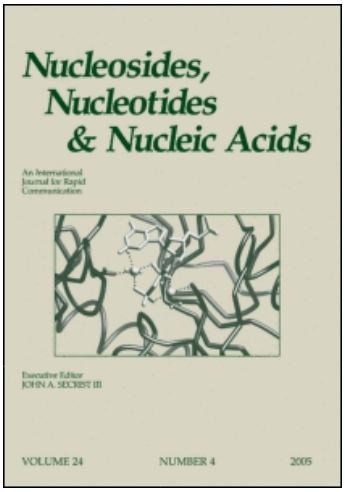

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Synthesis of the P^1, P^2-Methylene Analog of N^2- (*p*-n-Butylphenyl)-2'-deoxyguanosine 5'-Triphosphate: A Non-substrate Inhibitor of DNA Polymerases

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SYNTHESIS OF THE P^1,P^2-METHYLENE ANALOG OF N^2-(*p*-n-BUTYLPHENYL)-2'-DEOXYGUANOSINE 5'-TRIPHOSPHATE: A NON-SUBSTRATE INHIBITOR OF DNA POLYMERASES

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Abstract N^2-(*p*-n-Butylphenyl)-2'-deoxyguanosine 5'-(P^1,P^2-methylene)-triphosphate (BuPdGMPCH$_2$PP) has been synthesized. Displacement of the mesyloxy group of 5'-MesBuPdG by methanediphosphonate gave 30% of BuPdGMPCH$_2$P, but 68% of 3,5'-cycloBuPdG. Reaction of the nucleoside BuPdG with methanediphosphonate and DCC gave 62% of BuPdGMPCH$_2$PPCH$_2$P, which was hydrolyzed to BuPdGMPCH$_2$P in 77% yield. The title compound was obtained by reacting the imidazolide of BuPdGMPCH$_2$P with orthophosphate. BuPdGMPCH$_2$PP inhibited calf thymus DNA polymerase α with K_i = 9.5 nM, a potency only fivefold weaker than that of BuPdGTP itself.

N^2-(*p*-n-Butylphenyl)-2'-deoxyguanosine 5'-triphosphate (BuPdGTP, see structures) is a potent inhibitor of eukaryotic DNA polymerase α.[1,2] Its ability to inhibit DNA polymerase α selectively makes it a useful probe of the role of that enzyme in the background of other DNA polymerases (δ, ϵ) putatively involved in eukaryotic DNA replication. In addition to DNA polymerase α BuPdGTP has been found to inhibit several DNA polymerases belonging to the "B family",[3] although with generally lower potencies.[1,4] However, although BuPdGTP is a direct, non-substrate inhibitor of DNA polymerase α,[5] other members of the B family do incorporate BuPdGTP, for example DNA polymerases from the bacteriophages T4[5] and ϕ29[4] and that from *Herpes simplex* virus type 1 (K.-H. Knopf, personal communication). Consequently, it is difficult to interpret potencies of BuPdGTP against different DNA polymerases as resulting from changes in affinity as contrasted from those resulting from incorporation of the nucleotide. This is especially important in the use of BuPdGTP to study the effects of

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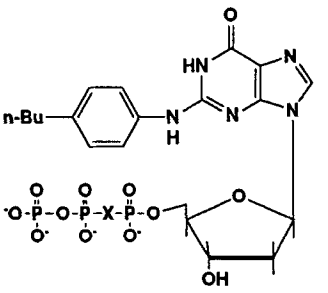
This paper is dedicated to the memory of Professor Roland Robins.

individual amino acid changes on inhibitor affinity for DNA polymerase mutants.

We decided to develop a non-substrate equivalent of BuPdGTP, focussing on the polyphosphate portion of the molecule. The fact that it is the α,β-phosphoanhydride bond that is cleaved during dNTP incorporation suggested that a phosphonate analog containing a P-C-P linkage at this position (P^1,P^2) would be stable to susceptible enzymes. Although the difluoromethylene group is a logical choice for such an analog, based on the suggestion that the CF_2 group is isopolar and isosteric with oxygen,[6] we found that a P^1,P^2-CF_2 derivative of a ribonucleoside triphosphate was unstable in aqueous buffer.[7] Therefore, we proposed to prepare the methylene derivative BuPdGMPCH$_2$PP, a compound expected to be both chemically and enzymatically stable, and we investigated two methods for its synthesis. We show that this compound is a potent inhibitor of DNA polymerase α, and preliminary results suggest that it is not a substrate for the T4 DNA polymerase.



X=O, BuPdGTP

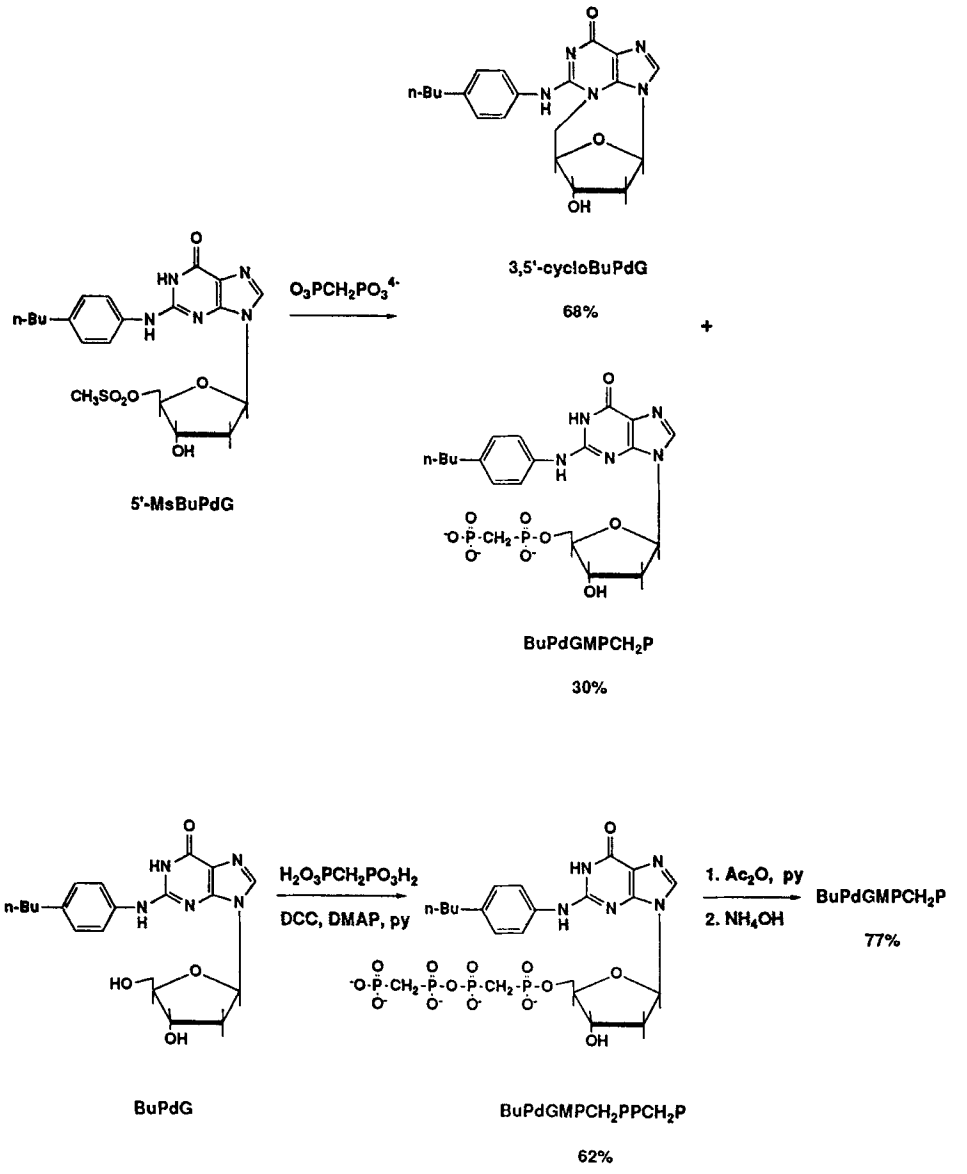
X=CH$_2$, BuPdGMPCH$_2$PP

The use of α,β [P^1,P^2] or β,γ [P^2, P^3] methylene or halomethylene analogs of nucleotides for enzymatic studies has been widely used since the first synthesis of the corresponding methylene-ATPs.[8,9] While the synthesis of P^2,P^3 analogs is straightforward, using the same chemistry as with natural triphosphates, the synthesis of P^1,P^2 analogs is somewhat more complicated. This generally requires the synthesis of the corresponding methylene-NDP followed by condensation with orthophosphate. Two general approaches have been used. In the first approach a protected nucleoside is condensed with a methanediphosphonic acid in the presence of DCC or trichloroacetonitrile in pyridine. The reaction is slow and causes

formation of polyphosphates which must be hydrolyzed at the end. This method has been used to prepare P^1,P^2-methylene analogs of ATP,[9] GTP[10] and dTTP,[11] 2-chloroADP,[12] guanosine 2',5'- and 3',5'-bis(methylene)-diphosphates[13] and P^1,P^2-(bromomethylene)ATP.[14] A potentially more attractive approach is nucleophilic displacement of 5'-tosyl nucleosides by pyrophosphate or methanediphosphonates, a method first used to prepare the dTTP analogs.[15] It is well known, however, that purine nucleosides bearing good leaving groups at C-5' (I, tosyl) tend to cyclize under conditions of nucleophilic substitution forming 3,5'-cyclonucleosides.[16] The group of Poulter overcame this problem by using very high concentrations of tetra-(n-butyl)ammonium pyrophosphate or methanediphosphonate in acetonitrile.[17] They reported the synthesis of methylene- and difluoromethylene-ADP and difluoromethylene-dADP in 50-70% yields.

We were unable to prepare the 5'-tosylate of BuPdG under standard conditions (TosCl, pyridine) used with other nucleosides. However reaction with mesyl chloride proceeded quickly to afford the 5'-mesyl derivative, 5'-MesBuPdG, in 67% yield accompanied by 9% of the 3',5'-dimesyl derivative. Because 5'-mesyl derivatives of unprotected guanine nucleosides have not been synthesized and tested for their tendency to cyclize under conditions of nucleophilic substitution, we tested the reactivity of 5'-MsBuPdG with azide ion. The reaction went smoothly to give 5'-azido-5'deoxyBuPdG in 98% yield without any evidence of cyclization. In addition to expected changes in the ^1H NMR spectrum, the characteristic IR stretching frequency of the azido group at 2100 cm^{-1} distinguished the product from the 5'-mesyl starting compound. The azide was reduced to 5'-amino-5'-deoxyBuPdG with triphenylphosphine and triethylamine[18] in 97% yield.[19]

Reaction of 5'-MesBuPdG with the tetra-(n-butyl)ammonium salt of methanediphosphonic acid under the conditions of Davisson et al.[17] (Scheme) gave 30% of the desired BuPdGMPCH$_2$P but 67% of a compound identified as 3,5'-cycloBuPdG (see below). BuPdGMPCH$_2$P was identified by ^1H and ^{31}P NMR. Only δ of 5',5" protons underwent a characteristic downfield shift (δ = 4.09) compared to the starting nucleoside, and the P^1,P^2-methylene group appeared as a triplet at δ 2.05 with a large coupling to ^{31}P (J = 19.5 Hz). The ^{31}P spectrum consisted of a multiplet arising from P^1 and two triplets for P^2, displaying J_{PH} = 19.5 Hz and J_{PCP} = 9.5 Hz. The latter value is



Scheme

typical for P-P coupling through carbon and smaller than the $J_{POP} \approx 20$ Hz typically observed in phosphoanhydrides.[6,7]

The ^1H NMR spectrum of the cyclonucleoside from the above reaction lacked an exchangeable downfield resonance corresponding to the purine 1-H, and the 5' and 5" proton resonances were much more non-equivalent ($\Delta\delta$ = 1.47 ppm) than in normal nucleosides, e.g., $\Delta\delta_{BuPdG}$ = 0.04 ppm,[20] indicating a rigid cyclic structure. A model of this compound revealed that one 5'-H/5"-H is in the plane (downfield) and another is perpendicular to the plane (upfield) of the purine ring. In addition, the 1'-H resonance was a doublet in which $J_{1',2'} = 5.81$ Hz but $J_{1',2''} \approx 0$. The torsion angle between 1'-H and 2'-H in a likely stable conformation of this model was about 20°, but that between 1'-H and 2"-H was close to 90°. Two dimensional NMR studies to confirm the conformational structure of 3,5'-cycloBuPdG are underway.

Use of DMF as solvent rather than MeCN increased the rate of this reaction but resulted exclusively in formation of the cyclonucleoside. This is in agreement with the results of Davisson et al.,[17] who found that a MeCN:DMSO solvent mixture increased the rate of consumption of 5'-tosyladenosine but reduced the yield of 5'-phosphates. Use of a solvent of lower polarity, $CHCl_3$, resulted (tlc) in relatively higher selectivity for $BuPdGMPCH_2P$, but the reaction was very slow: considerable starting material was present after two weeks at rt.

Direct condensation of unprotected BuPdG with tetra-(tri-n-butyl)ammonium methanediphosphonate was attempted under the conditions of Myers et al.[9] with dicyclohexylcarbodiimide (DCC) in pyridine. At rt the reaction was very slow, but at 60 °C there were signs of decomposition of the starting nucleoside. The reaction was faster in the presence of 4-(N,N-dimethylamino)pyridine (DMAP), and after 4 days at rt (Scheme), we isolated by DEAE-Sephadex chromatography a nucleotide which has been identified as the $P^1,P^2;P^3,P^4$-bis(methylene)tetraphosphate of BuPdG, $BuPdGMPCH_2PPCH_2P$, in 62% yield. The ^1H spectrum of this compound showed two triplets for the methylene groups, at δ 2.39 and 2.18, with J_{PH} = 20.6 and 20.2 Hz, respectively. Four ^{31}P resonances were observed. The downfield multiplet at δ 17.92 was assigned to P^1 based upon its simplification to a doublet of triplets during selective ^1H irradiation of H-5',5" at δ 4.09. The doublet of triplets at δ 11.98, which became a doublet in the fully ^1H-decoupled spectrum, was assigned to P^4. The other

two resonances, quartets of triplets at δ 13.31 and 7.71, respectively, could not be unequivocally assigned to P^2 or P^3. In the fully ^1H-decoupled spectrum, however, doublets of doublets displaying values typical of geminal J_{POP} and J_{PCP} were observed.

The tetraphosphonate was unusually resistant to hydrolysis. Conditions for cleaving polyphosphates have involved, for example, 88% formic acid at 25 °C for 16 hours[13] or 20% formic acid at 4 °C for 50 hours.[14] After storing the tetraphosphonate in 20% formic acid at rt for 3 days, we isolated only the base, BuPG, in quantitative yield, while the ^{31}P spectrum of the filtrate indicated that the tetraphosphonate moiety was intact. BuPdGMPCH$_2$PPCH$_2$P was stable in the presence of 1 M $Bu_4N^+F^-$ in 50% pyridine at rt for 3 days and in 0.2 M NaOH or 1.5 M pyridine-2-aldoxime in 50% dioxane[21] at rt for 3 days. The tetraphosphonate was successfully cleaved to the desired diphosphonate by the method of Khorana et al.[22] Treatment with acetic anhydride in pyridine followed by stirring of the mixed anhydride in 25% ammonium hydroxide gave BuPdGMPCH$_2$P in 77% yield. An attempt to cleave the tetraphosphonate by this method *in situ*, i.e. without its prior isolation, failed.

The target compound BuPdGMPCH$_2$PP was prepared in 36% yield in the usual way[23] by reaction of the imidazolide of BuPdGMPCH$_2$P with orthophosphate in hexamethylphosphoric triamide (HMPA). ^1H NMR showed the methylene triplet at δ 2.39 with J_{PH} = 20.3 Hz. The ^{31}P spectrum consisted of three resonances, a downfield multiplet for P^1 at δ 19.07 and an upfield doublet at δ -5.98 (J_{POP} = 24.4 Hz) assigned to P^3. The intermediate multiplet at δ 6.15, assigned to P^2, displayed couplings to P^1 (J = 8.0 Hz), P^3 (J = 24.4 Hz) and the methylene protons (J = 20.9 Hz).

BuPdGMPCH$_2$PP was compared with BuPdGTP as an inhibitor of immunopurified calf thymus DNA polymerase α. The enzyme was assayed[5] with activated DNA, dATP, dCTP and [^{3}H]dTTP, but lacking the competitor dGTP. Such a "truncated" assay has been shown[2] to provide a direct measure of K_i for a reversible inhibitor that is competitive with the omitted substrate. The phosphonate BuPdGMPCH$_2$PP inhibited DNA polymerase α in this assay with a K_i of 9.5 nM, about fivefold higher than that of the triphosphate BuPdGTP itself (K_i = 2 nM). This result shows that the replacement of the P^1,P^2 phosphoanhydride oxygen by a methylene group reduced affinity of the phosphonate to DNA polymerase α, but the compound is still a potent inhibitor of the enzyme. Significantly, BuPdGMPCH$_2$PP was found **not** to be

a substrate for bacteriophage T4 DNA polymerase (ms in preparation) under conditions where BuPdGTP itself was readily incorporated into a defined primer:template by this enzyme.[5] Consequently, BuPdGMPCH$_2$PP should prove to be a useful, non-substrate inhibitor:probe of B family DNA polymerases.

EXPERIMENTAL PROCEDURES

General. Melting points were determined on a Mel-temp apparatus and are uncorrected. ^{1}H and ^{31}P NMR spectra were recorded on a Varian Unity 300 instrument. Chemical shifts are referenced as follows: ^{1}H in Me_2SO-d_6 and $CDCl_3$, internal TMS; in D_2O, internal DSS; ^{31}P, external 1% phosphoric acid. Analyses for C, H and N were done by the Microanalysis Laboratory, University of Massachusetts, Amherst, and analyses for P were done as described.[24] Preparative HPLC was done with a Waters model 600 gradient system and a Lambda-max model 481 detector using a Synchropak AX-100 column (Rainin). Thin layer chromatography was done with Merck Kieselgel 60F-254 analytical plates, and column chromatography employed silica gel 230-400 mesh from EM Science. Pyridine was refluxed and distilled over calcium hydride. Hexamethylphosphoric triamide (HMPA) was distilled over calcium hydride under reduced pressure. Acetonitrile (MeCN) was "Sure-seal" obtained from Aldrich Chemical Co. All dry solvents were stored in the dark over molecular sieves (4 Å). DEAE-Sephadex was from Pharmacia.

5'-Mesyl-N^2-(*p*-n-butylphenyl)-2'-deoxyguanosine. Mesyl chloride (87 μL, 1.13 mmol) was added dropwise during 2 h to a stirred solution of N^2-(*p*-n-butylphenyl)-2'-deoxyguanosine[20] (300 mg, 0.75 mmol) in dry pyridine (3 mL) at 0 °C. After 0.5 h methanol (0.5 mL) was added, and the mixture was evaporated *in vacuo*. The residue was chromatographed on a silica gel column (4x17 cm) with 8:1 chloroform:methanol. The first fractions contained 3',5'-dimesyl-N^2-(*p*-n-butylphenyl)-2'-deoxyguanosine (38.3 mg, 9.2%), m.p. 172-174 °C (dec). NMR (Me_2SO-d_6) δ 10.67 (s, 1H, H-1), 8.83 (s, 1H, 2-NH), 8.06 (s, 1H, H-8), 7.47, 7.19 (d, 2H ea, Ph), 6.31 (t, 1H, H-1'; $J_{1'2'} = J_{1'2''} = 7.31$ Hz), 5.38 (m, 1H, H-3'), 4.46 (m, 1H, H-4'), 4.40-4.33 (m, 2H, H-5',5"), 3.35, 3.12 (s, 3H ea, $MeSO_2$), 3.05, 2.80 (m, 1H ea, H-2',2"), 2.56 (t, 2H, α-CH_2), 1.55, 1.31 (m, 2H ea, β,γ-CH_2), 0.90 (t, 3H, δ-CH_3). Anal. Calcd. for $C_{22}H_{29}N_5O_8S_2$: C, 47.56; H, 5.26; N, 12.60. Found: C, 47.54; H, 5.17, N, 12.51. Further elution gave the title compound, 5'-MesBuPdG, which was purified by slow evaporation of a $CHCl_3$:MeOH solution

with addition of petroleum ether to give colorless crystals (240 mg, 67%), m.p. 168-174 °C. NMR (Me_2SO-d_6) δ 10.59 (s, 1H, H-1), 8.74 (s, 1H, 2-NH), 8.02 (s, 1H, H-8), 7.47, 7.18 (d, 2H ea, Ph), 6.27 (t, 1H, H-1'; $J_{1'2'} = J_{1'2''} = 6.9$ Hz), 5.57 (d, 1H, OH; J = 4.15 Hz), 4.39 (m, 1H, H-3'), 4.35-4.20 (m, 2H, H-5',5"), 4.05 (m, 1H, H-4'), 3.11 (s, 3H, $MeSO_2$), 2.73, 2.36 (m, 1H ea, H-2',2"), 2.55 (t, 2H, α-CH_2), 1.55, 1.31 (m, 2H ea, β,γ-CH_2), 0.90 (t, 3H, δ-CH_3). Anal. Calcd. for $C_{21}H_{27}N_5O_6S \cdot 0.5H_2O$: C, 51.84; H, 5.80; N, 14.48. Found: C, 51.92; H, 5.68; N, 14.48.

Reaction of 5'-MesBuPdG with methanediphosphonate. A solution of methanediphosphonic acid (184 mg, 1.05 mmol) in water (5 mL) was brought to pH 7 with 40% tetra-(n-butyl)ammonium hydroxide. After evaporation and drying of the residue for 1 h at rt and 0.2 torr, and coevaporation of the latter twice with MeCN (10 mL), the residue was dissolved in dry MeCN (0.2 mL). 5'-MsBuPdG (100 mg, 0.21 mmol) was added, and the slurry was stirred for 3 days under nitrogen at rt. Dilution of the mixture with water (5 mL) and filtration gave, after washing with water and drying, colorless crystals (54.1 mg, 67.8%) of 3,5'-cycloBuPdG, m.p. 229-232 °C. ^{1}H NMR ($CDCl_3$:MeSO-d_6 4:1) δ 7.51 (s, 1H, H-8), 7.17, 6.77 (d, 2H each, Ph), 6.38 (d, 1H, H-1'; $J_{1'2'} = 5.81$ Hz, $J_{1'2''} = 0$), 5.44 (d, 1H, 3'-OH), 5.18 (dd, 1H, 5'-H, $J_{5'5''} = 14.6$ Hz, $J_{4'5'} = 2.71$ Hz), 4.70 (m, 1H, 3'-H), 4.58 (m, 1H, 4'-H), 3.71 (dd, 1H, 5"-H, $J_{5'5''} = 14.6$ Hz, $J_{4'5''} = 2.45$ Hz), 2.58 (t, 2H, α-CH_2), 2.48-2.25 (m, 2H, 2',2"-H), 1.60 (quint, 2H, β-CH_2), 1.39 (sext, 2H, γ-CH_2), 0.95 (t, 3H, CH_3). Anal. Calcd. for $C_{20}H_{23}N_5O_3$: C, 62.98; H, 6.08; N, 18.36. Found: C, 62.69; H, 5.99; N, 17.71. The filtrate and washings were loaded on a DEAE-Sephadex column (2x20 cm), and products were eluted with a linear gradient of 0.2-1.3 M triethylammonium bicarbonate (TEAB) during 16 h at a flow rate of 2.67 mL/min. Fractions 76-88 (15 mL each) were coevaporated with n-BuOH, and the residue was lyophilized giving 54.8 mg (30.4%) of N^2-(*p*-n-butylphenyl)-2'-deoxyguanosine 5'-(methylene)diphosphate, BuPdGMPCH$_2$P, as the bis(TEA) salt. A solution of this compound (11 mg) in water was passed through a column of Chelex 100 (0.5x5 cm) in the Na^+ form. The product was eluted with water (2 mL), and lyophilization of the eluate gave 9.0 mg of BuPdGMPCH$_2$P as the disodium salt. ^{1}H NMR (D_2O) δ 8.08 (s, 1H, 8-H), 7.55, 7.27 (d, 2H ea, Ph), 6.42 (t, 1H, H-1'; $J_{1'2'} = J_{1'2''} = 6.64$ Hz), 4.77 (m, 1H, 3'-H), 4.22 (m, 1H, 4'-H), 4.18-3.99 (m, 2H, 5',5"-H), 2.81 (m, 1H, 2'-H), 2.63 (t, 2H, α-CH_2), 2.52 (m, 1H, 2"-H), 2.05 (t, 2H, PCH_2P; $J_{PH} = 19.5$ Hz), 1.61 (quint, 2H,

β-CH_2), 1.35 (sext, 2H, γ-CH_2), 0.93 (t, 3H, δ-CH_3). ^{31}P NMR (D_2O) δ 22.71 (m, P^1), 11.50 (d of t, P^2; J_{PCP} = 9.5 Hz, J_{PH} = 19.1 Hz). Anal. Calcd. for $C_{21}H_{27}N_5O_9P_2Na_2$: P, 10.30. Found: P, 10.23.

Condensation between BuPdG and methanediphosphonic acid. BuPdG (100 mg, 0.25 mmol) was added to a solution of methanediphosphonic acid (176 mg, 1 mmol) in a mixture of dry pyridine (5 mL) and tri-(n-butyl)amine (0.95 mL, 4 mmol) under nitrogen. Dicyclohexylcarbodiimide (826 mg, 4 mmol) was added with stirring followed by the addition of a solution of 4-(N,N-dimethylamino)pyridine (122 mg, 1 mmol) in dry pyridine (1 mL). A precipitate formed immediately, and the suspension was stirred at rt under nitrogen until no starting material remained (tlc), about 2-4 days in several experiments. The mixture was diluted with water (100 mL) and shaken with 200 mL of diethyl ether. The mixture was filtered and the solid was washed with a mixture of water and ether. The combined filtrates were separated by centrifugation, and the aqueous layer was concentrated *in vacuo* and loaded on a DEAE-Sephadex column (2x20 cm). The products were eluted by a linear gradient of 0.2-1.3 M TEAB during 16 h at a flow rate of 2.67 mL/min. Fractions 108-124 (16 mL each) were combined and coevaporated with n-BuOH and lyophilized to give 173 mg (62%) of N^2-(*p*-n-butylphenyl)-2'-deoxyguanosine 5'-(P^1,P^2;P^3,P^4-bis[methylene])-tetraphosphate, BuPdGMPCH_2PPCH_2P, as the TEA salt. A sample was converted to the sodium salt as described above. ^1H NMR (D_2O) δ 4.09 (m, 2H, H-5',5"), 2.39 (t, 2H, $P^1CH_2P^2$; J_{PH} = 20.6 Hz), 2.18 (t, 2H, $P^3CH_2P^4$; J_{PH} = 20.2 Hz), all other resonances essentially identical to those of BuPdGMPCH_2P. ^{31}P NMR (D_2O) δ 17.92 (m, P^1; ^1H irradiation at δ 4.09 gave d of t, J_{PH} = 19.8 Hz, J_{PCP} = 8.05 Hz), 13.31 (q of t, P^3; J_{PH} = 21.2 Hz, J_{PCP} = 7.6 Hz, J_{POP} = 28.6 Hz), 11.98 (t of d, P^4; J_{PH} = 18.7 Hz, J_{PCP} = 7.7 Hz), 7.71 (q of t, P^2; J_{PH} = 20.9 Hz, J_{PCP} = 8.1 Hz, J_{POP} = 28.7 Hz). Anal. Calcd. for $C_{22}H_{29}N_5O_{14}P_4Na_4 \cdot H_2O$: P, 15.08. Found: P, 15.11.

Hydrolysis of BuPdGMPCH_2PPC_2P. Acetic anhydride (2 mL) was added to a stirred solution of the TEA salt of BuPdGMPCH_2PPCH_2P (99.4 mg, 88.7 μmol). After stirring for 24 h at rt under nitrogen the reddish solution was evaporated at rt and 0.3 torr. The residue was evaporated once with water, and then dissolved in 25% aqueous ammonia (3 mL). After stirring for 24 h the solution was evaporated *in vacuo* and the residue dissolved in 0.3 M TEAB (3 mL), and chromatographed on a DEAE-Sephadex column with a linear TEAB gradient as described above. Product was isolated from

fractions 70-83 giving 52 mg (77.2%) of BuPdGMPCH$_2$P as the TEA salt. This product, and a sample of the sodium salt prepared as above, were identical (chromatography, ^{1}H, ^{31}P NMR) to the corresponding product from direct displacement of 5'-MesBuPdG (see above).

5'-Azido-N^2-(*p*-n-butylphenyl)-2',5'-dideoxyguanosine (5'-N$_3$BuPdG). 5'-MesBuPdG (70 mg, 0.147 mmol) and lithium azide (21.5 mg, 0.44 mmol) were dissolved in dry DMF (0.42 mL), and the sealed solution was stored at 60 °C for 24 h. The mixture was diluted with water (4 mL), and the resulting precipitate was filtered and washed with water to give 61 mg (98%) of 5'-N$_3$BuPdG as a colorless solid, mp 231-232 °C (dec). ^{1}H NMR (Me$_2$SO-d$_6$) δ 10.68 (bd s, 1H, 1-H), 8.82 (bd s, 1H, 2-NH), 8.02 (s, 1H, 8-H), 3.47-3.26 (m, 2H, 5',5"-H), all other resonances essentially identical to those of 5'-MesBuPdG. IR (KBr) 2100 cm^{-1}. Anal. Calcd. for C$_{20}$H$_{24}$N$_8$O$_3$·0.5H$_2$O: C, 55.42; H, 5.81; N, 25.85. Found: C, 55.51; N, 5.67; N, 25.76.

5'-Amino-N^2-(*p*-n-butylphenyl)-2',5'-dideoxyguanosine (5'-NH$_2$BuPdG). Triphenylphosphine (97.5 mg, 0.37 mmol) was added to a solution of 5'-N$_3$BuPdG (50.5 mg, 0.12 mmol) in methanol (7.5 mL) containing TEA (1.5 mL). After stirring at rt for 30 h and evaporation of solvents, the residue was isolated by chromatography on silica gel. Elution with CHCl$_3$:MeOH:25% ammonia (4:1:0.2) gave 44.7 mg (97%) of 5'-NH$_2$BuPdG, m.p. 210-213 °C (dec). ^{1}H NMR (Me$_2$SO-d$_6$) 6.22 (t, 1H, 1'-H; $J_{1'2'} = J_{1'2"} = 6.84$ Hz), 5.5 (v bd s + bd s, 3H, OH and NH$_2$), 2.74 (m, 3H, 2',5',5"-H), all other resonances essentially identical to those of 5'-MesBuPdG. Anal. Calcd. for C$_{20}$H$_{26}$N$_6$O$_3$·0.5H$_2$O: C, 58.95; H, 6.68; N, 20.62. Found: C, 58.69; H, 6.52; N, 20.34.

N^2-(*p*-n-butylphenyl)-2'-deoxyguanosine 5'-(P^1,P^2-methylene)triphosphate, BuPdGMPCH$_2$PP. The TEA salt of BuPdGMPCH$_2$P (39.5 mg, 52 μmol) was converted to the TBA salt by passing an aqueous solution through a column of Dowex 50, pyridinium form, (0.5x5 cm), elution with water (5 mL), and coevaporation of the eluate with tri(n-butyl)amine (37.2 μL, 156 μmol). After lyophilization a solution of the residue in HMPA (1 mL) was stirred at rt with a solution of 1,1'-carbonyldiimidazole (76.4 mg, 416 μmol) in HMPA (0.5 mL). After 2.5 h a solution of mono(tri-n-butyl)ammonium phosphate (147 mg, 520 μmol) in HMPA (1 mL) was added, and the solution was stirred under nitrogen at rt for 20 h. Ice/water (5 mL) was added slowly to the chilled reaction mixture. The product was isolated by chromatography on DEAE-Sephadex (2x20 cm) using a linear gradient of 0.2-

1.3 M TEAB at a flow rate of 2.67 mL/min over 24 h. Fractions 119-136 (18.5 mL each) were combined and coevaporated with n-BuOH, and the residue was lyophilized. The product was converted to the sodium salt as described above yielding 13.1 mg (36%) of BuPdGMPCH$_2$PP contaminated with about 10% of starting material. This product was purified by HPLC on a Synchropak AX-100 column (1x25 cm) in a gradient of 30% MeCN in water to 30% MeCN in 0.2 M ammonium bicarbonate during 60 min at a flow rate of 8 mL/min. Fractions containing the product (at 0.08-0.1 M ammonium bicarbonate) were lyophilized and converted to the sodium salt as described above to give 6.6 mg of pure product. ^{1}H NMR (D_2O) δ 6.42 (t, 1H, 1'-H; $J_{1'2'} = J_{1'2''} = 6.82$ Hz), 2.39 (t, 2H, PCH$_2$P; $J_{PH} = 20.3$ Hz), all other resonances essentially identical to those of BuPdGMPCH$_2$P. ^{31}P (D_2O) δ 19.07 (m, P^1), 6.15 (q of t, P^2; $J_{PH} = 20.9$ Hz, $J_{PCP} = 8.0$ Hz, $J_{POP} = 24.4$ Hz), -5.98 (d, P^3; $J_{POP} = 24.4$ Hz). Anal. Calcd. for $C_{21}H_{27}N_5O_{12}P_3Na_3 \cdot 0.5H_2O$: P, 13.04. Found: P, 13.07.

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