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Anti-hepatitis C virus activity of novel β-d-2′-*C*-methyl-4′-azido pyrimidine nucleoside phosphoramidate prodrugs

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Abstract

Background: 2′-*C*-methyl and 4′-azido nucleosides have previously demonstrated inhibition of hepatitis C virus (HCV) replication by targeting the RNA-dependent RNA polymerase NS5B. In an effort to discover new and more potent anti-HCV agents, we envisioned synthesizing nucleoside analogues by combining the 2′-*C*-methylmoiety with the 4′-azido-moiety into one molecule.

Methods: 2′-*C*-methyl-4′-azido pyrimidine nucleosides were synthesized by first converting 2′-*C*-methyl ribonucleosides to the corresponding 4′-exocyclic methylene nucleosides. Treatment with iodine azide, benzoylation of the 2′- and 3′-hydroxy groups, oxidative displacement of the 5′-iodo group with meta-chloroperoxybenzoic acid, and debenzoylation gave the desired 2′-*C*-methyl-4′-azido uridine and thymidine analogues in good yield. Standard conversion of uridine to cytidine via the 4-triazole yielded 2′-*C*-methyl-4′-azido cytidine. In addition, 5′-phosphoramidate derivatives of 2′-C-methyl-4′-azido uridine and cytidine were synthesized to bypass the initial phosphorylation step.

Results: The prepared nucleosides and their 5′- monophosphate prodrugs were evaluated for their ability to inhibit replication of the hepatitis C virus in a subgenomic replicon cell based assay. Cytotoxicity in Huh7 cells was determined simultaneously with anti-HCV activity by extraction and amplification of both HCV RNA and ribosomal RNA. Among the newly synthesized compounds, only the 5′-monophosphate nucleoside prodrugs had modest and selective anti-HCV activity. All prepared pyrimidine nucleosides and 5′-monophosphate nucleoside prodrugs displayed no evidence of cytotoxicity at high concentrations.

Conclusions: This work is the first example of both inactive uridine and cytidine analogues of a nucleoside being converted to active anti-HCV nucleosides via 5′-monophosphate prodrugs.

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Disclosure statement

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Introduction

Twenty years ago, hepatitis C virus (HCV) was discovered by scientists at Chiron (now Novartis) and the Centers for Disease Control and Prevention to be the causative agent of non-A and non-B hepatitis in humans. Today, this virus has infected an estimated 3% of the global population and 3–4 million individuals become newly infected each year [1,2]. HCV infections often lead to reduced liver function, cirrhosis, and hepatocellular carcinoma, and eventually liver transplantation. The current approved therapy based on pegylated interferon alone or in combination with ribavirin is effective in only approximately half of the genotype 1 population [3,4]. Moreover, this limited efficacy is often associated with significant side effects leading to discontinuation of treatment [5–7]. Therefore, there is a need for the development of more effective therapeutic agents for the treatment of HCV infection.

The HCV RNA-dependent RNA polymerase (NS5B) and NS3/4A protease are currently the most promising targets for the development of novel treatments [8–11]. The activity of these virally encoded enzymes is essential for HCV replication, and antiviral agents targeting these enzymes are in both preclinical and clinical development. To date, most of the reported nucleoside analogues that inhibit HCV polymerase have modifications at the 2′ or 4′ positions of the sugar [1–12]. Several 2′-*C*-methyl nucleoside analogues [13–16] and 4′-azido cytidine analogues [17,18] have been identified as potent inhibitors of HCV NS5B polymerase (Figure 1).

In an effort to discover new and more potent anti-HCV agents, we envisioned synthesizing a nucleoside analogue by combining the 2′-*C*-methyl moiety of NM-107 (**1**) [15] with the 4′-azido moiety of R-1479 (**5**) [17,18] into one molecule. Herein, we report the synthesis, anti-HCV activity and toxicity data for several 2′-*C*-methyl-4′-azido pyrimidines, which were synthesized as intermediates for the synthesis of the corresponding phosphoramidate derivatives. The main potential advantage of these phosphoramidate prodrugs is their ability to bypass the first phosphorylation step that is usually the rate-limiting step towards the 5′-triphosphate necessary for antiviral activity [19].

Methods

Chemistry

Thin layer chromatography was carried out on precoated silica gel thin layer sheets 60 F254 from EMD Merck (Darmstadt, Germany). Plate layer chromatography (PLC) from Analtech, Inc. (Newark, DE, USA) was employed for purification of products. ^{1}H NMR (400.14 MHz) was recorded on Varian VNMR 400 spectrometer (Varian, Inc., Palo Alto, CA, USA). Mass spectral analyses were performed on a Micromass TOF instrument (Waters Corp., Milford, MA, USA) and HPLC (Hewlett–Packard, Palo Alto, CA, USA) driven electrospray MS instrument. Analytical HPLC analyses were performed on a Hewlett–Packard HPLC with a Phenomenex Gemini-NX column (2×50 mm, 3 μm, C18, 110 Å). Mobile phase flow was 0.7 ml/min with a 3.5 min gradient from 96% aqueous media (0.05% formic acid) to 96% CH_3CN (0.05% formic acid) with a 5.5 min total acquisition time and 190–360 nm photodiode array detection).

1-(2-Methyl-5-iodo-β-d-ribofuranosyl)uracil (9a) and 1-(2-methyl-5-iodo-β-d-ribofuranosyl)thymine (9b)—2′-*C*-Methyluridine (5 g, 200 mmol), triphenylphosphine (8 g, 307 mmol) and imidazole (2.09 g, 307 mmol) were slurried in anhydrous tetrahydrofuran (THF). A solution of I_2 (5.7 g, 220 mmol) in THF was added slowly to the slurry while the reaction temperature was maintained below 28°C. The reaction mixture was stirred at room temperature for 18 h. The reaction was quenched with water and extracted with ethyl acetate. After evaporation of the solvent, the residue was purified by column chromatography using 5% methanol in dichloromethane as eluent to obtain **9a** in 85% yield. ^{1}H NMR (CD_3OD), δ: 1.12 (s, 3H, $-CH_3$), 3.48–3.76 (m, 4H, H-3′, H-4′, H-5′), 5.73 (d, 1H, *J*=8.0 Hz, H-5), 5.93 (s, 1H, H-1′), 7.74 (d, 1H, *J*=8.0 Hz, H-6); ^{13}C NMR δ: 4.8, 19.5, 78.0, 78.6, 80.1, 92.4, 101.5, 141.4, 150.9, 164.6.

In a similar manner from **8b** we obtained **9b** in 81% yield. ^{1}H NMR (CD_3OD), δ: 1.12 (s, 3H, $-CH_3$), 1.88 (s, 3H, $-CH_3$), 3.53–3.71 (m, 4H, H-3′, H-4′, H-5′), 5.92 (s, 1H, H-1′), 7.62 (d, 1H, H-6); ^{13}C NMR δ: 5.5, 11.2, 19.4, 78.0, 78.4, 79.4, 92.3, 110.3, 137.0, 151.1, 164.9.

1-(4-Azido-2,3-O-dibenzoyl-2-methyl-5-iodo-β-d-ribofuranosyl)uracil (12a) and 1-(4-azido-2,3-O-dibenzoyl-2-methyl-5-iodo-β-d-ribofuranosyl)thymine (12b)— A solution of **9a** (210 mg, 0.57 mmol) and 0.5 M sodium methoxide solution (2.84 ml, 1.42 mmol) was stirred at 60°C for 2 h. The reaction mixture was then added to a solution of *N*-methylmorpholinium mesylate in MeOH [prepared *in situ* by adding *N*-methylmorpholine (0.15 ml, 1.42 mmol) to a solution of methanesulfonic acid (0.09 ml, 1.42 mmol)]. The reaction mixture was concentrated under reduced pressure and the residue was partially purified by silica gel column chromatography (10% MeOH in CH_2Cl_2) to give a white solid product **10a** (76%). A mixture of benzyl triethylammonium chloride (194 mg, 0.855 mmol) and NaN_3 (55 mg, 0.855 mmol) was slurried in acetonitrile (5 ml). The insoluble NaCl was removed by filtration and the filtrate washed with acetonitrile. The acetonitrile solution of benzyltriethylammonium azide was added to a solution of **10a** and *N*-methylmorpholine (0.02 ml) in THF (5 ml), and a clear solution was formed. A solution of I_2 (217 mg, 0.855 mmol) in THF was added slowly at 0°C. The reaction mixture was stirred at 5–10°C for 2 h. After removal of the solvents, the residue was purified by silica gel column chromatography with 10% MeOH in CH_2Cl_2 to afford the product **11a** (65% yield). The benzoylation of **11a** was carried out by reaction with benzoyl chloride (0.23 ml, 1.995 mmol), triethylamine (0.277 ml, 1.995 mmol) and DMAP (14 mg, 0.114 mmol) in THF (10 ml) at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (1–5% MeOH in CH_2Cl_2) to give **12a** as a foam (82%). ^{1}H NMR (CD_3OD), δ 1.30 (s, 3H, $-CH_3$), 3.32 (s, 1H, H-3′), 3.70 (d, 1H, *J*=11.2 Hz, H-5′), 3.79 (d, 1H, *J*=11.2 Hz, H-5″), 5.74 (d, 1H, *J*=8.0 Hz, H-5), 5.98 (s, 1H, H-1′), 7.41–7.62 (m, 6H, Ar-H), 7.65 (d, 1H, *J*=8.0 Hz, H-6), 7.93–8.16 (m, 4H, Ar-H). **12b** was obtained using similar procedures in good yield, as shown in Figure 2, from **9b**. ^{1}H NMR (CD_3OD), δ 1.82 (s, 3H, $-CH_3$), 2.02 (s, 3H, $-CH_3$), 3.34 (s, 1H, H-3′), 3.76 (d, 1H, *J*=11.2 Hz, H-5′), 3.83 (d, 1H, *J*=11.2 Hz, H-5″), 6.22 (s, 1H, H-1′), 7.38–8.16 (m, 11H, Ar-H, H-6).

1-(4-Azido-5-O-(4-chloro)benzoyl-2,3-O-dibenzoyl-2-methyl-β-d-ribofuranosyl)uracil (13a) and 1-(4-azido-5-O-(4-chloro)benzoyl-2,3-O-dibenzoyl-2-methyl-β-d-ribofuranosyl)thymine (13b)—To a solution of compound **12a** (170 mg, 0.275 mmol) in CH_2Cl_2-H_2O (5:1, 24 ml), tetrabutyl ammonium sulfate (94 mg, 0.275 mmol), potassium hydrogen phosphate (94 mg, 0.412 mmol) and *m*-chloroperbenzoic acid (308 mg, 1.37 mmol) were added. The reaction mixture was stirred at room temperature overnight and then quenched with a solution of sodium thiosulfate. After stirring 5 min, a solution of sodium carbonate was added. The organic layer was separated and evaporated. The residue was purified by silica gel column chromatography using 2% MeOH in CH_2Cl_2 to give **13a** (62% yield). ^{1}H NMR (CD_3OD), δ 1.31 (s, 3H, $-CH_3$), 3.32 (s, 1H, H-3′), 4.72 (s, 2H, H-5′), 5.65 (d, 1H, *J*=8.0 Hz, H-5), 5.81 (s, 1H, H-1′), 7.22–7.61 (m, 10H, Ar-H), 7.66 (d, 1H, *J*=8.0 Hz, H-6), 7.74–8.10 (m, 4H, Ar-H). **13b** was obtained by using same procedure with 66% yield. ^{1}H NMR (CD_3OD), δ: 1.80 (s, 3H, $-CH_3$), 2.01 (s, 3H, $-CH_3$), 3.33 (s, 1H, H-3′), 3.78 (d, 1H, *J*=11.2 Hz, H-5′), 3.84 (d, 1H, *J*=11.2 Hz, H-5″), 6.23 (s, 1H, H-1′), 7.39–8.14 (m, 15H, Ar-H, H-6).

1-(4-Azido-2-methyl-β-d-ribofuranosyl)uracil (14a) and 1-(4-azido-2-methyl-β-d-ribofuranosyl)thymine (14b)—Compound **13a** (100 mg, 0.15 mmol) was dissolved in saturated NH_3 in MeOH (20 ml) and the mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using 10% MeOH in CH_2Cl_2 to give white foam solid (40 mg, 86%). ^{1}H NMR (CD_3OD), δ 1.19 (s, 3H, $-CH_3$), 3.67 (d, 1H, *J*=12.0 Hz, H-5′), 3.78 (d, 1H, *J*=12.0 Hz, H-5″), 4.03 (s, 1H, H-4′), 5.70 (d, 1H, *J*=8.0 Hz, H-5), 6.22 (s, 1H, H-1′), 8.04 (d, 1H, *J*=8.0 Hz, H-6); ^{13}C NMR δ 19.9, 61.8, 73.2, 77.7, 93.2, 98.2, 101.7, 140.9, 151.0, 164.6. MS (ESI): 298 $(M-H)^-$. **14b** was obtained with same method in 83% yield. ^{1}H NMR (CD_3OD), δ 1.15 (s, 3H, $-CH_3$), 1.84 (s, 3H, $-CH_3$), 3.64 (d, 1H, *J*=12.4 Hz, H-5′), 3.78 (d, 1H, *J*=11.6 Hz, H-5″), 4.06 (s, 1H, H-4′), 6.21 (s, 1H, H-1′), 7.93(s, 1H, H-6); ^{13}C NMR δ 11.3, 19.8, 61.7, 73.1, 77.7, 93.1, 98.2, 110.5, 136.5, 151.2, 164.9. MS (ESI): 312 $(M-H)^-$.

1-(4-Azido-2-methyl-β-d-ribofuranosyl)cytosine (15)—To a solution of **13a** (200 mg, 0.31 mmol), 1,2,4-triazole (339 mg, 4.91 mmol) and triethylamine (0.68 ml, 4.91 mmol) in anhydrous CH_2Cl_2 (30 ml) was added $POCl_3$ (0.09 ml, 0.98 mmol). The reaction mixture was stirred at room temperature for 48 h and then quenched with water. The organic layer was separated. After evaporation of the solvent under reduced pressure, the residue was dissolved in acetonitrile. A quantity of 10 ml of concentrated NH_4OH was added. The reaction mixture was stirred for 2 h. The solvent was evaporated and the residue was dissolved in MeOH. A quantity of 10 ml of concentrated NH_4OH was added and the reaction mixture was stirred overnight. After removed the solvent, the residue was purified by silica gel column chromatography by using MeOH/CH_2Cl_2 (5–30%) as eluent to give **15** in 42% yield. ^{1}H NMR (CD_3OD), δ 1.20 (s, 3H, $-CH_3$), 3.70 (d, 1H, J=12.0 Hz, H-5′), 3.80 (d, 1H, *J*=12.0 Hz, H-5′), 4.05 (s, 1H, H-4′), 6.15 (d, 1H, *J*=7.6 Hz, H-5), 6.20 (s, 1H, H-1′), 8.50 (d, 1H, *J*=7.6 Hz, H-6); ^{13}C NMR δ 19.7, 61.6, 72.8, 78.0, 93.7, 94.0, 98.6, 144.6, 147.4, 159.8. MS (ESI): 299 $(M+H)^+$.

4-Azido-2-C-methyluridine-5′-O-[Phenyl(ethyloxyl-alaninyl)]phosphate (16) and 4-azido-2-C-methylcytidine-5′-O-[phenyl(ethyloxy-l-alaninyl)]phosphate (17)—To a solution of **14a** (70 mg, 0.23 mmol) in THF at 0°C, *t*-BuMgCl (0.47 ml, 0.47 mmol) was added and the mixture was stirred at room temperature for 20 min. A solution of phenyl-(ethoxy-L-alaninyl)-phosphorochloridate in THF (0.47 ml, 0.47 mmol) was added at 0°C and stirring was continued for 72 h. The reaction was quenched with methanol followed by aqueous NH_4Cl. The solvents were evaporated and the residue was purified by PLC to give prodrug **16** (3 mg, 2.3%) and 30 mg of recovered **14a**. ^{1}H NMR (CD_3OD), δ 1.14–1.22 (m, 6H, CH_3-ethyl, CH_3-alanine), 1.33 (s, 3H, CH_3-2′), 3.92–4.29 (m, 6H, CH_2-ethyl, CH-alanine, H-3′, H-5′), 5.61 (d, 1H, *J*=8.0 Hz, H-5), 6.17 (s, 1H, H-1′), 7.17–7.37 (m, 5H, phenyl-H), 7.58 (d, 1H, *J*=8.0 Hz, H-6). MS (ESI): 553 $(M-H)^-$. The same procedure was employed for **15** (35 mg, 0.12 mmol) to prepared prodrug **17** (3 mg, 4.6%). ^{1}H NMR (CD_3OD), δ 1.12–1.33 (m, 9H, CH_3-ethyl, CH_3-alanine, CH_3-2′), 3.89–4.48 (m, 6H, CH_2-ethyl, CH-alanine, H-3′, H-5′), 5.83 (d, 1H, *J*=7.6 Hz, H-5), 6.28 (s, 1H, H-1′), 7.17–7.38 (m, 5H, phenyl-H), 7.60 (d, 1H, *J*=7.6 Hz, H-6). MS (ESI): 554 $(M+H)^+$.

Virology

HCV replicon assays—Huh 7 clone B cells containing HCV replicon RNA were seeded in a 96-well plate at 5,000 cells/well, and the compounds tested initially at 10 μM in triplicate immediately after seeding. Following 5 days incubation (37°C, 5% CO_2), total cellular RNA was isolated by using the VersaGene RNA purification kit (Gentra, Minneapolis, MN, USA). Replicon RNA and an internal control (TaqMan rRNA control reagents, Applied Biosystems, Foster City, CA, USA) were amplified in a single step multiplex real time reverse transcriptase (RT)-PCR assay. A dose–response curve was determined for nucleosides demonstrating antiviral activity below 10 μM. The antiviral effectiveness of the nucleosides was calculated by subtracting the threshold RT-PCR cycle of the test compound from the threshold RT-PCR cycle of the no-drug control (ΔCt HCV). A ΔCt of 3.3 equals a 1 $\log_{10}$ reduction (equal to 90% less starting material) in replicon RNA levels. The cytotoxicity of the compounds was also calculated by using the ΔCt ribosomal RNA (rRNA) values. (2′-Me-*C*) was used as the control. To determine 90% effective concentration, 50% effective concetration and 50% cytotoxicity concentrations, ΔCt values were first converted into fraction of starting material and were then used to calculate the percentage inhibition.

Results

During the preparation of this paper, an alternate synthesis appeared for 2′-*C*-methyl-4′-azido cytidine (**15**) [20]. The general procedure used for the synthesis of 2′-*C*-methyl-4′-azido nucleosides was an extension of that previously reported in the literature (Figure 2) [17,18,21,22].

2′-*C*-Methyl ribonucleosides **8a**–**b** [23] were converted to the corresponding 5′-iodo compounds **9a**–**b** and then treated with sodium methoxide to yield the 4′-exocyclic methylene nucleosides **10a**–**b**. 4′-Azido-nucleosides **11a**–**b** were obtained in good yield by treatment of olefins **10a**–**b** with *in situ* generated iodine azide. Benzoylation of the 2′ and

3′-hydroxy groups followed by oxidative displacement of 5′-iodo group with meta-chloroperoxybenzoic acid afforded the protected 4′-azido nucleosides **13a**–**b**. Deprotection with NH_3 in MeOH gave the desired 2′-*C*-methyl-4′-azido uridine **14a** and thymidine analogue **14b** in good yield. 2′-*C*-Methyl-4′-azido cytidine (**15**) was prepared, for comparison, in two steps by treatment of compound **13a** with 1,2,4-triazole and phosphorous oxychloride in the presence of triethyl amine. Subsequent exposure of the crude 4-(*1H*-1,2,4-triazol-1-yl)-2′-*C*-methyl-4′-azido pyrimidinone to NH_4OH furnished the desired 2′-*C*-methyl-4′-azido cytidine, (**15;** Figure 2). This synthesis represents an alternate and higher yielding approach to this class of compounds when compared with previous work [20].

The 2′-*C*-methyl-4′-azido nucleosides **14a** and **14b** were evaluated for their ability to inhibit HCV RNA replication using a Huh7-cell-based subgenomic replicon assay [24] and compared with the known cytidine analogue **15**. Cytotoxicity in Huh7 cells was determined simultaneously with anti-HCV activity by extraction and amplification of both HCV RNA and rRNA. The anti-HCV replicon activity and cytotoxicity of these compounds are summarized in Table 1. It is interesting to note that NM-107 displayed low toxicity in peripheral blood mononuclear (PBM) and CEM cells, whereas R-1479 showed some toxicity only in the more intensively proliferative CEM cell line. Although the lack of anti-HCV activity might have been predicted for the uridine analogue **14a** and the thymidine analogue **14b**, we were surprised to find that the cytidine analogue **15** was also inactive at concentrations up to 10 μM [20]. The inactivity of the cytidine analogue **15** is in clear contrast to the trend that is seen in the R-1479 [25], NM-107 [26] and PSI-6130 [27,28] compound families where the cytidine nucleoside analogues display anti-HCV activity, whereas the uridine nucleosides are devoid of anti-HCV activity. Indeed, these three new analogues displayed no inhibition of HCV replicon RNA replication or toxicity to Huh7. Further evaluation in PBM, CEM, and Vero cells up to 100 μM indicated no cytotoxicity. The lack of cytotoxicity or activity of these nucleosides as anti-HCV agents might be the result of inefficient uptake and/or their inability to be anabolized to the corresponding nucleoside triphosphates.

Because the cytidine analogue **15** is a combination of the 2′-*C*-methyl moiety of NM107 (**1**) [15] and the 4′-azido moiety of R1479 (**5**) [17,18] and it has been reported that the initial phosphorylation of each is the result of different cellular enzymes [18], we hypothesized that disruption in the phosphorylation pathway was a likely cause for loss of anti-HCV activity. To address this possibility, the 5′-monophosphate aryloxy phosphoramidate derivatives of uridine analogue **14a** and cytidine analogue **15** were synthesized. This phosphoramidate prodrug approach was successfully applied to various nucleosides [19,29–31] and allowed the nucleoside analogue to bypass the first phosphorylation step by intracellular delivery of monophosphorylated nucleoside. HCV phosphoramidate prodrugs have been successfully utilized to convert the inactive uridine analogue of R-1479 [25]; adenosine analogue of R-1479 [32]; uridine analogue of PSI-6130 [27,28]; and uridine analogue of NM-107 [26] into active compounds *in the* HCV replicon system.

The target monophosphate prodrugs **16** and **17** were prepared as diastereoisomeric mixtures at the phosphorous centre by the addition of phenyl-(ethoxy-L-alaninyl)-phosphorochloridate to the preformed 5′-alkoxide of nucleosides **14a** and **15** in THF (Figure 3).

In contrast to the parent nucleosides **14a** and **15** that were inactive, the prodrugs **16**–**17** exhibited anti-HCV activity with 50% effective concentration values of 3.0 μM and 4.9 μM, respectively. Additionally, these 5′-monophosphate prodrugs display no observable toxicity toward Huh7, PBM, CEM, or Vero cells up to 100 μM. These results suggest that the poor activity of parent nucleosides was probably the result of insufficient conversion to their corresponding 5′-monophosphate nucleoside.

Discussion

Three 2′-*C*-methyl-4′-azido-pyrimidine nucleosides and two novel 5′-monophosphate prodrugs were synthesized and evaluated for their antiviral activity in an HCV replicon system. As expected, 2′-*C*-methyl-4′-azido cytidine **15** did not exhibit anti-HCV activity up to 10 μM [20]. Furthermore, we prepared and tested the corresponding uridine and thymidine 2′-*C*-methyl-4′-azido analogues and found them also to be devoid of anti-HCV activity and all three pyrimidine analogues displayed no evidence of cytotoxicity at high concentrations. We demonstrated that the 5′-monophosphate prodrugs of the uridine analogue **14a** and cytidine analogue **15** exhibited good anti-HCV activity without apparent cytotoxicity. This work is the first example of both inactive analogues of a nucleoside being converted to active anti-HCV nucleosides via 5′-monophosphate prodrugs. Ongoing work is focused on optimizing the 5′-monophosphate prodrug portion of these novel nucleoside analogues to improve their potency.

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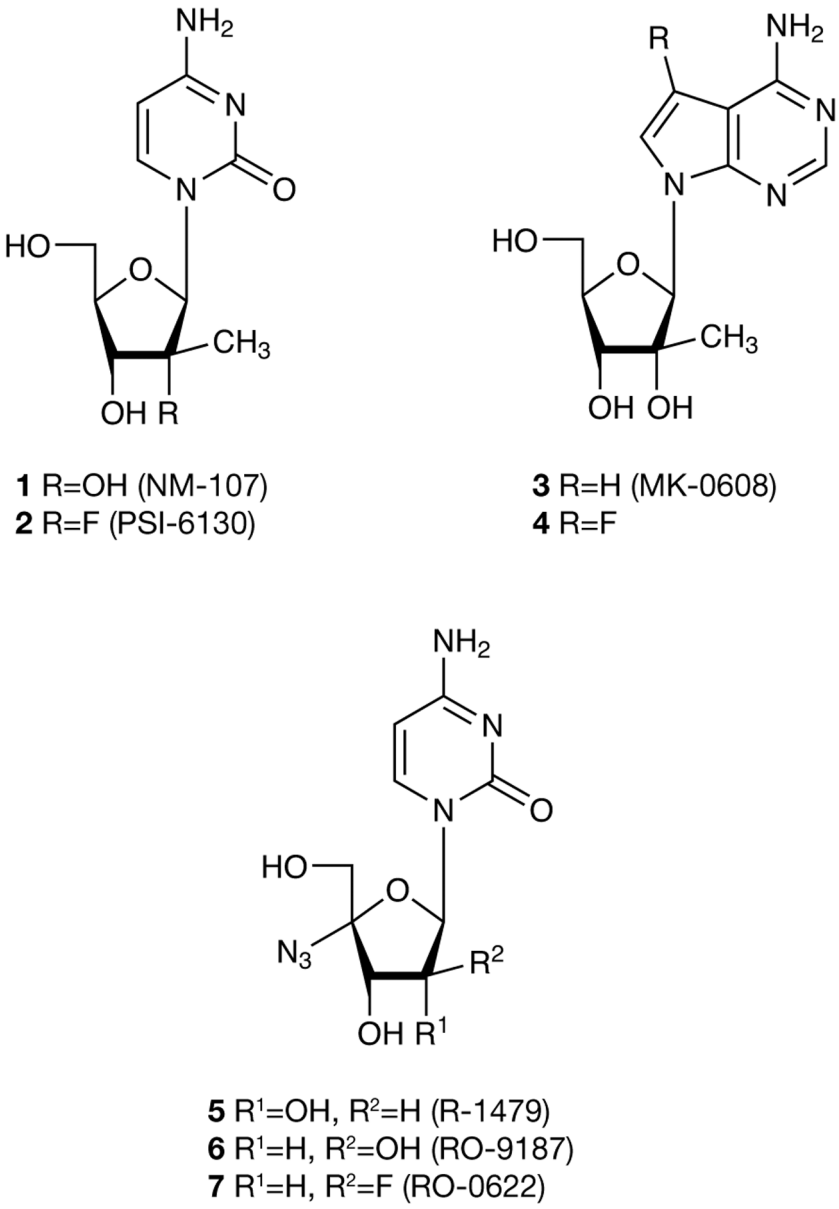

Figure 1.
Structures of 2′-*C*-methyl and 4′-azido nucleosides

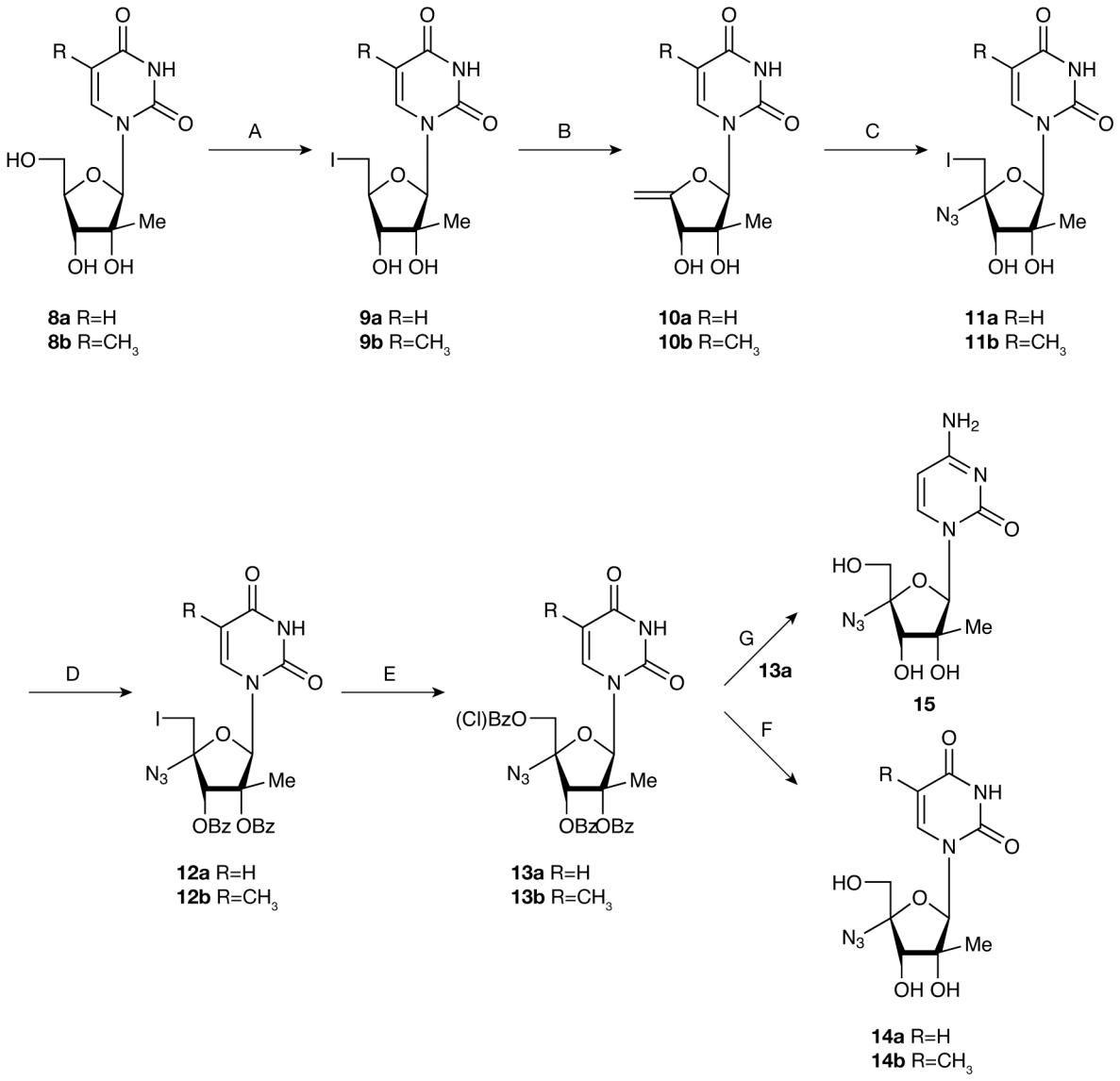

Figure 2.

Synthetic route to 2′ *C*-methyl-4′azido pyrimidine nucleosides

A. I_2, Ph_3P, imidazole, tetrahydrofuran (THF), rt, (85%; **9a**), (81%; **9b**). B. $NaOCH_3$, CH_3OH, 60°C, (76%; **10a**), (78%; **10b**). C. ICI, NaN_3, THF, (65%; **11a**), (61%; **11b**). D. BzCl, DMAP, Et_3N, CH_3CN, rt, (82%; **12a**), (85%; **12b**). E. meta-Chloroperoxybenzoic acid, CH_2Cl_2, rt, (62%; **13a**), (66%; **13b**). F. NH_3/MeOH, rt, (86%; **14a**), (83%; **14b**). G. Step 1: 1,2,4-triazole, Et N, $POCl_3$, CH_2Cl_2, 0°C to rt. Step 2: NH_4OH/CH_3CN/MeOH, rt, 42%.

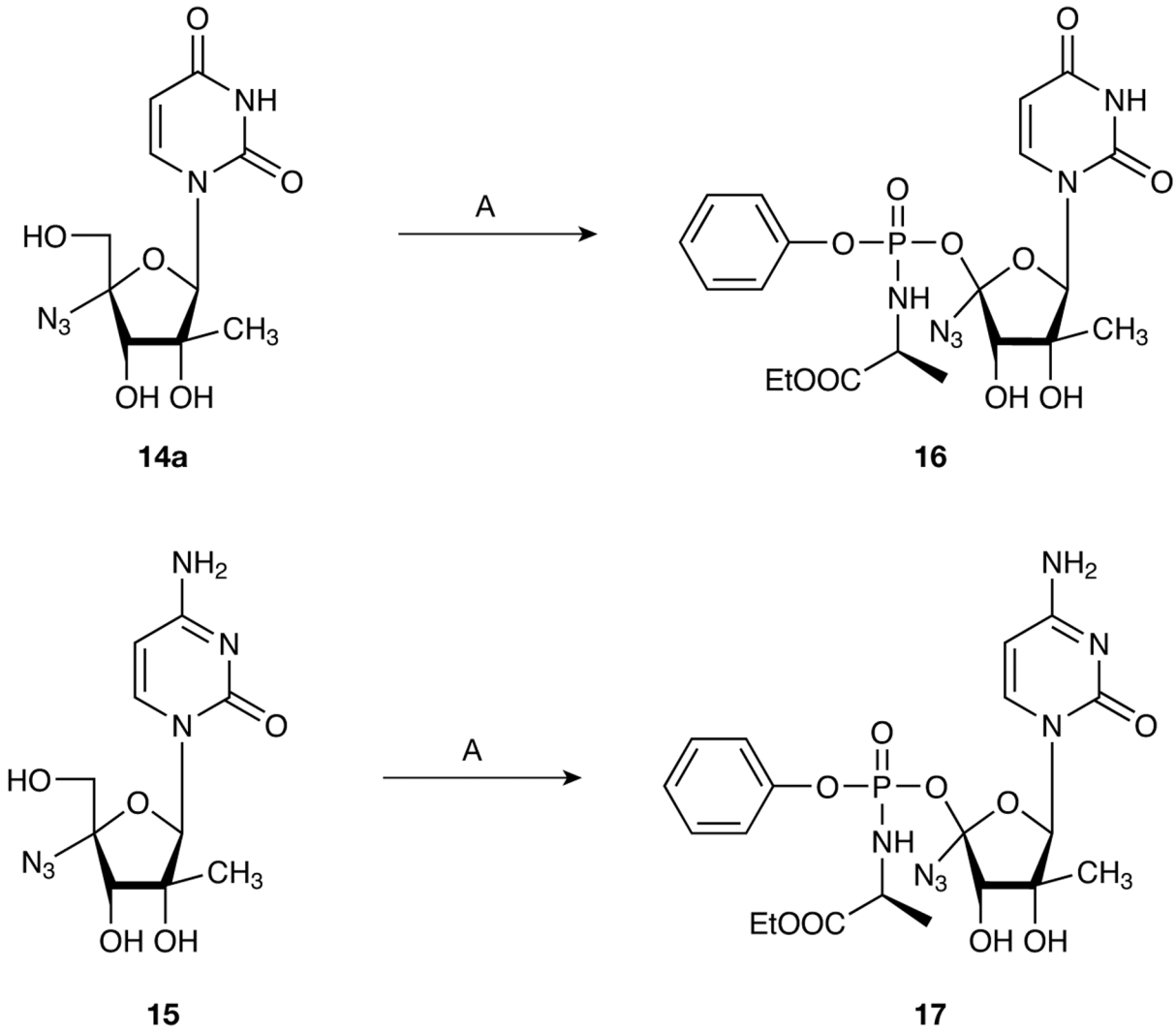

Figure 3.

Reagents and conditions

A. *t*-BuMgCl, phenyl-(ethoxy-L-alaninyl)-phosphorochloridate, tetrahydrofuran, 0°C to rt, 72 h (2.3%; **16**), (4.6%; **17**).

Table 1.

Anti-HCV replicon activity and cellular toxicity of synthesized nucleoside and nucleotide analogues[a]

			CC_{50}, μM			
Compound	**HCV EC_{50}, μM**	**HCV EC_{90}, μM**	**Huh7**	**PBM**	**CEM**	**Vero**
NM-107	2.8	9.6	>10	29.4	24.5	>100
R-1479	10.6	31.8	>10	>100	10.5	>100
14a	>10	>10	>10	>100	>100	>100
14b	>10	>10	>10	>100	>100	>100
15	>10	>10	>10	>100	>100	>100
16	3.0 ±1.1[b]	7.6 ±1.7[b]	>100	>100	>100	>100
17	4.9 ±2.3[b]	>10[b]	>100	>100	>100	>100

[a]Values are means of triplicate measurements.

[b]Means of two experiments ±SD. CC_{50}, 50% cytotoxicity concentration; EC_{50}, 50% effective concentration; EC_{90}, 90% effective concentration; HCV, hepatitis C virus; PBM, peripheral blood mononuclear cell.