ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

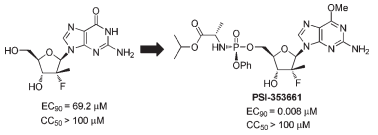
Discovery of PSI-353661, a Novel Purine Nucleotide Prodrug for the Treatment of HCV Infection

Wonsuk Chang, Donghui Bao, Byoung-Kwon Chun, Devan Naduthambi, Dhanapalan Nagarathnam, Suguna Rachakonda, P. Ganapati Reddy, Bruce S. Ross, Hai-Ren Zhang, Shalini Bansal, Christine L. Espiritu, Meg Keilman, Angela M. Lam, Congrong Niu, Holly Micolochick Steuer, Phillip A. Furman, Michael J. Otto, and Michael J. Sofia*

Pharmasset, Inc., 303A College Road East, Princeton, New Jersey 08540-6608, United States

ABSTRACT Hepatitis C virus afflicts approximately 180 million people worldwide, and the development of direct acting antivirals may offer substantial benefit compared to the current standard of care. Accordingly, prodrugs of 2′-deoxy-2′-fluoro-2′-*C*-methylguanosine monophosphate analogues were prepared and evaluated for their anti-HCV efficacy and tolerability. These prodrugs demonstrated >1000 fold greater potency than the parent nucleoside in a cell-based replicon assay as a result of higher intracellular triphosphate levels. Further optimization led to the discovery of the clinical candidate PSI-353661, which has demonstrated strong *in vitro* inhibition against HCV without cytotoxicity and equipotent activity against both the wild type and the known S282T nucleoside/tide resistant replicon. PSI-353661 is currently in preclinical development for the treatment of HCV.

KEYWORDS PSI-353661, hepatitis C virus, NS5B polymerase, antivirals, nucleoside, prodrug, phosphoramidate, triphosphate



Hepatitis C virus is a 9.6 kb positive-sense single-stranded RNA virus of the *Flaviviridae* family. Nearly 1.6% of the U.S. population and an estimated 180 million people worldwide are infected with HCV.[1] Approximately 80% of infected individuals become chronically infected. Untreated HCV infections can progress to cirrhosis, hepatocellular carcinoma, and liver failure, a primary cause for liver transplantation.[2] The current standard of care (SOC) for chronic HCV infection, PEG-IFNα and ribavirin, provides limited sustained virologic response (SVR) rates (e.g., ≤50% for genotype 1 patients) and can produce various undesirable side effects ranging from flu-like symptoms to severe adverse effects, including anemia, cardiovascular events, and psychiatric problems such as suicidal ideation.[3] Consequently, there is a clear unmet medical need to discover new direct-acting antivirals (DAA) to effectively and safely treat chronic HCV infection.[4]

Three first generation nucleoside prodrugs (NM283, R1626, and RG7128) have been reported to show anti-HCV activity as direct-acting antiviral agents in clinical trials.[5,6] Among them, only RG7128 is currently under development (phase IIb). RG7128 is a 3′,5′-diisobutyrate ester prodrug of β-D-2′-deoxy-2′-α-fluoro-2′-β-*C*-methylcytidine (PSI-6130) and has already established proof-of-concept in the clinic.[7,8] In a 28 days combination study with SOC, RG7128 demonstrated efficacy in genotype 1, 2, and 3 patients and was the first direct-acting antiviral to show broad genotype coverage in the clinic.[9]

As part of our continuing efforts to discover novel anti-HCV agents, we were interested in finding second generation agents with improved potency, better resistance profile, enhanced pharmacokinetic properties to support QD dosing, and the potential for generating high concentrations of the active triphosphate in the liver. In addition to the cytidine analogue RG7128, we have investigated derivatives of 2-deoxy-2-α-fluoro-2-β-*C*-methylribofuranose with other nucleic acid bases.[10−13] Among them, the guanosine analogue **1** was shown to be only weakly active (EC_{90} = 69.2 μM) in a HCV replicon assay, but its triphosphate **2** was found to be a potent inhibitor of the HCV NS5B polymerase (IC_{50} = 5.94 μM) (Figure 1).

Nucleoside analogues need to be phosphorylated to their corresponding triphosphate by host cellular kinases before they can bind to the HCV NS5B polymerase and inhibit RNA replication. However, many nucleoside analogues are poor substrates for nucleoside kinases that are responsible for their phosphorylation to their active triphosphate derivative.[14] The tri-/di-/monophosphate species cannot be considered as possible drug candidates because of their instability and poor cellular permeability.[15] Phosphoramidate prodrugs have been shown to enhance nucleoside potency in cell culture,

Received Date: September 10, 2010
Accepted Date: December 8, 2010
Published on Web Date: December 17, 2010

DOI: 10.1021/ml100209f | *ACS Med. Chem. Lett.* **2011**, *2*, 130–135

presumably by increasing the intracellular concentrations of the nucleoside monophosphate, thus bypassing a potentially nonproductive first phosphorylation step.[16−18] We therefore decided to investigate phosphoramidate derivatives of 2′-deoxy-2′-α-fluoro-2′-β-*C*-methylguanosine monophosphate.

The study of phosphoramidate prodrugs began with the investigation of the anti-HCV structure activity relationship at the 6-position of the purine base, keeping the substitution on the phosphoramidate moiety constant. A series of 6-(un)-modified guanosine analogues **6a**−**k** was synthesized starting from the previously reported[19] ribonolactol **3** by preparing the 6-chloro nucleoside **5** as a key intermediate (Scheme 1).[20] The phosphoramidate moiety was introduced onto each nucleoside by treatment with a phosphorochloridate reagent in the presence of *N*-methylimidazole.[21] Each purine phosphoramidate derivative was prepared as a mixture of diastereomers at phosphorus.

Anti-HCV activity in the clone A replicon whole cell assay of the ethyl ester series **8**−**18** is summarized in Table 1.[22−24] The 6-unsubstituted guanine phosphoramidate **8** showed low micromolar activity: approximately a 25-fold increase in potency over nucleoside **1**. On the other hand, a number of the phosphoramidates having 6-alkoxy, -alkylsulfane, and -alkylamine substituents (double prodrug) demonstrated more dramatic improvements in potency against HCV. Especially, 6-methoxyguanosine analogue **9** and 6-amino substituted

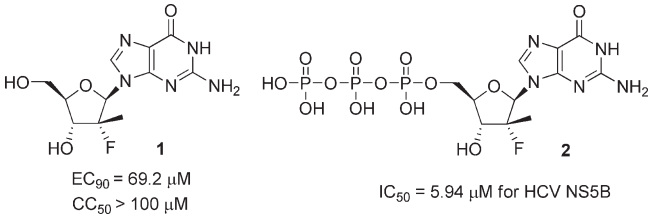


Figure 1. β-D-2′-Deoxy-2′-α-fluoro-2′-β-*C*-methylguanosine (**1**) and its anabolite triphosphate **2**.

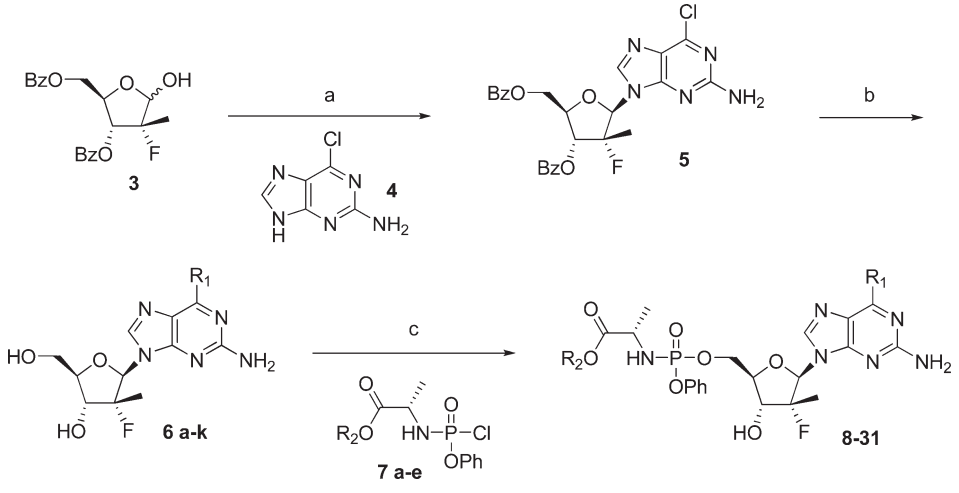
analogues **15** and **16** exhibited single digit nanomolar EC_{90} values, thus offering >5000-fold increased potency compared to the parent nucleoside **1**.[25]

Further SAR assessment focused on preferred C-6 substitutions on the base and small alkyl esters at the terminal carboxylate group of the phosphoramidate moiety (Table 2). A survey of substitutions at both the C-6 position of the base and the terminal ester showed that substituents that increased the overall lipophilicity of the molecule translated into increased cytotoxicity (data not shown). Consequently, we chose to minimize the overall lipophilicity and focus our efforts on the introduction of relatively small substituents both at C-6 of the base and at the terminal ester group. For the 6-unmodified guanosine series (**19**, **20**, and **21**), improved anti-HCV activity was observed when the terminal carboxylate ester substituent was either *i*Pr, cyclopentyl, or cyclohexyl, affording EC_{90} values of ≤0.75 μM. None of the 6-OH (natural guanosine) analogues showed cytotoxicity up to 100 μM against an expanded cell panel. Most of the 6-alkoxy analogues demonstrated a >1000-fold increase in potency compared to the guanosine analogue **1**. Among

Table 1. HCV Clone A Replicon Data

compd	R_1	R_2	EC_{90} (μM)
8	−OH	Et	2.38
9	−OMe	Et	0.0074
10	−OEt	Et	0.010
11	−OPr	Et	0.013
12	$-O(CH_2)_2OMe$	Et	0.020
13	$-O(CH_2)_2F$	Et	0.027
14	−SEt	Et	0.047
15	$-N(CH_2)_5$	Et	0.0070
16	−NH(*c*Pr)	Et	0.0098
17	−NH(*c*Bu)	Et	0.024
18	−NH(*c*Pentyl)	Et	0.026

Scheme 1. General Synthetic Pathway for the Phosphoramidates of Guanosine Analogues[a]



[a] Reagents and conditions: (a) **4**, DEAD, PPh_3, THF, rt, 16 h. (b) for **6a**, 2-mercaptoethanol, NaOMe, MeOH, Δ; for **6b** to **6g**, ROH, NaH or K_2CO_3, DME, Δ; for **6h**−**k**, NHR′R″, MeOH or EtOH, Δ. (c) **7**, NMI, THF, 0 °C to rt.

DOI: 10.1021/ml100209f | *ACS Med. Chem. Lett.* **2011**, *2*, 130–135

Table 2. Anti-HCV Activity and Cytotoxicity

			HCV inhibition EC_{90} (μM)		cytotoxicity CC_{50} (μM)			
compd	R_1	R_2	WT	S282T	Huh7	BxBC3	HepG2	CEM
8	−OH	Et	2.38		> 100	> 100	> 100	> 100
19	−OH	*i*Pr	0.75	0.61	> 100	> 100	> 100	> 100
20	−OH	*c*Pentyl	0.18	0.12	> 100	> 100	> 100	> 100
21	−OH	*c*Hexyl	0.446		> 100	> 100	> 100	> 100
22	−OMe	Me	0.0081		> 100	65	71	20
9	−OMe	Et	0.0074	0.011	> 100	67	62	55
23	−OMe	*i*Pr	0.020	0.024	> 100	> 100	> 100	> 100
24	−OEt	Me	0.016	0.016	> 100	> 100	> 100	74
10	−OEt	Et	0.010		47	68	61	37
25	−OEt	*i*Pr	0.020		59	> 100	> 100	93
26	−OPr	Me	0.0146		39	39	45	17
27	−OPr	*i*Pr	0.033		49	> 100	> 100	44
28	$-N(CH_2)_3$	Me	0.090	0.04	> 100	> 100	> 100	75
15	$-N(CH_2)_3$	Et	0.0070		84	92	75	39
29	$-N(CH_2)_3$	*i*Pr	0.019		53	74	100	74
30	−NH(*c*Pr)	Me	0.0082		> 100	> 100	> 100	34
16	−NH(*c*Pr)	Et	0.0098		> 100	> 100	> 100	45
31	−NH(*c*Pr)	*i*Pr	0.0088		96	98	> 100	> 100

the 6-alkoxy derivatives, isopropyl ester **23** and methyl ester **24** showed no significant cytotoxicity. Alkylamine substitution at the 6-position of the base also produced derivatives with potent anti-HCV activity. Of the alkylamine derivatives, the 6-*N*-azetidine **28** gave the best cytotoxicity profile relative to the other phosphoramidates in the alkylamine series. Several analogues (**9**, **19**, **20**, **23**, **24**, and **28**) were tested further against the known nucleoside resistant mutant, S282T, which has demonstrated reduced sensitivity to the 2′-methyl containing nucleoside analogues RG7128 and NM283.[26] It is noteworthy that all of these purine nucleosides were shown to be equipotent against both the wild type and the S282T mutant replicon (Table 2). Based on anti-HCV replicon potency, cytotoxicity profile, and structural diversity, a set of four guanosine phosphoramidate analogues, **20**, **23**, **24**, and **28**, were selected for further study.

Since some compounds previously reported to inhibit the HCV replicon also inhibited cell growth[27] and exhibited mitochondrial toxicity,[28] compounds **20**, **23**, **24**, and **28** were evaluated for effects on cell growth and for mitochondrial toxicity. When evaluated for their cytostatic potential, compounds **20**, **23**, and **28** showed no significant effect on cell doubling time up to 100 μM; however, the 6-ethoxy ether analogue **24** was shown to retard cell growth by 5 h at 50 μM. When mitochondrial toxicity (CC_{90}) was evaluated by incubating CEM and HepG2 cells with compounds **20**, **23**, and **28** for 14 days and then measuring the levels of mitochondrial COXII DNA (mtDNA) and rDNA (rDNA) using real time PCR, all three compounds showed no measurable mitochondrial toxicity up to 100 μM in both cell lines.

To assess the ability of prodrugs **20**, **23**, and **28** to survive exposure in the gastrointestinal tract and preferentially deliver the nucleotide monophosphate to the liver, stability in simulated gastrointestinal fluid (SGF), simulated intestinal fluid (SIF), human plasma, and human liver S9 fraction, a surrogate *in vitro* model for liver stability, was studied. As shown in Table 3, the derivatives with bulky ester groups, **20** and **23**, provided better stability in human plasma as well as in SGF and SIF when compared to the methyl ester analogue **28**. Each compound exhibited short half-lives in liver S9 fraction, indicating that each phosphoramidate had the potential for rapid conversion to the desired nucleotides in the liver.

Table 3. Stability of Key Compounds **20**, **23**, and **28**

	stability, $t_{1/2}$ (h)			
compd	SGF	SIF	human plasma	S9
20	15	> 20	> 24	0.2
23	14	> 20	> 24	0.2
28	> 20	> 20	11.6	0.4

Since triphosphate levels are correlated to the *in vivo* potency of nucleos(t)ide analogues,[29] *in vitro* triphosphate production of the remaining compounds **20**, **23**, and **28** in primary human hepatocytes was assessed and compared to that produced in the replicon clone A cells (Table 4). *In vitro* analysis of triphosphate **2** production was accomplished by incubating each compound and then extracting triphosphate **2** from the cells and quantifying by HPLC analysis. Of the three compounds studied, compound **23** produced the highest level of triphosphate in primary human hepatocytes. Triphosphate levels for each of the compounds in clone A cells correlated well with anti-HCV replicon potency. Therefore, one might anticipate that triphosphate levels observed in primary human hepatocytes *in vitro* provide an indication of anti-HCV potency in human hepatocytes *in vivo*.

DOI: 10.1021/ml100209f | *ACS Med. Chem. Lett.* **2011**, *2*, 130–135

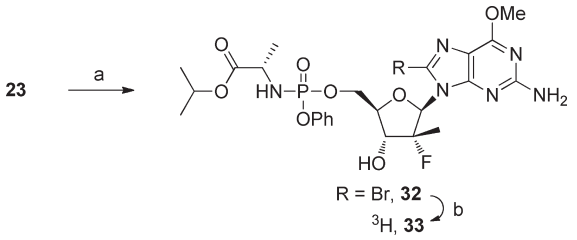
Since **23** was developed as a liver targeted nucleotide prodrug, the relative liver to plasma exposure and the production of active triphosphate **2** in the liver was evaluated when **23** was administered *in vivo*. To accomplish this, tritiated phosphoramidate nucleotide **33** was prepared by first bromination of **23** at C-8 of the purine base followed by reductive tritiation (Scheme 2). The tritiated derivative **33** was administered orally to rats (60 μCi total dose in peanut oil), and plasma samples were collected at 1 and 6 h after dosing. In addition, after administration of tritiated derivative **33**, livers were removed at 1 and 6 h postdosing and levels of triphosphate and key metabolites were determined from the liver extracts. Parent prodrug and metabolites were measured in plasma and liver extracts by HPLC using radiodetection. As has been described for other nucleotide phosphoramidates,[16] metabolism of phosphoramidate **23** proceeds first to the diacid intermediate **34** and then to the monophosphate **35**, to the diphosphate **36**, and finally to the active triphosphate **2** (Scheme 3). With radiolabeled **33**, we were able to measure the levels of each of these productive metabolites in both the liver and plasma (Table 5). In plasma after 1 h, each of the diastereomers of **23** is evident, as is the diacid **34** and the guanosine nucleoside **1**. Nucleoside **1** presumably arises from decomposition of the monophosphate. At the 6 h time point, the major metabolite in plasma is the nucleoside **1**, and very little of the parent prodrugs or intermediate metabolite **34** is present. In the liver at 1 h, the mono-, di-, and triphosphates are observed in addition to the intermediate metabolite **34** and the nucleoside **1**. At 6 h postdose, liver extracts still show the presence of the mono-, di-, and triphosphates and intermediate metabolite **34** in addition to large amounts of **1**. To determine the liver to plasma ratio, the total metabolites observed in the liver were compared to the total metabolites observed in plasma (Table 5). The liver to plasma ratio was determined to be 3.5/1 and 4.8/1 at 1 and 6 h postdose, respectively, thus supporting the liver targeting properties of **23**. In addition, the presence of diacid **34**, mono-, di-, and triphosphate in the liver indicates that **23** is able to reach the liver and achieve conversion to the active triphosphate as designed.

Table 4. Cellular Triphosphate Levels of Key Compounds **20**, **23**, and **28**

	cellular TP levels[a] (mM)	
compd	clone A (at 48 h)	primary human hepatocytes (at 24 h)
20	0.06	0.04
23	0.12	0.12
28	0.16	0.05

[a] Incubated at 100 μM compound concentration.

Scheme 2. Synthesis of Radiolabeled Prodrug[a]

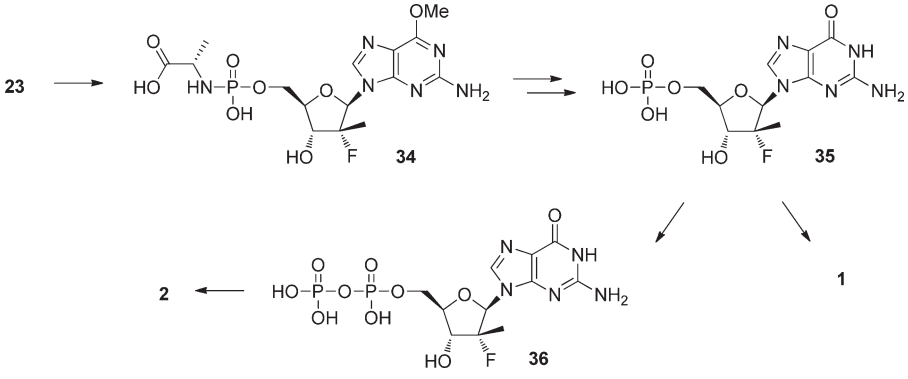


[a] Reagents and conditions: (a) NBS, dioxane, rt, 5 h. (b) $^3H_2$, 10% Pd/C, Et_3N, MeOH, rt, 1 h.

As a result of its strong *in vitro* potency against HCV, good metabolic stability, very clean *in vitro* toxicity profile, and high liver to plasma ratio in a rat *in vivo* study, the 6-methoxyguanosine analogue **23** was chosen for further study. Phosphoramidate **23** is a mixture of diastereomers (**23a**:**23b**, ~1:1.8 ratio) at the phosphorus center.[30] The diastereomers of **23** were separated by simulated moving bed chromatography to provide the two pure isomers **23a** and **23b**. The anti-HCV activity of each diastereomer against the wild type clone A replicon and the S282T mutant replicon was determined, and diastereomer **23b** was shown to be 3.4-fold more potent than diastereomer **23a** and again retained activity in the mutant replicon (Table 6). The more potent diastereomer **23b** also produced about 3-fold higher concentrations of triphosphate in both clone A and primary human hepatocytes than did **23a**, consistent with its *in vitro* potency in clone A cells.

Pure **23b** was crystallized in dichloromethane and hexane solution, and a single crystal X-ray structure of **23b** was obtained unambiguously, confirming the configuration of the phosphorus center as Sp (see Supporting Information). Subsequently, it was demonstrated that diastereomer **23b** could be selectively crystallized directly from the diastereomeric mixture without prior chromatographic separation. Based on its superior overall potency and safety profile,

Scheme 3. Activation Pathway for Prodrug **23**



DOI: 10.1021/ml100209f | *ACS Med. Chem. Lett.* **2011**, *2*, 130–135

Table 5. Radio Counts of Tritiated **23** (**33**) and Metabolites in Rat Plasma and Liver Samples

			peak area[b] (cpm)							
rat samples[a]		sample (volume or weight)	**1**	**34**	**35**	**36**	**2**	**23a**	**23b**	total (cpm/mL or cpm/g)
plasma	1 h	1.33 mL	1376	920	ND	ND	ND	1160	1112	3426
	6 h	1.00 mL	3848	288	ND	ND	ND	272	368	4776
liver	1 h	0.75 g	4224	1648	1828	988	384	ND	ND	12096
	6 h	0.70 g	11512	572	1920	1620	460	ND	ND	22977

[a] Dose: 60 μCi total dose in 2.9 mL of peanut oil. [b] ND = not detected.

Table 6. Comparison of the Diastereomers **23a** and **23b**

	HCV inhibition EC_{90} (μM)		cellular TP levels[a] (mM)	
compd	WT	S282T	clone A (at 48 h)	primary human hepatocytes (at 24 h)
23a	0.027	0.038	0.07	0.14
23b	0.008	0.011	0.17	0.44

[a] Incubated at 25 μM compound concentration for clone A cells and 100 μM compound concentration for primary human hepatocytes, respectively.

compound **23b** (PSI-353661) was selected as a clinical development candidate.

In conclusion, a series of β-D-2′-deoxy-2′-α-fluoro-2′-β-*C*-methylguanosine phosphoramidate analogues were prepared and their SAR as inhibitors of HCV replication was studied. Most of the prodrugs showed superior anti-HCV potency relative to the parent guanosine analogue **1**, often by more than 1000-fold. After a comprehensive evaluation that included cytotoxicity, resistance profile, cytostatic activity, metabolic stability, and cellular triphosphate production, the single diastereomer **23b** (PSI-353661) was chosen as a preclinical development candidate for the treatment of HCV infection. PSI-353661 exhibited strong inhibition of HCV in the cell-based replicon assay, equipotent activity against both the wild type and S282T resistant replicons, and ability to produce high concentrations of the active triphosphate in primary human hepatocytes.

SUPPORTING INFORMATION AVAILABLE

Experimental procedures and analytical data for all new compounds. X-ray structure and cif data for **23b**. HPLC radiochromatograms and triphosphate measurement of active prodrugs. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author: *Telephone: 609-613-4123. Fax: 609-613-4150. E-mail: michael.sofia@pharmasset.com.

ACKNOWLEDGMENT

We thank Dr. Patrick Carroll, University of Pennsylvania for X-ray analysis, and WuXi AppTec and Moravek Biochemicals for their contribution to the work described in this manuscript.

REFERENCES

(1) Lavanchy, D. The global burden of hepatitis C. *Liver Int.* **2009,** *29* (S1), 74–81.

(2) Zeuzem, S.; Berg, T.; Moeller, B.; Hinrichsen, H.; Mauss, S.; Wedemeyer, H.; Sarrazin, C.; Hueppe, D.; Zehnter, E.; Manns, M. P. Expert opinion on the treatment of patients with chronic hepatitis C. *J. Viral Hepatitis* **2009,** *16*, 75–90.

(3) Neukam, K.; Macias, J.; Mira, J. A.; Pineda, J. A. A review of current anti-HCV treatment regimens and possible future strategies. *Expert Opin. Pharmacother.* **2009,** *10*, 417–433.

(4) Schinazi, R. F.; Bassit, L.; Gavegnano, C. HCV drug discovery aimed at viral eradication. *J. Viral Hepatitis* **2010,** *17*, 77–90.

(5) Furman, P. A.; Lam, A. M.; Murakami, E. Nucleoside analogue inhibitors of hepatitis C viral replication: recent advances, challenges and trends. *Future Med. Chem.* **2009,** *1*, 1429–1452.

(6) Carroll, S. S.; La Femina, R. L. Nucleoside analogue inhibitors of hepatitis C viral replication. In *Antiviral Research*; La Femina, R. L., Ed.; American Society for Microbiology: Washington, DC, 2009; pp153−166.

(7) Stuyver, L. J.; McBrayer, T. R.; Tharnish, P. M.; Clark, J. L.; Hollecher, L.; Lostia, S.; Nachman, T.; Grier, J.; Bennett, M. A.; Xie, M.-Y.; Schinazi, R. F.; Morrey, J. D.; Julander, J. L.; Furman, P. A.; Otto, M. J. Inhibition of hepatitis C replicon RNA synthesis by β-D-2′-deoxy-2′-fluoro-2′-C-methylcytidine: a specific inhibitor of hepatitis C virus replication. *Antiviral Chem. Chemother.* **2006,** *17*, 79–87.

(8) Murakami, E.; Niu, C.; Bao, H.; Steuer, H. M.; Whitaker, T.; Nachman, T.; Sofia, M. A.; Wang, P.; Otto, M. J.; Furman, P. A. The mechanism of action of β-D-2′-deoxy-2′-fluoro-2′-*C*-methylcytidine involves a second metabolic pathway leading to β-D-2′-deoxy-2′-fluoro-2′-*C*-methyluridine 5′-triphosphate, a potent inhibitor of the hepatitis C virus RNA-dependent RNA polymerase. *Antimicrob. Agents Chemother.* **2008,** *52*, 458–464.

(9) Le Pogam, S.; Kang, H.; Seshaadri, A.; Kosaka, A.; Hu, S.; Ewing, A.; Yan, J.-M.; Beard, A.; Symons, J.; Cammack, N.; Nájera, I. No evidence of R7128 drug resistance after up to 4 weeks treatment of GT 1, 2 and 3 hepatitis C virus infected individuals. *44th Annual Meeting of the European Association for the Study of the Liver*; Copenhagen, Denmark, April, 2009; Abstr. No. 839.

(10) Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R. F.; Watanabe, K. A.; Otto, M. J.; Furman, P. A.; Stec, W. J.; Patterson, S. E.; Pankiewicz, K. W. Design, synthesis, and antiviral activity of

DOI: 10.1021/ml100209f | *ACS Med. Chem. Lett.* **2011**, *2*, 130–135

2′-deoxy-2′-fluoro-2′-C-methyl-cytidine, a potent inhibitor of hepatitis C virus replication. *J. Med. Chem.* **2005,** *48*, 5504–5508.

(11) Clark, J. L.; Mason, J. C.; Hollecker, L.; Stuyver, L. J.; Tharnish, P. M.; McBrayer, T. R.; Otto, M. J.; Furman, P. A.; Schinazi, R. F.; Watanabe, K. A. Synthesis and antiviral activity of 2′-deoxy-2′-fluoro-2′-*C*-methyl purine nucleosides as inhibitors of hepatitis C virus RNA replication. *Bioorg. Med. Chem. Lett.* **2006,** *16*, 1712–1715.

(12) Wang, P.; Sofia, M. J.; Chun, B.-K.; Du, J.; Rachakonda, S.; Steuer, H. M.; Murakami, E.; Bao, H.; Nagarathnam, D.; Otto, M. J.; Furman, P. A. D-2′-Deoxy-2′-fluoro-2′-*C*-methyl-ribofuranosyl nucleosides: Effect of base modifications on antihepatitis C virus activity. 238th ACS National Meeting, Washington, DC, August, 2009; MEDI 100.

(13) Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H.-R.; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Micolochick Steuer, H. M.; Niu, C.; Otto, M. J.; Furman, P. A. Discovery of a β-D-2′-deoxy-2′-α-fluoro-2′-β-*C*-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J. Med. Chem.* **2010,** *53*, 7202–7218.

(14) Oeberg, Bo. Rational design of polymerase inhibitors as antiviral drugs. *Antiviral Res.* **2006,** *71*, 90–95.

(15) Hecker, S. J.; Erion, M. D. Prodrugs of phosphates and phosphonates. *J. Med. Chem.* **2008,** *51*, 2328–2345.

(16) Mehellou, Y.; Balzarini, J.; McGuigan, C. Aryloxy phosphoramidate triesters: a technology for delivering monophosphorylated nucleosides and sugars into cells. *ChemMedChem* **2009,** *4*, 1779–1791.

(17) McGuigan, C.; Madela, K.; Aljarah, M.; Gilles, A.; Brancale, A.; Zonta, N.; Chamberlain, S.; Vernachio, J.; Hutchins, J.; Hall, A.; Ames, B.; Gorovits, E.; Ganguly, B.; Kolykhalov, A.; Wang, J.; Muhammad, J.; Patti, J. M.; Henson, G. Design, synthesis and evaluation of a novel double pro-drug: INX-08189. A new clinical candidate for hepatitis C virus. *Bioorg. Med. Chem. Lett.* **2010,** *20*, 4850–4854.

(18) McGuigan, C.; Gilles, A.; Madela, K.; Aljarah, M.; Holl, S.; Jones, S.; Vernachio, J.; Hutchins, J.; Ames, B.; Bryant, K. D.; Gorovits, E.; Ganguly, B.; Hunley, D.; Hall, A.; Kolykhalov, A.; Liu, Y.; Muhammad, J.; Raja, N.; Walters, R.; Wang, J.; Chamberlain, S.; Henson, G. Phosphoramidate ProTides of 2′-C-methylguanosine as highly potent inhibitors of hepatitis C virus. study of their *in vitro* and *in vivo* properties. *J. Med. Chem.* **2010,** *53*, 4949–4957.

(19) Wang, P.; Chun, B.-K.; Rachakonda, S.; Du, J.; Khan, N.; Shi, J.; Stec, W.; Cleary, D.; Ross, B. S.; Sofia, M. J. An efficient and diastereoselective synthesis of PSI-6130: a clinically efficacious inhibitor of HCV NS5B polymerase. *J. Org. Chem.* **2009,** *74*, 6819–6824.

(20) The improved synthesis of the intermediate **5** will be published elsewhere.

(21) McGuigan, C.; Hassan-Abdallah, A.; Srinivasan, S.; Wang, Y.; Siddiqui, A.; Daluge, S. M.; Gudmundsson, K. S.; Zhou, H.; McLean, E. W.; Peckham, J. P.; Burnette, T. C.; Marr, H.; Hazen, R.; Condreay, L. D.; Johnson, L.; Balzarini, J. Application of phosphoramidate ProTide technology significantly improves the antiviral potency of carbocyclic adenosine derivatives. *J. Med. Chem.* **2006,** *49*, 7215–7226.

(22) In addition to L-alanine, other amino acid derivatives were also prepared and studied (no data shown). However, the phosphoramidates of L-alanine were strongly preferred to the others in terms of anti-HCV potency *in vitro*.

(23) For previously reported 6-modified guanine nucleotide analogues with anti-HCV activity, see:Gunic, E.; Girardet, J.-L.; Ramasamy, K.; Stoisavljevic-Petkov, V.; Chow, S.; Yeh, L.-T.; Hamatake, R. K.; Raney, A.; Hong, Z. Cyclic monophosphate prodrugs of base-modified 2′-*C*-methyl ribonucleosides as potent inhibitors of hepatitis C virus RNA replication. *Bioorg. Med. Chem. Lett.* **2007,** *17*, 2452–2455.

(24) Lambe, C. U.; Averett, D. R.; Paff, M. T.; Reardon, J. E.; Wilson, J. G.; Krenitsky, T. A. 2-Amino-6-methoxypurine arabinoside: an agent for T-cell malignancies. *Cancer Res.* **1995,** *55*, 3352–3356.

(25) The 5′-triphosphate of **6b** (6-methoxyguanosine analogue) was synthesized according to the reported procedure: Ludwig, J. A new route to nucleoside 5′-triphosphates. *Acta Biochim. Biophys. Acad. Sci. Hung.* **1981,** *16*, 131–133. We found that the **6b-TP** did not significantly inhibit the HCV NS5B polymerase (IC_{50} > 100 μM), indicating the represented compounds are double prodrugs.

(26) Sarrazin, C.; Zeuzem, S. Resistance to direct antiviral agents in patients with hepatitis C virus infection. *Gastroenterology* **2010,** *138*, 447–462.

(27) Hocek, M.; Naus, P.; Pohl, R.; Votruba, I.; Furman, P. A.; Tharnish, P. M.; Otto, M. J. Cytostatic 6-arylpurine nucleosides. 6. SAR in anti-HCV and cytostatic activity of extended series of 6-hetarylpurine ribonucleosides. *J. Med. Chem.* **2005,** *48*, 5869–5873.

(28) Fleischer, R.; Boxwell, D.; Sherman, K. E. Nucleoside analogues and mitochondrial toxicity. *Clin. Infect. Dis.* **2004,** *38*, e79–80.

(29) Ma, H.; Jiang, W.-R.; Robledo, N.; Leveque, V.; Ali, S.; Lara-Jaime, T.; Masjedizadeh, M.; Smith, D. B.; Cammack, N.; Klumpp, K.; Symons, J. Characterization of the metabolic activation of hepatitis C virus nucleoside inhibitor β-D-2′-Deoxy-2′-fluoro-2′-C-methylcytidine (PSI-6130) and identification of a novel active 5′-triphosphate species. *J. Biol. Chem.* **2007,** *282*, 29812–29820.

(30) When compound **23** was prepared by the method described in the Supporting Information. The ratio varies depending on the reaction conditions. Selective synthetic preparation of **23b** will be reported elsewhere in the near future.

DOI: 10.1021/ml100209f | *ACS Med. Chem. Lett.* **2011**, *2*, 130–135