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# Synthesis and anti-HCV activity of 3′,4′-oxetane nucleosides

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ABSTRACT

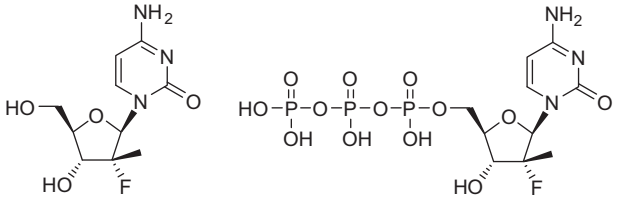
Hepatitis C virus afflicts approximately 180 million people worldwide and currently there are no direct acting antiviral agents available to treat this disease. Our first generation nucleoside HCV inhibitor, RG7128 has already established proof-of-concept in the clinic and is currently in phase IIb clinical trials. As part of our continuing efforts to discover novel anti-HCV agents, 3′,4′-oxetane cytidine and adenosine nucleosides were prepared as inhibitors of HCV RNA replication. These nucleosides were shown not to be inhibitors of HCV as determined in a whole cell subgenomic replicon assay. However, 2′-mono/difluoro analogs, **4**, **5**, and **6** were readily phosphorylated to their monophosphate metabolites by deoxycytidine kinase and their triphosphate derivatives were shown to be inhibitors of HCV NS5B polymerase in vitro. Lack of anti-HCV activity in the replicon assay may be due to the inability of the monophosphates to be converted to their corresponding diphosphates.



Hepatitis C virus (HCV) is a leading cause of chronic liver disease.[1] Nearly 1.6% of the U.S. population and an estimated 180 million people worldwide are infected with HCV.[2] Approximately 80% of infected individuals become chronically infected; this chronic infection can lead to liver cirrhosis and hepatocellular carcinoma in a significant number of patients.[3] The current standard of care (SOC) for those infected with HCV includes administration of pegylated interferon alpha and ribavirin. However, only 50% of patients infected with genotype 1 virus respond to treatment with SOC therapy. In addition, the SOC regimens are associated with various intolerable adverse side effects.[4] To date no vaccine has been developed to treat HCV due to the multiple genotypes of HCV and to the relatively high mutation rate associated with this virus.[5] Consequently, there is an urgent need for the identification of new small molecule direct acting antiviral agents to effectively treat chronic HCV infection.

RG7128 is a nucleoside prodrug of β-D-2′-deoxy-2′-α-fluoro-2′-*C*-methylcytidine **1** (PSI-6130, Fig. 1) and is a specific inhibitor of the HCV NS5B RNA dependent RNA polymerase (RdRp). Recently, we reported exceptional clinical efficacy of RG7128 as an anti-HCV direct acting antiviral agent.[6] Since **1** is a nucleoside, in order to inhibit the HCV RdRp, it must be phosphorylated by cellular kinases to its 5′-triphosphate form. The 5′-triphosphate of PSI-6130 (**2**) is an alternative substrate of the HCV RdRp and acts as a non-obligate chain terminator.[7,8]

Other than the 2′-α-fluoro-2′-*C*-methyl class of nucleosides represented by PSI-6130 (**1**), several other nucleoside classes have been reported to exhibit in vitro anti-HCV activity. These other nucleosides include 2′-deoxy-2′-α-fluorocytidine,[9] 2′-α-*O*-methyl substituted nucleosides, 2′-C-methyl ribonucleosides,[10,11] and 4′-substituted nucleoside derivatives where 2′-substitution can be either α-OH, β-OH, β-F or diF.[12,13] What is also known about these nucleosides is that the 4′-substituted nucleosides possess a different conformational preference (2′-endo) relative to the other classes which prefer a 3′-endo conformation (Fig. 2), yet they still demonstrate good in vitro potency as inhibitors of HCV.[7,12] Therefore, we were interested in taking key features of each of these




**1, PSI-6130**

$EC_{90}$ = 4.6 μM

**2**

$K_i$ = 0.059 μM for HCV NS5B

**Figure 1.**

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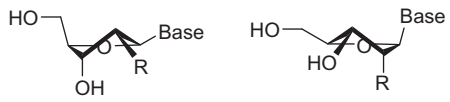



**Figure 2.** C2′- and C3′-endo conformations of nucleosides.


**3**, Base = cytosine, $R^1$ = F; $R^2$ = Me
**4**, Base = cytosine, $R^1$ = F; $R^2$ = H
**5**, Base = cytosine, $R^1$ = H; $R^2$ = F
**6**, Base = cytosine, $R^1$ = F; $R^2$ = F
**7**, Base = cytosine, $R^1$ = OMe; $R^2$ = H
**8**, Base = adenine, $R^1$ = F; $R^2$ = H

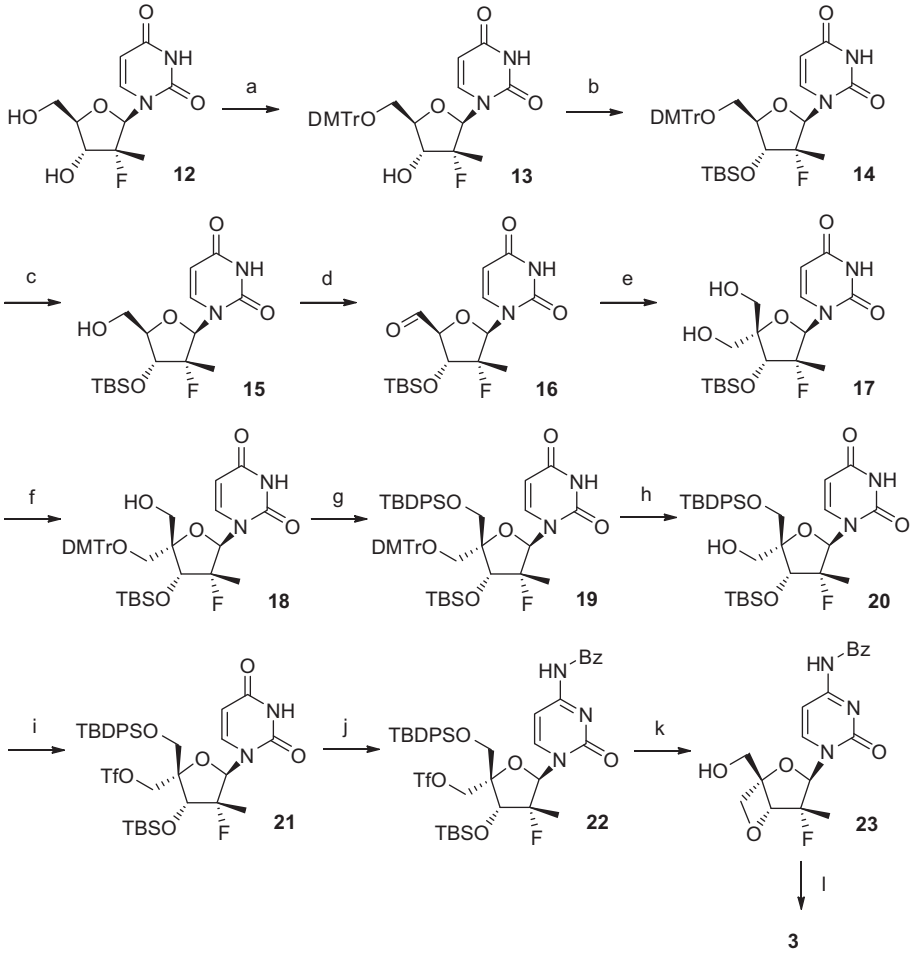
**9**, $R^1$ = F; $R^2$ = H
**10**, $R^1$ = H; $R^2$ = F
**11**, $R^1$ = F; $R^2$ = F

**Figure 3.**

nucleosides and developing a novel series of compounds as potential inhibitors of HCV replication.

In exploring the potential of novel nucleosides as anti-HCV agents, we were interested in investigating conformationally restricted nucleoside derivatives. Introducing a conformational restriction by the use of fused ring system is a well-established approach to enhance a specific biological activity of a nucleoside.[14] This modification has been extensively pursued not only for antisense oligonucleotides,[15,16] but also for direct-acting therapeutic agents such as antituberculosis,[17] antitumor,[18] and antivirals.[19] In pursuing this concept of conformationally restricted nucleosides we chose to investigate the 3′-*O*,4′-*C* methylene (3′,4′-oxetane) nucleoside template. The ribofuranose ring of a 3′,4′-oxetane nucleoside is known to prefer the C2′-endo (Southern-type) conformation similar to the 2′-deoxy-4′-azido substituted anti-HCV nucleosides.[12,16] However, the biological activity of this class of molecules against HCV remains unexplored. Therefore, we proceeded to investigate the anti-HCV activity of the 3′,4′-oxetane nucleosides introducing substitution at the 2′-position known to be compatible with anti-HCV activity (Fig. 3).

Oxetane nucleoside **3** was prepared from 2′-deoxy-2′-fluoro-2′-*C*-methyluridine **12** (Scheme 1). The 5′- and 3′-hydroxyl groups were sequentially protected with a dimethoxytrityl group and a *tert*-butyldimethylsilyl group. After deprotection under acidic conditions, the primary alcohol at C5′ was oxidized via the Pfitzner–Moffatt reaction[20] to give aldehyde **16**. Subsequent aldol reaction with formaldehyde followed by reduction of the aldehyde provided diol **17**. Then, the less hindered C5′-primary alcohol on the α-face of diol **17** was selectively protected with a dimethoxytrityl group. The other primary alcohol was then protected with TBDPS. Subse-




**Scheme 1.** Reagents and conditions: (a) DMTrCl, pyridine, 0 °C to rt, 8 h, 93%; (b) TBSCl, imidazole, rt, 16 h, 92%; (c) 3% TFA in DCM, rt, 3 h, 66%; (d) EDC, DMSO, pyridine, TFA, rt, 30 min, 50%; (e) $CH_2O$, NaOH, aq dioxane, rt, 30 min; $NaBH_4$, 0 °C to rt, 1 h, 45%; (f) DMTrCl, pyridine, 0–10 °C, 8 h, 67%; (g) TBDPSCl, imidazole, rt, 18 h, 46%; (h) CAN, MeCN, rt, 16 h, 83%; (i) $Tf_2O$, 2,6-lutidine, DCM, 16 h, 91%; (j) TIPBSCl, Hűnig's base, DMAP, $CH_3CN$, rt, 1 h; $NH_4OH$, rt, 0.5 h; BzCl, Hűnig's base, DMAP, rt, 1 h, 65%; (k) TBAF, THF, reflux, 3 h, 58%; (l) 7 N $NH_3$ in MeOH, rt, 6 h, 62%.