A Robust Synthetic Route to 2′-Deoxy-3′-nucleotide Derivatives of Modified Nucleobases

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Introduction

We required pure diastereomeric *cis*- and *trans*-3-(2′-deoxyguanosine-3′-phosphate-N^2-yl)-4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene (**7a,b**) as chromatographic standards to confirm the structures of the major in vivo DNA adducts of the ubiquitous environmental carcinogen cyclopenta[*cd*]pyrene (CPP)[1−6] detected by ^{32}P-postlabeling techniques. Although ^{32}P-postlabeling of DNA adducts followed by chromatographic separation is an extremely sensitive analytical technique,[7] only limited structural information about adducts can be derived in the absence of such standards. This need prompted us to pursue a convenient and robust synthetic route to 3′-deoxynucleotide derivatives of base-modified nucleosides. We report here the synthesis of diastereomeric *cis*- and *trans*-deoxyguanosine-3′-nucleotide adducts by a convenient route, using the stable, commercially available phosphitylating reagent 2-chloro-5,6-benzo-1,3,2-dioxaphosphorin-4-one (salicylchlorophosphine).[8,9] An advantage of the synthetic scheme is that 3′-*H*-phosphonate products of the phosphitylation reaction can be used directly as reagents for automated oligonucleotide syntheses due to their stability and ease of handling.[10] However, despite the importance of 3′-*H*-phosphonate and 3′-nucleotide derivatives of DNA adducts, synthetic routes to such derivatives are not generally available. Current approaches require the preliminary synthesis of phosphorylating agents and maintenance of strictly anhydrous reaction conditions, and yields may be highly variable.[11,12] Hence, the procedures described here should find wide applicability in the syntheses of modified 3′-deoxynucleotides and oligonucleotides containing modified bases at specific sites.

Results and Discussion

The key step in the strategy is phosphitylation of 2-fluoro-6-benzyloxypurine-9-(5′-*O*-[4,4′-dimethoxytrityl]-2′-deoxyribose) (**1**), summarized in Scheme 1.

Salicylchlorophosphine has been reported to phosphitylate O^6- and N^2-protected 2′-deoxyguanosine in high yield in a robust procedure.[8,9] On the basis of this report, the reaction was applied to the O^6-blocked fluorodeoxyinosine (**1**) to give the corresponding fluoroinosine 3′-*H*-phosphonate (**2**) in 81% yield as the triethylammonium salt. In accord with expectation, **2** could be readily identified by the characteristically large P−H coupling constant (∼600 Hz), which was confirmed in both ^{1}H and ^{31}P NMR spectra.

Cis- and trans N^2-adducted deoxynucleoside 3′-*H*-phosphonate diastereomers **6a**,**b** were obtained by coupling the corresponding racemic 3-amino-4-hydroxy-3,4-dihydro-CPP (CPP-aminohydrin) with O^6-deblocked phosphonate **5**. Coupling the racemic *cis*-CPP-aminohydrin with O^6-blocked fluoroinosine 3′-*H*-phosphonate **3** yielded the O^6-benzylated cis N^2-adducted 3′-*H*-phosphonate diastereomeric mixture **4**. The coupling reaction, which required no phosphorus protection, was accomplished by published procedures.[13] However, because the aminohydrin derivatives of CPP appear to degrade rapidly in an aerobic environment, this reaction was performed with the strict exclusion of air. Coupling via **3** provides O^6-protected phosphonate intermediates suitable for use in preparation of oligonucleotides containing the modified deoxyguanosine.

The *cis*-O^6-benzyl-protected phosphonate diastereomers **4** were resolved by reverse phase HPLC. The diastereomeric mixtures of O^6-deblocked CPP-modified deoxyguanosine 3′-*H*-phosphonates **6a,b** were not well-resolved by reverse phase HPLC. However, separation of **6a,b** was achieved following oxidation to the corresponding 3′-nucleotides **7a,b**. Although phosphonate monoester salts are more difficult than phosphodiesters to oxidize to phosphates, quantitative oxidation of the modified 3′-*H*-phosphonates was achieved by treatment of the persilylated compounds by I_2 in pyridine.[14] Use of triethylammonium bicarbonate buffer proved advantageous in reverse phase HPLC separations of the diastereomeric phosphates, since the buffer salts were easily removed from the collected HPLC fractions by repeated evaporation in the presence of water. In the case of the *cis*-N^2-deoxyguanosine adducts of CPP-oxide, the 3′-*H*-

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(1) Gold, A.; E. Eisenstadt *Cancer Res.* **1980**, *40*, 3940−3944.

(2) Gold, A.; Nesnow, S.; Moore, M.; Garland, H.; Curtis, G.; Howard, B.; Graham, D.; Eisenstadt, E. *Cancer Res.* **1980**, *40*, 4482−4484.

(3) Wood, A. W.; Levin, W.; Chang, R. L.; Huang, M.-T.; Ryan, D. E.; Thomas, P. E.; Lehr, R. E.; Kumar, S.; Koreeda, M.; Akagi, H.; Ittah, Y.; Dansette, P.; Yagi, H.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1980**, *40*, 642−649.

(4) Gold, A. *Anal. Chem.* 1**975**, *47*, 1469−1471.

(5) Wallcave, L.; Nagel, D.; Smith, J. W.; Waniska, R. D. *Environ. Sci. Technol.* **1975**, *9*, 143−145.

(6) Grimmer, G. In *Air Pollution and Cancer in Man*; Mohr, Y., Schmel, D., Tomatis, L., Eds.; IARC: Lyon, France, 1977; pp 193−199.

(7) Randerath, K.; Randerath, E.; Agrawal, H. P.; Gupta, R. C.; Schurdak, M. E.; Reddy, M. V. *Environ. Health Persp.* **1985**, *62*, 57−65.

(8) Hermans, J. P. G.; de Vroom, E.; Elie, C. J. J.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 510−511.

(9) Marugg, J. E.; Tromp, M.; Kuyl-Yeheskiely, E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2661−2664.

(10) Froehler, B. C. In *Methods in Molecular Biology: Protocols for Oligonucleotides and Analogues:* Agrawal, S., Ed.; Humana Press Inc.: Totowa, 1993; Vol. 20, Chapter 4, p 63−80.

(11) Pongracz, K.; Bodell, W. J. *Chem. Res. Toxicol.* **1995**, *9*, 593−598.

(12) Han, S.; Harris, C. M.; Harris, T. H.; Kim, H. H.; Kim, S. J. *J. Org. Chem.* **1996**, *61*, 174−178.

(13) Lakshman, M. K.; Sayer, J. M.; Yagi, H.; Jerina, D. M. *J. Org. Chem* **1992**, *57*, 4585−4589.

(14) Garegg, P. J.; Regberg, T.; Stawinski, J.; Stromberg, R. *Nucleosides Nucleotides* **1987**, *6*, 429−432.

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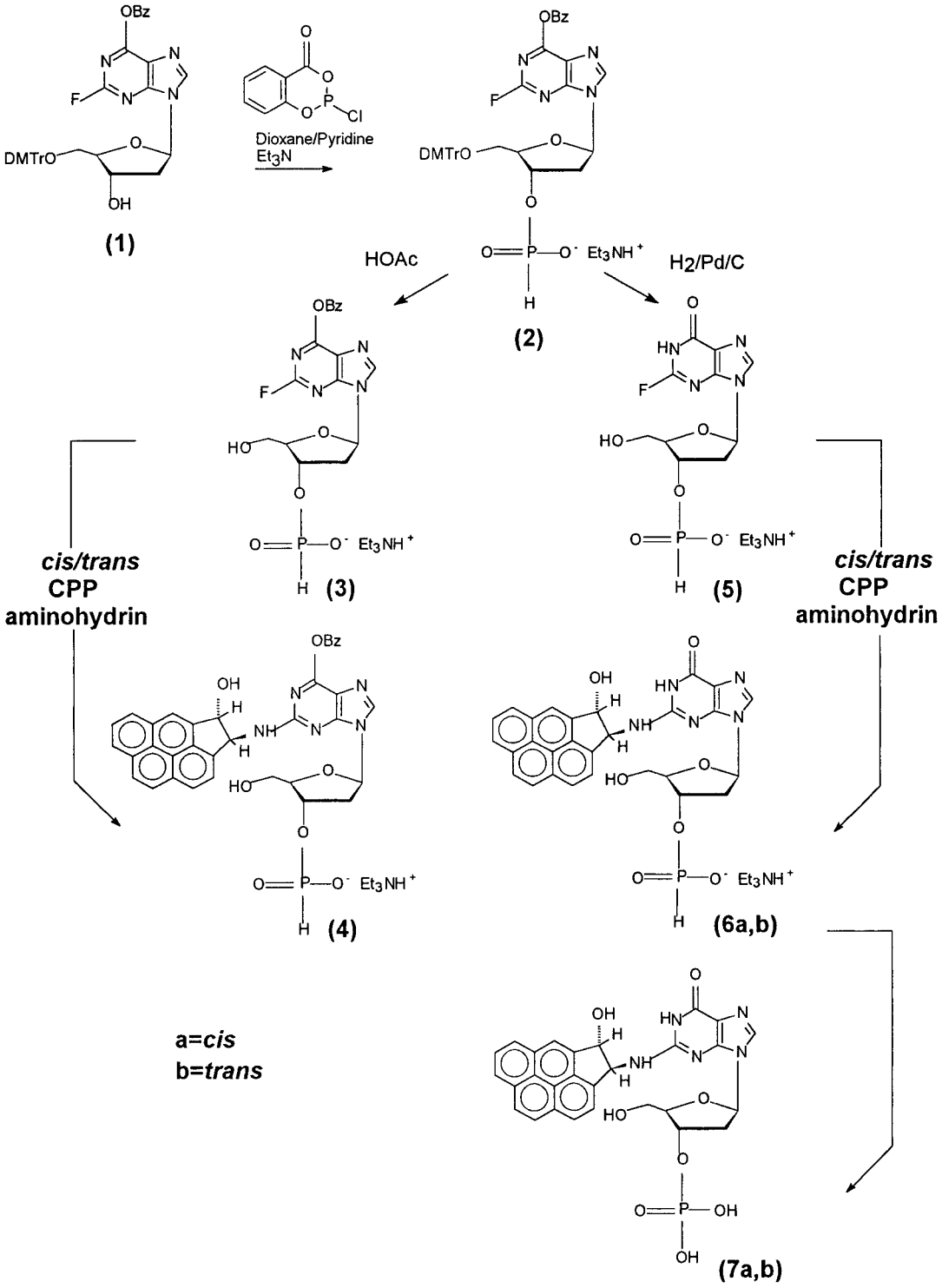
Scheme 1



phosphonate diastereomers appropriate for use as synthons in the preparation of oligodeoxynucleotides were more easily resolvable as the O^6-benzylated derivatives. In general, whether 3′-*H*-phosphonate diastereomers for oligodeoxynucleotide synthesis are more easily separated as the O^6-blocked derivatives or in deblocked form will depend on the specific polycyclic aromatic hydrocarbon and the relative cis/trans orientation of the aminohydrin intermediate.

The UV−vis spectra of both sets of 3′-phosphate diastereomers are dominated by the pyrene chromophore and are indistinguishable. Both ^{31}P and ^{1}H NMR are consistent with assigned structures. Formation of the 3′-*H*-phosphonates and oxidation to the corresponding 3′-phosphates is easily demonstrated by ^{31}P NMR. ^{31}P NMR signals of the 3′-*H*-phosphonates appear as doublets ($J \cong 600$ Hz) centered at 3.79 ppm downfield from the H_3PO_4 standard, and the phosphate signals as singlets at 0.01 ppm. In the ^{1}H NMR spectra of both *cis*- and *trans*-diastereomers, the proton resonances of the 3,4-dihydrocyclopenta[*cd*]pyrenyl moieties of the early and late-eluting nucleotides are identical. Peak assignments were made by comparison to the spectra of the corresponding nucleoside adducts.[15,16] Peak assignments for the sugar

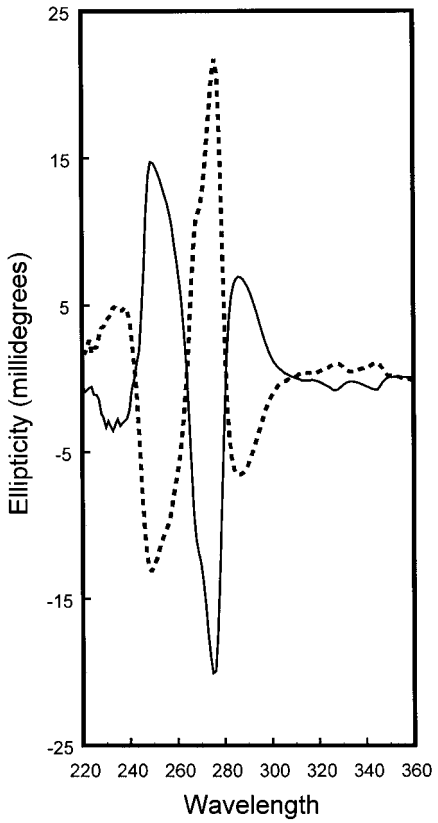


Figure 1. Circular dichroism spectra (methanol, 20 °C) of early (—) and late (- - -) eluting diastereomers of *cis*-3-(2′-deoxyguanosine-3′-phosphate-N^2-yl)-4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene.

and nucleobase resonances were made by comparison to spectra of nucleoside adducts, the most significant difference being a shift of the H3′ signal by ~0.4 ppm downfield in the nucleotides. Mass spectra of the target nucleotides, obtained by electrospray ionization operated in the negative mode, are characterized by the expected peak at *m*/*z* 588, corresponding to loss of a hydrogen from the molecular ion ($M^{\bullet -}$). Major fragment ions are consistent with the assigned structures: *m*/*z* 392, corresponding to loss of deoxyribose-3-phosphate; *m*/*z* 374, corresponding to loss of deoxyribose-3-phosphate and water; and *m*/*e* 265, corresponding to loss of both 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrenyl and PO_3.

The CD spectra of the adducts (Figure 1) show Davydov-split Cotton effects[17] arising from coupling of the in-plane purine and pyrene transitions. However, inspection of the CD traces suggests that exciton coupling may involve more than two bands for both cis and trans sets of diastereomers and identification of a first Cotton effect is not straightforward. Moreover, the relative orientation of the transition dipoles of the coupled bands cannot be unambiguously established. As a result, application of exciton chirality rules to assign configuration at the N-bonded benzylic carbon does not appear warranted. Tentative assignment of stereochemistry at the N-substituted benzylic carbon atom of bay region diolepoxide adducts has been reported on the basis of the sign of the major Cotton effect.[18] However, the cyclopenta[*cd*]pyrene—deoxyguanosine-3′-phosphate adducts lack the *trans*-dihydrodiol feature adjacent to the amine alcohol generated in bay region diol epoxide adducts, and there is no evidence to support application of this correlation in the absence of the dihydrodiol structural feature. Absolute stereochemical configurations can therefore not be assigned on the basis of CD spectroscopy of the adducts alone. The CD spectra of the cis diastereomers are virtually mirror images, indicating that the coupled chromophores are related by reflection in a mirror plane and that the relative orientation of the chromophores is not sensitive to the configuration at C3. The CD spectra of the trans diastereomers are similar to those of the cis diastereomers.

Experimental Section

General Information. ^{1}H NMR spectra were recorded at 500 MHz, and chemical shifts are reported in ppm relative to trimethylsilane. ^{31}P NMR spectra were recorded at 202.5 MHz using 5% H_3PO_4 in D_2O (A) or 0.1% H_3PO_4 in D_2O (B) as the external standard. Adducts were lyophilized prior to analysis. Electrospray mass spectra (ESMS) were acquired on a Finnegan 4000 quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (nonpneumatically assisted electrospray ionization) operating in the negative ion mode. The ESI voltage and current were −2.5−3 kV and 0.06−0.1 μA, respectively. Samples were run in the infusion mode and the flow rate of the solution was controlled using a syringe pump (Kd Scientific, Model 100, Fisher Scientific) at 1.5−2.0 μL/min. Coaxial flow of SF_6 was applied (2 psi) on the ESI needle to stabilize the electrospray. Mass spectra were acquired over a mass range of *m*/*z* 0−800 in 2 s and processed by a Technivet Vector/2 (St. Louis, MO) data system. The exact mass measurement was performed under negative ion electrospray conditions on a Micromass Q-TOF hybrid quadrupole/time-of-flight mass spectrometer (Altrincham, UK) using a nanoflow electrospray interface. The capillary voltage was 2895 V and the desolvation (cone) voltage was 30 eV. The solvent was 50:50 methanol/0.1% formic acid in water. The instrument was calibrated with the formic acid dimer (−H) and the 2′-deoxyguanosine-3′-monophosphate $(M-H)^-$ and $(2M-H)^-$ ions. Fast atom bombardment mass spectra (FABMS) were obtained on a VG 70−250SEQ mass spectrometer operating in the positive ion mode, with samples suspended in a 1:1 dithioerythritol/dithiothreitol matrix. Reverse phase HPLC was carried out using a Zorbax C_{18} 9.6 × 250 mm column (Dupont) eluted at a flow rate of 2.5 mL/min with a gradient program: 40% MeOH/60% 50 mM triethylammonium bicarbonate to 80% MeOH/20% 50 mM triethylammonium bicarbonate over 80 min (gradient A) or 30% MeOH/70% 50 mM triethylammonium bicarbonate to 60% MeOH/40% 50 mM triethylammonium bicarbonate over 60 min (gradient B). The UV absorbance of the HPLC eluate was monitored at 254 nm. Electronic (UV−vis) spectra were scanned between 400 and 200 nm. Circular dichroism spectra were recorded at 20 °C in methanol and normalized to 1.0 AUFS at 242 nm.

CPP, *cis*- and *trans*-3-amino-4-hydroxy-3,4-dihydro-CPP, and 2-fluoro-O^6-benzyl-2′-deoxyinosine were synthesized according to published procedures.[16,19−24] Chemicals were obtained from

(15) Hsu, C. H.; Skipper, P. L.; Harris, T. M.; Tannenbaum, S. R. *Chem. Res. Toxicol.* **1997**, *10*, 248−253.
(16) Sangaiah, R.; Gold, A.; Ball, L. M.; Matthews, D. L.; Toney, G. E. *Tetrahedron Lett.* **1992**, *33*, 5487−5490.
(17) Gaffield, W. In *Bioactive Natural Products. Detection, Isolation and Structural Determination*; Colegate, S. M., Molyneux, R. J., Eds.; CRC Press: Boca Raton, FL, 1993; pp 148−156.
(18) Agarwal, S. K.; Sayer, J. M.; Herman, J. C. Y.; Pannell, L. K.; Hilton, B. D.; Pigott, M. A., Dipple, A.; Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1987**, *109*, 2497−2504.
(19) Lakshman, M. K.; Sayer, J. M.; Jerina, D. M. *J. Org. Chem.* **1992**, *57*, 3438−3443.
(20) Konieczny, M.; Harvey, R. G. *J. Org. Chem.* **1979**, *44*, 2158−2160.
(21) Sangaiah, R.; Gold, A. *J. Org. Chem.* **1988**, *53*, 2620−2622.
(22) Robins, M. J.; Robins, R. K. *J. Org. Chem.* **1969**, *34*, 2160−2163.
(23) McManus, S. P.; Larson, C. A.; Hearn, R. A. *Synth. Commun.* **1973**, *3*, 177−180.

commercial sources (Aldrich Chemical Co. or Sigma Chemical Co.). HPLC solvents and normal phase and reverse phase preparative TLC plates (20 × 20 cm, 1000 μm, Whatman) were obtained from Fisher Scientific (Pittsburgh, PA). All solvents used were anhydrous unless otherwise noted.

5′-*O*-(4,4′-Dimethoxytrityl)-2-fluoro-*O*^{6}-benzyl-2′-deoxyinosine (1). 2-Fluoro-*O*^{6}-benzyl-2′-deoxyinosine (275 mg, 0.76 mmol) was coevaporated three times with dry pyridine and then dissolved in 5 mL of dry pyridine under an argon atmosphere at room temperature. Dimethoxytrityl chloride (414 mg, 1.2 mmol) and triethylamine (500 μL) were added, and the resulting solution was stirred at room temperature for 12 h, at which time TLC analysis (10% MeOH/CH_2Cl_2) indicated that no more starting material was present. Ethanol (1 mL) was added and the solution stirred for 10 min. Water (20 mL) was added and the mixture extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with 10% K_2CO_3, dried, and concentrated to produce an orange oil. Purification by column chromatography (98:2 CH_2Cl_2/Et_3N to 96:2:2 CH_2Cl_2/Et_3N/MeOH) yielded **1** as a yellow foamy solid (350 mg, 70% yield): R_f = 0.41 by TLC in 96:2:2 CH_2Cl_2/Et_3N/MeOH; ^{1}H NMR (acetone-d_6) δ 8.31(s, 1H, H8), 7.58 (m, 2H, aromatic), 7.14−7.43 (m, 12H, aromatic), 6.78 (m, 4H, aromatic), 6.45 (t, 1H, J = 6.5 Hz, H′1), 5.65 (s, 2H, CH_2), 4.69 (m, 1H, 3′-O*H*), 4.58 (m, 1H, H3′), 4.16 (m, 1H, H4′), 3.75 (s, 6H, OCH_3), 3.12 (m, 2H, H5′ and 5″), 2.94 (m, 1H, H2′), 2.50 (m, 1H, H2″); FABMS m/z 663 (M + H)$^+$.

5′-*O*-(4,4′-Dimethoxytrityl)-2-fluoro-*O*^{6}-benzyl-2′-deoxyinosine-3′-*H*-phosphonate (2). To a stirred solution of 5′-*O*-(4,4′-dimethoxytrityl)-2-fluoro-*O*^{6}-benzyl-2′-deoxyinosine **(1)** (187 mg, 0.282 mmol) in dioxane (1 mL) and pyridine (0.5 mL) under an argon atmosphere at room temperature was added salicylchlorophosphine reagent (69 mg, 0.362 mmol) dissolved in dioxane (1 mL). After 15 min, ^{31}P NMR analysis of an aliquot of the reaction mixture showed strong resonances at 123.82 and 125.35 ppm, confirming the presence of an intermediate containing phosphorus. Pyridine/water (1:1) (1 mL) was then added to the reaction mixture and a further aliquot analyzed by ^{31}P NMR. A doublet at 3.72 ppm (J = 725 Hz) indicated formation of the 3′-*H*-phosphonate. Triethylamine (500 μL) was added and the reaction mixture concentrated under a stream of argon. The resulting crude residue was purified by preparative reverse phase TLC (70:30:1 MeOH/H_2O/Et_3N) to give **2** (triethylammonium salt) as a white foamy oil (167 mg, 72% yield): R_f = 0.35; ^{1}H NMR (acetone-d_6) δ 8.29 (s, 1H, H8), 7.57−7.18 (m, 18H, benzyl and DMT aromatic), 7.05 (d, 1H, J = 725 Hz, P*H*), 6.43 (t, 1H, J = 6.5 Hz, H1′), 5.60 (s, 2H, CH_2), 4.97 (m, 1H, H3′), 4.33 (m, 1H, H4′), 3.77 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.34 (m, 2H, H5′and 5″, 2.98 (m, 1H, H2′), 2.67 (m, 1H, H2″), ^{31}P NMR (pyridine/dioxane/A) δ 3.72 (d, J = 725 Hz, *P*H); ESMS m/z 725 (M − N(Et_3)H)$^-$, 423 (M − DMT), 243 (M − [3′-*H*-phosphityl-5′-*O*-DMT-deoxyribose]).

2-Fluoro-*O*^{6}-benzyl-2′-deoxyinosine-3′-*H*-phosphonate (3). Compound **2** (75 mg, 0.91 mmol, triethylammonium salt) was treated with 80% acetic acid (3 mL) and stirred at room temperature for 60 min. The resulting orange solution was concentrated under reduced pressure at 30 °C. Water (10 mL) was added along with triethylamine until neutral pH. Excess triethylamine was removed under a stream of argon. Lyophilization followed by preparative reverse phase TLC using 70:30:1 MeOH/H_2O/Et_3N gave **3** (triethylammonium salt) as a clear oil (42 mg, 88% yield): R_f = 0.67; ^{1}H NMR (DMSO-d_6) δ 8.57 (s, 1H, H8), 7.55−7.35 (m, 5H, aromatic), 6.67 (d, 1H, J = 622 Hz, P*H*), 6.26 (t, 1H, J = 6.5 Hz, H1′), 5.59 (s, 3H, CH_2 and 5′-O*H*), 4.76 (m, 1H, H3′), 3.95 (m, 1H, H4′), 3.54 (m, 2H, H5′and 5″), 2.69 (m, 1H, H 2′), 2.47 (m, 1H, H2″); ^{31}P NMR (MeOH/B) δ 3.31 (d, J = 622 Hz, *P*H); ESMS m/z 423 (M − N(Et_3)H)$^-$, 243 (M − [3′-*H*-phosphityldeoxyribose]).

***cis*-3-(*O*^{6}-Benzyl-2′-deoxyguanosine-3′-*H*-phosphonate-*N*^{2}-yl)-4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene (4).** Phosphonate **3** (28 mg, 0.053 mmol, triethylammonium salt) and cyclopenta[*cd*]pyrene cis aminohydrin (20 mg, 0.077 mmol) in 1 mL of anhydrous DMSO and 40 μL of collidine were degassed by freeze−thaw (three cycles) and stirred under N_2 for 5 d at 85° C. The brown residue from lyophilization was chromatographed by reverse phase TLC (70:30:1 MeOH/H_2O/Et_3N). The mixture of diastereomeric 3′-*H*-phosphonate adducts was collected as a band at R_f = 0.44. Separation by HPLC using gradient program A gave diastereomers **4** (triethylammonium salts) eluting at 79.5 (2.4 mg, 5.9%) and 80.5 (2.1 mg, 5.2%) min, respectively. Late-eluting diastereomer (identical to early eluting diastereomer): ^{1}H NMR (DMSO-d_6, 50 °C) δ 8.33 (d, 1H J = 7.7 Hz, H8,), 8.25 (d, 1H, J = 7.7 Hz, H6 or H1), 8.24 (d, 1H, J = 7.7 Hz, H1 or H6), 8.17 (d, 1H, J = 9.3 Hz, H9 or H10), 8.15 (d, 1H, J = 9.3 Hz, H10 or H9), 8.13 (s, 1H, H5), 8.12 (d, 1H, J = 7.7 Hz, H2), 8.06 (t, 1H, J = 7.7 Hz, H7), 8.08 (bs, 1H, H8$_{dG}$), 7.46 (d, 2H, J = 7.3 Hz, *m*-phenyl), 7.40−7.32 (m, 3H, phenyl), 6.79 (d, 1H, C3N*H*, J = 8.5 Hz), 6.56 (d, 1H, J = 579 Hz, P*H*), 6.28, (t, 1H, J = 7.1 Hz, H1′), 5.84 (bt, 1H, J = 7.1 Hz, H3), 5.75 (bs, 1H, H4), 5.57 (d, 1H, J = 12.4 Hz, CH_2-Bz), 5.52 (d, 1H, J = 12.4 Hz, CH_2-Bz), 4.86 (bs, 1H, H3′), 3.96−3.94 (m, 2H, H5′, H5″), H2′, H2″ obscured by water and residual proton signal from DMSO-d_6; ESMS m/z 662 (M − N(Et_3)H)$^-$, 482 (M − H − [phosphonylated deoxyribose]), 373 (M − H − OBz − [phosphonylated deoxyribose]).

2-Fluoro-2′-deoxyinosine-3′-*H*-phosphonate (5). A solution of **2** (167 mg, 0.202 mmol, triethylammonium salt) and methanol (20 mL) containing 50 mg of 5% Pd/C was hydrogenated for 60 min at room temperature. The catalyst was filtered off and the filtrate, on concentration, gave a clear oil, which was purified by reverse phase TLC using 70:30:1 MeOH/H_2O/Et_3N. The product, eluting with the solvent front, was extracted with methanol, treated with decolorizing carbon, and concentrated to give the triethylammonium salt **5** as a clear oil (53 mg, 60% yield): ^{1}H NMR (DMSO-d_6) δ 7.74 (s, 1H, H8), 6.58 (d, J = 575 Hz, 1H, P*H*), 6.00 (t, J = 7 Hz, 1H, H1′), 5.83 (m, 1H, 5′-O*H*), 4.65 (m, 1H, H3′), 3.88 (m, 1H, H4′), 3.45 (m, 2H, H5′and 5″), 2.58 (m, 1H, H2′), 2.23 (m, 1H, H2″); ^{31}P NMR (MeOH/A) δ 1.01 (dd, J_{PH} = 575, $J_{PH3'}$ = 9.32 Hz, *P*H); ESMS m/z 333 (M − N(Et_3)H)$^-$, 166.

***trans*-CPP-N^2-deoxyguanosine-3′-*H*-phosphonate Diastereomers (6b).** *trans*-CPP-aminohydrin (25 mg, 0.096 mmol) and **5** (25 mg, 0.058 mmol, triethylammonium salt) were dissolved in DMSO (700 μL) and 2,6-lutidine (40 μL) under nitrogen. The reaction flask, degassed by three cycles of freeze−thaw under high vacuum, was stirred at 80 °C for 16−20 h under a nitrogen atmosphere. Lyophilization of the resulting brown solution and purification by reverse phase TLC (70:30:1 MeOH/H_2O/Et_3N) afforded the diastereomeric product mixture **6b** (triethylammonium salts) as a light yellow oil (4 mg, 10% yield based on **5**): R_f = 0.77. Definitive NMR (^{1}H NMR, DMSO-d_6) peak assignments were hindered by an inability to resolve the diastereomeric product mixture by reverse phase HPLC; ^{1}H NMR (DMSO-d_6) δ 8.31 (d, 1H, H8), 8.24−8.17 (m, 2H, H1 and H6), 8.15 (s, 2H, H9 and H10), 8.06−7.96 (m, 3H, H2, H5 and H7), 7.90−7.80 (m, 2H, C3N*H* and H8$_{dG}$), 6.31−5.9 (m, 2H, H1′ and H3), 5.70−5.55 (m, 1H, H4), 4.97 (m, 1H, H3′), 3.73 (m, 1H, H4′), 3.5 (m, 2H, H5′ and H5″); ^{31}P NMR (MeOH/B) δ 3.79 (d, J = 614 Hz, *P*H); ESMS m/z 572 (M − N(Et_3)H)$^-$, 392 (M − deoxyribose), 374 (M − deoxyribose − H_2O); UV−vis (MeOH) λ_{max} 342, 326, 277, 266, 242 nm.

***trans*-CPP-*N*^{2}-deoxyguanosine-3′-phosphate Diastereomers (7b).** *trans*-*N*^{2}-CPP-deoxyinosine-3′-*H*-phosphonate (1.5 mg, 0.0022 mmol, triethylammonium salt) was lyophilized and dissolved in pyridine (500 μL). TMSCl (200 μL) was added at room temperature under nitrogen and the mixture stirred for 5 min. An iodine solution (200 μL) (2 mg dissolved in 500 μL pyridine) was added and the mixture stirred for 5 min. Pyridine/water (1:1, 100 μL) was added followed by solid sodium sulfite until the yellow color dissipated. The resulting solution was concentrated at room temperature under a stream of argon. HPLC (gradient program B) showed no residual starting material and the two diastereomeric phosphates **7b** as the only products present, indicating complete conversion. The pure diastereomers were collected, eluting at 45.0 and 47.5 min. Early-eluting diastereomer (identical to late-eluting diastereomer):^{1}H NMR (DMSO-d_6, 50 °C) δ 8.34 (d, 1H, J = 7.5 Hz, H8), 8.28 (d, 1H, J = 7.5 Hz, H1 or H6), 8.24 (d, 1H, J = 7.5 Hz, H6 or H1), 8.17 (s, 2H, H9 and H10), 8.08 (s, 1H, H5), 8.06 (t, 1H, J = 7.5 Hz, H7), 8.02 (d, 1H, J = 7.5 Hz, H2), 7.96 (s, 1H, H8$_{dG}$), 7.47 (d, 1H, J = 8 Hz, C3N*H*), 6.18 (t, 1H, J = 7 Hz, H1′), 5.99

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(br m, 1H, H3), 5.60 (br s, 1H, H4), 4.89 (br s, 1H, *H*3′), 4.00 (br s, 1H, H4′), 3.50 (m, 2H, H5′, H5″) (H2′, and H2″ obscured by DMSO and water); ^{31}P NMR (MeOH/B) δ 0.01 (s); ESMS *m*/*z* 588 (M − H)$^-$, 392 (M − [deoxyribose-3′-phosphate]), 374 (M − [deoxyribose-3′-phosphate] − H_2O), 265 (deoxyguanosine); UV−vis (MeOH) λ_{max} 342, 326, 277, 266, 242 nm.

***cis*-CPP-*N*^{2}-deoxyguanosine-3′-*H*-phosphonate Diastereomers (6a).** Coupling of *cis*-CPP-aminohydrin (25 mg, 0.096 mmol) and **5** (25 mg, 0.057 mmol, triethylammonium salt) was carried out as described above for **6b**, with stirring for 18 h, to give the diastereomeric mixture **6a** as a light yellow solid (6.1 mg, 16% yield) R_f = 0.83. Diastereomeric mixture: ^{1}H NMR (DMSO-d_6) δ 8.36 (d, 1H, H8), 8.29−8.25 (m, 2H, H1 and H6), 8.18 (s, 2H, H9 and H10), 8.16−8.06 (m, 3H, H2, H5, H7), 7.96 (s, 1H, H8$_{dG}$), 7.06 (m, 1H, C3N*H*), 6.56 and 6.39 (2 d, 1H total, J = 578 Hz, P*H*), 6.21 (m, 2H, H1′ and H3), 5.88 (m, 1H, H4), 4.84 (br s, 1H, H3′), 3.89 (m, 1H, H4′), 3.53 (m, 2H, H5′and H5″), 2.79 (m, 1H, H2′), 2.40 (m, 1H, H2″); ^{31}P NMR (MeOH/B) δ 3.79 (d, J = 578 Hz, *P*H) ppm; ESMS *m*/*z* 572 (M − N(Et$_3$)H)$^-$, 392 (M − [deoxyribose-3′-phosphonate]), 374 (M − [deoxyribose-3′-phosphonate] − H_2O); UV−vis (MeOH) λ_{max} 342, 326, 277, 266, 242 nm.

***cis*-CPP-*N*^{2}-deoxyguanosine-3′-phosphate Diastereomers (7a).** Oxidation of *cis*-N^2-CPP-deoxyinosine-3′-*H*-phosphonate (6.0 mg, 0.0089 mmol, triethylammonium salt) was carried out as described for **7b**, giving complete conversion to the mixture of diastereomers **7a**. Separation by reverse phase HPLC using gradient program **B** gave the products eluting at 36.0 and 41.7 min. Late-eluting diastereomer (identical to the early-eluting diastereomer): ^{1}H NMR (DMSO-d_6) 8.35 (d, 1H, J = 8 Hz, H8), 8.28 (d, 1H, J = 8 Hz, H1), 8.26 (d, 1H, J = 8.6 Hz, H6), 8.18 (bs, 2H, H9, H10), 8.17 (d, 1H, J = 8 Hz, H2), 8.13 (s, 1H, H5), 8.07 (dd, 1H, J_{H7H6} = 8.6 Hz, J_{H7H8} = 8 Hz, H7), 7.97 (s, 1H, H8$_{dG}$), 7.08 (d, 1H, J = 7 Hz, C3N*H*), 6.23 (t, 1H, J = 6 Hz, H1′), 6.16 (m, 1H, H3), 5.88 (d, 1H, J = 6 Hz, H4), 4.72 (bs, 1H, H3′), 3.95 (bs, 1H, H4′), 3.53 (m, 2H, H5′, H5″), 2.72 (m, 1H, H2′), 2.06 (m, 1H, H2″); ^{31}P NMR (MeOH/B) δ 0.01 (s); HRESMS 588.1281 (M$^-$), calcd for $C_{28}H_{24}O_8N_5P$ 588.1284; ESMS *m*/*z* 588 (M − H)$^-$, 392 (M − [deoxyribose-3′-phosphate]), 374 (M − [deoxyribose-3′-phosphate] − H_2O), 265 (deoxyguanosine); UV−vis (MeOH) λ_{max} 342, 326, 277, 266, 242 nm.

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Supporting Information Available: ^{1}H NMR spectra are available for compounds **4**, **5**, **6a**, **6b**, **7a** and **7b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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