JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Rigid 2′,4′-Difluororibonucleosides: Synthesis, Conformational Analysis, and Incorporation into Nascent RNA by HCV Polymerase

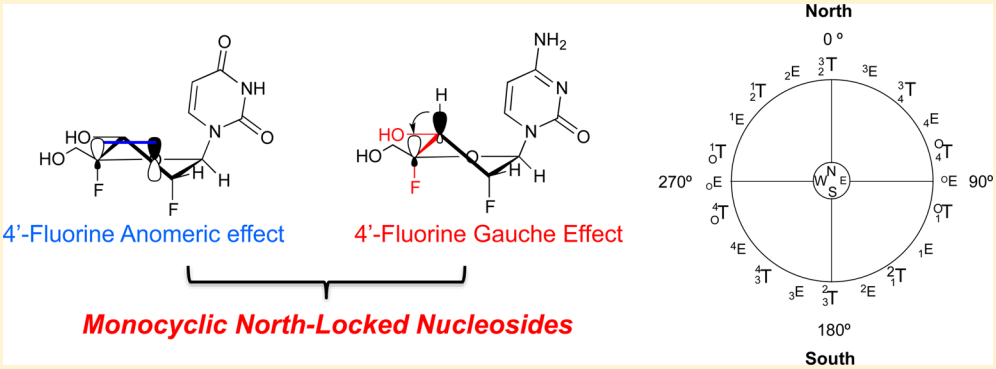
Saúl Martínez-Montero,[†] Glen F. Deleavey,[†] Anupriya Kulkarni,[‡] Nerea Martín-Pintado,[§] Petra Lindovska,[†] Michael Thomson,[†] Carlos González,[§] Matthias Götte,[‡] and Masad J. Damha*[,†]

[†]Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, QC Canada H3A 0B8

[‡]Department of Microbiology and Immunology, McGill University, 3775 University, Montreal, QC Canada H3A 2B4

[§]Instituto de Química Física Rocasolano, CSIC, C/. Serrano 119, 28006 Madrid, Spain

Supporting Information



ABSTRACT: We report on the synthesis and conformational properties of 2′-deoxy-2′,4′-difluorouridine (2′,4′-diF-rU) and cytidine (2′,4′-diF-rC) nucleosides. NMR analysis and quantum mechanical calculations show that the strong stereoelectronic effects induced by the two fluorines essentially "lock" the conformation of the sugar in the North region of the pseudorotational cycle. Our studies also demonstrate that NS5B HCV RNA polymerase was able to accommodate 2′,4′-diF-rU 5′-triphosphate (2′,4′-diF-rUTP) and to link the monophosphate to the RNA primer strand. 2′,4′-diF-rUTP inhibited RNA synthesis in dinucleotide-primed reactions, although with relatively high half-maximal inhibitory concentrations (IC_{50} > 50 μM). 2′,4′-diF-rU/C represents rare examples of "locked" ribonucleoside mimics that lack a bicyclic ring structure.

INTRODUCTION

Considerable research efforts have been concentrated to prepare chemically modified nucleoside derivatives as effective anticancer[1] and antiviral agents.[2,3] In this aspect, the conformational preferences of nucleosides can have a profound impact on their biological activity. The furanose ring of the nucleosides in solution can adopt two major antipodal conformations classified as North (*N*, C-3′*endo*, phase ≈ 0°) and South (*S*, C-2′*endo*, phase ≈ 180°) described in the pseudorotation cycle (Figure 1).[4] In the case of nucleoside antiviral compounds, the sugar conformation plays a crucial role in determining biological activity, which depends on kinase phosphorylation and polymerase-catalyzed incorporation steps.[5] Nucleosides with a sugar pucker in the South range of the pseudorotation cycle are preferentially phosphorylated by kinases, while North-type nucleosides are preferentially incorporated by polymerases.[6,7]

The level of understanding and control over nucleoside conformation has a significant impact on the ability to successfully develop therapeutic nucleosides and oligonucleotides. A variety of conformationally rigid "locked" nucleoside analogues have been used to expand the understanding of the structural and biological dependence on nucleoside conformation. In the examples provided in Figure 2A, conformational rigidity is conferred by the bicyclic nature of the nucleoside sugar moiety. Marquez and co-workers have studied the antiherpes activity of *N*-methanocarbathymidine and *S*-methanocarbathymidine.[6] The North isomer exhibited high antiherpes activity; meanwhile, the South isomer was found to be inactive, proving the important role of the sugar conformation on the biological activity of nucleoside analogues.[6,7] On the other hand, LNA (**3**, locked nucleic acid), features a methylene bridge joining the 2′-OH to C4′, restricting the sugar in the North region of the pseudorotational cycle. The rigid conformation shown by LNA and related derivatives is widely applied to increase thermal stability of duplexes and to improve gene-silencing properties of oligonucleotide-based therapeutics.[8−12]

A sugar modification that improves the biological properties of some nucleoside analogues is the introduction of fluorine, a common tool used in drug discovery efforts.[13,14] Our research

Received: April 8, 2014
Published: May 29, 2014

   dx.doi.org/10.1021/jo500794v | J. Org. Chem. 2014, 79, 5627−5635

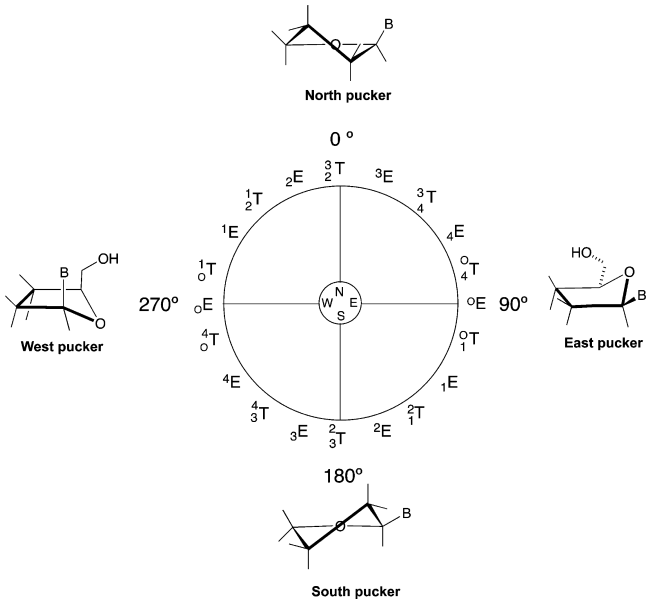


Figure 1. Pseudorotational cycle describing the sugar conformations of nucleosides; E = envelope, T = twist. Superscripts and subscripts indicate the specific atoms in the ribose ring that project away from the plane defined by the remaining ring atoms. Natural nucleosides have characteristic minima in the North (0–36°) and South regions (144–180°).

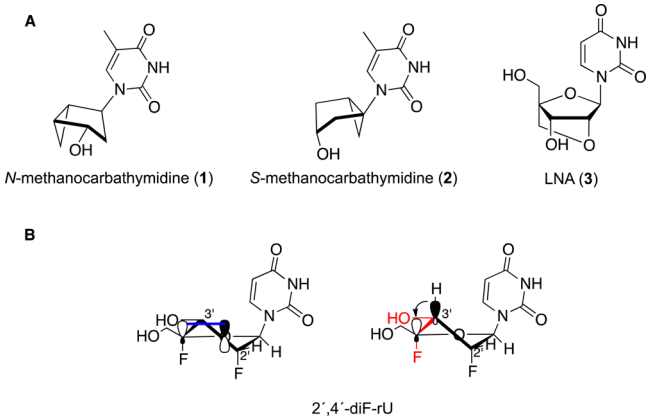


Figure 2. (A) Some examples of conformationally bicyclic "locked" nucleosides. (B) Anomeric effect (left) due to the overlap of a lone-pair orbital of O4′ (p-type) with the $\sigma^*_{C4'-F4'}$ antibonding orbital and gauche effect (right) due to the interaction between the $\sigma_{C3'-H3'}$ bonding orbital and the $\sigma^*_{C4'-F4'}$ antibonding orbital. The combined effects stabilize the North sugar pucker in 2,′4′-diF-rU.

group has focused on the development of fluorinated nucleoside derivatives for biological studies and oligonucleotide modification encouraged by the conformational changes that fluorine imparts in the ribose and arabinose moiety.[15−18] In the present study, we explore the utility of two fluorines as a tool for creating rigidified nucleoside structures similar to those in Figure 2A. The fluorine of 2′-deoxy-2′-fluorouridine (2′-F-rU) imparts a preference for the North pucker through gauche effects but does not provide the sufficient rigidity to compare with true locked analogues.[19] We reasoned that replacing the H4′ of 2′-F-rU with a fluorine, creating a 2′,4′-difluorinated analogue, would impart additional bias toward the North conformation without the need of a bicyclic scaffold. Although an energy penalty may be expected from dipole−dipole interactions from parallel aligned C−F bonds in these analogues,[20] the 4′-fluorine should introduce a strong anomeric effect favoring the North pucker and an additional gauche effect that reinforces such conformation (Figure 2B).

Since the discovery of Nucleocidin, a potent and toxic antibiotic 4′-fluorinated nucleoside derivative found in nature,[21,22] some other 4′-fluorinated nucleoside analogues have been prepared.[23−27] However, in nearly all cases, isolating the free 4′-fluorinated nucleoside was either not achieved or the isolated compound was very unstable to cleavage of the glycosidic linkage in water. This feature was deemed problematic for nucleoside-based antiviral applications. Since 2′-fluorination is known to enhance the stability of the glycosidic linkage, we hypothesized that 4′-fluorinated derivatives of 2′-F-rU nucleosides would be less prone to degradation in aqueous solution.[28] Additionally, the anticipated North conformation of these analogues featuring minimal modification to the natural ribose sugar of RNA makes them excellent candidates for incorporation into siRNAs and biological study. Herein we describe the synthesis, characterization, and conformational analysis of two novel 2′-deoxy-2′,4′-difluororibonucleosides, namely, 2′,4′-diF-rU and 2′,4′-diF-rC. We also report on preliminary incorporation studies of 2′,4′-diF-rU nucleotide by NS5B RNA polymerase.

RESULTS AND DISCUSSION

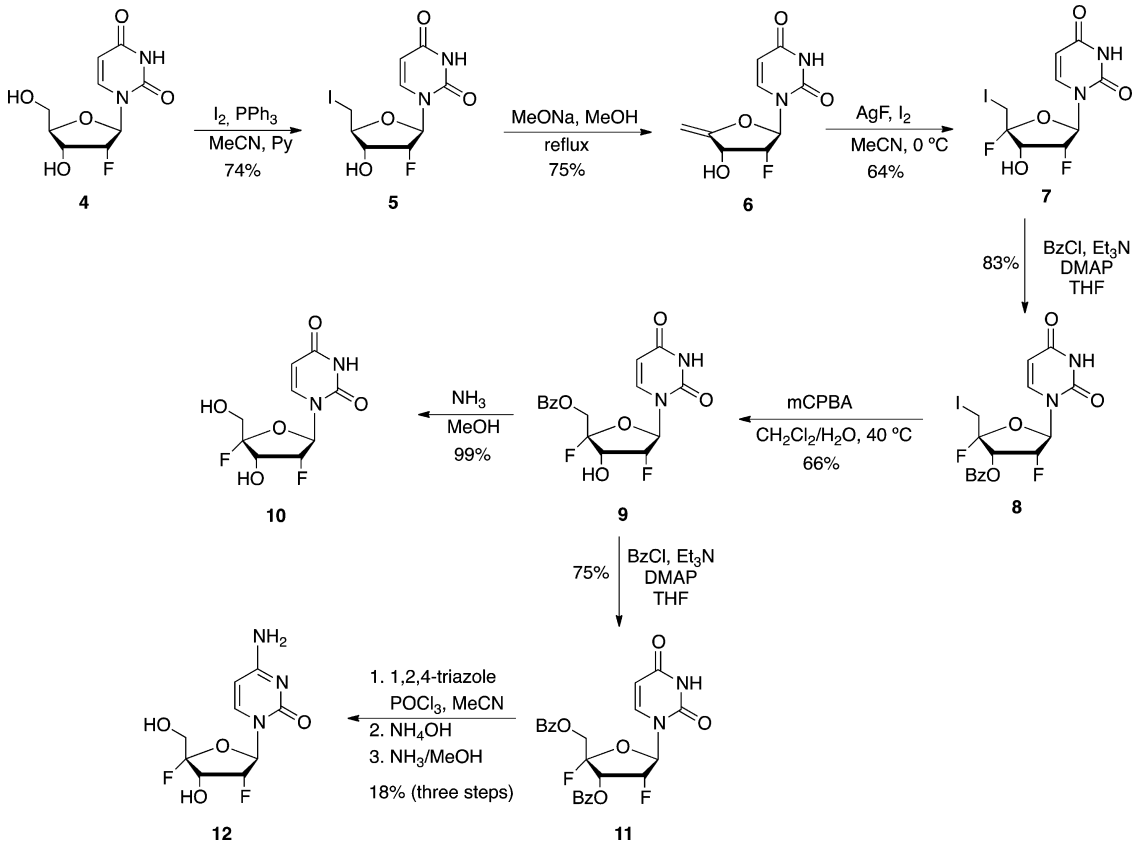
Our investigation of 2′,4′-difluorinated nucleoside analogues began with the synthesis of 2′-deoxy-2′,4′-difluorouridine (2′,4′-diF-rU) shown in Scheme 1. Our chosen synthetic route, inspired by reported syntheses of 4′-substituted nucleosides,[22,24−27] began with commercially available 2′-deoxy-2′-fluorouridine [2′-F-rU (**4**)]. Reaction of **4** with iodine and triphenylphosphine yielded the 5′-iodo derivative, **5**. This was followed by an elimination reaction in the presence of sodium methoxide to produce the 4′,5′-unsaturated nucleoside **6**. Both steps proceeded in high yields.

With compound **6** in hand, the next step required installation of the desired 4′-fluorine. The syntheses of all other reported examples of 4′-fluorinated nucleosides effect fluorination through addition of iodine fluoride across the 4′,5′-olefin.[22,24−27] The stereochemical outcome of this addition is highly dependent on the nucleobase identity and functionalities at the 2′ and 3′ positions. The uracil nucleobase likely participates in this reaction, with the C2 carbonyl of uracil opening the iodonium intermediate **13** to form the 4′-anhydro nucleoside **14** and subsequently yielding the right stereochemistry at C4′ upon attack by fluoride (Scheme 2A).[26] We have observed that addition of iodine monofluoride (I-F) to alkene **6** can produce unidentified reaction products. The best conditions we have found provide the 5′-iodo-4′-fluoro nucleoside **7** in 64% yield, obtained through the gradual addition of iodine/AgF in acetonitrile at 0 °C. The stereochemistry at C4′ of nucleoside **7** was assessed by 2D ^{1}H−^{1}H NOESY NMR experiments and later by extensive analysis of the ^{1}H NMR coupling constants of **4** and **10**. For example, the NOESY spectrum of **7** (and the parent compound **4**) shows a strong correlation between H5′ and H3′ as in naturally occurring ribonucleosides.

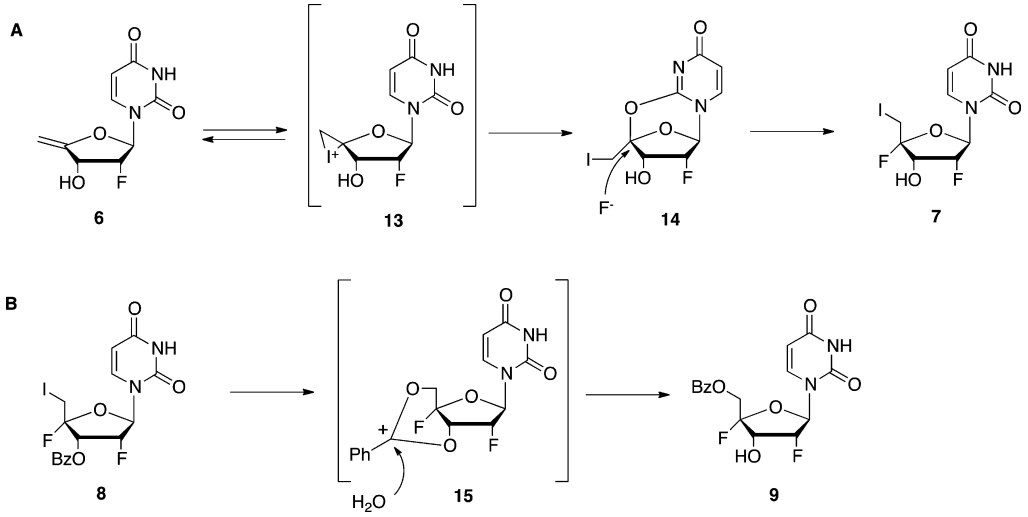
With the installation of the 4′-fluorine, substitution of the 5′-iodine was required to furnish the desired nucleoside analogue. However, displacement of the 5′-iodine from 4′-fluorinated compounds has proven surprisingly difficult in many instances.[22,24−27] A variety of oxygenated nucleophiles were

dx.doi.org/10.1021/jo500794v | J. Org. Chem. 2014, 79, 5627−5635

Scheme 1. Stereoselective Synthesis of 2′,4′-diF-rU (10) and 2′,4′-diF-rC (12)



Scheme 2. Postulated Mechanisms To Explain the Formation of Compounds 7 and 9



unsuccessful, and the presence of the 4′-fluorine has been estimated to decrease the ease of displacement of the 5′-iodine by about 1000-fold.[26] Activation of the iodine as hypoiodate was found to be effective,[24] especially when a 3′-benzoyl group is present, which may assist in the displacement through migration from the 3′ to the 5′ position (Scheme 2B). Thus, benzoylation of **7** using benzoyl chloride afforded **8** in 83% yield as a precursor for the substitution reaction. Indeed, displacement of the 5′-iodine was achieved with mCPBA in water-saturated dichloromethane with concomitant migration of the benzoyl group to the 5′ position to afford protected nucleoside **9** (Scheme 2B). A simple deprotection step to remove the 5′-*O*-benzoyl group produced the desired final product 2′,4′-diF-rU (**10**) in quantitative yield (Scheme 1).

To expand our collection of difluorinated nucleoside analogues, we next sought to prepare 2′,4′-diF-rC (**12**). We took advantage of 5′-protected intermediate **9**, which was used for the conversion of uracil to cytosine following benzoyl protection of the 3′-hydroxyl group, to form compound **11**. The triazole intermediate method[29] followed by full deprotection with ammonia was used to obtain 2′,4′-diF-rC (**12**) (Scheme 1). The NOESY NMR spectra of **12** show clear

dx.doi.org/10.1021/jo500794v | J. Org. Chem. 2014, 79, 5627−5635

Table 1. $^{1}H-^{1}H$ and $^{1}H-^{19}F$ Coupling Constant Values for Nucleosides 4 and 10 at 25 °C in D_2O and MeCN-d_3 (500 MHz)

2′,4′-diF-rU (**10**)	J (Hz) (±0.2) (D_2O)	J (Hz) (±0.2) (MeCN-d_3)	J (Hz) (±0.2) (MeCN-d_3)	J (Hz) (±0.2) (D_2O)	2′-F-rU (**4**)
H1′−H2′	0	0	1.9	1.4	H1′−H2′
H1′−F2′	21.5	20.0	17.2	19.6	H1′−F2′
H2′−H3′	5.8	5.5	4.4	4.8	H2′−H3′
H3′−F2′	22.1	25.6	20.7	21.2	H3′−F2′
H3′−F4′	20.7	21.0	7.5	8.4	H3′−H4′
F4′−H5′	6.5	4.3	2.1	2.4	H4′−H5′
F4′−H5″	10.0	4.3	2.8	4.4	H4′−H5′
H5′−H5″	12.8	12.4	12.8	13.2	H5′−H5″

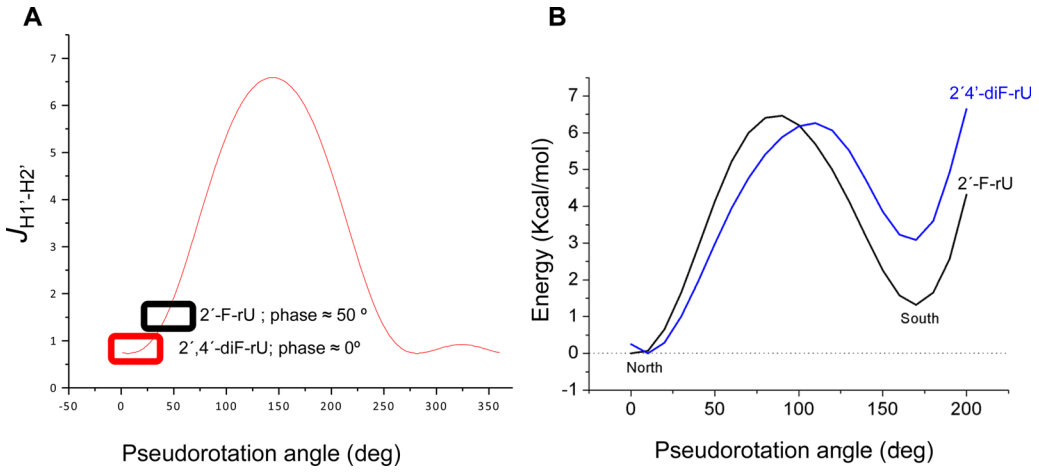


Figure 3. (A) $J_{H1'H2'}$ vs sugar pseudorotation angle. Sugar pucker curves of both nucleosides overlap. (B) Energy profiles of 2′-F-rU and 2′,4′-diF-rU.

correlations between H6 of the base and the H5′ protons as well as between H5′ protons and H3′, again supporting the right configuration at C4′ (see Supporting Information). No decomposition of these compounds was observed in aqueous solution after several days.

The percentage of North and South conformers in solution was calculated by applying the empirical equation $S(\%) = 10 \times J_{H1'H2'}$, where $J_{H1'H2'}$ is the $^{1}H-^{1}H$ coupling constant between the ribose ring H1′ and H2′ protons.[30,31] For 2′,4′-diF-rU (**10**), $J_{H1'H2'}$ is 0 Hz (Table 1), indicating a pure North conformation in aqueous solution. Furthermore, $J_{F4'H3'}$ is 20.7 Hz (Table 1), consistent with the pseudo-trans-diaxial orientation of H3′ and F4′ of a North puckered nucleoside (see Figure 2B). In the South conformer, these two atoms are pseudoequatorial, and hence a much smaller coupling constant value ($J_{H,F}$ = 4−10 Hz) would be expected.[22,32−34] In the case of 2′-F-rU, $J_{H1'H2'}$ is 1.4 Hz, consistent with the existence of both North (86%) and South (14%) conformers in aqueous solution. In MeCN, the fraction of the South conformer of 2′-F-rU increases to 19% ($J_{H1'H2'}$ = 1.9 Hz); meanwhile, 2′,4′-diF-rU adopts exclusively the North conformation ($J_{H1'H2'}$ = 0 Hz).

Since the presence of the electronegative 4′-fluorine can affect the $J_{H1'H2'}$ and mislead the comparison of coupling constant values,[35] we calculated $J_{H1'H2'}$ for 2′-F-rU and 2′,4′-diF-rU by applying a generalized Karplus equation that takes into account the inductive effect of the 4′-fluorine (see Supporting Information).[36−38] Figure 3A shows the dependence of $J_{H1'H2'}$ on the pseudorotation angle for these nucleosides. Both curves overlap, suggesting that the 4′-fluorine does not have a significant effect on $J_{H1'H2'}$. According to the Karplus equation, a $J_{H1'H2'}$ constant less than 1 Hz (as is the case for 2′,4′-diF-rU), is only consistent with a single sugar conformation with a pseudorotation phase angle of 0−10° (a pure North conformation) (Figure 1). However, the coupling constant of 1.4 Hz obtained for 2′-F-rU (**4**) in aqueous solution corresponds to either a single conformation with a higher pseudorotation phase angle of approximately 50° or a pure North sugar pucker ($P \sim 0°$) in a conformational equilibrium with a South pucker with a relative population of around 10%. The experimental J coupling data indicate that this minor conformer does not exist in the case of 2′,4′-diF-rU.

To further explore the impact of 4′-fluorination on the sugar puckering preference, quantum mechanical calculations were carried out using Gaussian 03 at the M062x/6-31+G(d,p) level.[39] The pseudorotation energetic profiles of 2′-F-rU and 2′,4′-diF-rU were calculated by means of a constrained energy optimization (see Supporting Information). These quantum mechanical calculations indicate a clear preference for the North pucker and, most importantly, that the energy of the minor South conformation (pseudorotation angle 175°) is significantly higher for 2′,4′-diF-rU than it is for 2′-F-rU. This result is in complete agreement with the experimental NMR data and further supports the notion that these monocyclic nucleoside analogues are true mimics of previously reported bicyclic nucleosides (Figure 3B).

The rigid RNA-like conformation of 2′,4′-diF-rU (**10**) led us to prepare the corresponding triphosphate and evaluate it as a substrate for an RNA polymerase. Among the polymerases available, we chose HCV NS5B RNA polymerase given the potent anti-HCV activity found in 4′-substituted nucleosides.[40−47] HCV NS5B is capable of initiating RNA synthesis either de novo or in the presence of short RNA primers. In this assay, we measured enzymatic activity from the extension of a radio-labeled dinucleotide (rGG) primer complementary to the 3′ end of a model 20 nt oligo-RNA template. Single nucleotide incorporation events were assessed during the elongation stage

dx.doi.org/10.1021/jo500794v | J. Org. Chem. 2014, 79, 5627−5635

and possible inhibition by 2′,4′-diF-rUTP and 3′-deoxyuridine triphosphate (3′-dUTP, an obligate chain terminator). The oligo template/primer duplex utilized in these studies is shown in Figure 4. Extension of the 2 nt primer by rGTP and rATP

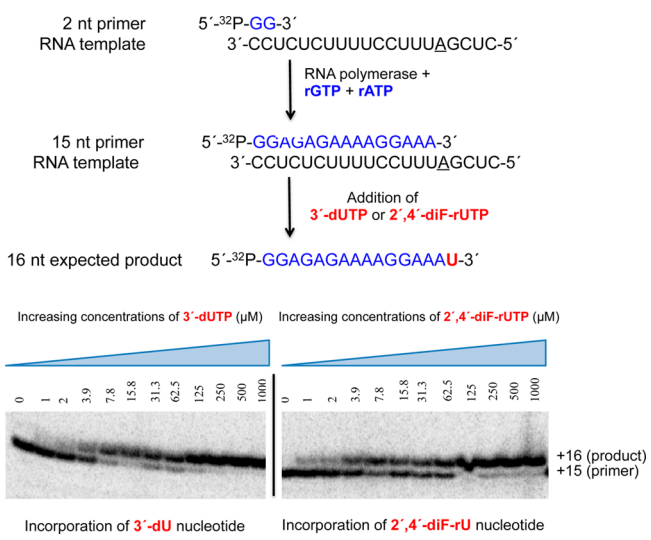


Figure 4. Incorporation assay of 3′-dU and 2′,4′-diF-rU nucleotides in the absence of competing rUTP and the next triphosphate, rCTP, leading to chain termination. Underlined A indicates the site for the incorporation of the modified nucleotide.

yields a stalled elongation complex with an extended 15 nt primer.[48,49] Incorporation of these analogues in the absence of competing rUTP and the next nucleoside triphosphate, rCTP, is demonstrated by a concentration-dependent increase in the formation of a 16 nt product (Figure 4; Supporting Information).

Next, chain elongation was assessed in the presence of multiple nucleotide incorporation events that permit competition with the inhibitors (Figure 5). In this experiment, 5 μM rNTPs and increasing concentrations (0−1000 μM) of 2′,4′-diF-rUTP (or 3′-dUTP) were added as the elongating nucleotides. Under these conditions, RNA synthesis was completely blocked at concentrations of 2′,4′-diF-rUTP of approximately 500 μM (IC_{50} 54.7, Figure S3, Supporting Information). The pattern of products observed in these assays suggests that this compound acts predominantly at the level of initiation (Figure 5). Chain termination at position +16 is not evident. In contrast, 3′-dUTP was able to inhibit RNA synthesis, as a chain terminator with increasing accumulation of the +16 oligonucleotide (IC_{50} 15.4, Figure S3, Supporting Information) and no significant effect during initiation.[49]

Additional nucleotide incorporation experiments were applied to assess whether 2′,4′-diF-rU may act as a chain terminator under conditions that ignore the initiation stage. Previous studies on nucleoside analogues with a 3′-hydroxyl group show that they may act as pseudo-obligate chain terminator if the 3′-OH group is sterically hindered to enter into a phosphodiester linkage with the incoming rNTP.[50,51] For evaluation of 2′,4′-diF-rUTP as a chain terminator, rATP and rGTP were added as the elongating nucleotides followed by addition of 10 μM 2′,4′-diF-rUTP (or 3′-dUTP) at their 3′-termini. Only the primer with the terminal 2′,4′-diF-rU underwent further extension to afford the full-length product (+20), demonstrating that 2′,4′-diF-rU nucleotide does not act as a chain terminator under these conditions (Figure S4, Supporting Information).

CONCLUSIONS

A new approach to "lock" the ribofuranose sugar without resorting to a bicyclic framework has been developed through the design and synthesis of 2′,4′-difluororibonucleosides. Placing a 4′-fluorine at C4′ of 2′-fluorouridine introduces strong stereoelectronic effects that lock the conformational equilibrium toward a pure North conformation as assessed by NMR and computational studies. 2′,4′-diF-rU nucleotide was incorporated into RNA by the NS5B HCV polymerase and the primer subsequently extended to the corresponding full-length product. Furthermore, 2′,4′-diF-rUTP inhibited RNA synthesis at early stages in dinucleotide-primed reactions, although with relatively high half-maximal inhibitory concentrations (IC_{50} > 50 μM).

EXPERIMENTAL SECTION

Procedures and Experimental Data. *1-(2-Deoxy-2-fluoro-5-iodo-β-D-furanosyl)uracil (**5**).* Iodine (4.03 g, 15.85 mmol) and triphenylphoshpine (4.47 g, 17.08 mmol) were added to a suspension of **4** (3.0 g, 12.2 mmol) in pyridine (12.8 mL) and anhydrous CH_3CN (240 mL). After being stirred at room temperature for 48 h, solvents were concentrated under vacuum and the residue was purified by column chromatography (1−4% MeOH in CH_2Cl_2) to give **5** as a pale yellow solid (3.2 g, 74%): R_f (10% MeOH/CH_2Cl_2) 0.41; ^{1}H NMR (MeOH-d_4, 300 MHz) δ 3.44 (dd, 1H, H-5′, J_{HH} = 6.1 Hz, J_{HH} = 11.2 Hz), 3.63 (dd, 1H, H-5″, J_{HH} = 3.6 Hz, J_{HH} = 11.2 Hz), 3.81 (m, 1H, H-4′), 4.16 (ddd, 1H, H-3′, J_{HH} = 5.0 Hz, J_{HH} = 8.0 Hz, J_{HF} = 19.9 Hz), 5.17 (ddd, 1H, H-2′, J_{HH} = 1.8 Hz, J_{HH} = 5.0 Hz, J_{HF} = 53.3 Hz), 5.72 (d, 1H, H-5, J_{HH} = 8.1 Hz), 5.89 (dd, 1H, H-1′, J_{HH} = 1.8 Hz, J_{HF} = 21.0 Hz), 7.71 (d, 1H, J_{HH} = 8.1 Hz); ^{13}C NMR (MeOH-d_4, 75.5 MHz) δ 3.5 (C-5′), 72.7 (d, C-3′, J_{CF} = 16.6 Hz), 80.9 (C-4′), 90.0 (d, C-1′, J_{CF} = 37.0 Hz), 93.4 (d, C-2′, J_{CF} = 187.2 Hz), 101.6 (C-5), 142.2 (C-6), 150.3 (C-2), 164.6 (C-4); HRMS (ESI$^+$) m/z calcd for $C_9H_{10}FIN_2NaO_4$ $[M + Na]^+$ 378.9562, found 378.9550.

*1-(2,5-Dideoxy-2-fluoroerythro-β-D-4-enofuranosyl)uracil (**6**).* A commercially available solution of 25% sodium methoxide in MeOH (7.6 mL, 35 mmol) was added to a suspension of compound **5** (2.5 g, 7.02 mmol) in anhydrous MeOH (66 mL). The reaction mixture was stirred at reflux for 24 h. MeOH was evaporated, and the residue was filtered over a small bed of silica gel using 10% MeOH/CH_2Cl_2 as eluent to remove the salts. The obtained solid was then purified by column chromatography (4% MeOH/CH_2Cl_2) to afford **6** as a white solid (1.20 g, 75%): R_f (10% MeOH/CH_2Cl_2) 0.38; ^{1}H NMR (MeOH-d_4, 300 MHz) δ 4.38 (s, 1H, H5′), 4.60 (s, 1H, H5″), 4.90 (m, 1H, H-3′), 5.17 (dd, 1H, H-2′, J_{HH} = 4.9 Hz, J_{HF} = 52.5 Hz), 5.71 (d, 1H, H-5, J_{HH} = 8.0 Hz), 6.02 (dd, 1H, H-1′, J_{HH} = 1.7 Hz, J_{HF} = 17.4 Hz), 7.48 (d, 1H, H-6, J_{HH} = 8.1 Hz); ^{13}C NMR (MeOH-d_4, 75.5 MHz) δ 68.4 (d, C-3′, J_{CF} = 16.6 Hz), 84.4 (C-5′), 90.4 (d, C-1′, J_{CF} = 35.5 Hz), 91.1 (d, C-2′, J_{CF} = 188.8 Hz), 101.9 (C-5), 141.1 (C-6), 150.3 (C-4′), 161.0 (C-2), 164.5 (C-4); HRMS (ESI$^+$) m/z calcd for $C_9H_9FN_2NaO_4$ $[M + Na]^+$ 251.0439, found 251.0439.

*1-(2-Deoxy-2,4-difluoro-5-iodo-β-D-ribofuranosyl)uracil (**7**).* A suspension of alkene **6** (350 mg, 1.53 mmol) and silver fluoride (6.12 mmol, 777 mg) in MeCN (20.4 mL) was vigorously stirred at 0 °C while a solution of iodine (777 mg, 3.06 mmol) in MeCN (12.4 mL) was added over 15 min. After completion, the reaction mixture was directly filtered over a small bed of silica gel using 50% MeOH/CH_2Cl_2 as eluent to remove the Ag salts. Fractions containing the product were collected; solvents were evaporated, and the resulting residue was purified by column chromatography (2% MeOH/CH_2Cl_2) to afford **7** as a white solid (371 mg, 64%): R_f (10% MeOH/CH_2Cl_2) 0.44; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.49 (dd, 1H, H-5′, J_{HH} = 11.7 Hz, J_{HF} = 23.4 Hz), 3.58 (dd, 1H, H-5″, J_{HF} = 4.3 Hz, J_{HH} = 11.7 Hz), 4.63 (dddd, 1H, H-3′, J_{HH} = 5.7 Hz, J_{HH} = 8.2 Hz, J_{HF} = 20.9 Hz,

dx.doi.org/10.1021/jo500794v | J. Org. Chem. 2014, 79, 5627−5635

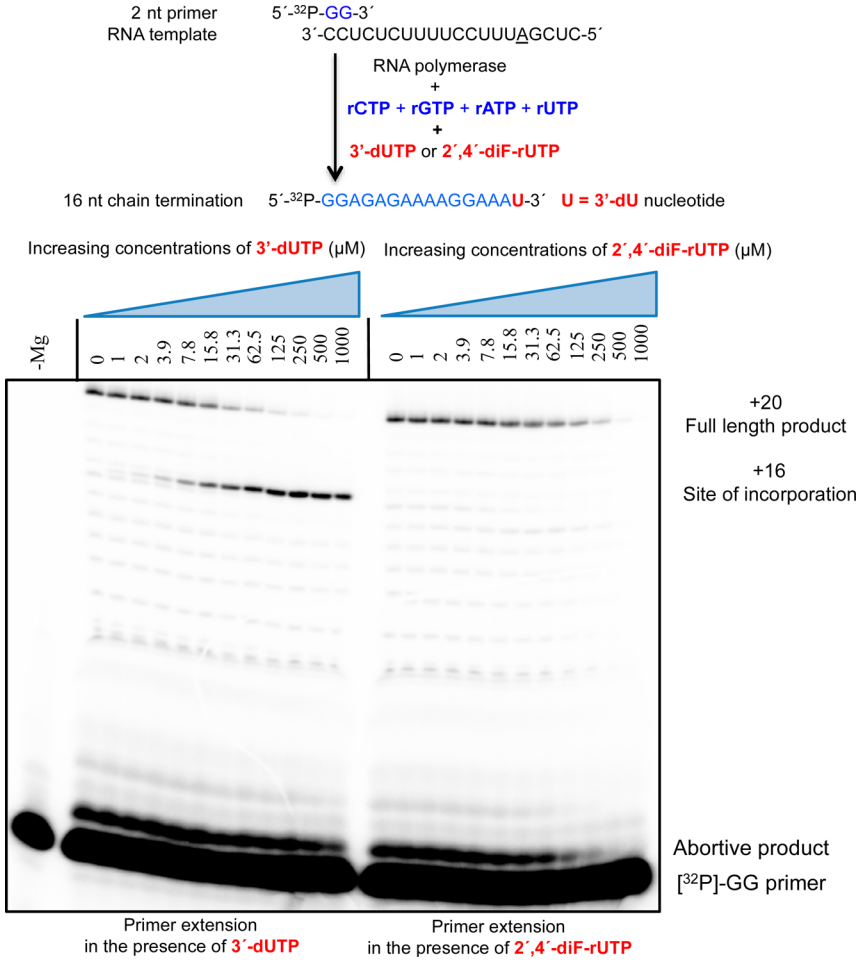


Figure 5. Primer extension assay from the dinucleotide primer [^{32}P]-rGG for 3′-dUTP (left) and 2′,4′-diF-rUTP (right). Underlined A indicates the site for the incorporation of the modified nucleotide.

J_{HF} = 23.8 Hz), 5.38 (ddd, 1H, H-2′, J_{HH} = 1.1 Hz, J_{HH} = 5.9 Hz, J_{HF} = 53.7 Hz), 5.72 (d, 1H, H-5, J_{HH} = 8.0 Hz), 5.89 (m, 2H, H-1′ + OH), 7.70 (d, 1H, J_{HH} = 8.0 Hz), 11.6 (br s, 1H, NH); ^{13}C NMR (MeCN-d_3, 75.5 MHz) δ 1.9 (d, C-5′, J_{CF} = 33.2 Hz), 71.3 (dd, C-3′, J_{CF} = 17.4, J_{CF} = 23.4 Hz), 91.4 (d, C-2′, J_{CF} = 186.5 Hz), 93.3 (d, C-1′, J_{CF} = 38.5 Hz), 102.4 (C-5), 113.2 (d, C-4′, J_{CF} = 258.2 Hz), 143.0 (C-6), 149.9 (C-2), 162.6 (C-4); ^{19}F NMR (DMSO-d_6, 470.35 MHz) δ −109.5 (dddd, F-4′, J_{HF} = 4.2 Hz, J_{FF} = 10.0 Hz, J_{HF} = 20.8 Hz, J_{HF} = 25.0 Hz), −193.7 (ddd, F-2′, J_{FF} 9.9 Hz, J_{HF} = 23.4 Hz, J_{HF} = 53.5 Hz); HRMS (ESI$^+$) m/z calcd for $C_9H_9F_2IN_2NaO_4$ [M + Na]$^+$ 396.9467, found 396.9467.

*1-(3-O-Benzoyl-2-deoxy-2,4-difluoro-5-iodo-β-D-ribofuranosyl)-uracil (**8**).* Benzoyl chloride (28 μL, 0.241 mmol) was added dropwise to a solution of compound **7** (90 mg, 0.241 mmol), triethylamine (169 μL, 1.212 mmol), and DMAP (0.5 mg, 0.004 mmol) in THF (6 mL). The reaction was stirred for 15 min at room temperature and was then quenched by addition of 3 mL of MeOH. Solvents were evaporated, and the residue obtained was purified by column chromatography (40% AcOEt/hexanes) to give benzoylated compound **8** (96 mg, 83%) as a white solid: R_f (70% AcOEt/hexanes) 0.57; ^{1}H NMR (MeCN-d_3, 300 MHz) δ 3.59 (dd, 1H, H-5′, J_{HH} = 11.9 Hz, J_{HF} = 21.9 Hz), 3.75 (dd, 1H, H-5″, J_{HF} = 8.2 Hz, J_{HH} = 11.9 Hz), 5.52−6.13 (m, 4H, H-1′ + H-2′ + H-3′ + H-5), 7,47 (d, 1H, H-6, J_{HH} = 8.1 Hz), 7.58 (t, 2H, Bz, J_{HH} = 8.0 Hz), 7.71 (t, 1H, Bz, J_{HH} = 7.7 Hz), 8.12 (m, 2H, Bz), 9.24 (br s, 1H, NH); ^{13}C NMR (MeCN-d_3, 75.5 MHz) δ 1.6 (d, C-5′, J_{CF} = 32.4 Hz), 71.5 (dd, C-3′, J_{CF} = 15.9, J_{CF} = 21.1 Hz), 89.9 (d, C-2′, J_{CF} = 190.2 Hz), 94.7 (d, C-1′, J_{CF} = 39.2 Hz), 102.6 (C-5), 114.4 (d, C-4′, J_{CF} = 234.1 Hz), 128.7 (Bz), 128.9 (Bz), 129.8 (Bz), 134.0 (Bz), 143.8 (C-6), 150.0 (C-2), 162.7 (C-4), 165.0 (Bz); HRMS (ESI$^+$) m/z calcd for $C_{16}H_{13}F_2IN_2NaO_5$ [M + Na]$^+$ 500.9729, found 500.9714.

*1-(5-O-Benzoyl-2-deoxy-2,4-difluoro-β-D-ribofuranosyl)uracil (**9**).* 3-Chloroperoxybenzoic acid (mCPBA) (77% purity, 449 mg, 2.00 mmol of mCPBA) was added to a suspension of **8** (248 mg, 0.52 mmol) in CH_2Cl_2 (14 mL) and H_2O (0.8 mL). The mixture was heated at 40 °C for 5 h, after which time solvents were concentrated and the residue was purified by flash column chromatography (40% AcOEt/hexanes) to afford **9** (126 mg, 66% yield) as a white solid: R_f (70% AcOEt/hexanes) 0.40; ^{1}H NMR (MeCN-d_3, 300 MHz) δ 3.91 (d, 1H, OH, J_{HH} = 10.7 Hz), 4.55 (dd, 1H, H-5′, J_{HF} = 6.9 Hz, J_{HH} = 12.3 Hz), 4.64 (dd, 1H, H-5″, J_{HF} = 7.5 Hz, J_{HH} = 12.3 Hz), 4.90 (m, 1H, H-3′), 5.35 (dd, 1H, H-2′, J_{HH} = 6.0 Hz, J_{HF} = 53.7 Hz), 5.58 (dd, 1H, H-5, J_{HH} = 1.9 Hz, J_{HH} = 8.0 Hz), 5.96 (d, 1H, H-1′, J_{HF} = 22.2 Hz), 7.40 (d, 1H, H-6, J_{HH} = 8.1 Hz), 7.55 (t, 2H, Bz, J_{HH} = 8.0 Hz), 7.68 (t, 1H, Bz, J_{HH} = 7.7 Hz), 8.07 (m, 2H, Bz), 9.16 (br s, 1H, NH); ^{13}C NMR (acetone-d_6, 75.5 MHz) δ 61.2 (d, C-5′, J_{CF} = 43.0 Hz), 70.5 (dd, C-3′, J_{CF} = 16.9, J_{CF} = 22.0 Hz), 90.5 (d, C-2′, J_{CF} = 185.7 Hz), 94.2 (d, C-1′, J_{CF} = 38.5 Hz), 102.4 (C-5), 115.7 (d, C-4′, J_{CF} = 229.5 Hz), 128.7 (Bz), 129.4 (Bz), 129.7 (Bz), 133.4 (Bz), 143.2 (C-6), 150.0 (C-2), 162.5 (C-4), 165.1 (Bz); HRMS (ESI$^+$) m/z calcd for $C_{16}H_{14}F_2N_2NaO_6$ [M + Na]$^+$ 391.0712, found 391.0696.

*1-(2-Deoxy-2,4-difluoro-β-D-ribofuranosyl)uracil (**10**).* Protected nucleoside **9** (47 mg, 0.128 mmol) was treated with 2 M NH_3 in MeOH, and the mixture was stirred overnight at room temperature and then evaporated to dryness under reduced pressure. Purification by column chromatography (1−10% MeOH/CH_2Cl_2) gave **10** (33.7 mg, 99%) as a white solid: R_f (10% MeOH/CH_2Cl_2) 0.22; ^{1}H NMR (D_2O, 50 °C, 500 MHz) δ 4.00 (dd, 1H, H-5′, J_{HF} = 7.1 Hz, J_{HF} = 12.8 Hz), 4.06 (dd, 1H, H-5″, J_{HF} = 10.1 Hz, J_{HH} = 12.8 Hz), 4.91 (ddd,

dx.doi.org/10.1021/jo500794v | J. Org. Chem. 2014, 79, 5627−5635

1H, H-3′, J_{HH} = 5.8 Hz, J_{HF} = 21.2 Hz, J_{HF} = 23.2 Hz), 5.53 (dd, 1H, H-2′, J_{HH} = 5.8 Hz, J_{HF} = 53.2 Hz), 6.02 (d, 1H, H-5, J_{HH} = 8.0 Hz), 6.30 (d, 1H, H-1′, J_{HF} = 21.5 Hz), 7.79 (d, 1H, H-6, J_{HH} = 8.0 Hz); ^{13}C NMR (MeOH-d_4, 75.5 MHz) δ 59.2 (d, C-5′, J_{CF} = 41.5 Hz), 68.7 (dd, C-3′, J_{CF} = 16.6, J_{CF} = 21.1 Hz), 91.2 (d, C-2′, J_{CF} = 188.0 Hz), 91.7 (d, C-1′, J_{CF} = 37.0 Hz), 101.6 (C-5), 117.1 (d, C-4′, J_{CF} = 232.5 Hz), 142.0 (C-6), 150.2 (C-2), 164.6 (C-4); ^{19}F NMR (D_2O, 470.35 MHz) δ −109.5 (ddt, F-4′, J_{HF} = 7.5 Hz, J_{FF} = 10.3 Hz, J_{HF} = 20.7 Hz), −193.7 (dddd, F-2′, J_{FF} = 9.9 Hz, J_{HF} = 21.1 Hz, J_{HF} = 22.5 Hz, J_{HF} = 52.2 Hz); HRMS (ESI$^+$) m/z calcd for $C_9H_{10}F_2N_2NaO_5$ $[M + Na]^+$ 287.0450, found 287.0448.

*1-(3,5-Di-O-benzoyl-2-deoxy-2,4-difluoro-β-D-ribofuranosyl)-uracil (**11**).* Benzoyl chloride (58 μL, 0.489 mmol) was added dropwise to a solution of compound **9** (180 mg, 0.489 mmol), Et_3N (346 μL, 2.48 mmol), and DMAP (1 mg, 0.008 mmol) in dry THF (13 mL). After 15 min, the crude reaction mixture was evaporated to dryness under reduce pressure. The resulting residue was purified by column chromatography (30% AcOEt/hexanes) to afford **11** (174 mg, 75%) as a white solid: R_f (70% AcOEt/hexanes) 0.68; ^{1}H NMR (acetone-d_6, 300 MHz) δ 4.70 (d, 1H, H-5′, J_{HF} = 1.6 Hz), 4.73 (s, 1H, H5″), 5.72 (d, 1H, H-5, J_{HH} = 8.1 Hz), 5.98 (ddd, 1H, H-2′, J_{HH} = 1.1 Hz, J_{HH} = 5.8 Hz, J_{HF} = 53.2 Hz), 6.13−6.34 (m, 2H, H-1′ + H-3′), 7.41 (t, 2H, Bz, J_{HH} = 7.8 Hz), 7.38−7.63 (m, 4H, Bz), 7.70 (t, 1H, J_{HH} = 7.5 Hz, Bz), 7.87 (d, 1H, H-6, J_{HH} = 8.1 Hz), 8.00−8.08 (m, 4H, Bz); ^{13}C NMR (acetone-d_6, 75.5 MHz) δ 62.3 (d, C-5′, J_{CF} = 39.3 Hz), 71.3 (dd, C-3′, J_{CF} = 15.1, J_{CF} = 18.9 Hz), 89.1 (d, C-2′, J_{CF} = 189.5 Hz), 95.8 (d, C-1′, J_{CF} = 40.0 Hz), 102.5 (C-5), 115.1 (d, C-4′, J_{CF} = 234.1 Hz), 128.5−133.8 (12C, Bz), 144.1 (C-6), 150.2 (C-2), 162.5 (C-4), 164.7 (Bz), 165.0 (Bz); HRMS (ESI$^+$) m/z calcd for $C_{23}H_{18}F_2N_2NaO_7$ $[M + Na]^+$ 495.0974, found 495.0964.

*1-(4-Fluoro-2-deoxy-2-fluoro-β-D-ribofuranosyl)cytosine (**12**).* Phosphoryl chloride (129.6 μL, 1.39 mmol) was added dropwise to a solution, cooled on a ice−water bath, of **11** (164 mg, 0.348 mmol), dry Et_3N (967 μL, 6.95 mmol), and 1,2,4-triazole (360 mg, 5.21 mmol) in dry MeCN (7 mL). After 2 h, the reaction mixture was evaporated to dryness and the residue was filtered through a small bed of silica gel (50% AcOEt/hexanes). The residue was then treated with aqueous NH_3 (33%, 5 mL), and the solution was stirred at room temperature for 30 min. After concentration under reduced pressure, the residue was dissolved in 2 M NH_3 in MeOH (5 mL) and stirred for 6 h at room temperature. Purification by silica gel chromatography (2−10% MeOH/CH_2Cl_2) affords the cytosine derivative **12** (16 mg, 18% overall yield): R_f (20% MeOH/CH_2Cl_2) 0.25; ^{1}H NMR (MeOH-d_4, 400 MHz) δ 3.78 (d, 2H, H-5′, J_{HF} = 4.8 Hz), 4.61 (ddd, 1H, H-3′, J_{HH} = 5.4 Hz, J_{HF} = 21.2 Hz, J_{HF} = 24.7 Hz), 5.08 (dd, 1H, H-2′, J_{HF} = 53.7 Hz, J_{HH} = 5.4 Hz), δ 5.89 (br s, 1H, H-5), 6.12 (d, 1H, H-1′, J_{HF} = 19.6 Hz), 7.81 (d, 1H, H-6, J_{HH} = 7.5 Hz); ^{13}C NMR (MeOH-d_4, 75.5 MHz) δ 59.4 (d, C-5′, J_{CF} = 41.9 Hz), 68.7 (dd, C-3′, J_{CF} = 16.8 Hz, J_{CF} 21.8 Hz), 92.3 (d, C-1′, J_{CF} = 36.2 Hz), 91.5 (d, C-2′, J_{CF} = 189.3 Hz), 94.9 (C-5), 117.1 (d, C-4′, J_{CF} = 231.9 Hz), 142.1 (C-6), 156.2 (C-2), 166.6 (C-4); ^{19}F NMR (MeOH-d_4, 282 MHz) δ −125.30 (dddd, F-4′, J_{HF} = 4.8 Hz, J_{HF} = 4.8 Hz, J_{FF} = 11.4 Hz, J_{HF} = 21.1 Hz), −197.62 (dddd, F-2′, J_{HF} = 53.7 Hz, J_{HF} = 24.7 Hz, J_{HF} = 19.6 Hz, J_{FF} = 11.4 Hz); HRMS (ESI$^+$) m/z calcd for $C_9H_{11}F_2N_3O_4Na$ $[M + Na]^+$ 286.0610, found 286.0609.

2′-Deoxy-2′,4′-difluorouridine triphosphate was synthesized by custom-made laboratory (ChemGenes): ^{31}P NMR (D_2O, 80 MHz) δ −7.4 (γ-P), −13.59 (α-P), −22,15 (β-P); HRMS (ESI$^+$) m/z calcd for $C_9H_9F_2Li_3N_2)_{14}P_3$ $[M + 3Li]^+$ 514.9639, found 514.9638.

HCV Polymerase Incorporation Reaction. The RNA sequence used for this study to mediate RNA synthesis was as follows: 5′-CUCGAUUUCCUUUUCUCUCC-3′ (Trilink). The sequence contained a single site for incorporation of the uridine analogue at position +16 and was PAGE-purified. A 5′-GG-3′ dinucleotide primer (Trilink) was used for primer extension. The 5′-end of the dinucleotide primer was labeled with [γ-^{32}P] ATP using the T4 polynucleotide kinase (Thermo Scientific). Ribonucleoside triphosphates were purchased from Thermo Scientific, and the 3′-dUTP was purchased from Trilink.

Incorporation Assay of 3′-dUTP and 2′,4′-diF-rUTP (Figure 4). The standard reaction mixture contained 500 nM of RNA template, 1 μM of HCV NS5B, 0.2 μM radio-labeled GG primer, and 5 μM of rGTP and rATP. Reactions were carried out in a buffer containing 40 mM HEPES (pH 8), 15 mM NaCl, 1 mM dithiothreitol, and 0.5 mM EDTA. All reactions were started using 6 mM $MgCl_2$ and allowed to proceed at room temperature for 40 min up to position +15. To these reactions were added increasing concentrations of 3′-dUTP or 2′,4′-diF-rUTP analogue to look at their incorporation against A at position +16. The compounds were allowed to incorporate for 5 min, and the reaction was then stopped using EDTA and formamide. The samples were heat-denatured at 95 °C, and the products were resolved on a 20% polyacrylamide gel and visualized using a Bio-Rad phosphorimager.

Primer Extension Assay in the Presence of 3′-dUTP and 2′,4′-diF-rUTP from the Dinucleotide Primer (Figure 5). The standard reaction mixture contained 500 nM of RNA template, 1 μM of HCV NS5B, 0.2 μM radio-labeled GG primer, and 5 μM of rGTP, rATP, rCTP, and 0.1 μM of UTP as the competing nucleotide. Reactions were carried out in a buffer containing 40 mM HEPES (pH 8), 15 mM NaCl, 1 mM dithiothreitol, and 0.5 mM EDTA. Increasing concentrations of 3′-dUTP or 2′,4′-diF-rUTP were added, and the reactions were started using 6 mM $MgCl_2$ and allowed to proceed at room temperature for 40 min. The reactions were then stopped using EDTA and formamide, samples heat-denatured at 95 °C, and the products resolved on a 20% polyacrylamide gel and visualized using a Bio-Rad phosphorimager.

Primer Extension Reaction to Full-Length Product. The standard reaction mixture contained 500 nM of RNA template, 1 μM of HCV NS5B, 0.2 μM radio-labeled rGG primer, and 5 μM of rGTP and rATP. Reactions were carried out in a buffer containing 40 mM HEPES (pH 8), 15 mM NaCl, 1 mM dithiothreitol, and 0.5 mM EDTA. All reactions were started using 6 mM $MgCl_2$ and allowed to proceed at room temperature for 40 min up to position +15. 3′-dUTP or 2′,4′-diF-rUTP was then added to the reactions at a concentration of 10 μM and allowed to incorporate for 10 min to confirm full incorporation of the analogue. To these reactions were added increasing concentrations of the next rNTP and rCTP, and the reactions were allowed to proceed for an additional 10 min and then stopped using EDTA and formamide. The samples were heat-denatured at 95 °C, and the products were resolved on a 20% polyacrylamide gel and visualized using a Bio-Rad phosphorimager (see Figure S4, Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, some ^{19}F, and 2D NMR spectra for compounds **4**−**12**. Additional computational data. Full gel images for HCV NS5B polymerase-catalyzed incorporation assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: masad.damha@mcgill.ca.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support was provided by Natural Sciences and Engineering Research Council of Canada (M.J.D.), the McGill CIHR Drug Development Training Program (S.M.M.), a Vanier NSERC Scholarship (G.F.D), the Canadian Institutes of Health Research and Fonds du Recherche du Québec (M.G.), the National CIHR Research Training Program in Hepatitis C (A.K.). N.M.P. and C.G. thank Spanish MICINN (CTQ2010-21567-C02-02).

DEDICATION

This paper is dedicated to John A. ("Jack") Secrist on the occasion of his retirement, after 34 years of seminal contributions and service at the Southern Research Institute, Alabama.

REFERENCES

(1) Parker, W. B. *Chem. Rev.* **2009**, *109*, 2880−2893.
(2) Morris, P. E.; Kamath, V. P. *Modified Nucleosides in Biochemistry, Biotechnology and Medicine*; Wiley-VCH: Weinheim, Germany, 2008.
(3) De Clercq, E. *J. Clin. Virol.* **2004**, *30*, 115−133.
(4) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205−8212.
(5) Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J.; Wagner, R. W.; Matteucci, M. D. *J. Med. Chem.* **1996**, *39*, 3739−3747.
(6) Marquez, V. E.; Ben-Kasus, T.; Barchi, J. J., Jr.; Green, K. M.; Nicklaus, M. C.; Agbaria, R. *J. Am. Chem. Soc.* **2004**, *126*, 543−549.
(7) Marquez, V. E.; Ezzitouni, A.; Russ, P.; Siddiqui, M. A.; Ford, H.; Feldman, R. J.; Mitsuya, H.; George, C.; Barchi, J. J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 2780−2789.
(8) Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.-I.; Morio, K.-I.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401−5404.
(9) Wengel, J. *Acc. Chem. Res.* **1999**, *32*, 301−310.
(10) Seth, P. P.; Allerson, C. R.; Berdeja, A.; Siwkowski, A.; Pallan, P. S.; Gaus, H.; Prakash, T. P.; Watt, A. T.; Egli, M.; Swayze, E. E. *J. Am. Chem. Soc.* **2010**, *132*, 14942−14950.
(11) Seth, P. P.; Allerson, C. R.; Siwkowski, A.; Vasquez, G.; Berdeja, A.; Migawa, M. T.; Gaus, H.; Prakash, T. P.; Bhat, B.; Swayze, E. E. *J. Med. Chem.* **2010**, *53*, 8309−8318.
(12) Migawa, M. T.; Prakash, T. P.; Vasquez, G.; Seth, P. P.; Swayze, E. E. *Org. Lett.* **2013**, *15*, 4316−4319.
(13) Qiu, X.-L.; Xu, X.-H.; Qing, F.-L. *Tetrahedron* **2010**, *66*, 789−843.
(14) Liu, P.; Sharon, A.; Chu, C. K. *J. Fluorine Chem.* **2008**, *129*, 743−766.
(15) Martin-Pintado, N.; Deleavey, G. F.; Portella, G.; Campos-Olivas, R.; Orozco, M.; Damha, M. J.; Gonzalez, C. *Angew. Chem., Int. Ed.* **2013**, *52*, 12065−12068.
(16) Anzhaee, M. Y.; Watts, J. K.; Alla, N. R.; Nicholson, A. W.; Damha, M. J. *J. Am. Chem. Soc.* **2011**, *133*, 728−731.
(17) Watts, J. K.; Sadalapure, K.; Choubdar, N.; Pinto, B. M.; Damha, M. J. *J. Org. Chem.* **2006**, *71*, 921−925.
(18) Damha, M. J.; Wilds, C. J.; Noronha, A. M.; Brukner, I.; Borkow, G.; Arion, D.; Parniak, M. A. *J. Am. Chem. Soc.* **1998**, *120*, 12976−12977.
(19) Thibaudeau, C.; Acharya, P.; Chattopadhyaya, J. *Stereoelectronic Effects in Nucleosides and Nucleotides and Their Structural Implications*, 2nd ed.; Uppsala University Press: Uppsala, Sweden, 2005.
(20) Wu, D.; Tian, A. *J. Phys. Chem. A* **1998**, *102*, 9901−9905.
(21) Thomas, S. O.; Lowery, J. A.; Sharpe, R. W.; Pruess, L. M.; Porter, J. N.; Mowat, J. H.; Bohonos, N. *Antibiot. Annu.* **1956**, 716−721.
(22) Jenkins, I. D.; Verheyden, J. P.; Moffatt, J. G. *J. Am. Chem. Soc.* **1976**, *98*, 3346−3357.
(23) Lee, S.; Uttamapinant, C.; Verdine, G. L. *Org. Lett.* **2007**, *9*, 5007−5009.
(24) Ivanov, M. A.; Ludva, G. S.; Mukovnya, A. V.; Kochetkov, S. N.; Tunitskaya, V. L.; Alexandrova, L. A. *Russ. J. Bioorg. Chem.* **2010**, *36*, 488−496.
(25) Guillerm, D.; Muzard, M.; Allart, B.; Guillerm, G. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1455−1460.
(26) Owen, G. R.; Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1976**, *41*, 3010−3017.
(27) Verheyden, J. P. H.; Jenkins, I. D.; Owen, G. R.; Dimitrijevich, S. D.; Richards, C. M.; Srivastava, P. C.; Le-Hong, N.; Moffatt, J. G. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 151−165.
(28) Marquez, V. E.; Tseng, C. K. H.; Mitsuya, H.; Aoki, S.; Kelley, J. A.; Ford, H., Jr.; Roth, J. S.; Broder, S.; Johns, D. G.; Driscoll, J. S. *J. Med. Chem.* **1990**, *33*, 978−985.
(29) Divakar, K. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171−1176.
(30) de Leeuw, F. A. A. M.; Altona, C. *J. Chem. Soc., Perkin Trans. 2* **1982**, 375−384.
(31) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333−2344.
(32) Hall, L. H.; Steiner, P. R.; Pedersen, C. *Can. J. Chem.* **1970**, *48*, 1155−1165.
(33) Hall, L. H.; Steiner, P. R.; Pedersen, C. *Can. J. Chem.* **1970**, *48*, 3937−3945.
(34) Inch, T. D. *Annu. Rep. Nucl. Magn. Reson. Spectrosc.* **1969**, *2*, 35−82.
(35) Thibaudeau, C.; Plavec, J.; Chattopadhyaya, J. *J. Org. Chem.* **1998**, *63*, 4967−4984.
(36) Haasnot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783−2792.
(37) De Leeuw, F. A. A. M.; Beuzekom, A. A.; Altona, C. *J. Comput. Chem.* **1983**, *4*, 438−448.
(38) Huggins, M. L. *J. Am. Chem. Soc.* **1953**, *75*, 4123−4126.
(39) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.
(40) For a review on 4′-substituted nucleosides, see: Alexandrova, L. A. *Russ. J. Bioorg. Chem.* **2011**, *37*, 651−671.
(41) O-Yang, C.; Wu, H.; Fraser-Smith, E. *Tetrahedron Lett.* **1992**, *33*, 37−40.
(42) Nomura, M.; Shuto, S.; Tanaka, M.; Sasaki, T.; Mori, S.; Shigeta, S.; Matsuda, A. *J. Med. Chem.* **1999**, *42*, 2901−2908.
(43) Sugimoto, I.; Shuto, S.; Mori, S.; Shigeta, S.; Matsuda, A. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 385−388.
(44) Hayakawa, H.; Kohgo, S.; Kitano, K.; Ashida, N.; Kodama, E.; Mitsuya, H.; Ohrui, H. *Antiviral Chem. Chemother.* **2004**, *15*, 169−187.
(45) Smith, D. B.; Kalayanov, G.; Sund, C.; Winqvist, A.; Maltseva, T.; Leveque, V.; Rajyaguru, S.; Le Pogam, S.; Najera, I.; Benkestock, K.; Zhou, X.-X.; Kaiser, A. C.; Maag, H.; Cammack, N.; Martin, J. A.; Swallow, S.; Johansson, N. G.; Klumpp, K.; Smith, M. *J. Med. Chem.* **2009**, *52*, 2971−2978.
(46) Smith, D. B.; Martin, J. A.; Klumpp, K.; Baker, S. J.; Blomgren, P. A.; Devos, R.; Granycome, C.; Hang, J.; Hobbs, C. J.; Jiang, W.-R.; Laxton, C.; Le Pogam, S.; Leveque, V.; Ma, H.; Maile, G.; Merrett, J. H.; Pichota, A.; Sarma, K.; Smith, M.; Swallow, S.; Symons, J.; Vesey, D.; Najera, I.; Cammack, N. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2570−2576.
(47) Klumpp, K.; Leveque, V.; Le Pogam, S.; Ma, H.; Jiang, W.-R.; Kang, H.; Granycome, C.; Singer, M.; Laxton, C.; Qi Hang, J.; Sarma, K.; Smith, D. B.; Heindl, D.; Hobbs, C. J.; Merrett, J. H.; Symons, J.; Cammack, N.; Martin, J. A.; Devos, R.; Najera, I. *J. Biol. Chem.* **2006**, *281*, 3793−3799.
(48) Powdrill, M. H.; Deval, J.; Narjes, F.; De Francesco, R.; Götte, M. *Antimicrob. Agents Chemother.* **2010**, *54*, 977−983.

(49) Deval, J.; Powdrill, M. H.; D'Abramo, C. M.; Cellai, L.; Gotte, M. *Antimicrob. Agents Chemother.* **2007**, *51*, 2920−2928.
(50) Deval, J. *Drugs* **2009**, *69*, 151−166.
(51) Eldrup, A. B.; Allerson, C. R.; Bennett, C. F.; Bera, S.; Bhat, B.; Bhat, N.; Bosserman, M. R.; Brooks, J.; Burlein, C.; Carroll, S. S.; Cook, P. D.; Getty, K. L.; MacCoss, M.; McMasters, D. R.; Olsen, D. B.; Prakash, T. P.; Prhavc, M.; Song, Q.; Tomassini, J. E.; Xia, J. *J. Med. Chem.* **2004**, *47*, 2283−2295.

dx.doi.org/10.1021/jo500794v | *J. Org. Chem.* 2014, 79, 5627−5635