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Synthesis and antiviral activity of 2′-deoxy-2′-fluoro-2′-*C*-methyl purine nucleosides as inhibitors of hepatitis C virus RNA replication

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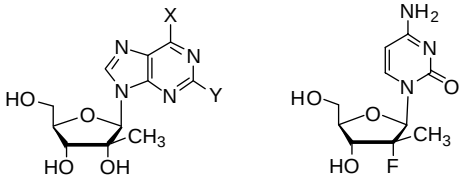
Abstract—A series of purine nucleosides containing the 2′-deoxy-2′-fluoro-2′-*C*-methylribofuranosyl moiety were synthesized and evaluated as potential inhibitors of the hepatitis C virus in vitro. Of the nucleosides that were synthesized, only those possessing a 2-amino group on the purine base reduced the levels of HCV RNA in a subgenomic replicon assay.

The current standard of care for chronic hepatitis C virus (HCV) infections is combination therapy with alpha interferon and ribavirin. Studies have shown that more patients with hepatitis C respond to pegylated interferon-α/ribavirin combination therapy than to combination therapy with unpegylated interferon alpha. Due to the low response rates as well as toxic side effects and unsustained viral load reductions, these therapies are inadequate and there is a need for improved therapies for treating chronic HCV infection.[1]

Several 2′-*C*-methyl purine nucleoside analogs with potent inhibitory activity against the HCV NS5B polymerase have been identified (Fig. 1).[2–5] The potent inhibitory activity against HCV replication of a novel pyrimidine nucleoside, β-D-2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (**3**), has recently been reported.[6] The synthesis and antiviral activity of several purine analogs containing the 2′-deoxy-2′-fluoro-2′-*C*-methylribofuranosyl moiety are reported herein.



1: X = OH, Y = NH_2
2: X = NH_2, Y = H

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Figure 1. Branched-chain nucleoside HCV polymerase inhibitors.

The general synthetic procedure used for 2′-deoxy-2′-fluoro-2′-*C*-methyl purine nucleoside analogs (**11**–**14**) described herein is similar to the linear synthesis described for 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (Scheme 1).[6] For the preparation of compounds **6a** and **6b**, the 3′,5′-silyl-protecting groups were removed with TBAF in THF and replaced with the acetyl groups in a one-pot desilylation/acetylation reaction. This one-pot reaction provided a convenient method that avoided the isolation of the polar 3′,5′-diol intermediate.

Upon treating **6a** and **6b** with DAST in toluene, the desired fluorinated products (**7a** and **7b**) were obtained in approximately 20% isolated yield after silica gel purifica-

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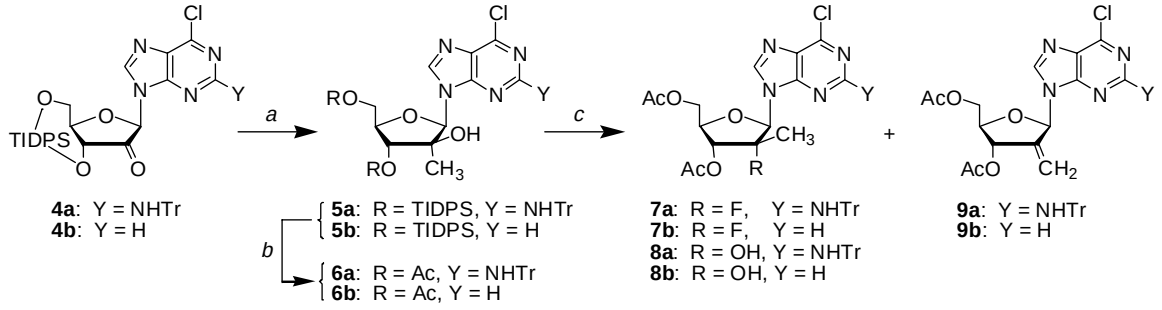


Scheme 1. Reagents and conditions: (a) MeLi, −78 °C; (b) i—1 M TBAF, concd AcOH, rt, ii—Ac_2O, TEA; (c) DAST, toluene, 0 °C to rt.

Table 1. ^{1}H NMR data for compounds **7b** and **10**–**14**[a]

Compound	2′-CH_3	H-1′	H-3′	H-4′	H-5′/H-5a′	H-2	H-8
7b	1.24 (d, J = 22.8)	6.37 (d, J = 17.6)	5.70 (dd, J = 9.1, 22.1)	4.41–4.55 (m)		8.82 (s)	8.45 (s)
10	1.26 (d, J = 22.5)	6.12 (d, J = 17.7)	5.84 (dd, J = 9.2, 22.8)	4.47 (m)	4.34, 4.55 (dd, J = 2.9, 12.4)	—	7.97 (s)
11	1.23 (d, J = 22.4)	6.37 (d, J = 17.4)	4.61 (dd, J = 9.2, 22.3)	4.17 (dd, J = 1.5, 9.2 Hz)	4.25, 4.04 (dd, J = 2.11, 12.5)	8.78 (s)	8.70 (s)
12	1.14 (d, J = 22.3)	6.28 (d, J = 17.4)	4.40 (dd, J = 9.4, 24.3)	4.03–4.09 (m), 3.87 (dd, J = 3.0, 12.6 Hz)		8.52 (s)	8.20 (s)
13	1.21 (d, J = 22.8)	6.19 (d, J = 17.3)	4.38 (dd, J = 9.5, 24.5)	4.02–4.07 (m), 3.87 (dd, J = 3.1, 12.6 Hz)		—	8.51 (s)
14	1.20 (d, J = 22.3)	6.10 (d, J = 17.8)	4.32 (dd, J = 8.9, 24.3)	3.85–4.04 (m)		—	8.15 (s)

[a] Spectra were obtained at 400 MHz. Chemical shifts are reported as δ (ppm) downfield with respect to an internal standard of tetramethylsilane. J values are in Hertz. Spectra were obtained in $CDCl_3$ for compounds **7b**, **10**, **11** and in CD_3OD for compounds **12**–**14**.

tion (Scheme 1).[10] The structures of the fluorinated products were determined by analyzing the ^{1}H and ^{13}C NMR multiplicities arising from the 2′-fluorine coupling and by nuclear Overhauser effect (NOE) ^{1}H NMR difference spectroscopy (Table 1). Upon irradiation of the doublet at δ 1.26 (2′-CH_3) in compound **10**, NOEs were observed with the doublet of doublets at δ 5.84 (H-3′) and the anomeric doublet at δ 6.12 (Fig. 2). Similar NOEs were observed with the 6-chloropurine analog **7b**.

The DAST fluorination of compounds **6a** and **6b** also provided two additional corresponding products that were isolated by silica gel chromatography and identified as the exomethylene products **9a** and **9b**, and the protected 2′-*C*-methyl nucleoside products **8a** and **8b**. Analysis of the ^{1}H NMR spectra of compounds **9a** and **9b** revealed two doublets (J = 3.9 Hz) at around δ 5.6 and 5.3, indicating the presence of an exomethylene containing diastereotopic protons. The structures of compounds **8a** and **8b** were confirmed by the transformation to the known compounds **1** and **2** using standard methods.[4] Compound **8a** was converted to 2′-*C*-methylguanosine (**1**) by the same methods used to prepare 2′-deoxy-2′-fluoro-2-*C*-methyl-guanosine **14** from **7a** (Scheme 2).

The N^2 trityl group in **7a** was removed using $CHCl_3$-TFA (9:1) to provide compound **10**. Treating compounds **7b** and **10** with methanolic ammonia at room temperature provided **11** and **13**, respectively; performing the aminolysis at 80 °C in a sealed tube for 24–36 h provided **12** from **7b**. 2′-deoxy-2′-fluoro-2′-*C*-methylguanosine (**14**) was prepared from **11** using an excess of mercaptoethanol and sodium methoxide.[7]

Although the yields of fluorinated products were essentially the same for both compounds **6a** and **6b**, the yields

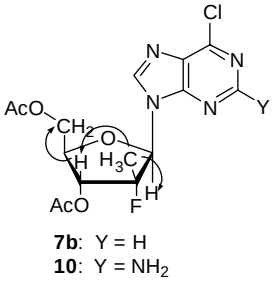
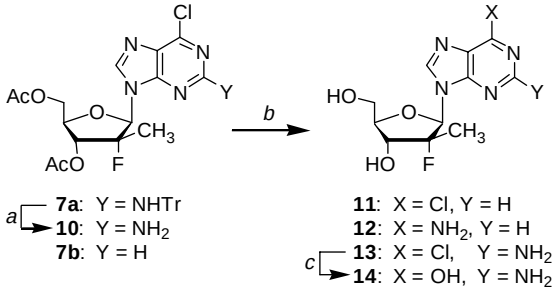


Figure 2. 1D NOE correlations for compounds **7b** and **10**.



Scheme 2. Reagents and conditions: (a) $CHCl_3$-TFA (9:1), rt; (b) MeOH/NH_3, rt for **10** → **13** and **7b** → **11**, 80 °C for **7b** → **12**; (c) mercaptoethanol, NaOMe, reflux.

Table 2. Anti-HCV activity and cellular toxicity of compounds **11**–**14**, 2′-*C*-methylguanosine (**1**) and 2′-*C*-methyladenosine (**2**)

Compound	HCV EC_{90}[a] (μM)	Cytotoxicity CC_{50}[b] (μM)
11	88.2 ± 3.7	27.1 ± 13.0
12	>100	>100
13	62.4 ± 22.6	>100
14	56.0 ± 31.7	>100
1	10.8	>100
2	1.40 ± 0.5	15.0 ± 1.6

[a] Average of at least two experiments.
[b] MTS CC_{50} was determined in a 96 h assay using the Celltiter 96 nonradioactive cell proliferation assay from Promega (Madison, WI).

of the by-products (**8a** and **8b**) were sensitive to the purine ring substituents. The DAST fluorination of **6a** provided the protected 2′-*C*-methylpurine (**8a**) in 72% isolated yield, suggesting that the N^3 moiety, made more nucleophilic by the exocyclic N^2 amino substituent, is involved in this epimerization.[8] In contrast, fluorination of compound **6b**, that contains no 2-amino group, consistently afforded a modest 20% yield of the C-2′ epimerized compound **8b**.

Compounds **11**–**14** were evaluated as inhibitors of the HCV in a subgenomic replicon assay as previously described.[9] The anti-HCV activity of 2′-*C*-methylguanosine (**1**) and 2′-*C*-methyladenosine (**2**), as obtained from the DAST fluorination, is shown for comparison. As indicated in Table 2, the 2′-deoxy-2′-fluoro-2′-*C*-methylpurine analogs **13** and **14** demonstrate modest inhibition against HCV replication. 2′-Deoxy-2′-fluoro-2′-*C*-methylguanosine (**14**) was ~5 times less potent than the corresponding 2′-*C*-methylguanosine (**1**), while 2′-deoxy-2′-fluoro-2′-*C*-methyladenosine (**12**) showed no activity or cytotoxicity when tested up to 100 μM.

The DAST fluorination of purine nucleosides such as **6a** and **6b** provides modest yields of the desired fluorinated nucleosides **7a** and **7b**. However, this synthetic route provides a direct method for the preparation of the corresponding 2′-*C*-methylnucleosides and thereby a concise method for determining the effect of fluorine substitution with respect to in vitro HCV inhibition. While the reason for the lack of potency of compound **12** is unclear, the modest activity of compounds **13** and **14** suggests that both the 2-amino and the 2′-β-methyl groups are important substituents for selective HCV inhibition in these molecules. The weak inhibition of compounds **13** and **14** in the cell-based replicon assay may be due to inefficient uptake and/or metabolism to the active 5′-triphosphate. The lack of efficient phosphorylation or uptake of 2′-*C*-methylguanosine (**1**) in a cell-based replicon assay has been suggested. Eldrup *et al.* reported a remarkable anti-HCV potency of the 5′-triphosphate of compound **1** in an NS5B enzyme assay even though the free nucleoside lacked the same level of potency in a cell-based replicon system.[4] The latter assay requires penetration of the parent nucleoside into the cells followed by metabolism to the active triphosphate species.

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10. Reaction of **6a** with DAST: To a stirred solution of **6a** (0.830 g, 1.29 mmol) in anhydrous toluene (30.0 mL) was added DAST (0.280 mL, 2.12 mmol) at 0 °C under argon. After the addition was complete, the cooling bath was removed and stirring was continued for 1 h. The reaction mixture was poured into satd $NaHCO_3$ (30.0 mL) and washed until gas evolution ceased. The organic phase was dried (Na_2SO_4), concentrated to dryness, and purified by silica gel chromatography washing with 1:1 Et_2O–petroleum ether and eluting with 1:7:7 EtOAc–Et_2O–petroleum ether to afford **7a** (0.171 g, 20.5%) as a syrup, followed by **9a** (0.062 g, 7.8%). Further elution afforded **8a** (0.598 g, 72.0%) as a syrup. The same procedure, starting from **6b**, was used to obtain **7b**, **8b**, and **9b**. ^1H, ^{19}F, and ^{13}C NMR spectra were obtained with a Varian Unity Plus 400 spectrometer at 400, 376, and 100 MHz, respectively. ^1H and ^{13}C NMR chemical shifts are reported as δ (ppm)

downfield with respect to an internal standard of tetramethylsilane, while ^{19}F chemical shifts are reported downfield from an external standard of hexafluorobenzene. **7a**: ^{1}H NMR ($CDCl_3$): δ 0.67 (d, 3 H, J = 22.5 Hz), 2.16 (s, 3H), 2.19 (s, 3H), 4.25–4.40 (m, 3H), 5.19 (dd, 1H, J = 8.5, 22.4 Hz), 5.52 (d, 1H, J = 17.3 Hz), 6.76 (s, 1H), 7.19–7.34 (m, 15H), 8.01 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 16.6 (d, J = 24.6 Hz), 20.6, 20.9, 61.1, 70.8 (d, J = 15.9 Hz), 71.0, 88.3 (d, J = 38.6 Hz), 99.9 (d, J = 186.0 Hz), 124.6, 127.0, 128.0, 129.0, 129.1, 138.4, 144.7, 151.1, 152.1, 157.7, 170.0, 170.3. ^{19}F NMR ($CDCl_3$): δ 5.10 (m); **8a**: ^{1}H NMR ($CDCl_3$): δ 0.63 (s, 3H), 2.14 (s, 3H), 2.17 (s, 3H), 4.25–4.37 (m, 3H), 5.04 (d, 1H, J = 7.7 Hz), 5.38 (s, 1H), 6.76 (s, 1H), 7.14–7.35 (m, 15H), 8.01 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 20.3, 20.7, 20.9, 61.9, 71.0, 73.4, 78.8, 90.6, 124.7, 125.4, 127.0, 127.9, 128.3, 129.07, 129.1, 138.8, 144.8, 151.0, 152.1, 157.5, 169.7, 170.4; **9a**: ^{1}H NMR ($CDCl_3$): δ 2.10 (s, 3H), 2.16 (s, 3H), 4.29 (d, 2H, J = 3.5 Hz), 4.79 (bs, 1H), 5.31 (bs, 1H), 5.32 (d, 1H, J = 3.5 Hz), 5.65 (d, 1H), 6.00 (bs, 1H), 6.69 (s, 1H), 7.21–7.34 (m, 15H), 7.80 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 20.9, 21.0, 63.3, 71.2, 72.2, 80.5, 83.2, 116.9, 126.9, 127.9, 128.0, 129.0, 129.1, 139.4, 143.4, 144.9, 157.8, 170.2, 170.4.