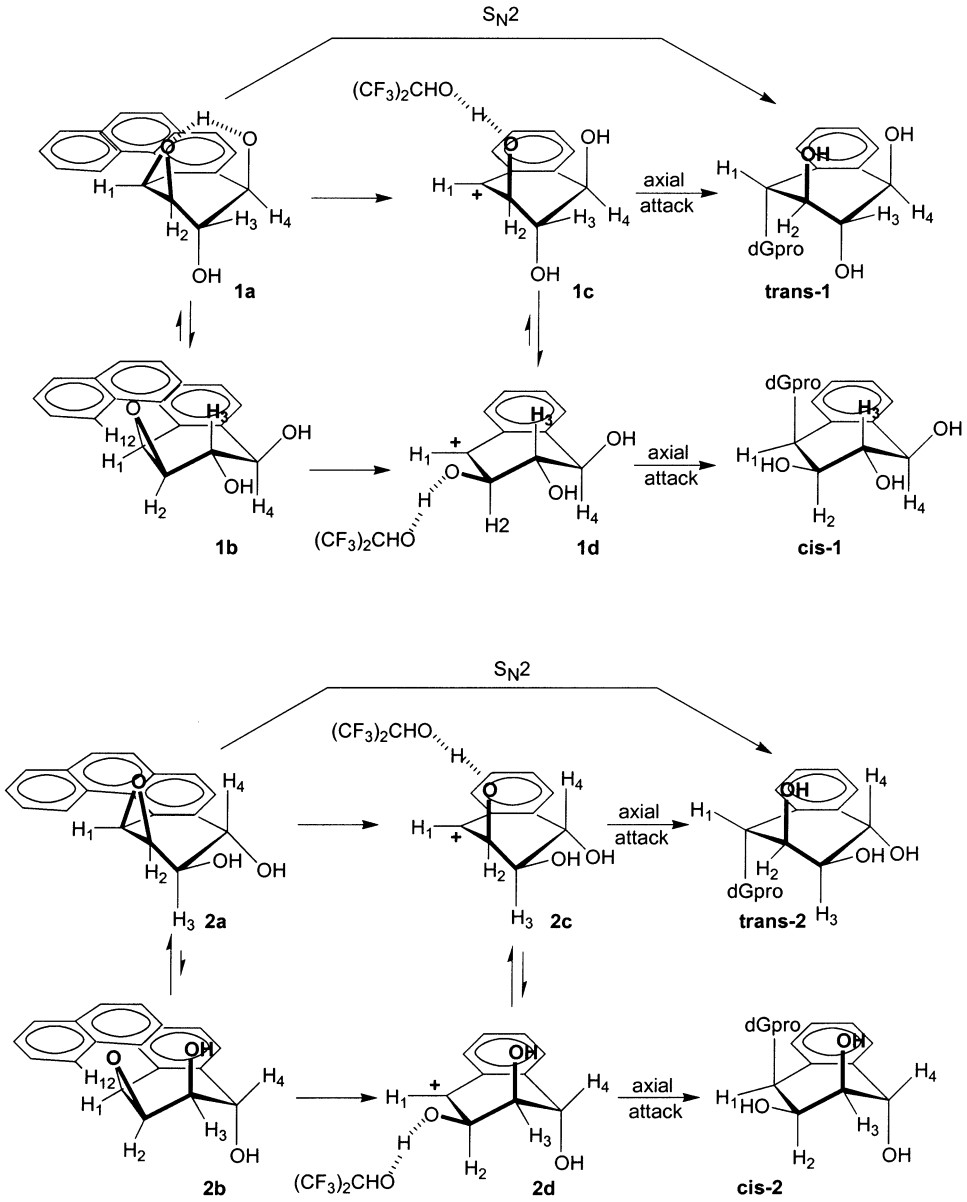
SCHEME 2



value for $J_{2,3}$ (9.3 Hz) and a small value for $J_{3,4}$ (3.3 Hz) compared to a small value of $J_{2,3}$ (2.4 Hz) and a large value of $J_{3,4}$ (7.8−8.2 Hz) for the trans-opened DE-1 products (i.e., **5a**,**b**). In the case of the cis-opened adducts of DE-1 (**4a**,**b**), the substituent at C-1 prefers a quasiaxial conformation (constrained by steric hindrance in the fjord region), which requires the acetoxy groups at C-2 and C-3 to be quasidiequatorial in a *twisted half chair* conformation; thus, $J_{2,3}$ is large (*cis*-**1** in Figure 3). For the trans adducts (**5a**,**b**), a *twisted half boat* conformation is preferred in which the substituent at C-1 is again quasiaxial (*trans*-**1** in Figure 3). Consequently, the C-3 acetoxy group adopts a quasiaxial conformation (*trans*-**1** in Figure 3). The dihedral angle between C_2-H and C_3-H is ∼110° ($J_{2,3}$ = 2.4 Hz), and the angle between C_3-H and C_4-H is large (J ≈ 8 Hz). A characteristic W-type long-range coupling ($J_{1,3}$ = 0.9 Hz) was observed only for the trans adducts (**5a**,**b**).

^{1}H NMR spectral data for the saturated benzo-ring protons of the HFP adducts and their triacetates are given in Table 3. In all cases (**8** from DE-1, **9** and **10** from DE-2), the most downfield doublets were assigned as H-1 on the carbon bearing the HFP substituent, as they underwent little downfield shift on acetylation in contrast to marked downfield shifts of the other signals. The large values of $J_{2,3}$ and the intermediate values of $J_{3,4}$ for the HFP adduct **8** and its acetate indicate cis addition and are in good agreement with those of the cis addition products of BcPh DE-1 (**1**) discussed above. HFP adduct **9** also results from cis addition but has somewhat unusual intermediate values for $J_{2,3}$ and $J_{3,4}$, which did not agree with the previously observed values for either cis or trans adducts derived from BcPh DE-2.[23] The *cis*-**2A** (half chair) and preferred *cis*-**2B** (half boat) conformations consistent with these coupling constants are shown in Figure 3. Notably, in the presence of 3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine, reaction of solvent TFE with BaP DEs occurred by exclusive cis addition.[13] A minor HFP adduct **10** derived from DE-2 had a large

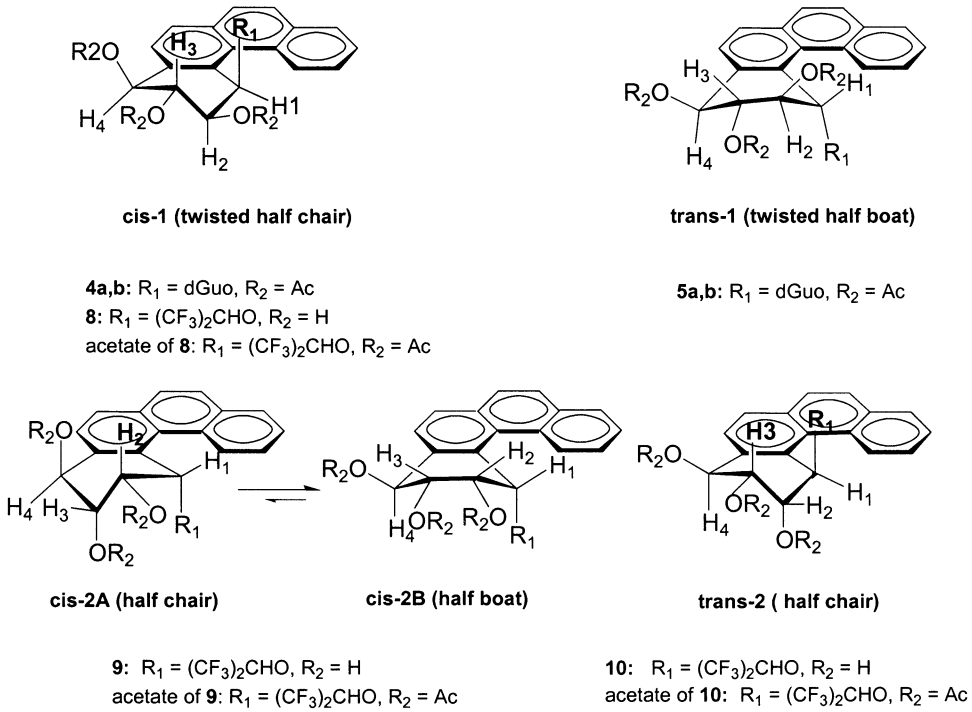


FIGURE 3. Preferred conformations of adducts derived from BcPh DEs.

TABLE 3. Comparison of the B*c*Ph DE-1 and DE-2 Benzo-Ring ^{1}H NMR Data for Disilyl HFP Adducts before and after Acetylation[a]

compd	H_1	H_2	H_3	H_4
8[b]	6.65	4.25	4.27	4.79
	$J_{1,2} = 1.8$	$J_{2,3} = 10.3$	$J_{3,4} = 7.3$	
triacetate of **8**[c]	6.82	5.55	5.95	6.47
	$J_{1,2} = 1.9$	$J_{2,3} = 9.6$	$J_{3,4} = 4.1$	
9[b]	6.52	4.44	3.95	5.16
	$J_{1,2} = 3.0$	$J_{2,3} = 8.2$	$J_{3,4} = 7.1$	
triacetate of **9**[d]	6.63	5.54	5.55	6.76
	$J_{1,2} = 1.7$	$J_{2,3} = 7.4$	$J_{3,4} = 5.1$	
10[e]	6.82	4.64	4.20	5.10
	$J_{1,2} = 4.6$	$J_{2,3} = 2.7$	$J_{3,4} = 8.4$	
triacetate of **10**[c]	6.72	6.03	5.74	6.63
	$J_{1,2} = 4.7$	$J_{2,3} = 2.9$	$J_{3,4} = 8.6$	

[a] Assignments and coupling constants were confirmed by decoupling experiments. [b] Measured at 500 MHz ($CDCl_3$−CD_3OD). [c] Measured at 300 MHz ($CDCl_3$). [d] Measured at 500 MHz ($CDCl_3$). [e] Measured at 300 MHz ($CDCl_3$−CD_3OD).

value for $J_{3,4}$ and a small value for $J_{2,3}$ and $J_{1,2}$ compatible[23] with the *trans*-**2** (half chair) form shown in Figure 3.

Previously, we had established the absolute configuration of the O^6-allyl disilyl triacetyl N^2-dGuo adducts (**6a**,**b** and **7a**,**b**) derived from BcPh DE-2 by removal of the O^6-allyl protecting group and comparison of the CD spectra of the resulting disilyl triacetates[8b] with those of the corresponding unprotected nucleoside adducts of known absolute configuration.[23] For BcPh DE N^2-dGuo adducts, a negative CD band at ~258 nm is typical of an *R* absolute configuration at C-1 of the hydrocarbon. The nonchromophoric TBDMS and acetyl groups have little effect on the CD spectra of unprotected nucleoside adducts.[12b] In contrast, O^6-allyl protection significantly alters the guanine chromophore and causes marked changes in the exciton CD spectra.[8] The same strategy has been used here to determine the absolute configuration of the BcPh DE-1 O^6-allyl disilyl triacetyl N^2-dGuo adducts (**4a**,**b** and **5a**,**b**) by removal of the O^6-allyl protecting group and determination of the CD spectra (Figure 4) of the resultant disilyl triacetates of the N^2-dGuo adducts (**11a**,**b** and **12a**,**b**). Thus, the positive CD band at 258 nm for (1*S*)-**12a**, which was obtained on O^6-allyl deprotection of (1*S*)-**5a**, establishes that these configurationally related compounds have a 1*S* absolute configuration and were formed from (−)-[1*R*,2*S*,3*R*,4*S*]-BcPh DE-1 by inversion of the configuration at C-1 (Scheme 1).

In the preceding paper,[8b] we have reported the synthesis of 5′-dimethoxytrityl (DMT)-protected 3′-phosphoramidite building blocks of the N^2-dGuo adducts derived from cis and trans opening of BcPh DE-2 (**6a**,**b** and **7a**,**b**). Those phosphoramidites are 1*R*/1*S* diastereomeric mixtures at C-1. Their use in oligonucleotide synthesis results in a mixture of 1*R*/1*S* constructs. Although separation of the two adducted oligonucleotides has generally been possible, we have recently encountered a case in which the *R*/*S* pair of oligonucleotides could not be separated by HPLC under a variety of conditions.[24] In the present study, each of the pure diastereomers at C-1, **6a**,**b** and **7a**,**b** derived from cis and trans opening of BcPh DE-2, as well as newly synthesized N^2-dGuo adducts (**4a**,**b** and **5a**,**b**) derived from DE-1 were separately used as the starting materials for the synthesis of the corresponding phosphoramidites (Scheme 3). Although more work synthetically, use of single phosphoramidite diastereomers with a known absolute configuration greatly simplifies the HPLC purification of the resultant oligonucleotides since only one of two closely

(24) We have been unable to separate a pair of 16-mer oligonucleotides containing the *trans*-DE-2 adducts **7a** and **7b**. See: Ramos, L. A.; Pontén, I.; Yagi, H.; Sayer, J. M.; Kroth, H.; Kalena, G.; Kumar, S.; Dipple, A.; Jerina, D. M. *Chem. Res. Toxicol.*, submitted for publication.

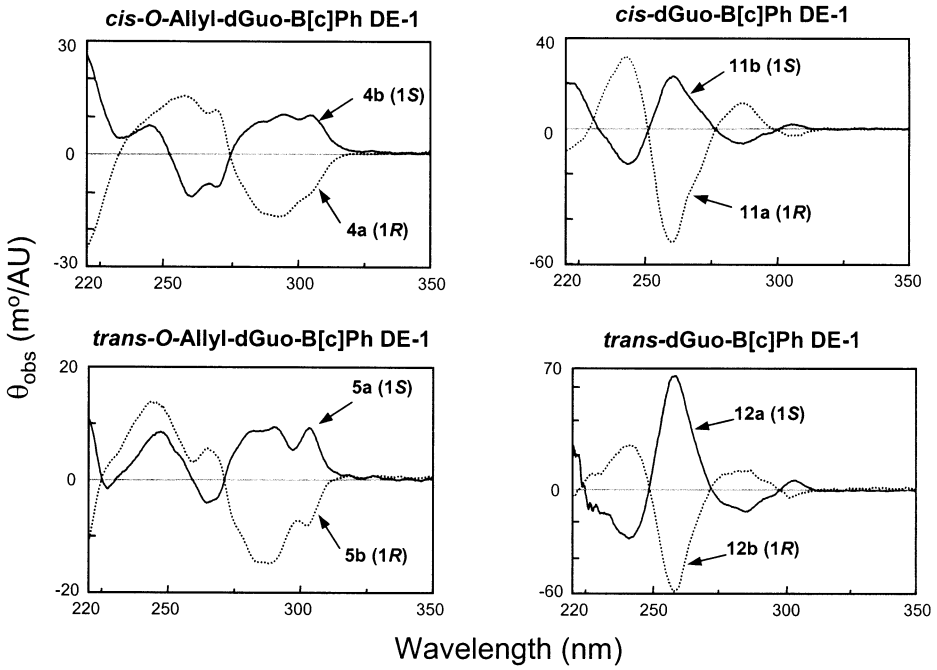


FIGURE 4. Circular dichroism spectra (normalized to 1.0 absorbance unit at λ_{max} in methanol) of the cis- and trans-opened O^6-allyl-*protected* N^2-dGuo adducts **4a**,**b** and **5a**,**b** and of the O^6-allyl-*deprotected* N^2-dGuo adducts **11a**,**b** and **12a**,**b**, all as disilyl triacetetes. The strong CD bands at 261 nm for the O^6-allyl-deprotected cis-opened N^2-dGuo adducts **11a**,**b** (solid line, 1*S*) and at 258 nm for the O^6-allyl-deprotected trans-opened N^2-dGuo adducts **12a**,**b** (solid lines, 1*S*) are diagnostic of their absolute configuration. See text and Scheme 1.

eluting adducted oligonucleotide peaks is present and the adduct configuration is known in advance. In addition, there is twice as much of this adduct-containing peak relative to failure sequences. Also, separation of *R*/*S* pairs of adducted oligonucleotides is likely to become increasingly more difficult as the sequence length increases.

Previously, desilylation of **6a**,**b** and **7a**,**b** was achieved with 2% HF in a mixture of acetonitrile and pyridine (4:1).[8b] Although the yields for desilylation under these mild conditions were reasonably high (70−75%), conversion of starting material into desilylated products often required recycling recovered starting material (24 h) two to three times. This time-consuming recycling procedure could be avoided by simply using 7% HF in pyridine for 12 h at room temperature. Thus, **4a**,**b**, **5a**,**b**, **6a**,**b**, and **7a**,**b** were converted to the sugar desilylated derivatives **13a**,**b**, **14a**,**b**, **15a**,**b**, and **16a**,**b**, respectively, in >95% yield (Scheme 3).

To date, two methods have been utilized for the selective introduction of the 5′-*O*-dimethoxytrityl (DMT) protecting group into polycyclic aromatic hydrocarbon-adducted nucleotide building blocks. The first method uses DMT chloride in pyridine under strictly anhydrous conditions.[25] Although this method has seen considerable use,[8b,9b,c] yields can be variable and sometimes low. Some improvements have been observed when a 2.25-fold excess of DMT chloride and DMAP are present.[8b] The second method uses DMT fluoroborate[26] with lutidine and Li_2CO_3 as bases in THF.[10,12,27] We have had generally good results with the second method when the reaction proceeds normally (>90% conversion within 2 h). However, when extended reaction times are required (> 20 h), substantial byproducts form. For example on tritylation of **14a** in THF (Scheme 3), a major secondary reaction occurs in which the desired product (**19a**) reacts with THF at the 3′-hydroxyl group to produce a diastereomeric pair of acetals (**17a**,**b**) on consumption of the starting material. Presumably, hydride abstraction from the C-2 position of THF by the DMT carbocation occurred,[28] and the resultant carbocation alkylated the 3′-hydroxyl group of **19a**. In fact, when DMT fluoroborate was stored in THF, the characteristic reddish color of the DMT carbocation gradually faded and the hydride-transfer product, bis-(4-methoxyphenyl)phenylmethane, was produced. Simply replacing THF with CH_2Cl_2 as solvent solved the problem. The tritylation reactions were completed within 2−3 h without formation of any significant side products. Thus, the required 5′-DMT derivatives **18a**,**b**, **19a**,**b**, **20a**,**b**, and **21a**,**b** were obtained from **13a**,**b**, **14a**,**b**, **15a**,**b**, and **16a**,**b**, respectively, in nearly quantitative yield (>95%).

Finally, introduction of the phosphoramidite function at the 3′-deoxyribose hydroxyl group in the 5′-DMT derivatives (**18a**,**b**, **19a**,**b**, **20a**,**b**, and **21a**,**b**) was achieved with 2-cyanoethyl tetraisopropylphosphorodiamidite and *N*,*N*-diisopropylammonium tetrazolide[29] in CH_2Cl_2 to give **22a**,**b**, **23a**,**b**, **24a**,**b**, and **25a**,**b**, respectively, in 80−90%

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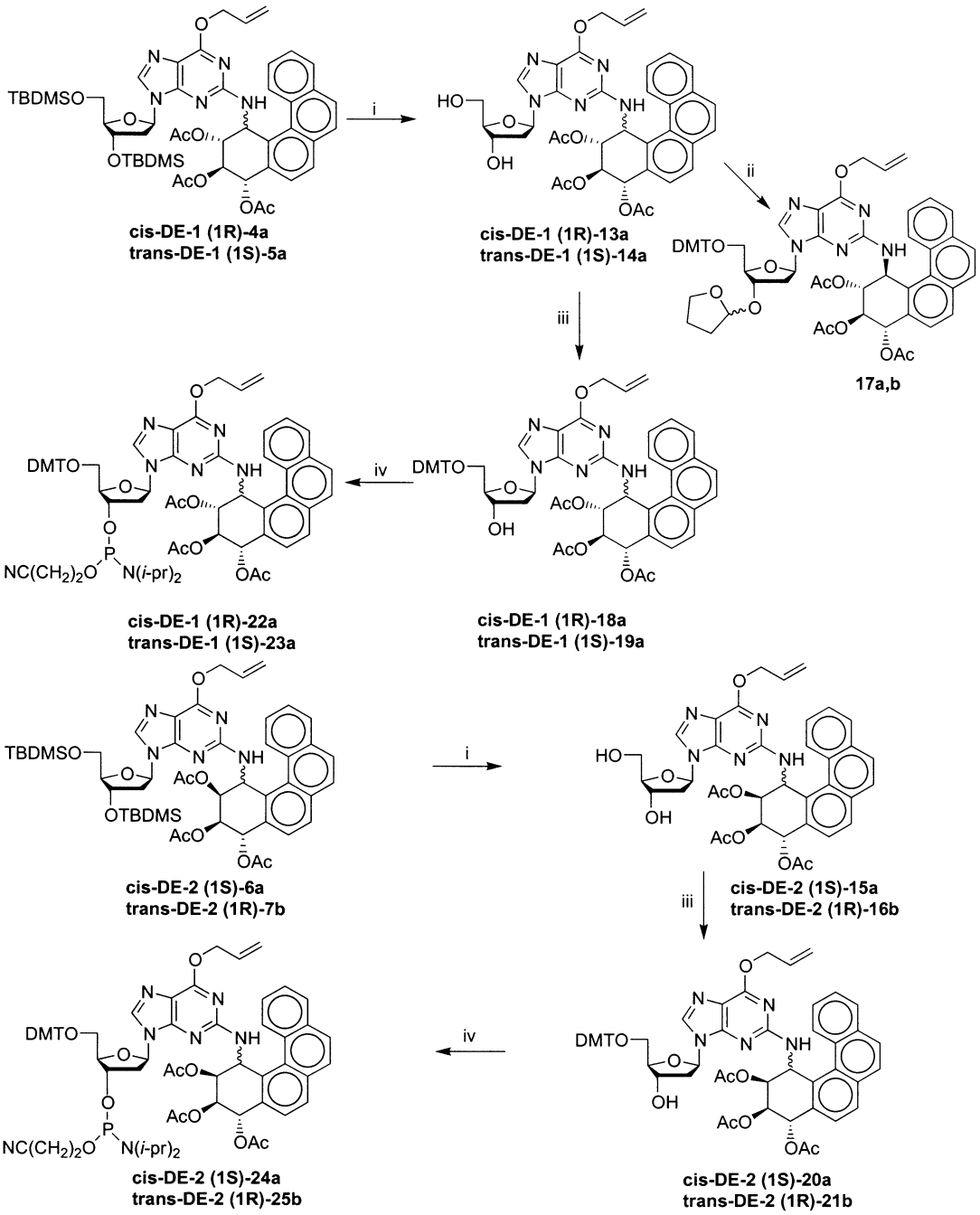
(29) Beaucage, S. L. In *Methods in Molecular Biology*: *Protocols for Oligonucleotides and Analogues*; Agrawal, S., Ed.; Humana Press: Totowa, NJ, 1993; Vol. 20, pp 33−61.

SCHEME 3[a]



[a] Key. (i) 7%/HF/pyridine; (ii) $DMT^+BF_4^-$−THF; (iii) $DMT^+BF_4^-$−CH_2Cl_2; (iv) 2-cyanoethyltetraisopropylphosphoramidite.

yield. Each of the diastereomeric phosphoramidites thus obtained consisted of a mixture of two diastereomers due to the asymmetry of the phosphorus. In general the mixed phosphorus diastereomers exhibit two peaks with equal intensities on ^{31}P NMR spectroscopy. Although these phosphorus diastereomers could be separated by HPLC (see Experimental Section), such separation was not routinely done prior to their use in oligonucleotide synthesis.

In summary, novel and efficient syntheses of O^6-allyl-protected N^2-dGuo phosphoramidite building blocks **22a**,**b**, **23a**,**b**, **24a**,**b**, and **25a**,**b** (cf. Scheme 3) in diastereomerically pure forms are described. Simply heating the individual DEs with O^6-allyl- and di-*O*-TBDMS-protected dGuo (**3**) in the absence of solvent provided almost exclusively the trans adducts in 60−74% yield. Through the use of appropriate amounts of HFP, the cis/trans ratio can be varied from 1/1 to as much as 91/9 for DE-1 (66% yield). For DE-2, the highest cis/trans ratio was 61/39 in 78% yield. The high polarity and higher acidity of HFP relative to that of TFE are thought to account for the marked catalytic activity of HFP through hydrogen bonding and/or protonation of the epoxide oxygen. HFP may also enhance the nucleophilicity of the exocyclic amino group of guanine.[30]

(30) Moon, K.-Y.; Moschel, R. C. *Chem. Res. Toxicol.* **1998**, *11*, 696−702.

Experimental Section

***Caution*:** *Benzo[c]phenanthrene 3,4-dihydrodiol and diol epoxides DE-1 and DE-2 are mutagenic and carcinogenic and must be handled carefully in accordance with NIH guidelines*.[31]

General Methods. ^{1}H NMR spectra were recorded at 300 or 500 MHz as indicated. Chemical shifts are reported in parts per million (δ) downfield from TMS. Coupling constants (J) are given in hertz, and spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Assignments are based on decoupling experiments in all cases. 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 H_v. ^{31}P NMR spectra were recorded at 300 MHz in CD_3CN with 85% H_3PO_4 as an external standard. High-resolution mass spectroscopy (HRMS) was performed using standard methods. Methylene chloride (CH_2Cl_2), lutidine, triethylamine (TEA), and pyridine were dried over 4 Å molecular sieves after distillation. Tetrahydrofuran (THF) was distilled over $LiAlH_4$ prior to use. O^6-Allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**3**) was prepared from 2′-deoxyguanosine as described.[32] The diol epoxides, DE-1 and DE-2 of BcPh, were synthesized from the BcPh 3,4-dihydrodiol as described.[17] Flash column chromatography was performed using thick-walled glass columns with 230−400 mesh silica gel 60. Melting points were not corrected.

Reaction of BcPh DE-1 (1) with O^6-Allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (3) in HFP. To a solution of O^6-allyl dGuo di-TBDMS ether (**3**) (2.6 g, 4.85 mmol) in HFP (4.9 g, 29.2 mmol, 30 molar equiv) was added BcPh DE-1 (**1**) (270 mg, 0.97 mmol), and the mixture was stirred at 50 °C in a sealed vial filled with Ar gas. The crystals of **1** gradually dissolved, and the mixture was stirred at room temperature for 3 h or until no starting material was detected by HPLC. If 100 or more molar equiv of HFP was used, the sealed reaction mixture was stirred for 3 h at room temperature. If less than 100 molar equiv was used, the sealed reaction mixture was heated with stirring at various temperatures (45−140 °C) for 3 h, at which time a uniform solution was obtained (see Table 1). When no solvent HFP was used, a mixture of **1** and 20 molar equiv of **3** was melted in a sealed vial under Ar gas with stirring at 140 °C for 3 h.

The solvent HFP was evaporated in vacuo, and the residue was subjected to column chromatography on silica gel (2.5 × 30 cm) eluted with 40% *n*-hexane in EtOAc to give unreacted **3** (1.8 g, 61% recovery). The column was then eluted with 5% MeOH in CH_2Cl_2, and the solvent was evaporated to give a colorless glass (550 mg). A portion (∼5 mg) of this crude product was subjected to HPLC on two coupled Axxiom silica gel columns (9.5 × 250 mm, 5 μm) using 25% *n*-hexane in EtOAc as a solvent at a flow rate of 6 mL/min (detected at 260 nm) and separated into two fractions (t_R = 9.2 and 10.4 min). Evaporation of the early fraction gave **(±)-1β-hexafluoro-2-propyloxy-2β,3α,4β-trihydroxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (8)** as a colorless solid (0.5 mg). ^{1}H NMR (500 MHz, $CDCl_3$−CD_3OD) δ: 3.81 (m, 1H, $(CF_3)_2$ CHO), 4.22 (dd, 1H, H_2, J = 10.3, 1.8), 4.27 (dd, 1H, H_3, J = 10.3, 7.3), 4.79 (d, 1H, H_4, J = 7.3), 6.65 (d, 1H, H_1, J = 1.8), 7.48−7.90 (m, 7H, aromatic protons), 8.27 (br d, 1H, H_{12}, J = 8.0). HRMS (FAB+) calcd for $C_{21}H_{16}O_7F_6$ (M^+): 446.0953. Found: 446.0941.

Evaporation of the late fraction gave a mixture of two *cis*- and two *trans*-N^2-dGuo adduct diastereomers as a colorless glass (4.5 mg). The ratio of the cis to trans adducts was estimated to be ∼1/1 by ^{1}H NMR (300 MHz, acetone-d_6) on the basis of the H_{12} aromatic proton signals of the cis diastereomers (δ 8.87 ppm) and trans diastereomers (δ 8.70 ppm). The remainder of the crude product was acetylated with Ac_2O (1 mL) in pyridine (5 mL) in the presence of DMAP (20 mg) at room temperature for 18 h. After removal of volatiles in vacuo, the residue was taken up in EtOAc (100 mL) and washed with 5% $NaHCO_3$ (2 × 30 mL) and water (50 mL). Evaporation of the solvent gave a gum, which was separated by HPLC on a Vertex column (LiChrosorb Si-60, 2 × 25 cm, Sonntek, Inc., Upper Saddle River, NJ) eluted with 20% EtOAc in *n*-hexane at a flow rate of 25 mL/min (detected at 300 nm). The triacetate of the *cis*-HFP adduct **8** had t_R = 8.4 min (10% as detected at 300 nm; 45 mg, 8% isolated yield). The triacetate of the *cis*-N^2-dGuo adducts had $t_{R(early)}$ = 19.1 min [(1*R*)-**4a**, 25% (300 nm); 158 mg, 17.3% isolated yield] and $t_{R(late)}$ = 25.6 min [(1S)-**4b**, 22% (300 nm); 114 mg, 13% isolated yield]. The triacetate of the *trans*-N^2-dGuo adducts had $t_{R(early)}$ = 31.3 min [(1*S*)-**5a**, 18% (300 nm); 145 mg, 16% isolated yield] and $t_{R(late)}$ = 34.1 min [(1*R*)-**5b**, 26% (300 nm); 205 mg, 23% isolated yield]. **(±)-1β-Hexafluoro-2-propyloxy- 2β,3α,4β-triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (Triacetate of 8).** Colorless prisms; mp 141 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.16, 2.17 and 2.23 (each s, each 3H, 3 × CH_3CO), 3.33 (m, 1H, $(CF_3)_2CHO$), 5.55 (dd, 1H, H_2, J = 9.6, 1.9), 5.95 (dd, 1H, H_3, J = 9.6, 4.1), 6.47 (d, 1H, H_4, J = 4.1), 6.82 (d, 1H, H_1, J = 1.9), 7.50−8.00 (m, 7H, aromatic protons), 8.45 (m,1H, H_{12}). HRMS (FAB+) calcd for $C_{27}H_{22}O_7F_6$ (M^+): 572.1270. Found: 572.1258. ***N*^{2}-[1*R*-(2*R*,3*R*,4*S*-Triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrenyl)]-*O*^{6}-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (4a).** ^{1}H NMR (500 MHz, acetone-d_6) δ: 0.11(s, 6H, Si bonded Me), 0.14 (s, 6H, Si bonded Me), 0.88 (br s, 9H, *tert*-butyl Me), 0.94 (s, 9H, *tert*-butyl Me), 1.80, 2.06 and 2.23 (each s, each 3H, 3 x CH_3CO), 2.50 (m, 1H, $H_{2'}$), 2.80 (m, 1H, $H_{2'}$), 3.86 (m, 2H, $H_{5',5'}$), 3.98 (m, 1H, $H_{4'}$), 4.72 (br s, 1H, $H_{3'}$), 4.90 (dd, 1H, H_2, J = 9.3, 3.3), 5.03 (m, 2H, $CH_{2(allyl)}$), 5.26 (br d, 1H, H_c, J = 10.5), 5.47 (br d, 1H, H_t, J = 16.8), 5.97 (dd, 1H, H_3, J = 9.3, 3.3), 6.16 (m, 1H, H_v), 6.17 (dd, 1H, H_4, J = 3.3), 6.50 (m, 1H, $H_{1'}$), 6.74 (1H, d, NH, J = 10.0), 6.92 (d, 1H, H_1, J = 10.0, 3.3), 7.32 (m, 1H, H_{11}), 7.60 (br t, 1H, H_{10}), 7.71 (d, 1H, H_5, J = 8.2), 7.86 (AB q, 2H, H_7/H_8, J = 7.8), 8.00 (dd, 1H, H_9, J = 8.8, 0.9), 8.07 (d, 1H, H_6, J = 8.2), 8.12 (s, 1H, $H_{8''}$), 8.70 (br s, 1H, H_{12}). HRMS (FAB+) calcd. for $C_{49}H_{66}O_{10}N_5Si_2$ (M^+): 940.4348. Found: 940.4342. CD spectrum (see Figure 4). ***N*^{2}-[1*S*-(2*S*,-3*S*,4*R*-Triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrenyl)]-*O*^{6}-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (4b).** ^{1}H NMR (500 MHz, acetone-d_6) δ: 0.00−0.20 (m, 12H, Si bonded Me), 0.90 (br s, 18H, *tert*-butyl Me), 1.76, 2.06 and 2.24 (each s, each 3H, 3 × CH_3CO), 2.50 (m, 1H, $H_{2'}$), 2.80 (m, 1H, $H_{2'}$), 3.88 (m, 2H, $H_{5',5'}$), 3.97 (m, 1H, $H_{4'}$), 4.72 (br s, 1H, $H_{3'}$), 4.92 (dd, 1H, H_2, J = 9.3, 3.3), 5.03 (m, 2H, $CH_{2(allyl)}$), 5.28 (br d, 1H, H_c, J = 10.5), 5.48 (br d, 1H, H_t, J = 16.8), 5.98 (dd, 1H, H_3, J = 9.3, 3.4), 6.20 (m, 1H, H_v), 6.18 (d, 1H, H_4, J = 3.4), 6.48 (m, 1H, $H_{1'}$), 6.75 (1H, d, NH, J = 10.0), 6.95 (dd, 1H, H_1, J = 10.0, 3.3), 7.46 (m, 1H, H_{11}), 7.62 (m, 1H, H_{10}), 7.71 (d, 1H, H_5, J = 8.2), 7.88 (AB q, 2H, H_7/H_8, J = 7.8), 8.00 (dd, 1H, H_9, J = 7.6, 0.9), 8.08 (d, 1H, H_6, J = 8.2), 8.11 (s, 1H, $H_{8''}$), 8.82 (br d, 1H, H_{12}, J = 8.5). HRMS (FAB+) calcd for $C_{49}H_{66}O_{10}N_5Si_2$ (M^+): 940.4348. Found: 940.4358. CD spectrum (see Figure 4). ***N*^{2}-[1*S*-(2*R*,-3*R*,4*S*-Triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrenyl)]-*O*^{6}-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (5a).** ^{1}H NMR (500 MHz, acetone-d_6) δ: 0.00−0.20 (m, 12H, Si bonded Me), 0.85−0.92 (m, 9H, *tert*-butyl Me), 0.94 (s, 9H, *tert*-butyl Me), 1.78, 1.94 and 2.18 (each s, each 3H, 3 × CH_3CO), 2.34 (m, 1H, $H_{2'}$), 2.92 (m, 1H, $H_{2'}$), 3.78 (m, 2H, $H_{5',5'}$), 3.92 (m, 1H, $H_{4'}$), 4.62 (br s, 1H, $H_{3'}$), 5.00 (m, 2H, $CH_{2(allyl)}$), 5.12 (ddd, 1H, H_3, J = 7.8, 2.4, 0.9), 5.20 (br d, 1H, H_c, J = 10.5), 5.41 (dd, 1H, H_t, J = 17.2, 2.6), 5.84 (dd, 1H, H_2, J = 3.3, 2.4), 6.14 (m, 1H, H_v), 6.19 (dd, 1H, H_1, J = 3.3,

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

0.9), 6.37 (dd, 1H, $H_{1'}$, J = 7.7, 5.7), 6.88 (d, 1H, H_4, J = 7.8), 7.30 (m, 2H, NH and H_{11}), 7.60 (dt, 1H, H_{10}, J = 7.8, 1.1), 7.72 (d, 1H, H_5, J = 7.9), 7.86 (app. s, 2H, H_7/H_8), 8.00 (dd, 1H, H_9, J = 8.8, 1.2), 8.08 (d, 1H, H_6, J = 8.1), 8.12 (s, 1H, $H_{8''}$), 8.44 (br d, 1H, H_{12}, J = 8.2). HRMS (FAB+) calcd for $C_{49}H_{66}O_{10}N_5Si_2$ (M^+): 940.4348. Found: 940.4358. CD spectrum (see Figure 4). **N^2-[1*R*-(2*S*,3*S*,4*R*-Triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrenyl)]-O^6-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (5b).** ^{1}H NMR (500 MHz, acetone-d_6) δ: (−0.38)−0.12 (m, 12H, Si bonded Me), 0.86 (m, 9H, *tert*-butyl Me), 0.89 (m, 9H, *tert*-butyl Me), 1.72, 1.96 and 2.27 (each s, each 3H, 3 × CH_3CO), 2.35 (m, 1H, $H_{2'}$), 2.95 (m, 1H, $H_{2'}$), 3.75 (m, 2H, $H_{5',5'}$), 3.90 (m, 1H, $H_{4'}$), 4.60 (br s, 1H, $H_{3'}$), 5.00 (m, 2H, $CH_{2(allyl)}$), 5.13 (ddd, 1H, H_3, J = 8.2, 2.4, 0.9), 5.20 (br d, 1H, H_c, J = 10.5), 5.41 (dd, 1H, H_t, J = 17.2, 2.6), 5.84 (dd, 1H, H_2, J = 3.3, 2.4), 6.14 (m, 1H, H_v), 6.19 (dd, 1H, H_1, J = 3.3, 0.9), 6.37 (dd, 1H, $H_{1'}$, J = 7.7, 5.7), 6.85 (d, 1H, H_4, J = 8.2), 7.30 (dt, 1H, H_{11}), 7.43 (br s, 1H, NH), 7.58 (br t, 1H, H_{10}, J = 7.8, 1.1), 7.72 (d, 1H, H_5, J = 8.2), 7.86 (app. s, 2H, H_7/H_8,), 8.00 (dd, 1H, H_9, J = 8.8, 1.1), 8.07 (d, 1H, H_6, J = 8.2), 8.09 (s, 1H, $H_{8''}$), 8.49 (br d, 1H, H_{12}, J = 8.6). HRMS (FAB+) calcd for $C_{49}H_{66}O_{10}N_5Si_2$ (M^+): 940.4348. Found: 940.4359. CD spectrum (see Figure 4).

Reaction of BcPh DE-2 (2) with O^6-Allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (3) in HFP. To a solution of O^6-allyl dGuo di-TBDMS ether (**3**) (783 mg, 1.46 mmol) in HFP (3.69 g, 21.93 mmol, 75 molar equiv) was added BcPh DE-2 (**2**) (81.4 mg, 0.29 mmol) with stirring at 60 °C in a sealed vial filled with Ar gas. The crystals of **2** gradually dissolved, and the reaction mixture was stirred at room temperature for 3 h or until no starting material was detected by HPLC. If more than 100 molar equiv was used, the sealed reaction mixture was heated with stirring at various temperatures (60−140 °C) for 3 h, at which time uniform solutions were obtained (see Table 1). When no HFP was used, a mixture of **1** and 20 molar equiv of **3** was melted and stirred in a sealed vial under Ar gas at 140 °C for 3 h.

The solvent HFP was evaporated in vacuo, and the residue was subjected to HPLC separation on an Axxiom silica gel column (9.5 × 250 mm) eluted with a mixture of TEA (1%), *n*-hexane (29%), and EtOAc (70%) as a solvent at a flow rate of 10 mL/min (detected at 300 nm). Evaporation of the combined fraction at t_R = 1.8 min gave recovered **3** (600 mg, 77% recovery). Evaporation of the combined fraction at t_R = 2.57 min gave **(±)-1α-hexafluoro-2-propyloxy-2α,3α,4β-trihydroxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (9**) as a colorless solid (8 mg, 6%). ^{1}H NMR (500 MHz, $CDCl_3$−CD_3OD) δ: 3.93 (m, 1H, $(CF_3)_2CHO$), 3.95 (dd, 1H, H_3, J = 8.2, 7.1), 4.44 (dd, 1H, H_2, J = 8.2, 3.0), 5.16 (d, 1H, H_4, J = 7.1), 6.52 (d, 1H, H_1, J = 3.0), 7.48−7.90 (m, 7H, aromatic protons), 8.10 (br d, 1H H_{12}, J = 8.0). HRMS (FAB+) calcd for $C_{21}H_{16}O_7F_6$ (M^+): 446.0953. Found: 446.0948. Acetylation of **9** by standard methods afforded **(±)-1α-hexafluoro-2-propyloxy-2α,3α,4β-triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]-phenanthrene** (**triacetate of 9**) as colorless prisms from Et_2O−*n*-hexane (mp 163 °C, 95% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.15, 2.17 and 2.27 (each s, each 3H, 3 × CH_3CO), 4.08 (m, 1H, $(CF_3)_2CHO$), 5.54 (dd, 1H, H_2, J = 7.4, 1.7), 5.55 (dd, 1H, H_3, J = 7.4, 5.1), 6.63 (d, 1H, H_1, J = 1.7), 6.76 (d, 1H, H_1, J = 5.1), 7.50−7.93 (m, 7H, aromatic protons), 8.42 (m, 1H, H_{12}). HRMS (FAB+) calcd for $C_{27}H_{22}O_7F_6$ (M^+): 572.1270. Found: 572.1255.

Evaporation of the combined fraction of t_R = 2.63 min gave **(±)-1β-hexafluoro-2-propyloxy-2α,3α,4β-trihydroxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (10**) as a colorless solid (2 mg, 1.5%). ^{1}H NMR (300 MHz, $CDCl_3$−CD_3OD) δ: 3.31 (m, 1H, $(CF_3)_2CHO$), 4.20 (dd, 1H, H_3, J = 8.4, 2.7), 4.64 (dd, 1H, H_2, J = 4.6, 2.7), 5.10 (d, 1H, H_4, J = 8.4), 6.82 (d, 1H, H_1, J = 4.6), 7.50−8.01 (m, 7H, aromatic protons), 8.51 (br d, 1H H_{12}, J = 8.1). HRMS (FAB+) calcd for $C_{21}H_{16}O_7F_6$ (M^+): 446.0953. Found: 446.0956. Acetylation of **10** by standard methods afforded **(±)-1β-hexafluoro-2-propyloxy-2α,3α,4β-triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene** (**triacetate of 10**) as colorless solid (95% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.10, 2.13 and 2.27 (each s, each 3H, 3 × CH_3CO), 4.42 (m, 1H, $(CF_3)_2CHO$), 5.74 (dd, 1H, H_3, J = 8.6, 2.9), 6.03 (dd, 1H, H_2, J = 4.7, 2.9), 6.63 (d, 1H, H_4, J = 8.6), 6.72 (d, 1H, H_1, J = 4.7), 7.55−8.10 (m, 7H, aromatic protons), 8.30 (br d, 1H, H_{12}, J = 8.2). HRMS (FAB+) calcd for $C_{27}H_{22}O_7F_6$ (M^+): 572.1270. Found: 572.1265.

Evaporation of the combined fraction at t_R = 4.4 min afforded a mixture of the two *trans*-N^2-dGuo diastereomers as a colorless solid (128 mg, 54%). Evaporation of the combined fraction at t_R = 5.1 min afforded a mixture of the two *cis*-N^2-dGuo diastereomers as a colorless solid (69 mg, 30%). The cis/trans adduct ratio obtained from HPLC (300 nm) was 36/64. These adduct mixtures were separately acetylated and separated by HPLC into the diastereomerically pure triacetates (1*S*)-**6a** and (1*R*)-**6b** and (1*S*)-**7a** and (1*R*)-**7b**, respectively, according to the conditions previously reported,[8b] and their structures were confirmed by comparison of their ^{1}H NMR, HRMS, and CD spectra with those of the authentic samples.[8b]

N^2-[1*R*-(2*R*,3*R*,4*S*-Triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrenyl)]-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (11a) and N^2-[1*S*-(2*S*,3*S*,4*R*-Triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrenyl)]-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (11b). Palladium(0) catalysts are effective reagents for the removal of *O*-allyl protecting groups.[33] To a solution of either **4a** (7 OD at 260 nm) or **4b** (8 OD at 260 nm) in CH_2Cl_2 (3 mL) was added a solution of $Pd(Ph_3)_4$ (10.2 mg) and morpholine (620 μL) in CH_2Cl_2 (1 mL), and the mixture was stirred at room temperature under dry Ar gas for 1 h. The reaction mixture was washed with brine (2 mL), saturated $NaHCO_3$ (2 mL), and water (2 mL). The extract was dried ($MgSO_4$) and evaporated to give a solid, which was purified by HPLC on an Axxiom silica gel column (9.5 × 250 mm, 5 μm) eluted with 10% *n*-hexane in EtOAc at a flow rate of 5 mL/min (detected at 260 nm) to give **11a** (4.2 OD at 260 nm; t_R = 9.1 min) or **11b** (5.0 OD at 260 nm; t_R = 10.7 min). CD spectrum (see Figure 4). HRMS (FAB+) calcd for $C_{46}H_{61}N_5O_{10}Si_2Cs$ (M^+ + Cs): 1032.3011. Found: 1032.3029 (**11a**); 1032.3007 (**11b**).

N^2-[1*S*-(2*R*,3*R*,4*S*-Triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrenyl)]-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12a) and N^2-[1*R*-(2*S*,3*S*,4*R*-Triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrenyl)]-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (12b). To a solution of either **5a** (3 OD at 260 nm) or **5b** (3 OD at 260 nm) in CH_2Cl_2 (3 mL) was added a solution of $Pd(Ph_3)_4$ (10.2 mg) and morpholine (620 μL) in CH_2Cl_2 (1 mL), and the mixture was stirred at room temperature under dry Ar gas for 1 h. The reaction mixture was washed with brine (2 mL), saturated $NaHCO_3$ (2 mL), and water (2 mL). The extract was dried ($MgSO_4$) and evaporated to give a solid, which was purified by HPLC on an Axxiom silica gel column (2.0 × 250 mm, 5 μm) eluted with 35% *n*-hexane in EtOAc at a flow rate of 0.95 mL/min (detected at 260 nm) to give **12a** (1.5 OD at 260 nm; t_R = 9.4 min) or **12b** (0.42 OD at 260 nm; t_R = 9.1 min). CD spectrum (see Figure 4). HRMS (FAB+) calcd for $C_{46}H_{61}N_5O_{10}Si_2Cs$ (M^+ + Cs): 1032.3011. Found: 1032.3005 (**12a**); 1032.3029 (**12b**).

Synthesis of O^6-Allyl-dGuo Phosphoramidite Building Blocks. (1) Desilylation of the Sugar Hydroxyl Groups. The diastereomerically pure O^6-allyl-protected *cis*-(1*R*)-**4a** or *cis*-(1*S*)-**4b** and *trans*-(1*S*)-**5a** or *trans*-(1*R*)-**5b**, derived from BcPh DE-1 (**1**), as well as *cis*-(1*S*)-**6a** or *cis*-(1*R*)-**6b** and *trans*-(1*S*)-**7a** and *trans*-(1*R*)-**7b**, derived from BcPh DE-2 (**2**) (each on a 120 mg scale), were separately dissolved in 7% HF-pyridine (14 mL) under cooling at 5 °C in a polyethylene vial. The reaction mixture was stirred for 12 h at room temperature

(33) (a) Kunz, H.; Waldmann, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 71−73. (b) Schmittberger, T.; Cottè, A.; Waldmann, H. *J. Chem. Soc., Chem. Commun.* **1998**, 937−938.

and diluted with EtOAc (300 mL), which was washed with saturated $NaHCO_3$ (50 mL) and water (3 × 50 mL). The extract was dried (Na_2SO_4) and evaporated in vacuo to give a colorless solid (>95% yield). HRMS (FAB+) calcd for $C_{37}H_{37}N_5O_{10}$ (M^+): 712.2619. Found: 712.2634 [(1*R*)-**13a**]; 712.2603 [(1*S*)-**13b**]; 712.2612 [(1*S*)-**14a**]; 712.2593 [(1*R*)-**14b**]; 712.2632 [(1*S*)-**15a**]; 712.2613 [(1*R*)-**15b**]; 712.2622 [(1*S*)-**16a**]; 712.2599 [(1*R*)-**16b**].

(2) Synthesis of 5′-*O*-Dimethoxytrityl Derivatives. (i) The desilylated triacetates **13a**,**b**, **14a**,**b**, **15a**,**b**, and **16a**,**b** were transformed into the corresponding 5′-*O*-DMT-triacetates by the treatment with a 2.6-fold excess of $DMT^+BF_4^-$, a 5-fold excess of lutidine, and a 4-fold excess of Li_2CO_3 in dry CH_2Cl_2 (2 mL). Reaction mixtures were stirred under Ar gas at room temperature for 2−4 h until no starting material was detected by TLC or HPLC. The reaction mixtures were purified by silica gel column chromatography using 0−40% *n*-hexane in EtOAc (with a trace of TEA) as the mobile phase to give colorless solids (>95% yield). HRMS (FAB+) calcd for $C_{58}H_{55}N_5O_{12}$-Cs (M^+ + Cs): 1146.2902. Found: 1146.2904 [(1*R*)-**18a**]; 1146.2931 [(1*S*)-**18b**]; 1146.2899 [(1*S*)-**19a**]; 1146.2898 [(1*R*)-**19b**]; 1146.2870 [(1*S*)-**20a**]; 1146.2921 [(1*R*)-**20b**]; 1146.2908 [(1*S*)-**21a**]; 1146.2902 [(1*R*)-**21b**].

(ii) *N*^{2}-[1*S*-(2*R*,3*R*,4*S*-Triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrenyl)]-*O*^{6}-allyl-3′-*O*-(2*R*-tetrahydrofuranyl)-5′-*O*-(dimethoxytrityl)-2′-deoxyguanosine (17a) and/or *N*^{2}-[1*S*-(2*R*,3*R*,4*S*-Triacetoxy-1,2,3,4-tetrahydrobenzo[*c*]-phenanthrenyl)]-*O*^{6}-allyl-3′-*O*-(2*S*-tetrahydrofuranyl)-5′-*O*-(dimethoxytrityl)-2′-deoxyguanosine (17b). Tritylation of **14a (**147 mg, 0.207 mmol) was carried out the same way as above except that THF (2 mL) was used instead of CH_2Cl_2. The reaction mixture was stirred for 20 h until ∼90% of the starting material was consumed as judged by HPLC. The crude product was subjected to chromatography on a silica gel column eluted with 50% *n*-hexane in EtOAc (with a trace of TEA) to give a glass (240 mg), which was separated by HPLC on an Axxiom silica gel column (9.5 × 250 mm) eluted with 50% *n*-hexane in EtOAc (with a trace of TEA) at a flow rate of 10 mL/min (detected at 300 nm). The combined fraction of t_R = 8.8 min afforded a glass (**17a** or **17b**; 88 mg, 39%). ^{1}H NMR (300 MHz, acetone-d_6) δ: 1.73−2.01 (m, 4H, 2 × C-3 and C-4 methine protons of the THF acetal), 1.71, 2.02 and 2.14 (each s, each 3H, 3 × $COCH_3$), 2.70 (m, 1H, $H_{2'}$), 3.00 (m, 1H, $H_{2'}$), 3.25−3.50 (m, 1H, $H_{5',5'}$), 3.85 and 3.84 (each s, each 3H, 2 × OCH3), 3.95 (m, 2H, C-5 methylene protons of the THF acetal), 4.02 (m, 1H, $H_{4'}$), 4.70 (m, 1H, $H_{3'}$), 5.10 (m, 2H, $CH_{2(allyl)}$), 5.24 (dd, 1H, H_3, J = 8.1, 2.5), 5.28−5.35 (m, 2H, H_2 and C-2 methine proton of the THF acetal), 5.54 (d, 1H, H_t, J = 17.5), 5.96 (m, 1H, H_2), 6.26 (m, 1H, H_v), 6.34 (dd, 1H, H_1, J = 3.3, 0.9), 6.46 (t, 1H, $H_{1'}$, J = 6.5), 6.90−7.70 (m, 15H, aromatic protons), 7.18 (d, 1H, H_4, J = 8.1), 7.84 (d, 1H, H_5, J = 8.3), 7.98 (app. s, 2H, H_7/H_8), 8.12 (br d, 1H, H_9, J = 8.0), 8.14 (s, 1H, $H_{8''}$), 8.20 (d, 1H, H_6, J = 8.3), 8.65 (br d, 1H. H_{12}, J = 8.4). HRMS (FAB+) calcd for $C_{62}H_{62}N_5O_{13}$ (M^+ + 1): 1084.4349. Found: 1084.4384.

The combined fraction at t_R = 10.5 min afforded a glass (**17a** or **17b**; 80 mg, 36%). ^{1}H NMR (300 MHz, acetone-d_6) δ: 1.73−2.02 (m, 4H, 2 × C-3 and C-4 methine protons of the THF acetal), 1.80, 2.02 and 2.10 (each s, each 3H, 3 × $COCH_3$), 2.67 (m, 1H, $H_{2'}$), 2.98 (m, 1H, $H_{2'}$), 3.40 (m, 2H, $H_{5',5'}$), 3.77 (m, 2H, C-5 methylene protons of the THF acetal), 3.85 and 3.87 (each s, each 3H, 2 × OCH_3), 4.26 (m, 1H, $H_{4'}$), 4.74 (m, 1H, $H_{3'}$), 5.15 (m, 2H, $CH_{2(allyl)}$), 5.22 (dd, 1H, H_3, J = 8.1, 2.5), 5.28−5.36 (m, 2H, H_2 and C-2 methine proton of THF acetal), 5.54 (d, 1H, H_t, J = 17.5), 5.98 (m, 1H, H_2), 6.26 (m, 1H, H_v), 6.30 (dd, 1H, H_1, J = 3.3, 0.9), 6.46 (t, 1H, $H_{1'}$, J = 6.5), 6.80−7.72 (m, 15H, aromatic protons), 6.98 (d, 1H, H_4, J = 8.1), 7.84 (d, 1H, H_5, J = 8.2), 7.98 (app. s, 2H, H_7/H_8), 8.12 (br d, 1H, H_9, J = 8.0), 8.14 (s, 1H, $H_{8''}$), 8.20 (d, 1H, H_6, J = 8.3), 8.65 (br d, 1H. H_{12}, J = 8.4). HRMS (FAB+) calcd for $C_{62}H_{62}N_5O_{13}$ (M^+ + 1): 1084.4349. Found: 1084.4353.

(iii) Reaction of $DMT^+BF_4^-$ in THF. A solution of $DMT^+BF_4^-$ (390 mg, 1.0 mmol) in THF (10 mL) was stirred at room temperature for 48 h. The bright red color of the reaction mixture gradually faded to give a yellowish solution. The reaction mixture was evaporated in vacuo, and the residue was loaded onto a silica gel column and eluted with 20% EtOAc in *n*-hexane (with trace of TEA) to give ***bis*-(4-methoxyphenyl)-phenylmethane** as a slightly yellowish oil (216 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.76 (s, 5H, 2 × CH_3O), 5.18 (s, 1H, C-1 methine proton), 6.80−7.30 (m, 9H, aromatic protons). HRMS (EI) calcd for $C_{21}H_{20}O_2$ (M^+): 304. 1463. Found: 304.1461.

(3) 3′-(2-Cyanoethyl-*N*,*N*-diisopropyl)phosphoramidites. Each of the 5′-*O*-DMT-derivatives **18a**,**b**, **19a**,**b**, **20a**,**b**, and **21a**,**b** was combined with a 10-fold excess of *N*,*N*-diisopropylammonium tetrazolide and dried for 6 h under high vacuum (0.3 mmHg). To this mixture was added a 10-fold excess of 2-cyanoethyl tetraisopropylphosphorodiamidite in dry CH_2Cl_2 (10 mL). The mixture was stirred under Ar gas at room temperature for 3−4 h until no starting material was detected by HPLC. The reaction mixture was evaporated in vacuo, and the residue was purified by silica gel column chromatography under Ar gas using 40% EtOAc in *n*-hexane (with 0.1% TEA) as a solvent. Fractions containing the desired products were pooled and evaporated to give a colorless glass to which *n*-hexane (∼2 mL) was added. The mixture was sonicated until the products formed colorless powders. The solvent was decanted, and the resulting powders were washed several times with *n*-hexane and dried in vacuo. Yields were 80−90%. HPLC on an Axxiom silica gel column (9.5 × 250 mm) eluted at 8 mL/min with the proportions of EtOAc in *n*-hexane designated in parentheses showed the following two peaks of equal areas (260 nm) corresponding to diastereomers at phosphorus: (1*R*)-**22a**, t_R = 7.7 and 8.6 min (40%); (1*S*)-**22b**, t_R = 12.4 and 20.4 min (40%); (1*S*)-**23a**, t_R = 5.4 and 7.4 min (50%); (1*R*)-**23b**, t_R = 5.5 and 6.4 min (50%); (1*S*)-**24a**, t_R = 7.2 and 10.7 min (45%); (1*R*)-**24b**, t_R = 9.6 and 25.8 min (45%). HPLC on a Vertex LiChrosorb Si-60 column (20 × 250 mm) eluted at 9 mL/min with a linear gradient of 30% −70% EtOAc in *n*-hexane over 60 min gave two peaks with equal areas: (1*S*)-**25a**, t_R = 33.8 and 43.3 min; (1*R*)-**25b**, t_R = 30.5 and 36.2 min. ^{31}P NMR (300 MHz, CD_3CN) δ: 148.810 (s) [(1*R*)-**22a**]; 149.212 (s) [(1*S*)-**22b**]; 148.885 (s) and 149.288 (s) [(1*S*)-**23a**]; 149.086 (s) [(1*R*)-**23b**]; 149.272 (s) [(1*S*)-**24a**]; 149.020 (s) [(1*R*)-**24b**]; 148.793 (s) and 149.185 (s) [(1*S*)-**25a**]; 148.974 (s) [(1*R*)-**25b**]. HRMS (FAB+) calcd for $C_{67}H_{72}N_7O_{13}Cs$ (M^+ + Cs): 1346.3980. Found: 1346.3962 [(1*R*)-**22a**]; 1346.3970 [(1*S*)-**22b**]; 1346.3991 [(1*S*)-**23a**]; 1346.3978 [(1*R*)-**23a**]; 1346.3942 [(1*S*)-**24a**]; 1346.3998 [(1*R*)-**24b**]; 1346.3993 [(1*S*)-**25a**]; 1346.3988 [(1*R*)-**25b**].

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