2-(4-Nitrophenyl)ethyl Methylenebis(phosphonate): A Versatile Reagent for the Synthesis of Nucleoside 5′-Methylenebis(phosphonate)s

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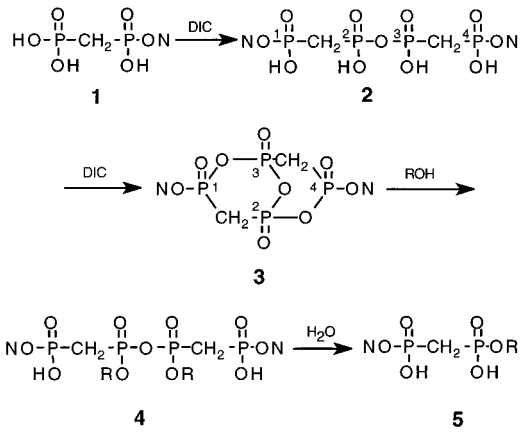
2-(4-Nitrophenyl)ethyl methylenebis(phosphonate) (**6**) was prepared by reaction of equimolar amounts of 2-(4-nitrophenyl)ethyl alcohol and methylenebis(phosphonyl) tetrachloride in the presence of tetrazole. Compound **6** was further converted into the corresponding 4-nitrophenylethyl trisanhydride intermediate **7** by dehydration with diisopropylcarbodiimide (DIC)**.** Reaction of **7** with either 2′,3′-*O*-isopropylideneadenosine (**8a**) or 2′,3′-*O*-isopropylideneguanosine (**8b**) afforded, after hydrolysis, the desired P^1-[2-(4-nitrophenyl)ethyl]-P^2-(2′,3′-*O*-isopropylideneadenosin-5′-yl) methylenebis(phosphonate) (**9a**) and guanosine analogue **9b**, respectively. A similar treatment of intermediate **7** with 3′-*O*-acetylthymidine (**12a**), 3′-*O*-acetyl-2′-deoxy-N^4-benzoylcytidine (**12b**), 3′-*O*-acetyl-2′-deoxy-N^6-benzoyladenosine (**12c**), and 3′-*O*-acetyl-2′-deoxy-N^2-isobutyrylguanosine (**12d**) gave the corresponding 2-(4-nitrophenyl)ethyl methylenebis(phosphonate)s **13a**−**d**. These compounds as well as **9a**,**b** were treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) which caused elimination of the 2-(4-nitrophenyl)ethyl group. The base labile 3′-*O*-acetyl, N^4-acetyl, N^6-benzoyl, and N^2-isobutyryl groups of **12a**−**d** were also removed during the DBU treatment. Thus, the 5′-methylenebis(phosphonate)s of 2′,3′-*O*-isopropylideneadenosine (**10a**), 2′,3′-*O*-isopropylideneguanosine (**10b**), thymidine (**14a**), 2′-deoxycytidine (**14b**), 2′-deoxyadenosine (**14c**), and 2′-deoxyguanosine (**14d**) were prepared in good yield. De-*O*-isopropylidenation of **10a** and **10b** afforded adenosine 5′-methylenebis(phosphonate) (**11a**) and guanosine 5′-methylenebis(phosphonate) (**11b**), respectively.

Introduction

Recently, we reported[1] the synthesis of several P^1,P^2-disubstituted methylenebis(phosphonate) analogues of biologically important P^1,P^2-disubstituted pyrophosphates using a new method of activation of nucleoside 5′-methylenebis(phosphonate)s **1** with dehydrating agents such as dicyclohexylcarbodiimide (DCC) or diisopropylcarbodiimide (DIC). We found that reaction of **1** (Scheme 1) with DIC led to the formation of P^1,P^4-dinucleoside tetraphosphonates **2** which further underwent P^1,P^3 and P^2,P^4-dehydration with DIC to give the corresponding bicyclic trisanhydrides **3** as major products. Compounds **3** were found to be potent phosphonylating reagents, reacting with protected nucleosides, carbohydrates, and alcohols to form the corresponding P^2,P^3-disubstituted P^1,P^4-dinucleoside tetraphosphonates **4**. After hydrolysis and deprotection the desired methylenebis(phosphonate) analogues **5** of such biologically important pyrophosphates as benzamide adenine dinucleotide (BAD), flavine adenine dinucleotide (FAD), ADP-ribose, CDP-diacylglycerol, and CDP-ethanolamine were obtained.[1,2] Although our DIC-promoted coupling of nucleoside 5′-methylenebis(phosphonate)s with nucleosides was efficient, as we recently prepared an analogue of benzamide adenine dinucleotide, β-methylene-BAD, in 95% overall yield,[2] the synthesis of the starting nucleoside 5′-methylenebis(phosphonate)s is rather cumbersome. It is known that the DCC coupling of nucleosides with methylenebis(phosphonic acid) is inefficient due to formation of polyphosphonates.[3] The more attractive approach, described by Poulter,[4] was a nucleophilic displacement of the 5′-*O*-tosyl group of nucleosides with the tris(tetrabutylammonium) salt of methylenebis(phosphonic acid). In the case of purine nucleosides, however, such 5′-tosylates readily form the 3,5′-cyclic nucleosides, giving a low yield of the desired nucleoside 5′-methylenebis(phosphonate)s. Purine nucleosides protected as the 2′,3′-*O*-isopropylidene-5′-*O*-tosyl derivatives are known to be

Scheme 1



1 2 3 4 5

(1) Pankiewicz, K. W.; Lesiak, K.; Watanabe, K. A. *J. Am. Chem. Soc.* **1997**, *119*, 3691.

(2) Pankiewicz, K. W.; Lesiak, K.; Zatorski, A.; Goldstein, B. M.; Carr, S. F.; Sochacki, M.; Majumdar, A.; Seidman, M.; Watanabe, K. A. *J. Med. Chem.* **1997**, *40*, 1287.

(3) Yanachkov, I.; Wright, G. E. *Nucleosides Nucleotides* **1994**, *13*, 339 and references herein.

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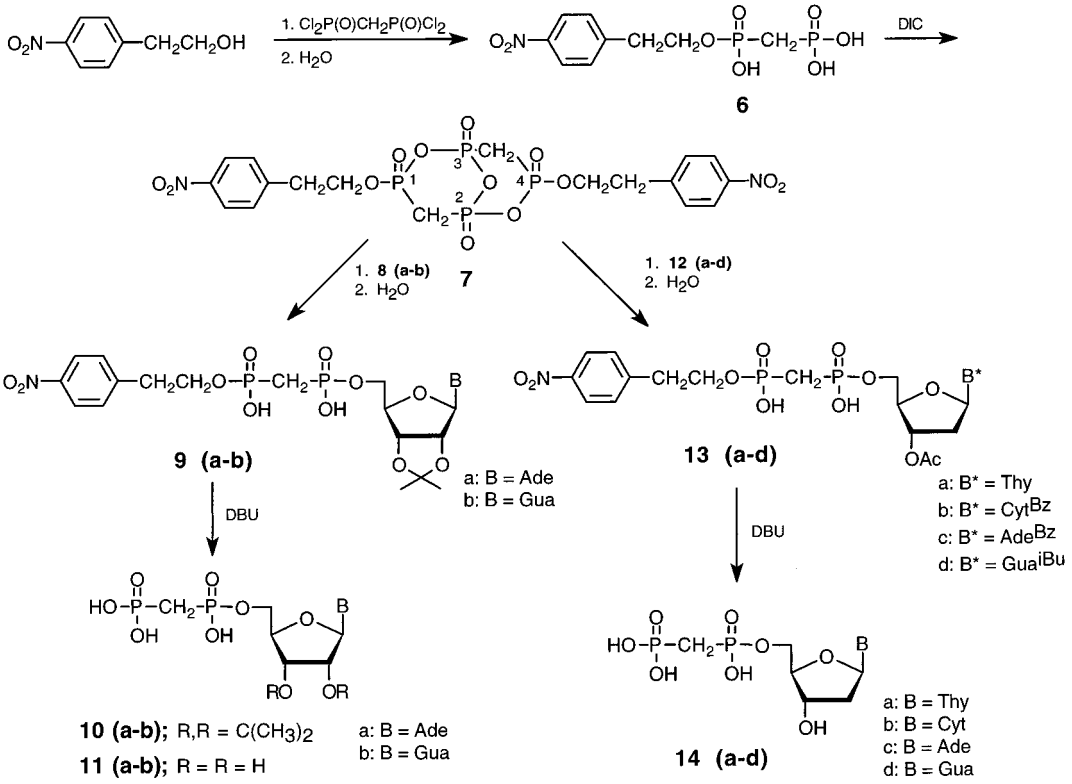
Scheme 2



converted into the 3,5′-cyclic nucleosides spontaneously. Such conversion has been used as a chemical verification of the β-configuration assignment of purine ribofuranosides.[5]

It has been reported that an almost exclusive formation of 3,5′-cyclic purine nucleosides occurred in attempted phosphorylation of the 5′-hydroxyl group of purine nucleosides via the Mitsunobu reaction in DMF or HMPA.[6] Recently, however, an efficient phosphorylation/phosphonylation of purine nucleosides in pyridine under Mitsunobu conditions was described in the literature.[7] This approach requires the synthesis of the tribenzyl ester of methylenebis(phosphonic acid)[8] and consecutive debenzylation of the bis(phosphonate) products.

Herein we report a general synthesis of nucleoside and deoxynucleoside 5′-methylenebis(phosphonate)s using anhydride **7** prepared from 2-(4-nitrophenyl)ethyl methylenebis(phosphonate) (**6**, Scheme 2).

Results and Discussion

2-(4-Nitrophenyl)ethyl methylenebis(phosphonate) (**6**) was prepared from equimolar amounts of 2-(4-nitrophenyl)ethyl alcohol and methylenebis(phosphonyl) tetrachloride.[9] After hydrolysis with 1 M triethylammonium bicarbonate, a mixture of mono-, di-, and trinitrophenylethyl-substituted methylenebis(phosphonate)s was obtained. The major product, the desired monosubstituted derivative **6**, was isolated by HPLC in 36% yield.

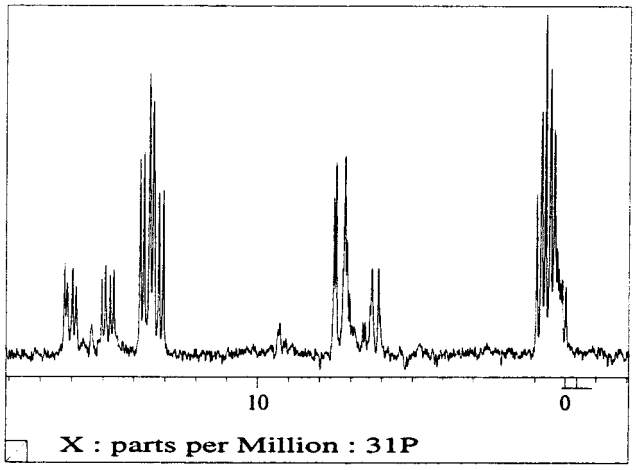


Figure 1. ^{31}P NMR of the reaction mixture of 2-(4-nitrophenyl)ethyl methylenebis(phosphonate) with DIC in pyridine.

Treatment of **6** with DIC in pyridine afforded **7** as a mixture of diastereomers due to the presence of four chiral phosphorus atoms as evidenced by ^{31}P NMR spectrum (Figure 1).

Addition of 2′,3′-*O*-isopropylideneadenosine (**8a**) or 2′,3′-*O*-isopropylideneguanosine (**8b**) at this stage of the reaction caused gradual simplification of the ^{31}P NMR spectrum, which showed two narrow multiplets at δ 7 and 18 ppm. Finally, addition of water to the reaction mixture resulted in the ^{31}P NMR showing an AB system (as the major signal) of the desired product **9a** or **9b**, respectively. Treatment of **9a** and **9b** separately with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) caused removal of the 4-nitrophenylethyl group by β-elimination and provided the desired 2′,3′-isopropylidene-protected methylenebis(phosphonate)s **10a** and **10b** in overall yields of

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32% and 51%, respectively. De-*O*-isopropylidenation of **10a** and **10b** with Dowex 50WX8/H^+ afforded the desired adenosine 5′-methylenebis(phosphonate) (**11a**) and guanosine 5′-methylenebis(phosphonate) (**11b**), respectively.

In a similar manner, reaction of **7** with the protected nucleosides[10] 3′-*O*-acetylthymidine (**12a**), 3′-*O*-acetyl-2′-deoxy-N^4-benzoylcytidine (**12b**), 3′-*O*-acetyl-2′-deoxy-N^6-benzoyladenosine (**12c**), and 3′-*O*-acetyl-2′-deoxy-N^2-isobutyrylguanosine (**12d**) afforded the corresponding 2-(4-nitrophenyl)ethyl-protected nucleotide analogues **13a**−**d** in good yields. Treatment of **13a**−**d** with DBU removed the 4-nitrophenylethyl group as well as the acetyl, benzoyl, or isobutyryl protecting groups. Thus the desired 5′-methylenebis(phosphonate) analogues of thymidine **14a**, 2′-deoxycytidine **14b**, 2′-deoxyadenosine **14c**, and 2′-deoxyguanosine **14d** were obtained efficiently in two steps from **6** in overall yield of 53%, 53%, 45% and 31%, respectively.

On the basis of the known reactivity of nucleoside bicyclic trisanhydrides **3**,[1] it is reasonable to assume that our 2-(4-nitrophenyl)ethyl intermediate **7** would also react with a variety of nucleosides, alcohols, and carbohydrates. Therefore, 2-(4-nitrophenyl)ethyl methylenebis(phosphonate) (**6**), as a precursor of **7**, is expected to have a broad application as a versatile reagent for the synthesis of methylenebis(phosphonate) analogues of a variety of nucleoside 5′-diphosphates and related derivatives.

Experimental Section

General Methods. For a description, see ref 1.

2-(4-Nitrophenyl)ethyl Methylenebis(phosphonate) (6). Methylenebis(phosphonyl) tetrachloride[9] (1.5 g, 6.0 mmol) was added to a solution of 2-(4-nitrophenyl)ethyl alcohol (1.0 g, 6.0 mmol) and tetrazole (490 mg, 7.0 mmol) in chloroform (10 mL) (tetrazole did not dissolve completely). The mixture was left for 16 h at room temperature with stirring, during which tetrazolium chloride gradually precipitated. The reaction was quenched by addition to 1 M TEAB (50 mL), and the resulting two-phase solution was vigorously stirred for 4 h. The aqueous layer was separated and concentrated, and the product was purified by preparative HPLC. Compound **6** was obtained as the bis(triethylammonium) salt. Yield: 1.06 g (33.5%, lightly yellow oil). ^{1}H NMR (D_2O) δ: 1.20 (t, 18H, Et_3N, J = 7.4 Hz), 1.97 (t, 2H, PCH_2P $J_{P,H}$ =19.8 Hz), 3.02 (t, 2H, $CH_2CH_2PhNO_2$, J = 6.7 Hz), 3.14 (q, 12H, Et_3N, J = 7.4 Hz), 4.08 (q, 2H, NO_2-$PhCH_2CH_2OP$, J = 6.7 Hz), 7.49 (d, 2H, H2, H6, J = 8.3 Hz), 8.14 (d, 2H, H3, H5, J = 8.3 Hz). ^{31}P NMR (D_2O) δ: 14.7 (d, 1P, J = 8.8 Hz) and 19.8 [d, 1P, P(NPE), J = 8.8 Hz].

***P*^{1}-[2-(4-Nitrophenyl)ethyl]-*P*^{2}-(2′,3′-*O*-isopropylideneadenosin-5′-yl) Methylenebis(phosphonate) (9a).** 2-(4-Nitrophenyl)ethyl methylenebis(phosphonate) (**6**, 117 mg, 0.22 mmol) was dissolved in anhydrous pyridine (0.8 mL) in an NMR tube. DIC (156 μL, 1.0 mmol) was added to the tube and the mixture was kept at room temperature for 2−3 h until the ^{31}P NMR showed multisignal resonances (see Figure 1). At this stage of the reaction **8a** (0.25 mmol) was added and the mixture was kept at 55−60 °C for approximately 16−24 h until the ^{31}P NMR spectrum showed two broad signals at δ 7 and 18 ppm. After addition of water (200 μL) and Et_3N (100 μL) the reaction mixture was incubated at 75 °C for 1 h. The mixture was then diluted with water (up to 5 mL), extracted with ethyl acetate, concentrated to dryness, and purified by HPLC to give 2′,3′-*O*-isopropylideneadenosine 5′-methylenebis(phosphonate) (63 mg, 35%). ^{1}H NMR (D_2O) δ: 1.26 (t, 18H, Et_3N, J = 7.3 Hz), 1.43 (s, 3H, CH_3C), 1.66 (s, 3H, CH_3C), 1.10 (t, 2H, PCH_2P, $J_{H,P}$ = 19.9 Hz), 2.87 (t, 2H, $CH_2CH_2PhNO_2$, J = 6.9 Hz), 3.18 (q, 12H, Et_3N, J = 7.3 Hz), 4.01 (q, 2H, NO_2-$PhCH_2CH_2OP$, J = 6.9 Hz), 4.10 (dd, 2H, H5′ and H5″, $J_{4',5'}$ = 3.4 Hz and $J_{5',P}$ = 5.4 Hz), 4.58 (m, 1H, H4′), 5.16 (dd, 1H, H3′, $J_{2',3'}$ = 6.2 Hz, $J_{3',4'}$ = 2.2 Hz), 5.23 (dd, 1H, H2′, $J_{1',2'}$ = 3.6 Hz, $J_{2',3'}$ = 6.2 Hz), 6.14 (d, 1H, H1′, $J_{1',2'}$ = 3.6 Hz), 7.25 [d, 2H, H2 and H6, (NO_2Ph) J = 8.7 Hz], 7.88 [d, 2H, H3 and H5, (NO_2Ph) J = 8.7 Hz], 8.11 [s, 1H, H2 (Ade)], 8.25 [s, 1H, H8 (Ade)]. ^{31}P NMR (D_2O) δ: 17.57 [d, 1P, P(NPE), J_{P^1,P^2} = 12 Hz], 18.08 [d, 1P, P(Ade), J_{P^1,P^2} = 12.0 Hz].

***P*^{1}-[2-(4-Nitrophenyl)ethyl]-*P*^{2}-(2′,3′-*O*-isopropylideneguanosin-5′-yl) methylenebis(phosphonate) (9b)** was obtained in the same manner starting from **6** (90 mg, 0.165 mmol) and **8a** (58 mg, 0.18 mmol). Yield: 86 mg [63%, as bis(triethylammonium) salt]. ^{1}H NMR (D_2O) δ: 1.26 (t, 18H, Et_3N, J = 7.3 Hz), 1.42 (s, 3H, CH_3C), 1.63 (s, 3H, CH_3C), 2.10 (t, 2H, PCH_2P, $J_{P,H}$ = 19.9 Hz), 2.90 (t, 2H, CH_2CH_2-$PhNO_2$, J = 6.8 Hz), 3,19 (q, 12H, Et_3N, J = 7.3 Hz), 4.04 (q, 2H, $CH_2CH_2PhNO_2$, J = 6.8 Hz), 4.10 (m, 2H, H5′, H5″), 4.48 (m, 1H, H4′), 5.16 (dd, 1H, H3′, $J_{2',3'}$ = 6.3 Hz, $J_{3',4'}$ = 2.2 Hz), 5.20 (dd, 1H, H2′, $J_{2',3'}$ = 6.3 Hz, $J_{1',2'}$ = 2.8 Hz), 5.96 (d, 1H, H1′, $J_{1',2'}$ = 2.8 Hz), 7.32 [d, 2H, H2, H6 ($PhNO_2$)], 7.95 [s, 1H, H8 (Gua)], 7.96 [d, 2H, H3, H5 ($PhNO_2$)]. ^{31}P NMR (D_2O) δ: 17.64 [d, 1P, P(NPE), $J_{P,P}$ =12 Hz], 18.05 [d, 1P, P(Gua), $J_{P,P}$ = 12 Hz].

***P*^{1}-[2-(4-Nitrophenyl)ethyl]-*P*^{2}-(2′-deoxynucleos-5′-yl) Methylenebis(phosphonate)s (13a−d).** Compounds **13a**−**d** were prepared according to the procedure described for compound **9a**, but a smaller amount of triethylamine (25 μL) was used in the hydrolysis step. The following compounds were obtained in this manner:

***P*^{1}-[2-(4-Nitrophenyl)ethyl]-*P*^{2}-(3′-*O*-acetylthymidin-5′-yl) Methylenebis(phosphonate) (13a).** Yield: 113 mg [65%, as bis(triethylammonium) salt]. ^{1}H NMR (D_2O) δ: 1.24 (t, 18H, Et_3N, J = 7.3 Hz), 1.74 [s, 3H, CH_3 (Thy)], 2.06 (t, 2H, PCH_2P, J = 19.9 Hz), 2.10 (s, 3H, OAc), 2.30 (m, 2H, H2′ and H2″), 3.02 (t, 2H, $CH_2CH_2PhNO_2$, J = 6.7 Hz), 3.16 (q, 12H, Et_3N, J = 7.3 Hz), 4.04 (m, 2H, H5′ and H5″), 4.12 (q, 2H, $CH_2CH_2PhNO_2$, J = 6.7 Hz), 4.25 (m, 1H, H4′), 5.29 (m, 1H, H3′), 6.26 (dd, 1H, H1′, $J_{1',2'}$ = 7.1 Hz, $J_{1',2''}$ = 7.6 Hz), 7.47 [d, 2H, H2, H6 ($PhNO_2$), J = 8.6 Hz], 7.59 [s, 1H, H6, (Thy)], 8.08 [d, 2H, H3, H5 ($PhNO_2$), J = 8.6 Hz]. ^{31}P NMR (D_2O) δ: 17.62 [d, 1P, P(NPE), J = 11.2 Hz], 17.73 [d, 1P, P(Thy), J = 11.6 Hz].

***P*^{1}-[2-(4-Nitrophenyl)ethyl]-*P*^{2}-(2′-deoxy-3′-*O*-acetyl-N^4-benzoylcytidin-5′-yl) Methylenebis(phosphonate) (13b).** Yield: 136 mg [70%, as bis(triethylammonium) salt]. ^{1}H NMR (D_2O) δ: 1.24 (t, 18H, Et_3N, J = 7.3 Hz), 2.11 (t, 2H, PCH_2P, J_{HP} = 20.0 Hz), 2.12 (s, 3H, OAc), 2.35 (ddd, 1H, H2″, $J_{1'2''}$ = 8.0 Hz, $J_{2'',3'}$ = 6.3 Hz, $J_{2',2''}$ = 14.3 Hz), 2.60 (ddd, 1H, H2′, $J_{1',2'}$ = 5.9 Hz, $J_{2',3'}$ = 2.0 Hz, $J_{2',2''}$ = 14.3 Hz), 2.99 (t, 2H, $CH_2CH_2PhNO_2$, J = 6.6 Hz), 3.16 (q, 12H, Et_3N, J = 7.3 Hz), 4.13 (m, 4H, H5′, H5″, $CH_2CH_2PhNO_2$), 4.42 (m, 1H, H4′), 5.32 (m, 1H, H3′), 6.26 (dd, 1H, H1′, $J_{1',2''}$ = 8.0 Hz, $J_{1',2'}$ = 5.9 Hz), 7.22 [d, 1H, H6 (Cyt), J = 7.4 Hz], 7.40 [d, 2H, H2, H6 ($PhNO_2$), J = 8.8 Hz], 7.5−7.9 (m, 5H, N^4-Bz), 7.97 [d, 2H, H3, H5 ($PhNO_2$), J = 8.8 Hz], 8.34 [d, 1H, H5 (Cyt), J = 7.4 Hz]. ^{31}P NMR (D_2O) δ: 17.45 [d, 1P, P(NPE), J = 11.6 Hz] and 18.22 [d, 1P, P(Cyt), J = 11.6 Hz].

***P*^{1}-[2(4-Nitrophenyl)ethyl]-*P*^{2}-(2′-deoxy-3′-*O*-acetyl-N^6-benzoyladenosin-5′yl) Methylenebis(phosphonate) (13c)**. Yield: 111 mg [56%, as bis(triethylammonium) salt]. ^{1}H NMR (D_2O) δ: 1.26 (t,18H, Et_3N, J = 7.3 Hz), 2.12 (t, 2H, PCH_2P, J = 19.9 Hz), 2.18 (s, 3H, OAc), 2.66 (ddd, 2H, H2″, $J_{1',2''}$ = 5.9 Hz, $J_{2'',3'}$ = 1.7 Hz, $J_{2',2''}$ = 14.5 Hz), 2.82 (t, 2H, CH_2CH_2-$PhNO_2$, J = 6.7 Hz), 2.96 (ddd, 1H, H2′, $J_{1',2'}$ = 8.4 Hz, $J_{2',3'}$ = 6.0 Hz, $J_{2',2''}$ = 14.5 Hz), 3.18 (q, 12H, Et_3N, J = 7.3 Hz), 4.00 (dt, 2H, $J_{H,H}$ = 6.7 Hz, $J_{H,P}$ = 7.3 Hz, $CH_2CH_2PhNO_2$), 4.14 (m, 2H, H5′, H5″), 4.46 (m, 1H, H4′), 5.50 (m, 1H, H3′), 6.55 (dd, 1H, H1′, $J_{1'2''}$ = 5.9 Hz, $J_{1',2'}$ = 8.4 Hz), 7.22 [d, 2H, H2, H6 ($PhNO_2$), J = 8.9 Hz], 7.5−7.75 (m, 5H, N^6-Bz), 7.82 [d, 2H, H3, H5 ($PhNO_2$), J = 8.7 Hz], 8.64 and 8.72 [s, 1H each,

(10) Nucleosides **12a**−**d** were synthesized from the commercially available 5′-*O*-dimethoxytrityl derivatives of thymidine, N^4-benzoylcytidine, N^6-benzoyladenosine, and N^2-isobutyrylguanosine by acetylation with acetic anhydride in pyridine followed by removal of the dimethoxytrityl group by treatment with 80% acetic acid.

Table 1. ^{13}C NMR of Nucleoside 5′-Methylenebis(phosphonate)s

compd	C1'	C2'	C3'	C4'	C5'	PCH_2P	purine or pyrimidine
10a[a]	90.3	84.0	81.5	85.1 $J = 7.8$	64.2 $J = 4.5$	27.3 $J = 126$	118.7, 140.4, 148.7, 152.5, 155.3
10b[a]	90.2	83.9	81.5	85.3 $J = 7.9$	64.1 $J = 5.0$	27.5 $J = 125$	116.2, 137.9, 151.4, 154.0, 158.9
14a[b]	85.1	38.5	71.1	85.6 $J = 7.7$	64.0 $J = 4.6$	27.5 $J = 124$	118.8, 137.6, 151.9, 166.7
14b	86.3	39.3	70.8	86.0 $J = 7.7$	63.7 $J = 5.1$	27.6 $J = 125$	96.1, 142.9, 154.5, 163.8
14c	83.7	39.0	71.2	86.0 $J = 7.8$	63.9, $J = 5.0$	27.6 $J = 123$	118.5, 139.9, 148.5, 152.6, 155.4
14d	83.9	38.8	71.3	85.9 $J = 7.4$	63.9 $J = 5.0$	27.5 $J = 124$	115.5, 137.5, 151.2, 154.0, 158.5

[a] Chemical shifts of the carbons of the 2′,3′-*O*-ispropylidene group of **10a** and **10b** are 24.6, 26.3, 115.1; and 24.5, 26.2, 114.9, respectively. [b] Thymidine CH_3 group, 11.8 ppm.

H2 and H8 (Ade)]. ^{31}P NMR (D_2O) δ: 17.63 [d, 1P, P(NPE), J = 11.6 Hz] and 18.16 [d, 1P, P(Ade), J = 12.0 Hz].

***P*^{1}-[2-(4-Nitrophenyl)ethyl]-*P*^{2}-(2′deoxy-3′-*O*-acetyl-*N*^{2}-isobutyrylguanosin-5′-yl) Methylenebis(phosphonate) (13d)**. Yield: 73 mg [37%, as bis(triethylammonium) salt]. ^{1}H NMR (D_2O) δ: 1.21 (t, 6H, N^2-^{i}Bu, J = 6.5 Hz), 1.26 (t, 18H, Et_3N, J = 7.3 Hz), 2.12 (t, 2H, PCH_2P, J = 20.0 Hz), 2.15 (s, 3H, OAc), 2.54 (ddd, 1H, H2″, $J_{1',2''}$ = 5.9 Hz, $J_{2'',3'}$ = 2.0 Hz, $J_{2',2''}$ = 14.5 Hz), 2.81 (t, 2H, $CH_2CH_2PhNO_2$, J = 7.0 Hz, overlapped with m, 1H, N^2-^{i}Bu), 2.98 (ddd, 1H, H2′, $J_{1',2'}$ = 8.5 Hz, $J_{2',3'}$ = 6.0 Hz, $J_{2',2''}$ = 14.5 Hz), 3.18 (q, 12H, Et_3N, J = 7.3 Hz), 3.99 (q, 2H, $CH_2CH_2PhNO_2$, J = 7.0 Hz), 4.16 (m, 2H, H5′,H5″), 4.39 (m, 1H, H4′), 5.48 (m, 1H, H3′), 6.32 (dd, 1H, H1′, $J_{1',2''}$ = 5.9 Hz, $J_{1',2'}$ = 8.5 Hz), 7.25 [d, 2H, H2, H6 ($PhNO_2$), J = 8.8 Hz], 7.91 [d, 2H, H3, H5, ($PhNO_2$), J = 8.8 Hz], 8.24 [s, 1H, H8 (Gua)]. ^{31}P NMR (D_2O) δ: 17.53 [d, 1P, P(NPE), J = 12.0 Hz] and 18.11 [d, 1P, P(Gua), J = 12.4 Hz].

DBU Deprotection of 9a,b and 13a−d. General Procedure. The 2-(4-nitrophenyl)ethyl-protected methylenebis(phosphonate)s **9a,b** and **13a−d** (about 0.2 mmol) were dissolved in 2 M aqueous DBU (1 mL) and were kept for 4 days at 75−80 °C. The resulting deep orange solutions were diluted (up to 3 mL) with water, extracted with ethyl acetate, neutralized with 1 M HCl, and purified by HPLC. Triethylammonium salts of compounds **10** and **14** were then converted into corresponding sodium salts by passing through a column of Dowex 50WX8 (Na^+ form). The following compounds were obtained.

2′,3′-*O*-Isopropylideneadenosine 5′-Methylenebis(phosphonate) (10a). A yield of 34 mg (91%, as disodium salt) was obtained from **9a** [60 mg, bis(triethylammonium) salt]. ^{1}H NMR (D_2O) δ: 1.45 (s, 3H, CH_3C), 1.67 (s, 3H, CH_3C), 2.076 and 2.081 (1:1 ratio, t, 2H, PCH_2P, $J_{P,H}$ = 19.9 Hz), 4.09 (m, 2H, H5′,H5″), 4.66 (m, 1H, H4′), 5.22 (dd, 1H, H3′, $J_{2',3'}$ = 6.2 Hz, $J_{3',4'}$ = 2.2 Hz), 5.43 (dd, 1H, H2′, $J_{2',3'}$ = 6.2 Hz, $J_{1',2'}$ = 3.3 Hz), 6.27 (d, 1H, H1′, $J_{1',2'}$ = 3.3 Hz), 8.25 (s, 1H, H2), 8.47 (s, 1H, H8). See Table 1 for ^{13}C NMR data. ^{31}P NMR (D_2O) δ: 15.47 (d, 1P, $J_{P,P}$ = 9.2 Hz), 19.02 [d, 1P, P(Ade), $J_{P,P}$ = 9.2 Hz].

2′,3′-*O*-Isopropylideneguanosine 5′-Methylenebis(phosphonate) (10b). A yield of 41 mg [81%, as bis(triethylammonium) salt] was obtained from **9b** [66 mg, bis(triethylammonium) salt]. ^{1}H NMR (D_2O) δ: 1.21 (t, 18H, Et_3N, J = 7.3 Hz), 1.39 (s, 3H, CH_3C), 1.59 (s, 3H, CH_3C), 2.06 (t, 2H, PCH_2P, $J_{P,H}$ = 19.8 Hz), 3.13 (q, 12H, Et_3N, J = 7.3 Hz), 4.05 (m, 2H, H5′, H5″), 4.52 (m, 1H, H4′), 5.16 (dd, 1H, H3′, $J_{2',3'}$ = 6.2 Hz, $J_{3',4'}$ = 2.4 Hz), 5.31 (dd, 1H, H2′, $J_{1',2'}$ = 3.0 Hz, $J_{2',3'}$ = 6.2 Hz), 6.00 (d, 1H, H1′, $J_{1',2'}$ = 3.0 Hz), 7.99 (s, 1H, H8). ^{31}P NMR (D_2O) δ: 15.41 (d, 1P, $J_{P,P}$ = 9.4 Hz), 18.92 [d, 1P, P(Gua), $J_{P,P}$ = 9.4 Hz].

Thymidine 5′-Methylenebis(phosphonate) (14a). A yield of 50 mg (82%, as disodium salt) was obtained from the bis(triethylammonium) salt of **13a** (103 mg). ^{1}H NMR (D_2O) δ: 1.91 [d, 3H, CH_3(Thy), J = 1.1 Hz], 2.17 (t, 2H, PCH_2P, J = 19.8 Hz), 2.28−2.45 (m, 2H, H2′, H2″), 4.09 (m, 2H, H5′, H5″), 4.14 (m, 1H, H4′), 4.60 (m, 1H, H3′), 6.32 (t, 1H, H1′, J = 7.0 Hz), 7.72 [d, 1H, H6 (Thy), J = 1.1 Hz]. ^{31}P NMR (D_2O) δ: 15.61 (d, 1P, J = 9.9 Hz) and 18.85 [d, 1P, P(Thy), J = 9.9 Hz].

2′-Deoxycytidine 5′-Methylenebis(phosphonate) (14b). From **13b** [142 mg, bis(triethylammonium salt)] was obtained **14b** (46 mg, 76%, as the disodium salt). ^{1}H NMR (D_2O) δ: 2.18 (t, 2H, J = 19.9 Hz, PCH_2P), 2.38 (dt, 1H, H2″, $J_{1',2''}$ = 6.6 Hz, $J_{2'',3'}$ = 3.7 Hz, $J_{2',2''}$ = 14.0 Hz), 2.45 (td, 1H, H2′, $J_{1',2'}$ = 6.3 Hz, $J_{2',3'}$ = 4.1 Hz, $J_{2',2''}$ = 14.1 Hz), 4.13 (m, 2H, H5′, H5″), 4.21 (m, 1H, H4′), 4.61 (dt, 1H, H3′, $J_{2',3'}$ = 4.1 Hz, $J_{2'',3'}$ = 3.7 Hz, $J_{3',4'}$ = 6.0 Hz), 6.18 (d, 1H, $J_{5,6}$ = 7.6 Hz, H6), 6.31 (t, 1H, H1′, J = 6.6 Hz), 8.10 (d, 1H, $J_{5,6}$ = 7.6 Hz, H5). ^{31}P NMR (D_2O) δ: 15.46 (d, 1P, J = 9.8 Hz) and 19.05 [d, 1P, P(Cyt), J = 9.8 Hz].

2′-Deoxyadenosine 5′-Methylenebis(phosphonate) (14c). Compound **14c** (50 mg, 81%, as disodium salt) was prepared from the bis(triethylammonium) salt of **13c** (101 mg). ^{1}H NMR (D_2O) δ: 2.13 (t, 2H, PCH_2P, J = 19.9 Hz), 2.58 (ddd, 1H, H2″, $J_{1',2''}$ = 6.5 Hz, $J_{2'',3'}$ = 4.7 Hz, $J_{2',2''}$ = 13.9 Hz), 2.85 (ddd, 1H, H2′, $J_{1',2'}$ = 6.5 Hz, $J_{2',3'}$ = 7.2 Hz, $J_{2',2''}$ = 13.9 Hz), 4.00−4.15 (m, 2H, H5′, H5″), 4.26 (m, 1H, H4′), 4.75 (m, overlapped with HDO, H3′), 6.49 (t, 1H, H1′, $J_{1',2'}$ = $J_{1',2''}$ = 6.5 Hz), 8.26 (s, 1H, H2), 8.51 (s, 1H, H8). ^{31}P NMR (D_2O) δ: 15.68 (d, 1P, J = 9.2 Hz) and 22.66 [d, 1P, P(Ade), J = 9.7 Hz].

2′-Deoxyguanosine 5′-Methylenebis(phosphonate) (14d). Starting from **13d** [66 mg, bis(triethylammonium) salt] was prepared **14d** (29 mg, 83%, as disodium salt). ^{1}H NMR (D_2O) δ: 2.16 (t, 2H, PCH_2P, J = 19.9 Hz), 2.53 (ddd, 1H, H2″, $J_{1',2''}$ = 6.3 Hz, $J_{2'',3'}$ = 3.6 Hz, $J_{2',2''}$ = 14.0 Hz), 2.84 (ddd, 1H, H2′, $J_{1',2'}$ = 6.5 Hz, $J_{2',3'}$ = 7.4 Hz, $J_{2',2''}$ = 14.0 Hz), 4.10 (m, 2H, H5′, H5″), 4.25 (m, 1H, H4′), 4.75 (m, 1H, H3′, overlapped with HDO), 6.33 (t, 1H, H1′, $J_{1',2'}$ = $J_{1',2''}$ = 6.4 Hz), 8.20 (s, 1H, H8). ^{31}P NMR (D_2O) δ: 15.68 (d, 1P, J = 10.0 Hz) and 19.10 [d, 1P, P(Gua), J = 9.7 Hz].

Adenosine 5′-Methylenebis(phosphonate) (11a). To a solution of **10a** (6 mg, 172 OD_{260}, 0.0115 mmol) in water was added Dowex50WX8/H^+ (1 mL of suspension in water) and the mixture was left at room temperature for 24 h, with occasional stirring. Then the mixture was applied on the top of a Dowex 50WX8/Na^+ column (1 × 3 cm) and eluted with water. UV-absorbing fractions were combined and lyophilized to give **11a** (disodium salt, 158 OD_{260}, 5 mg, 0.0105 mmol) in 92% yield. ^{1}H NMR (D_2O) δ: 2.17 (t, 2H, PCH_2P, J = 19.8 Hz), 4.16 (two d, 2H, H5′ and H5″, $J_{4',5'}$ = $J_{4',5''}$ = 3.4 Hz), 4.37 (m, 1H, H4′), 4.55 (dd, 1H, H3′, $J_{2',3'}$ = 5.3 Hz, $J_{3',4'}$ = 3.8 Hz), 4.79 (dd, 1H, H2′, $J_{1',2'}$ = 5.6 Hz, $J_{2',3'}$ = 5.3 Hz), 6.12 (d, 1H, H1′, $J_{1',2'}$ = 5.6 Hz), 8.25 (s, 1H, H2), 8.52 (s, 1H, H8). ^{31}P NMR (D_2O) δ: 15.36 (d, 1P, J = 9.6 Hz) and 19.57 [d, 1P, P(Ade), J = 9.6 Hz].

Guanosine 5′-Methylenebis(phosphonate) (11b). The compound was obtained in the same manner as **11a**, starting from 14 mg (274 OD_{253}, 0.02 mmol) of **10b**. The yield was 10 mg (disodium salt, 266 OD_{253}, 0.0194 mmol, 97%). ^{1} H NMR (D_2O) δ: 2.17 (t, 2H, PCH_2P, J = 19.8 Hz), 4.14 and 4.16 (2d, 1H each, H5′ and H5″, $J_{4',5'}$ = $J_{4',5''}$ = 3.9 Hz), 4.33 (m, 1H, H4), 4.53 (dd, 1H, H3′, $J_{2',3'}$ = 5.5 Hz, $J_{3',4'}$ = 3.8 Hz), 4.79 (pseudo t, 1H, H2′, $J_{1',2'}$ = 5.8, $J_{2',3'}$ = 5.5 Hz), 5.92 (d, 1H, H1′, $J_{1',2'}$ = 5.8 Hz), 8.11 (s, 1H, H8). ^{31}P NMR (D_2O) δ: 15.53 (d, 1P, J = 9.2 Hz) and 19.35 [d, 1P, P(Gua), J = 9.2 Hz].

Supporting Information Available: Copies of the ^{1}H NMR spectra of **11a**, **11b**, **14a**, **14b**, **14c,** and **14d** (6 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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