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Synthesis and antiviral activity of 2′-deoxy-2′-fluoro-2′-*C*-methyl-7-deazapurine nucleosides, their phosphoramidate prodrugs and 5′-triphosphates

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Abstract

Thirty novel α- and β-*D*-2′-deoxy-2′-fluoro-2′-*C*-methyl-7-deazapurine nucleoside analogs were synthesized and evaluated for *in vitro* antiviral activity. Several α- and β-7-deazapurine nucleoside analogs exhibited modest anti-HCV activity and cytotoxicity. Four synthesized 7-deazapurine nucleoside phosphoramidate prodrugs (**18**–**21**) showed no anti-HCV activity, whereas the nucleoside triphosphates (**22**–**24**) demonstrated potent inhibitory effects against both wild-type and S282T mutant HCV polymerases. Cellular pharmacology studies in Huh-7 cells revealed that the 5′-triphosphates were not formed at significant levels from either the nucleoside or the phosphoramidate prodrugs, indicating that insufficient phosphorylation was responsible for the lack of anti-HCV activity. Evaluation of anti-HIV-1 activity revealed that an unusual α-form of 7-carbomethoxyvinyl substituted nucleoside (**10**) had good anti-HIV-1 activity (EC_{50} = 0.71 ± 0.25 μM; EC_{90} = 9.5 ± 3.3 μM) with no observed cytotoxicity up to 100 μM in four different cell lines.

Keywords

HCV; antiviral; 7-deazapurine; nucleoside; nucleotide; prodrug; Mitsunobu

Hepatitis C virus (HCV) is an important pathogen affecting nearly 170 million people worldwide.[1] HCV infections become chronic in about 50% of cases,[2] and about 20% of these chronic patients develop liver cirrhosis that can lead to hepatocellular carcinoma.[3] Because current therapies such as interferon-alpha (IFN-α) and ribavirin carry limited efficacy and are associated with significant side-effects even when used with the newly approved HCV protease inhibitors Incivek and Victrelis, there is a need for more effective anti-HCV agents that can be used in IFN-α/ribavirin sparing regimens.[4]

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Nucleosides that target HCV NS5B have demonstrated advantages of broader activity against various HCV genotypes and a higher barrier to development of resistant viruses when compared to known HCV protease inhibitors.[5] A number of modified nucleoside analogs have been reported to have anti-HCV activity.[6] Among the sugar-modifications, 2′-*C*-methyl featured nucleosides have shown the most promise, and several of these nucleosides and nucleoside monophosphate prodrugs are at various stages of clinical trials as anti-HCV agents.[7] Although the base-modifications have not produced any clinically significant nucleosides, 7-deazapurine base-modified nucleosides have exhibited some usefulness with 2′-*C*-methyl-7-deazapurine nucleosides demonstrating the most potent anti-HCV activity in an HCV replicon.[8] Based on the potent activity of some 2′-deoxy-2′-fluoro-2′-*C*-methyl nucleosides,[9] we hypothesized that the combination of 2′-deoxy-2′-fluoro-2′-*C*-methyl ribosyl sugars and 7-deazapurine bases could produce potent anti-HCV nucleosides. Therefore, a series of novel 2′-deoxy-2′-fluoro-2′-*C*-methyl-7-deazapurine nucleoside analogs were designed and synthesized. To better understand the anti-HCV assay data, some phosphoramidate prodrugs and triphosphates of selected nucleosides were also prepared. These nucleosides and their phosphoramidate prodrugs were evaluated in a Huh-7 cell-based HCV replicon for anti-HCV activity and cytotoxicity. The compounds were also tested for anti-HIV-1 activity and cytotoxicity in three additional cell lines. In addition, some selected nucleosides were evaluated for anti-HBV activity. Herein we report the synthesis and biological evaluation of a series of 2′-deoxy-2′-fluoro-2′-*C*-methyl-7-deazapurine nucleoside analogs along with the phosphoramidate prodrugs and triphosphates of selected compounds.

The synthesis of 2′-deoxy-2′-fluoro-2′-*C*-methyl-7-deazapurine nucleosides was challenging due to either a lengthy synthesis or poor yields followed by a difficult separation based on using two different synthetic approaches. One approach is more linear in which the 2′-*C*-methyl and 2′-fluoro substituent are added to a 7-deazapurine ribonucleoside, and the other approach is more convergent where the completed moieties are brought together by a sugar-base condensation. Since the 2′-*C*-methyl-2′-fluoro sugar lactone **1** can be readily prepared using a recently published method,[10] it appeared advantageous to choose the sugar-base condensation approach. We first attempted to utilize Vörbrügen sugar-base condensations under various conditions. Two sugars (1-*O*-acetyl-3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-2-*C*-methylriboside and its 1-bromo analog) and two 7-deazapurine bases (6-chloro-7-deazapurine and 6-chloro-7-flouro-7-deazapurine) were used for the condensation, and the silylating agent was either *N*,*O*-bis(trimethylsilyl)acetamide (BSA) or hexamethyldisilazane (HMDS). The solvent was chosen from CH_2Cl_2, $CHCl_3$, $ClCH_2CH_2Cl$, or CH_3CN, in the presence or absence of a Lewis acid catalyst (trimethylsilyl trifluoromethanesulfonate (TMSOTf), $SnCl_4$, or trimethylsilyl iodide). The reaction temperature ranged from room temperature to 70°C, and the reaction time varied from a few hours to several days. However, all these condensation conditions failed to generate detectable amounts of the desired product. Another reported method[11] for condensation of 7-deazapurines was tested using 1-chloro-3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-2-*C*-methylriboside with the catalysts of tris(2-(2-methoxyethoxy)ethyl)amine (TDA-1) and KOH, without success. The direct SN_2 substitution using 1-bromo-3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-2-*C*-methylriboside and 7-deazapurine under basic conditions (NaH) also did not work. Ultimately, Mitsunobu reaction conditions were explored, which resulted in successful couplings (Scheme 1). The required lactol **2** was prepared from lactone **1** by careful $LiAl(t\text{-}BuO)_3H$ reduction. Under relatively standard Mitsunobu conditions,[12] the condensation of several different 7-substituted 6-chloro-7-deazapurines[11b,13] with lactol **2** produced 6-chloro-7-deazapurine nucleosides **3**. In the case that the 7-deazapurine contained an amino moiety, the amino group was protected with a pivaloyl group. The coupling products were obtained in about 40–60% yield, as an approximate 1:1 mixture of α and β isomers. Heating of **3** with NH_4OH and 1,4-dioxane in a sealed steel vessel resulted in amination and deprotection, giving rise to the 7-deazapurine

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nucleosides **4a**–**g** and **5a**–**g**.[14] When $MeNH_2$ was used instead of NH_4OH, 6-methylamino-7-deazapurine nucleosides **4h** and **5h** were obtained. Deprotection of **3** with NaOMe/MeOH produced 6-methoxy products **4i** and **5i**. The α and β isomers were isolated by flash chromatography, and the configuration was assigned based on NOE experiments. The ^{1}H-NMR signals for 2′-*C*-methyl group are also very distinctive between the α and β isomers: all the α-isomers had a doublet at around 1.29 ppm with a coupling constant $J_{H,F}$ of 21.95 Hz, whereas the β-isomers doublets were around 0.94 ppm with $J_{H,F}$ of 22.33 Hz.

7-Iodo-7-deazapurine nucleosides (**4e**, **4g**, **5e** and **5g**) were used as building blocks for the preparation of 7-carbon substituted nucleosides under the catalytic effects of various palladium reagents. For example, using $Pd(PPh_3)_4$ as a catalyst,[15] **4e** and **5e** were reacted with tributyl(vinyl)tin, giving 7-vinyl substituted nucleosides **6** and **7**, respectively, in moderate to good yield (Scheme 2). Hydrogenation of the vinyl compound (**6** or **7**) resulted in the 7-ethyl nucleoside (**8** or **9**). Using $Pd(OAc)_2$ and methyl acrylate, **4e** and **5e** were converted to 7-carbomethoxyvinyl-7-deazapurine nucleosides **10** and **11** in excellent yield (Scheme 3). Coupling of 7-iodo compounds (**4e**, **4g**, **5e**, or **5g**) with triethylsilylacetelene in the presence of $Pd(Ph_3P)_2Cl_2$ and CuI produced, upon desilylation with K_2CO_3 in methanol, moderate yield of 7-ethynyl-substituted nucleosides **12**–**15**. Similarly, 7-phenylethynyl-substituted nucleosides **16** and **17** were also prepared in very good yield by replacing triethylsilylacetelene with phenylacetelene (Scheme 4).

Some nucleosides were selected for preparation of phenyl phosphoramidate prodrugs using a published procedure.[16] Thus, two 6-amino-7-deazapurine nucleosides (**5b** and **5e**) and two 2,6-diamino-7-deazapurine nucleosides (**5f** and **5g**) were converted to their corresponding phosphoramidates **18**–**21** (Scheme 5). For an HCV polymerase enzyme kinetics study, three 5′-triphosphates (**22**–**24**) were also prepared (Scheme 6) and purified by HPLC [DIONEX NucleoPac PA-100 (9 mm × 250 mm) column; buffer A, 0.05 M TEAB; buffer B, 0.5 M TEAB; flow rate, 7.5 mL/min; gradient, increasing buffer B from 0% at 0 min to 50% at 10 min, and then 100% at 12 min and maintained to 17 min].

All synthesized nucleosides and phosphoramidate prodrugs were evaluated for *in vitro* anti-HIV and anti-HCV activities, and toxicity. For determination of anti-HCV activity and toxicity (decreased rRNA), the compounds were tested in an HCV replicon system (96 h) using a standard published protocol.[17] The anti-HIV activity and cytotoxicity were assayed as previous described.[18] The three synthesized triphosphates (**22**–**24**) were tested against both wild-type and S282T mutant HCV NS5B polymerases.[19] In addition, some selected compounds were also evaluated against HBV in hepatocytes.[20]

The results of antiviral activities and toxicities of these nucleoside analogs and prodrugs are summarized in Table 1. Among the 34 compounds tested in HCV replicon cells, 12 compounds showed modest anti-HCV activity with EC_{50} values less than 10 μM. However, their EC_{90} values were all greater than 10 μM, except one compound, 7-vinyl-7-deaza-adenine nucleoside (**7**, β-form), which demonstrated anti-HCV activity with an EC_{90} of 7.6 μM, comparable to the control compound, 2′-*C*-methyl-cytidine (NM-107). However, compound **7** also exhibited toxicity in the ribosomal RNA assay and in three independent cell lines, so it appears that the antiviral activity is secondary to cytotoxic effects. Interestingly, its α-isomer **6**, also showed moderate anti-HCV activity and strong toxicity across all cell lines. Similarly, both α and β forms of 7-iodo substituted nucleosides (**4e** and **5e**) also showed modest anti-HCV activity and toxicity. It seems that the activity and toxicity are associated mainly with the 7-position substituent, and not related to the α or β configuration. Moreover, these antiviral activities are almost certainly due to the secondary toxicity of the compounds, which could cause cell death or cytostatic effects, thus slowing down or stopping viral replication.

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Some 7-substituted nucleosides demonstrated anti-HIV-1 activity albeit again with cytotoxic effects in PBM cells, and in most cases all cell lines tested. These include 7-vinyl (**6** and **7**, α and β-isomers, respectively), 7-ethynyl (**14**, α-isomer), and 7-phenylethynyl (**16** and **17**, α and β-isomers, respectively) substituted nucleoside analogs. Only one compound, 7-carbomethoxyvinyl substituted nucleoside (**10**, α-isomer), exhibited strong anti-HIV-1 activity without any apparent cytotoxicity up to 100 μM. Again, it showed that the anti-HIV-1 activity and cytotoxicity are not related to the α- or β-configuration of these nucleoside analogs.

The enzymatic assay results of the nucleoside triphosphates (**22**–**24**) with HCV RNA-dependent-RNA-polymerases are presented in Table 2. These triphosphates were potent inhibitors of HCV NS5B polymerase. It is worth noting that these compounds had almost equal potency against wild-type and S282T mutant polymerases. This is in contrast to 2′-*C*-methylcytidine-TP (NM-107-TP), where a 17-fold increase in the S282T mutant compared to wild-type virus was observed.

Five selected nucleosides (**4e**, **10**, **14**, **16**, and **17**) were evaluated for their *in vitro* antiviral activity against wild-type HBV. Compounds **4e** and **17** exhibited weak anti-HBV activity and toxicity to HepG2 cells (data not shown). Compounds **10**, **14** and **16** showed no anti-HBV activity.

Since the selected nucleoside 5′-triphosphates demonstrated potent inhibitory activity against HCV polymerases, it is surprising that the nucleoside phosphoramidate prodrugs **18–21** did not show any anti-HCV activity. In many cases, the phosphorylation step of nucleoside analogs to the monophosphate is the rate-limiting step. Therefore, a monophosphate prodrug approach would bypass initial phosphorylation and potentially increase the amount of nucleoside 5′-triphosphate produced intracellularly. In order to understand the mechanism of these nucleoside analogs, a cellular pharmacology study was conducted. The nucleoside **5f** and its phosphoramidate prodrug **20** were separately incubated at 50 μM with Huh-7 cells at 37°C for 4 h, and then the cells were washed with phosphate-buffered saline. The intracellular metabolites were extracted with 60% methanol in water and identified by LC-MS/MS. In these experiments, only a trace amount of nucleoside triphosphate was found following incubation of the nucleoside **5f** or its phosphoramidate prodrug **20**. In the case of compound **5f**, neither the 5′-monophosphate nor the diphosphate was detected. In contrast, with the prodrug **20** a large amount of the monophosphate and the parent nucleoside **5f** were detected. In addition, no corresponding 7-deazaguanosine nucleoside or nucleoside phosphate was found after incubation of the two compounds, suggesting that no deamination had occurred. These results provide an explanation for the lack of antiviral activity of these nucleoside analogs and phosphoramidate prodrugs. This work confirmed that not only the first phosphorylation step from the nucleoside analog to monophosphate, but also the second phosphorylation step from the monophosphate to the diphosphate was problematic in Huh-7 cells at least for nucleoside **5f**, and probably for all other nucleoside analogs synthesized. It is not clear if the third step of the phosphorylation, from the diphosphate to the triphosphate was also a problem. Nonetheless, a monophosphate prodrug approach is not sufficient, and a diphosphate prodrug, or most likely a triphosphate prodrug would be needed to exert the anti-HCV activity of these nucleoside analogs.

It is unusual that some α-nucleoside analogs exhibited antiviral activity and/or toxicity, as these nucleoside analogs are not typically efficiently phosphorylated to the triphosphate.[21] Although it is possible that these compounds are acting as non-nucleoside inhibitors, it is more likely that they are unstable and degrade to the 7-deazapurine base. Moreover, the fact that both α and β nucleosides with the same 7-substitutent had similar antiviral activity and toxicity profiles suggests that the biological activity may be associated with the 7-substituent

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moieties of the nucleosides. The only compound with good anti-HIV-1 activity and no cytotoxicity, α-7-carbomethoxyvinyl nucleoside **10**, is worth further investigation to determine its mechanism of action.

In conclusion, thirty novel α and β-*D*-2′-deoxy-2′-fluoro-2′-*C*-methyl-7-deazapurine nucleoside analogs were synthesized and evaluated for *in vitro* anti-HCV activity in a replicon assay. The key step in the synthesis, the sugar-base condensation, was achieved *via* a Mitsunobu reaction. Several α- and β-7-deazapurine nucleoside analogs exhibited modest anti-HCV activity however this activity was most likely due to their intrinsic toxicity. The four synthesized 7-deazapurine nucleoside phosphoramidate prodrugs **18**–**21** showed no anti-HCV activity, whereas the nucleoside triphosphates **22–24** demonstrated good inhibitory activity against both wild-type and S282T mutant HCV polymerases (IC_{50}: 3–15 μM). These 7-deazapurine nucleosides and prodrugs were also evaluated for *in vitro* anti-HIV-1 activity and cytotoxicity. Only one compound, an α-form of 7-carbomethoxyvinyl substituted nucleoside (**10**), showed good anti-HIV-1 activity (EC_{50} and EC_{90} of 0.71 ± 0.25 μM and 9.5 ± 3.3 μM, respectively) with no cytotoxicity up to 100 μM. Some selected compounds were evaluated against HBV, and none showed significant *in vitro* anti-HBV activity. A cellular pharmacology study of the nucleosides and prodrugs in Huh-7 cells utilizing LC-MS/MS revealed that the triphosphates were not formed at therapeutically significant levels, indicating problems in the first and second phosphorylation steps for these nucleoside analogs by the cellular phosphorylation enzymes.

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13. Wang X, Seth PP, Ranken R, Swayze EE, Migawa MT. Nucleosides Nucleotides Nucleic Acids. 2004; 23:161. [PubMed: 15043144]
14. *Selected synthetic procedures and spectroscopic data*: 4-amino-7-iodo-1-(2-deoxy-2-fluoro-2-*C*-methyl-α-D-ribofuranosyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine (**4e**) and 4-amino-7-iodo-1-(2-deoxy-2-fluoro-2-*C*-methyl-β-D-ribofuranosyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine (**5e**). To a solution of 4-chloro-7-iodo-1H-pyrrolo[2,3-d]pyrimidine (339 mg, 1.21 mmol), 2-deoxy-2-fluoro-2-*C*-methyl-α/β-D-ribofuranose (500 mg, 1.34 mmol) and triphenylphosphine (698 mg, 2.66 mmol) in anhydrous THF (5 mL) was added diisopropyl azodicarboxylate (DIAD) (0.53 mL, 2.66 mmol), and the reaction mixture was stirred at rt for 2 d. The solvent was evaporated, and the residue was purified by flash chromatography on silica gel eluting with hexane-EtOAc (9:1 to 2:1) to give 390 mg (51%) of the nucleoside **3e** as a white solid, which contained a 1:1 ratio of α and β anomers. The compound **3e** (390 mg, 0.61 mmol) was placed in a steel vessel, and 1,4-dioxane (10 mL) was added followed by NH_4OH (28%, 20 mL). The steel vessel was sealed and heated at 120°C for 14 h. After cooling to rt, the solvent was evaporated and the residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2-MeOH (95:5 to 9:1) to give 108 mg (43%) of **4e** and 82 mg (33%) of **5e** both as a white solid. **4e**: R_f 0.30 (CH_2Cl_2-MeOH 9:1); ^{1}H-NMR (DMSO-d_6) δ 8.12 (s, 1H, H-2), 7.38 (d, J = 3.47 Hz, 1H, H-6), 6.70 (brs, 2H, NH_2), 6.38 (d, J = 20.79 Hz, 1H, H-1′), 5.71 (d, J = 6.93 Hz, 1H, OH-3′), 4.88 (t, J = 5.78 Hz, 1H, OH-5′), 4.12–4.10 (m, 1H, H-3′), 4.07–4.05 (m, 1H, H-4′), 3.70, 3.50 (2m, 2H, H-5′), 1.29 (d, J = 21.95 Hz, 3H, CH_3). LC-MS calcd for $C_{12}H_{15}FIN_4O_3$ (M+1): 409.0, found: 409.0. **5e**: R_f 0.42 (CH_2Cl_2-MeOH 9:1); ^{1}H-NMR (DMSO-d_6) δ 8.13 (s, 1H, H-2), 7.77 (s, 1H, H-6), 6.75 (brs, 2H, NH_2), 6.32 (d, J = 18.10 Hz, 1H, H-1′), 5.65 (d, J = 6.93 Hz, 1H, OH-3′), 5.31 (t, J = 4.62 Hz, 1H, OH-5′), 4.14–4.12 (m, 1H, H-3′), 3.87, 3.67 (2m, 3H, H-4′, H-5′), 0.94 (d, J = 22.72 Hz, 3H, CH_3). LC-MS calcd for $C_{12}H_{15}FIN_4O_3$ (M+1): 409.0, found: 409.0.
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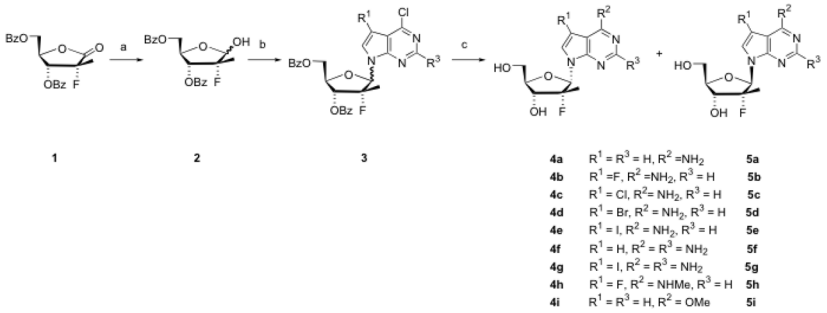

Scheme 1. Synthesis of α- and β-D-2′-deoxy-2-′-fluoro-2′-*C*-methyl-7-deazapurine nucleosides

Reagents and conditions: (a) LiAl(*t*-BuO)$_3$H, THF, 0 °C, 3 h, 99%; (b) 7-deazapurine (R^3 = H or NHPiv), Ph_3P, DEAD, THF, rt, 2 d, 40–60%; (c) i) NH_4OH, 1.4-dioxane, 120 °C, 20 h, 60–80%; or ii) $MeNH_2$, 1,4-dioxane, 120 °C, 20 h, 63–67%; or iii) MeOH, NaOMe, reflux, 3 h, 55–91%.

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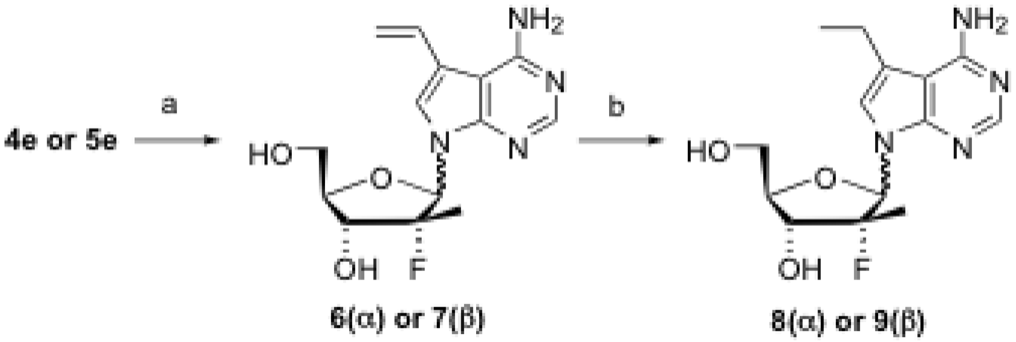

Scheme 2. Synthesis of 7-deaza-7-vinyl and -7-ethyl-purine nucleosides

Reagents and conditions: (a) Tributyl(vinyl)tin, $Pd(PPh_3)_4$. DMF, 100 °C, 5 h, 53–75%; (b) Pd-C, H_2, MeOH, rt, 20 h, 60–70%.

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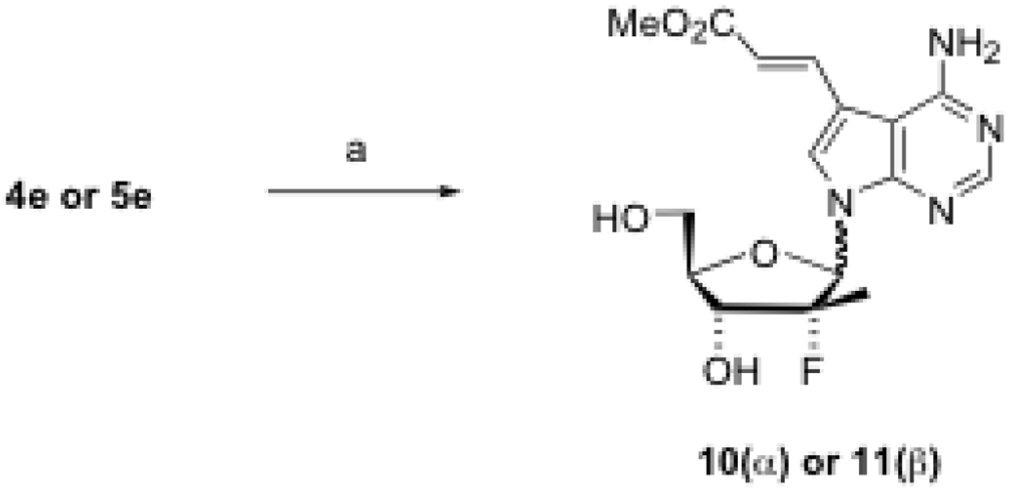

Scheme 3. Synthesis of 7-carbomethoxyvinyl-7-deazapurine nucleosides

Reagents and conditions: (a) $Pd(OAc)_2$, PPh_3, 1,4-dioxane, Et_3N, methyl acrylate, reflux, 16 h, 83–100%.

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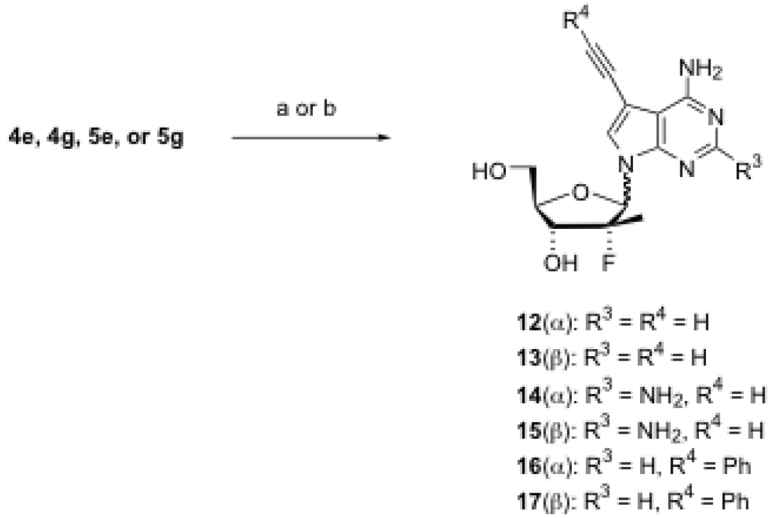
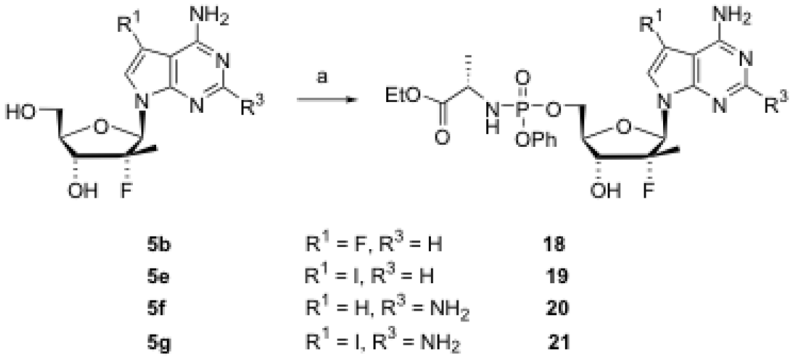


Scheme 4. Synthesis of 7-ethynyl-substituted nucleosides

Reagents and conditions: (a) i) $Pd(Ph_3P)_2Cl_2$, THF, CuI, Et_3N, triethylsilylacetelene, 45 °C, 20 h, 78–86%; ii) K_2CO_3, MeCO, rt, 20 h, 30–71%; (b) $Pd(Ph_3P)_2Cl_2$, THF, CuI, Et_3N, phenylacetelene, 45 °C, 20 h, 89–95%.



5b	R^1 = F, R^3 = H	**18**
5e	R^1 = I, R^3 = H	**19**
5f	R^1 = H, R^3 = NH_2	**20**
5g	R^1 = I, R^3 = NH_2	**21**

Scheme 5. Synthesis of nucleoside phosphoramidate prodrugs

Reagents and conditions: (a) Phenyl L-ethoxyalaninyl phosphorochloridate, 1-methylimidazole,THF, rt, 14 h, 11–47%.

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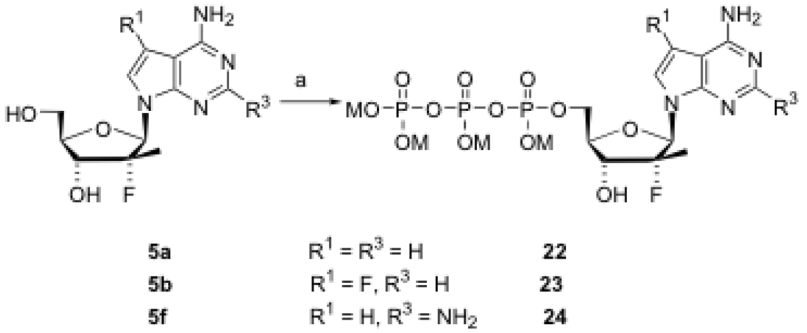

Scheme 6. Synthesis of nucleoside triphosphates

Reagents and conditions: M = NH(*n*-Bu)$_3$; (a) i) $POCl_3$, $PO(OCH_3)_3$, 2,4,6-collidine, 0 °C, 2 h; ii) $(Bu_3N)_2P_2O_7H_2$, Bu_3N, rt, 30 min; iii) TEAB, rt, 45 min.

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Table 1

In vitro antiviral activity and cytotoxicity of 2′-deoxy-2′-fluoro-2′-*C*-methyl-7-deazapurine nucleosides (**4a–17**) and phosphoramidate prodrugs (**18–21**).[a]

Compd #	Anti-HCV (μM)		rRNA (μM)	Anti-HIV-1 (μM)		Cytotoxicity, IC_{50} (μM) in:		
	EC_{50}	EC_{90}	CC_{50}[b]	EC_{50}	EC_{90}	PBM	CEM	VERO
4a	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
4b	> 10	> 10	> 10	> 100	> 100	> 100	8.1	> 100
4c	< 10	> 10	> 10	> 100	> 100	> 100	96.0	> 100
4d	< 10	> 10	> 10	> 100	> 100	89.1	76.0	71.1
4e	7.0	24.9	> 33	> 100	> 100	> 100	40.9	65.9
4f	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
4g	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
4h	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
4i	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
5a	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
5b	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
5c	< 10	> 10	> 10	82.4	> 100	18.5	> 100	> 100
5d	< 10	> 10	< 10	> 100	> 100	74.2	50.5	53.1
5e	1.1	> 33	10.4	94.0	> 100	53.2	24.0	21.4
5f	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
5g	> 10	> 10	> 10	99.5	>100	72.9	85.4	62.8
5h	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
5i	> 10	> 10	> 10	24.8	85.6	> 100	> 100	> 100
6	5.6	18.4	8.3	35.5	65.2	21.0	16.0	14.6
7	**1.8**	**7.6**	**4.5**	**14.7**	**44.8**	**4.8**	**3.5**	**10.1**
8	> 10	> 10	> 10	95.5	> 100	> 100	> 100	> 100
9	< 10	> 10	> 10	19.0	> 100	20.7	> 100	> 100
10	> 10	> 10	> 10	**0.71**	**9.5**	> 100	> 100	> 100
11	> 10	> 10	> 10	> 100	> 100	> 100	> 100	> 100
12	< 10	> 10	> 10	95.2	> 100	> 100	> 100	> 100
13	< 10	> 10	> 10	59.1	> 100	94.1	> 100	> 100
14	> 10	> 10	> 10	7.1	59.1	54.0	26.5	61.7

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Compd #	Anti-HCV (μM)		rRNA (μM)	Anti-HIV-1 (μM)		Cytotoxicity, IC_{50} (μM) in:		
	EC_{50}	EC_{90}	CC_{50}[b]	EC_{50}	EC_{90}	PBM	CEM	VERO
15	> 10	> 10	> 10	84.4	> 100	> 100	> 100	> 100
16	> 10	> 10	> 10	7.7	38.0	40.8	15.7	51.3
17	< 10	> 10	< 10	10.7	31.1	41.1	11.6	45.7
18	> 10	> 10	> 10	39.4	98.8	> 100	> 100	> 100
19	> 10	> 10	> 10	58.1	> 100	17.4	13.5	17.8
20	> 10	> 10	> 10	88.0	> 100	> 100	> 100	> 100
21	> 10	> 10	> 10	26.7	> 100	67.4	> 100	65.1
NM-107	2.8	9.6	> 10	47.5	100.0	47.4	54.4	> 100
AZT	> 10	> 10	> 10	0.0033	0.028	> 100	14.3	56.0

[a] All values are based on mean of replicate assays (N ≤ 2).

[b] CC_{50}: cytotoxic concentration that reduced the rRNA levels by 50% in 96 h.

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Table 2

Inhibitory activity of 2′-deoxy-2′-fluoro-2′-*C*-methyl-7-deazapurine nucleoside triphosphates against wild-type and S282T mutant HCV NS5B polymerases

Nucleotide #	HCV NS5B pol IC_{50} (μM)		
	Wild-type	S282T	Fold resistance relative to wild-type HCV pol
22	15	15	1
23	4	6	1.5
24	3	6	2
NM-107-TP	3	51	17