Synthesis of 5′-*O*-Amino-2′-deoxypyrimidine and Purine Nucleosides: Building-Blocks for Antisense Oligonucleosides[1,†]

Michel Perbost,[2] Tomonori Hoshiko,[3] François Morvan,[4] Eric Swayze, Richard H. Griffey, and Yogesh S. Sanghvi*

Medicinal Chemistry Department, Isis Pharmaceuticals, 2292 Faraday Avenue, Carlsbad, California 92008

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An efficient synthesis of 5′-*O*-amino-2′-deoxy analogs of uridine **1**, thymidine **2**, cytidine **3**, 5-methylcytidine **3a**, adenosine **4**, and guanosine **5** was accomplished. The key step of 5′-O-N-bond formation in 2′-deoxynucleosides **1–5** was achieved *via* a Mitsunobu reaction in excellent yields. The 5′-*O*-amino nucleosides **1–5** are useful building-blocks for the synthesis of nucleoside dimers linked by a methylene(methylimino) (MMI) bridge. MMI is a novel phosphate surrogate for antisense oligonucleosides.

Introduction

The replacement of the phosphodiester group in DNA and RNA by neutral, achiral and nonhydrolyzable linkages has recently gained much attention due to the usefulness of this strategy in the design of novel antisense oligonucleotides (AO).[5] Our research in the antisense field has focused on the synthesis of oligonucleosides in which phosphate linkages are replaced by *m*ethylene (*m*ethyl*i*mino) (MMI)[6] as shown in Figure 1.

Until recently, we had prepared only thymidine dimers containing the MMI linkage due to its synthetic ease.[7] For similar reasons, other research groups have reported syntheses of various backbone modified thymidine dimers[8] and as a result the chemical and biological information on AO containing backbone modifications employing mixed base dimers is limited.[9] In order to expand and strengthen our knowledge of MMI-linked AO, we wish to synthesize various mixed dimers containing all nucleic acid base residues. The retrosynthetic pathway of MMI-linked dimers indicated that the 5′-*O*-amino-2′-deoxynucleosides **1-5** could serve as the common building-blocks (lower-half) for such nucleosidic dimers (Figure 1). The synthesis of 2′,3′-dideoxy-3′-α-*C*-formyl nucleosides which constitutes the upper-half of these MMI dimers have been recently reported by us.[10]

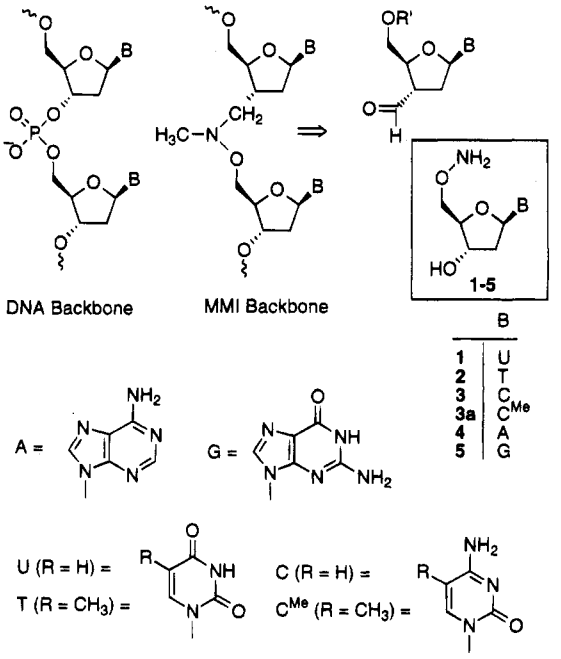

Figure 1.

A literature search revealed that the main routes to 5′-*O*-amino 2′-deoxynucleosides have been either a condensation of the ethyl ester of potassium hydroxycarbamate with 5′-*O*-tosylate followed by base hydrolysis of the urethane (13% overall yield)[11] or by treatment of 5′-*O*-tosylate with the sodium salt of ethyl acetohydroxamate followed by deprotection with aqueous acetic acid

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(1) We refer to modified oligonucleotides that lack the phosphorus atom in the backbone linkage as oligonulceosides.

(2) Present address: GENSET, 1, Passage Etienne Delaunay, 75011 Paris, France.

(3) Visiting Scientist: Eisai Co., Ltd., 1–3 Tokodai 5-chome, Tsukuva-Shi, Ibaraki 300–26, Japan.

(4) Visiting Scientist: Laboratoire de Chimie Bio-Organique, Université de Montpellier, Cedex, France.

(5) List of recent review articles on backbone-modified antisense oligonucleotides: (a) Sanghvi, Y. S.; Cook, P. D. In *Nucleosides and Nulceotides as Antitumor and Antiviral Agents*; Chu, C. K., Baker, D. C., Eds; Plenum Press: New York, 1993; p 311. (b) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123. (c) Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923. (d) Uhlmann, E.; Peyman, A. In *Protocols for Oligonulcleotides and Analogs*; Agrawal, S., Ed.; Humana Press: Totowa, New Jersey, 1993; p 355. (e) Varma, R. S. *Synlett* **1993**, 621.

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0022-3263/95/1960-5150$09.00/0

(undisclosed yields).[12] A two-step conversion of an alcohol (ROH) to an aminooxy alcohol ($RONH_2$) was first reported by Mitsunobu *et al.*[13] and subsequently utilized by others.[14] The Mitsunobu route appeared to be practical and valuable to us for the conversion of 5′-OH group of a 2′-deoxynucleoside to a 5′-*O*-NH_2 functionality. One of the salient features of this approach is the commercial availability of 2′-deoxynucleosides.[15] In addition, various Mitsunobu reactions have shown high regioselectivity toward the 5′-OH group in a variety of nucleosides.[16]

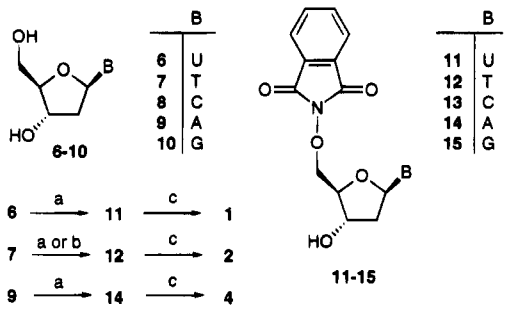
As an example, Kuroda *et al.*[14] reacted 2′-deoxy-5-fluorouridine with *N*-hydroxyphthalimide (HONPhth), Ph_3P, and dimethyl azodicarboxylate in THF to furnish 2′-deoxy-5-fluoro-5′-*O*-phthalimidouridine in 26% yield. In a preliminary communication[17] we described a high yield transformation of thymidine **7** to 5′-*O*-phthalimidothymidine **12** via a Mitsunobu reaction in DMF.

We believe that the solvent plays a major role in the improvements of the product yield. In the literature[18] aprotic solvents have found use with carbohydrates and nucleosides that are insoluble in the typical nonpolar solvents and thus furnishing better yields. We now report on the regioselective synthesis of 5′-*O*-phthalimidonucleoside derivatives in high yields and high purity employing the Mitsunobu reaction.

Results and Discussion

The synthesis of 5′-*O*-phthalimidonucleosides is illustrated in Schemes 1–4. Treatment of 2′-deoxyuridine (**6**) and -thymidine (**7**) in HONPhth, Ph_3P, and diisopropyl azodicarboxylate (DIPAD) in DMF at ambient temperature gave the 5′-*O*-phthalimido **11** (62%) and **12** (60%), respectively. In both cases, an extensive column chromatography was required to separate the byproducts (Ph_3P=O) from the desired products. We were able to overcome this problem by utilizing [*p*-(dimethylamino)-phenyl]diphenylphosphine[19] in the preparation of pure **11** and **12**, without chromatography and maintaining similar yields. An acidic workup was employed to remove the Ph_3P=O formed during the reaction, and the hydrazine byproduct was washed with CH_2Cl_2 to furnish crystalline **11** and **12**. Since the aminophosphine is not commercially available and must be converted back to the phosphine for next reaction, scaleup of this improved methodology became unattractive for our needs in an antisense project. Therefore, we sought a cheap and convenient workup of the Mitsunobu reaction on 2′-deoxynucleosides.

Scheme 1[a]



[a] **Reagents and conditions**: (a) Ph_3P/DIPAD/DMF, rt, 6–12 h; (b) *p*-(Me_2N)PhP(Ph)$_2$/PhthNOH/DIPAD/DMF; rt, 6 h; H_3CNHNH_2/CH_2Cl_2, rt, 0.5–1 h; (d) *t*-$BuPh_2SiCl$/imidazole/DMF, rt, 12–14 h; (e) Cl_3CO_2H/CH_2Cl_2, rt, 3 h; (f) PhthNOH/Ph_3P/DIPAD/THF, rt, 5–10 h; (g) *n*Bu_4NF/THF, rt, 6 h; (h) NH_3/MeOH rt, 14 h; (i) 1,2,4-triazole/$POCl_3/Et_3N/CH_3CN$, 0 °C to rt, 6 h; (j) $PhCONH_2$/NaH/1,4-dioxane, rt, 1 h; (k) TMSTf/CH_2Cl_2, 0 °C, 4 h. (For details see methods a–k in the experimental section).

Taking advantage of the solubilities of these nucleosides in various solvents, we have developed a biphasic extraction system (workup) which provided easy isolation of 5′-*O*-phthalimidonucleoside derivatives. In brief, after completion of the Mitsunobu reaction, the mixture was concentrated under vacuum to provide a syrupy residue. The residue was diluted with cold CH_2Cl_2 and poured into ice–water to precipitate the desired product. In general, the precipitated products were sufficiently pure (>95%) for subsequent reactions. Crystallization from alcohol furnished the analytical samples of **11** and **12**.

Attempted synthesis of 5′-*O*-phthalimido derivative **13** from unprotected dC (**8**) under similar Mitsunobu conditions gave little (<10%) or no product, while recovering the starting material. The Mitsunobu reaction on **8** was repeated under forcing conditions with a 2-fold excess of all reagents to give an unexpectedly polar product in 65% yield. The polar compound was characterized as 2,3′-anhydro nucleoside **23** on the basis of elemental analysis and spectral data. We rationalize the formation of 2,3′-anhydro-5′-*O*-phthalimido (*β-D-threo*-pentofuranosyl)cytosine hydrate in the following manner (Scheme 2). Under the Mitsunobu conditions, first, **8** was transformed to 5′-*O*-phthalimido derivative **13**, which then further reacted at the 3′-hydroxyl group to form the corresponding phosphonium salt **22**. The intermediate **22** then undergoes an intramolecular displacement leading to 2,3′-anhydro nucleoside **23**. This hypothesis is in good agreement with similar reports of intramolecular cyclization of pyrimidine nucleosides.[20] A notable feature of the structure of **23** was that it existed almost completely in the protonated form (**23** H^+) even in DMSO-d_6 (neutral p*H*).

The ^{1}H NMR of **23** clearly exhibited two resolved NH protons (δ 9.07 and 9.29 ppm) and one of these proton had a strong NOE with *C*5H of the pyrimidine base. This observation may be further supported by the structural similarity between **23** and the stable "para quinonoidal" structure of protonated 1,4-dihydro-4-imino-1-methylpyrimidine.[21]

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(13) Mitsunobu, O. *Synthesis* **1981**, 1.
(14) (a) Grochowski, E.; Jurczak, J. *Synthesis* **1976**, 682. (b) Kuroda, T.; Hisamura, K.; Matsukuma, I.; Nishikawa, H.; Morimoto, M.; Ashizawa, N.; Otsuji, Y. *J. Heterocycl. Chem.* **1992**, *29*, 1133. (c) Tronchet, J. M.; Zosimo-Landolfo, G.; Galland-Barrera, G.; Dolatshahi, N. *Carbohydr. Res.* **1990**, *204*, 145. (d) Grochowski, E.; Stepowska, H. *Synthesis* **1988**, 795. (e) Nashed, E. M.; Grochowski, E.; Czyzewska, E. *Carbohydr. Res.* **1990**, *196*, 184. (f) Grochowski, E.; Jurczak, J. *J. Org. Chem.* **1978**, *43*, 2541. (g) Chemla, P. *Tetrahedron Lett.* **1993**, *34*, 7391.
(15) Purchased from Chem-Impex International Inc., Wood Dale, IL 80191 (1-800-869-9290).
(16) (a) Mitsunobu, O.; Kimura, J.; Fujisawa, Y. *Bull. Chem. Soc. Jpn.* **1972**, 45, 245. (b) Montero, J.-L.; Criton, M.; Dewynter, G.-F.; Imbach, J.-L. *Tetrahedron Lett.* **1991**, *32*, 5357.
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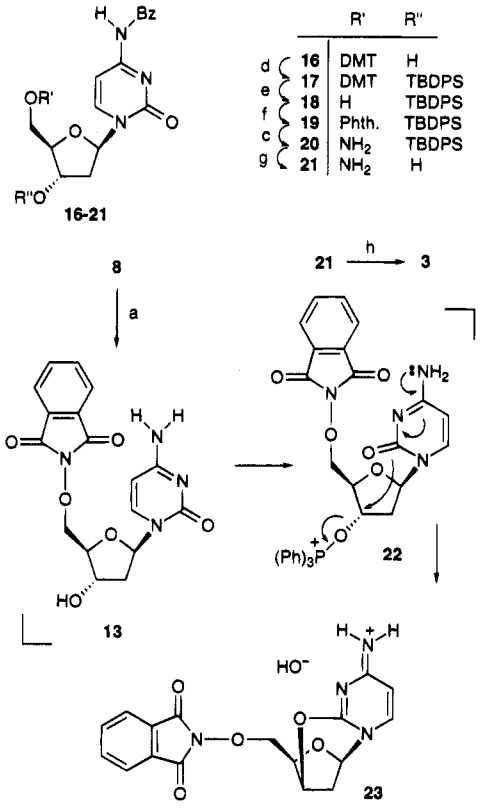
(21) (a) Fujii, T.; Saito, T.; Nakasaka, T. *Chem. Pharm. Bull.* **1989**, *37*, 2601.(b) Brown, D. J.; Hoerger, E.; Mason, S. F. *J. Chem. Soc.* **1955**, 4035. (c) Albert, A. *Synthetic Procedures in Nucleic Acid Chemistry:* Zorbach, W. W., Tipson, R. S., Eds.; Wiley Interscience: New York, 1993; Vol. 2, Chapter 1.

Scheme 2[a]

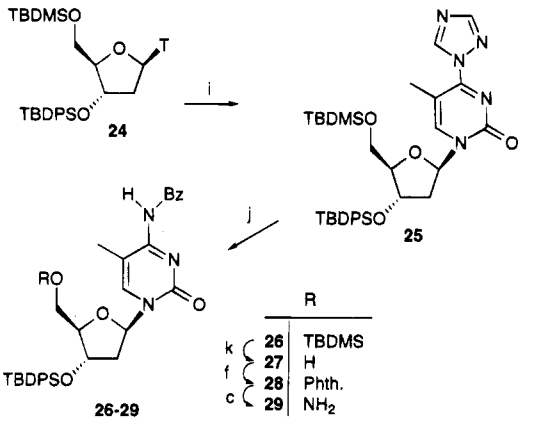


[a] See Scheme 1 legend for reagents and conditions.

The synthesis of 5′-*O*-amino-2′-deoxycytidine analog **21** was then attempted from 3′-*O*-TBDPS and N^4-benzoyl protected compound **18**, thus reducing the possibilities of participation of these groups during the Mitsunobu reaction. Conceptually N^4-benzoyl protected compound **18** still bears an acidic proton which can undergo Mitsunobu reaction. However, empirical results demonstrate that this side raction can be avoided when both the 3′-OH and N^4-NH_2 groups are protected. Commercially available **16**[15] was silylated[17] with TBDMS-Cl to furnish **17** (95%), which on treatment with trichloroacetic acid [22] gave **18** in 60% yield. Appropriately protected nucleoside **18** underwent Mitsunobu reaction to provide **19** in good yields (81%). Deprotection of **19** with *N*-methylhydrazine (NMH) at room temperature resulted in the formation of **20** (66%). We observed that the deprotection of a phthalimido group with NMH had a clear advantage over the traditionally used hydrazine for two reasons. First, deprotections with NMH formed a thick precipitate of 1,2-dihydro-4-hydroxy-2-methyl-1-oxophthalazine in most of the cases which can be separated by simple filtration. Secondly, excess NMH can be removed from the products easily because of its low boiling point. The benzoylated **20** was then desilylated[23] and the product **21** treated with saturated (0 °C) methanolic ammonia to furnish 5′-*O*-amino-2′-deoxycytidine (**3**) in good yield.

It has been demonstrated[24] that presence of a 5-methyl group on the cytosine residue improved the antisense properties of oligonucleotides compared to unsubstituted dC. Therefore, synthesis of 5′-*O*-amino-N^4-benzoyl-2′-deoxy-5-methylcytidine **29** was next investigated (Scheme 3). The silylated starting material **24** was readily prepared from thymidine following the literature procedures.[25] Triazolation of **24** with 1,2,4-triazole, $POCl_3$, and Et_3N according to the procedure of Reese *et al.*[26] furnished 68% of **25**. Recently, we developed[27] an efficient procedure for the displacement of *C*-4-triazolo group of a pyrimidine nucleoside with sodium salt of benzamide in 1,4-dioxane to provide *C*-4-*N*-benzoylated nucleosides. This displacement procedure was utilized to convert **25** into **26** (95%) in one step. Selective deprotection of the *t*-butyldimethylsilyl group from **26** was accomplished by treatment with TMSTf according to the reported[28] method to provide **27**. Deblocked **27** undergoes Mitsunobu reaction in a standard manner to furnish **28** in essentially quantitative yield, which upon treatment with NMH provided **29** (83%). The 5-methyl dC **29** was then successfully coupled with 3′-*C*-formyl nucleosides to provide MMI linked dimers.[17c] We made no attempts to deblock **29** to provide the unprotected nucleoside **3a**.

Scheme 3[a]



[a] See Scheme 1 legend for reagents and conditions.

Among Mitsunobu reactions on purine nucleosides, a regioselective aroylation of 5′-hydroxy group of unprotected adenosine using DEAD and Ph_3P has been reported[29] in the literature. It was suggested that the nucleophilic attack of the 5′-hydroxyl group of the adenosine on the sterically crowded phosphorus cation of the betaine was more favorable than the 3′-hydroxyl group affording the high regioselectivity. In another example, Kolb *et al.* reported[30] a similar reaction without any protection of the *C*-6-amino group of adenosine. In view of these and our observations, reaction of unprotected 2′-deoxyadenosine (**9**) with HONPhth/Ph_3P/DEAD in DMF afforded **14** in 62% yield (Scheme 1). Hydrazinolysis of **14** in a manner described earlier gave 5′-*O*-amino-2′-deoxyadenosine (**4**) in 80% yield.

Our success with the conversion of unprotected nucleosides **6**, **7**, and **9** to **11**, **12**, and **14**, respectively, encouraged us to utilize unprotected 2′-deoxyguanosine (**10**) for

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(27) Perbost, M.; Sanghvi, Y. S. *J. Chem. Soc. Perkin Trans. 1* **1994**, 2051.
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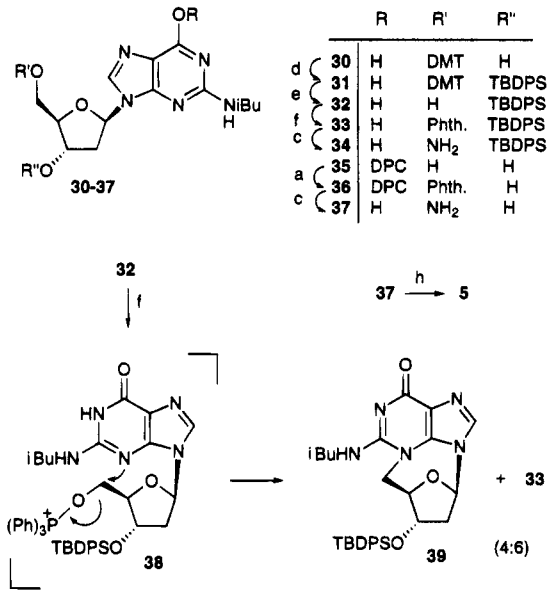
(30) Kolb, M.; Barth, *J. Liebigs Ann. Chem.* **1985**, 1036.

Scheme 4[a]



[a] See Scheme 1 legend for reagents and conditions.

its transformation to 5′-*O*-amino-2′-deoxyguanosine (**15**). Treatment of **10** with HONPhth/Ph_3P/DEAD in DMF under standard conditions failed to give the desired product **15**. Close examination of this reaction under various conditions including (a) changing the solvent to HMPT;[31] (b) using a new redox system[32] [1,1′-(azodicarbonyl)dipiperidine−tributylphosphine]; (c) higher reaction temperature (up to 100 °C);[18] and (d) altering the order of addition of reagents did not give **15**. Kimura *et al.* have reported[33] similar unsuccessful attempts to carry out a Mitsunobu reaction on dG (**10**). We believe that the principle reason of unreactivity of dG (**10**) is due to its poor solubility in most of the solvents.

Therefore an appropriately protected dG derivative **32** was prepared with the expectation that it would be soluble in most of the necessary solvents. Commercially available[15] nucleoside **30** was 3′-*O*-silylated, followed by acidic 5′-*O*-deprotection of the product **31** resulted in good yields (72%) of dG derivative **32**. As expected, **32** reacted with HONPhth/Ph_3P/DIPAD under standard conditions to provide a mixture of two products in a 4:6 ratio (total 70%). The two products were separated by fractional crystallization from EtOH/ether. The major (less polar on TLC) product was characterized as the desired 5′-*O*-phthalimidonucleoside **33**, based on its elemental analysis, NMR, and mass spectral data. NMH treatment of **33** provided **34** in good yield (71%).

Assignment of each proton resonance of the side product (more polar on TLC) indicated an N^3-5′-cyclonucleoside structure **39**, as shown in Scheme 4. The molecular modeling results (Figure 2) indicated that N^3-5′-cyclonucleoside **39** adopted a ^1E conformation, an *N*-sugar pucker. As a result, the H1′−2′ dihedral is 78°, near value for minimum H1′−H2′ three-bond coupling constant. Therefore, the signal from the H1′ proton is expected to appear as a doublet from the H1′−H2′ coupling, rather than the pseudo triplet or quartet

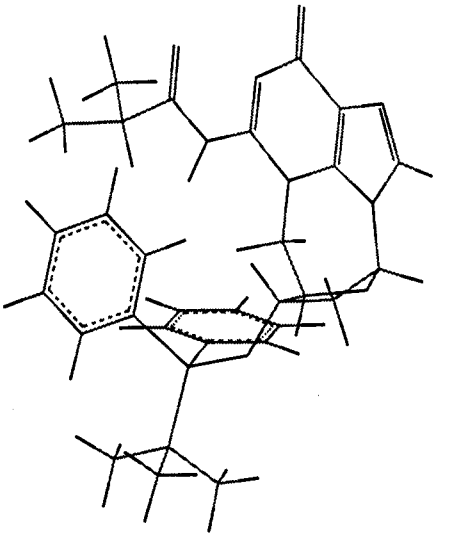


Figure 2. Structure of 9-[3-*O*-(*tert*-butyldiphenylsilyl)-5,N^3-cyclo-2′-deoxy-β-D-*erythro*-pentofuranosyl]-2-*N*-isobutyrylguanine (**39**). The minimum energy conformation was determined using a distance geometry minimization with the Biosym (San Diego) Insight and Discover software. A total of 2000 fs steps were employed using a conjugate gradient minimization routine (Crippen, G. M. *Distance Geometry and Conformationa Calculations*; John Wiley & Sons: New York, 1981).

structure normally seen for the H1′ signal in deoxynucleosides. The H5′ and H5″ protons have identical 2.6 Hz couplings to the H4′ proton, consistent with the bifurcation of the two protons by the plane of the purine ring. However, the H5′ proton was shifted considerably to 4.85 ppm (downfield), while the H5″ proton appears upfield at 3.59 ppm. In addition, the resonance of the H3′ proton also appears upfield at 4.46 ppm. These chemical shift changes result from the proximity of the H3′ and H5′ protons to the phenyl rings of the 3′-TBDPS group. Satisfactory elemental analysis and mass spectra were also obtained on the cyclonucleoside **39**.

The formation of **39** can be rationalized by assuming intermediacy of a quaternary phosphonium salt **38**, which could be cyclized by nucleophilic attack of N^3 lone-pair to the 5′-carbon atom, eliminating Ph_3P=O (Scheme 4). An analogous observation of the formation of exclusively N^3-5′-cyclonucleoside was made by Kimura *et al.* while preparing 5′-phosphates of adenosine and guanosine.[33] They postulated that the preponderance of cyclonucleoside formation was due to electrostatic interaction between the phosphorus cation and purine base of adenine or guanine and it was in close proximity to favor a cyclization. The cyclization results indicated that the intramolecular elimination may be avoided if the lone pair of N^3 was delocalized by aromatization of the purine ring. In order to check this hypothesis, we synthesized a base−protected analog **35** following a modified literature procedure.[34] Mitsunobu reaction of **35** with HONPhth/Ph_3P/DIPAD in a mixture of THF/DMF provided 69% yield of 5′-*O*-phthalimido derivative **36** as the sole product. No cyclonucleoside was detected under the described conditions. Hydrazinolysis of **36** followed by ammonolysis of the product **37** afforded **5** in 45% overall yield.

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Conclusions

In summary, we believe that the present syntheses constitutes a convenient approach for the conversion of the 5'-hydroxyl group of a 2'-deoxynucleoside into the corresponding 5'-*O*-amino substituted derivatives. Among unprotected nucleosides the Mitsunobu reaction on T and dA represents a regioselective 5'-O−N-bond formation. The methodology described herein is attractive because it utilizes readily available reagents. Indeed, it is reasonable to conclude that the described synthetic strategy is likely to be valuable in the preparation of various ribonucleosides[35] and sugar/base modified nucleosides as well. To the best of our knowledge, this is for the first time that all four natural 2'-deoxynucleosides (*i.e.* T, dC, dA and dG) have been transformed to their 5'-*O*-amino derivatives, which may prove to be useful as antiviral agents,[36] as interesting antiviral activity of 3'-*O*-amino nucleosides has been reported.[37] The coupling of these 5'-*O*-amino-2'-deoxynucleosides with 3'-*C*-formyl nucleosides to provide MMI linked mixed base dimers for antisense oligonucleosides is in progress and will be a subject matter of another publication.[38]

Experimental Section

Starting Materials, Reagents, and Abbreviations. N^4-Benzoyl-2'-deoxy-5'-*O*-(dimethoxytrityl)cytosine (**16**), 2'-Deoxy-5'-*O*-(dimethoxytrityl)-N^2-isobutyryl guanosine (**30**), 2'-deoxyuridine (dU), thymidine (T), 2'-deoxycytidine (dC), 2'-deoxyadenosine (dA), and 2'-deoxyguanosine (dG) were purchased from Chem-Impex International Inc., Wood Dale, IL; 5'-*O*-(*tert*-Butyldimethylsilyl)thymidine was prepared according to the procedure reported by Nair *et al.*;[39] 2'-deoxy-O^6-(diphenylcarbamoyl)-N^2-isobutyrylguanosine was prepared according to the procedure reported by Kamimura *et al.*;[34] *N*-hydroxyphthalimide (HONPhth), triphenylphosphine (Ph_3P), diisopropyl azodicarboxylate (DIPAD), dimethylformamide (DMF), *N*-methylhydrazine (NMH), dichloromethane (DCM), *tert*-butyldiphenylsilyl chloride (TBDPS-Cl), *tert*-butyldimethylsilyl chloride (TBDMS-Cl), triethylamine (TEA), and trimethylsilyl trifluoromethanesulfonate (TMSTf) were purchased from Aldrich Chemical Co.

Details of the general experimental procedures have been published elsewhere.[40]

Experimental Methods a−k Related to Reaction Schemes 1−4. Method a. Mitsunobu Reaction (e.g. **6** → **11**, **7** → **12**, and **9** → **14**) (**7** → **12**: a representative example). To a stirred mixture of thymidine (**7**, 72.6 g, 0.30 mol), NONPhth (65.25 g, 0.39 mol), and Ph_3P (102.0 g, 0.39 mol) in DMF (1000 mL) was added DEAD (78.3 g, 0.45 mol) over a period of 3 h at ~0 °C under inert (argon) atmosphere. The reaction mixture was then stirred at room temperature for 18 h. The TLC (CH_2Cl_2:MeOH, 9:1, v/v) of the reaction mixture indicated complete consumption of the starting material and formation of a less polar product (R_f 0.62). The solution was concentrated under vacuum to remove most of the DMF. The syrupy residue was dissolved in CH_2Cl_2 (1000 mL) and poured into vigorously stirred cold water (~10 °C, 5000 mL). After 1 h, the precipitate was filtered and washed with cold water (3 × 1000 mL) and ether (2 × 500 mL). The precipitated product was dried (P_2O_5) to furnish 12 (69.6 g, 60%, homogenous on TLC). Crystallization of this material from EtOH gave **12** an analytically pure sample. However, precipitated **12** was found to be pure enough for subsequent reactions.

Method b. An alternate Mitsunobu reaction (e.g. **7** → **12**). The reaction was carried out in a similar manner as described in method a above where Ph_3P was replaced by *p*-$(Me_2N)PhP(Ph)_2$ and in workup cold aqueous HCl (0.1 N) was employed to furnish 67% of **12**.

Method c. Hydrazinolysis (e.g., **11** → **1**, **12** → **2**, **14** → **4**, **19** → **20**, **28** → **29**, **33** → **34**, **36** → **37**) **14** → **4**: a representative example). 2'-Deoxy-5'-phthalimidoadenosine (**14**, 3.65 g, 10 mmol) was dissolved in MeOH:CH_2Cl_2 (100 mL, 2:8, v/v) with slight warming. Cold (5 °C) NMH (0.74 mL, 14 mmol) was added over a period of 5 min to above solution at room temperature under an argon atmosphere while stirring. The clear reaction mixture was stirred for 1−2 h at room temperature. During this time a thick precipitation of 1,2-dihydro-4-hydroxy-2-methyl-1-oxophthalazine occurred which is indicative of complete hydrazinolysis. TLC (CH_2Cl_2/MeOH, 9:1, v/v) of the reaction mixture indicated that all of the starting material was consumed and only one major spot for the product (R_f 0.18) was observed. The reaction mixture was then cooled (~ 15 °C), whereupon most of the phthalazine falls out of the solution. Filtration of the mixture provided a clear solution free (almost) of phthalazine derivative. The solids were further washed with cold (~15 °C) CH_2Cl_2 (2 × 25 mL) to recover the product. The combined filtrates were found to be pure enough for the next step. However, the solvent was evaporated in vacuum and the residue purified by silica gel chromatography (CH_2Cl_2/MeOH, 9:1 → 6:4). Pooling of appropriate fractions and concentration furnished **4** as colorless solid (2.13 g, 80%).

Method d. Silylation (e.g. **16** → **17**, **30** → **31**) (**16** → **17**: a representative example). To a stirred mixture of N^4-benzoyl-2'-deoxy-5'-*O*-(dimethyoxytrityl)cytosine (**16**, 20.0 g, 31.6 mmol) and imidazole (6.53 g, 97 mmol) in DMF (100 mL) was added dropwise TBDPS-Cl (9.9 mL, 38.7 mmol) over 1 h at room temperature under an argon atmosphere. The reactants were allowed to stir at room temperature for 16 h, and MeOH (10 mL) was added to quench the excess TBDPS-Cl. The solution was concentrated (> 45 °C; 2 mmHg) to obtain a mobile syrupy residue. The syrup was poured into ice−water (500 mL) and extracted with EtOAc (2 × 300 mL). The combined extracts were washed with water (4 × 250 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuum, and the crude product **17** (~27 g, quantitative) was found to be pure enough for the next step (detritylation).

Silylation of commercially available nucleoside **30** in a similar manner provided 75% yield of protected **31**.

Method e. Detritylation (e.g. **17** → **18**, **31** → **32**) (**17** → **18**: a representative example). To a stirred solution of **17** (27.0 g, 31 mmol) in DCM (250 mL) was added trichloroacetic acid (10.27 g, 63 mmol) under an argon atmosphere at room temperature. After 2−3 h, TLC (CH_2Cl_2/MeOH, 95:5) indicated complete consumption of the starting material. The red colored solution was poured into ice-water (500 mL) containing $NaHCO_3$ (25 g). The suspension was stirred for 1 h and extracted with DCM (2 × 250 mL). The combined extracts were washed with cold aqueous $NaHCO_3$ (5%, 2 × 100 mL) and water (2 × 250 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuum and the residue purified by column chromatography (silica gel, EtOAc/hexanes, 0 → 50%), Appropriate fractions were pooled and concentrated to provide **18** (10.0 g, 65%) as a pale foam.

Detritylation of **31** in a similar manner gave **32** in 72% yield.

Method f. Mitsunobu Reaction (e.g., **18** → **19**, **32** → **33**) (**18** → **19**: a representative example). To a stirred mixture of **18** (10.0 g, 17.5 mmol), HONPhth (3.25 g, 19.3 mmol), and Ph_3P (5.12 g, 19.3 mmol) in anhydrous THF (200 mL) was added DIPAD (4.0 mL, 19.3 mmol) over a period of 15 min. at

(35) We have also prepared 5'-*O*-aminouridine, 5'-*O*-amino-5-methyluridine, 5'-*O*-amino-2'-*O*-methyluridine, 5'-*O*-amino-2'-*O*-methyl-5-methyluridine, and 5'-*O*-amino-2'-deoxy-2'-fluoro-5-methyluridine following the Mitsunobu reaction described in this manuscript.

(36) Preliminary screening of 5'-*O*-aminothymidine **12** against HSV-2 and influenza A indicated no antiviral activity. The antiviral screening of remaining nucleosides is in progress.

(37) De Clercq, E.; Inoue, I.; Kazuhiko, K. European Patent 0381335 A1, 1990.

(38) Preliminary results have been presented; Hoshiko, T.; Fraser, A.; Perbost, M.; Dimock, S.; Cook, P. D.; Sanghvi, Y. S. 207th American Chemical Society National Meeting, San Diego, CA, March 13−17, 1994, CARB 35. Sanghvi, Y. S.; Bellon, L.; Morvan, F.; Hoshiko, T.; Swayze, E.; Cummins, L.; Freier, S.; Dean, N.; Monia, B.; Cook, P. D. 11th IRT Nucleosides, Nucleotides & Their Biological Applications, Leuven, Belgium, September 7−11, 1994.

(39) Nair, V.; Buenger, G. *Org. Prep. Proc. Int.* **1990**, *22*, 57.

(40) Fraser, A.; Wheeler, P.; Cook, P. D.; Sanghvi, Y. S. *J. Heterocycl. Chem.* **1993**, *30*, 1277.

room temperature under argon atmosphere. On complete addition, the reaction mixture turned orange/red in color, which faded to pale yellow at the end of the reaction. TLC ($MeOH/CH_2Cl_2$, 5:95) after 16 h indicated the reaction to be complete. The products were concentrated and the residue was dissolved in diethyl ether/hexanes. The desired product **19** (10.15 g, 81%) was crystallized as white needles (~10% contaminated with $Ph_3P{=}O$).

Treatment of **32** in an analogous manner provided a mixture of **33** and **39** in a 6:4 ratio (70%), respectively.

Method g. Desilylation (20 → 21). To a stirred solution of **20** (0.82 g, 1.4 mmol) in the THF (10 mL) was added TBAF (1 M in THF; 1.5 mL) under the argon atmosphere at room temperature. After 3 h, TLC (CH_2Cl_2/MeOH, 95:5) indicated complete consumption of the starting material and formation of a less polar spot (R_f 0.11) as the sole product. The solvent was evaporated under vacuum and the residue purified by silica gel chromatography (CH_2Cl_2/MeOH, 9:1). Pooling of the appropriate fractions and evaporation furnished **21** as a white powder (0.40 g, 82%).

Method h. Debenzoylation (21 → 3). A solution of **21** (0.35 g, 1 mmol) in saturated (0 °C) methanolic NH_3 (20 mL) was stirred in a pressure bottle for 12 h at room temperature. The bottle was cooled (0 °C) and opened and solvent evaporated to dryness. The residue was washed with diethyl ether (2 × 25 mL), EtOAc (25 mL), and DCM (25 mL) to remove benzamide. The product **3** (0.11 g, 45%) was dried and found to be pure for all practical purpose (Attempted crystallization of **3** resulted in decomposition).

Method i. Triazolation[26] (24 → 25). To a stirred solution of 1,2,4-triazole (41.1 g, 0.58 mol) in anhydrous CH_3CN (400 mL) at 0 °C was added freshly distilled $POCl_3$ (12.0 mL, 0.13 mol) over a period of 1 h, followed by TEA (90.36 mL, 0.64 mol) over a period of 1 h under argon atmosphere. The stirring was continued for 1 h, before a solution of **24** (77.0 g, 0.13 mol) in anhydrous CH_3CN (200 mL) was added dropwise (1 h) to the reaction mixture at 0 °C. After addition, solution was allowed to warm to room temperature and stirred for 3 h. The reaction was quenched by addition of aqueous TEA (30 mL, 1:1, TEA/H_2O) at 0 °C and concentrated under vacuum to afford a syrup. The residue was diluted with EtOAc (1 Lt), extracted with aqueous $NaHCO_3$ (2 × 500 mL), water (2 × 250 mL), and brine solution (2 × 200 mL), dried ($MgSO_4$), filtered, and concentrated to provide a foam. The crude product **25** was purified silica gel chromatography (16 × 60 cm, 1 kg). The appropriate fractions were eluted with EtOAc/hexanes (0:1 → 1:1), pooled, and concentrated to furnish the product **25** (57.3 g, 68%) as white solid.

Method j. Displacement Reaction[27] (25 → 26). To benzamide (15.75 g, 130 mmol) in dry dioxane (325 mL) was added sodium hydride (60% w/w, 5.2 g, 130 mmol), and the resulting suspension was stirred at room temperature for 1 h. This mixture was then transferred via cannula into a well stirred solution of the triazolide **25** (21 g, 32.5 mmol) in dry dioxane (325 mL). The resulting mixture was stirred at room temperature under an inert atmosphere for 2 h, and acetic acid (7.3 mL, 130 mmol) was added to quench the reaction. The mixture was then diluted with 900 mL of 33% EtOAc/hexane, washed with water (4 × 400 mL), filtered through magnesium sulfate, and concentrated in vacuum to a syrup. This material was chromatographed (silica, 11 × 10 cm, hexane to 30% EtOAc/hexane) to provide 21.7 g (96%) of **26** as a foam.

Method k. Selective Desilylation[28] (26 → 27). To N^4-benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-(*tert*-butyldiphenylsilyl)-5-methyl-2′-deoxycytidine (**26**, 23.8 g, 34 mmol) in dry CH_2Cl_2 (340 mL) at 0 °C was added TMSTf (36 mL, 186 mmol) dropwise over 3 h. The reaction was stirred an additional 1 h at 0 °C and poured into 500 mL of ice cold saturated aqueous $NaHCO_3$, and the mixture was allowed to come to room temperature with vigorous stirring over 1 h. The organic layer was separated, washed with water, dried over magnesium sulfate, concentrated, dissolved in the minimum amount of CH_2Cl_2, and loaded onto a column (silica, 11 × 15 cm) packed in 10% EtOAc/hexane. Elution with 10 to 50% EtOAc/hexane and concentration of the appropriate fractions provided 16.4 g (83%) of **27** as a foam.

5′-*O*-Amino-2′-deoxyuridine (1). Prepared by method c in 85% yield; mp 150−152 °C; ^{1}H NMR (DMSO-d_6) δ 2.07, 3.68, 3.90, 4.17, 5.30, 5.62, 6.12, 6.14, 7.66, 11.29; ^{13}C NMR (DMSO-d_6) δ 39.1, 70.7, 75.5, 84.3, 84.6, 101.9, 140.5, 163.1, 150.4. Anal. Calcd for $C_9H_{13}N_2O_5$: C, 44.44; H, 5.38; N, 17.27. Found: C, 44.26; H, 5.38; N, 16.90.

5′-*O*-Aminothymidine (2). Prepared by method c in 77% yield; mp 195−197 °C; ^{1}H NMR (DMSO-d_6) δ 1.80, 2.10, 3.70, 3.96, 4.22, 5.37, 6.14, 6.17, 7.53, 11.37; ^{13}C NMR (DMSO-d_6) δ 12.2, 38.8, 70.7, 75.5, 81.4, 83.7, 109.6, 135.7, 150.4, 163.7. Anal. Calcd for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.33. Found: C, 46.67; H, 5.83; N, 16.18.

5′-*O*-Amino-2′-deoxycytidine (3). Prepared by method h in 45% yield; mp 155−158 °C; ^{1}H NMR (DMSO-d_6) δ 1.91, 2.06, 3.65, 3.90, 4.12, 5.23, 5.77, 6.12, 6.25, 7.10, 7.18, 7.60; mass: $[M + H]^+$ *m/z* calcd 243.24, found 243.3.

5′-*O*-Amino-2′-deoxyadenosine (4). Prepared by method c in 80% yield; mp 135−136 °C; ^{1}H NMR (DMSO-d_6) δ 2.23−2.35, 2.72, 2.86, 3.60−3.81, 3.99−4.07, 4.36−4.46, 5.39, 6.09, 6.34, 7.29, 8.15, 8.31; ^{13}C-NMR (DMSO-d_6) δ 71.89, 75.77, 83.16, 84.65, 119.13, 139.41, 149.18, 152.61, 156.05. Anal. Calcd for $C_{10}H_{14}N_6O_3{\cdot}^2/_3$MeOH: C, 44.54; H, 5.84; N, 29.22. Found: C, 44.37; H, 5.60; N, 29.35.

5′-O-Amino-2′-deoxyguanosine (5). Prepared by method c in 80% yield; ^{1}H NMR (DMSO-d_6) δ 2.20, 2.56, 3.67, 3.98, 4.31, 5.34, 6.12, 6.51, 7.89, 9.90. Anal. Calcd for $C_{10}H_{14}N_6O_4{\cdot}0.2H_2O{\cdot}0.2$AcOH: C, 41.93; H, 5.14; N, 28.21. Found: 41.79; H, 5.02; 28.32.

5′-*O*-Phthalimido-2′-deoxyuridine (11). Prepared by method a in 62% yield; mp 240 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.15, 4.09, 4.36, 5.49, 6.2, 5.62, 7.6, 7.88, 11.34; ^{13}C NMR (DMSO-d_6) δ 38.7, 70.5, 77.6, 84.0, 84.6, 101.9, 140.6, 150.4, 163.0, 120.8, 123.3. Anal. Calcd for $C_{17}H_{15}N_3O_7$: C, 54.69; H, 4.05; N, 11.26. Found: C, 54.61; H, 3.95; N, 11.15.

5′-*O*-Phthalimidothymidine (12). Prepared by method a in 80% yield or method b in 67% yield; mp 233−235 °C dec; ^{1}H NMR (DMSO-d_6) δ 11.29, 7.85, 7.58, 6.20, 2.09−21.13, 1.79. Anal. Calcd for $C_{18}H_{17}O_7N_3{\cdot}0.7H_2O$: C, 54.05; H, 4.64; N, 10.51. Found: C, 53.81; H, 4.25; N, 10.39.

2′-Deoxy-5′-*O*-phthalimidoadenosine (14). Prepared by method a in 62% yield; mp 140−142 °C; ^{1}H NMR (DMSO-d_6) δ 2.28−2.40, 2.76−2.90, 4.18−4.28, 4.41, 4.51−4.61, 5.54, 6.38, 7.27, 7.78−7.90, 8.12, 8.33; ^{13}C-NMR (DMSO-d_6) δ 71.0, 77.8, 83.6, 84.4, 119.1, 123.2, 134.2, 128.5, 139.5, 149.1, 152.6, 156.0, 163.0. Anal. Calcd for $C_{18}H_{16}N_6O_5{\cdot}^1/_2CH_3OH$: C, 53.88; H, 4.40; N, 20.38. Found: C, 53.64; H, 4.21; N, 20.22. Mass: $[M + H]^+$ 397, $[A + 2H]^+$ 136.

N^4-Benzoyl-3′-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxycytidine (18). Prepared by method e in 65% yield as a foam; ^{1}H NMR ($CDCl_3$) δ 1.09, 2.06, 2.24, 2.60, 3.25, 3.67, 4.03, 4.45, 6.27, 7.15 - 7.35, 7.35 - 7.70, 8.15, 8.67; ^{13}C ($CDCl_3$) δ 39.0, 74.0, 79.1, 85.0, 87.5, 94.0, 127.9, 130., 132.9, 135.2, 161.0, 140.8, 155.0, 165.5.

N^4-Benzoyl-3′-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxy-5′-*O*-phthalimidocytidine (19). Prepared by method f in 81% yield; mp 175−176 °C; ^{1}H NMR ($CDCl_3$) δ 1.09, 1.95, 2.83, 3.68, 4.03, 4.17, 4.70, 6.56, 7.30−8.00, 8.61. Anal. Calcd for $C_{40}H_{38}N_4O_7Si{\cdot}0.25H_2O$: C, 66.54; H, 5.42; N, 7.76. Found: C, 66.79; H, 5.37, N, 7.71.

5′-*O*-Amino-N^4-benzoyl-3′-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxycytidine (20). Prepared by method c in 66% yield; mp 158 °C; ^{1}H NMR ($CDCl_3$) δ 1.07, 1.95, 2.75, 3.31, 3.36, 4.16, 4.35, 5.26, 8.1, 6.36, 7.3−7.9, 8.71.

5′-*O*-Amino-N^4-benzoyl-2′-deoxycytidine (21). Prepared by method g in 82% yield as a foam; ^{1}H NMR (DMSO-d_6) δ 2.05, 2.31, 3.74, 4.03, 4.20, 5.33, 6.13, 6.22, 7.39, 7.50, 7.61, 7.98, 8.20, 11.24. Anal. Calcd for $C_{16}H_{18}N_4O_5{\cdot}H_2O$: C, 54.08; H, 5.38; N, 15.76. Found: C, 54.19; H, 5.22; N, 15.74.

2,3′-Anhydro-5′-*O*-phthalimido-(β-D-*threo*-pentofuranosyl)cytosine Hydrate (23). Prepared by method a in 65% yield (using double the amount of all reagents); mp 208−210 °C dec; UV_{max} (pH 7) 258 nm (ε 13,000), 302 nm (ε 3,140); ^{1}H NMR (DMSO-d_6) δ 2.68, 2.8, 4.32, 4.55, 4.72, 5.66, 6.25, 6.52, 7.89, 8.16, 9.07, 9.29; ^{13}C NMR (DMSO-d_6) δ 31.8, 76.0, 79.6,

83.1, 88.4, 101.3, 123.3, 136.5, 163.0, 143.9, 154.5, 166.0. Anal. Calcd for $C_{17}H_{15}N_4O_6$: C, 54.99; H, 4.07; N, 15.09. Found: C, 52.15; H, 3.72; N, 14.24. Mass: m/z 371 $(M)^+$, 355 $(M-NH_2)^+$.

5′-*O*-(*tert*-Butyldimethylsilyl)-3′-*O*-(*tert*-butyldiphenylsilyl)thymidine (24). 5′-*O*-(*tert*-Butyldimethylsilyl)thymidine[39] was silylated by method d to provide **24** (86%) as a foam. ^{1}H NMR ($CDCl_3$) δ −0.08, 0.10, 1.08, 1.60, 1.82, 2.31, 3.13, 3.63, 3.99, 4.32, 6.48, 7.37−7.65, 8.15. Anal. Calcd for $C_{32}H_{46}N_2O_5Si_2$: C, 64.60; H, 7.79; N, 4.70. Found: C, 64.31; H, 7.79; N, 4.63.

5′-*O*-(*tert*-Butyldimethylsilyl)-3′-*O*-(*tert*-butyldiphenylsilyl)-4-(1,2,4-triazolyl)thymidine (25). Prepared by method i in 68% yield; mp 135−136 °C; ^{1}H NMR (DMSO-d_6) δ 0.71, 1.06, 1.89, 2.32, 2.59, 3.20, 3.63, 4.10, 4.32, 6.32, 7.33−7.47, 7.58−7.62, 8.13−8.15, 9.20. Anal. Calcd for $C_{34}H_{47}N_5O_4Si_2$: C, 63.22; H, 7.33; N, 10.84. Found: C, 63.18; H, 7.18; N, 10.78.

N^4-Benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-(*tert*-butyldiphenylsilyl)-5-methyl-2-deoxycytidine (26). The cytidine analog **26** was prepared from **25** via method j in 96% yield as a foam: R_f 0.73 (20% EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 13.36, 8.33, 7.70−7.36, 6.26, 4.38, 4.06, 3.66, 3.15, 2.45, 2.08, 1.90, 1.28, 0.82, 0.03, 0.08; ^{13}C NMR (DMSO-d_6) δ 12.9, 17.7, 18.5, 25.4, 26.5, 62.7, 73.7, 84.9, 87.5, 110.2, 127.6, 127.9, 129.2, 129.8, 132.1, 132.4, 135.1, 137.1, 147.1, 158.9, 178.2. Anal. Calcd for $C_{39}H_{51}N_3O_5Si_2$: C, 67.11; H, 7.36; N, 6.02. Found: C, 66.91; H, 7.31; N, 5.67.

N^4-Benzoyl-3′-*O*-(*tert*-butyldiphenylsilyl)-5-methyl-2′-deoxycytidine (27). The cytidine analog **27** was prepared from **26** in 83% yield by method k as a foam: R_f 0.42 (30% EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 8.30, 7.69−7.36, 6.26, 4.46, 4.00, 3.68, 3.27, 2.45−1.92, 2.04, 1.09. Anal. Calcd for $C_{33}H_{37}N_3O_5Si$: C, 67.90; H, 6.39; N, 7.20. Found: C, 67.60; H, 6.34; N, 7.15.

5′-*O*-Phthlimido-N^4-benzoyl-3′-*O*-(*tert*-butyldiphenylsilyl)-5-methyl-2′-deoxycytidine (28). The cytidine analog **28** was prepared from **27** via method f utilizing diethyl azodicarboxylate in place of DIPAD in essentially quantitative yield. A sample was precipitated from ether/hexane (83% recovery) for analysis; R_f 0.47 (30% EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 13.32, 8.33, 7.90−7.35, 6.56, 4.80, 4.15−3.55, 2.60−1.95, 2.16, 1.13. Anal. Calcd for $C_{41}H_{40}N_4O_7Si{\cdot}H_2O$: C, 65.93; H, 5.67; N, 7.50. Found: C, 65.59; H, 5.56; N, 7.39.

5′-*O*-Amino-N^4-benzoyl-3′-*O*-(*tert*-butyldiphenylsilyl)-5-methyl-2′-deoxycytidine (29). The cytidine analog **29** was prepared from **28** using method c. Chromatographic purification (30% EtOAc/hexane) afforded **29** (83%) as a foam; R_f 0.37 (30% EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 13.28, 8.32, 7.75−7.35, 6.41, 5.31, 4.39, 4.11, 3.51, 2.43, 2.07, 1.89, 1.09. Anal. Calcd for $C_{33}H_{38}N_4O_5Si{\cdot}^1/_4H_2O$: C, 65.70; H, 6.43; N, 9.29. Found: C, 65.66; H, 6.35; N, 9.23.

9-[3-*O*-(*tert*-butyldiphenylsilyl)-5-*O*-(4,4′-dimethoxytrityl)-2-deoxy-β-D-*erythro*-pentofuranosyl]-2-*N*-isobutyrylguanine (31). Commercially available[15] nucleoside **30** was silylated following the general protocol d to furnish **31** (75%). The crude **31** was deprotected by method e without characterization.

9-[3-*O*-(*tert*-Butyldiphenylsilyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]-2-*N*-isobutyrylguanine (32). Deprotection of crude **31** by method e gave **32** (72%) as a foam. ^{1}H NMR ($CDCl_3$) δ 1.19, 1.22, 2.29, 2.59, 3.13, 3.68, 4.60, 4.09, 4.60, 6.22, 7.35−7.50, 7.60−7.72, 8.60, 12.07; ^{13}C NMR ($CDCl_3$) δ 18.9, 26.9, 40.9, 62.6, 74.5, 86.8, 89.1, 122.5, 138.0, 130.1, 133.0, 135.7, 138.7, 147.1, 147.5, 155.1, 179.1. Anal. Calcd for $C_{30}H_{37}N_5O_5Si{\cdot}0.6H_2O$: C, 61.43; H, 6.56; N, 11.94. Found: C, 61.14; H, 6.66; N, 11.86.

9-[3-*O*-(*tert*-Butyldiphenylsilyl)-5′-*O*-(*N*-phthalimidyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]-2-*N*-isobutyrylguanine (33). The nucleoside **32** was transformed to **33** (and **39** in 6:4 ratio, total 70%) following the method f. Mp 132−134 °C; ^{1}H NMR ($CDCl_3$) δ 1.09, 1.19, 1.22, 2.29, 2.60, 2.74, 4.01−4.20, 4.39, 4.72, 6.24, 7.4, 7.71, 8.63, 11.98. Anal. Calcd for $C_{38}H_{40}N_6O_7Si{\cdot}1.5H_2O$: C, 61.03; H, 5.80; N, 11.24. Found: C, 61.22; H, 5.87; N, 10.96.

9-[5-*O*-Amino-3-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]-2-*N*-isobutyrylguanine (34). Hydrazinolysis of **33** by method c gave **34** (71%) as a foam. ^{1}H NMR ($CDCl_3$) δ 1.08, 1.20, 2.38, 2.66, 3.45, 3.62, 4.13, 4.49, 6.23, 7.31−7.73, 9.28; ^{13}C NMR ($CDCl_3$) δ 19.0, 26.8, 40.6, 73.5, 75.3, 84.0, 85.9, 121.6, 127.9, 130.1, 133.0, 135.7, 137.1, 147.4, 148, 155.5. Anal. Calcd for $C_{30}H_{38}N_6O_5Si{\cdot}H_2O$: C, 60.26; H, 6.54; N, 14.05. Found: C, 60.07; H, 6.37, N, 13.80.

9-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-6-*O*-(diphenylcarbamoyl)-2-*N*-isobutyrylguanine (35). Modified procedure:[34] To a stirred solution of 2′-deoxyguanosine (50 g, 0.175 mol) in anhydrous pyridine (900 mL) was added TEA (145 mL, 0.96 mol) at room temperature. After 15 min, isobutyl chloride (65.0 g, 0.61 mol) in pyridine (100 mL) was added to the reaction mixture over a period of 1 h at 0 °C. After 3 h, diphenylcarbamoyl chloride (88.5 g, 0.35 mol) was added dropwise (1 h) to the reaction mixture at 0 °C. Subsequently, TEA (60 mL, 0.36 mol) was added to the reaction mixture at 0 °C and the solution was stored at 4 °C for 16 h. A solution of 2 M NaOH/EtOH/pyridine (2000 mL, 5:5:2) was prepared and added to the reaction mixture at 0 °C while stirring vigorously. After 30 min, the reaction mixture was passed through Dowex 50W8 (H^+) (1 kg) and the resin washed with EtOH (2 × 250 mL). The combined filtrates were concentrated under vacuum to provide a dark colored syrupy residue, which crystallized from EtOAc on standing (5 °C) to furnish **35** (59.5 g, 64%) as of white powder. ^{1}H NMR (DMSO-d_6) δ 1.10, 1.15, 2.33, 2.80, 3.56, 3.88, 4.42, 4.95, 5.45, 6.38, 7.3−7.7, 8.63, 10.63; ^{13}C NMR (DMSO-d_6) δ 19.0, 34.2, 40.1, 61.9, 70.5, 83.8, 120.2, 126.5, 127.0, 128.3, 129.2, 131.3, 141.2, 144.0, 150.0, 152.0, 154.2, 154.6, 174.6. Anal. Calcd for $C_{27}H_{28}N_6O_6$: C, 60.89; H, 5.29; N, 15.78. Found: C, 60.49; H, 5.12; N, 15.59.

9-[5-*O*-(*N*-Phthalimidyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]-6-*O*-(diphenylcarbamoyl)-2-*N*-isobutyrylguanine (36). Base-protected **35** was converted into the title compound **36** (69%) following method a, except that DMF/THF (3:1) was used as solvent for the reaction; ^{1}H NMR ($CDCl_3$) δ 0.95, 2.30, 3.05, 4.23, 4.55, 4.56, 4.66, 5.55, 6.41, 7.3−7.7, 8.6, 10.65; ^{13}C NMR (DMSO-d_6) δ 19.0, 34.5, 45.2, 71.2, 77.7, 84.8, 120.8, 123.0, 126.8, 127.2, 128.3, 128.8, 129.4, 131.3, 131.5, 132.0, 134.5, 141.5, 144.6, 150.1, 152.0, 154.1, 154.9, 162.8, 174.7. Anal. Calcd for $C_{35}H_{31}N_7O_8{\cdot}1.5H_2O$: C, 59.65; H, 4.70; N, 13.90. Found: C, 59.72; H, 4.39; N, 13.16.

9-(3-*O*-(*tert*-Butyldiphenylsilyl)-5,N^3-cyclo-2′-deoxy-β-D-*erythro*-pentofuranosyl)-2-*N*-isobutyrylguanine (39). The title compound was obtained as a side product during preparation of **33** via Mitsunobu reaction. Mp 214−215 °C; UV (90% aqueous EtOH) λ_{max} 284 nm (ϵ 27,430); ^{1}H NMR ($CDCl_3$) δ 1.05, 2.15, 2.18, 2.07, 2.52, 2.55, 3.58, 4.89, 4.47, 4.73, 6.23, 7.35−7.65; ^{13}C NMR ($CDCl_3$) 19.3, 26.7, 45.6, 52.5, 7.25, 85.7, 88.6, 121.0, 133.8, 128.0, 130.3, 132.2, 135.5, 139.2, 152.6, 154.5, 190.9. Anal. Calcd for $C_{30}H_{35}N_5O_4Si$: C, 64.61; H, 6.33; N, 12.56. Found: C, 64.48; H, 6.29; N, 12.51.

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