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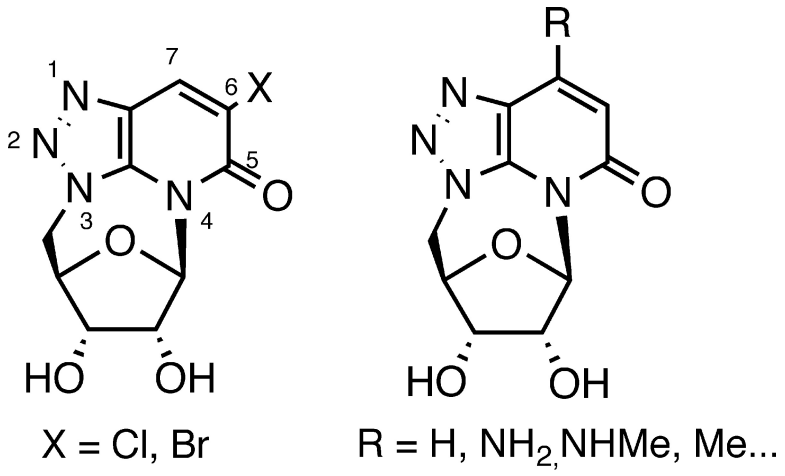

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Synthesis and Structure−Activity Relationships of Novel Anti-hepatitis C Agents: N^3,5′-Cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one Derivatives

Peiyuan Wang,*,† Jinfa Du,† Suguna Rachakonda,† Byoung-Kwon Chun,† Phillip M. Tharnish,† Lieven J. Stuyver,† Michael J. Otto,† Raymond F. Schinazi,‡ and Kyoichi A. Watanabe†

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Several 6- and 7-monosubstituted N^3,5′-cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one derivatives as well as the 5-thiono analogue were synthesized, providing structure−anti-hepatitis C virus (HCV) activity relationships for the series. Among the compounds synthesized, the 6-bromo, 7-methylamino, and 5-thiono analogues exhibited more potent anti-HCV activity in an HCV subgenomic replicon cell based assay (EC_{90} = 1.9, 7.4, and 10.0 μM, respectively) than the lead compound N^3,5′-cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (EC_{90} = 79.8 μM).

Introduction

The hepatitis C virus (HCV) has infected approximately 2% of the worldwide population.[1] It is estimated that there are up to 230 000 new HCV infections every year, and in the United States, this infection is responsible for over 8000 deaths annually. Chronic hepatitis C is predicted to become a major burden on the health care system as individuals that are currently asymptomatic with relatively mild disease progress to end-stage liver disease and develop hepatocellular carcinoma. Furthermore, the number of deaths due to HCV infection is predicted to triple in the next 10−20 years.[2]

The HCV is a single-stranded, enveloped, positive-sense RNA virus in the *flaviviridae* family. It contains a genome of RNA with approximately 9600 nucleotides, a capsid, a matrix, and an envelope. It encodes a single polyprotein precursor which is processed into three structural (C, E1, and E2) and into several nonstructural (NS1, NS2, NS3, NS4, and NS5) viral proteins.[3] Currently, the only treatment available for patients with chronic HCV is α-interferon (IFN-α), either alone or in combination with ribavirin (RBV).[4] The overall sustained response rate to treatment by the combination therapy with RBV and IFN-α for 6−12 months is around 40% in patients infected with HCV genotype 1. Response rates are better in genotype 2 or 3. Anemia is the most common adverse event associated with RBV, and neuropsychiatric adverse effects due to IFN-α can lead to premature cessation of therapy in 10−20% of patients. This treatment is far from satisfactory, as the response rate is low and adverse side effects are common.[4,5] Therefore, it is critical to discover new, more potent anti-HCV agents.

Recently, we discovered that the novel molecule N^3,5′-cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (**8**) exhibited moderate anti-HCV activity in a subgenomic RNA replicon system.[6] In an effort to discover better anti-HCV agents, a series of 6- or 7-substituted derivatives were synthesized and their potency was compared to that of the lead compound, **8**. The effect of substitution on the heterocyclic moiety of the molecule on the biological activity was explored, in order to gain some insight into the mode of action. The facile one-pot synthesis of **8**, synthesis of derivatives thereof, structural determination, synthesis of novel 5-thiono, 6-halo, 7-alkylamino, and 7-methyl analogues of **8**, and anti-HCV and cytotoxicity evaluation of these compounds are reported.

Results and Discussion

Chemistry. We first attempted to improve the synthesis of **8**, which was synthesized from 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5-nitropyridin-2-one (**1**, Scheme 1). It is known that 2′,3′-*O*-isopropylidene pyrimidine nucleosides undergo cyclization to form 5′,2-anhydropyrimidine much more readily than the corresponding 2′,3′-unprotected nucleosides.[7] We, therefore, synthesized 4-(1-β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one[8] (**3**) in two steps by de-O-benzoylation to **2**, followed by treatment with a mixture of acetone and 2,2-dimethoxypropane in the presence of acid to afford the 2′,3′-*O*-isopropylidene derivative **4**. Upon treatment of **4** with diethyl azodicarboxylate and triphenylphosphine under Mitsunobu conditions, cyclization occurred to give the desired 3,5′-cyclonucleoside **5**. Acid hydrolysis of **5** gave the desired product **8** in 83% yield. We found, however, that this procedure was not suitable for preparative scale synthesis because three of the five steps require chromatographic purification and the overall yield was less than 20%.

A more efficient two-step procedure was developed. Treatment of **1**[6] with NaN_3 at 110−120 °C in dimethylformamide (DMF) for 3 days afforded the 3,5′-cyclo product **6** in 60% yield. After saponification of **6**, compound **8** was obtained in good yield (78%). The overall yield from **1** was 47%. Treatment of **6** with

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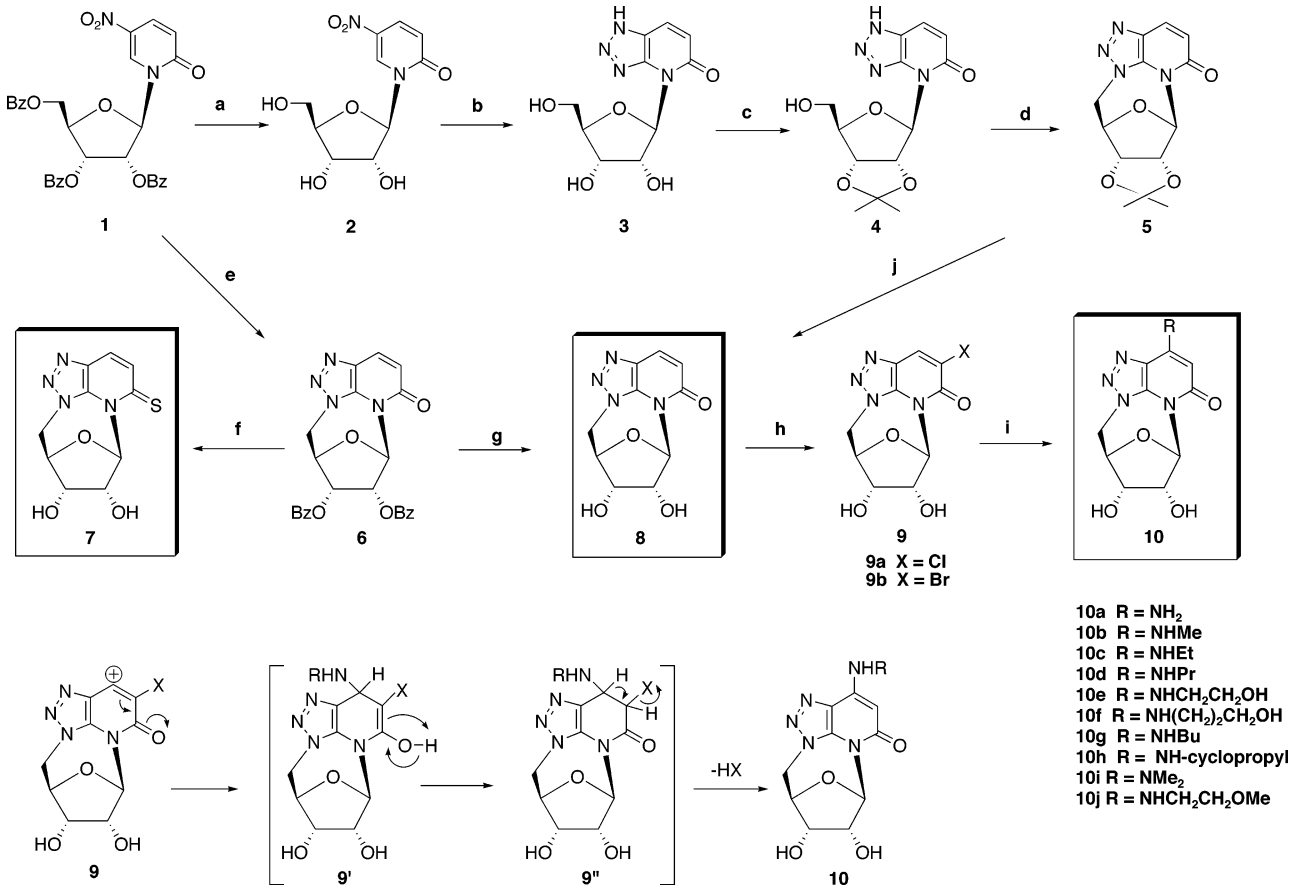
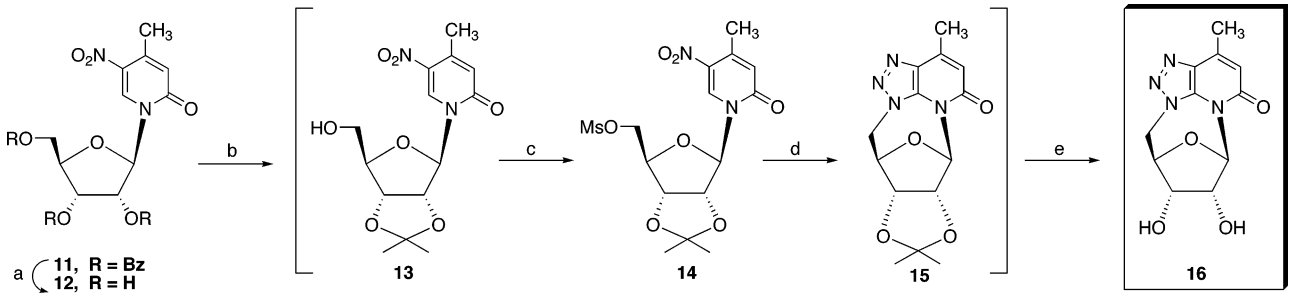
Scheme 1



Reagents: (a) NH_3/MeOH, rt; (b) NaN_3, DMF, 110−120 °C, 12 h; (c) DMP/DMF/acetone/p-TsOH, rt; (d) Ph_3P/DEAD/DMF, rt; (e) NaN_3, DMF, 110−120 °C, 48 h; (f) i. Lawesson's reagent/DCE, reflux; ii. NH_3/MeOH, rt; (g) 0.5 M NaOMe in MeOH, rt; (h) NCS/AcOH, reflux to **9a**; Br_2/H_2O to **9b**; (i) RNH_2 or R_2NH, heated; (j) HCl/dioxane, 50 °C, 12 h.

Scheme 2



Reagents: (a) *n*-$BuNH_2$/MeOH, rt; (b) HCl in Et_2O/acetone, rt; (c) MsCl/Pyr/CH_2Cl_2, rt; (d) NaN_3, DMF, 120 °C, 3 days; (e) concentrated HCl/MeOH, 50 °C, 20 min.

Lawesson's reagent in toluene afforded the 5-thio derivative, which, without purification, was treated with MeONa/MeOH at room temperature. The free nucleoside **7** that precipitated was crystallized from MeOH.

Bromination with Br_2 in water or chlorination with NCS in acetic acid of **8** afforded the 6-substituted halides **9a** and **9b**. Treatment of **9b** with various amines yielded the corresponding 7-substituted products **10a**−**f**. The structure of **9** can readily be verified by ^{1}H NMR spectroscopy. In structure **8**, 6-H and 7-H appeared at δ 6.36 and 8.05, respectively, as doublets. On halogenation, the higher field 6-H signal disappeared, and 7-H stayed at δ 8.54 for **9a** and δ 8.69 for **9b** as a singlet. On treatment of **9b** with a nucleophile, such as $MeNH_2$, the 7-H singlet disappeared and the 6-H singlet emerged at δ 6.18. Apparently, halogenation of **8** occurred at the α-position of the carbonyl group on the heterocyclic ring to form **9**; Michael addition of an amine on **9** took place, giving rise to the saturated intermediates **9′** and **9″**, and from the latter, HX came off, leading to the thermodynamic product **10**.

The 7-methyl analogue **16** was prepared by a different method from the one used for the synthesis of **8**, since, due to the presence of the 7-methyl group, the nucleophilicity of N-3 is apparently reduced, resisting anhydrolinkage formation. Thus, we prepared the 5′-azido-2′,3′-*O*-isopropylidene derivative **14** (Scheme 2) first and then tried to add the azido group across the 5,6-double bond with concomitant elimination of the 5-nitro group. We condensed 4-methyl-5-nitropyridin-2-one with 1-*O*-acetyl-tri-*O*-benzoyl-D-ribofuranose under Vorbrüggen's conditions and obtained **11** in 57% yield. Compound **11** was

Table 1. Anti-HCV Activity of Synthesized Compounds in the Huh-7 Replicon Cells

compound	EC_{90}[a] (μM)	CC_{50}[b] (μM)
7	10.0	11.86
8	79.8	30.6
9a	2.1	0.06
9b	1.9	1.5
10a	29.7	74.2
10b	7.4	4.6
10c	>100	>100
10d	>100	>100
10e	>100	>100
10f	>100	>100
10g	>100	>100
10h	>100	>100
10i	86.1	97.3
10j	>100	>100
16	15.4	22.6

[a] EC_{90} = effective concentration to reduce the HCV RNA by 90%. [b] CC_{50} = concentration at which cell number as detected by the MTS assay was reduced by 50%.

subjected to de-O-benzoylation to give **12**, which was treated with acetone and acid to afford 2′,3′-*O*-isopropylidene derivative **13**, followed by mesylation to **14**. Treatment of **14** with NaN_3 afforded the desired triazolopyridine cyclonucleoside **15** in 53% crude overall yield. Acid hydrolysis of **15** afforded the desired product **16** in 77% yield in crystalline form.

Anti-HCV Activity. Compounds **7**, **8**, **9a**, **9b**, **10a**−**j**, and **16** were assayed for their ability to inhibit HCV RNA replication in a subgenomic replicon Huh7 cell line, as described previously.[9] The anti-HCV replicon activity and cytotoxicity of these compounds are summarized in Table 1. The 5-thiono (**7**), 6-chloro (**9a**), 6-bromo (**9b**), 7-amino (**10a**), 7-methylamino (**10b**), and 7-methyl (**16**) derivatives exhibited more potent anti-HCV activity than **8**.[6] Among them, the 6-bromo and 6-chloro derivatives, **9b** and **9a**, exhibited the most potent anti-HCV activity with EC_{90} values of 1.9 and 2.1 μM, respectively (Table 1). The 7-methylamino derivative (**10b**) was found to exhibit significant anti-HCV activities with an EC_{90} value of 7.4 μM. The 5-thiono and 7-methyl derivatives, **7** (EC_{90} = 10.0 μM) and **16** (EC_{90} = 15.4 μM) (part A of Figures 1−3), are the next most active group, followed by 7-methoxyethylamino derivatives, i.e., **10i** (EC_{90} = 86.1 μM). The 7-amino derivative **10a** (EC_{90} = 29.7 μM) exhibited more potent anti-HCV activity than **8**. Other 7-alkylamino derivatives **10c**−**h** were neither active nor cytotoxic up to 100 μM. From the biological results, the most active compound was the 6-bromo analogue **9b**, but unfortunately, it was also toxic. Among 7-amino substituted derivatives, the most active compound was the methylamino analogue **10b**. Only **10a** showed activity at a concentration significantly lower than the CC_{50}. It appears that the activity would be reduced as the size of the substituent on the amino group at C-7 grows larger. The ribosomal RNA (a cellular marker for potential toxicity) was also reduced, but to a lesser extent than the amount of HCV RNA was reduced (Figures 1−3). To evaluate the specific antiviral effect of compounds **7**, **10b**, and **16** over a longer exposure time, replicon cells were kept in culture for 7 days, in either the presence (100 μM) or absence of the compound (part B of Figures 1−3). These experiments suggest that these compounds caused a cytostatic effect at 100 μM, since the treated cells did not proliferate with the same dynamics as the no-treatment control. Concomitantly with the slower cell proliferation, a significant decrease in intracellular HCV RNA was observed, consistent with a previous report that examined cytostatic agents.[9] Other synthesized compounds also showed similar results (data not shown). These observations suggest that the anti-HCV activity may be associated with the cytostatic effects.

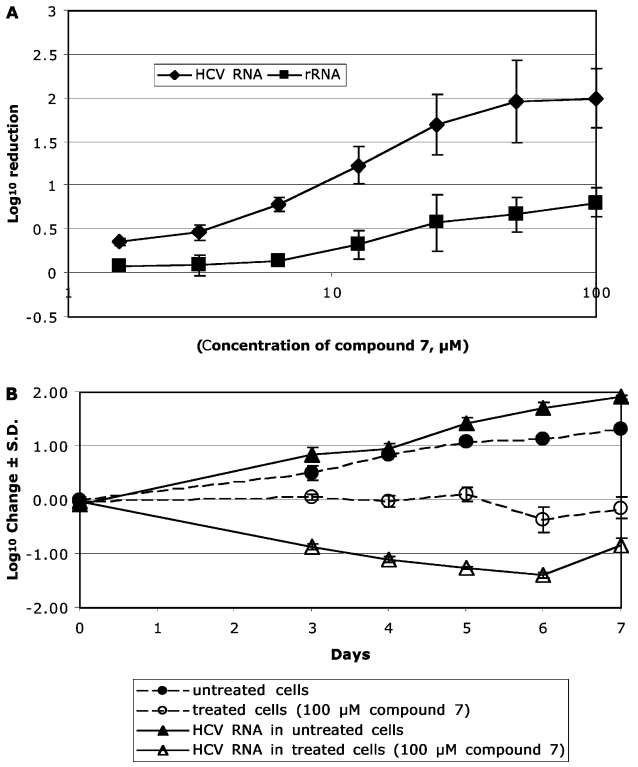


Figure 1. (A) Dose response of compound **7** and dose-dependent antiviral effect on HCV replicon RNA containing Huh-7 cells. Cells were seeded at 1000 cells per well in a 96 well plate in the presence of the compound. After 96 h of incubation, replicon HCV RNA and rRNA levels were quantified by real-time reverse transcription-PCR. (B) Comparison of the effects of compound **7** on cell growth and on HCV replicon dynamics over 7 days.

This class of compounds did not inhibit purified HCV RNA-dependent RNA polymerase (NS5B) in vitro when tested in cell-free systems.[6,10] It is not surprising that they do not appear to inhibit HCV RNA-dependent RNA polymerase since there is no 5′-OH present in these compounds. The mode of anti-HCV action is now the subject of further studies.

In conclusion, a new class of 5-thiono, 6-halo, 7-alkylamino, and 7-methyl analogues of N^3,5′-cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one was synthesized and evaluated for its biological activity in a HCV replicon system. Several synthesized compounds were found to exhibit significant anti-HCV activity. In view of these interesting preliminary antiviral data, further chemical synthesis and biological studies are warranted.

Experimental Section

General. Melting points were determined on an electrothermal digital melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Unity Plus 400 spectrometer at room temperature, with Me_4-

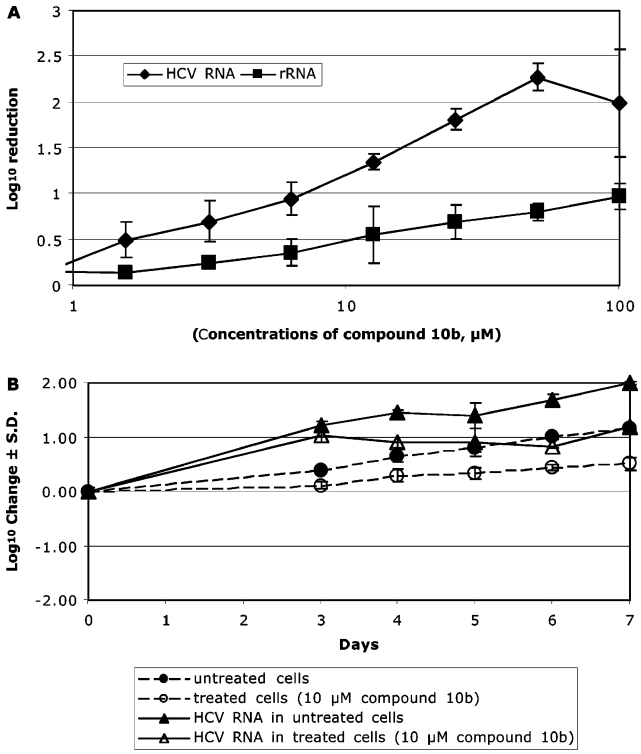


Figure 2. (A) Dose response of compound **10b** and dose-dependent antiviral effect on HCV replicon RNA containing Huh-7 cells. Cells were seeded at 1000 cells per well in a 96 well plate in the presence of the compound. After 96 h of incubation, replicon HCV RNA and rRNA levels were quantified by real-time reverse transcription-PCR. (B) Comparison of the effects of compound **10b** on cell growth and on HCV replicon dynamics over 7 days.

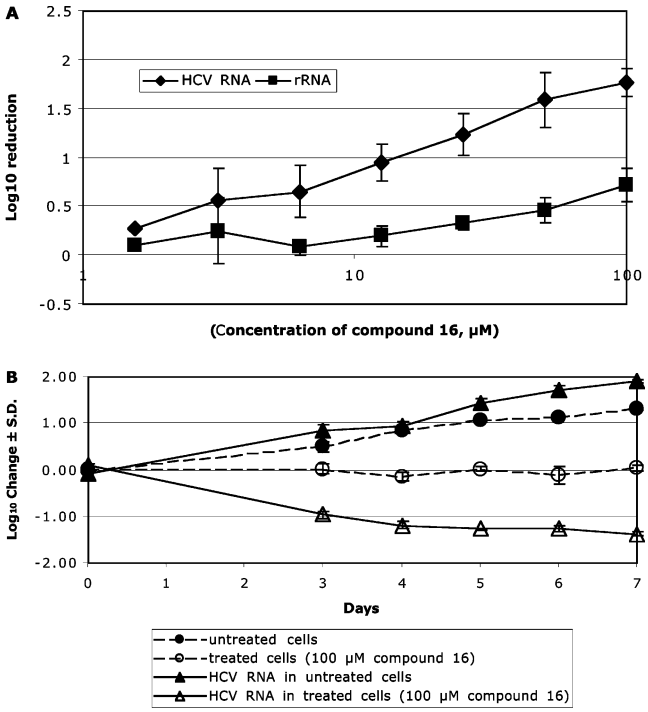


Figure 3. (A) Dose response of compound **16** and dose-dependent antiviral effect on HCV replicon RNA containing Huh-7 cells. Cells were seeded at 1000 cells per well in a 96 well plate in the presence of the compound. After 96 h of incubation, replicon HCV RNA and rRNA levels were quantified by real-time reverse transcription-PCR. (B) Comparison of the effects of compound **16** on cell growth and on HCV replicon dynamics over 7 days.

Si as the internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and signals as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), or br s (broad singlet). Values given for coupling constants are first order, and UV spectra were recorded on a Varian CARY 50 Bio UV−vis spectrophotometer. Fast atom bombardment (FAB) mass spectroscopic data were obtained at the Emory University Mass Spectrometry Center. Thin-layer chromatography (TLC) was performed on Uniplates (silica gel) from Analtech Co., and column chromatography on silica gel (60 Å) purchased from Sorbent Technologies, Atlanta, GA. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

5-Nitro-1-(β-D-ribofuranosyl)pyridin-2-one (2). A mixture of 5-nitro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyridone (**1**, 100 mg, 0.17 mmol)[8] and saturated NH_3/MeOH (10 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated to dryness, and the residue was triturated with EtOH until precipitation was completed. The precipitates were collected and recrystallized with water to give **2** (37 mg, 81%) as a white solid. ^{1}H NMR (DMSO-d_6 + D_2O) δ 3.64 (m, 1H), 3.90 (m, 1H), 4.01 (m, 3H), 5.89 (s, 1H), 6.48 (d, J = 10.4 Hz, 1H), 8.12 (dd, J = 3.2, 10 Hz, 1H), 9.65 (d, J = 3.2 Hz, 1H).

4-(β-D-Ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (3). A mixture of **2** (54 mg, 0.2 mmol) and NaN_3 (20 mg, 0.3 mmol) in DMF (20 mL) was stirred at 110−120 °C for 12 h. The reaction mixture was concentrated to dryness, and the residue was purified on a silica gel column with 15% MeOH in CH_2Cl_2 to give **3** (32 mg, 60%) as a solid. ^{1}H NMR (DMSO-d_6) δ 3.53 (m, 1H), 3.66 (m, 1H), 3.88 (m, 1H), 4.16 (dd, J = 5.2, 9.2 Hz, 1H), 4.79 (m, 1H), 5.04 (d, J = 5.2 Hz, 1H), 5.17 (d, J = 6 Hz, 1H), 6.33 (d, J = 6 Hz, 1H), 6.46 (d, J = 9.6 Hz, 1H), 8.01 (d, J = 9.6 Hz, 1H). Anal. Calcd for $C_{10}H_{12}N_4O_5$: C, 44.78; H, 4.51; N, 20.89. Found: C, 44.60; H, 4.53; N, 20.60.

4-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-*vic*-triazolo-[4,5-*b*]pyridin-5-one (4). To a solution of **3** (2.68 g, 1.0 mmol) in a mixture of 2,2-dimethoxypropane (10 mL), DMF (10 mL), and dry acetone (30 mL) was added *p*-TsOH·H_2O (100 mg), and the mixture was stirred overnight at room temperature. The reaction mixture was neutralized with excess $NaHCO_3$ and filtered, and the solvent was removed in vacuo to give a pale yellow residue, which was passed through a column of silica gel eluting with 30% ethyl acetate (EtOAc) in hexane to give **4** (2.24 g, 72.5%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H), 1.64 (s, 3H), 3.97 (d, J = 12 Hz, 1H), 4.06 (d, J = 12 Hz, 1H), 4.45 (s, 1H), 5.08 (m, 1H), 5.14 (m, 1H), 5.25 (m, OH), 6.63 (d, J = 10 Hz, 1H), 6.72 (d, J = 4.8 Hz, 1H), 7.81 (s, J = 10 Hz, 1H). This product was used directly in the next step.

3,5′-Cyclo-4-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (5). Compound **4** (0.5 g, 1.61 mmol) was dissolved in 15 mL of anhydrous DMF along with triphenylphosphine (1.27 g, 4.85 mmol) and diethyl azadicarboxylate (0.84 g, 4.85 mmol), and the mixture was stirred at room temperature overnight. The mixture was concentrated to dryness, and the residue was partitioned between EtOAc and water. The organic layer was washed with water (2 × 20 mL), dried (Na_2SO_4), and concentrated *in vacuo* to give a yellow residue that was purified by column chromatography using 5−10% MeOH in CH_2Cl_2 to give **5** (306 mg, 65.4%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3H), 1.53 (s, 3H), 4.52 (d, J = 5.2 Hz, 1H), 4.70 (m, 1H), 4.79 (d, J = 5.6 Hz, 1H), 4.89 (d, J = 4.0 Hz, 1H), 5.06 (d, J = 14 Hz, 1H), 6.40 (d, J = 10 Hz, 1H), 6.83 (1H), 7.89 (d, J = 10 Hz, 1H). HRMS (FAB) obsd: *m/z* 291.1100. Calcd for $C_{13}H_{14}N_4O_4$ + H: *m/z* 291.1093 $(M + H)^+$.

3,5′-Cyclo-4-(2,3-di-*O*-benzoyl-β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (6). A mixture of **1** (25 g, 42.8

mmol), NaN_3 (4.17 g, 64.2 mmol), and DMF (700 mL) was stirred at 110−120° C for 3 days. The mixture was cooled, the insoluble material was filtered off, and the filtrate was evaporated in vacuo to give a residue, which was extracted with EtOAc (3 × 500 mL) in the presence of water (500 mL). The ethyl acetate solution was back washed with water, dried (Na_2SO_4), and evaporated to give a crystalline residue. Recrystallization from a mixture of EtOAc and EtOH gave **6** (11.8 g, 60.2%). ^{1}H NMR (DMSO-d_6) δ 5.00 (dd, J = 4.4, 14.0 Hz, 1H), 5.33 (m, 2H), 5.79 (t, J = 4.8 Hz, 1H), 5.88 (d, J = 5.2 Hz, 1H), 6.44 (d, J = 9.6 Hz, 1H), 6.75 (s, 1H), 7.32−8.08 (m, 10H), 8.14 (d, J = 9.6, 1H). Anal. Calcd for $C_{24}H_{18}N_4O_3 \cdot 0.5H_2O$: C, 61.67; H, 4.10; N, 11.99. Found: C, 61.41; H, 4.10; N, 12.39.

3,5′-Cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (8). Compound **6** (2.2 g, 4.8 mmol) was treated with 0.5 M MeONa/MeOH (44 mL), and the mixture was stirred at room temperature for 3 h. After neutralization with HOAc, the mixture was concentrated to dryness, and the residue was chromatographed on a silica gel column with 5% MeOH in CH_2Cl_2 to give a solid which was triturated with EtOH to afford pure **8** (0.93 g, 77.5%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 3.98 (t, J = 4.4 Hz, 1H), 4.13 (m, 1H), 4.61 (t, J = 4.2 Hz, 1H), 4.83 (dd, J = 4, 13.6 Hz, 1H), 5.02 (d, J = 13.6 Hz, 1H), 5.37 (d, J = 7.2 Hz, 1H), 5.76 (d, J = 4.8 Hz, 1H), 6.21 (s, 1H), 6.36 (d, J = 9.6 Hz, 1H), 8.05 (d, J = 9.6 Hz, 1H). Anal. Calcd for $C_{10}H_{10}N_4O_4 \cdot 0.2H_2O$: C, 47.32; H, 4.13; N, 22.07. Found: C, 47.10; H, 4.09; N, 21.97.

3,5′-Cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-thione (7). A mixture of **6** (2.0 g, 4.36 mmol) and Lawesson's reagent (2.0 g) in anhydrous dichloroethane (85 mL) was heated under reflux for 16 h, after which it was concentrated to dryness. The residue was treated with saturated NH_3/MeOH (80 mL), and the mixture was stirred at room temperature for 12 h. Yellowish precipitates were collected by suction and washed with MeOH. Recrystallization of the precipitate from hot water gave **7** (910 mg, 78%) as a yellowish solid. ^{1}H NMR (DMSO-d_6) δ 4.13 (t, J = 4.4 Hz, 1H), 4.23 (m, 1H), 4.70 (t, J = 4.8 Hz, 1H), 4.97 (dd, J = 4.4, 13.6 Hz, 1H), 5.11 (d, J = 14.0 Hz, 1H), 5.43 (d, J = 7.2 Hz, 1H), 5.84 (d, J = 5.2 Hz, 1H), 6.88 (s, 1H), 7.31 (d, J = 8.8 Hz, 1H), 7.96 (d, J = 9.6 Hz, 1H). Anal. Calcd for $C_{10}H_{10}N_4O_3S \cdot 0.2H_2O$: C, 44.50; H, 3.88; N, 20.76. Found: C, 44.25; H, 3.94; N, 20.58.

3,5′-Cyclo-6-chloro-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (9a). A mixture of **8** (94 mg, 0.38 mmol) and *N*-chlorosuccinimide (69 mg) in 3.5 mL of glacial HOAc was refluxed for 30 min. The mixture was concentrated to dryness, and the residue was purified by silica gel column chromatography with 1−2% MeOH in CH_2Cl_2 to give **9a** (43 mg, 40.2%) as a solid: mp 192−195 °C; UV (MeOH) λ_{max} 319.9 nm; ^{1}H NMR (DMSO-d_6) δ 4.06 (t, J = 5 Hz, 1H), 4.15 (m, 1H), 4.62 (t, J = 4.2 Hz, 1H), 4.89 (dd, J = 4, 13.6 Hz, 1H), 5.05 (d, J = 14.0 Hz, 1H), 5.42 (d, J = 7.6 Hz, 1H, D_2O exchangeable), 5.84 (d, J = 4.8 Hz, 1H, D_2O exchangeable), 6.23 (s, 1H), 8.54 (s, 1H). HRMS (FAB) Obsd: *m/z* 283.0222. Calcd for $C_{10}H_8N_4O_4Cl$: *m/z* 283.0234 $(M - H)^-$.

3,5′-Cyclo-6-bromo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (9b). To a stirred solution of **8** (3 g, 11.99 mmol) in H_2O (60 mL) was added Br_2 (0.9 mL) dropwise at room temperature. The product **9b** that precipitated while stirring at room temperature was collected by suction and washed with MeOH to give **9b** (3.35 g, 85.1%): mp 199−201 °C; UV (MeOH) λ_{max} 325 nm; ^{1}H NMR (DMSO-d_6 + D_2O) δ 4.04 (d, J = 5.2 Hz, 1H), 4.15 (t, J = 4.8 Hz, 1H), 4.62 (t, J = 4.4 Hz, 1H), 4.87 (dd, J = 4, 14 Hz, 1H), 5.04 (d, J = 14.0 Hz, 1H), 6.22 (s, 1H), 8.69 (s, 1H). Anal. Calcd for $C_{10}H_9N_4O_4Br \cdot 0.75H_2O$: C, 35.06; H, 3.09; N, 16.35. Found: C, 34.71; H, 3.13; N, 16.03.

7-Amino-3,5′-cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (10a). A mixture of **9b** (150 mg, 0.456 mmol) and liquid ammonia (10 mL) was heated at 70 °C in a sealed vessel for 15 h. The vessel was cooled and opened at −78 °C, and 5 mL of MeOH was added. The mixture was warmed to room temperature, and the amount of **10a** that precipitated was collected (135 mg): mp 294−296 °C; UV (MeOH) λ_{max} 295 nm; ^{1}H NMR (DMSO-d_6) δ 3.86 (t, J = 4.8 Hz, 1H), 4.07 (dd, J = 5.2, 11.6 Hz, 1H), 4.53 (t, J = 4 Hz, 1H), 4.74 (dd, J = 4.0, 14.0 Hz, 1H), 4.96 (d, J = 13.6 Hz, 1H), 5.16 (s, 1H), 5.34 (d, J = 6.8 Hz, 1H, D_2O exchangeable), 5.66 (d, J = 4.8 Hz, 1H, D_2O exchangeable), 6.16 (s, 1H), 6.98 (s, 2H, D_2O exchangeable). Anal. Calcd for $C_{10}H_{11}N_5O_4 \cdot H_2O$: C, 42.40; H, 4.63; N, 24.73. Found: C, 42.17; H, 4.64; N, 24.43.

3,5′-Cyclo-7-methylamino-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (10b). A mixture of **9b** (150 mg, 0.456 mmol) and 33% $MeNH_2$ in EtOH (15 mL) was heated at 110 °C in a sealed bottle for 16 h. The mixture was concentrated in vacuo, and the residue was triturated with EtOH. The precipitated product was collected by suction (35 mg, 27.5%): mp 264−266 °C; UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6) δ 2.77 (d, J = 4.4 Hz, 3H), 3.87 (t, J = 4.8 Hz, 1H), 4.07 (dd, J = 5.2, 12 Hz, 1H), 4.54 (t, J = 4.4 Hz, 1H), 4.75 (dd, J = 3.6, 13.6 Hz, 1H), 4.96 (d, J = 13.6 Hz, 1H), 5.05 (s, 1H), 5.34 (d, J = 7.6 Hz, 1H, D_2O exchangeable), 5.68 (d, J = 4.8 Hz, 1H, D_2O exchangeable), 6.18 (s, 1H), 7.56 (s, 1H, D_2O exchangeable). Anal. Calcd for $C_{11}H_{13}N_5O_4 \cdot 0.5H_2O$: C, 45.83; H, 4.89; N, 24.29. Found: C, 45.56; H, 4.96; N, 23.97.

3,5′-Cyclo-7-(ethylamino)-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (10c). In a similar manner but using 75% $EtNH_2$ instead of 33% $MeNH_2$, the corresponding 7-ethylamino product **10c** was obtained in 36% yield from **9b**: mp 265−267 °C; UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6) δ 1.16 (t, J = 4.4 Hz, 3H), 3.20 (brs, 2H), 3.87 (t, J = 4.4 Hz, 1H), 4.08 (dd, J = 5.2, 12.8 Hz, 1H), 4.54 (t, J = 4.0 Hz, 1H), 4.74 (dd, J = 4.0, 13.6 Hz, 1H), 4.96 (d, J = 13.6 Hz, 1H), 5.11 (s, 1H), 5.33 (d, J = 7.2 Hz, 1H, D_2O exchangeable), 5.66 (d, J = 4.8 Hz, 1H, D_2O exchangeable), 6.18 (s, 1H), 7.52 (t, J = 5.2 Hz, 1H, D_2O exchangeable). Anal. Calcd for $C_{12}H_{15}N_5O_4 \cdot 0.25H_2O$: C, 48.40; H, 5.25; N, 23.51. Found: C, 48.17; H, 5.31; N, 23.33. Similarly, the following compounds were synthesized.

3,5′-Cyclo-7-propylamino-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (10d) in 32% yield: mp 269−272 °C; UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6) δ 0.89 (t, J = 7.2 Hz, 3H), 1.57 (dd, J = 8.0, 14.8 Hz, 2H), 3.12 (m, 2H), 3.87 (t, J = 4.4 Hz, 1H), 4.08 (dd, J = 5.2, 12 Hz, 1H), 4.53 (t, J = 4 Hz, 1H), 4.74 (dd, J = 4.0, 14.0 Hz, 1H), 4.96 (d, J = 13.2 Hz, 1H), 5.11 (s, 1H), 5.33 (d, J = 7.2 Hz, 1H, D_2O exchangeable), 5.67 (d, J = 4.8 Hz, 1H, D_2O exchangeable), 6.17 (s, 1H), 7.56 (s, 1H, D_2O exchangeable). Anal. Calcd for $C_{13}H_{17}N_5O_4$: C, 50.81; H, 5.58; N, 22.79. Found: C, 50.87; H, 5.74; N, 22.69.

3,5′-Cyclo-7-(2-hydroxyethylamino)-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]-pyridin-5-one (10e) in 41% yield: mp 253−256 °C; UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6) δ 3.23 (brs, 2H), 3.56 (dd, J = 6.4, 12.0 Hz, 2H), 3.87 (t, J = 4.8 Hz, 1H), 4.07 (dd, J = 5.2, 12 Hz, 1H), 4.54 (t, J = 4.0 Hz, 1H), 4.75 (dd, J = 4.0, 14.0 Hz, 1H), 4.79 (t, J = 6.0 Hz, 1H, D_2O exchangeable), 4.97 (d, J =14.0 Hz, 1H), 5.17 (s, 1H), 5.34 (d, J = 7.6 Hz, 1H, D_2O exchangeable), 5.68 (d, J = 4.4 Hz, 1H, D_2O exchangeable), 6.18 (s, 1H), 7.32 (t, J = 6 Hz, 1H, D_2O exchangeable). Anal. Calcd for $C_{12}H_{15}N_5O_5$: C, 46.60; H, 4.89; N, 22.64. Found: C, 46.49; H, 5.05; N, 22.48.

3,5′-Cyclo-7-hydroxypropylamino-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (10f) in 39.7% yield: mp 279−281 °C; UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6) δ 1.73 (m, 2H), 3.24 (m, 2H), 3.48 (dd, J = 6.0, 11.6 Hz, 2H), 3.89 (t, J = 4.8 Hz, 1H), 4.09 (m, 1H), 4.56 (m, 2H), 4.76 (dd, J = 3.6, 13.6 Hz, 1H), 4.97 (d, J = 14.0 Hz, 1H), 5.14 (s, 1H), 5.35 (d, J = 7.2 Hz, 1H), 5.69 (d, J = 4.4 Hz, 1H), 6.19 (s, 1H), 7.53 (t, J = 5.6 Hz, 1H). Anal. Calcd for $C_{13}H_{17}N_5O_5$: C, 48.29; H, 5.30; N, 21.66. Found: C, 48.44; H, 5.49; N, 21.56.

7-*n*-Butylamino-3,5′-cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (10g) in 32.8% yield: mp 228−232 °C; UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6) δ 0.89 (t, J = 7.2 Hz, 3H), 1.33 (m, 2H), 1.53 (m, 2H), 3.15 (brs, 2H), 3.87 (t, J = 4 Hz, 1H), 4.08 (dd, J = 5.2, 11.6 Hz, 1H), 4.53 (t, J = 3.6 Hz, 1H), 4.74 (dd, J = 4, 14 Hz, 1H), 4.96 (d, J = 13.6 Hz, 1H), 5.10 (s, 1H), 5.34 (d, J = 7.2 Hz, 1H, D_2O exchangeable), 5.67 (d, J = 4.4 Hz, 1H, D_2O exchangeable), 6.17 (s, 1H), 7.55

(br s, 1H, D_2O exchangeable). Anal. Calcd for $C_{14}H_{19}N_5O_4 \cdot 0.4MeOH$: C, 51.76; H, 6.21; N, 20.96. Found: C, 51.51; H, 6.53; N, 20.63.

3,5′-Cyclo-7-cyclopropylamino-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (10h) in 45.8% yield: mp 258−260 °C; UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6) δ 0.56 (m, 2H), 0.73 (m, 2H), 3.26 (m, 1H), 3.88 (t, J = 4.8 Hz, 1H), 4.07 (dd, J = 5.2, 12.0 Hz, 1H), 4.54 (t, J = 4.0 Hz, 1H), 4.74 (dd, J = 4.0, 14.0 Hz, 1H), 4.96 (d, J = 13.6 Hz, 1H), 5.34 (d, J = 7.2 Hz, 1H, D_2O exchangeable), 5.37 (s, 1H), 5.69 (d, J = 4.8 Hz, 1H, D_2O exchangeable), 6.19 (s, 1H), 7.87 (s, 1H, D_2O exchangeable). Anal. Calcd for $C_{12}H_{15}N_5O_4 \cdot 0.9H_2O$: C, 48.57; H, 5.26; N, 21.78. Found: C, 48.37; H, 5.22; N, 21.65.

3,5′-Cyclo-7-(dimethylamino)-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (10i) in 35% yield: mp 292−294 °C (dec); UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6) δ 2.48 and 2.49 (2 s, 2 × 3H), 3.86 (t, J = 4.8 Hz, 1H), 4.07 (m, J = 1H), 4.55 (t, J = 4.0 Hz, 1H), 4.74 (dd, J = 4.0, 13.6 Hz, 1H), 4.99 (d, J = 14.0 Hz, 1H), 5.10 (s, 1H), 5.35 (d, J = 7.2 Hz, 1H, D_2O exchangeable), 5.68 (d, J = 4.8 Hz, 1H, D_2O exchangeable), 6.22 (s, 1H). Anal. Calcd for $C_{12}H_{15}N_5O_4 \cdot 0.9H_2O$: C, 46.57; H, 5.47; N, 22.63. Found: C, 46.80; H, 5.22; N, 22.71.

3,5′-Cyclo-7-methoxyethylamino-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (10j) in 29.5% yield: mp 227−228 °C; UV (MeOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6) δ 3.25 (s, 3H), 3.34 (m, 2H), 3.50 (t, J = 5.6 Hz, 1H), 3.87 (t, J = 4.8 Hz, 1H), 4.08 (m, 1H), 4.54 (t, J = 3.6 Hz, 1H), 4.75 (dd, J = 3.6, 13.6 Hz, 1H), 4.97 (d, J = 14.0 Hz, 1H), 5.18 (s, 1H), 5.35 (d, J = 7.2 Hz, 1H), 5.69 (d, J = 4.8 Hz, 1H), 6.17 (s, 1H), 7.41 (t, J = 5.6 Hz, 1H). Anal. Calcd for $C_{13}H_{17}N_5O_5 \cdot 0.85H_2O$: C, 46.11; H, 5.57; N, 20.68. Found: C, 45.85; H, 5.64; N, 20.47.

4-Methyl-5-nitro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyridin-2-one (11). A mixture of 2-hydroxy-4-methyl-5-nitropyridine (3.08 g, 20 mmol) and $(NH_4)_2SO_4$ (100 mg) in HMDS (20 mL) was refluxed for 2 h, and the clear solution was concentrated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (60 mL). To the solution were added 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (7.56 g, 15 mmol) and TMSOTf (3.99 g, 18 mmol), and the solution was stirred at room temperature for 16 h. To the solution were added $NaHCO_3$ (2.27 g, 27 mmol) and then H_2O (15 mL) slowly, and the mixture was stirred at room temperature for 30 min. The aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic solutions were washed with brine and dried (Na_2SO_4). The solvent was removed, and the resulting solid was recrystallized from EtOAc−hexanes to give compound **11** (6.80 g, 56.8%): mp 170−171.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 1H), 8.10−7.34 (m, 15H), 6.49 (d, J = 4.8 Hz, 1H), 6.37 (s, 1H), 5.90 (t, J = 5.2 Hz, 1H), 5.84 (t, J = 5.6 Hz, 1H), 4.84 (m, 3H), 2.52 (s, 3H). Anal. Calcd for $C_{32}H_{26}N_2O_{10}$: C, 64.21; H, 4.38; N, 4.68. Found: C, 64.37; H, 4.41; N, 4.65.

4-Methyl-5-nitro-1-(β-D-ribofuranosyl)pyridin-2-one (12). A mixture of **11** (2.99 g, 5 mmol) and excess *n*-$BuNH_2$ (10 mL) in MeOH (100 mL) was stirred at room temperature for 48 h, and the solvent was removed under reduced pressure. The residue was triturated with CH_2Cl_2, and the resulting solid was collected by filtration to give nucleoside **12** (1.28 g, 89.5%): mp 136−137.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H), 3.60 (dd, J = 1.6, 12.0 Hz, 1H), 3.80 (dd, J = 2.4, 12.4 Hz, 1H), 3.98 (m, 3H), 5.08 (d, J = 6.4 Hz, 1H, D_2O exchangeable), 5.33 (t, J = 4.2 Hz, 1H, D_2O exchangeable), 5.62 (d, J = 4.8 Hz, 1H, D_2O exchangeable), 5.88 (d, J = 1.6 Hz, 1H), 6.38 (s, 1H), 9.48 (s, 1H). Anal. Calcd for $C_{11}H_{14}N_2O_7$: C, 46.16; H, 4.93; N, 9.79. Found: C, 46.19; H, 4.95; N, 9.68. HRMS (FAB) Obsd: *m/z* 287.0883. Calcd for $C_{11}H_{14}N_2O_7$ + H: *m/z* 286.0879 $(M + H)^+$.

3,5′-Cyclo-7-methyl-4-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]-pyridin-5-one (15). To a mixture of compound **12** (572 mg, 2 mmol) in acetone (20 mL) was added HCl (2 M in Et_2O, 2 mL, 4 mmol), and the resulting mixture was stirred at room temperature for 3 h. Pyridine (2 mL) was added, and the resulting solution was concentrated to dryness under reduced pressure to give crude **13**. To **13** were added pyridine (5 mL), CH_2Cl_2 (5 mL), and MsCl (0.5 mL, 4.5 mmol), and the resulting solution was stirred at room temperature for 16 h. CH_2Cl_2 (50 mL) was added, and the solution was washed with brine and dried (Na_2SO_4). Solvent was removed to give mesylate **14** as a foam [^{1}H NMR ($CDCl_3$) δ 1.36 (s, 3H), 1.60 (s, 3H), 2.55 (s, 3H), 3.09 (s, 3H), 4.53 (m, 3H), 4.94 (m, 2H), 5.92 (d, J = 1.2 Hz, 1H), 6.37 (s, 1H), 8.78 (s, 1H)], which was dissolved in DMF (10 mL). To the solution was added NaN_3 (390 mg, 6 mmol), and the mixture was heated at 120 °C for 3 days. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography (50% EtOAc in hexanes) to give 2′,3′-*O*-isopropylidene-5′,9-anhydronucleoside (**15**, 320 mg, 52.6%) as a white solid: mp 247−249 °C; ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3H), 1.58 (s, 3H), 2.53 (s, 3H), 4.53 (d, J = 5.6 Hz, 1H), 4.67 (dd, J = 5.6, 14.0 Hz, 1H), 4.78 (d, J = 5.6 Hz, 1H), 4.91 (d, J = 4.4 Hz, 1H), 5.10 (d, J = 14.0 Hz, 1H), 6.25 (d, J = 0.8 Hz, 1H), 6.88 (s, 1H). HRMS (FAB) Obsd: *m/z* 305.1263. Calcd for $C_{14}H_{16}N_4O_4$ + H: *m/z* 305.1250 $(M + H)^+$.

3,5′-Cyclo-7-methyl-4-(β-D-ribofuranosyl)-*vic*-triazolo-[4,5-*b*]-pyridin-5-one (16). To a solution of **15** (300 mg, 0.99 mmol) in MeOH (10 mL) was added concentrated HCl (2 mL), and the solution was heated at 50 °C for 20 min. The solvent was removed in vacuo to give a solid which was washed with MeOH to provide pure **16** (200 mg, 76.6%): mp 252−253.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H), 3.94 (t, J = 4.8 Hz, 1H), 4.11 (m, 1H), 4.68 (t, J = 4.8 Hz, 1H), 4.81 (dd, J = 4.4, 14.0 Hz, 1H), 5.00 (d, J = 14.0 Hz, 1H), 5.37 (d, J = 7.6 Hz, 1H, D_2O exchangeable), 5.76 (d, J = 4.4 Hz, 1H, D_2O exchangeable), 6.18 (s, 1H), 6.20 (s, 1H). Anal. Calcd for $C_{11}H_{12}N_4O_4 \cdot 0.25H_2O$: C, 49.12; H, 4.65; N, 20.84. Found: C, 48.85; H, 4.67; N, 20.58. HRMS (FAB) Obsd: *m/z* 265.0977. Calcd for $C_{11}H_{12}N_4O_4$ + H: *m/z* 265.0937 $(M + H)^+$.

Antiviral Assay. Briefly, HCV replicon cells (Clone A cells; Apath LLC, St. Louis, MO) in log phase growth were exposed to various concentrations of the test compounds for 3 days; after this time, the HCV RNA was extracted, and the amount produced was quantified by real-time PCR. The potency of the compounds against HCV replicon is expressed as EC_{90} (effective concentration to reduce the HCV RNA by 90%).[6,11] MTS was utilized to determine the associated potential toxicity (CC_{50}) as described previously.[12,13]

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