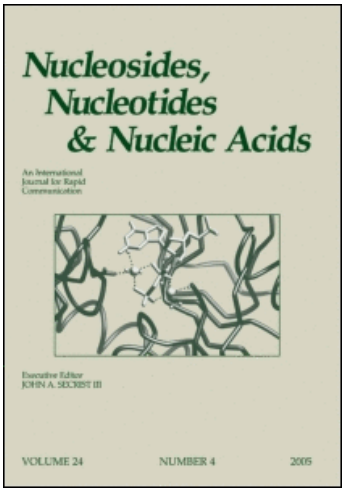

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Synthetic Approaches to Nuclease-Resistant, Nonnatural Dinucleotides of Anti-Hiv Integrase Interest

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SYNTHETIC APPROACHES TO NUCLEASE-RESISTANT, NONNATURAL DINUCLEOTIDES OF ANTI-HIV INTEGRASE INTEREST

Guochen Chi and Vasu Nair □ *Department of Pharmaceutical and Biomedical Sciences and The Center for Drug Discovery, University of Georgia, Athens, Georgia, USA*

□ *New, nonnatural dinucleotide 5′-monophosphates with a surrogate isonucleoside component of* L*-related stereochemistry, have been synthesized. Structures of the target compounds were confirmed by multinuclear NMR spectra (^{1}H, ^{13}C, ^{31}P, COSY), UV hypochromicity, FAB HRMS data and X-ray crystallography. These compounds are totally resistant to cleavage by 3′- and 5′-exonucleases. Dinucleotides of this study with a terminal* L*-isonucleoside component showed remarkable selectivity for inhibition of the strand transfer step of HIV-1 integrase. To the best of our knowledge, these compounds represent only the second example of this type of selectivity of inhibition of the strand transfer step.*

Keywords Synthesis; Phosphorylation; Exonuclease; Inhibitors; HIV integrase

INTRODUCTION

The retroviral enzyme, HIV integrase, is essential for the replication of HIV. It incorporates HIV double helical DNA into host chromosomal DNA.[1–7] The viral enzyme first catalyzes the enzymatic removal of two terminal nucleotides at the 3′-end of each strand of viral DNA (3′-processing) leaving recessed ends that terminate with xxCA-OH (Figure 1). In the next steps (strand transfer, integration) nucleophilic attack of the terminal 3′-OH of the tailored HIV DNA on a specific internucleotide phosphodiester

In honor and celebration of the life and career of Dr. John A. Montgomery.

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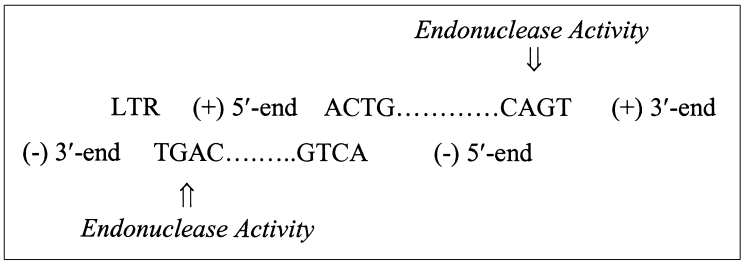


FIGURE 1 Processing of viral DNA prior to integration into host DNA.

functionality results in cleavage of host DNA and this is followed by integration of the tailored HIV DNA into host DNA.[1–3] The integration process is essential for the replication of HIV and there is apparently no functional equivalent of HIV integrase in human cells.

Some small oligonucleotides of natural origin are capable of interfering with the integration process by competing with viral DNA for binding to HIV integrase.[8] Protein-nucleotide interactions appear to be of importance in other steps of the replication cycle of HIV such as the recognition and binding of Tat protein to HIV-1 TAR RNA.[9] However, small oligonucleotides of natural origin are rapidly cleaved by cellular nuclease activity. In addition, increasing nuclease resistance by chemical alteration of the internucleotide phosphate bond results in decreased integrase activity.[8] A nonnatural dinucleotide with a conformationally unusual internucleotide phosphodiester bond that joins a D-deoxynucleoside and an L-related isodeoxynucleoside (pIsodApdC, Figure 2), previously synthesized by us,[10] exhibits resistance to mammalian 3′- and 5′-exonucleases.[11] This compound is an inhibitor of wild-type HIV-1 integrase, inhibiting both the 3′-processing and strand transfer steps.[10] We report here some new results that suggest that changing the

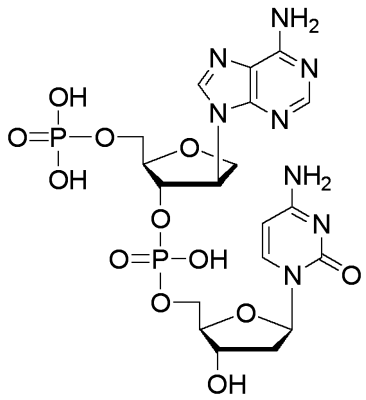


FIGURE 2 Structure of pIsodApdC.

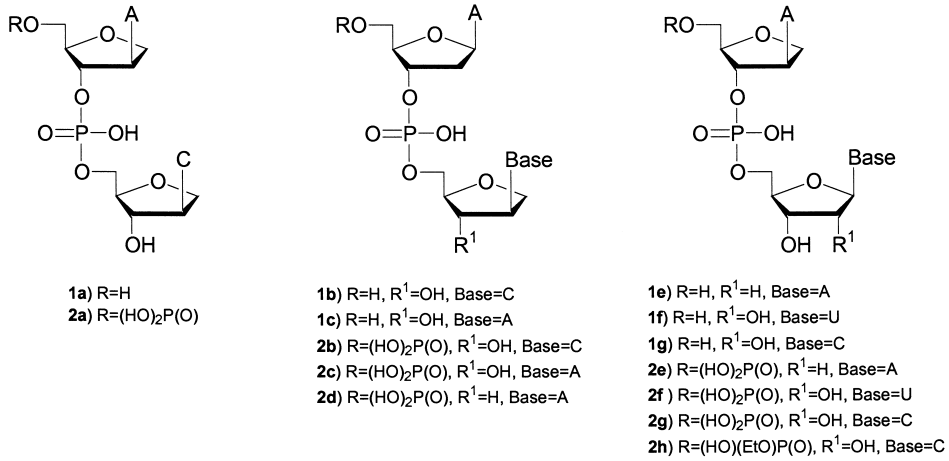
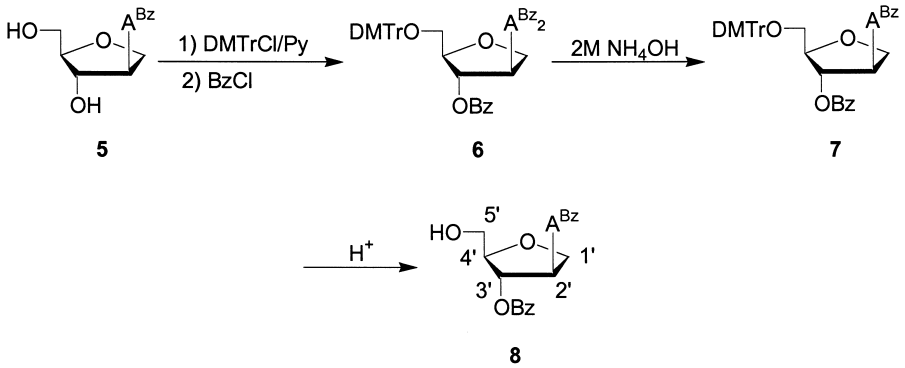


FIGURE 3 Structures of title nonnatural dinucleotides.

position of the surrogate nucleoside component of the dinucleotide (as in compound **2b**, Figure 3) can dramatically change the mode of inhibitory activity from both key steps of integrase action to just the strand transfer step. This article will describe the synthesis and anti-HIV integrase activity of these compounds (Figure 3).

RESULTS AND DISCUSSION

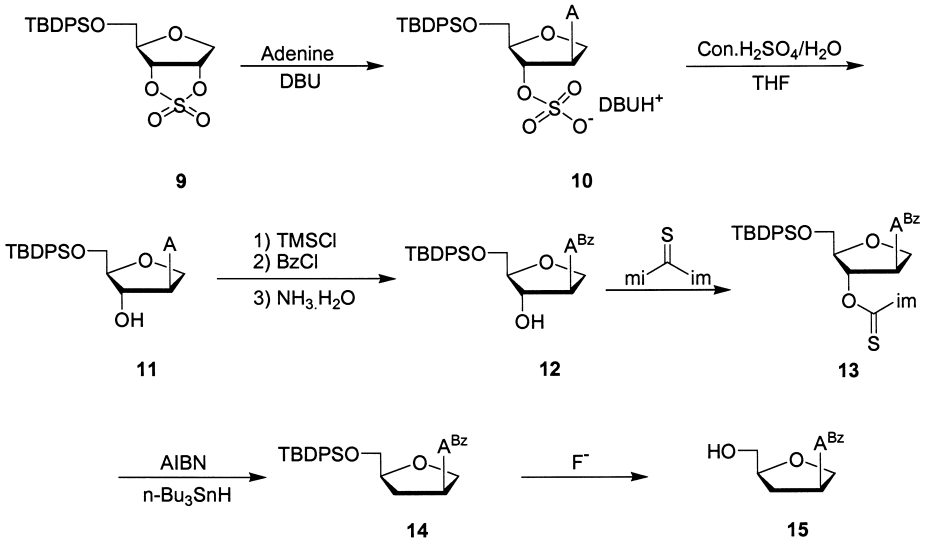
The preparation of dinucleotides utilized protected isodeoxy- or isodideoxy- nucleosides as starting materials. Thus, 6-N-benzoylisodeoxyadenosine **5**[12] was 5′-protected using dimethoxytrityl chloride in pyridine and benzoylated with benzoyl chloride to give intermediate **6** (Scheme 1). Addition of concentrated ammonium hydroxide selectively removed one 6-N-benzoyl



SCHEME 1 Synthesis of protected isodeoxyadenosine **8**.

group in the tribenzoyl intermediate **6** but the benzoate group at the 3′-position was not affected. Detritylation of the resulting compound **7** gave intermediate **8** (73% yield from **5**). 6-N, 3′-O-dibenzoyl-2′-deoxyadenosine[13] was prepared by a similar method from natural 2′-deoxyadenosine in 40% yield.

Although 6-N-benzoyl-isodideoxyadenosine **15** (Scheme 2) can be synthesized by deoxygenation and detritylation reactions from 6-N-benzoyl-5′-O-DMTr-isodeoxyadenosine, synthesis of the latter from isodeoxyadenosine was difficult on a gram scale because of the difficulty associated with purifying isodeoxyadenosine due to its relatively high polarity. An alternative procedure was through the cyclic sulfate **9**. Treatment of **9** with adenine and DBU gave **10** which, when treated with HCl in methanol, removed both the sulfate and silyl groups simultaneously to give isodeoxyadenosine.[14] Selective hydrolysis of the sulfate group was desirable as the protecting group at the 5′-position was required for the next step. Maintaining silyl protection at the 5′-position also simplified purification at this stage. Thus, after the cyclic sulfate **9** and adenine were heated in the presence of DBU in anhydrous CH_3CN for 2 h, the solvent was evaporated. The residue, in THF and MeOH, was treated with 2 equivalents of concentrated sulfuric acid in THF with 2 equivalents of water for 1.5 h.[15] Under these conditions, the reaction proceeded smoothly and the sulfate group was hydrolyzed selectively without cleavage of the silyl protecting group. Compound **11** was easily purified (64% yield from **9**) and was converted to **12** (88% yield) by the conventional method.[16] Compound **12** was deoxygenated[17,18] at the 3′-position

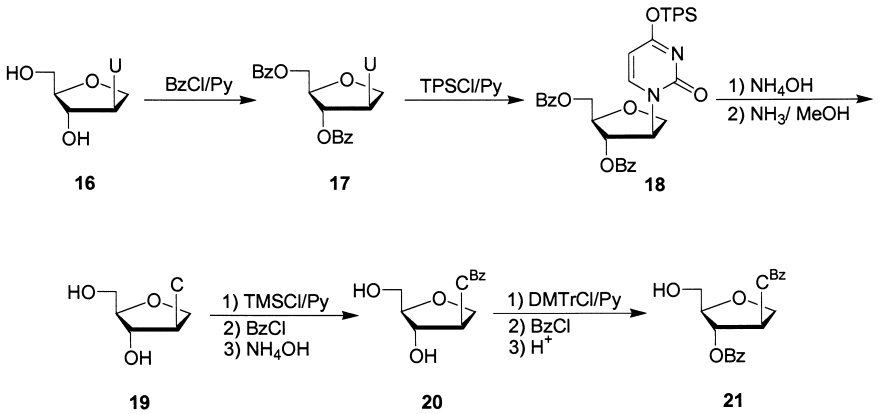


SCHEME 2 Synthesis of isodideoxyadenosine **15**.

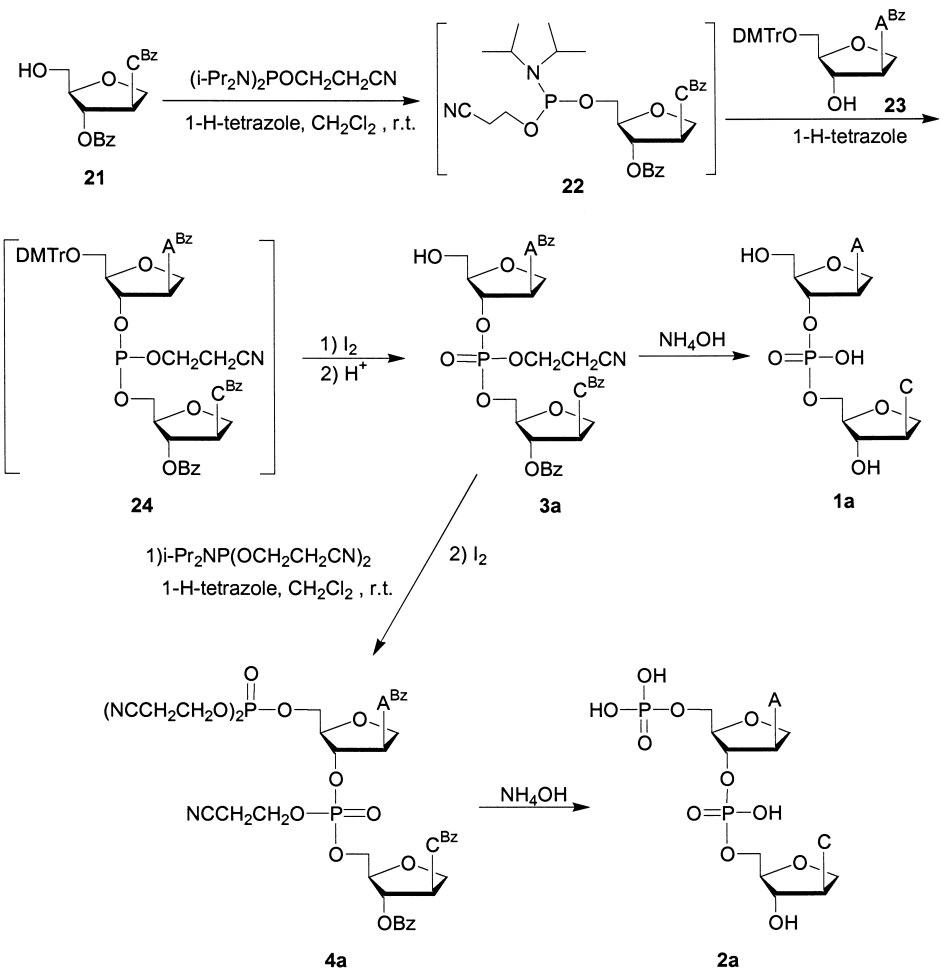
by conversion of the 3′-hydroxyl group to its imidazole thiocarbonyl ester (compound **13**) which was treated with tributyltin hydride and AIBN in refluxing toluene to give **14** in 63% yield. Desilylation with fluoride ions gave intermediate **15** (77% yield).

Isodeoxycytidine **19** (Scheme 3), another key intermediate, cannot be synthesized from the direct reaction of the sulfate **9** and cytosine, because O-alkylation and not N-alkylation of cytosine is the predominant reaction. However, it was synthesized from isodeoxyuridine **16** as previously described.[19] Thus, the dibenzoyl derivative **17** of isodeoxyuridine was converted to its corresponding 4-O-triisopropylbenzenesulfonyl derivative **18**. Ammonolysis of this intermediate and subsequent deprotection of the benzoyl group with methanolic ammonia afforded isodeoxycytidine **19**, which was fully characterized through its 4-N-benzoyl derivative **20** (76% yield from **16)**. Compound **20** can be tailored for coupling by its conversion to **21** in three steps (5′-O-tritylation, benzoylation and detritylation, 73% yield).

The dinucleotides were synthesized by the phosphoramidite method (Scheme 4).[20] Thus, for example, the free 5′-hydroxyl group of isonucleoside **21** was condensed with the reagent, 2-cyanoethyl tetraisopropylphosphorodiamidite, in the presence of 1H-tetrazole to give the intermediate **22** which was directly coupled with nucleoside **23**.[12] Subsequent oxidation with iodine and detritylation provided the phosphotriester **3a** (62% yield from **21**). 5′-Phosphorylation of **3a** was performed using *bis*(2-cyanoethyl) N, N-diisopropyl-phosphoramidite and 1-H-tetrazole. Compound **4a** was obtained in 85% yield after oxidation with iodine. The other protected dinucleotides, **3b-g** and **4b-g**, were synthesized using a similar approach as for **3a** and **4a** (Figure 4). For example, coupling isonucleoside **8** or **15** with 6-N-benzoyl-5′-O-DMTr-deoxyadenosine afforded **3c** or **3d**, respectively. Compound **4h** was synthesized from **3g** in 63% yield (Scheme 5).

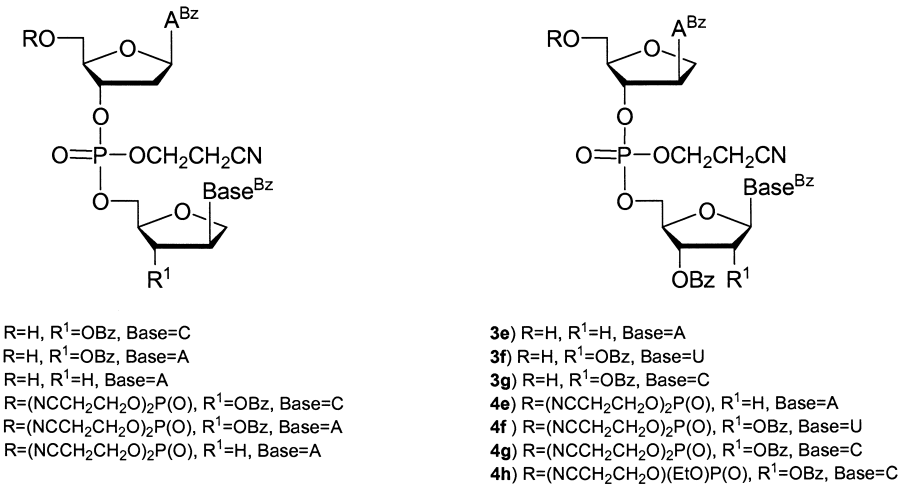


SCHEME 3 Synthesis of protected isodeoxycytidine **21**.



SCHEME 4 Synthetic route to nonnatural dinucleotide **2a**.

The protected dinucleotides, **3a-g** and **4a-h** (Scheme 4 and Figure 4), were deprotected using concentrated ammonium hydroxide at room temperature for 24 h. The benzoyl group and the 2-cyanoethyl group were simultaneously removed. Purification was performed by HPLC using a C-18 column with elution involving MeOH and 10 mM aqueous AcOH. The residual acetic acid was removed completely by coevaporation several times with water. Lyophilization produced the target compounds as white spongy solids. The yields of the target compounds **1** and **2** (Figure 3) were in the range of 58–89% for the deprotection step. The compounds were characterized by their ^{1}H, ^{13}C and ^{31}P NMR spectra, HRMS data, single-crystal X-ray crystallography[21] and quantitative UV spectral data.



3b) R=H, R^1=OBz, Base=C
3c) R=H, R^1=OBz, Base=A
3d) R=H, R^1=H, Base=A
4b) R=$(NCCH_2CH_2O)_2P(O)$, R^1=OBz, Base=C
4c) R=$(NCCH_2CH_2O)_2P(O)$, R^1=OBz, Base=A
4d) R=$(NCCH_2CH_2O)_2P(O)$, R^1=H, Base=A

3e) R=H, R^1=H, Base=A
3f) R=H, R^1=OBz, Base=U
3g) R=H, R^1=OBz, Base=C
4e) R=$(NCCH_2CH_2O)_2P(O)$, R^1=H, Base=A
4f) R=$(NCCH_2CH_2O)_2P(O)$, R^1=OBz, Base=U
4g) R=$(NCCH_2CH_2O)_2P(O)$, R^1=OBz, Base=C
4h) R=$(NCCH_2CH_2O)(EtO)P(O)$, R^1=OBz, Base=C

FIGURE 4 Structures of fully protected nonnatural dinucleotides.

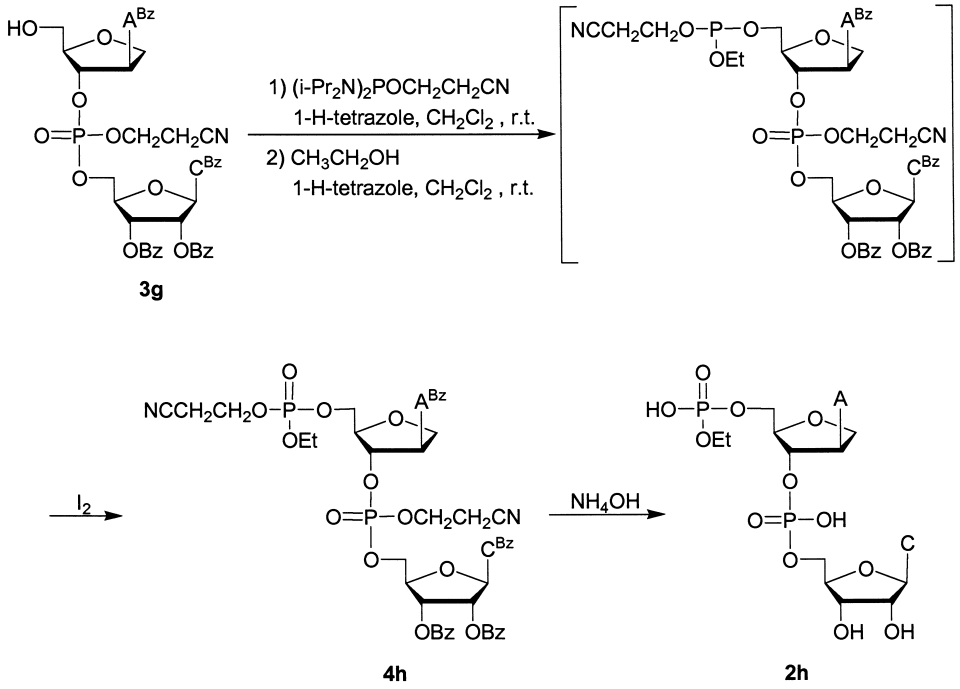
Integrase inhibition assays were conducted with purified recombinant HIV-1 integrase (wild type) using a 21-mer oligonucleotide substrate.[22] The data (Table 1) clearly showed that compounds **2b-d** have strand transfer inhibitory activity against wild-type HIV-1 integrase but do not exhibit inhibition of the 3′-processing step. This is in sharp contrast to compound pIsodApdC (Figure 2, L-related, D-stereochemistry) which showed strong inhibition of both key steps of the integrase mechanism of action.[10,23] The major structural difference between dinucleotide, pIsodApdC, and

TABLE 1 Anti-HIV-1 Integrase (Wild-type) Inhibition Data for Dinucleotides[22]

Compounds	3′-Processing IC_{50}, μM	Strand transfer IC_{50}, μM
pIsodApdC (Figure 2)[10]	19	25
pIsodApd5MeC[10]	60	50
pIsodApIsodC (**2a**)	>1000	>1000
pdApIsodC (**2b**)	>1000	65
pdApIsodA (**2c**)	>1000	41
pdApIsoddA (**2d**)	>1000	114
pIsodApdA (**2e**)	>1000	>1000
pIsodApU (**2f**)	>1000	>1000
pIsodApC (**2g**)	>1000	>1000
pdCpIsodT (**25**)[a]	405	58
pdCpIsodU (**26**)[a]	164	43

[a]Synthesized as described for compounds of the series **1** and **2** (see experimental for data).

Preliminary data on a few of the compounds were reported in the communication cited in Chi et al.[23]



SCHEME 5 Synthetic route to nonnatural dinucleotide **2h**.

its counterpart **2b** (or the related compound **2c**) is the position and accompanying stereochemistry of the surrogate isonucleoside component. The inhibition of integrase by pIsodApdC (Figure 2) is likely the result of base recognition and binding by the viral enzyme. Thus, it is remarkable that this apparently small structural and accompanying stereochemical change in the counterpart of the compound of Figure 3 (i.e., compounds **2b**, **2c**) can produce such a major impact on the mode of inhibition of integrase. Related results were also observed with pdCpIsodT and pdCpIsodU (Table 1). The only other reported selective inhibitor of strand transfer is a class of diketo containing compounds,[24–26] and, to the best of our knowledge, the compounds described here represent the second examples. Also, in comparing integrase activities of **2g** (pIsodApC) with pIsodApdC (Figure 2), it should be noted that the presence of 2′-OH in the cytidine moiety of **2g** resulted in complete loss of the activity. The other counterpart of the anti-HIV integrase active compound, pIsodApdC (Figure 2), one with two L-related isonucleoside components, pIsodApIsodC, **2a**, was also not an inhibitor. Finally, the internucleotide phosphodiester linkage of all dinucleotides with isonucleoside components were resistant to cleavage by mammalian 3′- and 5′-exonucleases.[11]

EXPERIMENTAL SECTION

General

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on Varian Mercury Plus 400 MHz or Varian Inova 500 MHz NMR spectrometers. High-resolution ESI or FAB mass spectral data were obtained through the Nebraska Center for Mass Spectrometry. UV spectra were recorded on a Varian Cary 3 UV-visible spectrometer. Column chromatographic separations were carried out using 230–400 mesh silica gel. HPLC separations were performed on a Beckman Gold HPLC using a Waters C_{18} column (300 × 30 mm). The solvent system was methanol and 10 mM aqueous HOAc with a flow rate of 5 mL/min (linear gradient from 0 to 8–25% methanol, depending on the polarity of the compound and then 8–25% methanol).

6-N, 3′-O-Dibenzoyl-1′-deoxy-2′-isoadenosine (8). A suspension of 6-N-benzoyl-1′-deoxy-2′-isoadenosine **5**[12] (2.13 g, 6 mmol) in anhydrous pyridine (60 mL) was treated with DMTrCl (4.07 g, 12 mmol). After 2 h, BzCl (2.79 mL, 24 mmol) was added gradually under ice-bath temperatures. The mixture was stirred overnight at room temperature, cooled in an ice bath, and then treated with water (5 mL). After 5 min, concentrated ammonium hydroxide (10 mL) was added (the concentration of NH_4OH in the mixture was about 2 M). After stirring at rt for 30 min, the pyridine was evaporated under vacuum and co-evaporated with toluene (2 × 40 mL). The residue was dissolved in CH_2Cl_2 (200 mL), washed with brine (80 mL) and saturated aqueous $NaHCO_3$ (80 mL). The methylene chloride layer was dried, filtered, and concentrated. The residue in CH_2Cl_2 (10 mL) was treated with 3% CF_3COOH in CH_2Cl_2 (60 mL) under ice-bath temperatures and then stirred at room temperature for 30 min. Additional CH_2Cl_2 (100 mL) was added and the solution was washed with saturated aqueous $NaHCO_3$ (60 mL × 2). The methylene chloride layer was dried, filtered, concentrated and purified on a silica gel column (CH_2Cl_2:CH_3OH, 30:1). The product (2.01 g) was obtained as a white solid (yield 73.0%). ^{1}HNMR (DMSO-d_6): 11.20 (s, 1H), 8.73 (s, 1H), 8.61 (s, 1H), 8.02 (m, 4H), 7.53–7.71 (m, 6H), 5.64 (dd, 1H, $J = 5.0, 3.0$), 5.46 (m, 1H), 5.17 (t, 1H, $J =$ 5.5, OH), 4.41 (dd, 1H, $J = 10.5, 4.0$), 4.34 (dd, 1H, $J = 10.5, 6.0$), 4.15 (m, 1H), 3.80 (m, 2H). ^{13}CNMR (DMSO-d_6): 166.1, 165.7, 152.7, 151.9, 150.7, 143.6, 134.3, 133.9, 132.9, 130.0, 129.4, 129.3, 129.0, 125.8, 84.9, 79.6, 70.9, 60.8, 60.7. FAB-HRMS: $[M+H]^+$ calcd. for $C_{24}H_{22}N_5O_5$ 460.1621, found 460.1606.

5′-O-(*t*-Butyldiphenylsilyl)-1′-deoxy-2′-isoadenosine (11). To a suspension of adenine (1.35 g, 10 mmol) in anhydrous CH_3CN (100 mL), DBU (1.60 g, 10.5 mmol) was added and the suspension was stirred at room tem-

perature for 0.5 h. A solution of cyclic sulfate **9** (4.35 g, 10 mmol) in CH_3CN (100 mL) was added and the reaction mixture was then heated at 75°C for 2 h. The solvent was then evaporated and the residue in THF (150 mL) and CH_3OH (10 mL) was treated with sulfuric acid solution in THF (20 mL, 1 mmol H_2SO_4/1 mmol H_2O in 1 mL THF) at 0°C and the reaction mixture was stirred at room temperature for 1.5 h. Triethylamine (6.07 g, 60 mmol) was added to neutralize the acid and the solvent was removed and the residue was purified on a silica gel column (CH_2Cl_2:CH_3OH, 25:1) to give **11** (3.12 g, 63.7%). ^{1}HNMR ($CDCl_3$): 8.20 (s, 1H), 7.80 (s, 1H), 7.65 (m, 4H), 7.32–7.41 (m, 6H), 6.40 (s, 2H, NH_2), 4.91 (m, 1H), 4.66 (dd, 1H, J = 5.5, 5.0), 4.45 (dd, 1H, J = 9.5, 6.5), 4.31 (dd, 1H, J = 9.5, 6.0), 4.05 (m, 1H), 3.93 (dd, 1H, J = 11.5, 3.5), 3.88 (dd, 1H, J = 11.5, 4.5), 1.01 (s, 9H). ^{13}CNMR ($CDCl_3$): 155.9, 152.7, 149.9, 138.7, 135.8, 133.3, 130.0, 127.9, 119.6, 85.4, 77.0, 69.9, 63.9, 63.3, 27.0, 19.5. ESI-MS: $[M+H]^+$ 490.

6-N-Benzoyl-5′-O-(*t*-butyldiphenylsilyl)-1′-deoxy-2′-isoadenosine (12) was synthesized in 87.5% yield from **11** by known methods.[16] ^{1}HNMR ($CDCl_3$): 9.21 (br, 1H), 8.67 (s, 1H), 8.05 (s, 1H), 7.98 (m, 2H), 7.32–7.67 (m, 13H), 5.37 (br, 1H, OH), 5.03 (m, 1H), 4.80 (t, 1H, J = 6.0), 4.43 (dd, 1H, J = 9.5, 6.5), 4.30 (dd, 1H, J = 9.5, 6.0), 4.04 (m, 1H), 3.96 (dd, 1H, J = 11.0, 3.0), 3.90 (dd, 1H, J = 11.0, 4.0), 1.02 (s, 9H). ^{13}CNMR ($CDCl_3$): 165.0, 152.5, 151.9, 149.6, 141.5, 135.7, 133.7, 133.3, 133.2, 133.1, 130.1, 130.0, 129.1, 128.1, 128.0, 127.9, 122.6, 85.1, 76.8, 70.0, 63.8, 63.2, 27.1, 19.5. ESI-MS: $[M+H]^+$ 594.

6-N-Benzoyl-1′, 3′-Dideoxy-2′-isoadenosine (15). A solution of 6-N-benzoyl-5′-O-tert-butyl-diphenylsilyl-1′-deoxy-2′-isoadenosine **12** (1.78 g, 3.0 mmol) and 1,1′-thiocarbonyl-diimidazole (802 mg, 4.5 mmol) in dry 1,2-dichloroethane (20 mL) was stirred under reflux for 4h. The solvent was evaporated and the residue was purified on a silica gel column ($CHCl_3$:CH_3OH, 50:1) to give 1.90 g (2.70 mmol, 90%) of **13**. To a refluxing solution of **13** in anhydrous toluene (20 mL), a nitrogen-purged solution of tributyltin hydride (1.21 mL, 4.5 mmol) and 2, 2′-azobis(2-methylpropionitrile) (394 mg, 2.4 mmol) in anhydrous toluene (15 mL) was added dropwise over a period of 15 min. The reaction mixture was stirred at reflux for 2 h and then the solvent was evaporated under reduced pressure. The resulting residue was purified on a silica gel column ($CHCl_3$:CH_3OH, 50:1) to afford 1.10 g (1.90 mmol) of **14**. Compound **14** was dissolved in THF (15 mL). Acetic acid (0.22 mL, 3.8 mmol) and TBAF solution in THF (3.8 mL, 3.8 mmol) were added under ice-bath temperatures. The resulting mixture was stirred at room temperature for 3 h. The solvent was removed and the residue was purified on a silica gel column ($CHCl_3$:CH_3OH, 40:1) to give 0.50 g of **15** as an amorphous solid (total yield from **12** was 49.1%). ^{1}HNMR ($CDCl_3$):

9.57 (br, 1H), 8.76 (s, 1H), 8.50 (s, 1H), 8.00 (d, 2H, $J = 7.5$), 7.58 (m, 1H), 7.47 (t, 2H, $J = 7.5$), 5.38 (m, 1H), 4.19 (d, 1H, $J = 10.5$), 4.11 (m, 1H), 4.03 (dd, 1H, $J = 10.5, 5.5$), 3.99 (dd, 1H, $J = 12.5, 2.0$), 3.62 (dd, 1H, $J = 12.5, 3.0$), 2.65 (m, 1H), 2.30 (m, 1H). ^{13}CNMR ($CDCl_3$): 165.0, 152.4, 151.5, 149.5, 141.7, 138.7, 132.6, 128.7, 127.9, 122.5, 79.8, 73.2, 62.5, 54.9, 33.8. FAB-HRMS: $[M+H]^+$ calcd. For $C_{17}H_{18}N_5O_3P$ 340.1410, found 340.1401.

1′-Deoxy-2′-isocytidine (19) was prepared from the dibenzoyl derivative of its uracil counterpart 1′-deoxy-2′-isouridine using the literature method.[14,19]

4-N-Benzoyl-1′-deoxy-2′-isocytidine (20) was synthesized in 86.5% yield from 1′-deoxy-2′-isocytidine by the standard method.[16] ^{1}HNMR (DMSO-d_6): 11.21 (s, 1H), 8.16 (d, 1H, $J = 7.0$), 7.99 (d, 2H, $J = 7.5$), 7.49–7.63 (m, 3H), 7.32 (d, 1H, $J = 7.0$), 5.71 (d, 1H, $J = 5.5$), 4.90 (m, 2H), 4.17 (m, 1H), 4.07 (dd, 1H, $J = 10.0, 6.5$), 3.96 (dd, 1H, $J = 10.0, 3.5$), 3.59–3.65 (m, 2H), 3.53 (m, 1H). ^{13}CNMR (DMSO-d_6): 167.7, 163.0, 155.6, 147.7, 133.6, 133.2, 128.94, 128.9, 96.7, 86.8, 76.4, 70.2, 65.3, 60.9. ESI-HRMS: $[M+H]^+$ calcd. for $C_{16}H_{18}N_3O_5$ 332.1246, found 332.1260.

4-N, 3′-O-Dibenzoyl-1′-deoxy-2′-isocytidine (21) was synthesized in 73.0% yield from **20** by a similar method to that for **8**, but without the addition of concentrated ammonia solution. ^{1}HNMR (DMSO-d_6): 11.26 (s, 1H), 8.24 (d, 1H, $J = 7.5$), 8.00 (m, 4H), 7.49–7.71 (m, 6H), 7.36 (d, 1H, $J = 7.5$), 5.47 (dd, 1H, $J = 5.5, 3.5$), 5.21 (m, 1H), 5.09 (t, 1H, $J = 5.5$), 4.22 (m, 2H), 4.05 (m, 1H), 3.73 (m, 2H). ^{13}CNMR (DMSO-d_6): 167.8, 165.7, 163.4, 155.4, 148.2, 134.3, 133.6, 133.2, 129.9, 129.5, 129.3, 128.9, 96.8, 85.1, 79.4, 70.2, 63.9, 60.7. ESI-HRMS: $[M+H]^+$ calcd. for $C_{23}H_{22}N_3O_6$ 436.1509, found 436.1511.

6-N-Benzoyl-P-(2-cyanoethyl)-1′-deoxy-2′-isoadenylyl-(3′→5′)-4-N, 3′-O-Dibenzoyl-1′-deoxy-2′-isocytidine (3a). To a suspension of **21** (261 mg, 0.60 mmol) and 1H-tetrazole (63 mg, 0.90 mmol) in dry CH_2Cl_2 (5 mL), 2-cyanoethyl N, N, N′,N′-tetraisopropylphosphoro-diamidite (271 mg, 0.90 mmol) was added. After stirring for 2 h, 1H-tetrazole (63 mg, 0.90 mmol) and **23**[12] (513 mg, 0.78 mmol) were added. The reaction mixture was stirred for 4 h and then iodine (460 mg, 1.81 mmol) in THF-H_2O-pyridine (66:33:1, 4.6 mL) was added. After 10 min, the mixture was poured into CH_2Cl_2 (100 mL) and washed with 0.2 M sodium sulfite (30 mL × 2). The organic layer was dried, filtered, and concentrated. The residue in 5 mL of dichloromethane was stirred with 2% dichloroacetic acid in CH_2Cl_2 (20 mL) for 20 min. The reaction mixture was poured into CH_2Cl_2 (100 mL) and

washed with saturated aqueous sodium bicarbonate (50 mL). The organic layer was dried, filtered, and concentrated. The residue was purified over silica gel column (CH_2Cl_2:CH_3OH, 25:1) to give **3a** (340 mg, 62.5%) as an amorphous solid. ^{1}H NMR ($CDCl_3$): 9.30 (br, 2H), 8.70 and 8.68 (s and s, 1H), 8.45 and 8.44 (s and s, 1H), 7.77–8.02 (m, 7H), 7.38–7.60 (m, 10H), 5.32–5.58 (m, 3H), 5.14 (m, 1H), 3.98–4.58 (m, 12H), 2.76 (m, 2H). ^{31}P NMR ($CDCl_3$): −1.57, −2.28. ESI-HRMS: $[M+H]^+$ calcd. for $C_{43}H_{41}N_9O_{12}P$ 906.2612, found 906.2627.

Compounds **3b-g** (data given below) were synthesized using a similar procedure to that described above for **3a**.

6-N-Benzoyl-P-(2-cyanoethyl)-2′-deoxyadenylyl-(3′→5′)-4-N, 3′-O-Dibenzoyl-1′-deoxy-2′-isocytidine (3b) (68.0% yield). ^{1}HNMR ($CDCl_3$): 9.35 and 9.21 (br and br, 2H), 8.69 and 8.57 (s and s, 1H), 8.32 and 8.30 (s and s, 1H), 7.85–8.02 (m, 7H), 7.39–7.62 (m, 10H), 6.49 (m, 1H), 5.95 (m, 1H, OH), 5.61 (m, 1H), 5.26–5.40 (m, 2H), 4.21–4.70 (m, 8H), 3.75–3.99 (m, 2H), 3.10 (m, 1H), 2.60–2.91 (m, 3H). ^{31}PNMR ($CDCl_3$): −1.64, −2.28. ESI-HRMS: $[M+Na]^+$ calcd. for $C_{43}H_{40}N_9NaO_{12}P$ 928.2432, found 928.2442.

6-N-Benzoyl-P-(2-cyanoethyl)-2′-deoxyadenylyl-(3′→5′)-6-N, 3′-O-Dibenzoyl-1′-deoxy-2′-isoadenosine (3c) (48.7% yield). ^{1}HNMR ($CDCl_3$): 9.03 (br, 2H), 8.80, 8.77, 8.74, and 8.71 (s, s, s and s, 2H), 8.43 and 8.41 (s and s, 1H), 8.21 and 8.16 (s and s, 1H), 7.96–8.07 (m, 6H), 7.42–7.66 (m, 9H), 6.51 and 6.26 (m and m, 1H), 5.68 and 5.64 (m and m, 1H), 5.51–5.55 (m, 1H), 5.37 and 5.30 (m and m, 1H), 4.25–4.70 (m, 8H), 3.80–4.00 (m, 2H), 3.07–3.23 (m, 1H), 2.59–2.94 (m, 3H). ^{31}PNMR ($CDCl_3$): −2.18, −2.39. FAB-HRMS: $[M+H]^+$ calcd. for $C_{44}H_{41}N_{11}O_{11}P$ 930.2725, found 930.2690.

6-N-Benzoyl-P-(2-cyanoethyl)-2′-deoxyadenylyl-(3′→5′)-6-N-benzoyl-1′, 3′-dideoxy-2′-isoadenosine (3d) (58.0% yield). ^{1}HNMR ($CDCl_3$): 8.79, 8.77, 8.76 and 8.73 (s, s, s and s, 2H), 8.42, 8.38, 8.25, and 8.19 (s, s, s and s, 2H), 8.03 (m, 4H), 7.47-7.65 (m, 6H), 6.52 and 6.40 (m and m, 1H), 5.45 (m, 1H), 5.39 and 5.36 (t and t, 1H, J = 5.5 and 5.5), 4.44–4.55 (m, 2H), 4.27–4.38 (m, 5H), 4.17 (m, 1H), 3.98 (m, 1H), 3.88 (m, 1H), 3.22 and 3.15 (m and m, 1H), 2.64–2.87 (m, 4H), 2.23 (m, 1H). ^{31}PNMR ($CDCl_3$): −1.29, −1.50. FAB-HRMS: $[M+H]^+$ calcd. For $C_{37}H_{37}N_{11}O_9P$ 810.2513, found 810.2505.

6-N-Benzoyl-P-(2-cyanoethyl)-1′-deoxy-2′-isoadenylyl-(3′→5′)-6-N, 3′-O-Dibenzoyl-2′-deoxyadenosine (3e) (69.9% yield). ^{1}HNMR ($CDCl_3$): 9.38 (br, 2H), 8.73, 8.69, 8.63 and 8.61 (s, s, s and s, 2H), 8.39, 8.38, 8.37 and 8.34 (s, s, s and s, 2H), 8.00–8.03 (m, 6H), 7.44–7.62 (m, 9H), 6.55 and

6.47 (m and m, 1H), 5.72 and 5.68 (m and m, 1H), 5.35 (m, 1H), 5.18 and 5.09 (m and m, 1H), 4.22–4.51 (m, 7H), 4.03 (m, 1H), 3.80–3.91 (m, 2H), 3.08–3.15 (m, 1H), 2.71–2.81 (m, 3H). ^{31}PNMR ($CDCl_3$): −2.57. FAB-HRMS: $[M+H]^+$ calcd. for $C_{44}H_{41}N_{11}O_{11}P$ 930.2725, found 930.2716.

6-N-Benzoyl-P-(2-cyanoethyl)-1′-deoxy-2′-isoadenylyl-(3′→5′)-3-N, 2′-O, 3′-O-Tribenzoyl Uridine (3f) (68.1% yield). ^{1}HNMR ($CDCl_3$): 9.15 (br, 1H), 8.78 and 8.75 (s and s, 1H), 8.47 and 8.42 (s and s, 1H), 7.88–8.00 (m, 8H), 7.26–7.81 (m, 13H), 6.05–6.10 (m, 1H), 5.94 (m, 1H), 5.77 (m, 1H), 5.61 (m, 1H), 5.51 (m, 1H), 5.29 (m, 1H), 4.46–4.55 (m, 2H), 4.32–4.40 (m, 4H), 4.25 (m, 1H), 4.15 (m, 1H), 3.97–4.06 (m, 2H), 2.79 (t, 2H, $J = 6.0$). ^{31}PNMR ($CDCl_3$): −1.23, −2.16. FAB-HRMS: $[M+H]^+$ calcd. for $C_{50}H_{44}N_8O_{15}P$ 1027.2664, found 1027.2633.

6-N-Benzoyl-P-(2-cyanoethyl)-1′-deoxy-2′-isoadenylyl(3′→5′)-4-N, 2′-O, 3′-O-Tribenzoyl Cytidine (3g) (66.5% yield). ^{1}HNMR ($CDCl_3$): 9.22 (br, 2H), 8,72, 8.68, 8.66, and 8.50 (s, s, s and s, 2H), 7.73–7.98 (m, 9H), 7.28–7.58 (m, 13H), 5.77–6.04 (m, 3H), 5.50 (m, 1H), 5.42 and 5.30 (m and m, 1H), 4.28–4.67 (m, 7H), 4.17 (m, 1H), 3.96–4.09 (m, 2H), 2.73–2.88 (m, 2H). ^{31}PNMR ($CDCl_3$): −1.67, −2.64. FAB-HRMS: $[M+H]^+$ calcd. for $C_{50}H_{45}N_9O_{14}P$ 1026.2824, found 1026.2809.

6-N-Benzoyl-5′-O-[di(2-cyanoethoxy)phosphinyl]-P-(2-cyanoethyl)-1′-deoxy-2′-isoadenylyl-(3′→5′)-4-N, 3′-O-Dibenzoyl-1′-deoxy-2′-isocytidine (4a). To a solution of **3a** (272 mg, 0.30 mmol) and 1H-tetrazole(42 mg, 0.60 mmol) in CH_2Cl_2 (5 mL), di(2-cyanolethyl) N, N-diisopropylphosphoramidite (163 mg, 0.60 mmol) was added. The resulting mixture was stirred at room temperature for 4 h. Iodine (300 mg, 1.18 mmol) in THF-H_2O-pyridine (66:33:1, 3.0 mL) was added. After 10 min, the mixture was poured into CH_2Cl_2 (100 mL) and washed with 0.2 M sodium sulfite (30 mL × 2). The organic layer was dried, filtered and concentrated. The residue was purified on a silica gel column (CH_2Cl_2:CH_3OH, 20:1) to give **4a** (279 mg, 85.1%) as an amorphous solid. ^{1}HNMR ($CDCl_3$): 9.11 (br, 2H), 8.74 and 8.72 (s and s, 1H), 8.36 and 8.32 (s and s, 1H), 7.85–8.03 (m, 7H), 7.39–7.63 (m, 10H), 5.43–5.60 (m, 2H), 5.16–5.31 (m, 2H), 4.07–4.65 (m, 16H), 2.67–2.85 (m, 6H). ^{31}PNMR ($CDCl_3$): −0.70, −1.75, −1.85, −2.27. ESI-HRMS: $[M+H]^+$ calcd. for $C_{49}H_{48}N_{11}O_{15}P_2$ 1092.2807, found 1092.2798.

Compounds 4b-g were synthesized using a similar procedure to that described for **4a**.

6-N-Benzoyl-5′-O-[di(2-cyanoethoxy)phosphinyl]-P-(2-cyanoethyl)-2′-deoxyadenylyl-(3′→5′)-4-N, 3′-O-Dibenzoyl-1′-deoxy-2′-isocytidine (4b) (90.3% yield). ^{1}HNMR ($CDCl_3$): 9.00 (br, 2H), 8.76 and 8.72 (s and s,

1H), 8.30 and 8.24 (s and s, 1H), 7.88–8.06 (m, 7H), 7.45–7.64 (m, 10H), 6.57 (m, 1H), 5.65 (m, 1H), 5.21–5.46 (m, 2H), 4.16–4.70 (m, 14H), 3.27 (m, 1H), 2.70–2.90 (m, 7H). ^{31}PNMR ($CDCl_3$): −0.89, −1.87, −1.92, −2.03. ESI-HRMS: $[M+Na]^+$ calcd. for $C_{49}H_{47}N_{11}NaO_{15}P_2$ 1114.2626, found 1114.2605.

6-N-Benzoyl-5′-O-[di(2-cyanoethoxy)phosphinyl]-P-(2-cyanoethyl)-2′-deoxyadenylyl-(3′→5′)-6-N, 3′-O-Dibenzoyl-1′-deoxy-2′-isoadenosine (4c) (93.1% yield). ^{1}HNMR ($CDCl_3$): 9.27 and 9.04 (br, 2H), 8.78, 8.76, 8.74, and 8.69 (s, s, s and s, 2H), 8.43 and 8.40 (s and s, 1H), 8.27 and 8.22 (s and s, 1H), 7.97–8.07 (m, 6H), 7.45–7.65 (m, 9H), 6.60 and 6.45 (m and m, 1H), 5.68 (m, 1H), 5.54 (m, 1H), 5.42 and 5.35 (m and m, 1H), 4.22–4.71 (m, 14H), 3.14–3.26 (m, 1H), 2.70–2.92 (m, 7H). ^{31}PNMR ($CDCl_3$): −2.13, −2.39, −2.45, −2.50. FAB-HRMS: $[M+H]^+$ calcd. for $C_{50}H_{48}N_{13}O_{14}P_2$ 1116.2919, found 1116.2865.

6-N-Benzoyl-5′-O-[di(2-cyanoethoxy)phosphinyl]-P-(2-cyanoethyl)-2′-deoxyadenylyl-(3′→5′)-6-N-benzoyl-1′, 3′-Dideoxy-2′-isoadenosine (4d) (60.3% yield). ^{1}HNMR ($CDCl_3$): 8.79, 8.77, and 8.73 (s, s and s, 2H), 8.45, 8.44, 8.31, and 8.27 (s, s, s and s, 2H), 8.03 (m, 4H), 7.49–7.63 (m, 6H), 6.60 and 6.50 (t and t, 1H, $J = 6.5$), 5.44 (s, br, 2H), 4.15–4.58 (m, 14H), 3.25 and 3.18 (m and m, 1H), 2.78–2.90 (m, 8H), 2.24 (m, 1H). ^{31}PNMR ($CDCl_3$): −1.32, −1.35, −1.57. FAB-HRMS: $[M+H]^+$ calcd. For $C_{43}H_{44}N_{13}O_{12}P_2$ 996.2708, found 996.2666.

6-N-Benzoyl-5′-O-[di(2-cyanoethoxy)phosphinyl]-P-(2-cyanoethyl)-1′-deoxy-2′-isoadenylyl-(3′→5′)-6-N, 3′-O-Dibenzoyl-2′-deoxyadenosine (4e) (83.6% yield). ^{1}HNMR ($CDCl_3$): 9.20 (br, 2H), 8.79, 8.76, 8.74, and 8.68 (s, s, s and s, 2H), 8.43, 8.40, 8.33, and 8.24 (s, s, s and s, 2H), 7.99–8.07 (m, 6H), 7.45–7.64 (m, 9H), 6.61 and 6.54 (m and m, 1H), 5.69–5.76 (m, 1H), 5.45 (m, 1H), 5.19–5.26 (m, 1H), 4.29–4.51 (m, 14H), 3.06–3.18 (m, 1H), 2.67–2.85 (m, 7H). ^{31}PNMR ($CDCl_3$): −2.31, −2.37, −2.41, −2.56. FAB-HRMS: $[M+H]^+$ calcd. for $C_{50}H_{48}N_{13}O_{14}P_2$ 1116.2919, found 1116.2895.

6-N-Benzoyl-5′-O-[di(2-cyanoethoxy)phosphinyl]-P-(2-cyanoethyl)-1′-deoxy-2′-isoadenylyl-(3′→5′)-3-N, 2′-O, 3′-O-Tribenzoyluridine (4f) (84.9% yield). ^{1}HNMR ($CDCl_3$): 9.21 (br, 1H), 8.82 and 8.79 (s and s, 1H), 8.45 and 8.40 (s and s, 1H), 7.84–8.02 (m, 8H), 7.27–7.73 (m, 13H), 6.24 and 6.17 (d and d, 1H, $J = 5.6$ and 6.0), 5.99 and 5.94 (d and d, 1H, $J = 8.4$ and 8.8), 5.82 (m, 1H), 5.53–5.67 (m, 2H), 5.29 (m, 1H), 4.28–4.59 (m, 14H), 2.75–2.85 (m, 6H). ^{31}PNMR ($CDCl_3$): −1.02, −1.15, −1.94. FAB-HRMS: $[M+H]^+$ calcd. for $C_{56}H_{51}N_{10}O_{18}P_2$ 1213.2858, found 1213.2812.

6-N-Benzoyl-5′-O-[di(2-cyanoethoxy)phosphinyl]-P-(2-cyanoethyl)-1′-deoxy-2′-isoadenylyl-(3′→5′)-4-N, 2′-O, 3′-O-Tribenzoylcytidine (4g) (84.6% yield). ^{1}HNMR ($CDCl_3$): 9.80 (br, 1H), 9.20 (br, 1H), 8.78 and 8.73 (s and s, 1H), 8.32 and 8.27 (s and s, 1H), 7.88–8.10 (m, 9H), 7.31–7.72 (m, 13H), 6.10 (m, 1H), 5.76–5.92 (m, 2H), 5.49 (m, 1H), 5.33 and 5.12 (m and m, 1H), 4.27–4.73 (m, 14H), 2.75–2.93 (m, 6H). ^{31}PNMR ($CDCl_3$): −1.38, −1.42, −1.50, −2.71. FAB-HRMS: $[M+H]^+$ calcd. for $C_{56}H_{52}N_{11}O_{17}P_2$ 1212.3018, found 1212.2981.

6-N-Benzoyl-5′-O-[(2-cyanoethoxy)(ethoxy)phosphinyl]-P-(2-cyanoethyl)-1′-deoxy-2′-isoadenylyl-(3′→5′)-4-N, 2′, 3′-Tribenzoylcytidine (4h). To a solution of **3a** (190 mg, 0.185 mmol) and 2-cyanoethyl N, N, N′, N′-tetraisopropylphosphorodiamidite (112 mg, 0.370 mmol) in CH_2Cl_2 (8 mL), 1H-tetrazole solution in acetonitrile (0.83 mL, 0.370 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. Then ethanol (22 μl, 0.37 mmol) and 1H-tetrazole solution in acetonitrile (0.83 mL, 0.370 mmol) were added. After 3 h, iodine (180 mg, 0.71 mmol) in THF-H_2O-pyridine (66:33:1, 1.8 mL) was added. After 10 min, the mixture was poured into CH_2Cl_2 (100 mL), washed with 0.2 M sodium sulfite (30 mL × 2), dried, filtered, and concentrated. The residue was purified by on a silica gel column ($CHCl_3$:CH_3OH, 30:1) to give **4h** (140 mg, 63.7%) as an amorphous solid. ^{1}HNMR ($CDCl_3$): 10.1 and 9.68 (br and br, 1H), 9.20 (br, 1H), 8.73 (s, 1H), 8.23 and 8.20 (s and s, 1H), 7.28–8.04 (m, 22H), 6.04 and 5.99 (t and t, 1H, J = 6.5 and 6.5), 5.59–5.80 (m, 2H), 5.45 (m, 1H), 5.11 and 5.05 (s and s, 1H), 4.32–4.63 (m, 11H), 4.14 (m, 3H), 2.67–2.83 (m, 4H), 1.28 (m, 3H). ^{31}PNMR ($CDCl_3$): −0.68, −1.23, −2.87, −3.12. FAB-HRMS: $[M+H]^+$ calcd. for $C_{55}H_{53}N_{10}O_{17}P_2$ 1187.3065, found 1187.3087.

General Procedure for Deprotection Reaction

The protected dinucleotide (100–200 mg) in concentrated ammonium hydroxide (8 mL) was capped and stirred at room temperature for 24 h. Then the reaction solution was evaporated to dryness and the residue was dissolved in water (50 mL) and washed with ether (30 mL). The aqueous layer was concentrated and purified by HPLC to give the product as a white spongy solid after lyophilization.

1′-Deoxy-2′-isoadenylyl-(3′→5′)-1′-deoxy-2′-isocytidine (1a) (75.4% yield). ^{1}HNMR (D_2O): 8.13 (s, 1H), 8.02 (s, 1H), 7.22 (d, 1H, J = 7.5), 5.65 (d, 1H, J = 7.5), 5.08 (m, 1H), 4.81 (m, 1H), 4.68 (m, 1H), 4.24 (dd, 1H, J = 10.5, 7.5), 4.10 (dd, 1H, J = 10.5, 5.0), 3.96 (m, 2H), 3.81–3.87 (m, 2H), 3.63–3.77 (m, 4H), 3.47 (s, br, 1H). ^{13}CNMR (D_2O): 165.2, 157.4, 155.3, 152.3, 148.8, 142.9, 140.5, 118.5, 96.0, 84.3, 83.7, 78.5, 76.0, 70.0,

69.9, 64.1, 64.0, 61.1, 60.3. ^{31}PNMR (D_2O): −0.10. FAB-HRMS: $[M+H]^+$ calcd. For $C_{19}H_{26}N_8O_9P$ 541.1560, found 541.1552. UV (H_2O): λ_{max} 264(ε 19,300).

2′-Deoxyadenylyl-(3′→5′)-1′-deoxy-2′-isocytidine (1b) (83.9% yield). ^{1}HNMR (D_2O): 8.11 (s, 1H), 7.96 (s, 1H), 7.64 (d, 1H, J = 7.5), 6.21 (dd, 1H, J = 7.5, 6.5), 5.90 (d, 1H, J = 7.5), 4.74 (m, 2H), 4.26 (dd, 1H, J = 6.0, 3.0), 4.13 (q, 1H, J = 3.5), 4.07 (dd, 1H, J = 11.0, 6.5), 3.97–4.00 (m, 2H), 3.89–3.94 (m, 1H), 3.80 (m, 1H), 3.66 (dd, 1H, J = 12.5, 3.0), 3.61 (dd, 1H, J = 12.5, 4.5), 2.64 (m, 1H), 2.52 (m, 1H). ^{13}CNMR (D_2O): 160.9, 154.0, 151.6, 150.4, 148.1, 145.5, 141.1, 118.8, 95.5, 86.7, 85.0, 84.1, 76.0, 75.8, 69.2, 65.1, 64.1, 61.6, 38.4. ^{31}PNMR (D_2O): 0.17. ESI-HRMS: $[M+H]^+$ calcd. For $C_{19}H_{26}N_8O_9P$ 541.1560, found 541.1575. UV (H_2O): λ_{max} 262(ε 19,000).

2′-Deoxyadenylyl-(3′→5′)-1′-deoxy-2′-isoadenosine (1c) (85.6% yield). ^{1}HNMR (D_2O): 8.11 (s, 1H), 8.09 (s, 1H), 7.95 (s, 1H), 7.90 (s, 1H), 6.10 (t, 1H, J = 6.5), 4.86 (m, 1H), 4.70 (m, 1H), 4.43 (dd, 1H, J = 10.5, 4.0), 4.20 (m, 2H), 4.04 (m, 1H), 3.95–4.02 (m, 2H), 3.91 (m, 1H), 3.62 (dd, 1H, J = 13.0, 3.0), 3.55 (dd, 1H, J = 13.0, 4.5), 2.52 (m, 1H), 2.43 (m, 1H). ^{13}CNMR (D_2O): 152.8, 152.1, 148.6, 148.4, 148.0, 147.8, 141.5 (two carbons), 118.7, 117.9, 86.3, 84.6, 83.8, 76.1, 75.2, 69.9, 63.9, 62.5, 61.3, 38.1. ^{31}PNMR (D_2O): −0.41. FAB-HRMS: $[M+H]^+$ calcd. for $C_{20}H_{26}N_{10}O_8P$ 565.1673, found 565.1663. UV (H_2O): λ_{max} 259(ε 26,300).

1′-Deoxy-2′-isoadenylyl-(3′→5′)-2′-deoxyadenosine (1e) (89.8% yield). ^{1}HNMR (D_2O): 8.09 (s, 1H), 8.07 (s, 1H), 7.99 (s, 1H), 7.87 (s, 1H), 6.09 (t, 1H, J = 6.5), 5.01 (m, 1H), 4.51 (m, 1H), 4.42 (m, 1H), 4.15 (dd, 1H, J = 11.0, 6.0), 4.05 (dd, 1H, J = 11.0, 3.0), 3.94–3.98 (m, 2H), 3.88–3.92 (m, 2H), 3.81 (dd, 1H, J = 12.5, 2.5), 3.70 (dd, 1H, J = 12.5, 5.0), 2.41 (m, 1H), 2.29 (m, 1H). ^{13}CNMR (D_2O): 152.8, 151.8, 149.2, 148.0, 147.8 (two carbons), 141.5, 140.4, 117.9, 117.6, 85.8, 85.6, 83.6, 79.6, 70.8, 70.4, 65.0, 61.6, 60.2, 39.5. ^{31}PNMR (D_2O): −1.43. FAB-HRMS: $[M+H]^+$ calcd. for $C_{20}H_{26}N_{10}O_8P$ 565.1673, found 565.1649. UV (H_2O): λ_{max} 259(ε 23,600).

1′-Deoxy-2′-isoadenylyl-(3′→5′)-uridine (1f) (81.8% yield). ^{1}HNMR (D_2O): 8.24 (s, 1H), 8.21 (s, 1H), 7.23 (d, 1H, J = 8.0), 5.49 (d, 1H, J = 4.0), 5.44 (d, 1H, J = 8.0), 5.22 (m, 1H), 4.57 (m, 1H), 4.23 (dd, 1H, J = 11.0, 6.5), 4.11 (dd, 1H, J = 11.0, 3.5), 3.92–3.99 (m, 4H), 3.82–3.87 (m, 3H), 3.76 (dd, 1H, J = 12.0, 5.0). ^{13}CNMR (D_2O): 165.6, 151.2, 150.5, 148.4, 145.7, 142.6, 140.5, 117.9, 102.2, 88.1, 85.4, 82.3, 79.8, 73.6, 70.6, 69.0, 64.1, 61.9, 59.8. ^{31}PNMR (D_2O): −0.32. FAB-HRMS: $[M+H]^+$ calcd. for $C_{19}H_{25}N_7O_{11}P$ 558.1350, found 558.1336. UV (H_2O): λ_{max} 261(ε 20,300).

1′-Deoxy-2′-isoadenylyl-(3′→5′)-cytidine (1g) (89.9% yield). ^{1}HNMR (D_2O): 8.16 (s, 1H), 8.03 (s, 1H), 7.31 (d, 1H, $J = 8.0$), 5.59 (d, 1H, $J = 8.0$), 5.44 (d, 1H, $J = 3.0$), 5.15 (m, 1H), 4.69 (m, 1H), 4.22 (dd, 1H, $J = 10.0, 7.0$), 4.07 (dd, 1H, $J = 10.0, 3.5$), 3.83–3.98 (m, 7H), 3.78 (dd, 1H, $J = 12.5, 4.5$). ^{13}CNMR (D_2O): 161.6, 153.5, 151.6, 149.9, 148.5, 141.7, 141.6, 118.4, 95.1, 89.4, 85.2, 81.8, 79.6, 74.3, 70.6, 68.2, 63.5, 61.8, 60.1. ^{31}PNMR (D_2O): −0.42. FAB-HRMS: $[M+H]^+$ calcd. for $C_{19}H_{26}N_8O_{10}P$ 557.1510, found 557.1498. UV (H_2O): λ_{max} 263(ε 20,200).

5′-O-Phosphoryl-1′-deoxy-2′-isoadenylyl-(3′→5′)-1′-deoxy-2′-isocytidine (2a) (61.7% yield). ^{1}HNMR (D_2O): 8.34 (s, 1H), 8.24 (s, 1H), 7.46 (d, 1H, $J = 7.5$), 5.98 (d, 1H, $J = 7.5$), 5.20 (m, 1H), 4.78 (m, 1H), 4.63 (m, 1H, partly hid in water peak), 4.25 (dd, 1H, $J = 10.0, 6.5$), 4.18 (dd, 1H, $J = 10.0, 4.0$), 4.14 (dd, 1H, $J = 5.5, 2.5$), 4.05–4.09 (m, 2H), 4.00 (m, 1H), 3.90 (dd, 1H, $J = 11.5, 6.0$), 3.78–3.83 (m, 3H), 3.58 (m, 1H). ^{13}CNMR (D_2O): 159.2, 150.6, 149.2, 148.7, 146.0, 145.4, 143.0, 118.3, 95.1, 84.3, 83.8, 79.4, 75.9, 70.6, 69.0, 64.8, 64.0, 63.9, 61.9. ^{31}PNMR (D_2O): 0.96, −0.33. FAB-HRMS: $[M+H]^+$ calcd. for $C_{19}H_{27}N_8O_{12}P_2$ 621.1224, found 621.1234. UV (H_2O): λ_{max} 265(ε 19,700).

5′-O-Phosphoryl-2′-deoxyadenylyl-(3′→5′)-1′-deoxy-2′-isocytidine (2b) (58.8% yield). ^{1}HNMR (D_2O): 8.40 (s, 1H), 8.20 (s, 1H), 7.74 (d, 1H, $J = 7.5$), 6.37 (t, 1H, $J = 6.5$), 6.04 (d, 1H, $J = 7.5$), 4.82 (m, 1H), 4.73 (m, 1H), 4.28 (m, 2H), 3.98–4.09 (m, 3H), 3.89-3.93 (m, 3H), 3.81 (m, 1H), 2.71 (m, 1H), 2.61 (m, 1H). ^{13}CNMR (D_2O): 159.2, 150.5, 149.2, 148.3, 146.4, 145.5, 142.4, 118.5, 95.2, 85.5, 84.7, 84.3, 75.98, 75.92, 69.0, 65.1, 64.8, 64.1, 38.8. ^{31}PNMR (D_2O): 0.94, −0.030. FAB-HRMS: $[M+H]^+$ calcd. for $C_{19}H_{27}N_8O_{12}P_2$ 621.1224, found 621.1217. UV (H_2O): λ_{max} 262(ε 19,900).

5′-O-Phosphoryl-2′-deoxyadenylyl-(3′→5′)-1′-deoxy-2′-isoadenosine (2c) (69.4% yield). ^{1}HNMR (D_2O): 8.37 (s, 1H), 8.26 (s, 1H), 8.19 (s, 1H), 8.16 (s, 1H), 6.29 (t, 1H, $J = 7.0$), 4.98 (m, 1H), 4.80 (s, br, 1H), 4.47 (m, 1H), 4.21–4.28 (m, 3H), 3.90–4.04 (m, 5H), 2.66 (m, 1H), 2.58 (m, 1H). ^{13}CNMR (D_2O): 150.2, 150.1, 148.5, 148.0, 145.1, 144.8, 142.8, 142.4, 118.4, 118.1, 85.3, 84.6, 84.0, 76.1, 75.8, 69.8, 64.6, 64.1, 62.8, 38.7. ^{31}PNMR (D_2O): 0.065, −0.75. FAB-HRMS: $[M+H]^+$ calcd. for $C_{20}H_{27}N_{10}O_{11}P_2$ 645.1336, found 645.1325. UV (H_2O): λ_{max} 259(ε 26,400).

5′-O-Phosphoryl-2′-deoxyadenylyl-(3′→5′)-1′, 3′-Dideoxy-2′-isoadenosine (2d) (72.5% yield). ^{1}HNMR (D_2O): 8.39 (s, 1H), 8.34 (s, 1H), 8.19 (s, 1H), 8.16 (s, 1H), 6.30 (t, 1H, $J = 6.5$), 5.21 (m, 1H), 4.82 (s, br, 1H), 4.27 (s, br, 1H), 4.21 (s, br, 1H), 4.14 (d, 1H, $J = 10.5$), 4.04 (m, 1H), 3.98 (dd, 1H, $J = 10.5, 5.5$), 3.86–3.92 (m, 3H), 2.67 (m, 2H), 2.58

(m, 1H), 2.11 (m, 1H). ^{13}CNMR (D_2O): 150.4, 150.1, 148.4, 148.2, 145.3, 144.8, 142.9, 142.4, 118.5, 118.1, 85.5, 84.7, 78.3, 75.9, 72.3, 65.9, 64.8, 55.7, 38.9, 33.6. ^{31}PNMR (D_2O): 1.00, 0.25. FAB-HRMS: $[M+H]^+$ calcd. For $C_{20}H_{27}N_{10}O_{10}P_2$ 629.1387, found 629.1412. UV (H_2O): λ_{max} 259(ε 24,500).

5′-O-Phosphoryl-1′-deoxy-2′-isoadenylyl-(3′→5′)-2′-deoxyadenosine (2e) (69.2% yield). ^{1}HNMR (D_2O): 8.28 (s, 1H), 8.23 (s, 1H), 8.19 (s, 1H), 8.05 (s, 1H), 6.20 (t, 1H, $J = 7.0$), 5.09 (m, 1H), 4.50 (m, 2H), 3.92–4.20 (m, 8H), 2.57 (m, 1H), 2.34 (m, 1H). ^{13}CNMR (D_2O): 150.5, 149.8, 148.0, 147.8, 146.2, 145.0, 142.6, 141.9, 117.4, 117.1, 86.3, 84.9, 84.2, 79.9, 71.5, 70.9, 65.3, 63.6, 61.6, 39.2. ^{31}PNMR (D_2O): 0.49, −1.43. FAB-HRMS: $[M+H]^+$ calcd. for $C_{20}H_{27}N_{10}O_{11}P_2$ 645.1336, found 645.1333. UV (H_2O): λ_{max} 259(ε 23,500).

5′-O-Phosphoryl-1′-deoxy-2′-isoadenylyl-(3′→5′)-uridine (2f) (60.0% yield). ^{1}HNMR (D_2O): 8.28 (s, 1H), 8.23 (s, 1H), 7.26 (d, 1H, $J = 8.0$), 5.53 (d, 1H, $J = 4.4$), 5.50 (d, 1H, $J = 8.0$), 5.24 (m, 1H), 4.24 (dd, 1H, $J = 10.4, 6.4$), 4.07–4.16 (m, 4H), 3.84–3.99 (m, 5H), one proton hid in water peak. ^{13}CNMR (D_2O): 151.3, 149.6, 148.4, 144.4, 143.1, 140.6, 117.9, 102.4, 88.2, 83.9, 82.5, 79.7, 73.6, 70.8, 69.1, 64.3, 63.8, 61.8, one carbon not observable. ^{31}PNMR (D_2O): 1.09, −0.59. FAB-HRMS: $[M+H]^+$ calcd. for $C_{19}H_{26}N_7O_{14}P_2$ 638.1013, found 638.0983. UV (H_2O): λ_{max} 261(ε 21,000).

5′-O-Phosphoryl-1′-deoxy-2′-isoadenylyl-(3′→5′)-cytidine (2g) (68.0% yield). ^{1}HNMR (D_2O): 8.28 (s, 1H), 8.23 (s, 1H), 7.50 (d, 1H, $J = 8.0$), 5.96 (d, 1H, $J = 8.0$), 5.49 (d, 1H, $J = 4.5$), 5.24 (m, 1H), 4.52 (m, 1H), 4.26 (dd, 1H, $J = 11.0, 6.0$), 3.87–4.20 (m, 9H). ^{13}CNMR (D_2O): 159.3, 150.7, 148.6, 148.5, 146.3, 142.5, 117.7, 95.4, 89.1, 84.8, 82.8, 80.4, 74.1, 71.1, 69.0, 64.2, 63.7, 61.7, one carbon not observable. ^{31}PNMR (D_2O): 0.86, −0.44. FAB-HRMS: $[M+H]^+$ calcd. for $C_{19}H_{27}N_8O_{13}P_2$ 637.1173, found 637.1170. UV (H_2O): λ_{max} 264(ε 19,800).

5′-O-Ethoxyphosphonyl-1′-deoxy-2′-isoadenylyl-(3′→5′)-cytidine (2h) (66.0% yield). ^{1}HNMR (D_2O): 8.27 (s, 1H), 8.24 (s, 1H), 7.56 (d, 1H, $J = 7.5$), 5.93 (d, 1H, $J = 7.5$), 5.51 (d, 1H, $J = 4.0$), 5.24 (m, 1H), 4.52 (m, 1H), 4.26 (dd, 1H, $J = 11.0, 6.0$), 4.21 (dd, 1H, $J = 11.0, 3.0$), 3.99–4.16 (m, 6H), 3.88–3.95 (m, 2H), 3.81 (m, 2H), 1.10 (t, 3H, $J = 7.0$). ^{13}CNMR (D_2O): 159.2, 150.4, 148.54, 148.48, 145.9, 142.8, 142.7, 117.8, 95.3, 89.2, 84.7, 82.9, 80.3, 74.1, 71.0, 69.0, 64.2, 64.1, 62.7, 61.8, 15.8. ^{31}PNMR (D_2O): 0.95, −0.52. FAB-HRMS: $[M+H]^+$ calcd. for $C_{21}H_{31}N_8O_{13}P_2$ 665.1486, found 665.1460. UV (H_2O): λ_{max} 264(ε 18,000).

5′-O-Phosphoryl-2′-deoxycytidylyl-(3′→5′)-1′-deoxy-2′-isothymidine (25). ^{1}HNMR (D_2O): 7.98 (d, 1H, J = 8.0), 7.37 (s, 1H), 6.07 (m, 2H), 4.80 (m, 1H), 4.69 (m, 1H), 4.26 (m, 1H), 4.24 (dd, 1H, J = 6.5, 4.0), 4.07 (dd, 1H, J = 10.5, 7.0), 3.88–4.02 (m, 5H), 3.79 (m, 1H), 2.48 (m, 1H), 2.19 (m, 1H), 1.71 (s, 3H). ^{13}CNMR (D_2O): 166.4, 159.0, 152.2, 148.1, 144.2, 139.2, 111.5, 94.9, 86.6, 85.2, 83.2, 75.7, 75.6, 69.1, 64.6, 64.2, 63.3, 38.8, 11.5. ^{31}PNMR (D_2O): 0.58, 0.002. FAB-HRMS: $[M+H]^+$ calcd. for $C_{19}H_{28}N_5O_{14}P_2$ 612.1108, found 612.1090. UV (H_2O): λ_{max} 274(ε 20,300).

5′-O-Phosphoryl-2′-deoxycytidylyl-(3′→5′)-1′-deoxy-2′-isouridine (26). ^{1}HNMR (D_2O): 7.99 (d, 1H, J = 8.0), 7.56 (d, 1H, J = 8.0), 6.10 (m, 2H), 5.71 (d, 1H, J = 8.0), 4.78 (m, 1H), 4.69 (m, 1H), 4.27 (m, 1H), 4.24 (dd, 1H, J = 6.0, 4.0), 4.09 (dd, 1H, J = 11.0, 7.0), 3.87-4.01 (m, 5H), 3.81 (m, 1H), 2.49 (m, 1H), 2.21 (m, 1H). ^{13}CNMR (D_2O): 166.2, 159.1, 152.1, 148.2, 144.2, 143.7, 102.2, 95.0, 86.6, 85.3, 83.5, 75.7, 75.6, 69.0, 64.6, 64.2, 63.8, 38.8. ^{31}PNMR (D_2O): −0.20, −0.81. FAB-HRMS: $[M+H]^+$ calcd. for $C_{18}H_{26}N_5O_{14}P_2$ 598.0952, found 598.0930. UV (H_2O): λ_{max} 271(ε 19,000).

X-Ray Crystallographic Data

Crystallographic data (excluding structure factors) for compound **2g** described in this article have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 254123. Copies of the data can be obtained, free of charge, on application to the CCDC (deposit@ccdc.cam.ac.uk).

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