Note

Synthesis and Anti-HCV Activity of a Novel 2',3'-Dideoxy-2'-#-fluoro-2'-#-C-methyl Guanosine Phosphoramidate Prodrug

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Synthesis and Anti-HCV Activity of a Novel 2',3'-Dideoxy-2'-α-fluoro-2'-β-*C*-methyl Guanosine Phosphoramidate Prodrug

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KEYWORDS. 2',3'-dideoxy guanosine; 2'-α-fluoro-2'-β-C-methyl; phosphoramidate prodrug; anti-HCV activity; NS5B RdRp inhibitor

ABSTRACT: A novel 2',3'-dideoxy-2'-α-fluoro-2'-β-*C*-methyl-6-methoxy guanosine (**8**) and its phosphoramidate prodrug (**1**) have been designed and synthesized. Their biological activity was evaluated in both cytotoxicity and cell-based HCV replicon assays. Neither of compounds exhibited cytotoxicity up to the highest concentration tested (100 μM) in the Huh-7 cell line. The prodrug (**1**) displayed nanomolar level antiviral activity (EC_{50} = 0.39~1.1 μM) against the HCV genotype (GT) 1a, 1b, 2a, and 1b S282T replicons.

Hepatitis C virus (HCV), a member of the Flaviviridea family, is a positive-sense single-stranded RNA virus. HCV infection affects over 170 million people worldwide[1] and is a major cause of hepatocellular carcinoma (HCC). So far, a series of potential molecular targets have been indentified for anti-HCV drug discovery, including NS2-NS3 autoprotease, the N3 protease, the N3 helicase, and the NS5B polymerase. The NS5B, one of nonstructural proteins serving as the HCV RNA-dependent RNA polymerase (RdRp), is essential for the replication of the viral RNA genome and has attracted significant interest among medicinal chemists.[2-5] Nucleosides active against HCV are NS5B RdRp inhibitors, and they need to be converted to the corresponding triphosphates that can incorporate into viral RNA at the 3'-terminal so as to stop viral RNA elongation as chain terminators. Some nucleosides are weakly active because they cannot be efficiently phosphorylated by specific nucleoside kinases or are not substrates of the kinases at all. On the other hand, nucleoside phosphates (nucleotides) per se cannot be used as drugs very often because they are chemically less stable and/or too polar to enter the cells.[6, 7] In order to bypass the rate-limiting first-step phosphorylation as well as to improve the chemical stability and biological activity, various nucleoside phosphate prodrugs have been designed and synthesized,[7-10] Recently, the phosphoramidate prodrug strategy has been demonstrated as an effective approach for intracellular delivery of the monophosphorylated nucleosides. The monophosphate can be further phosphorylated to di-, and then the biologically active triphosphate.

Among various antiviral nucleos(t)ide compounds, the 2'-*C*-methyl substituted analogs[11-17] display potent anti-HCV activity in vitro, in vivo, and in clinic (Figure 1). These nucleotides act as non-obligate chain terminators of HCV RdRp as they all possess a 3'-OH moiety. 2',3'-Dideoxy nucleosides, such as Zidovudine and Emtricitabine exhibit good antiviral activity against HIV and HBV because they cannot support the elongation of the newly synthesized viral polynucleotide due to the lack of the 3'-hydroxy group. However, such analogs were rarely reported in the literature with good anti-HCV activity, a major reason for which could be *ribo*-nucleosides with 3'-OH may not be good substrates of phosphorylation kinases.[18] Herein, we designed and synthesized such a novel 2',3'-dideoxy-2'-α-fluoro-2'-β-*C*-methyl nucleoside analog (**8**) and its phosphoramidate prodrug (**1**) for the treatment of HCV infection.[19-20]

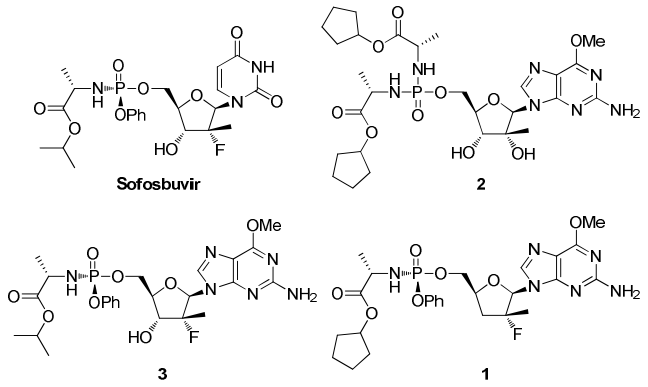
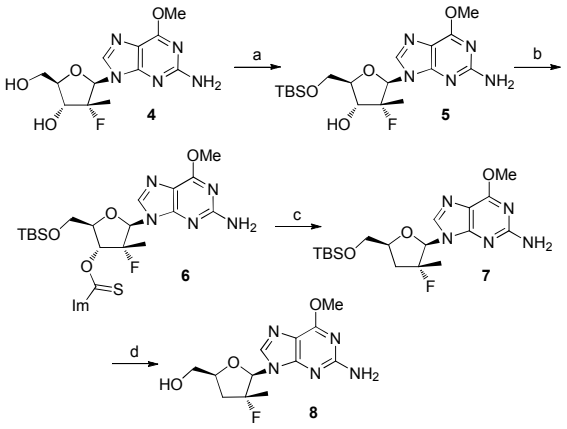


Figure 1. Representative 2'-*C*-methyl substituted nucleotide analogs with potent anti-HCV activity.

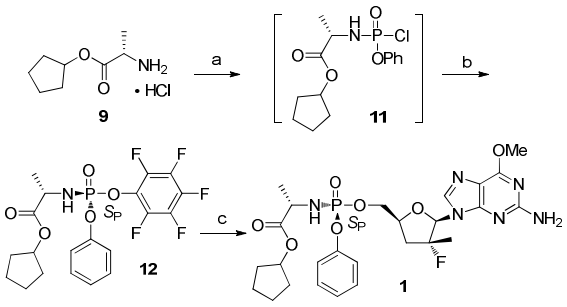
The synthesis of the target 2',3'-dideoxy-2'-α-fluoro-2'-β-C-methyl-6-methoxy guanosine (**8**) is described in Scheme 1. The starting material **4** was readily obtained according to a previously reported synthetic route.[13] Selective protection of the 5'-OH group in nucleoside **4** with *tert*-butyldimethylsilyl chloride in the presence of imidazole resulted in compound **5**, which was further converted into intermediate **6** by the treatment with 1,1-thiocarbonyldiimidazole in acetonitrile. Bu_3SnH/AIBN-mediated 3'-deoxylation of compound **6** and subsequent removal of the silyl protecting group with TBAF afforded the 2',3'-dideoxy guanosine **8**.

Scheme 1. Synthesis of 2',3'-Dideoxy-2'-α-fluoro-2'-β-*C*-methyl-6-methoxy Guanosine 8



(a) TBDMSCl, imidazole, DMF, rt, 81%; (b) 1,1-thiocarbonyldiimidazole, MeCN, rt, 69%; (c) (n-Bu)$_3$SnH, AIBN, toluene, 80 ºC, 75%; (d) TBAF, THF, rt, 64%.

Scheme 2. Synthesis of the Phosphoramidate Prodrug 1



(a) PO(OPh)Cl$_2$ (**10**), NEt$_3$, CH$_2$Cl$_2$, -78 ºC ~ rt; (b) pentafluorophenol, NEt$_3$, CH$_2$Cl$_2$, rt; then recrystallized in EtOAc/hexanes, 39% (over two steps); (c) guanosine **8**, ^{t}BuMgCl, THF, rt, 83%.

To synthesize the phosphoramidate prodrug **1**, the corresponding perfluorophenyl phosphoramidate (**12**) was prepared (Scheme 2). Treatment of phenyl dichlorophosphate (**10**) with L-alanyl cyclopentyl ester hydrochloride (**9**) and triethylamine at -78 °C followed by reaction of the resulting monochlorophosphate **11** with pentafluorophenol and triethylamine gave the S_P-cyclopentyl ester **12** after the recrystallization from a mixture of ethyl acetate and hexanes. Reaction of nucleoside **8** with compound **12** in the presence of *tert*-butyl magnesium chloride afforded the desired prodrug **1** as the pure S_P-enantiomer.

The biological evaluation was carried out in cytotoxicity and cell-based HCV replicon assays, respectively. As summarized in Table 1, neither of guanosine **8** and its phosphoramidate prodrug (**1**) exhibited cytotoxicity at the highest concentration tested (100 μM) in Huh-7 cells. In the same cell line containing replicating HCV genotype (GT) 1b replicon, although the nucleoside **8** was inactive, its prodrug **1** inhibited the replication of the replicon with an EC_{50} of 0.58 μM. In light of these encouraging results, the antiviral activity of compound **1** was further profiled using different HCV replicon cells. This prodrug also displayed nanomolar antiviral activity (EC_{50} = 0.81 μM) against the HCV GT 1a, 2a, and 1b S282T replicons.

Table 1. Cytotoxicity and anti-HCV activity of guanosine 8 and its phosphoramidate prodrug 1[a]

compd	CC_{50} (μM)	HCV 1b EC_{50} (μM)	HCV 1a EC_{50} (μM)	HCV 2a EC_{50} (μM)	HCV 1b S282T EC_{50} (μM)
8	> 100	> 100	ND	ND	ND
1	> 100	0.58	1.1	0.39	0.81

[a]EC_{50}: 50% effective concentration; CC_{50}: 50% cytotoxic concentration.

In summary, we have designed and synthesized a novel 2',3'-dideoxy-2'-α-fluoro-2'-β-*C*-methyl-6-methoxy guanosine (**8**) and its phosphoramidate prodrug (**1**). Neither of compounds is cytotoxic in the Huh-7 cell line at the highest tested concentration up to 100 μM. Although guanosine **8** is inactive, the corresponding prodrug (**1**) displayed potent anti-HCV activity against the genotype (GT) 1a, 1b, 2a, and 1b S282T replicons with similar EC_{50}s (between 0.39 and 1.1 μM). For the first time, we have discovered a 2',3'-dideoxy nucleotide analog possessing potent antiviral activity against HCV infection. These encouraging results warrant further investigation towards the development of compound **1** as a potential anti-HCV agent.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.
Experimental procedures, characterization data, and biological assays (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

HCV, hepatitis C virus; HCC, hepatocellular carcinoma; RdRp, RNA-dependent RNA polymerase; TBDMSCl, *tert*-butyldimethylsilyl chloride; DMF, *N*,*N*-dimethylformamide; AIBN, azobisisobutyronitrile; TBAF, tetrabutylammonium fluoride; THF, tetrahydrofuran; GT, genotype; EC_{50}, 50% effective concentration; CC_{50}, 50% cytotoxic concentration; PE, petroleum ether; LTR, long terminal repeat; DMEM, Dulbecco's minimum essential medium; FBS, fetal bovine serum.

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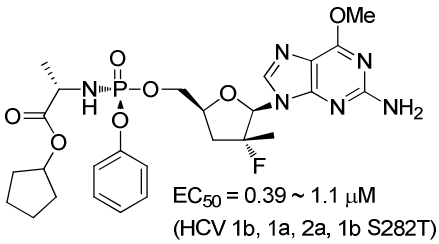

EC_{50} = 0.39 ~ 1.1 μM
(HCV 1b, 1a, 2a, 1b S282T)
CC_{50} > 100 μM