# Naphthalene, Phenanthrene, and Pyrene as DNA Base Analogues: Synthesis, Structure, and Fluorescence in DNA

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**Abstract:** We describe the synthesis, structures, and DNA incorporation of deoxyribonucleosides carrying polycyclic aromatic hydrocarbons as the DNA "base" analogue. The new polycyclic compounds are 1-naphthyl, 2-naphthyl, 9-phenanthrenyl, and 1-pyrenyl deoxynucleosides. The compounds are synthesized using a recently developed C-glycosidic bond formation method involving organocadmium derivatives of the aromatic compounds coupling with a 1α-chlorodeoxyribose precursor. The principal products of this coupling are the α-anomers of the deoxyribosides. An efficient method has also been developed for epimerization of the α-anomers to *β*-anomers by acid-catalyzed equilibration; this isomerization is successfully carried out on the four polycyclic nucleosides as well as two substituted phenyl nucleosides. The geometry of the anomeric substitution is derived from $^1$H NOE experiments and is also correlated with a single-crystal X-ray structure of one α-isomer. Three of the polycyclic C-nucleoside derivatives are incorporated into DNA oligonucleotides via their phosphoramidite derivatives; the pyrenyl and phenanthrenyl derivatives are shown to be fluorescent in a DNA sequence. The results (1) broaden the scope of our C-glycoside coupling reaction, (2) demonstrate that (using a new acid-catalyzed epimerization) both α- and *β*-anomers are easily synthesized, and (3) constitute a new class of deoxynucleoside derivatives. Such nucleoside analogues may be useful as biophysical probes for the study of noncovalent interactions such as aromatic *π*-stacking in DNA. In addition, the fluorescence of the phenanthrene and pyrene nucleosides may make them especially useful as structural probes.

## Introduction

A large number of nonnatural analogues of DNA nucleosides have been synthesized in recent years. Changing the structure of the base moiety attached to deoxyribose has been a useful strategy for probing structure and function in DNA. For example, a number of base analogues have been used to test the importance of specific hydrogen bonding interactions which may be important for function of the natural nucleic acid bases.[1−3] Using this strategy, workers have examined the importance of hydrogen bonding in stabilizing DNA and RNA structure,[1] in protein-DNA interactions,[2] and in the fidelity of enzymatic DNA and RNA synthesis.[3]

Modified DNA bases have also been synthesized with the purpose of serving as reporter groups in physical and biochemical studies of structure and function. Examples of reporter groups which have been attached to DNA bases include biotin[4] and digoxigenin[5] groups, spin-label groups,[6] and DNA-cleaving moieties.[7] Among the most prominent class of reporters used in DNA are fluorescent-tagged DNA bases which can serve as probes in biophysical and biochemical studies.[8] In contrast to placement of such reporter groups at the end of a DNA strand using nonnucleotide linkers, the attachment of a reporter to a DNA base allows for placement and probing even near the middle of a stretch of DNA. Such a strategy has found considerable practical use in fluorescence-based automated DNA sequencing.[9] An alternative approach to the conjugation of a fluorophore to a natural DNA base is the more direct modification of a DNA base itself to render it fluorescent. A number of modified DNA bases with useful fluorescence properties have been reported recently; among the most widely used nucleosides of this type are 2-aminopurine[10] and ethenoadenosine.[11]

We have undertaken a program to develop a new class of DNA base analogues which are designed to serve as biophysical probes.[12] The molecules we have chosen to synthesize and study are nucleosides having "base" moieties which are nonpolar, weakly hydrogen bonding aromatic groups. We have described the synthesis[13] and study[14] of substituted benzene-derived nucleosides as nonpolar isosteres of pyrimidine DNA bases; these compounds were intended as ideal steric mimics of the natural analogs but with little or no hydrogen bonding

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potential. Similarly, we have synthesized indole- and benzimidazole-derived nucleosides as purine isosteric mimics.[12]

We have recently begun to focus on base stacking interactions as an important noncovalent interaction which has received very little experimental attention in the context of DNA. Since surface area, electrostatics, polarizability, and hydrophobicity are all factors which may influence $\pi$-stacking interactions in aqueous solution,[15] we felt that a useful test of these effects might be probed with a simple series of aromatic compounds standing in for DNA bases in nucleic acid structures.

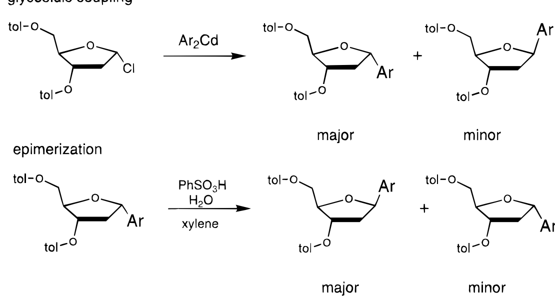
With those aims in mind we describe here the synthesis of a series of DNA nucleoside analogues which contain polycyclic aromatic hydrocarbons as the DNA "base" equivalents. New analogues containing pyrene, phenanthrene, and two naphthalene isomers are described. The aromatic rings are coupled to the sugar using a modification of our recently developed organocadmium strategy.[14] Also described is a new method for isomerization of such C-nucleosides from α- to naturally-configured $\beta$-epimers. The title compounds are synthesized in a concise manner and in relatively good yields, thus establishing the relatively broad scope of both the coupling and epimerization methods for C-nucleoside synthesis in general. We further demonstrate the incorporation into DNA of naphthalene, phenanthrene, and pyrene nucleosides using standard automated methods. In addition to the potential utility of the analogues in aromatic stacking studies, it is found that the pyrene and phenanthrene nucleosides in particular show fluorescence emission properties in DNA which may enhance their utility as probes of structure and dynamics.

## Results

Unsubstituted polycyclic aromatic hydrocarbons have not commonly been incorporated into deoxyribonucleosides as the directly coupled C-nucleosides, although substituted[16a] and unsubstituted[16b] naphthyl ribonucleosides have been reported.[16] A naphthyl deoxynucleoside was synthesized for use in polymerase chain reaction experiments, although its structure was not characterized in detail.[17] The smallest monocyclic member of the series, benzene, has been incorporated previously as a ribonucleoside[18] and also as a deoxyribonucleoside,[19] and the latter was briefly studied when incorporated into a DNA strand. We recently described a relatively high-yield strategy for coupling of aromatic benzene-derived compounds to the glycosidic position of deoxyribose.[13] Subsequent studies showed that the primary products derived from these reactions are α-configured isomers, with the $\beta$-isomers appearing as minor products.[13b] However, recent work has shown (see below) that it is possible to equilibrate these α-C-nucleosides to $\beta$-isomers in good yield, and so the coupling method can now be applied generally to the synthesis of both anomers of deoxynucleosides.

**Synthesis.** The previously described method of C-nucleoside coupling[13] was utilized to generate the new aromatic nucleosides **1**−**4** as their bis-toluoyl esters (Scheme 1). The method involves the reaction of organocadmium derivatives of the aromatic species with the well-known α-chlorosugar synthon of Hoffer[20] (Scheme 1). As seen in reactions with benzene

**Scheme 1**




derivatives,[13] this coupling with the larger polycyclic aromatics yields a mixture of α- and $\beta$-anomers in good overall yields (54−81% isolated yields). The major isomer in all four cases is formed with retention of configuration; thus, α-anomeric C-nucleosides (the *p*-toluoyl esters of **1a**, **2a**, **3a**, and **4a**) are the primary products. Measured ratios of the two isomers (by NMR integration) ranged from 5:1 (α:$\beta$) for the 1-naphthyl derivative to 10:1 for the 9-phenanthrenyl derivative. The configuration at the C-1′ carbons of all isomers was determined by analysis of H1′−H2′ coupling constants for the protected nucleosides, by $^1$H nuclear Overhauser experiments on the deprotected nucleosides, and by correlation with an X-ray crystal structure of one of the α-anomeric compounds (see below).

Although the desired $\beta$-anomers (the toluoyl esters of **1**−**4**) could also be isolated from this coupling reaction, the yields were less than ideal. Studies were thus undertaken to find conditions under which the predominant α-anomers could be converted to the desired $\beta$-configuration. It was anticipated that acidic conditions might allow epimerization at the C-1 position by reversible ring-opening. Experimentation with several sets of conditions revealed that benzenesulfonic acid in refluxing xylene, in the presence of a small amount of water, did indeed result in ready equilibration of the α-anomers to mixtures of $\beta$- and α-isomers after several hours. Addition of a small amount of water was found to be necessary for the isomerization. The equilibration was then carried out for all four α-isomers (**1a**, **2a**, **3a**, and **4a**) as their bis-toluoyl esters. We also tested the isomerization on two previously reported substituted benzene nucleosides (the toluoyl esters of **5a** and **6a**) to test the scope of the reaction. Significantly, the major component of each mixture after equilibration was in all cases the desired $\beta$-anomer (**1**−**6**). The ratios of $\beta$- to α-isomers ranged from 4:1 for the trimethylbenzene derivative to 2.5:1 for the 1-naphthyl derivative. Isolated yields of the desired $\beta$-anomers after column chromatography ranged from 28 to 54%. The α-anomers and mixed fractions could be reisolated and recycled in the isomerization if desired. Interestingly, the deprotected free nucleosides themselves did not undergo any observable isomerization under these conditions, even at extended reaction times.

With the new method for epimerization to $\beta$-anomeric configuration in hand, the synthetic scheme made possible the facile generation of the six aromatic C-nucleosides (**1**−**6**) in generally good yields (Scheme 2). The toluoyl protecting groups were removed in methanolic base with yields ranging from 50 to 78%. Following this overall scheme, the free unprotected nucleosides were produced in a total of only three steps (aromatic coupling, isomerization, ester deprotection).

**Structural Assignments.** The structural assignment of anomeric configuration for compounds **1**−**6** (and **1a**−**6a**) was made by $^1$H NOE studies of all compounds, by examination of coupling constants for H1′ and H2′ protons, and by correlation with an X-ray crystal structure of the α-1-naphthyl nucleoside **3a** (below). In addition, the compounds were characterized by

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**1** (**1a**: α-anomer) **2** (**2a**: α-anomer) **3** (**3a**: α-anomer)

**4** (**4a**: α-anomer) **5** (**5a**: α-anomer) **6** (**6a**: α-anomer)

their $^{1}H$ and $^{13}C$ NMR spectra and by high-resolution mass spectrometry.

Proton nuclear Overhauser effects were used to examine the geometries of the anomeric isomers of compounds **1**−**6**. The approach used was to separately irradiate the H-2′ proton resonances situated at $\delta$ 1.7−2.7 and observe enhancements at vicinal 1′ and 3′ protons (see Figure 1 and Tables 1 and 2). Although specific assignment of which resonance corresponds to 2′-α and which to 2′-$\beta$ could not be made *a priori*, analysis of predicted NOE effects makes possible a simple approach to assignment of stereochemistry at the 1′ position. Examination of the structures of α- and $\beta$-nucleosides (Figure 1) shows that for α-anomers the 2′-$\beta$ proton is in close proximity to both the 1′ and 3′ protons, while the C-2′-α proton is not near either one of these protons. In $\beta$-anomers, on the other hand, the 2′-$\beta$ proton is near only the 3′ proton, while the 2′-α proton is only near the 1′ proton. Thus, in an α-anomer, separate irradiation of each of the C-2′ protons should lead to two and zero NOE enhancements at the vicinal protons, while in a $\beta$-anomer these two irradiations would lead to one significant enhancement for each irradiation.

To test this prediction we carried out these experiments on naphthyl nucleoside isomers **3** and **3a** (Tables 1 and 2). The diester of **3a** is the principal product of the glycosidic coupling reaction (with the diester of **3** being a minor product). Irradiation of one of the 2′ protons of the nucleoside gave significant nuclear Overhauser enhancements of 8 and 7% at the 1′ and 3′ protons; however, irradiation of the other 2′ proton gave no significant enhancement at either 1′ or 3′ positions. Using the analysis above, this indicates that this compound is an α anomer. This assignment was confirmed by a single-crystal X-ray structure obtained for the compound (below). To complete the analysis of the two isomers, we carried out the same experiments on the isomeric nucleoside **3**, which is the major product after

α-anomer β-anomer

Irradiation at :

Hα: 0 enhancements; Hβ: 2 enhancements (α-anomer)

Hα: 1 enhancement; Hβ: 1 enhancement (β-anomer)

**Figure 1.** Illustration of qualitative differences in nuclear Overhauser enhancements observed for α- (left) and $\beta$-anomers (right) of compounds **1**−**6**. In α−anomers, irradiation of the C-2′-$\beta$ proton gives enhancements in both the C-1′ and C-3′ protons, while irradiation of the C-2′-α proton gives little or no enhancement for either. In the $\beta$-anomers, irradiation of the C-2′-$\beta$ proton gives enhancement only in the C-3′ proton, while irradiation of the C-2′-α proton gives enhancement only in the C-1′ proton.

**Table 1.** H1′−H2′ Coupling Constants and Proton NOE Data for $\beta$-Isomers of Aryl Nucleosides **1**−**6** in $CD_3OD$

| Aryl substituent | J values,[a] H1' - H2' | NOE observed: | Irradiation at: H1' | H2'α | H2'β |
|---|---|---|---|---|---|
| | 7.2, 10.8 Hz | H1' | -- % | 8 % | 0 % |
| | | H2'α | 7 | -- | -- |
| | | H3' | 0 | 0 | 0 |
| | | H4' | 6 | -- | -- |
| | 7.0, 10.5 Hz | H1' | -- % | 8 % | 0 % |
| | | H2'α | 8 | -- | -- |
| | | H3' | 0 | 0 | 12 |
| | | H4' | 6 | -- | -- |
| | 6.4, 10.7 Hz | H1' | -- % | 8 % | 0 % |
| | | H2'α | 7 | -- | -- |
| | | H3' | 0 | 0 | 5 |
| | | H4' | 6 | -- | -- |
| | 5.2, 10.9 Hz | H1' | -- % | 10 % | 0 % |
| | | H2'α | 7 | -- | -- |
| | | H3' | 0 | 0 | 12 |
| | | H4' | 6 | -- | -- |
| | 5.0, 10.8 Hz | H1' | -- % | 8 % | -- |
| | | H2'α | 9 | -- | -- |
| | | H3' | 0 | 0 | -- |
| | | H4' | 6 | -- | -- |
| | 4.6, 10.2 Hz | H1' | -- % | -- | -- |
| | | H2'α | 9 | -- | -- |
| | | H3' | 0 | -- | -- |
| | | H4' | 6 | -- | -- |

[a]Coupling constants are for bis-toluoyl ester derivatives of **1-6** in $CDCl_3$.

**Scheme 2**

a → **1 - 6** b,c → DMT—O ... P—O-$CH_2CH_2CN$

**1**: Ar = 2,4-difluorotoluyl
**2**: Ar = 2,4,5-trimethylphenyl
**3**: Ar = 1-naphthyl
**4**: Ar = 2-naphthyl
**5**: Ar = 2-phenanthryl
**6**: Ar = 1-pyrenyl

(a) NaOMe / methanol, 23°C; (b) 4,4'-dimethoxytrityl chloride, DMAP, pyridine, $CH_2Cl_2$, 23°C; (c) N,N'-diisopropyl-2-O-cyanoethyl phosphonamidic chloride, DIPEA, $CH_2Cl_2$, 23°C.

**Table 2.** H1′−H2′ Coupling Constants and NOE Data for α-Isomers of Aryl Nucleosides (compounds **3a**, **5a**, and **6a**) in $CD_3OD$

| Aryl substituent | J values,[a] H1' - H2' | NOE observed: | NOE data: irradiation at: H1' | H2'α | H2'β | H3' |
|---|---|---|---|---|---|---|
| (alpha isomer) | 8.0, 6.0 Hz | H1' | -- % | 0 % | 8 % | 0 % |
| | | H2'α | 0 | -- | -- | 0 |
| | | H2'β | 8 | -- | -- | 6 |
| | | H3' | 3 | 0 | 7 | -- |
| F, F, $CH_3$ (alpha isomer) | 7.6, 7.6 Hz | H1' | -- % | 0 % | 9 % | 0 % |
| | | H2'α | 0 | -- | -- | 0 |
| | | H2'β | 8 | -- | -- | 8 |
| | | H3' | -- | 0 | 8 | -- |
| $H_3C$, $CH_3$, $CH_3$ (alpha isomer) | 6.6, 6.6 Hz | H1' | -- % | 2 % | 12 % | -- % |
| | | H2'α | 0 | -- | -- | -- |
| | | H2'β | 6 | -- | -- | -- |
| | | H3' | 2 | 4 | 12 | -- |

[a] Coupling constants are for toluoyl ester derivatives in $CDCl_3$.

epimerization of **3a**. Irradiation of one of the 2′ protons gave an 8% enhancement of the 1′ proton (and none at the 3′ proton), while irradiation of the other 2′ proton gave a 5% enhancement of the 3′ proton (and none for the 1′ proton). Thus, this compound is assigned to the $\beta$ configuration. Also consistent with this assignment is a separate experiment in which the 1′ proton was irradiated; here we observed a 6% enhancement at the 4′ proton (Table 1), while the α-isomer shows no such enhancement (data not shown).

The NOE experiments were then carried out for the isomers of **1**, **2**, and **4**−**6**. The results are shown in Tables 1 and 2. The results were all consistent with the model, in that one isomer of each pair gave two and ∼zero enhancements of the vicinal protons for the two H-2′ irradiations while the other clearly gave one significant enhancement for each of the two irradiations. The isomers which gave two and zero enhancements were assigned as α-anomers, and those with one and one enhancements were assigned to be $\beta$-anomers. Also consistent with these assignments were NOE enhancements in the H-4′ positions on irradiation of the H-1′ protons for the $\beta$-isomers (Table 1) which were absent in the α-isomers.

These assignments were also internally consistent in that the major isomers obtained from the glycosidic coupling reaction were all of the same anomeric configuration (α). Similarly, the major isomers isolated from the epimerization were all of the same anomeric configuration ($\beta$). In addition, all the isomers assigned as α had H-1′ resonances which qualitatively appeared as pseudotriplets (they are actually doublets of doublets), having both coupling constants near 6.0−8.0 Hz. The isomers assigned to the $\beta$ configuration had H-1′ resonances which appeared as nearly evenly spaced doublets of doublets ($J \approx 5$ and 10 Hz). These H-1′−H-2′ coupling constant trends are consistent with a literature report of similar coupling constants for a related $\beta$-C-nucleoside[19] (although they are reversed relative to observations for $\beta$-N-nucleosides; see Discussion).

Also useful in confirmation of these structural assignments were X-ray structural data obtained for 1-naphthyl compound **3a** (Figure 2). A crystal suitable for analysis was obtained by recrystallization from methylene chloride/hexane. The structure shows the α configuration and in analogy to natural nucleosides the naphthalene is in an anti conformation, with the aromatic group oriented away from the sugar. The deoxyribose ring is in a C-3′-exo (S-type) conformation.

Experimental H-1′ to H-2′ coupling constants for the ester of this compound (**3a**) in $CDCl_3$ were $J = 8.0$ and 6.0 Hz. The corresponding dihedral angles generated from the X-ray structure are found to be 8.1° and 124.5°. Application of the Karplus

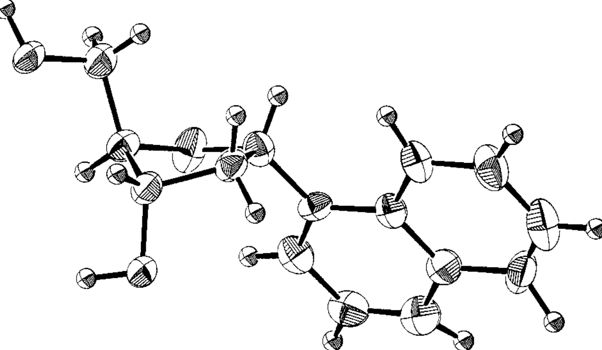

**Figure 2.** ORTEP drawing of 1-naphthyl nucleoside nucleoside **3a** from single-crystal X-ray structure. The structure has the α-anomeric configuration and a C-3′-*exo* S-type sugar conformation.

relationship empirically adjusted for nucleosides[21] predicts $J =$ 9.2 and 2.8 Hz, respectively, indicating a small change in ring conformation in solution relative to that in the crystal, or else a nonideal match of the empirical relationship for this C-nucleoside. Interestingly, although this compound clearly is an α-anomer, the experimentally measured coupling constants are more consistent with those commonly seen for $\beta$, rather than α, anomers of natural nucleosides (see below).

**Incorporation into DNA.** The $\beta$-C-deoxynucleosides **1**, **2**, and **3** were then carried on with the aim of incorporating them into DNA oligonucleotides by automated solid-phase methods (Scheme 2). The incorporation into DNA of substituted benzene nucleosides **5** and **6** has recently been reported elsewhere.[22] Standard methods were used to convert the unprotected nucleosides to 5′-dimethoxytrityl-protected derivatives in yields ranging from 59 to 92% after purification. These were then converted into cyanoethyl phosphoramidite derivatives, which were obtained in 50−89% yields after purification by column chromatography. Incorporation into oligodeoxynucleotides was carried out with standard coupling chemistry but with lengthened coupling times, and stepwise yields for coupling of these compounds were >95% by trityl monitoring. To test for intact incorporation, we synthesized trinucleotides having the sequence T-X-T and examined them by proton NMR (Figure 3); the spectra of the crude unpurified oligonucleotides show clear resonances very similar to those of the free nucleosides and having the expected aromatic integrations relative to anomeric C-1′ protons and thymine C-6 protons and C-5 methyl groups. This confirms both the presence of the intact structures (as expected for unreactive aromatic hydrocarbons) and the high coupling yields, since di- and mononucleotides which would result from incomplete coupling are not seen.

**Fluorescence Properties in DNA.** Since polycyclic aromatics such those in the nucleosides **1**, **2**, and **3** have been studied in other contexts as fluorescent probes,[23] we undertook the examination of possible fluorescence properties of oligonucleotides containing these structures in aqueous buffer. We synthesized heptamer oligodeoxynucleotides having the sequence 5′-dXCGCGCG, (where X = **1**, **2**, and **3**) which are self-complementary and form duplexes with the polycyclic aromatic nucleoside situated at the 5′ ends. These were purified by preparative denaturing gel electrophoresis.

Emission spectra were measured for the three sequences in a pH 7.0 buffer (10 mM PIPES buffer, 100 mM NaCl, 10 mM $MgCl_2$) at 25 °C, conditions under which they likely form

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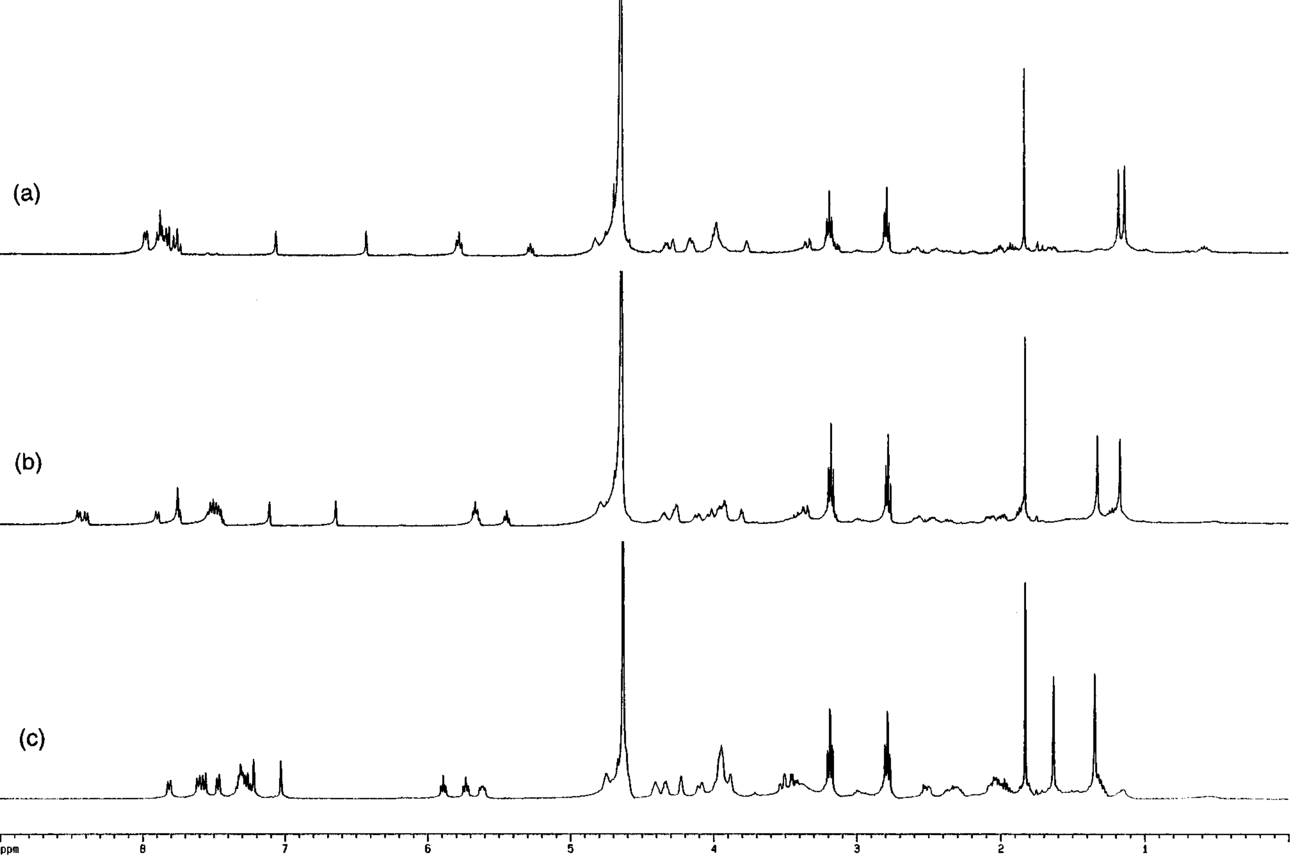


**Figure 3.** 400 MHz proton NMR spectra for trinucleotides (sequence T-X-T) containing (a) pyrenyl, (b) phenanthryl, and (c) naphthyl nucleosides **1**−**3** at the X position.

duplexes (data not shown). The naphthalene-containing sequence showed no emission detectable above background. The other compounds showed structured fluorescence emission profiles consistent with published spectra for the polycyclic aromatic parent structures.[23] The phenanthrene-containing oligonucleotide had the most intense emission (not shown), with the strongest peak at 370 nm. The pyrene-modified sequence showed a similar emission profile but with an emission maximum at 395 nm and with peak intensity considerably lower (by ca. 700-fold in peak height) than that for the phenanthrene case, suggesting considerable quenching by the DNA under these conditions and in this sequence.

## Discussion

**Utility of the C-Nucleoside Synthesis and Epimerization Methods.** The transition metal-mediated coupling reaction used here to generate aromatic C-nucleosides has been previously found to be useful in the generation of α-deoxynucleosides (and small amounts of β-deoxynucleosides) of substituted benzene derivatives.[13] The present results establish that the reactions can easily be extended to larger aromatic hydrocarbons without a penalty in yield. Since the only apparent requirement appears to be the ability for a given aromatic halide to be converted to the corresponding Grignard species, it seems likely that the method may well be generally useful in the synthesis of many different C-deoxynucleosides.

With the addition of the present method for epimerization at the anomeric center, the scheme for nucleoside assembly becomes more generally useful. After this equilibration all nonpolar aromatic nucleosides studied to date (benzenes, naphthalenes, phenanthrene, and pyrene) give predominantly the β-anomer as the more stable isomer. Thus, if α-anomers of C-nucleosides are desired, the coupling reaction will generate them directly in good yield; however, if β-anomers are desired, the epimerization will convert these initial adducts into the β-isomers, also in generally good yield.

It is interesting that the cadmium- or zinc-mediated reactions give primarily α-anomers. This corresponds to retention of configuration since the starting sugar synthon contains only the α-chloro isomer with no β-isomer observable by proton NMR. One possible explanation for this result is that the chloride is lost in a dissociative process and that the subsequent bond formation occurs with selective α-face attack by the organometallic species. Further studies will be required to better elucidate this mechanism.

**The Anomeric Effect and Deoxyribose Conformation.** Proton NMR studies of the natural N-nucleosides found in DNA have shown that the 2′-deoxynucleosides in normal anomeric β-orientation generally have very similar coupling constants between the H-1′ proton and the vicinal H-2′ protons.[21,24] The two coupling constants are of the same magnitude; for example, those of β-thymidine are 7.0 and 7.0 Hz,[24] and thus the H-1′ resonance usually appears as a pseudotriplet. This has also held true for other N-nucleosides containing base moieties not found in DNA.[12] By comparison, the α-anomers of the natural DNA N-nucleosides have considerably different coupling constants for this same H1′ resonance; for α-thymidine these are found to be 8.0 and 3.0 Hz, and the resonance appears as a doublet of doublets.[24] Virtually the same coupling constants are found for β- and α-deoxyadenosine as well.[25] This difference between the two isomers arises from the different dihedral angles which are present in the two anomeric isomers.

A few reports on C-nucleosides have described quite different H1′−H2′ coupling behavior for the two anomers.[26] These compounds often appear to have coupling constants which show

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different trends for α- and *β*-isomers relative to N-nucleosides. Such differences may arise in part from the presence or absence of the electronegative nitrogen at the C-1 position. However, the comparisons between C- and N-nucleosides have generally been made between pairs of nucleosides which differ in more than just the presence or absence of polar nitrogens in the rings. It is possible that steric differences between in a series of compounds might affect ring geometry as well.

In that light it is useful to compare these H1′−H2′ coupling constants (and thus ring conformations) for two compounds—one an N-nucleoside and one a C-nucleoside—in which steric differences are very small. This helps to eliminate steric effects in the analysis of anomeric effects. Comparison of *β*- and α-thymidine and *β*- and α-difluorotoluene nucleosides has shown that they are sterically virtually identical in the base moieties.[12,27] Comparison of the H1′−H2′ coupling constants shows considerable differences, however. The *β*-thymidine values (7.0 and 7.0 Hz)[24] are similar to those for the α-anomer of the difluorotoluene nucleoside (7.6 and 7.6 Hz), and both resonances appear as pseudotriplets. Conversely, the α-anomer of thymidine has coupling constants (8.0 and 3.0 Hz) which are similar to those for the *β*-anomer of the difluorotoluene nucleoside (10.4 and 4.6 Hz), and these appear as doublets of doublets. Thus, our comparison underscores this inverse relationship and also points out the strong influence of the polar nitrogen at C-1′ in natural nucleosides on influencing deoxyribose ring geometry.

Indeed, every C-nucleoside we have synthesized to date (including more than ten different base analogues derived from aromatic hydrocarbons) has shown the same inverse relationship for coupling constants relative to N-nucleosides. The $^1$H NOE experiments and the X-ray crystal data described herein have since clarified and solidified the inverse relationship.

**Potential Utility of Polycyclic Aromatic Nucleosides as Probes in DNA.** While a good deal of experimental data have been examined on the relative effects of base stacking and hydrogen bonding on the stability of RNA helices,[28] much less data have been generated on the topic of base stacking in DNA. "Dangling end" effects have been studied as measures of stacking of all four bases in RNA helices,[28] but in DNA only thymine has been briefly examined using this approach.[29] Since it is likely that electrostatics, van der Waals effects, and hydrophobicity may all play a role in base stacking in DNA, we synthesized the polycyclic aromatic compounds described here as possible probes of some of those effects. A detailed study of base stacking in DNA using both natural and nonnatural nucleosides will be reported in due course.

Both the pyrene and phenanthrene nucleosides are also of interest for their fluorescence properties. Pyrene in particular has been incorporated into DNA in a number of studies as a potential reporter group.[30] In most previous cases the pyrene has been incorporated at the end of strands by a simple flexible linker chain. This flexibility allows the pyrene chromophore to adopt a number of different conformations. The present approach allows pyrene or phenanthrene to be inserted within a DNA strand at any position and potentially to remain more rigidly stacked within the helix. This closer contact with the DNA may give more sensitive information about structure and dynamics of nucleic acid helices. Experiments directed to this possibility are now underway.

## Conclusions

We have developed a convenient synthesis of nucleoside analogues containing aromatic hydrocarbons as the "base" moiety. The synthetic approach involves coupling of organocadmium derivatives of aromatic hydrocarbons to a widely-used α-chlorosugar synthon. This coupling works efficiently and generates predominantly α-anomers for compounds **1**−**6**. *β*-Anomers can be obtained as the major product by a new acid-catalyzed isomerization of the α-compounds. The overall scheme thus allows generation of either α- or *β*-anomer of C-nucleosides, as desired, in good yields. The polycyclic compounds are quite stable and can be incorporated into DNA oligonucleotides using standard procedures. Such compounds may be useful as probes in DNA, and the pyrene and phenanthrene nucleosides in particular have fluorescence emission properties which make them potentially useful as reporter groups in nucleic acids.

## Experimental Section

$^1$H, $^{13}$C, and $^{31}$P NMR spectra were recorded with a 300 MHz spectrometer unless otherwise noted, chemical shifts are given in $\delta$ (ppm) using solvent as internal reference, and the coupling constants are in hertz (Hz). NOE difference spectra were also performed on a 300 MHz instrument. The mass spectra were performed using electron impact or chemical ionization. All reactions were monitored by thin-layer chromatography (TLC) using EM Reagents plates with fluorescence indicator ($SiO_2$-60, F-254). Flash column chromatography was conducted using EM Science silica gel 60 (230−400 mesh). Mass spectral analyses were performed by the University of California, Riverside Mass Spectrometry Facility, Riverside, CA. All reactions were carried out under a nitrogen atmosphere in dry, freshly distilled solvents under anhydrous conditions unless otherwise specified. THF was distilled from sodium metal/benzophenone, methylene chloride was distilled from NaH, and pyridine was distilled from BaO prior to use.

**Procedure for Glycosidic Coupling Reaction and Isolation of Major α-Epimers as Bis-*p*-toluoyl esters of 1-6a.** Dry THF (5 mL) was placed in a round-bottomed flask equipped with a condenser, drying tube, and addition funnel. Magnesium turnings (0.3 g, 1.2 mmol) and a few crystals of iodine were added. 1-Bromopyrene (0.35 g, 1.2 mmol) was added to the mixture. Slight heating was needed (40 °C) to drive the reaction to completion. After formation of the Grignard reagent was complete (∼1 h), dry $CdCl_2$ (110 mg, 0.6 mmol) was added and the reaction mixture was continuously heated under reflux for 1 h. 1′α-Chloro-3′,5′-di-*O*-toluoyl-2′-deoxyribose[20] (0.51 g, 1.3 mmol) was then added to the above mixture in one portion. The solution was stirred at room temperature for 4 h under an atmosphere of $N_2$. The solution was poured into 10% ammonium chloride (2 × 50 mL) and extracted with methylene chloride. The organic layers were washed with saturated sodium bicarbonate and brine and dried over anhydrous magnesium sulfate. The solution was filtered, concentrated, and purified by flash silica gel chromatography, eluting with hexanes−ethyl acetate (9:1). The major product **1a bis-toluoyl ester** was obtained as a pale yellow oil (α-epimer, 48% isolated yield): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 8.80 (2H, d, $J$ = 8.0), 8.72 (2H, d, $J$ = 8.0), 8.05 (1H, s), 7.92−8.00 (2H, m), 7.72−7.60 (4H, m), 7.58 (2H, d, $J$ = 8.0), 7.32 (2H, d, $J$ = 8.0), 6.96 (2H, d, $J$ = 8.0), 6.15 (1H, dd, $J$ = 8.2, 6.0), 5.76 (1H, m), 4.98 (1H, m), 4.75−4.65 (2H, m), 3.30−3.22 (1H, m),

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3.50−3.45 (1H, m), 3.44 (3H, s), 3.38 (3H, s); HRMS (FAB, 3-NBA matrix) calcd for $C_{37}H_{31}O_5$ (M + 1) 554.2093, found 554.2069.

**2a bis-toluoyl ester** (α-epimer, 43% isolated yield)**:** $^1$H NMR ($CDCl_3$, ppm) δ 8.80 (2H, d, *J* = 8.0), 8.72 (2H, d, *J* = 8.0), 8.05 (1H, s), 7.92−8.00 (2H, m), 7.72−7.60 (4H, m), 7.58 (2H, d, *J* = 8.0), 7.32 (2H, d, *J* = 8.0), 6.96 (2H, d, *J* = 8.0), 6.15 (1H, dd, *J* = 8.2, 6.0), 5.76 (1H, m), 4.98 (1H, m), 4.75−4.65 (2H, m), 3.30−3.22 (1H, m), 3.50−3.45 (1H, m), 3.44 (3H, s), 3.38 (3H, s); HRMS (FAB, 3-NBA matrix) calcd for $C_{35}H_{31}O_5$ (M + 1) 531.2172, found 531.2174.

**3a bis-toluoyl ester** (α-epimer, 52% isolated yield)**:** $^1$H NMR ($CDCl_3$, ppm) δ 8.05 (2H, d, *J* = 8.0), 7.95 (2H, m), 7.83 (2H, overlapped d), 7.71 (2H, d, *J* = 8.0), 7.55 (3H, m), 7.32 (2H, d, *J* = 8.0), 7.19 (2H, d, *J* = 8.0), 6.10 (1H, dd, *J* = 8.0, 6.0), 5.69 (1H, m), 4.90 (1H, m), 4.76−4.65 (2H, m), 3.28−3.18 (1H, m), 2.52−2.45 (1H, m), 2.48 (3H, s), 2.42 (3H, s); HRMS ((FAB, 3-NBA matrix) calcd for $C_{31}H_{29}O_5$ (M + 1) 481.2015, found 481.2025.

**4a bis-toluoyl ester** (α-epimer, 31% isolated yield)**:** $^1$H NMR ($CDCl_3$, ppm) δ 8.02 (2H, d, *J* = 8.0), 7.92−7.97 (4H, m), 7.83 (2H, d, *J* = 8.0), 7.52−7.60 (3H, m), 7.32 (2H, d, *J* = 8.0), 7.05 (2H, d, *J* = 8.0), 5.72 (1H, m), 5.62 (1H, dd, *J* = 8.2, 6.0), 4.85 (1H, m), 4.76−4.65 (2H, m), 3.12−3.02 (1H, m), 2.52−2.45 (1H, m), 2.42 (3H, s), 2.38 (3H, s); HRMS (FAB, 3-NBA matrix) calcd for $C_{31}H_{29}O_5$ (M + 1) 481.2043, found 481.2015.

**5a bis-toluoyl ester** (α-epimer, 13% isolated yield)**:** $^1$H NMR ($CDCl_3$, ppm) δ 8.02 (2H, d, *J* = 8.0), 7.86 (1H, d, *J* = 8.0), 7.45 (1H, s), 7.23−7.28 (5H, m), 6.95 (1H, s), 5.69 (1H, br s), 5.54 (1H, dd, *J* = 8.0, 6.0), 4.81 (1H, br s), 4.69−4.56 (2H, m), 3.07−2.98 (1H, m), 2.43 (6H, s), 1.35 (3H, s); HRMS (FAB, 3-NBA matrix) calcd for $C_{30}H_{33}O_5$ (M + 1) 472.2250, found 472.2234.

**6a bis-toluoyl ester** (α-epimer, 16% isolated yield)**:** $^1$H NMR ($CDCl_3$, ppm) δ 8.0 (2H, d, *J* = 8.0), 7.72 (2H, d, *J* = 8.0), 7.43 (1H, t, *J* = 8.5), 7.27 (2H, d, *J* = 8.0), 7.19 (2H, d, *J* = 8.0), 6.76 (1H, d, *J* = 8.0), 5.61 (1H, br s), 5.57 (1H, dd, *J* = 8.0, 6.0), 4.74 (1H, br s), 4.57 (2H, t, *J* = 5.0), 3.02−2.93 (1H, m), 2.43 (3H, S), 2.23 (3H, s); HRMS (FAB, 3-NBA matrix) calcd for $C_{28}H_{26}F_2O_5Na$ 503.1646, found 503.1636.

**Procedure For Epimerization of 1′,2′-Dideoxy-1′α-aryl-3′,5′-di-*O*-toluoyl-D-ribofuranoses and Isolation of *β*-Epimers.** To a solution of **6a bis-toluoyl ester** (780 mg, 1.62 mmol) in toluene (50 mL) were added a catalytic amount of benzenesulfonic acid (∼10%), 1 drop of concentrated $H_2SO_4$, and 2−4 drops of $H_2O$. The reaction mixture was refluxed under vigorous stirring for 4−6 h. The mixture was then poured into 5% aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and evaporated. Flash column chromatography (eluent solution 8:1 to 2:1 hexanes: EtOAc) of the crude mixture gave 430 mg of **6 bis-toluoyl ester** (*β*-epimer, 46% isolated yield): $^1$H NMR ($CDCl_3$, ppm) δ 8.0 (4H, 2 × d, *J* = 8.0), 7.35−7.25 (5H, m), 6.76 (1H, t, *J* = 10.0), 5.64 (1H, d, *J* = 5.2), 5.46 (1H, dd, *J* = 10.2, 4.6), 4.78 (1H, dd, *J* = 3.8, 11.8), 4.63 (1H, dd, *J* = 3.8, 11.8), 4.54 (1H, m), 2.64 (1H, dd, *J* = 5.2, 11.8), 2.43 (3H, s), 2.46 (3H, s), 2.23 (1H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{28}H_{26}F_2O_5$ 481.1827, found 481.1853.

**1 bis-toluoyl ester** (*β*-epimer, 38% isolated yield): $^1$H NMR ($CDCl_3$, ppm) δ 8.36 (1H, d, *J* = 7.9), 8.31 (1H, d, *J* = 7.9), 8.20−8.17 (3H, m), 8.13−8.05 (6H, m), 8.02 (2H, d, *J* = 8.0), 7.37 (2H, d, *J* = 8.0), 7.26 (2H, d, *J* = 8.0), 6.34 (1H, dd, *J* = 7.2, 10.8), 5.78 (1H, d, *J* = 5.4), 4.84−4.88 (2H, m), 4.78−4.76 (1H, m), 2.94 (1H, dd, *J* = 5.0, 13.9), 2.50 (3H, s), 2.46 (1H, m), 2.40 (3H, s); HRMS (FAB, 3-NBA matrix) calcd for $C_{37}H_{31}O_5$ (M + 1) 554.2093, found 554.2069.

**2 bis-toluoyl ester** (*β*-epimer, 28% isolated yield): $^1$H NMR ($CDCl_3$, ppm) δ 8.78 (1H, d, *J* = 7.9), 8.70 (1H, d, *J* = 7.9), 8.13−8.09 (4H, m), 8.03 (2H, d, *J* = 8.0), 7.84 (1H, d, *J* = 7.9), 7.77−7.60 (4H, m), 7.36 (2H, d, *J* = 8.0), 7.18 (2H, d, *J* = 8.0), 6.60 (1H, dd, *J* = 7.0, 10.5), 4.90 (1H, dd, *J* = 3.8, 11.8), 4,84 (1H, dd, *J* = 3.8, 11.8), 2.94 (1H, dd, *J* = 5.1, 13.7), 2.49 (3H, s), 2.44−2.41 (1H, m), 2.38 (3H, s); HRMS (FAB, 3-NBA matrix) calcd for $C_{35}H_{31}O_5$ (M + 1) 531.2172, found 531.2174.

**3 bis-toluoyl ester** (*β*-epimer, 37% isolated yield): $^1$H NMR ($CDCl_3$, ppm) δ 8.09−8.04 (3H, m), 7.97 (1H, d, *J* = 8.0), 7.91 (1H, overlapped d, *J* = 6.3, 6.2), 7.88 (1H, d, *J* = 8.0), 7.51 (2H, overlapped d, *J* = 6.7, 6.5), 7.46 (1H, d, *J* = 7.9), 7.34 (2H, d, *J* = 8.0), 7.22 (2H, d, *J* = 8.0), 6.02 (1H, dd, *J* = 6.4, 10.7), 5.71(1H, d, *J* = 5.9), 4.78−4.78 (2H, m), 4.70−4.71 (1H, m), 2.85 (1H, dd, *J* = 5.0, 13.8), 2.48 (3H, s), 2.47 (3H, s), 2.39−2.37 (1H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{31}H_{29}O_5$ (M + 1) 481.2015, found 481.2025.

**4 bis-toluoyl ester** (*β*-epimer, 41% isolated yield): $^1$H NMR ($CDCl_3$, ppm) δ 8.06 (2H, d, *J* = 8.0), 8.02 (2H, d, *J* = 8.0), 7.91−7.77 (4H, m), 7.57−7.48 (3H, m), 7.32 (2H, d, *J* = 8.0), 7.23 (2H, d, *J* = 8.0), 5.70 (1H, d, *J* = 5.7), 5.46 (1H, dd, *J* = 3.2, 10.9), 4.77−4.75 (2H, m), 4.66−4.65 (1H, m), 2.66 (1H, dd, *J* = 5.2, 13.8), 2.48 (3H, s), 2.42 (3H, s); HRMS (FAB, 3-NBA matrix) calcd for $C_{31}H_{29}O_5$ (M + 1) 481.2043, found 481.2015.

**5 bis-toluoyl ester** (*β*-epimer, 54% isolated yield): $^1$H NMR ($CDCl_3$, ppm) δ 8.02 (4H, 2 × d, *J* = 8.0), 7.35−7.23 (5H, m), 6.92 (1H, s), 5.62 (1H, d, *J* = 5.6), 5.42 (1H, dd, *J* = 7.0, 10.8), 4.78 (1H, dd, *J* = 3.8, 11.8), 4.70 (1H, dd, *J* = 3.8, 11.8), 4.55 (1H, m), 2.56 (1H, dd, *J* = 5.0, 14.0), 2.43 (3H, s), 2.46 (3H, s), 2.23 (1H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{30}H_{32}O_5$ 472.2250, found 472.2234.

**General Procedure for Deprotection of 1′,2′-dideoxy-1′-aryl-3′,5′-di-*O*-toluoyl-*β*-D-ribofuranoses.** To a solution of **1 bis-toluoyl ester** (360 mg, 0.65 mmol) in methanol (5 mL) was added NaOMe (in methanol, 25%, 0.5 mL, 3 equiv). The reaction mixture was stirred for 4−6 h. Solid ammonium chloride was added until the pH was 8. The mixture was then poured into water and extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and evaporated. Flash column chromatography (eluent EtOAc) of the crude mixture gave 165 mg of nucleoside **1** (*β*-epimer, 78%): $^1$H NMR ($CDCl_3$, ppm) δ 8.35 (1H, d, *J* = 8.0), 8.31−8.14 (4H, m), 8.08−8.02 (3H, m), 6.25 (1H, dd, *J* = 5.6, 10.4), 4.62 (1H, m), 4.28 (1H, m), 4.02−3.98 (2H, m), 2.64 (1H, ddd, *J* = 2.0, 2.6, 13.4), 2.02 (2H, broad s, 2 × OH); HRMS (FAB, 3-NBA matrix) calcd for $C_{23}H_{20}O_3$ 318.1256, found 318.1251.

**Nucleoside 2** (*β*-epimer, 74%): $^1$H NMR ($CDCl_3$, ppm) δ 8.78 (1H, d, *J* = 8.0), 8.68 (1H, d, *J* = 8.0), 8.12 (1H, d, *J* = 8.0), 7.90 (2H, m), 7.77−7.62 (4H, m), 5.95 (1H, dd, *J* = 5.6, 10.4), 4.59 (1H, m), 4.22 (1H, m), 4.0 (1H, dd, *J* = 6.4, 13.2), 3.95 (1H, dd, *J* = 6.2, 13.4), 2.62 (1H, ddd, *J* = 2.0, 5.2, 13.4), 2.25 (1H, m), 1.6 (2H, broad s, 2 × OH); HRMS (FAB, 3-NBA matrix) calcd for $C_{19}H_{18}O_3$ 294.1256, found 294.1250.

**Nucleoside 3** (*β*-epimer, 50%): $^1$H NMR ($CDCl_3$, ppm) δ 8.06 (1H, d, *J* = 8.0), 7.88 (1H, d, *J* = 8.0), 7.80 (1H, d, *J* = 8.0), 7.66 (1H, d, *J* = 8.0), 7.55−7.46 (3H, m), 5.92 (1H, dd, *J* = 5.2, 10.0), 4.52 (1H, m), 4.15 (1H, m), 3.92−3.86 (2H, m), 2.54 (1H, dd, *J* = 5.6, 13.3), 2.18 (1H, m), 2.02 (2H, broad s, 2 × OH); HRMS (FAB, 3-NBA matrix) calcd for $C_{15}H_{16}O_3$ 244.1099, found 244.1105.

**Nucleoside 4** (*β*-epimer, 68%): $^1$H NMR ($CDCl_3$, ppm) δ 7.85−7.80 (4H, m), 7.50−7.42 (3H, m), 5.35 (1H, dd, *J* = 5.6, 10.2), 4.43 (1H, m), 4.06 (1H, m), 3.77 (2H, m), 2.6 (2H, broad s, 2 × OH), 2.33 (1H, ddd, *J* = 2.0, 5.6, 13.4), 2.02 (1H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{15}H_{16}O_3$ 244.1099, found 244.1110.

**Nucleoside 5** (*β*-epimer, 93%): $^1$H NMR ($CDCl_3$, ppm) δ 7.20 (1H, s), 6.97 (1H, s), 5.38 (1H, dd, *J* = 5.6, 10.4), 4.43 (1H, m), 4.01 (1H, m), 3.82 (2H, m), 2.32 (3H, s), 2.26 (3H, s), 2.24 (3H, s), 1.99 (1H, m), 1.90 (2H, broad s, 2 × OH); HRMS (FAB, 3-NBA matrix) calcd for $C_{14}H_{20}O_3$ 237.1491, found 237.1484.

**Nucleoside 6** (*β*-epimer, 89%): $^1$H NMR ($CDCl_3$, ppm) δ 7.46 (1H, t, *J* = 10), 6.82 (1H, t, *J* = 10), 5.31 (1H, dd, *J* = 5.6, 10.4), 4.32 (1H, m), 3.92 (1H, m), 3.68 (2H, m), 2.22 (3H, s), 1.89 (1H, m), 1.78 (2H, broad s, 2 × OH); HRMS (FAB, 3-NBA matrix) calcd for $C_{12}H_{14}F_2O_3Na$ 267.0809, found 267.0812.

**General Procedure for Preparation of 5′-*O*-tritylated *β*-C-Nucleosides.** The above-synthesized nucleoside **1** (165 mg, 0.52 mmol) was coevaporated with dry pyridine (4 mL) twice and dissolved in 5 mL of pyridine and 4 mL of methylene chloride. To the above mixture were added catalytic amount of DMAP, diisopropylethylamine (0.14 mL, 1.5 equiv) and 4,4′-dimethoxytrityl (DMT) chloride (320 mg, 1.8 equiv). The mixture was stirred at room temperature for 8 h. Hexanes (5 mL) was added, and the mixture was loaded onto a flash silica gel column (pre-equilbrated with 5% triethylamine in hexanes) and eluted (5:1 hexanes:EtOAc to 2:1 hexanes: EtOAc). The product **1 DMT ether** was obtained as a yellowish foam in 64% yield (200 mg, 0.32 mmol): $^1$H NMR ($CDCl_3$, ppm) δ 8.34 (2H, overlapped d, *J* = 8.0), 8.24−8.02 (7H, m), 7.56 (2H, overlapped d, *J* = 8.0), 7.45−7.27 (7H, m), 6.86 (4H, d, *J* = 8.0), 6.52 (1H, d, *J* = 6.2), 6.24 (1H, dd, *J* = 5.2, 10.4), 4.60 (1H, m), 4.30 (1H, m), 3.81 (6H, s), 3.56 (2H, m), 2.64

(1H, m), 2.30 (1H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{42}H_{36}O_5$ 620.2564, found 620.2563.

**2 DMT ether** (280 mg, 59%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 8.78 (1H, d, $J$ = 8.0), 8.68 (1H, d, $J$ = 8.0), 8.07 (2H, m), 7.8 (1H, d, $J$ = 8.0), 7.80−7.24 (12H, m), 6.84 (4H, overlapped d, $J$ = 8.0), 5.94 (1H, dd, $J$ = 5.8, 10), 4.52 (1H, m), 4.22 (1H, m), 3.8 (6H, s), 3.50 (2H, m), 2.61 (1H, ddd, $J$ = 2.0, 5.2, 13.4), 2.25 (1H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{40}H_{36}O_5$ 596.2564, found 596.2563.

**3 DMT ether** (50 mg, 52%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 8.15 (1H, d, $J$ = 8.0), 7.9 (1H, d, $J$ = 8.0), 7.8 (1H, d, $J$ = 8.0), 7.65 (1H, d, $J$ = 8.0), 7.49−7.45 (3H, m), 5.94 (1H, dd, $J$ = 5.8, 10), 4.53 (1H, m), 4.25 (1H, m), 3.8 (3H, s), 3.42 (2H, m), 3.02 (3H, s), 2.58 (1H, ddd, $J$ = 2.0, 5.2, 13.4), 2.18 (1H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{36}H_{34}O_5$ 546.2407, found 546.2406.

**4 DMT ether** (200 mg, 66%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 7.83−7.94 (4H, m), 7.56−7.27 (11H, m), 6.87 (4H, overlapped d, $J$ = 8.0), 5.41 (1H, dd, $J$ = 5.8, 10), 4.52 (1H, m), 3.82 (6H, s), 3.42 (2H, m), 2.38 (1H, dd, $J$ = 5.4, 13.4), 2.21 (1H, m).

**5 DMT ether** (311 mg, 92%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 7.52 (2H, d, $J$ = 8.0), 7.43−7.24 (7H, m), 6.94−6.84 (6H, m), 5.34 (1H, dd, $J$ = 5.8, 9.8), 4.42 (1H, m), 4.18 (1H, m), 3.80 (6H, s), 3.40 (2H, m), 2.64 (1H, m), 2.29 (3H, s), 2.23 (3H, s), 2.18 (3H, s), 2.0 (1H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{35}H_{37}O_5$ 538.2719, found 538.2690.

**6 DMT ether** (350 mg, 88%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 7.46 (1H, d, $J$ = 8.0), 7.39−7.24 (5H, m), 6.83 (2H, overlapped d, $J$ = 8.0), 6.74 (1H, dd, $J$ = 9.8, 8.6), 5.38 (1H, dd, $J$ = 5.8, 9.9), 4.42 (1H, m), 4.06 (1H, m), 3.80 (6H, s), 3.38 (2H, m), 2.38 (1H, dd, $J$ = 5.0, 13.4), 2.2 (1H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{33}H_{32}F_2O_5$ 569.2115, found 569.2131.

**General Procedure for Preparation of 3′-*O*-Phosphoramidites.** The 5′-*O*-tritylated compound **1 DMT ether** (200 mg, 0.32 mmol) was dissolved in 4 mL of dry methylene chloride, and to this were added diisopropylethylamine (0.22 mL, 1.2 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.11 mL, 0.48 mmol). The reaction mixture was stirred at room temperature for 4 h. Hexanes (4 mL) was added, and the mixture was loaded to the flash silica gel column (pre-equilibrated with 5% triethylamine in hexanes) and eluted. The product was obtained as an oil, **DMT phosphoramidite 1** (210 mg, 81%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 8.44−8.33 (2H, m), 8.25−8.00 (7H, m), 7.62−7.22 (9H, m), 6.92−6.79 (4H, m), 6.28−6.20 (1H unresolved m), 4.69 (1H, m), 4.45 (1H, m), 4.0−3.2 (12H, m), 2.80 (1H, m), 2.69 (2H, t), 2.32 (1H, m), 1.15 (12H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{51}H_{54}N_2O_6P$ (M + H) 821.3722, found 821.3720.

**2 DMT phosphoramidite** (280 mg, 77%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 8.78 (1H, d, $J$ = 8.0), 8.68 (1H, d, $J$ = 8.0), 8.07 (2H, m), 7.8 (1H, d, $J$ = 8.0), 7.80−7.24 (12H, m), 6.84 (4H, overlapped d, $J$ = 8.0), 5.94 (1H, overlapped dd, $J$ = 5.8, 10), 4.62 (1H, m), 4.40 (1H, m), 3.9 (2H, m), 3.8 (6H, s), 3.50 (2H, m), 3.83 (3H, s), 3.77 (3H, s), 3.66−3.45 (3H, m), 3.42−3.38 (2H, m), 2.82 (2H, t, $J$ = 5.6), 2.52 (3H, t, $J$ = 5.6), 2.16 (1H, m), 1.20−1.05 (12H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{49}H_{53}N_2O_6PNa$ 819.3537, found 819.3539.

**3 DMT phosphoramidite** (48 mg, 50%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 8.10 (1H, d, $J$ = 8.0), 7.9 (1H, d, $J$ = 8.0), 7.8 (1H, d, $J$ = 8.0), 7.52−7.24 (9H, m), 6.82 (4H, overlapped d, $J$ = 8.0), 5.91 (1H, overlapped dd, 2 × isomers), 4.6 (1H, m), 4.38 (1H, m), 3.83 (3H, s), 3.77 (3H, s), 3.66−3.45 (3H, m), 3.42−3.38 (2H, m), 2.82 (2H, t, $J$ = 5.6), 2.52 (3H, t, $J$ = 5.6), 2.16 (1H, m), 1.20−1.05 (12H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{45}H_{52}N_2O_6P$ (M + H) 747.3565, found 747.3563.

**4 DMT phosphoramidite** (170 mg, 65%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 7.91 (1H, s), 7.83−7.78 (4H, m), 7.56−7.27 (11H, m), 6.87 (4H, overlapped d, $J$ = 8.0), 5.39 (1H, dd, $J$ = 5.8, 10), 4.58 (1H, m), 4.30 (1H, m), 3.90 (2H, m), 3.83 (3H, s), 3.77 (3H, s), 3.66−3.45 (3H, m), 3.42−3.38 (2H, m), 2.82 (2H, t, $J$ = 5.6), 2.52 (3H, t, $J$ = 5.6), 2.16 (1H, m), 1.20−1.05 (12H, m).

**5 DMT phosphoramidite** (380 mg, 89%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 7.54 (2H, overlapped d, $J$ = 8.0), 7.45−7.40 (4H, m), 7.36 −7.22 (3H, m), 6.98 (1H, s), 6.90−6.82 (4H, m), 5.37 (1H, dd, $J$ = 5.8, 10), 4.56 (1H, m), 4.22 (1H, m), 3.82 (6H, s), 3.66−3.45 (3H, m), 3.42−3.38 (2H, m), 2.82 (2H, t, $J$ = 5.6), 2.48 (1H, m), 2.29, (3H, s), 2.24 (3H, s), 2.18 (3H, s), 1.99 (1H, m), 1.28 (1H, m), 1.20−1.05 (12H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{44}H_{55}N_2O_6P$ 739.3876, found 739.3870.

**6 DMT phosphoramidite** (420 mg, 84%): $^1$H NMR ($CDCl_3$, ppm) $\delta$ 7.51 (2H, m), 7.42−7.22 (9H, m), 6.84 (4H, overlapped d, $J$ = 8.0), 6.78 (1H, dd, $J$ = 9.0, 8.6), 5.38 (1H, dd, $J$ = 2.9, 10), 4.54 (1H, m), 4.22 (1H, m), 3.82 (6H, s), 3.66−3.45 (3H, m), 3.42−3.38 (2H, m), 2.82 (2H, t, $J$ = 5.6), 2.50 (2H, t, $J$ = 5.6), 2.05 (1H, m), 1.20−1.05 (12H, m); HRMS (FAB, 3-NBA matrix) calcd for $C_{42}H_{49}F_2N_2O_6P$ 769.3194, found 769.3209.

**Oligonucleotide Synthesis.** DNA oligonucleotides were synthesized on an Applied Biosystems 392 synthesizer using standard $\beta$-cyanoethyl phosphoramidite chemistry but with extended (10 min) coupling cycles for the nonnatural residues. Stepwise coupling yields for the nonnatural residues were all greater than 95% as determined by trityl cation monitoring. Oligomers were purified by preparative 20% denaturing polyacrylamide gel electrophoresis and isolated by the crush and soak method and were quantitated by absorbance at 260 nm. Molar extinction coefficients were calculated by the nearest neighbor method. Values for oligonucleotides containing nonnatural residues were estimated in the following way: each of the new nucleosides was measured for its extinction coefficient at 260 nm. The molar extinction coefficients for **2** and **3** were found to be 8990 and 154, respectively, and these values were added to the value for the core sequence dCGCGCG. For **1** in DNA we measured the absorbance at 350 nm and subtracted 0.48 of this value from the total absorbance at 260 nm to get the absorbance of the core DNA alone. Oligodeoxynucleotides were obtained after purification as the sodium salt. Intact incorporation of residues **1**−**3** was confirmed by synthesis of short oligomers of sequence T-X-T (where X = **1**−**3**); proton NMR (400 MHz) indicated the presence of the intact structures with the expected integration.

**Fluorescence Measurements.** Fluorescence spectra were recorded on a SPEX-Fluorolog-2 series fluorometer. A xenon lamp was used as the source of radiation. The fluorescence measurements were taken in the right angle mode using 0.1−0.15 $\mu$M DNA solutions in a pH 7.0 buffer (10 mM Na•PIPES, 10 mM $MgCl_2$, 100 mM NaCl). Five scans were averaged at 23 °C. The excitation slits were set to 6mm and the emission slits to 2 mm. All emission spectra were corrected using a reference dye (rhodamine-B) to compensate for instrument fluctuations and also by subtraction of data for buffer alone. Excitation wavelengths of 233, 251, and 341 nm (the absorbance maxima) were used to excite the compounds containing naphthalene, phenanthrene, and pyrene, respectively.

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**Supporting Information Available:** Carbon-13 and phosphorus-31 spectral data for all compounds (where appropriate), fluorescence emission spectra, and a description of crystallographic methods (6 pages). See any current masthead page for ordering information and Internet access instructions.

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