Synthesis of Deoxyadenosine 3′-Phosphates Bearing *Cis* and *Trans* Adducts of 7*β*,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene: Standards for ^{32}P-Postlabeling Assays

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Deoxyadenosine 3′-phosphates bearing *cis* and *trans* N^6 adducts of (7*R*) and (7*S*)-*anti* 7*β*,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenes have been prepared in good yield by reaction of 6-fluoropurinyl 2′-deoxyriboside 3′-(bis(2-[4-nitrophenyl]ethyl)phosphate with the (±)-(7*β*,8α,9α,10*β*)- and (±)-(7*β*,8α,9α,10α)-10-amino-7,8,9,10-tetrahydrobenzo[*a*]pyrene-7,8,9-triols. The protected phosphates are easily prepared as diasteromeric mixtures, readily resolved by reversed phase HPLC, and efficiently deprotected with DBU to give the adducted 3′-phosphates. These nucleotides are of value as standards for the ^{32}P-postlabeling procedure of Randerath for determination of benzo[*a*]pyrene adducts in DNA (Reddy *et al. Carcinogenesis* **1984**, *5*, 231).

Introduction

The ^{32}P-postlabeling assay developed by Randerath and co-workers[1] is a versatile method for detection of DNA adducts and is widely used for analysis of adducts in DNA isolated from living systems.[2] In this method adducted DNA is enzymatically hydrolyzed to nucleoside 3′-phosphates which are converted to radioactive 3′,5′-diphosphates by [^{32}P] transfer from [γ-^{32}P]ATP catalyzed by T4 polynucleotide kinase. The diphosphates are analyzed by high-resolution thin layer chromatography and visualized by radioautography. [γ-^{32}P]ATP is commercially available in very high specific activity and as a consequence the method has extraordinarily high sensitivity such that it is possible to detect adducts at the level of approximately 1 base in 10^{10} using no more than 10 μg of DNA.[1] The high sensitivity presents a problem: adducts can readily be detected at lower levels than can be assayed by other analytical methods. Consequently, authentic standards of the adducted nucleoside 3′-phosphates are required for identification of the individual adducts. Use of the methodology for the analysis of polycyclic aromatic hydrocarbon adducts has been hindered by the poor availability of such standards. The 3′-nucleotides can be isolated from the reaction of the PAH diol epoxides with DNA followed by enzymatic hydrolysis and chromatographic separation of the various products,[3a] but the procedure is frequently inefficient, the chromatographic separations can be difficult, and the adducts of interest may be only minor products. Alternatively, the adducted 3′-nucleotides can be formed by reaction of the unadducted 3′-nucleotides with the diol epoxides. For example, **2**−**5** can be prepared by the reaction of deoxyadenosine 3′-phosphate with 7*R* and 7*S* forms of 7*β*,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (**1a** and **1b**, commonly called (+)- and (−)-*anti*-BPDE, respectively) (Chemical Abstracts Service designates BPDE as a derivative of benzo[10,11]chryseno-[3,4-*b*]oxirene) as shown in Scheme 1. The procedure leads to both cis and trans opening of the epoxide, but the separations are easier since they only involve the adducts of a single nucleotide.[3b] With both methods, hydrolysis of the diol epoxides is a major side reaction. We report herein an efficient and straightforward method for preparing regio- and stereospecific deoxyadenosine 3′-phosphates bearing N^6 adducts of the PAH diol epoxides by a strategy in which the electrophile−nucleophile relationship of PAH and nucleotide are reversed. The examples described in this paper involve the *cis* and *trans* opening products **2**-**5** of (+)- and (−)-*anti*-BPDE. The corresponding adducted nucleosides have been isolated from BPDE-treated calf thymus and plasmid DNA.[4] This indirect method is of particular value for the adenine adducts which are formed in very low yields by reaction of the epoxides with DNA; the N^2 guanine adduct arising by trans opening of the *anti*-BPDE is the predominant product. Interestingly, there is evidence that despite the low yields, the adenine adducts may have disproportionate importance in mutagenic and carcinogenic processes.[5]

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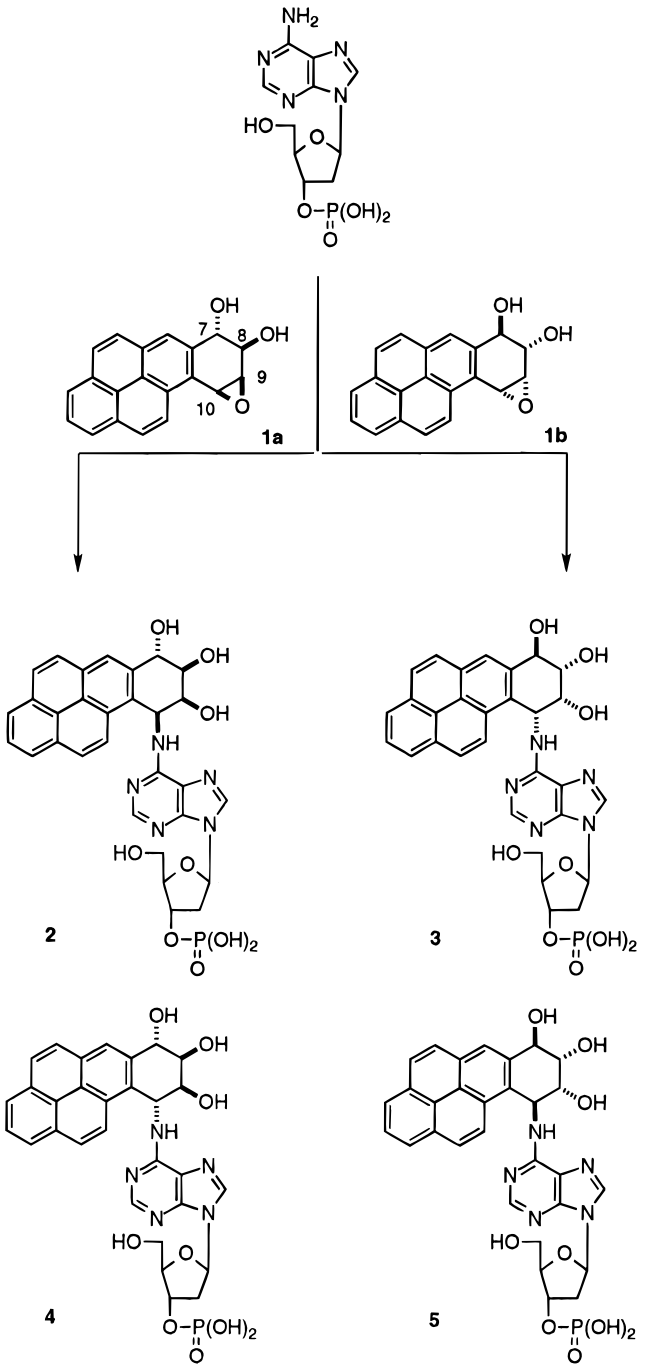
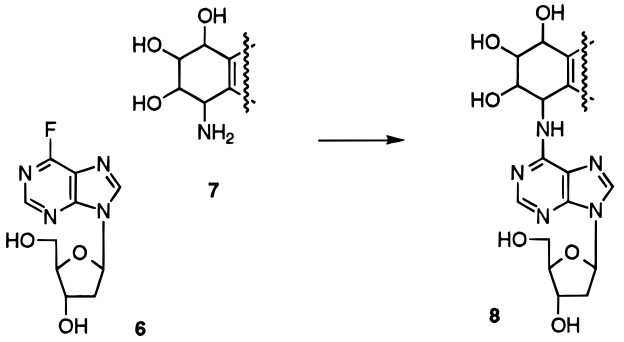
Scheme 1



Scheme 2



Results and Discussion

We have recently developed an attractive procedure for preparation of the adducted nucleosides **8** involving reaction of 6-fluoropurine 2′-deoxyribonucleoside (**6**) with amino triols **7** derived from ammonolysis of PAH diol epoxides (Scheme 2).[6a] Others have also found this strategy, which reverses the electrophile−nucleophile relationship of the PAH and nucleoside, to be highly efficient.[6b−h] The yields are high, and the stereochemical arrangement of the PAH functional groups is determined exclusively by the structure of the amino triol. Excess amino triol, used to drive the reaction, can be recovered. The initial approach we considered for preparation of the nucleotides involved direct introduction of phosphate on the 3′-hydroxyl group of the N^6-adducted deoxyadenosines. However, use of these nucleosides for synthesis of 3′-nucleotides would be somewhat cumbersome since selective protection of the hydroxyl groups on the PAH and the 5′-hydroxyl of deoxyribose would be required before reaction with a phosphorylating agent.

Phosphorylation prior to introduction of the PAH appeared more attractive. In addition, there would be considerable advantage to using a protected form of the phosphate to promote solubility in organic solvents since the solubility of PAH amino triols in water is very low and the fluoropurine is subject to hydrolysis in aqueous solvents. A protective group for phosphate was needed which was relatively stable to the conditions required for the preparation and reaction of the 6-fluoro-3′-nucleotide but could be removed under conditions which did not perturb the PAH or deoxyribose moieties.

With these requirements in mind, we examined several possibilities, finally settling on 2-(4-nitrophenyl)ethyl (NPE) as the protective group for phosphate. NPE esters are relatively stable but can be removed by β-elimination using DBU. An uncertainty concerning its use was whether the group would be stable to the relatively harsh conditions employed for the condensation of PAH amino triols with 6-fluoropurine derivatives. However, even before this potential problem could be addressed, difficulties arose in the use of bis-NPE phosphorochloridate to phosphorylate the 3′-hydroxyl group. The phosphorochloridate is very sensitive to moisture, and reproducible yields were difficult to obtain in the phosphorylation reaction.[7]

To circumvent the moisture sensitivity problem, the trivalent bis(NPE-O)(diisopropylamino)phosphine (**10**) was used instead. Phosphoramidite **10** is readily prepared from PCl_3 via dichloro(diisopropylamino)phosphine (**9**).[8−10] Phosphitylation of the 5′-(dimethoxytrityl)-protected 6-chloropurine nucleoside[11] **11** in the presence of tetrazole followed by *in situ* oxidation of the resulting

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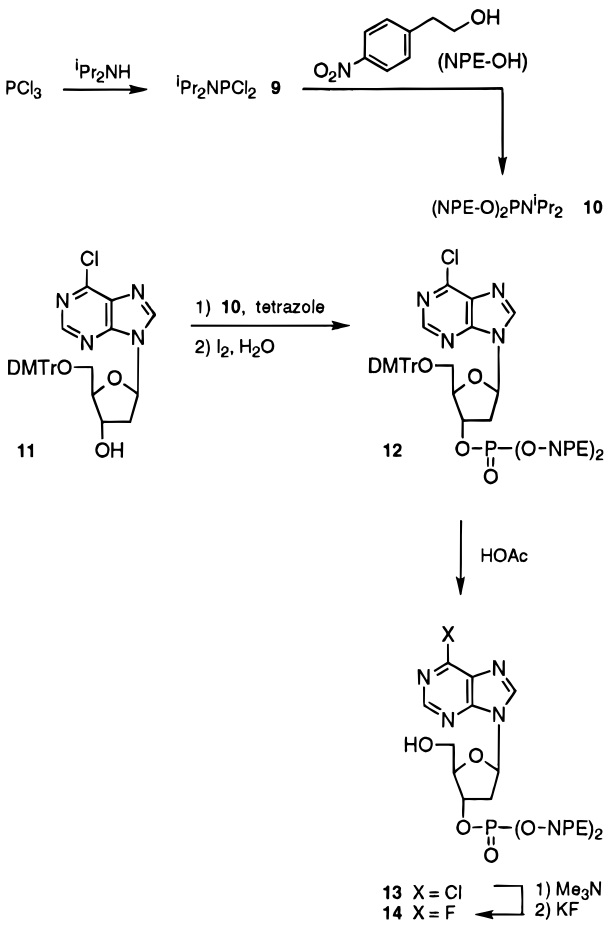
Scheme 3



phosphite ester with aqueous iodine afforded phosphotriester **12** in 86% yield (Scheme 3). After detritylation with aqueous AcOH (86% yield), 6-chloronucleotide **13** was converted to the 6-fluoro analog **14** in 85% yield by treatment with trimethylamine in glyme followed by KF in DMF.[12]

Condensation of **14** with *cis* and *trans* amino triols (**15** and **16**) derived from racemic *anti*-BPDE[6a,c,13] afforded the corresponding diastereomeric mixtures of N^6-adducted deoxyadenosine 3′-phosphotriesters **17**/**18** and **19**/**20**, respectively, in yields of ~50% (Scheme 4). The diastereomeric mixtures were separated by preparative reversed-phase HPLC. The NPE protecting groups were removed with DBU in pyridine to give adducts **2**−**5** in yields of ~78%. Final purification of the deprotected 3′-phosphates was accomplished by reversed-phase HPLC. A cursory examination was made of whether it would be preferable to separate the diastereomeric mixtures after deprotection, but separations appeared to be easier with the NPE-protected nucleotides.

The adducted nucleotides were characterized by FAB mass spectrometry and ^{1}H and ^{31}P NMR. Stereochemical assignments were made by comparison of circular dichroism spectra with published spectra.[4,14] For both the *trans* and *cis* adducts the isomers with the 10*S* configuration (**2** and **5**) eluted first on HPLC. To our knowledge, PAH-adducted 3′-nucleotides have never been prepared previously on sufficient scale to permit extensive spectroscopic characterization. The PAH adducted 3′-nucleotides have only limited solubility in aqueous buffers and cling to surfaces on account of the hydrophobic PAH moieties, so care has to be taken in handling them to avoid losses.

We initially investigated the 2,2,2-trichloroethyl group for protection of the phosphate; the bis-ester can be prepared from the commercially available bis(2,2,2-trichloroethyl) phosphorochloridate.[15] The 5′-(dimethoxytrityl) derivative **11** of 6-chloropurine 2′-deoxyriboside[11] was acylated with the phosphorochloridate to give the protected nucleotide; the dimethoxytrityl group was removed by treatment with acetic acid, the chloro substituent replaced by fluoro by treatment with trimethylamine followed by anhydrous KF.[12] The bis(2,2,2-trichloroethyl)phosphate group was fully resistant to the conditions used to prepare the 6-fluoro synthon. Reaction with racemic *cis*-amino triol **15** to give diastereomeric adducts analogous to **17**/**18** went efficiently but difficulties arose with the deprotection step. In literature examples, bis(2,2,2-trichloroethyl) groups have been removed from phosphates by reductive cleavage using Zn−Cu couple or tributylphosphine.[16−18] The procedure of Imai[18] using a highly activated Zn−Cu couple was successful in model deprotection reactions with bis(2,2,2-trichloroethyl)-protected 3′-phosphate of thymidine and bis(2,2,2-trichloroethyl)-protected 3′-phosphate of N^6-(styrene oxide)-adducted deoxyadenosine. Unfortunately, the reported deprotection methods were unsuccessful with the bis(2,2,2-trichloroethyl)-protected 3′-phosphate of benzo[*a*]pyrene-adducted deoxyadenosine. One trichloroethyl group could be removed, but the second was resistant to reductive elimination.

6-Fluoropurine 3′-nucleotide **14** represents a valuable synthon for preparation of the N^6-PAH-adducted deoxyadenosine 3′-nucleotides since the adduction reaction should proceed equally well with amino alcohols derived from the epoxides and diol epoxides of many other PAHs. Indeed, with sterically unencumbered amines, the chloropurine nucleotide would be equally satisfactory. The 6-chloronucleoside is significantly easier to prepare since moisture must be scrupulously excluded during the KF reaction used to prepare **14**.

The reactions described herein have been carried out with racemic amino triols. The use of racemic diol epoxides is much more cost-effective than the individual enantiomers, only a few of which are commercially available, and even those are often in short supply. In the cases presented here, separation of the resulting diastereomers has been relatively straightforward during the HPLC purification of the adducts.

Experimental Section

General. (±)-7*β*,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene used for synthesis of racemic benzo[*a*]pyrenetriol amines (**15** and **16**) was purchased from Midwest Research Institute or Chemsyn. ^{1}H NMR spectra were obtained at 300 and 400 MHz. Phosphorus spectra were standardized to external 85% H_3PO_4 ($\delta = 0$). Fluorine spectra were standardized to $CFCl_3$ ($\delta = 0$). Low and high resolution mass spectra were obtained in either negative or positive FAB mode; matrices are indicated with individual compounds.

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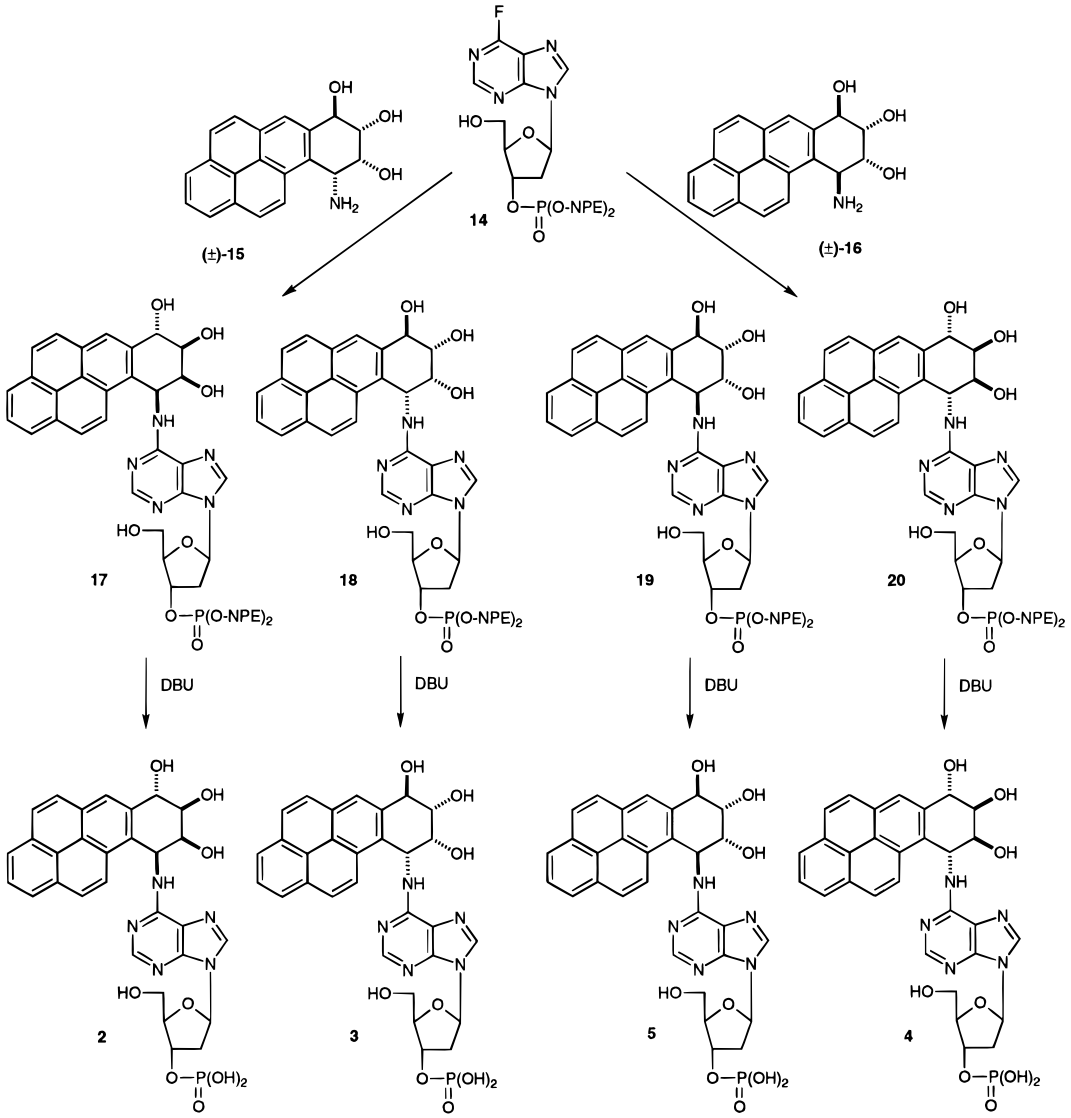
Scheme 4



Reactions were monitored by TLC on silica gel plates (EM Science, Kieselgel 60, F254). TLC plates were visualized by UV or anisaldehyde stain.[19] Column chromatography was conducted on silica gel 60 (70−230 mesh) from EM Science. HPLC analyses and isolations of nucleosides were carried out on a gradient HPLC system equipped with a diode array detector. Analytical runs were performed on a C-18 reverse-phase column (4.6 × 250 mm) at a flow rate of 1.0 mL/min; preparative scale isolations were carried out on a 10 × 250 mm C-18 column at a flow rate of 2.0 mL/min.

(Diisopropylamino)dichlorophosphine (9).[8] To a solution of phosphorus trichloride (1.83 mL, 21 mmol) in anhydrous ether (20 mL) was added a solution of diisopropylamine (5.8 mL, 42 mmol) in anhydrous ether (10 mL) with stirring at −10 °C for 2.5 h followed by an additional 1 h at room temperature. After the precipitated salt was filtered off, the reaction mixture was distilled at ambient temperature and 0.5 mmHg to give **9** in 76% yield. ^{31}P NMR ($CDCl_3$) δ 177; lit.[8] ^{31}P NMR ($CDCl_3$) δ 164.3.

Bis(2-[4-nitrophenyl]ethyl) *N*,*N*-Diisopropylphosphoramidite (10).[9] A solution of 2-(4-nitrophenyl)ethanol (3.34 g, 0.02 mol) and diisopropylethylamine (3.87 g, 0.03 mol) in anhydrous THF (10 mL) was cooled to 0 °C. To this solution was added 1.52 g (0.01 mol) of (diisopropylamino)dichlorophosphine (**9**) with vigorous stirring over 15 min. After stirring for a further 30 min at room temperature, the solution was filtered to remove amine hydrochloride. The filtrate was diluted with 200 mL of acid-free ethyl acetate, and the organic layer was extracted three times with 30 mL of phosphate buffer, pH 7, dried over sodium sulfate, and concentrated in vacuo. The oily product was purified by short column chromatography in the presence of Et_3N in 94% yield. ^{1}H NMR ($CDCl_3$) δ 8.10−8.15 (d, 4H), 7.33−7.36 (d, 4H), 3.72−3.84 (m, 4H), 3.46−3.56 (m, 1H), 2.95−2.98 (t, 4H), 1.07 (s, 6H), 1.05 (s, 6H). ^{31}P NMR ($CDCl_3$) δ 147; lit.[9] (no solvent given) δ 149.

6-Chloropurinyl-5′-*O*-(dimethoxytrityl)-2′-deoxyriboside 3′-(Bis(2-[4-nitrophenyl]ethyl) phosphate) (12). To 0.5 g (0.87 mmol) of 5′-*O*-(dimethoxytrityl)-6-chloro-2′-deoxyriboside **11**[11] and 0.57 g (1.32 mmol) of **5** was added 3.0 mL (0.5 M) of 1*H*-tetrazole in CH_3CN at room temperature. After addition of 20 mL of CH_3CN and 10 mL of CH_2Cl_2 at room temperature, the mixture was stirred for 1 h at which time TLC showed complete disappearance of **11** (silica gel, EtOAc/CH_2Cl_2 70:30, R_f 0.68). For oxidation, a solution of 16 mL of iodine (0.5 M in pyridine/water/acetonitrile 3:1:1) was added to the mixture over 5 min at room temperature. TLC (silica gel, EtOAc/CH_2Cl_2 70:30, R_f 0.22) after 2 h showed complete conversion to compound **12**. The pure product was obtained by short column chromatography in 86% yield. ^{1}H NMR ($CDCl_3$) δ 8.61 (s, 1H), 8.2 (s, 1H), 6.74−8.14 (m, 13H), 6.37−6.41 (dd, 1H), 5.12−5.16 (t, 1H), 4.30 (br, 1H), 4.15−4.26 (m, 4H), 3.76 (s, 6H), 2.96−3.05 (m, 4H), 2.92 and 2.68 (m, 2H). ^{31}P NMR δ −1.5. LRMS (FAB^-, Et_3N/DMSO) 950 ($M^{\bullet -}$); HRMS (FAB^-, Et_3N/DMSO) 950.2456; calcd for $C_{47}H_{44}ClN_6O_{12}P$ 950.2443 ($M^{\bullet -}$).

(19) *Thin Layer Chromatography*; Stahl, E., Ed.; Springer-Verlag: New York, 1969; p 587.

6-Chloropurinyl-2′-Deoxyriboside 3′-(Bis(2-[4-nitrophenyl]ethyl) phosphate) (13). Compound **12** was stirred with aqueous 85% AcOH for 3 h at room temperature. After neutralization with aqueous Na_2CO_3, the mixture was extracted with $CHCl_3$ (50 mL × 3), followed by rotary evaporation. Column chromatography afforded **13** as a yellow oil in 86% yield: R_f 0.21 (8% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.73 (s, 1H), 8.17 (s, 1H), 8.15 (d, 4H), 7.38 (d, 4H), 6.23−6.28 (dd, 1H), 5.04 (t, 1H), 4.98 (d, 1H), 4.21−4.29 (m, 4H), 3.83 and 3.65 (dd, 2H), 3.08 (t, 4H), 3.0 and 2.5 (m, 2H). ^{31}P NMR ($CDCl_3$) δ −5.3. LRMS (FAB$^-$, Et_3N/DMSO) 648.1 ($M^{\bullet-}$); (FAB$^+$, glycerol/DMSO) 649.2 ($M + H^+$); calcd for $C_{26}H_{26}ClN_6O_{10}P$ 648.

6-Fluoropurinyl-2′-Deoxyriboside 3′-(Bis(2-[4-nitrophenyl]ethyl) phosphate) (14). To a solution of 127 mg (0.2 mmol) of 6-chloropurinyl 2′-deoxyriboside 3′-(bis(2-[4-nitrophenyl]ethyl)phosphate) (**13**) in glyme (5.0 mL) was added 0.5 mL of liquid Me_3N.[12] The reaction mixture was stirred for 40 min at room temperature, evaporated, and dried to give a yellow foam. The foam was dissolved in dry DMF, and 116 mg (2 mmol) of anhydrous KF was added at room temperature. The mixture was stirred for 30 min at room temperature under a nitrogen atmosphere, followed by rotary evaporation. The residue was purified by column chromatography to afford **14** as a yellow solid in 85% yield: R_f 0.28 (MeOH/CH_2Cl_2, 10:90). ^{1}H NMR ($CDCl_3$) δ 8.63 (s, 1H), 8.18 (s, 1H), 8.17 (d, 4H), 7.38 (d, 4H), 6.25−6.30 (dd, 1H), 5.05 (t, 1H), 5.01 (br, 1H), 4.21−4.29 (m, 4H) 3.88 and 3.67 (m, 2H), 3.1 (t, 4H), 3.0 and 2.5 (m, 2H). ^{31}P NMR ($CDCl_3$) δ −1.5. ^{19}F ($CDCl_3$) δ −66.8. LRMS (FAB$^-$, Et_3N/DMSO) 632.2 ($M^{\bullet-}$); HRMS (FAB$^-$, Et_3N/DMSO) 632.1441 ($M^{\bullet-}$); calcd for $C_{26}H_{26}FN_6O_{10}P$ 632.1432 ($M^{\bullet-}$).

(7*S*)- and (7*R*)-(7*β*,8α,9α,10α)-2′-Deoxy-*N*^{6}-(7,8,9,10-tetrahydro-7,8,9-trihydroxybenzo[*a*]pyren-10-yl)adenosine 3′-(Bis(2-[4-nitrophenyl]ethyl) phosphate) (17 and 18, respectively). (±)-Amino triol **15** was prepared from *anti*-BPDE via an azide intermediate.[13] A solution of **15** (6 mg, 0.02 mmol) and excess nucleotide **14** (17.8 mg, 0.03 mmol) in anhydrous dimethylacetamide (0.3 mL) containing distilled triisobutylamine (0.01 mL, 0.04 mmol) was stirred at 60 °C for 5 days under nitrogen. The reaction mixture was evaporated to give a brown residue which was purified by column chromatography on silica gel (MeOH/CH_2Cl_2, 3:97) to give **17**/**18** in 55% yield: R_f 0.58 (MeOH/$CHCl_3$, 20:80). ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.81 (s, 1H), 7.99−8.53 (m, 13H), 7.53 (d, 4H), 6.67 (s, 1H), 6.30 (t, 1H), 5.11 (d, 1H), 5.00 (br, 1H), 4.56 (d, 1H), 4.23 (m, 5H), 4.02 (s, 1H), 3.56 and 3.52 (m, 2H), 3.05 (t, 4H), 2.98 and 2.43 (m, 2H). ^{31}P NMR (DMSO-d_6) δ −0.1. LRMS (FAB$^+$, glycerol/DMSO/TFA) 932.3 ($M + H^+$); HRMS (FAB$^-$, Et_3N/DMSO) calcd for $C_{46}H_{42}N_7O_{13}P$ 931.2578 ($M^{\bullet-}$); found 931.2580 ($M^{\bullet-}$). The diastereomeric mixture was resolved by preparative reversed phase HPLC (C-18 column) with isocratic elution (H_2O−acetonitrile, 48:52). The early and late eluting adducts had retention times of 39.0 min (**17**) and 43.4 min (**18**).

(7*R*)- and (7*S*)-(7*β*,8α,9α,10*β*)-2′-Deoxy-*N*^{6}-(7,8,9,10-tetrahydro-7,8,9-trihydroxybenzo[*a*]pyren-10-yl)adenosine 3′-(Bis(2-[4-nitrophenyl]ethyl)phosphate) (19 and 20, respectively). (±)-Amino triol **16** was prepared from (±)-*anti*-BPDE by ammonolysis.[6a,c] Amino triol **16** (12 mg, 0.04 mmol) and excess nucleotide **14** (35.6 mg, 0.06 mmol) in anhydrous dimethylacetamide (0.6 mL) containing distilled triisobutylamine (0.02 mL, 0.08 mmol) was stirred at 55 °C for 4−5 days under nitrogen. The reaction mixture was evaporated to give a brown residue. Column chromatography (silica gel, CH_3OH/CH_2Cl_2, 3:97) afforded **19** and **20** in 46% yield: R_f 0.58 (MeOH/$CHCl_3$, 20:80). ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.52 (s, 1H), 7.99−8.25 (m, 13H), 7.54 (d, 4H), 6.42 (s, 1H), 6.30 (t, 1H), 4.99 (d, 1H), 4.88 (br, 1H), 4.89 (br, 1H), 4.24 (m, 6H), 4.02 (s, 1H), 3.54 and 3.40 (m, 2H), 3.06 (t, 4H), 2.87 and 2.42 (m, 2H). ^{31}P NMR (DMSO-d_6) δ −1.4. LRMS (FAB$^+$, glycerol/DMSO) 932 ($M + H^+$); HRMS (FAB$^-$, Et_3N/DMSO) 931.2593 ($M^{\bullet-}$); calcd for $C_{46}H_{42}N_7O_{13}P$ 931.2578 ($M^{\bullet-}$). The diastereomeric mixture was resolved by preparative reversed phase HPLC (C-18 column) with isocratic elution (H_2O/acetonitrile, 48:52). The early and late eluting adducts had retention times of 39.6 min (**19**) and 42.5 min (**20**).

(7*S*)- and (7*R*)-(7*β*,8α,9α,10α)-2′-Deoxy-*N*^{6}-(7,8,9,10-tetrahydro-7,8,9-trihydroxybenzo[*a*]pyren-10-yl)adenosine 3′-Phosphate (2 and 3, respectively). Compound **17** (1 mg) was treated with 0.4 mL of 0.5 M DBU in pyridine for 24 h at room temperature; TLC analysis showed complete disappearance of the protecting groups. The mixture was neutralized by addition of 0.1 mL of 1 M AcOH in anhydrous pyridine and evaporated. The residue was purified by HPLC on a preparative reversed-phase C-18 HPLC column eluted at 3.0 mL/min with a gradient of 15−35% B over 20 min (solvent A, 0.05 M triethylammonium bicarbonate buffer, pH 7.45, and solvent B, CH_3CN). The elution profile exhibited one major peak eluting at 18.2 min, and this component was collected (75% yield). The fraction was evaporated and coevaporated 4−5 times with H_2O to remove the buffer. Lyophilization gave **2**, which was assigned the 10*S* configuration on the basis of the positive CD signal at 282 nm.[4,14] NMR (DMSO-d_6 + D_2O) δ 8.80 (d, 1H), 8.53 (s, 1H), 8.30 (s, 1H), 8.00−8.25 (m, 7H), 6.67 (s, 1H), 6.30 (t, 1H), 5.10 (d, 1H), 4.76 (br, 1H), 4.55 (d, 1H), 4.20 (br, 1H), 4.04 (dd, 1H), 3.42−3.57 (m, 2H), 2.49−2.70 (m, 2H). ^{31}P NMR (DMSO-d_6 +D_2O) δ 0.1. LRMS (FAB$^-$, glycerol/DMSO) 632.3 ($M - H^+$). HRMS (FAB$^-$, 3-nitrobenzyl alcohol/DMSO/PEG) 632.1546 ($M - H^+$); calcd for $C_{30}H_{27}N_5O_9P$ 632.1546 ($M - H^+$). The adduct **3** was prepared from **18** by the same procedure. The configuration at C10 was assigned as *R* on the basis of the negative CD peak at 282 nm. ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.80 (d, 1H), 8.53 (s, 1H), 8.30 (s, 1H), 8.00−8.25 (m, 7H), 6.67 (s, 1H), 6.30 (t, 1H), 5.10 (d, 1H), 4.76 (br, 1H), 4.55 (d, 1H), 4.20 (br, 1H), 4.04 (dd, 1H), 3.42−3.57 (m, 2H), 2.49−2.70 (m, 2H). ^{31}P NMR (DMSO-d_6 + D_2O) δ −0.2. LRMS (FAB$^-$, glycerol/DMSO) 632.7 ($M - H^+$); HRMS (FAB$^-$, 3-nitrobenzyl alcohol/DMSO/PEG) found 632.1546 ($M - H^+$); calcd for $C_{30}H_{27}N_5O_9P$ 632.1546 ($M - H^+$).

(7*R*)- and (7*S*)-(7*β*,8α,9α,10*β*)-2′-Deoxy-*N*^{6}-(7,8,9,10-tetrahydro-7,8,9-trihydroxybenzo[*a*]pyren-10-yl)adenosine 3′-Phosphate (4 and 5). Similarly, treatment of **19** with DBU gave **5**. The compound was purified by HPLC on a preparative reversed-phase C-18 HPLC column eluted at 3.0 mL/min with a gradient of 15−35% over 20 min (solvent A, 0.05 M triethylammonium bicarbonate buffer, pH 7.45, and solvent B, CH_3CN). The elution profile showed one major peak eluting at 17.8 min (75% yield). The collected fraction was evaporated and coevaporated 4−5 times with H_2O to remove the buffer. Compound **5** exhibited a positive CD signal at 286 nm and was assigned the *S* configuration at C10 by comparison with literature spectra.[4,14] ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.50 (s, 1H), 7.99−8.25 (m, 9H), 6.41 (s, 1H), 6.31 (t, 1H), 4.97 (d, 1H), 4.77 (br, 1H), 4.21−4.25 (m, 2H), 4.02 (dd, 1H), 3.43−3.55 (m, 2H), 2.38−2.64 (m, 2H). ^{31}P NMR (DMSO-d_6 + D_2O) δ 0.1. LRMS (FAB$^-$, Et_3N/DMSO) 632.3 ($M - H^+$). HRMS (FAB$^-$, Et_3N/DMSO) found 632.1556 ($M - H^+$); calcd for $C_{30}H_{27}N_5O_9P$ 632.1546 ($M - H^+$). Compound **4** was prepared from **20** by the same procedure. It showed a negative CD peak at 286 nm and was assigned the 10*R* configuration. ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.50 (s, 1H), 7.99−8.25 (m, 9H), 6.41 (s, 1H), 6.31 (t, 1H), 4.97 (d, 1H), 4.77 (br, 1H), 4.21−4.25 (m, 2H), 4.02 (dd, 1H), 3.43−3.55 (m, 2H), 2.38−2.64 (m, 2H). ^{31}P NMR (DMSO-d_6 + D_2O) δ 0.1. LRMS (FAB$^-$, glycerol/DMSO) 632.0 ($M - H^+$); HRMS (FAB$^-$, 3-nitrobenzyl alcohol/PEG) 632.1550 ($M - H^+$); calcd for $C_{30}H_{27}N_5O_9P$ 632.1546 ($M - H^+$).

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Supporting Information Available: ^{1}H NMR spectra of compounds **2-5**, **9**, **10**, **12-14**, **17**/**18**, and **19**/**20** and ^{31}P NMR spectra of **2-5**, **10**, **12-14**, **17**/**18**, and **19**/**20** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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