Discovery of 2'- α -C-Methyl-2'- β -C-fluorouridine Phosphoramidate Prodrugs as Inhibitors of Hepatitis C Virus

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S Supporting Information

ABSTRACT: 2'- α -C-Methyl-2'- β -C-fluorouridine and its phosphoramidate prodrugs were synthesized and evaluated for their inhibitory activity against HCV. The structure–activity relationship analysis of the phosphoramidate moiety found that **17m**, **17q**, and **17r** exhibit potent activities against HCV, with EC₅₀ values of 1.82 ± 0.19 , 0.88 ± 0.12 , and 2.24 ± 0.22 μ M, respectively. The docking study revealed that the recognition of the 2'- β -F by Arg158, 3'-OH by N291, and the Watson–Crick pairing with the template allowed **23** to form the in-line conformation necessary for its incorporation into the viral RNA chain.

KEYWORDS: hepatitis C virus, phosphoramidate prodrugs, docking, inhibitor, Hint 1

Hepatitis C virus (HCV) is a small, single-stranded positive-sense RNA virus that was explicitly identified in 1989.¹ The 9.6 kb RNA genome shares similarities with the genomes of flaviviruses and pestiviruses.² HCV infection is a global health problem that impacts approximately 180 million individuals, of which approximately 150 million people may proceed to develop chronic liver disease. A significant percentage of patients may ultimately progress to liver cirrhosis and eventually develop hepatocellular carcinoma.³ HCV infection is also the primary reason for liver transplants among adults. The therapy for HCV infection has long been regular injections of pegylated α -interferon with the daily oral administration of ribavirin (RBV).⁴ This standard of care (SOC) functions by enhancing the host immune system rather than acting directly on the virus. However, HCV patients undergoing this therapy may also suffer significant adverse effects, including fatigue, hemolytic anemia, depression, and flu-like symptoms, which are poorly tolerated. In 2011, boceprevir and telaprevir, as HCV protease inhibitors, became available to treat HCV infection with genotype 1 in combination with ribavirin and pegylated interferon.⁵ In 2013, two new direct-acting antivirals (DAAs), simeprevir and sofosbuvir, were approved by the FDA, which significantly enhanced the available armory.⁶ However, the high cost for a course of treatment with sofosbuvir creates a considerable economic burden for patients.⁷ Hence, the search for novel DAAs that are safe and effective remains a necessary endeavor.

The HCV NSSB RNA-dependent RNA polymerase is a key enzyme in the replication of the virus. Therefore, it has become an attractive target for the development of small molecule inhibitors of viral replication. Nucleoside and nucleotide inhibitors of HCV (NIs) are a class of DAAs that show broad activity across HCV genotypes and a high barrier to the

emergence of viral resistance.⁸ Structural modifications have been made to both the base and ribose sugar portions of a ribonucleoside to develop potent and selective anti-HCV inhibitors.⁹ Structural modifications of the ribose sugar portion focused primarily on the 1'-, 2'-, and 4'-positions. Structural modifications of the 2'-position mainly consisted of introducing an alkyl or including a methyl at the β -position and the substitution of 2'-OH, for instance, NM107, EC₅₀ = 1.23 μ M, PSI-6130, EC₅₀ = 4.5 μ M, PSI-7977 (sofosbuvir), EC₉₀ = 0.42 μ M (Figure 1).^{10–12} Additionally, some NIs containing 2'-

Figure 1. Nucleoside/nucleotide inhibitors of HCV.

spirocycloalkyl or 2'-spirocyclic ether groups showed anti-HCV activities. 2'-Deoxy-2'-spirocyclopropylcytidine (**1**, EC₅₀ = 7.3 μ M, Figure 1) was discovered as a new member of the class of 2'-modified nucleoside derivatives and showed potent anti-HCV activity.¹³ A 2'-spiroetheruridine analogue (**2**, EC₅₀ =

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14.9 μM , Figure 1) and its phosphoramidate prodrug (3, EC_{50} = 20.6 μM , Figure 1) exhibited medium activity against HCV.¹⁴ This may suggest that the 2'- α -C-alkyl in nucleosides/nucleotides might be tolerated and that the 2'- α -OH or 2'- α -F may not be essential for nucleosides/nucleotides to exhibit anti-HCV activity. Sofosbuvir, with the modification of 2'- β -C-CH₃-2'- α -C-F, shows potent activity against HCV, with few adverse effects. Inspired by 2'-spiro nucleosides/nucleotides and sofosbuvir, 2'- α -C-CH₃-2'- β -C-F uridine, and its phosphoramidate prodrugs were synthesized and evaluated to determine their inhibitory activity against HCV and investigate the impact of stereochemistry at the 2'-position on the anti-HCV activity.

The synthesis of intermediate 2'- α -C-CH₃-2'- β -C-F uridine 13 was illustrated in Scheme 1. D-Ribose was treated with

Scheme 1. Preparation of 13^a

^aReagents and conditions: (a) H₂SO₄, MeOH, r.t., 24 h; (b) 2,4-dichlorobenzyl chloride (DCBCl), NaH, DMF, 80 °C, 3 h, 60% for two steps; (c) SnCl₄, DCM, r.t., 3 h, 85%; (d) 2,2,6,6-tetramethylpiperidinoxy, trichloroisocyanuric acid, DCM, r.t., 3 h, 90%; (e) CH₃MgCl, THF, -20 °C, 4 h, 85%; (f) BzCl, Et₃N, DMAP, DCM, r.t., 2 h, 88%; (g) uracil, *N,O*-bis(trimethylsilyl)acetamide, SnCl₄, CH₃CN, 50 °C, 4 h, 88%; (h) LiOH, CH₃OH, r.t., 4 h, 95%; (i) diethylaminosulfur trifluoride, DCM, -40 °C, 2 h, 75%; (j) BCl₃, DCM, -78 °C to -30 °C, 2 h, 85%.

concentrated sulfuric acid in methanol to obtain 1-*O*-methyl-D-ribofuranose 4. Then, the hydroxyl groups of 4 were protected by 2,4-dichlorobenzyl (DCB) groups to obtain 5, followed by the selective removal of the 2-*O*-DCB group in the presence of tin(IV) tetrachloride to obtain the alcohol 6. Ketone 7, obtained by the oxidation of the 2-OH of alcohol 6, was treated with methylmagnesium chloride to obtain 8, followed by the protection of the 2-OH with benzoyl chloride to obtain 9. The treatment of the resulting intermediate with a silylated base and tin(IV) tetrachloride gave the uridine derivative 10. Then, the benzoyl group was removed to obtain alcohol 11, followed by the fluorination of the 2'-OH to obtain the 2'- α -C-CH₃-2'- β -C-F uridine derivative 12. Finally, the DCB groups were removed to obtain the desired 2'- α -C-CH₃-2'- β -C-F uridine 13.

The anti-HCV activity of 13 was evaluated by an in vitro HCV replicon cell system,¹⁵ and the results were expressed as EC_{50} values for antiviral activity and CC_{50} values for cytotoxicity. Uridine 13 presented medium anti-HCV activity, with an EC_{50} value of 14.65 $\pm$ 1.21 μM (Table 1). It was very interesting to find that the reverse stereochemistry of the 2'-carbon on the ribonucleoside compared to sofosbuvir was still active against HCV.

Table 1. Structures of Phosphoramidate Prodrugs 17a–17i and in Vitro Anti-HCV Activities and Cytotoxicities

Cmpd No.	R ¹	EC ₅₀ ^a (μM)	CC ₅₀ ^a (μM)
sofosbuvir		0.12 $\pm$ 0.02	>100
13	-	14.65 $\pm$ 1.21	>100
17a	isopropyl	> 50	>100
17b	sec-butyl	> 50	>100
17c	tert-butyl	> 50	>100
17d	2-methylbutyl	34.21 $\pm$ 1.88	>100
17e	3-methylbutyl	> 50	>100
17f	2,2-dimethylbutyl	> 50	>100
17g	3,3-dimethylbutyl	> 50	>100
17h	1-cyclohexylethyl	> 50	>100
17i	1-phenylethyl	> 50	>100

^aMeasurements of in vitro activity and cytotoxicity were performed in triplicate and represent the mean $\pm$ SD of at least three experiment sets.

Generally, nucleoside inhibitors of antiviral were believed to work as a chain terminator and needed to be converted intracellularly to their 5'-triphosphate derivatives to be active inhibitors of viral polymerases. So a series of phosphoramidate prodrugs based on 2'- α -C-CH₃-2'- β -C-F uridine 13 were synthesized. As illustrated in Scheme 2, amino acid 14 was

Scheme 2. Preparation of Phosphoramidate Prodrugs^a

^aReagents and conditions: (a) SOCl₂, alcohol, reflux, 2–24 h, 75–95%; (b) phenyl dichlorophosphate, Et₃N, 2,3,4,5,6-pentafluorophenol, DCM -78 °C to rt, 4 h, 60–85%; (c) *t*-BuMgCl, THF, 0 °C, 12 h, 28–35%.

reacted with an alcohol and thionyl chloride to obtain the amino acid ester hydrochloride 15, which reacted with phenyl dichlorophosphate and 2,3,4,5,6-pentafluorophenol under basic conditions to obtain phosphoramidate 16. The reaction of intermediates 13 and 16 under basic conditions gave the desired phosphoramidate prodrugs 17.

To explore the SAR of R¹, L-alanine was chosen as the amino acid, and various phosphoramidate prodrugs containing different ester groups were synthesized and evaluated (Table 1). With sofosbuvir (EC_{50} = 0.12 $\pm$ 0.02 μM) as the control, the results showed that phosphoramidate prodrugs presented

decreased activities compared to 13. The phosphoramidate prodrugs all exhibited weak activities ($EC_{50} > 50 \mu M$), except that 17d showed medium activity, with an EC_{50} value of $34.21 \pm 1.88 \mu M$. Nevertheless, low toxicity ($CC_{50} > 100 \mu M$) was observed for all phosphoramidate prodrugs. The isopropyl group of 17d as the fixed R^1 moiety for phosphoramidate prodrugs was investigated in the following study.

To explore the structure–activity relationship (SAR) of R^2 , various phosphoramidate prodrugs containing different amino acids with different stereochemistries were synthesized and evaluated (Table 2). The results showed that most amino acid

Table 2. Structures of Phosphoramidate Prodrugs 17j–17s and in Vitro Anti-HCV Activities and Cytotoxicities

Cmpd No.	R^{2a}	EC_{50}^b (μM)	CC_{50}^b (μM)
17j	H	22.88 ± 1.65	>100
17d	(L) Me	34.21 ± 1.70	>100
17k	(D) Me	34.34 ± 1.85	>100
17l	(L) Me_2CH	>50	>100
17m	(D) Me_2CH	1.82 ± 0.19	>100
17n	(L) $PhCH_2$	33.33 ± 1.54	>100
17o	(D) $PhCH_2$	35.34 ± 1.85	>100
17p	(L) indole-3- CH_2	>50	>100
17q	(D) indole-3- CH_2	0.88 ± 0.12	>100
17r	(L) $MeSCH_2CH_2$	2.24 ± 0.22	>100
17s	(D) $MeSCH_2CH_2$	>50	>100

^aL means from L-amino acid, D means from D-amino acid.

^bMeasurements of in vitro activity were performed in triplicate and represent the mean $\pm$ SD of at least three experiment sets.

side chains were accommodated. Phosphoramidate prodrugs with hydrogen, methyl, or benzyl groups at R^2 (17j, 17d, 17k, 17n, and 17o) exhibited comparable anti-HCV activities, with EC_{50} values of from 22.88 ± 1.65 to $35.34 \pm 1.85 \mu M$, indicating that the chirality of the α -carbon of the amino acid moieties has little effect on the anti-HCV activities of these phosphoramidate prodrugs. Interestingly, it was found that phosphoramidate prodrugs (17l and 17p) containing an L-valine or L-tryptophan moiety exhibited weak activities against HCV at the maximum testing concentration ($50 \mu M$). However, phosphoramidate prodrugs (17n and 17q) with a D-valine or D-tryptophan moiety displayed a significant improvement in activity, with EC_{50} values of 2.24 ± 0.22 and $0.88 \pm 0.12 \mu M$, respectively. Surprisingly, phosphoramidate prodrugs containing a methionine moiety preferred an L-amino acid moiety (EC_{50} values: $2.24 \pm 0.22 \mu M$ for 17r, $>50 \mu M$ for 17s). It was reported that sofosbuvir and its analogues containing a modification of 2'- β -C- CH_3 -2'- α -C-F preferred L-amino acid moieties,¹⁶ suggesting that the conformation inversion of the 2'-chiral carbon might influence the preference for the chirality of the α -carbon of the amino acid moieties. Favorable CC_{50} values ($CC_{50} > 100 \mu M$) were observed for all of the phosphoramidate prodrugs inhibitors in the in vitro cytotoxicity assay.

Phosphoramidate prodrugs cannot directly exhibit anti-HCV activities. According to the mechanism of sofosbuvir against HCV,¹² phosphoramidate prodrugs 17 must be metabolized to the active triphosphate form by a metabolic pathway that may

contain five steps (Figure 2A). The process from 17 to its corresponding 5'-monophosphate 21 involves two types of

Figure 2. Formation of NTP. (A) Metabolic pathway for nucleoside phosphoramidates 17. (B) Catalytic mechanism for Hint 1.

enzyme, human cathepsin A (CatA)/carboxylesterase 1 (CES1) and histidine triad nucleotide-binding protein 1 (Hint 1).¹² Compounds 17a–17c and 17e–17i displayed weak activities against HCV, probably because of the weak substrates for CatA/CES1. In 2007, Chou et al.¹⁷ reported that indole-containing nucleoside phosphoramidates could be the substrates for Hint 1, and the k_{cat} value reached as high as approximately $2.5 s^{-1}$ for uridine phosphoramidates. Moreover, it was found that Hint 1 was sensitive to the configurations at the α -carbon of the amino acid, preferring D- over L-tryptophan. This may be the reason why 17q was far more active against HCV than 17p. To provide insight into the interactions between the phosphoramidate prodrugs and Hint 1, a docking study was performed using Autodock software.

In Hint 1, His110 and His112 play important roles in the hydrolyzing the P–N bond of the phosphoramidate prodrugs according to the histidine triad (HIT) protein family¹⁸ (Figure 2B). The carbonyl of His110 formed a hydrogen bond with the imidazole of His112 to increase the basicity of the nitrogen atom, which attacked the phosphorus atom of the nucleoside phosphoramidates in the next step. The formation of a hydrogen bond between the NH of the phosphoramidates and Hint 1 was useful for the cleavage of the P–N bond. In the docking study, the corresponding metabolites 20 of the phosphoramidate prodrugs were docked into Hint 1. According to the docking models, 20j, 20d, 20k, 20n, and 20o interacted with Hint 1 in almost the same pattern (models not shown). The distance between the nitrogen atoms of His112 and the phosphorus atom was between 3.8 and 4.1 Å. Ser107 formed a hydrogen bond with the NH of the phosphoramidates. The interaction pattern of 20m and Hint 1 is illustrated in Figure 3. The distance between the nitrogen atom of His112 and phosphorus atom was 3.9 Å. The side chain of Asn99 interacted with the NH of phosphoramidate (3.3 Å), promoting the formation of monophosphate 21. Moreover, the oxygen atom of the P–O bonds formed hydrogen bonds with the side chain of His114 and the backbone of Ser107 (2.8 and 3.0 Å, respectively). Additionally, the backbone of Ile44 donated a

Figure 3. Docking models of **20l** (smudge) and **20m** (cyan) bound to Hint 1 (PDB code: 1KPF).

hydrogen bond to carbonyl of the base (3.0 Å). Compound **20l** bonded to Hint 1 in almost the same pattern as **20m**, except that no hydrogen bond interaction existed between the NH of **20l** and Hint 1 because of the chirality of the α carbon (Figure 3), which might be the reason why **20l** has a lower activity against HCV. The situations of **20p** and **20q** were similar to those of **20l** and **20m**. However, the chirality of the α carbon resulted in a great difference in the interactions between phosphoramidate prodrugs containing methionine and Hint 1 (Figure 4). The distance between the nitrogen atom of His112

Figure 4. Docking models of **20r** (cyan) and **20s** (yellow) bound to Hint 1 (PDB code: 1KPF).

and the phosphorus atom of **20r** was 3.4 Å, and the NH of the L-methionine moiety interacted with the side chain of Ser107 (3.3 Å). However, the distance between His112 and the phosphorus atom of **20s** containing the D-methionine moiety was as great as 6.8 Å, which resulted in a loss of activity against HCV.

In 2015, Appleby reported the crystal structure for HCV 2a NSSB triple mutant $\Delta 8$ elongation state assemblies with sofosbuvir and proposed a model of HCV replication by NSSB,¹⁹ which provided us with insight into the interactions between the nucleotide and NSSB polymerase. Herein, triphosphate 2'- α -C-CH₃-2'- β -C-F UTP **23**, the metabolite of phosphoramidate prodrugs **17**, was docked into NSSB polymerase using MOE software. Compound **23** and 2'- β -C-Me-2'- α -C-F UDP (**24**) were overlaid to show the differences

clearly (Figure 5A). It was reported that the recognition of the 2'-F by N291 and the Watson–Crick pairing with the template

Figure 5. Docking models of **23** (cyan) bound to NSSB and cocrystal structure of **24** (magenta)/NSSB (PDB code: 4WTG). (A) Compounds **23** and **24** were overlaid to show the differences. (B) Recognition of **23** by NSSB.

allow **24** to form the in-line conformation necessary for incorporation into the growing chain. The docking model of **23** with NSSB revealed that the 3'-OH of **23** moved close to the side chain of Asn291 and formed a hydrogen bond (2.5 Å), compared to **24**. Moreover, 2'- β -F was recognized by Arg158 (2.7 Å). Along with the Watson–Crick pairing with the template, **23** could be identified by NSSB polymerase. The conserved catalytic residues Asp220, Asp 318, and Asp 319 coordinated the two catalytic Mn²⁺ ions, which in turn coordinated the α and β phosphates of **23** (Figure 5B).

In summary, 2'- α -C-CH₃-2'- β -C-F uridine **13** and a series of phosphoramidate prodrugs **17** were synthesized, and their anti-HCV activities were evaluated. Phosphoramidates **17m**, **17q**, and **17r** exhibited potent activities against HCV, with EC₅₀ values of 1.82 ± 0.19 , 0.88 ± 0.12 , and 2.24 ± 0.22 μ M, respectively. The results revealed that conformation inversion of the 2'-chiral carbon might influence the preference for the chirality of the α -carbon of the amino acid moieties compared to sofosbuvir. Favorable CC₅₀ values (CC₅₀ > 100 μ M) were observed for all of the phosphoramidate inhibitors in the in vitro cytotoxicity assay. A docking study showed that the formation of a hydrogen bond between the NH of the phosphoramidates and Hint 1 had a significant impact on the cleavage of the P–N bond, thus influencing the activities of the phosphoramidate prodrugs against HCV. Finally, the triphosphate **23** of phosphoramidate prodrugs **17** docked into NSSB polymerase. This revealed that the recognition of the 2'- β -F by Arg158 and 3'-OH by N291 and the Watson–Crick pairing

with the template allowed **23** to form the in-line conformation necessary for incorporation into the viral RNA chain.

■ ASSOCIATED CONTENT

Supporting Information

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Full experimental details, in vitro antiviral activity and cytotoxicity assay (PDF)

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Author Contributions

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Notes

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■ REFERENCES

- (1) Choo, Q. L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* **1989**, *244*, 359–362.
- (2) Bartenschlager, R. Candidate targets for hepatitis C virus-specific antiviral therapy. *Intervirology* **1997**, *40*, 378–393.
- (3) Lavanchy, D. The global burden of hepatitis C. *Liver Int.* **2009**, *29*, 74–81.
- (4) Sofia, M. J. Nucleotide prodrugs for HCV therapy. *Antiviral Chem. Chemother.* **2011**, *22*, 23–49.
- (5) Poordad, F.; Dieterich, D. Treating hepatitis C: current standard of care and emerging direct-acting antiviral agents. *J. Viral Hepatitis* **2012**, *19*, 449–464.
- (6) Rehermann, B. Advances in hepatitis C research and treatment. *Nat. Rev. Gastroenterol. Hepatol.* **2016**, *13*, 70–71.
- (7) Keating, G. M. Sofosbuvir: a review of its use in patients with chronic hepatitis C. *Drugs* **2014**, *74*, 1127–1146.
- (8) Jensen, D. M.; Wedemeyer, H.; Herring, R. W.; Ferenci, P.; Ma, M. M.; Zeuzem, S.; Rodriguez-Torres, M.; Bzowej, N. H.; Pockros, P.; Vierling, J. M.; Ipe, D.; Hill, G. Z. High rates of early viral response, promising safety profile and lack of resistance-related breakthrough in HCV GT 1/4 patients treated with RG7128 plus PegIFN α -2a (40KD)/RBV: Planned Week 12 interim analysis from the Propel study. *Hepatology* **2010**, *52*, 360–361.
- (9) Sofia, M. J.; Chang, W.; Furman, P. A.; Mosley, R. T.; Ross, B. S. Nucleoside, nucleotide, and non-nucleoside inhibitors of hepatitis C virus NS5B RNA-dependent RNA-polymerase. *J. Med. Chem.* **2012**, *55*, 2481–2531.
- (10) Eldrup, A. B.; Allerson, C. R.; Bennett, C. F.; Bera, S.; Bhat, B.; Bhat, N.; Bosserman, M. R.; Brooks, J.; Burlein, C.; Carroll, S. S.; Cook, P. D.; Getty, K. L.; MacCoss, M.; McMasters, D. R.; Olsen, D. B.; Prakash, T. P.; Prhac, M.; Song, Q.; Tomassini, J. E.; Xia, J. Structure–activity relationship of purine ribonucleosides for inhibition

of hepatitis C virus RNA-dependent RNA polymerase. *J. Med. Chem.* **2004**, *47*, 2283–2295.

(11) 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 2'-deoxy-2'-fluoro-2'-C-methylcytidine, a potent inhibitor of hepatitis C virus replication. *J. Med. Chem.* **2005**, *48*, 5504–5508.

(12) Murakami, E.; Tolstykh, T.; Bao, H.; Niu, C.; Steuer, H. M. M.; Bao, D.; Chang, W.; Espiritu, C.; Bansal, S.; Lam, A. M.; Otto, M. J.; Sofia, M. J.; Furman, P. A. Mechanism of activation of PSI-7851 and its diastereoisomer PSI-7977. *J. Biol. Chem.* **2010**, *285*, 34337–34347.

(13) Jonckers, T. H. M.; Lin, T.-I.; Buyck, C.; Lachau-Durand, S.; Vandeyck, K.; Van Hoof, S.; Vandekerckhove, L. A. M.; Hu, L.; Berke, J. M.; Vijgen, L.; Dillen, L. L. A.; Cummings, M. D.; de Kock, H.; Nilsson, M.; Sund, C.; Rydegård, C.; Samuelsson, B.; Rosenquist, Å.; Fanning, G.; Van Emelen, K.; Simmen, K.; Raboisson, P. 2'-Deoxy-2'-spirocyclopropylcytidine revisited: a new and selective inhibitor of the hepatitis C virus NS5B polymerase. *J. Med. Chem.* **2010**, *53*, 8150–8160.

(14) Du, J.; Chun, B.-K.; Mosley, R. T.; Bansal, S.; Bao, H.; Espiritu, C.; Lam, A. M.; Murakami, E.; Niu, C.; Micolochick Steuer, H. M.; Furman, P. A.; Sofia, M. J. Use of 2'-spirocyclic ethers in HCV nucleoside design. *J. Med. Chem.* **2013**, *57*, 1826–1835.

(15) Cui, H.-K.; Qing, J.; Guo, Y.; Wang, Y.-J.; Cui, L.-J.; He, T.-H.; Zhang, L.; Liu, L. Stapled peptide-based membrane fusion inhibitors of hepatitis C virus. *Bioorg. Med. Chem.* **2013**, *21*, 3547–3554.

(16) 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.; Steuer, H. M. 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.

(17) Chou, T.-F.; Baraniak, J.; Kaczmarek, R.; Zhou, X.; Cheng, J.; Ghosh, B.; Wagner, C. R. Phosphoramidate pronucleotides: a comparison of the phosphoramidase substrate specificity of human and *Escherichia coli* histidine triad nucleotide binding proteins. *Mol. Pharmacol.* **2007**, *4*, 208–217.

(18) Lima, C. D.; Klein, M. G.; Hendrickson, W. A. Structure-based analysis of catalysis and substrate definition in the HIT protein family. *Science* **1997**, *278*, 286–290.

(19) Appleby, T. C.; Perry, J. K.; Murakami, E.; Barauskas, O.; Feng, J.; Cho, A.; Fox, D., III; Wetmore, D. R.; McGrath, M. E.; Ray, A. S.; Sofia, M. J.; Swaminathan, S.; Edwards, T. E. Structural basis for RNA replication by the hepatitis C virus polymerase. *Science* **2015**, *347*, 771–775.