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Article

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2'-Chloro,2'-Fluoro Ribonucleotide Prodrugs with Potent Pan-genotypic Activity against Hepatitis C Virus Replication in Culture

Shaoman Zhou,[1#] Sawsan Mahmoud,[1,2#] Peng, Liu,[1] Longhu Zhou,[1] Maryam Ehteshami,[1] Leda Bassit,[1] Sijia Tao,[1] Robert A. Domaoal,[1] Ozkan Sari,[1] Coralie De Schutter,[1] Sheida Amiralaei,[1] Ahmed Khalil,[1+] Olivia Ollinger Russell,[1] Tamara McBrayer,[3] Tony Whitaker,[3] Nageh Abou-Taleb,[2] Franck Amblard,[1] Steven J. Coats,[3]

Raymond F. Schinazi[1]*

[1] Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, USA; [2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Helwan University, Helwan, Egypt; [3] Cocrystal Pharma, Inc., Tucker, USA.

 # contributed equally – co-first authors

ABSTRACT

Pan-genotypic nucleoside HCV inhibitors display a high genetic barrier to drug resistance and are the preferred direct acting agents to achieve complete sustained virologic response in humans. Herein, we report, the discovery of a β-D-2'-Cl,2'-F-uridine phosphoramidate nucleotide **16**, as a non-toxic pan-genotypic anti-HCV agent. Phosphoramidate **16** in its 5'-triphosphate form specifically inhibited HCV NS5B polymerase with no marked inhibition of human polymerases and cellular mitochondrial

RNA polymerase. Studies on the intracellular half-life of phosphoramidate **16**-TP in live cells demonstrated favorable half-life of 11.6 h suggesting once a day dosing. Stability in human blood and favorable metabolism in human intestinal microsomes and liver microsomes make phosphoramidate **16** a prospective candidate for further studies to establish its potential value as a new anti-HCV agent.

KEYWORDS: Nucleoside, prodrug, hepatitis C, antiviral

INTRODUCTION:

Hepatitis C virus (HCV) presents a global health problem with approximately 180 million individuals infected worldwide with 80% of those progressing to chronic HCV infection.[1] Of those chronically infected individuals, approximately 30% will develop liver cirrhosis and 10% will go on to develop hepatocellular carcinoma.[2] Options comprising pegylated interferon-α (PEG-IFN) in combination with ribavirin (RBV) was the treatment of choice for HCV infection for many years; however, this treatment had demonstrated limited efficacy and generally intolerable side effects.[3,4] Recently, two newly approved once-daily combination hepatitis C drugs (Elbasvir + grazoprevir and ombitasvir + paritaprevir + ritonavir + dasabuvir) have demonstrated improved safety and efficacy.[5] However, without nucleoside NS5B inhibitors as backbone, these new agents are only approved for treating genotype 1 or 4 HCV-infected persons, thus excluding a large patient population infected with HCV genotypes 2, 3, 5 and 6.

The RNA-dependent RNA polymerase (RdRp) is essential to viral replication and has been clinically validated as target for therapeutic intervention by design of specific inhibitors. The HCV NS5B RdRp is part of a replication complex that is membrane bound and is responsible for HCV RNA genome replication.[3,6] Nucleoside inhibitors (NIs), in their 5'-triphosphate form, interact with the HCV NS5B polymerase and are attractive due to their high inhibitory potency, pan-genotypic profile, and high generic barrier to resistance.[7] So far, sofosbuvir is the only FDA approved nucleoside phosphoramidate prodrug for HCV and is the backbone of the first all-oral, pan-genotypic, single tablet regimen for the treatment of adults with genotype 1-6 chronic HCV infection (Combination of sofosbuvir with NS5A inhibitor, velpatasvir). However, because of long duration of treatment (8-12 weeks), there is still an urgent need to develop novel nucleoside analogs that are pan-genotypic and more efficacious, have an improved safety profile and a high barrier to resistance, which could lead to a new ultra-short combination therapy.[8]

The 2'-methyl substitution on nucleoside analogs has played a unique role in HCV drug discovery and clinical treatment.[9] Even though sofosbuvir is the only FDA approved nucleoside analog for HCV treatment so far, several 2'-methyl analogs have reached human clinical evaluation[10] (Figure 1). Based on these structures, we envisioned new anti-HCV nucleoside analogs by considering the isosteric replacement of the 2'-methyl group with groups or elements with similar van der Waals radius.[11] Isosteres are groups that exhibit some similarities in their chemical, physical and/or biological properties and

as a result, they have the potential to exhibit similar or superior pharmacokinetic and/or pharmacodynamic properties. Ultimately, we hypothesized that the replacement of the 2'-methyl group by a similarly sized chlorine atom could result in the formation of analogs with anti-HCV activity. Herein, we report the synthesis and biological evaluation of 2′-chloro,2'-fluoro ribonucleosides and their phosphate prodrugs as anti-HCV agents.[12]

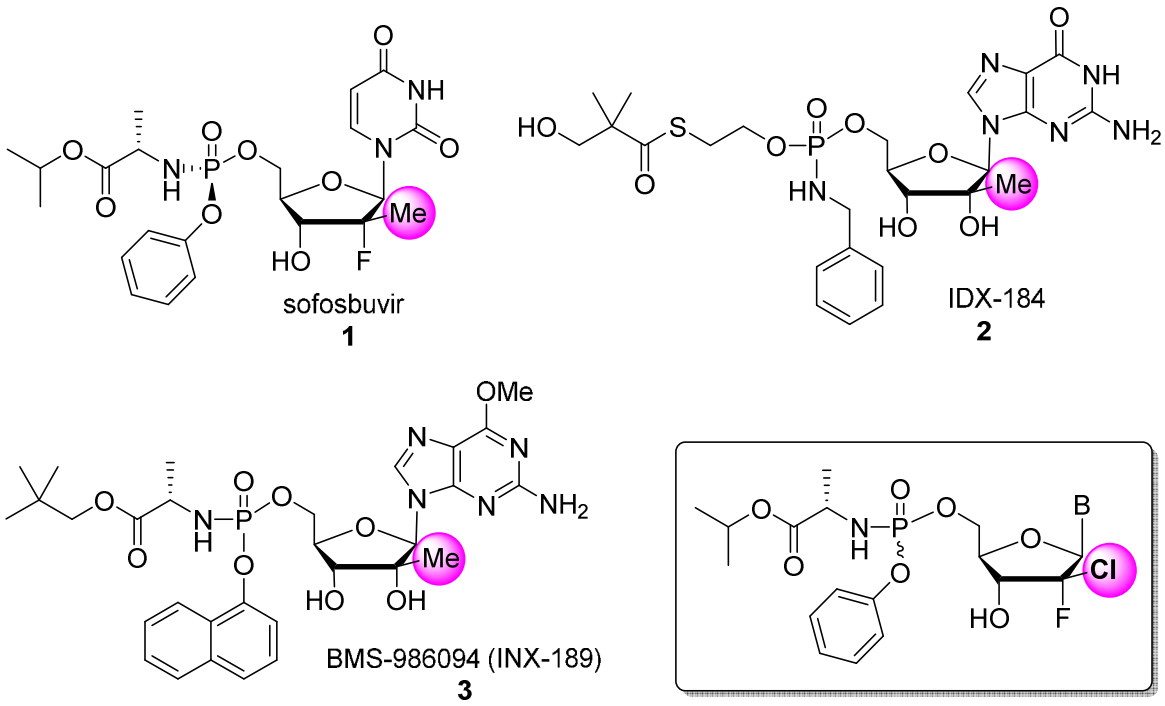


Figure 1. Selected clinical anti-HCV nucleoside analogs and targeted 2'-chloro, 2'-fluoro nucleoside analogs.

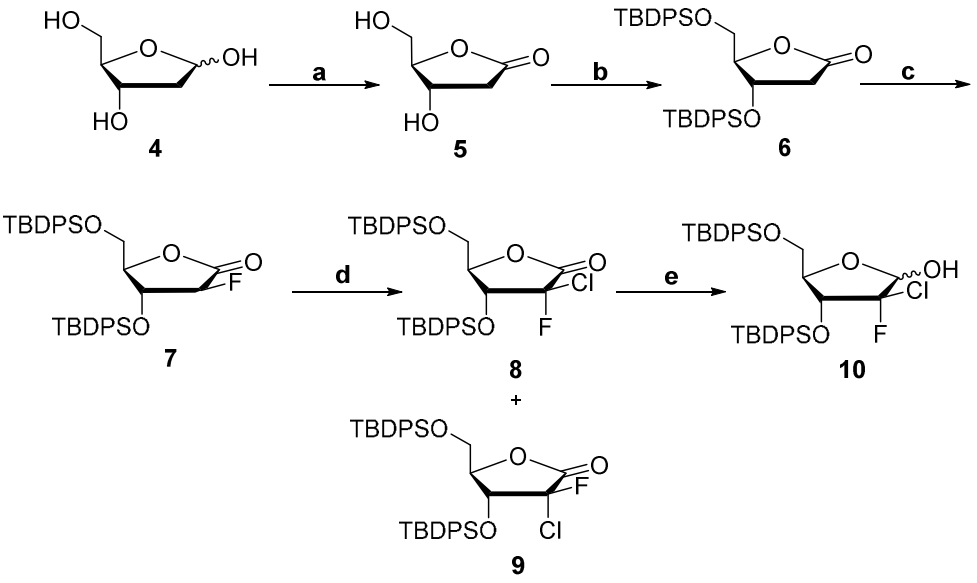
RESULTS AND DISCUSSION:

Chemistry:

The key protected lactol **10** was prepared using the chemistry described in Scheme 1.

Oxidation of commercially available 2-deoxy-D-ribose **4** with Br_2 gave lactone **5** which was then reacted with *tert*-butyldiphenylsilyl choride (TBDPSCl) to give the *bis*-protected lactone **6** in 83% yield. Reaction of **6** with fluorodibenezenesulfonimide (NFSI) in presence of LiHMDS resulted in the formation of **7** in 24% yield.[13] Chlorination of compound **7** with *N*-chlorosuccinimide (NCS) in presence of LiHMDS gave dihalogeno compounds **8** and **9** as a 4:1 mixture that were separated by flash chromatography on silica gel. Finally, reduction of isomer **8** with lithium tri-*tert*-butoxyaluminum hydride in THF gave the desired lactol **10** in 86% yield, as a α/β mixture (1.5:1).

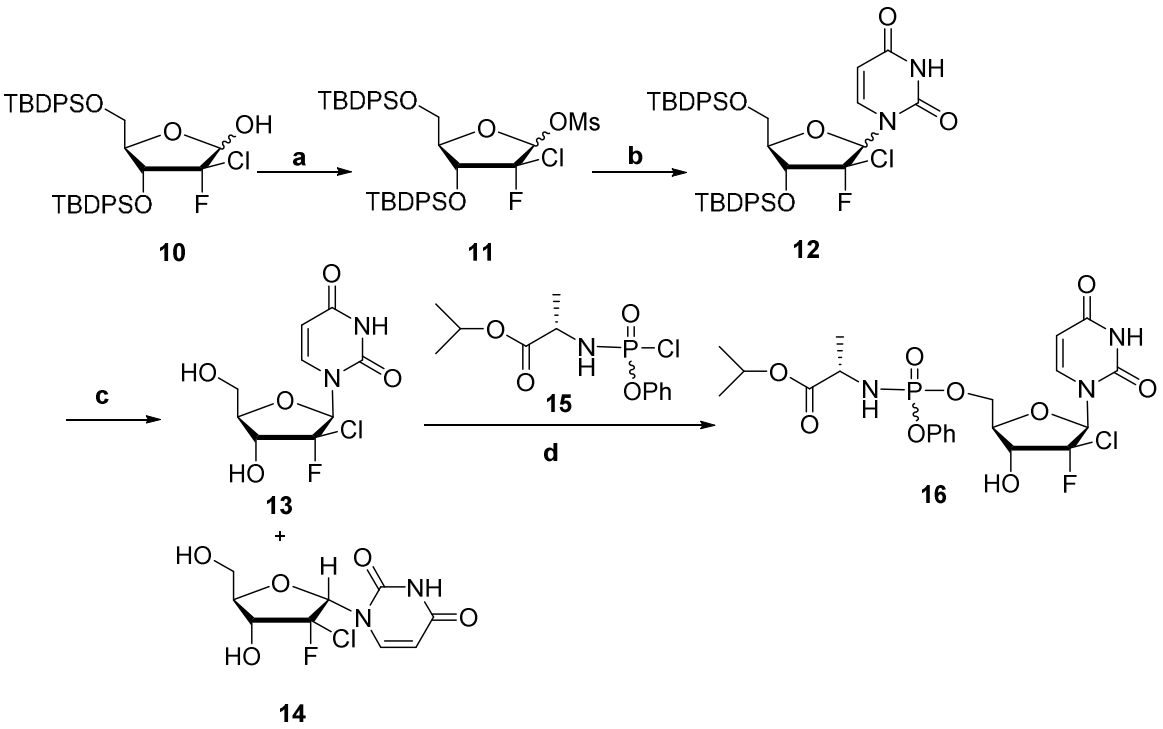
Scheme 1. Synthesis of protected lactone **10**.



Reagents and conditions: a) Br_2, H_2O, rt, 5 d, 86%; b) TBDPSCl, imidazole, DMF, rt, 24 h, 83%; c) NFSI, LiHMDS, THF, -78 ^oC, 1 h, 24%; d) NCS, LiHMDS, THF, -78 ^oC, 30 min, 57%; e) Li(*t*-BuO)$_3$AlH, THF, 4 h, rt, 86%.

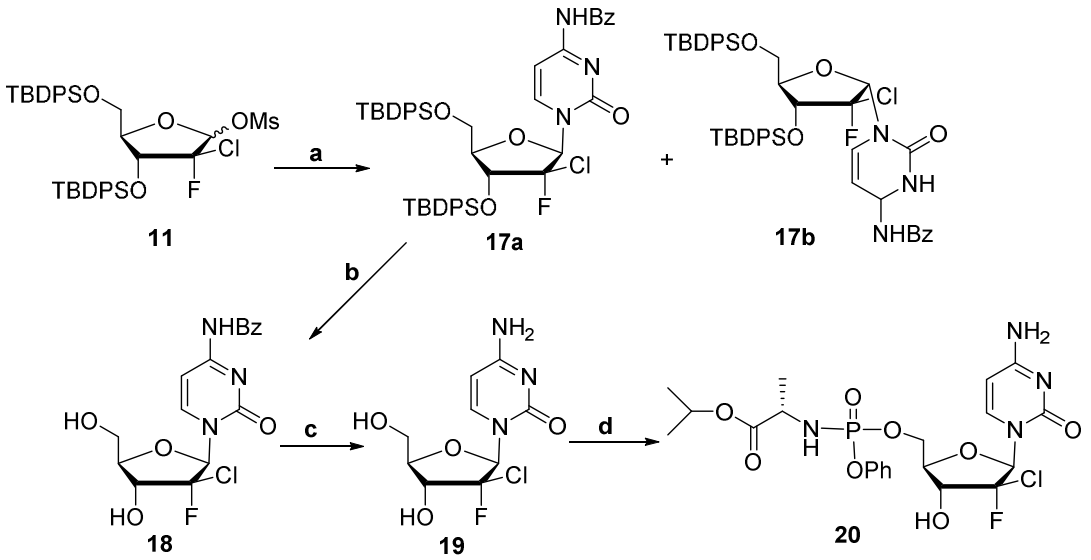
With key lactol **10** in hand, various pyrimidine, purine and 7-deaza purine nucleosides, along with their corresponding phosphoramidate prodrugs, were prepared as depicted in Schemes 2-7. Thus, 1-OMs derivative **11**, readily prepared from lactol **10** by mesylation, was reacted under Vorbrüggen conditions with persilylated uracil in presence of TMSOTf to give compound **12** as an inseparable mixture of α/β isomers (α/β ratio: 2:1). Desilylation with $Et_3N.3HF$ in THF and subsequent chromatographic separation gave the desired uridine nucleoside **13** (β-isomer, 32%) along with its α-isomer **14** (50%). Finally, nucleoside **13** was reacted with phenyl *L*-isopropylalaninyl phosphorochloridate **15**[14] in presence of NMI in THF to give phosphoramidate prodrug **16** as an approximate 1:1 mixture of *R*p/*S*p isomers (Scheme 2).

Scheme 2. Synthesis of compound 13 and its corresponding phosphoramidate prodrug 16.



Reagents and conditions: a) MsCl, Et_3N, DCM, 0 ^oC to rt; b) i) Uracil, BSA, 1,2-dichloroethane, 80 ^oC, 30 min; ii) TMSOTf, 1,2-dichloroethane, 80 ^oC, 4 h, 67%; c) $Et_3N.3HF$, THF, 0 ^oC to rt, 24 h, 82%; d) NMI, THF, rt, 4 h, 48%.

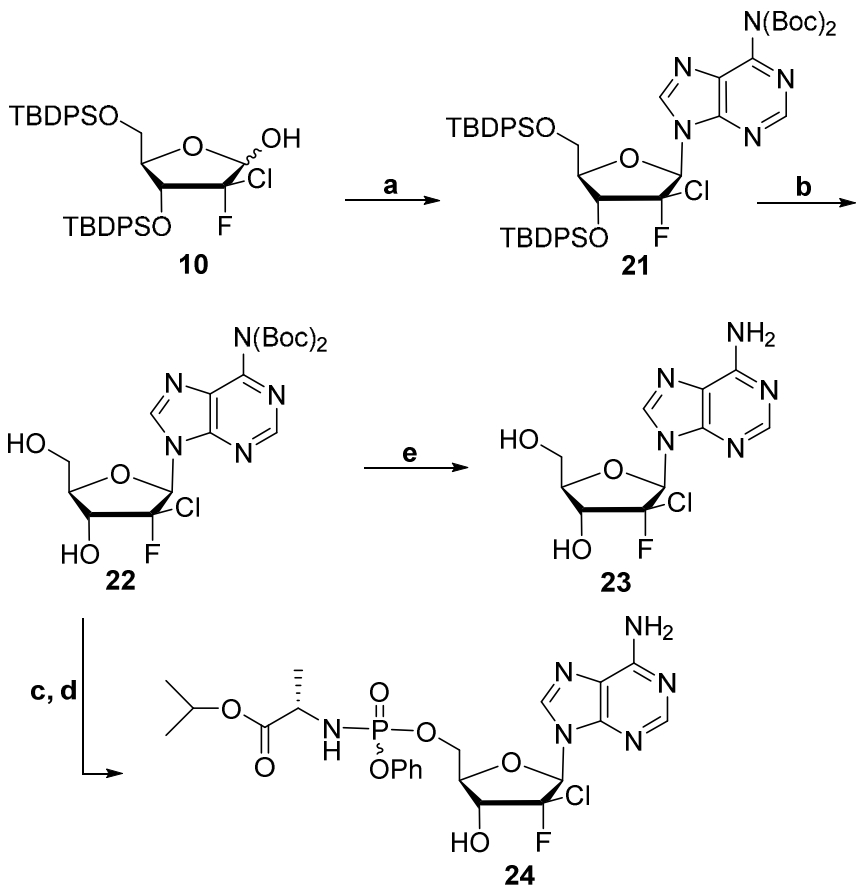
Scheme 3. Synthesis of nucleoside 19 and its monophosphate prodrug 20.



Reagents and conditions: a) i) N^4-benzoylcyctosine, BSA, 1,2-dichloroethane, 80 ^oC, 30 min; ii) TMSOTf, 1,2-dichloroethane, 80 ^oC, 5 h, 63%; b) $Et_3N.3HF$, THF, 0 ^oC to rt, 24 h; c) NH_3, MeOH, overnight, 88% over 2 steps; d) **15**, *t*-BuMgCl, THF, 0 ^oC to rt, 3 h, 18%.

Coupling of 1-mesyl sugar **11** with persilylated N^4-benzoylcytosine in the presence of TMSOTf in 1,2-dichloroethane at 80 ^oC gave compound **17a** along with its α isomer **17b** (α/β ratio is 2:1). Desilylation of **17** with $Et_3N.3HF$ and subsequent debenzoylation with a methanolic solution of ammonia gave cytosine analog **19** in 88% yield over 2 steps. Cytosine phosphoramidate prodrug **20** was finally prepared by reaction of compound **19** with phenyl *L*-isopropylalaninyl phosphorochloridate **15** in presence of *t*-BuMgCl (Scheme 3).

Scheme 4. Synthesis of adenine nucleoside **23** and its prodrug **24**.

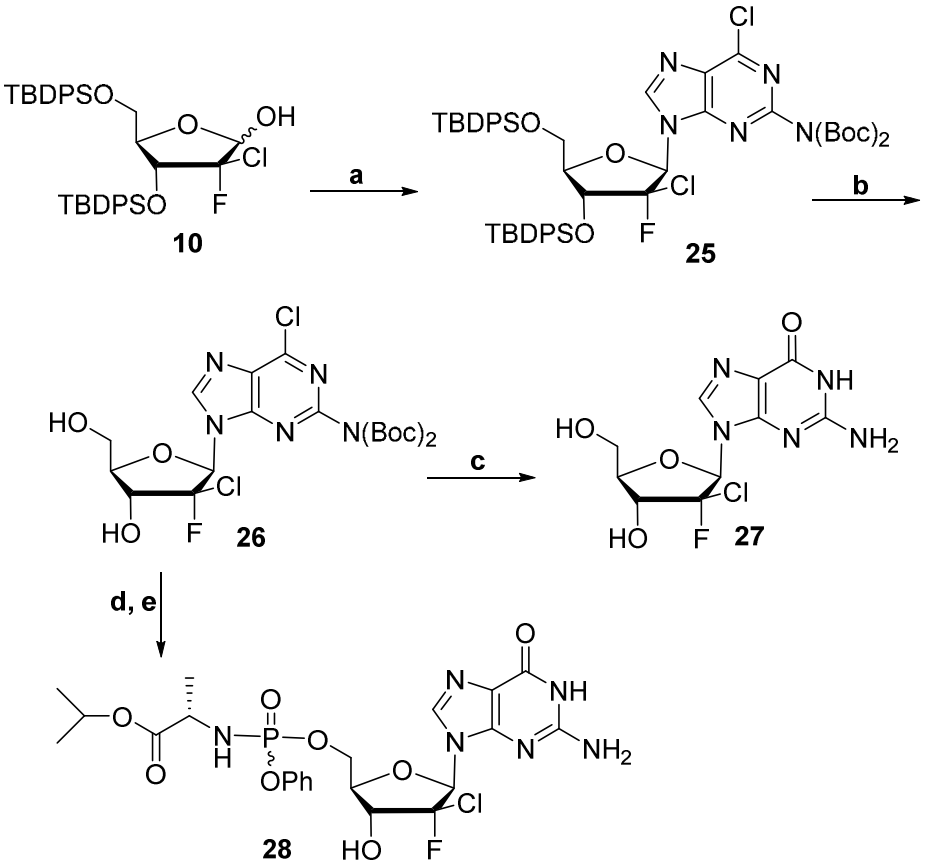


Reagents and conditions: a) bis-*N*-Boc adenine, DIAD, PPh_3, THF, rt, 24 h, 32%; b) $Et_3N.3HF$, rt, 24 h, 86%; c) **15**, NMI, THF, 0 ^oC to rt, 4 h; d) 10% TFA-H_2O, 38% over two steps; e) 4N HCl in dioxane, DCM, rt, 1 h, 72%.

Because the Vorbrüggen type coupling (S_N1) with the pyrimidine bases gave the corresponding α nucleosides as major compounds, we decided to prepare the purine and 7-deaza purine nucleosides under Mitsunobu conditions (S_N2). Thus, coupling of lactol **10** (α/β ratio is 1.5:1) with bis-*N*-Boc protected adenine in presence of DIAD and PPh_3[10,15,16] gave a mixture of α/β anomers (ratio 1:1.5) that was separated by flash silica gel column chromatography to give pure β–isomer **21** in 32% yield. Deprotection of β-isomer **21** with $Et_3N.3HF$ in THF resulted in bis-*N*-Boc protected adenine compound **22**,

which was converted to adenosine nucleoside **23** by treatment with 4M HCl in dioxane. On the other hand, reaction of compound **22** with phenyl *L*-isopropylalaninyl phosphorochloridate **15** in presence of NMI and finally, removal of the Boc group under acidic conditions (10% TFA in H_2O) afforded the corresponding phosphoramidate prodrug **24** (Scheme 4).

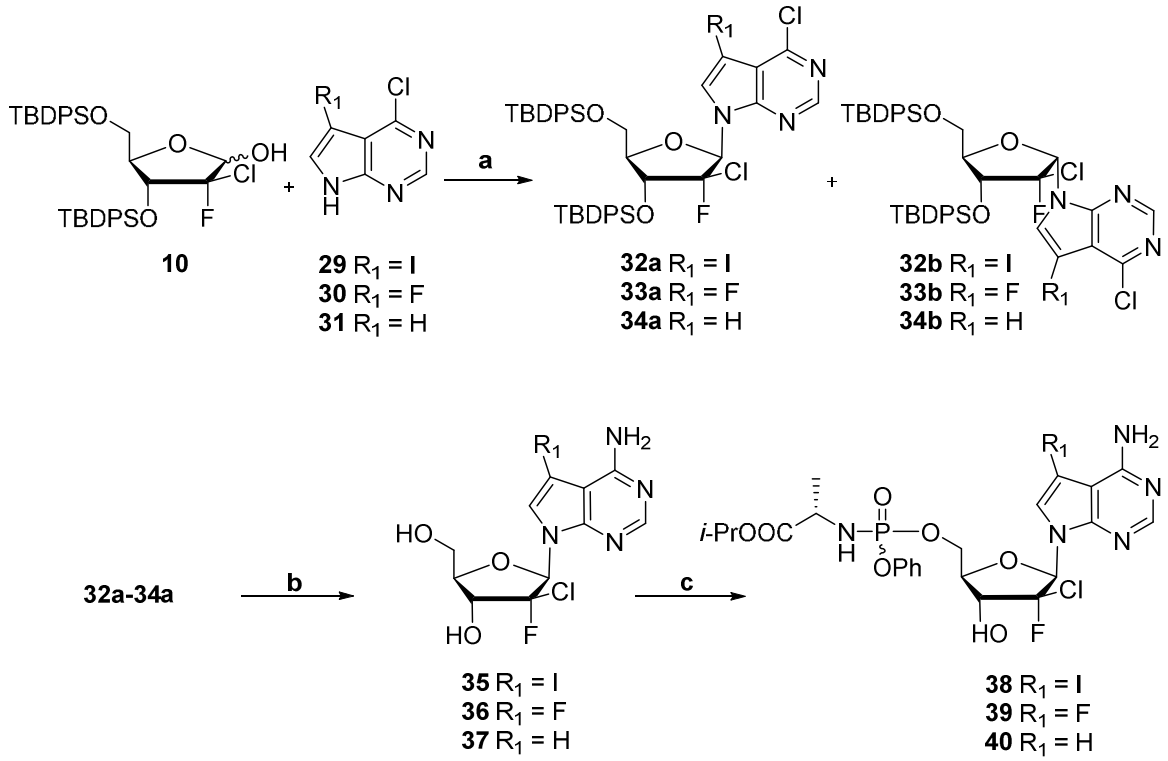
Scheme 5. Synthesis of guanine nucleoside 27 and its prodrugs 28.



Reagents and conditions: a) 6-chloro-2-*N*-Boc_2 purine, DIAD, PPh_3, THF, rt, 24 h, 35%; b) $Et_3N.3HF$, rt, 24 h, 93%; c) 80% TFA in H_2O, rt, 34 h, 47%; d) **15**, NMI, THF, 0 ^oC to rt, 3.5 h, 64%; e) 80% TFA in H_2O, rt, 34 h, 33%.

In a similar manner, coupling of 6-chloro-2-*N*-(Boc)$_2$ purine[11] with lactol **10** under Mitsunobu conditions (DIAD/PPh$_3$) gave a 1.4/1 mixture of β/α anomers which can be separated by column chromatography. Deprotection of β isomer **25** with Et$_3$N.3HF in THF afforded compound **26** in 93% yield. One-pot hydrolysis and deprotection of compound **26** with 80% aqueous TFA gave guanine analog **27** in 47% yield. Reaction of compound **26** with *L*-isopropylalaninyl phosphorochloridate **15** in presence of NMI and subsequent treatment with 80% aqueous TFA furnished guanine nucleoside prodrug **28** (Scheme 5).

Scheme 6. Synthesis of 7-deazapurine nucleosides **35**-**37** and their prodrugs **38**-**40**.



Reagents and conditions: a) Ph$_3$P, DIAD, THF, rt, 2 d, 34-36%; b) aq. NH$_4$OH,

1,4-dioxane, 120 ^{o}C, 20 h, 40-62%, c) Phenyl L-isopropylalaninyl phosphorochloridate **15**, NMI, THF, rt, 2-3 h, 38-43%.

We then turned our attention to the non-canonical 7-deazapurines bases which are known to be tolerated by the HCV polymerase in other series.[17] Thus, reaction of 7-iodo- or 7-fluoro- 6-chloro-7-deazapurine **29** and **30** or 6-chloro-7-deazapurine **31** with lactol **10** in presence of DIAD and PPh_3 in THF at rt provided a mixture of α:β isomers (ratio 1:1.2) that were separated by flash chromatography. Treatment of β-isomers **32a**-**34a** with NH_4OH resulted in 7-deazapurine nucleosides analogs **35**-**37**, which were converted to their corresponding phosphoramidate prodrug **38**, **39** and **40** by reaction with phenyl L-isopropylalaninyl phosphorochloridate **15** in presence of NMI (Scheme 6).

Assignment of Absolute Configuration and α/β Isomerism

The 1'-stereochemistry of our final compounds were assigned by using ^{1}H 2D-NOESY experiments. For instance, in the case of β-isomer **13**, NOE enhancements were observed between H_6 of the nucleobase and $H_{3'}$ as well as between $H_{1'}$ and $H_{4'}$; while interactions between the H_6 and $H_{4'}$ and $H_{1'}$ and $H_{3'}$ were observed for α-isomer compound **14** (Figure 2).

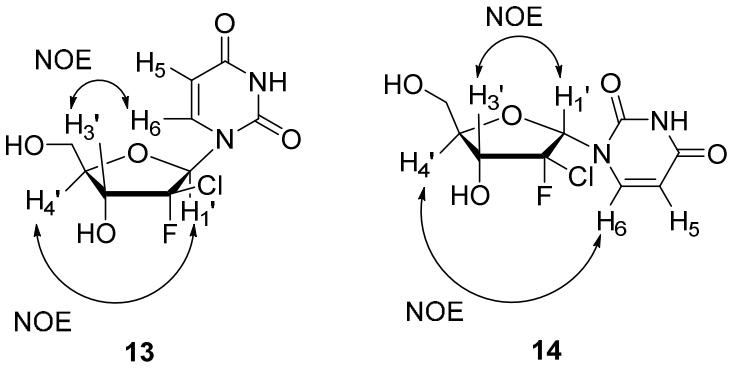
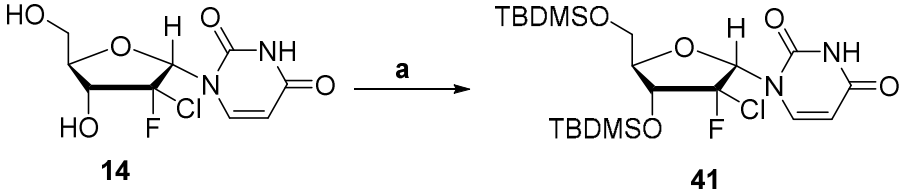


Figure 2. Anomer assignment for compounds **13** and **14** *via* NOE experiments

Because stereochemistry at the 2'-position could not be clearly established through common NMR experiments, we decided to use X-ray diffraction analysis. Unfortunately, we were not able to grow suitable crystals from any of the intermediates described above and only compound **41**, obtained by reaction of uracil derivative **14** with TBDMSCl in presence of imidazole (Scheme 7) gave us a single crystal and allowed us to confirm the (*S*)-configuration of C-2' of our nucleosides (Figure 3).

Scheme 7. Synthesis of compound **41**



Reagents and conditions: a) TBDMSCl, imidazole, DMF, rt, 16 h, 83%

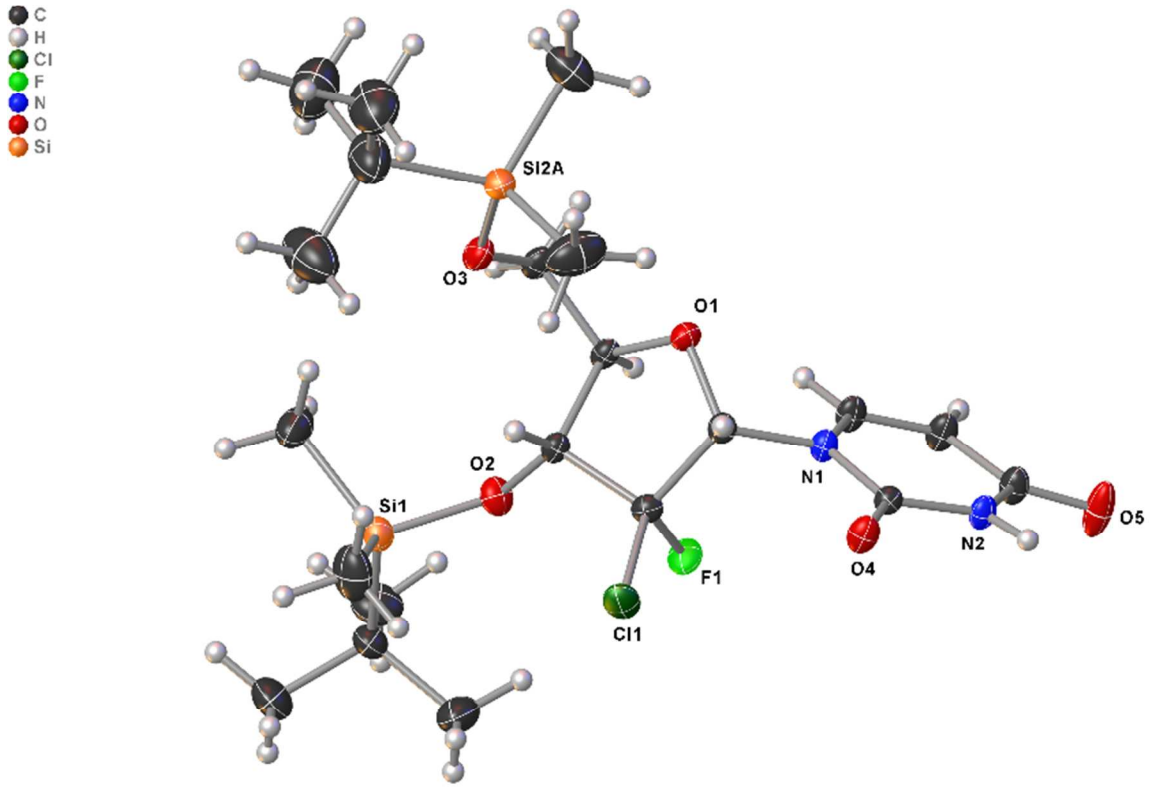


Figure 3. X-ray crystal structure of **41** crystallized from *i*-PrOAc

Antiviral activity and cytotoxicity

Compounds **13**, **19**, **23**, **27**, **35**, **36**, **37** and their corresponding phosphoramidate prodrugs **16**, **20**, **24**, **28**, **38**, **39**, **40** were evaluated for inhibition of HCV genotype 1b RNA replication in Huh-7 cells using a subgenomic HCV replicon system.[18] Cytotoxicity in Huh-7 cells was determined simultaneously by extraction and amplification of both HCV RNA and cellular ribosomal RNA (rRNA).[19] In addition, cytotoxicity was determined in primary human peripheral blood mononuclear (PBM) cells, human lymphoblastoid CEM, and African Green monkey Vero cells.[20,21] The results are summarized in Table 1. Uracil, adenine and guanine nucleosides **13**, **23** and **27** were devoid of anti-HCV activity at concentration up to 10 μM while they corresponding monophosphate prodrugs **16**, **24** and

28 were all active in the submicromolar range (EC_{50}'s of 0.1, 0.7 and 0.3 μM respectively). Interestingly, both cytosine nucleoside **19** and its prodrug **20** showed submicromolar activity in the HCV replicon assay. Compound **19** however, displayed cytotoxicity in PBM and Vero cells in the low micromolar range (CC_{50}'s of 12 and 8.4 μM respectively). Finally, none of the 7-deazapurine analogs displayed anti-HCV activity. Based on its potency and lack of cellular toxicity in the various cell line tested, we further investigated the potential of compound **16**.

Table 1. HCV genotype 1b replicon activity and cytotoxicity of nucleosides **13**, **19**, **23**, **27**, **35**, **36**, **37** and their phosphoramidate prodrugs **16**, **20**, **24**, **28**, **38**, **39**, **40**

Compound	Anti-HCV activity (μM)		Cytotoxicity, CC_{50} (μM)			
	EC_{50}	EC_{90}	Huh-7	PBM	CEM	Vero
13	> 100	> 100	> 100	> 100	> 100	> 100
16	0.2 ± 0.1	0.4 ± 0.2	>10	> 100	> 100	> 100
19	0.3 ± 0.03	0.9 ± 0.02	>10	12	> 100	8.4
20	0.1 ± 0.06	0.3 ± 0.02	>10	> 100	> 100	> 100
23	> 10	> 10	>10	> 100	> 100	> 100
24	0.6 ± 0.2	2.1 ± 0.6	> 33	84.8	36.3	53.9
27	19 ± 2.9	33 ± 0.5	> 33	> 100	> 100	> 100

28	0.3 ± 0.1	0.8 ± 0.2	>10	> 100	> 100	> 100
35	> 10	> 10	> 10	6.6	7.3	11.5
38	> 10	> 10	> 10	> 100	69.3	>100
36	> 10	> 10	>10	82.6	19.9	42.4
39	> 10	> 10	> 10	99.7	55.3	60.9
37	> 10	> 10	> 10	ND	ND	ND
40	> 10	> 10	> 10	> 100	69	> 100
2'*C*-MeC	1.7 ± 0.8	6.5 ± 1.9	> 10	> 100	> 100	> 100
Sofosbuvir	0.2	0.5 ± 0.3	>10	> 100	> 100	> 100

ND: Not determined. All assays were performed in replicates. Only means are shown.

Activity profile:

Compound **16** was tested against a panel of genetically diverse HCV replicons and showed anti-HCV activity in the submicromolar range (Table 2). Similar to SOF, compound **16**, which forms **16**-triphosphate intracellularly, displayed a 2-3 fold decrease in potency against genotype 1b polymerase mutations S96T (Table 3) and S282T (Table 4) associated with drug resistance.

Table 2. Effect of compound **16** in culture against HCV Genotypes 1-4 (4 day assay)

Genotype (GT): IC_{50} derived from data using patient isolates

Cmpd	1a	1b	2a/k	2b	3a	4
16, μM	0.13	0.15	0.05	0.09	0.15	0.17
SOF, μM	0.06	0.06	0.02	0.04	0.09	0.07
IFN, IU/mL	0.93	0.91	1.0	1.0	0.95	0.95
13, μM	> 60	> 60	> 60	> 60	> 60	> 60
PSI-6206,[a] μM	> 100	> 100	> 100	> 100	> 100	> 100

[a] 2'-deoxy-2'-fluoro-2'-*C*-methyl-uridine[22]

Table 3. 16-TP: IC_{50} with S96T mutant and various genotypes

NS5B	IC_{50}, μM	Fold-change relative to NS5B GT 1b WT	N
GT 1b WT	5.5 ± 2.8	1	3
GT 1b S96T	13 ± 2.2	2.4	2
GT 2a WT	6.1 ± 3.4	1.1	2
GT 4a WT	8.6 ± 7.0	1.6	2

Table 4. Effect of compound **16** in culture against HCV S282T mutant.

Cmpd	Wild-Type (μM)	S282T	Fold Increase

	(μM)					
	EC_{50}	EC_{90}	EC_{50}	EC_{90}	EC_{50}	EC_{90}
16	0.02 ± 0.001	0.07 ± 0.005	0.07 ± 0.002	0.2 ± 0.03	3.5	2.9
SOF	0.09 ± 0.01	0.3 ± 0.05	0.4 ± 0.12	1 ± 0.13	4.4	3.0

Toxicity profile: It has previously been reported that certain nucleoside analogs can exhibit mitochondrial toxicity.[23] In order to address this issue compounds **13** and **16** were evaluated for their effects on mitochondrial DNA levels. HepG2 cells were propagated in the presence of nucleotide analogs (up to 50 μM) for 14 days prior to quantification of mitochondrial COXII DNA (mtDNA) and β-actin DNA using real-time PCR. Lamivudine (3TC) and β-D-2',3'-dideoxycytidine (ddC) (at 10 μM) were included as negative and positive controls, respectively (Table 5). Over a 14 day period, neither the parent, nor the prodrug showed measurable mitochondrial toxicity up to 50 μM in HepG2 cell line (Table 5) whereas ddC, as anticipated, was highly toxic.

Table 5. Effect of compound **16** and **13** on mitochondrial (Mt), nuclear DNA levels and lactic acid production in HepG2 cells (14-day assay)

Cmpd	**Conc μM**	**% Inhibition MtDNA /nDNA**	**IC_{50}, μM MtDNA / nDNA**	**MtDNA Content (% of control)**	**Lactic acid production**

					(% of control)
16	10	< 1 / < 1	> 50 / > 50	130 (117-143)	60 ± 1.3
	50	27 / 13		84 (68-102)	56 ± 0.88
13	10	< 1 / 8.8	> 50 / > 50	160 (113-230)	85 ± 7.3
	50	< 1 / <1		120 (77-175)	120 ± 5.8
SOF	10	< 1 / 7.3	>50 / >50	176 (151 - 206)	
	25	<1 / 38.3		187 (170 - 205)	
	50	<1 / 38.8		255 (247 - 264)	
3TC	10	16 / 38	> 10 / > 10	140 (103-181)	120 ± 10
ddC	10	96 / 53	< 10 / < 10	9.5 (5.7-16)	330 ± 27
No drug control		0 / 0		100 (95-105)	100 ± 2.6

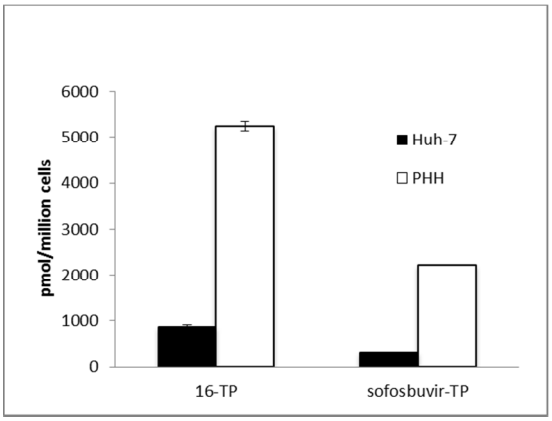
[a] CC_{50}, values obtained from HepG2 cells were generated from two separate experiments (± SD where applicable). mtDNA was measured and compared to nuclear β-actin DNA.

[b] Lactic acid measurements are the mean of triplicates from a single 96-well plate (± SD).

Lactic acid levels were also measured in the culture supernatant after 14 days of incubation with each drug. The total amount of lactic acid produced was determined for each sample. Increased production of lactic acid (generally above 100% when normalized to β-actin control) is associated with mitochondrial toxicity.[24] We did not observe increased lactic acid production with up to 50 μM of compound **13** and its prodrug **16**. As expected, treatment with ddC resulted in increased lactic acid production (Table 5). In addition, compound **16** was devoid of bone marrow toxicity at concentration up to 50 μM (data not shown).

Cellular pharmacology: The intracellular metabolism of prodrug **16** in Huh-7 cells was determined. Compound **16** was incubated in Huh-7 cells or primary human hepatocytes (PHH) at 50 μM for 4 h at 37 °C. Intracellular metabolites were extracted with 70% ice-cold methanol in water and subsequently identified by LC-MS/MS. Compound **16** generated markedly higher levels of nucleoside 5'-triphosphate (2.7-fold and 2.3-fold in Huh-7 cells and PHH, respectively) when compared to sofosbuvir (Figure 4).

Figure 4. Intracellular 5'-triphosphate levels for compound **16** and 2'-F,2'-*C*-Me nucleoside triphosphate derived from sofosbuvir in Huh-7 cells and PHH.



Selectivity: the potential off-target impact of **16**-TP on human DNA and RNA polymerases was evaluated.[25] Similar to 2'-F,2'-*C*-Me-UTP, the triphosphate metabolite of sofosbuvir, **16**-TP is not incorporated by RNA polII at concentration up to 100 μM and is only a weak substrate of POLRMT at this concentration (Table 6). In addition, **16**-TP has no major impact on either DNA polymerase α, β and γ (Table 7).

Table 6: Incorporation of **16**-TP by the human nuclear polymerase (RNA polII) and the mitochondrial RNA polymerase (POLRMT).

Inhibitor	**RNA POL II IC_{50}**	**POLRMT % Incorporation**
16-TP	> 100 μM	35 ± 0.5

2'-F,2'-*C*-Me-UTP	> 100 μM	3.1 ± 1.4
Alpha-amanitin (+ve CTRL)	2.5 ± 1.7 nM	NA

Table 7: Impact of **16**-TP on human DNA polymerase activity

Inhibitor	**IC_{50} (μM)**		
	DNA Pol α	**DNA Pol β**	**DNA Pol γ**
16-TP	20.2 ± 2.3	> 100	> 100
2'-F,2'-*C*-Me-UTP	> 100	> 100	> 100
Aphidicolin (+ve ctrl)	2.4 ± 1.0	NA	NA
ddTTP (+ve ctrl)	NA	19 ± 1.0	43

NA: Not Available

Stability and metabolism: Compound **16** was shown to be stable in both fresh human blood for up to 2 h while the positive control, eucatropine, was at 19% remaining at the same time point and in addition, human intestinal microsomes clearance was low (Table

8). However, as expected for a prodrug, compound **16** was unstable in human liver microsomes. It had a short half-life (11.6 min) and high clearance with 4.2% remaining at 60 min (Table 9)

Table 8. Stability of compound **16** in intestinal microsomes.

	Human Blood		**Human Intestinal Microsomes**	
Compound	**16**	**Eucatropine**	**16**	**Verapamil**
$t_{1/2}$ (min)			120	64
CL (µl/min/mg)			29	54
% Remaining	103 (2 h)	19 (2h)	79% (60 min)	56% (60 min)

Table 9. Stability of compound **16** in human liver microsomes.

	Human Liver Microsomes			
Compound	**16**	**Testosterone**	**Diclofenac**	**Propafenone**
$t_{1/2}$ (min)	11.6	15	8.9	5.3
CL (µl/min/mg)	120	92	156	260
% remaining at 60 min	4.2%	6.3%	1.2%	0%

PSI-7851 which is SOF except as the mixture of *R*p/*S*p phosphorous diastereomers, analogues to compound **16**, displayed a similar profile (human plasma $t_{1/2} > 24$ h; human liver S9 $t_{1/2} = 0.57$ h; simulated gastric fluid $t_{1/2} = 22$ h and simulated intestinal fluid $t_{1/2} > 24$ h).[26] Drug-drug interactions is an important issue in drug development and is often connected to the inhibition of hepatic enzymes such as cytochrome P450's (CYP450) by one of the drug. Therefore, the potential drug-drug interaction liabilities of compound **16** was investigated in a CYP450 reversible inhibition assay. As shown in table 10, compound **16** showed comparable inhibition to sofosbuvir with moderate inhibition of CYP450 3A4 and 2C9.

Table 10. Inhibition of Major Human CYP P450 Enzymes at Physiological Concentrations for **16**.

Compound	Cytochrome P450 Inhibition IC_{50} (μM)			
	3A4	**2C9**	**1A2**	**2D6**
16	6.8 ± 0.9	25 ± 4.4	> 100	> 100
Sofosbuvir	8.4 ± 1.6	60 ± 24	> 100	> 100

Conclusions:

We report herein for the first time the synthesis and biological evaluation of a unique series of 2'-fluoro,2'-chloro nucleosides. Among all the compounds synthesized, **16** is a

potent and specific inhibitor of HCV. It does not inhibit replication of major DNA and RNA viruses (At concentration up to 300 μM for vaccinia virus or 113 μM for rift valley fever virus, dengue virus, west nile virus, yellow fever virus, japanese encephalitis virus, chikungunya, influenza A H1N1 and respiratory syncytial virus; data not shown). Compound **16** had excellent pan-genotypic activity with anti-HCV replicon activity similar to SOF. Its nucleoside 5'-triphosphate was a specific inhibitor of HCV NS5B polymerase with no significant inhibition of human beta, gamma DNA polymerase and showed low incorporation by cellular mitochondrial RNA polymerase. Despite observing an IC_{50} of 20 μM versus polymerase alpha, we did not observe corresponding cytotoxicity nor cytostatic behavior in five different cell lines (PBM, CEM, Vero, Huh7 and HepG2) at concentrations up to 100 μM. No mitochondrial (MtDNA, nuclear DNA) including lactic acid and bone marrow toxicities were observed up to 50 μM. Compound **16** was stable in human blood for up to 2 h, rapidly metabolized in human hepatocytes and showed moderate to low metabolism in human intestinal microsomes. Moderate inhibition of CYP450 3A4 and 2C9 was observed similar to that of observed with SOF. The novel nucleotide analog **16** has an excellent preclinical profile suggesting further development to establish its potential value as a novel anti-HCV nucleotide phosphoramidate analog.

Experimental Section:

Anhydrous solvents were purchased from Aldrich Chemical Company, Inc. (Milwaukee). Reagents were purchased from commercial sources. Unless noted otherwise, the materials used in the examples were obtained from readily available commercial suppliers or synthesized by standard methods known to one skilled in the art of chemical synthesis. ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra were taken on a Bruker Ascend^{TM} 400 spectrometer at rt and reported in ppm downfield from internal tetramethylsilane (for ^{1}H-NMR). NMR processing was performed with MestReNova version 10.0.2-15465. Deuterium exchange and decoupling experiments were utilized to confirm proton assignments. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), br (broad), bs (broad singlet), m (multiplet). All J-values are in Hz and calculated by Mnova or MestReNova programs. Mass spectra were determined on a Micromass Platform LC spectrometer using electrospray ionization. Purity of final compounds was determined to be > 95%, using an UPLC analyses performed on a Waters Acquity UPLC System with a Kinetex LC column (2.1 mm x 50 mm, 1.7 μm, C18, 100 Å) and further supported by clean NMR spectra. Mobile phase flow was 0.4 mL/min with, a 1.20 min gradient from 95% aqueous media (0.05% formic acid) to 95% CH_3CN (0.05% formic acid), and a 4.5 min total acquisition time. Photo diode array detection was from 190 to 360 nm. Analytic TLC was performed on Analtech GHLF silica gel plates, and preparative TLC on Analtech GF silica gel plates. Column chromatography was performed on Combiflash R_f200 or via reverse-phase high performance liquid chromatography.

***2-Deoxy-3,5-di-O-(tert-butyldiphenylsilyl)*-D-*ribonolactone* (6)**

To a solution of 2-deoxyribono-lactone **5** (8.95 g, 66.80 mmol) in 300 mL of anhydrous DMF were added imidazole (22.7 g, 333 mmol, 5.0 eq) and *tert*-butyldiphenylsilyl chloride (38.4 g, 140 mmol, 2.1 eq). The reaction was stirred at rt for 24 h and quenched by addition of water. The water layer was extracted with hexanes (3 x 100 mL), and the combined organic layers were washed with brine, and dried over anhydrous Na_2SO_4. The crude product was concentrated and purified by flash chromatography (hexanes/ethyl acetate 50:1 to 30:1) to afford product **6** as a colorless oil (33.7 g, 83% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (m, 4H), 7.64-7.60 (m, 2H), 7.57-7.34 (m, 14H), 4.70-3.59 (m, 1H), 4.43 (d, *J* = 1.7 Hz, 1H), 3.68 (dd, *J* = 11.6, 2.7 Hz, 1H), 3.23 (dd, *J* = 11.6, 2.4 Hz, 1H), 2.90 (dd, *J* = 17.9, 6.8 Hz, 1H), 2.63 (dd, *J* = 17.9, 1.8 Hz, 1H), 1.15 (s, 9H), 1.01 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.1, 135.7, 135.6, 135.6, 135.4, 132.9, 132.8, 132.6, 132.0, 130.2, 130.2, 130.0, 129.9, 128.0, 128.04, 127.9, 127.9, 88.1, 77.5, 77.2, 76.8, 71.0, 63.3, 39.2, 26.8, 26.7, 19.1, 19.0.

***2-Deoxy-2-fluoro-3,5-di-O-(tert-butyldiphenylsilyl)-D-ribono-lacton*e (7).**

In a 1000 mL round-bottom flask, compound **6** (39.7 g, 65.2 mmol) and NFSi (30.84 g, 97.8 mmol, 1.5 eq) were dissolved in 320 mL of anhydrous THF. The solution was cooled to -78 °C, and 85 mL (85 mmol, 1.3 eq) of a 1 M solution of LiHMDS in THF was added dropwise over a period of 35 min. The reaction was allowed to stir at -78 °C for an

additional 1 h and was quenched with a saturated solution of NH_4Cl. Solid $KMnO_4$ (4.0 g) was added portion wise at 0 ^oC. After 1 h, the reaction mixture was filtrated through a pad of silica gel and washed with EtOAc (350 mL). The water layer was then extracted with hexanes (3 x 120 mL). The organic layers were finally combined, washed with a saturated solution of $NaHCO_3$, water, and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo.* The residue was purified by flash chromatography (hexanes/ethyl acetate 100:0 to 20:1) to afford a crude product that was purified again by flash chromatography (hexanes/DCM 10:1 to 3:1) to afford **7** (9.81 g, 24%) and starting material **6** (8.7 g).

^1H NMR (400 MHz, $CDCl_3$) δ 7.71-7.62 (m, 4H), 7.60-7.55 (m, 2H), 7.53-7.30 (m, 14H), 5.39 (d, *J* = 7.0 Hz, 1H), 5.26 (d, *J* = 7.0 Hz, 1H), 4.87-4.77 (m, 1H), 4.44-4.35 (m, 1H), 3.80-3.72 (m, 1H), 3.47 (dd, *J* = 12.2, 3.3 Hz, 1H), 1.12 (s, 9H), 0.93 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -202.70 (dd, *J* = 51.1, 18.6 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ168.7 (d, *J* = 22.9 Hz), 135.8, 135.7, 135.67, 135.5, 132.7, 132.2, 131.6, 130.4, 130.3, 129.9, 129.8, 128.0, 127.8, 127.7, 92.2 (d, *J* = 199.3 Hz), 81.95 (d, *J* = 9.8 Hz), 77.4, 77.2, 77.0, 76.76, 72.8 (d, *J* = 21.4 Hz), 61.2, 26.8, 26.6, 19.2, 19.1.

2-Deoxy-2-fluoro-2-chloro-3,5-di-O-(tert-butyldiphenylsilyl)-D-ribono-lactone (8).

In a 250 mL round-bottom flask compound **7** (9.70 g, 15.47 mmol) and NCS (4.17 g, 31.2 mmol, 2.0 eq) were dissolved in 75 mL of anhydrous THF. The solution was cooled to -78 °C, and 23.2 mL of a 1 M solution of LiHMDS in THF (23.2 mmol, 1.5 eq)

was added dropwise over a period of 20 min. The reaction mixture was allowed to stir at -78 °C for an additional 45 min and was quenched with a saturated solution of NH_4Cl. The water layer was extracted with hexanes (3 x 70 mL) and the combined organic layers were washed with a saturated solution of $NaHCO_3$, water and brine. The solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexanes/ethyl acetate 100:0 to 20:1) to afford a 4/1 mixture of compounds **8** and **9** (5.83 g, 57%). Additional flash chromatography on silica gel column (hexanes/ethyl acetate 100:0 to 20:1) afforded pure compounds **8** （3.76 g， 37%）and **9**（0.93g, 9%）.

Compound **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.58 (m, 4H), 7.58-7.40 (m, 8H), 7.43-7.29 (m, 8H), 4.65-4.49 (m, 2H), 3.70 (dd, *J* = 12.0, 3.7 Hz, 1H), 3.65-3.51 (m, 1H), 1.20-1.08 (s, 9H), 0.97-0.90 (s, 9H). ^{19}F NMR ($CDCl_3$, 376.3 MHz) δ (ppm): δ -133.22 (d, *J* = 7.7 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.2 (d, *J* = 26.6 Hz), 136.0, 135.7, 135.6, 135.4, 132.4, 132.1, 132.0, 131.2, 130.4, 129.9, 129.9, 128.0, 127.9, 127.8, 101.3 (d, *J* = 261.6 Hz), 84.4, 74.8 (d, *J* = 15.6 Hz), 61.2, 29.7, 26.7, 26.6, 19.4, 19.0.

Compound **9**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73-7.61 (m, 4H), 7.56-7.48 (m, 2H), 7.47-7.40 (m, 6H), 7.34 (tt, *J* = 10.3, 6.6 Hz, 8H), 4.79 (dd, *J* = 13.9, 7.8 Hz, 1H), 4.35-4.26 (m, 1H), 3.76 (d, *J* = 12.5 Hz, 1H), 3.50 (dd, *J* = 12.5, 3.4 Hz, 1H), 1.13 (s, 9H), 0.88 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -128.25 (d, *J* = 13.9 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ165.0 (d, *J* = 29.6 Hz), 136.0, 135.7, 135.6, 135.4, 132.6, 132.1, 131.9, 130.7, 130.6, 130.4, 129.9, 129.8, 128.0, 127.9, 104.2 (d, *J* = 267.1 Hz), 81.3 (d, *J* = 8.4

Hz), 72.8 (d, *J* = 22.6 Hz), 60.2, 29.7, 26.6, 26.6, 19.5, 19.0.

***1-Hydroxyl-2-deoxy-2-fluoro-2-chloro-3,5-di-O-(tert-butyldiphenylsilyl)-D-ribofuranose* (10).**

To a solution of compound **8** (3.93 g, 5.94 mmol) in 30 mL of anhydrous THF was added dropwise 13.1 mL of a 1 M solution of Li(*t*BuO)$_3$AlH in THF (13.1 mmol, 2.2 eq) at 0 °C. After stirring for 3 h at rt, the reaction mixture was quenched with a saturated solution of NH_4Cl at 0 °C. The mixture was then allowed to warm slowly to rt and stirred for another 2 h. The reaction mixture was filtered through a pad of silica gel and washed with ethyl acetate. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with a saturated solution of $NaHCO_3$, water, and brine. The solution was dried over Na_2SO_4, and concentrated *in vacuo* to give compound **10** (α/β ratio 1.5/1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82-7.60 (m, 8H), 7.57-7.30 (m, 32H), 5.24 (m, 2H), 4.65 (dd, *J* = 15.6, 6.1 Hz, 1.2H), 4.47 (dd, *J* = 9.5, 5.2 Hz, 0.8H), 4.40-4.30 (m, 0.8H), 4.19 (dt, *J* = 6.2, 2.5 Hz, 1.2H), 3.66-3.57 (m, 3H), 3.52-3.43 (m, 1.8H), 3.28 (dd, *J* = 11.5, 2.5 Hz, 1.2H), 1.10 (d, *J* = 10.8 Hz, 18H), 0.92 (d, *J* = 1.4 Hz, 18H). ^{19}F NMR (377 MHz, $CDCl_3$)) δ -132.85 (dd, *J* = 16.9, 6.2 Hz), -139.71 (d, *J* = 9.6 Hz).

(*3S, 4R, 5R*)-4-((*tert*-Butyldiphenylsilyl)oxy-5-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-chloro-

3-fluorotetrahydrofuran-2-yl methanesulfonate (11)

To a solution of compound **10** (3.5 g, 5.3 mmol) in DCM (25 mL) was added Et_3N (1.44 mL, 10.6 mmol) and MsCl (0.62 mL, 8.0 mmol) at 0 ^oC. The reaction mixture was stirred 1 h at 0 ^oC and another hour at rt. The reaction was then diluted with DCM (100 mL) and washed with 1 N HCl, 5% $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum to give **11** (3.71 g, 95%), as mixture of α:β isomers (1:1.5). **11** was dried overnight under high vacuum and used in the next step without further purification.

^1H NMR (400 MHz, $CDCl_3$) δ 7.71-7.61 (m, 10H), 7.54-7.51 (m, 9H), 7.47-7.28 (m, 34H), 6.06 (s, 1.6H), 5.97 (d, *J* = 7.5 Hz, 1H), 4.47-4.34 (m, 4.2H), 4.24 (dd, *J* = 18.6, 7.8 Hz, 1.2H), 3.73-3.65 (m, 1H), 3.56 (dt, *J* = 9.5, 4.7 Hz, 1.6H), 3.51-3.40 (m, 3.65H), 3.16 (s, 5H), 2.74 (s, 3H), 1.11(s, 15H) and 1.08 (s, 10H), 0.96 (s, 10H), 0.94 (s, 15H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -131.05 (s), -133.93 (dd, *J* = 18.8, 7.54 Hz).

1-(4-((tert-Butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione **(12)**

A solution of uracil (225 mg, 2.0 mmol) and BSA (0.98 mL, 4.0 mmol) in 1,2 dichloroethane (10 mL) was stirred for 30 min at 80 ^oC. After being cooled to rt, compound **11** (735 mg, 0.99 mmol) in 1,2 dichloroethane (2.0 mL) and TMSOTf (0.36 mL, 2.0 mmol) were added to the reaction mixture. The reaction mixture was then stirred at 80 ^oC for 4 h. After being cooled down to 0 ^oC, the reaction was quenched by

addition of a 5% aqueous solution of $NaHCO_3$ (20 mL), filtered through celite and washed with EtOAc. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with a saturated solution of $NaHCO_3$ and brine. The solution was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by flash chromatography (hexanes/EtOAc 5:1 to 2:1) to afford **12** (506 mg, 67 %) as an α:β mixture (2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (d, *J* = 9.8 Hz, 2H), 7.90-7.64 (m, 6H), 7.61-7.30 (m, 34H), 6.56-6.31 (m, 2H), 5.85 (dd, *J* = 8.2, 1.4 Hz, 1H), 4.88 (d, *J* = 8.2 Hz, 1H), 4.73 (td, *J* = 14.5, 6.0 Hz, 1H), 4.58-4.46 (m, 1H), 4.39 (d, *J* = 4.7 Hz, 1H), 4.17 (dt, *J* = 8.6, 4.9 Hz, 1H), 4.04 (dd, *J* = 12.1, 2.0 Hz, 1H), 3.89 (dd, *J* = 12.1, 2.1 Hz, 1H), 3.82-3.68 (m, 1H), 3.54 (dd, *J* = 11.8, 3.5 Hz, 1H), 1.16 (s, 9H), 1.14 (s, 9H), 0.99 (s, 18H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -121.78 (t, *J* = 14.0 Hz), -137.66 (t, *J* = 14.8 Hz). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{41}H_{47}ClFN_2O_5Si_2$: 757.2696, found: 757.2678.

***1-((2R,3S,4R,5R)-3-Chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl) pyrimidine-2,4(1H,3H)-dione* (13) *and 1-((2S,3S,4R,5R)-3-chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl) pyrimidine-2,4(1H,3H)-dione* (14)**

To a solution of compound **12** (450 mg, 0.59 mmol) in 3.0 mL of anhydrous THF was added, dropwise, 1.26 mL of $Et_3N.3HF$ (2.36 mmol) at rt. The reaction mixture was stirred for 24 h and the volatiles removed under reduced pressure. The residue was

finally purified by flash chromatography (DCM/MeOH 40:1 to 10:1 v/v) to afford **13** (β-isomer, 53 mg, 32%) and **14** (α-isomer, 63 mg, 50%).

Compound **13**: ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.95 (d, J = 8.1 Hz, 1H), 6.35 (d, J = 15.8 Hz, 1H), 5.75 (d, J = 8.1 Hz, 1H), 4.33 (dd, J = 18.0, 9.2 Hz, 1H), 4.05-3.89 (m, 2H), 3.81 (dd, J = 12.7, 2.8 Hz, 1H). ^{19}F NMR (377 MHz, MeOD-d_4) δ -124.31 (s). ^{13}C NMR (101 MHz, MeOD-d_4) δ 165.6, 151.6, 139.9, 114.2 (d, J = 252.4 Hz), 101.8, 87.8 (d, J = 41.6 Hz), 81.3, 74.0 (d, J = 17.8 Hz), 58.6. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_9H_{11}ClFN_2O_5$ 281.0340, found: 281.0330.

Compound **14**: ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.63 (dd, J = 8.2, 3.2 Hz, 1H), 6.50 (d, J = 16.6 Hz, 1H), 5.82-5.75 (m, 1H), 4.55 (dd, J = 19.0, 8.7 Hz, 1H), 4.30-4.19 (m, 1H), 3.91 (dd, J = 12.7, 2.5 Hz, 1H), 3.72 (dd, J = 12.7, 3.5 Hz, 1H). ^{19}F NMR (377 MHz, MeOD-d_4) δ -140.34 (t, J = 17.9Hz). ^{13}C NMR (101 MHz, MeOD-d_4) δ 164.3, 150.7, 141.3, 141.3, 110.4 (d, J = 258.2 Hz), 101.6, 86.6 (d, J = 15.9 Hz), 83.1, 74.2 (t, J = 64.9 Hz), 59.9. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_9H_{11}ClFN_2O_5$: 281.0340, found: 281.0329.

(2S)-Isopropyl-2-(((4-chloro-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate **(16)**

To a solution of **13** (47 mg, 0.167 mmol) and phenyl *L*-isopropylalaninyl phosphorochloridate **15** (77 mg, 0.25 mmol, 1.5 eq) in anhydrous THF (1 mL) was added 1-methylimidazole (20 μL, 2.0 mmol) over 10 min at 0 °C. After stirring for 2 h

at 0 °C, the reaction was maintained for 4 h at rt. After quenching with isopropyl alcohol (0.5 mL), the solvent was removed under reduced pressure and the residue was purified by flash chromatography (DCM/MeOH = 50:1 to 20:1 v/v) to give **2** (44 mg, 48%), as a 1:1 diastereomeric (R_p/S_p) mixture.

^{1}H NMR (400 MHz, MeOD-d_4) δ 7.59 (t, J = 8.5 Hz, 1H), 7.40 (t, J = 7.7 Hz, 2H), 7.33-7.17 (m, 3H), 6.35 (dd, J = 15.7, 9.2 Hz, 1H), 5.70 (dd, J = 22.5, 8.2 Hz, 1H), 5.00 (td, J = 6.4, 4.8 Hz, 1H), 4.65-4.48 (m, 1H), 4.47-4.26 (m, 1H), 4.19-4.08 (m, 1H), 4.01-3.88 (m, 1H), 1.41-1.30 (m, 4H), 1.25 (dd, J = 6.2, 4.5 Hz, 6H). ^{19}F NMR (377 MHz, MeOD-d_4) δ -123.54. ^{31}P NMR (162 MHz, MeOD-d_4) δ 3.65, 3.55. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{21}H_{27}ClFN_3O_9P$: 550.1157, found 550.1144.

N-(1-((2R,3S,4R,5R)-4-((Tert-butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (17a) and N-(1-((2S,3S,4R,5R)-4-((tert-butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)-2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)benzamide (17b).

A mixture of N^4-benzoylcytosine (215 mg, 1.0 mmol) and BSA (0.49 mL, 2.0 mmol) in 1,2 dichloroethane (5 mL) was stirred for 30 min at 80 °C. Compound **11** (370 g, 0.5 mmol) in 1,2 dichloroethane (1.5 mL) and TMSOTf (0.18 mL, 1.0 mmol) were added at rt. The reaction mixture was stirred for 5 h at 80 °C and then quenched by addition of a 5%

aqueous solution of $NaHCO_3$ (15mL) at 0 ^oC. The aqueous layer was extracted with EtOAc, and the combined organic layers were washed with a saturated solution of $NaHCO_3$ and brine. The solution was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography (hexanes/EtOAc 5:1 to 2:1) to afford a **17a** (β isomer, 72 mg, 17%) and **17b** (α isomer, 138mg, 32%).

Compound **17a**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.6 Hz, 2H), 7.83-7.74 (m, 2H), 7.73-7.68 (m, 3H), 7.61 (d, *J* = 7.5 Hz, 1H), 7.56-7.47 (m, 6H), 7.44-7.31 (m, 9H), 6.62 (d, *J* = 13.4 Hz, 1H), 4.48 (dd, *J* = 11.6, 7.7 Hz, 1H), 4.21-4.16 (m, 1H), 3.94 (dd, *J* = 12.1, 2.4 Hz, 1H), 3.77 (dd, *J* = 12.0, 3.0 Hz, 1H), 1.12 (s, 9H), 0.98 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -122.05 (t, *J* = 12.9 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.2, 136.0, 135.6, 135.3, 133.2, 132.9, 132.4, 132.1, 131.6, 130.3, 130.1, 130.1, 129.0, 127.9, 127.9, 127.8, 127.8, 113.0 (d, *J* = 261.7 Hz), 88.1 (d, *J* = 39.5 Hz), 82.5, 61.3, 26.9, 26.8, 19.4, 19.3. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{48}H_{52}ClFN_3O_5Si_2$: 860.3118, found: 860.3095.

Compound **17b**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.6 Hz, 2H), 7.91-7.83 (m, 1H), 7.74-7.62 (m, 5H), 7.59-7.39 (m, 9H), 7.38-7.30 (m, 9H), 6.56 (d, *J* = 13.8 Hz, 1H), 4.65 (dd, *J* = 14.2, 6.7 Hz, 1H), 4.44 (dt, *J* = 6.0, 2.9 Hz, 1H), 3.73 (dd, *J* = 11.8, 3.1 Hz, 1H), 3.56 (dd, *J* = 11.8, 4.0 Hz, 1H), 1.10 (s, 9H), 0.95 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -136.97 (s). ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.1, 135.9, 135.6, 135.5, 133.3, 132.8, 132.5, 132.1, 131.5, 130.3, 130.3, 129.8, 129.8, 129.1, 127.9, 127.8, 127.7, 127.7, 110.8, 109.5 (d, *J* = 263.4 Hz), 87.9 (d, *J* = 16.7 Hz), 84.6, 62.5, 26.8, 26.7, 19.4, 19.1.

HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{48}H_{52}ClFN_3O_5Si_2$: 860.3118, found: 860.3096.

4-Amino-1-((*2R,3S,4R,5R*)-3-chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1*H*)-one (18)

To the solution of compound **17a** (70 mg, 0.81 mmol) in anhydrous THF (1.2 mL) was added, dropwise, $Et_3N.3HF$ (55 mg, 0.34 mmol). After addition, the reaction mixture was stirred for 24 h at rt. Solvents were evaporated under reduced pressure and the residue was filtered through a silica gel pad and eluted with DCM/MeOH (20:1 to 10:1) to give **18** (28 mg, 90%).

^{1}H NMR (400 MHz, MeOD-d_4) δ 8.48 (d, J = 7.6 Hz, 1H), 8.00 (dt, J = 8.5, 1.7 Hz, 2H), 7.72-7.60 (m, 2H), 7.60-7.46 (m, 3H), 6.51 (d, J = 15.1 Hz, 1H), 4.38 (dt, J = 29.2, 14.7 Hz, 1H), 4.12-3.93 (m, 2H), 3.89-3.77 (m, 1H). ^{19}F NMR (377 MHz, MeOD-d_4) δ -125.00. ^{13}C NMR (101 MHz, MeOD-d_4) δ 167.9, 163.9, 156.3, 144.4, 133.2, 132.7, 128.4, 127.8, 113.9 (d, J = 253.7 Hz), 97.4, 88.7 (d, J = 40.6 Hz), 81.6, 74.0 (d, J = 17.7 Hz), 58.6. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{16}H_{16}ClFN_3O_5$: 384.0762, Found: 384.0750.

4-Amino-1-((2R,3S,4R,5R)-3-chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1H)-one (19)

Compound **18** (28 mg, 0.073 mmol) was dissolved in 20% NH_3/MeOH (5 mL) and stirred overnight. After concentration under reduced pressure, the crude product was

purified by flash chromatography (DCM/MeOH 100:1 to 8:1) to afford compound **19** (20 mg, 98%). ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.95 (d, J = 7.6 Hz, 1H), 6.42 (d, J = 16.0 Hz, 1H), 5.95 (t, J = 5.9 Hz, 1H), 4.29 (dt, J = 41.3, 20.7 Hz, 1H), 4.00 (dd, J = 12.7, 2.2 Hz, 1H), 3.97-3.85 (m, 1H), 3.81 (dd, J = 12.7, 2.8 Hz, 1H). ^{19}F NMR (377 MHz, MeOD-d_4) δ -124.27. ^{13}C NMR (101 MHz, MeOD-d_4) δ 166.2, 156.6, 140.6, 114.3 (d, J = 252.5 Hz), 95.1, 88.4 (d, J = 41.0 Hz), 81.1, 74.1 (d, J = 17.8 Hz), 58.6. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_9H_{12}ClFN_3O_4$: 280.0500, found: 280.0489.

Isopropyl((((*2R,3R,4S,5R*)-5-(4-amino-2-oxopyrimidin-1(2*H*)-4-chloro-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (20)

To a solution of **19** (87 mg, 0.31 mmol) in 2 mL of anhydrous THF was added *t*-BuMgCl (0.47 mL, 0.47 mmol, 1.51 eq) at 0 °C. After stirring for 30 min at 0 °C, a solution of phenyl *L*-isopropylalaninyl phosphorochloridate **15** (95 mg, 0.33 mmol, 1.1 eq) in THF (0.5 mL) was added. The reaction was maintained for 3 h at rt, and then quenched with isopropyl alcohol (0.8 mL). The solvent was removed under reduced pressure and the residue was purified by preparative TLC (CH_2Cl_2/MeOH 10:1 v/v) to give **20** as a single phosphorous isomer (30 mg, 18%). The other phosphorous isomer could not be isolated in purity sufficient for biological evaluation. ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.56 (d, J = 7.6 Hz, 1H), 7.40 (dd, J = 8.6, 7.2 Hz, 2H), 7.32-7.21 (m, 3H), 6.42 (d, J = 16.0 Hz, 1H), 5.88 (d, J = 7.6 Hz, 1H), 4.99 (d, J = 6.3 Hz, 1H), 4.57-4.43 (m, 1H), 4.39 (ddd, J = 12.0, 6.4, 3.7 Hz, 1H), 4.28 (dd, J = 15.8, 8.8 Hz, 1H), 4.15-4.04 (m, 1H), 4.02-3.85 (m, 1H),

1.37 (dd, *J* = 7.1, 1.1 Hz, 3H), 1.25 and 1.23 (2s, 6H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 172.9, 172.9, 166.2, 156.3, 150.7, 150.6, 140.5, 129.5, 124.9, 120.0, 119.9, 113.7 (d, *J* = 253.8 Hz), 95.3, 78.9 (d, *J* = 7.9 Hz), 75.0 (d, *J* = 18.3 Hz), 68.7, 64.1, 50.3, 50.3, 20.6, 20.5, 19.1, 19.1. ^{19}F NMR (377 MHz, MeOD-d_4) δ -123.61 (s). ^{31}P NMR (162 MHz, MeOD-d_4) δ 3.49 (s). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{21}H_{28}ClFN_4O_8P$: 549.1308, found: 549.1306.

1,3-Bis(1,1-dimethylethyl)-(9-((2R,3S,4R,5R)-4-((tert-butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)-9H-purin-6-yl)imidodicarbonate (22)

To a solution of compound **10** (260 mg, 0.39 mmol), *N*-Boc_2-adenine (140 mg, 0.42 mmol), and triphenylphosphine (210 mg, 1.0 mmol) in THF (5 mL) was added DIAD (162 mg, 0.8 mmol) dropwise at 0 °C. The ice-bath was removed and the yellow suspension was stirred at rt for 24 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel (hexanes/EtOAc =10:1 to 5:1) to afford crude product **21**. To a solution of compound **21** (86 mg, 0.09 mmol) in THF (0.8 mL) was added $Et_3N.3HF$ (70 mg, 0.43 mmol) at rt and the reaction mixture was stirred for 24 h. The volatiles were removed *in vacuo* and the residue was purified on silica gel (DCM/MeOH = 30:1 to 15:1) to give compound **22** (38 mg, 86%) as a colorless foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96-8.87 (m, 1H), 8.80 (s, 1H), 6.56 (d, *J* = 14.0 Hz, 1H),

4.91 (dd, *J* = 19.4, 9.0 Hz, 1H), 4.27-4.13 (m, 2H), 4.08-3.99 (m, 1H), 1.39 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.6, 152.4, 150.4, 150.1, 143.6, 128.9, 113.9 (d, *J* = 252.1 Hz), 88.4 (d, *J* = 39.8 Hz), 84.5, 82.3, 73.1 (d, *J* = 17.8 Hz), 59.3, 27.7. ^{19}F NMR (377 MHz, $CDCl_3$) δ -125.45 (t, *J* = 15.7 Hz). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{20}H_{28}ClFN_5O_7$: 504.1662, found: 504.1653.

(2R,3R,4S,5R)-5-(6-Amino-9H-purin-9-yl)-4-chloro-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (23)

To a solution of compound **22** (20 mg, 0.04 mmol) in DCM (0.5 mL) was added a 4 N solution of HCl in dioxane (0.1 mL, 0.4 mmol) dropwise. The solution was then stirred at rt for 1 h before the volatiles were removed *in vacuo*. The residue was then dissolved in a 20% solution of NH_3 in methanol (0.5 mL) and the mixture was stirred for 4 h at rt. After concentration under reduced pressure, the residue was purified by preparative TLC (DCM/MeOH = 9:1) to afford product **23** (8.8 mg, 72%) as a white solid.

^{1}H NMR (400 MHz, MeOD-d_4) δ 8.52 (s, 1H), 8.23 (s, 1H), 6.50 (d, *J* = 14.6 Hz, 1H), 4.77 (dd, *J* = 19.0, 9.0 Hz, 1H), 4.20 – 4.01 (m, 2H), 3.91 (dd, *J* = 12.6, 3.0 Hz, 1H). ^{19}F NMR (377 MHz, MeOD-d_4) δ -126.5 (t, *J* = 18Hz, 1F). ^{13}C NMR (101 MHz, MeOD-d_4) δ 156.1, 152.7, 149.1, 139.2, 118.7, 114.1 (d, *J* = 251.3 Hz), 87.8 (d, *J* = 40.0 Hz), 82.0, 73.5 (d, *J* = 17.6 Hz), 59.2. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{10}H_{12}ClFN_5O_3$: 304.0612, found: 304.0601.

Isopropyl (((((2R,3R,4S,5R)-5-(6-amino-9H-purin-9-yl)-4-chloro-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (24)

To a solution of compound **22** (25 mg, 0.05 mmol) in THF (0.4 mL) were added phenyl *L*-isopropylalaninyl phosphorochloridate **15** (1 M in THF, 0.06 mL, 0.06 mmol) and NMI (20 mg, 0.24 mmol) at 0 ^{o}C. The ice-bath was removed and the reaction mixture was stirred at rt for 4 h. The reaction was quenched by addition of *i*PrOH (0.5 mL), and concentrated *in vacuo*. A 10% solution of TFA in water (1 mL) was added to the crude product and the mixture was stirred for 2 h at rt. Solvents were removed *in vacuo*. A 5% aqueous solution of $NaHCO_3$ (1 mL) was added and the mixture was stirred for another hour. Water was evaporated and the residue was purified by preparative TLC (DCM/MeOH = 10:1) to afford product **24** (11 mg, 38%) as a white solid.

^{1}H NMR (400 MHz, MeOD-d_4) δ 8.45 (d, J = 3.9 Hz, 1H), 8.39 (d, J = 5.0 Hz, 1H), 7.42-7.30 (m, 2H), 7.28-7.11 (m, 3H), 6.60 (dd, J = 14.5, 4.4 Hz, 1H), 4.84-4.71 (m, 2H), 4.65-4.42 (m, 2H), 4.39-4.23 (m, 1H), 3.96-3.82 (m, 1H), 1.36-1.27 (m, 3H), 1.20 (ddd, J = 11.2, 6.3, 3.8 Hz, 6H). ^{19}F NMR (377 MHz, MeOD-d_4) δ -126.19 (t, J = 15.7 Hz), -126.38 (t, J = 16.2 Hz); ^{31}P NMR (162 MHz, MeOD-d_4) δ 3.63 (s), 3.52 (s). HRMS (ESI): m/z calcd. for $C_{22}H_{28}ClFN_6O_7P$: 573.1430, Found: 573.1439.

1,3-Bis(1,1-Dimethylethyl)-(9-((2R,3S,4R,5R)-4-((tert-butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)-6-chloro-9H-p

urin-2-yl)imidodicarbonate (25)

To a solution of compound **10** (260 mg, 0.39 mmol), 6-Cl-2-*N*-Boc_2-purine (220 mg, 0.60 mmol), and triphenylphosphine (260 mg, 1 mmol) in THF (10 mL) was added DIAD (160 mg, 0.8 mmol), dropwise, at 0 °C. The ice-bath was removed and the yellow suspension was stirred at rt for 24 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel (hexanes/EtOAc =10:1) to afford pure nucleoside **25** (138 mg, 35%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1 H), 7.30-7.71 (m, 20 H), 6.61 (d, J = 10.2 Hz, 1 H), 4.58 (t, J = 6.2 Hz, 1 H), 4.30 (s, br, 1 H), 3.76-3.79 (m, 1 H), 3.62 - 3.66 (m, 1 H), 1.45 (s, 18 H), 1.13 (s, 9 H), 0.99 (s, 9 H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -125.53 (t, J = 8.96 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.3, 153.3, 152.6, 152.4, 150.6, 140.5, 136.1, 136.0, 135.9, 135.6, 135.5, 135.4, 132.6, 132.4, 130.4, 130.0, 129.8, 128.0, 127.9, 127.8, 127.7, 127.6, 123.6, 113.9 (d, J = 251.0 Hz), 87.3 (d, J = 40.0 Hz), 81.8, 77.6, 77.3, 72.8 (d, J = 17.8 Hz), 59.2, 28.2, 27.8, 26.8, 26.7, 19.4, 19.1; HRMS (ESI): m/z [M+H+] calcd. for $C_{52}H_{63}Cl_2FN_5O_7Si_2$ 1014.3627, found 1014.3640.

1,3-Bis(1,1-Dimethylethyl)-(6-chloro-9-((2R,3S,4R,5R)-3-chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-9H-purin-2-yl)imidodicarbonate (26)

To a solution of compound **25** (300 mg, 0.3 mmol) in THF (5 mL) was added $Et_3N.3HF$ (240 mg, 1.49 mmol) at rt. After 24 h at rt the volatiles were removed *in vacuo* and the residue was purified on silica gel (DCM/MeOH = 20:1) to give compound **26** (150 mg,

93%) as a colorless foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 6.47 (d, J = 14.0 Hz, 1H), 4.88-4.96 (m, 1 H), 4.48 (s, br, 1H), 4.01 - 4.22 (m, 3H), 3.83 (s, br, 1H), 1.43 (s, 18H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -125.58 (t, J = 18.0 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.1, 153.1, 152.3, 152.1, 150.0, 140.2, 123.4, 113.9 (d, J = 251.0 Hz), 87.3 (d, J = 40.0Hz), 81.8, 77.6, 77.3, 72.8 (d, J = 17.8 Hz), 59.2, 27.4, 27.2; HRMS (ESI): m/z [M+H$^+$] calcd. for $C_{20}H_{27}Cl_2FN_5O_7$ 538.1272, found 538.1266.

2-Amino-9-((2R,3S,4R,5R)-3-chloro-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydro furan-2-yl)-1,9-dihydro-6H-purin-6-one (27)

To a mixture of trifluoroacetic acid (2 mL) and H_2O (0.5 mL) was added compound **26** (20 mg) at 0 ^{o}C. The solution was then stirred at rt for 34 h. The volatiles were removed *in vacuo*, and the residue was purified by preparative TLC (DCM/MeOH = 5:1) to afford product **27** (7 mg, 47%) as a white solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.12 (s, 1H), 6.31 (d, J = 14.9 Hz, 1H), 4.67 (dd, J = 18.9, 8.9 Hz, 1H), 4.08-3.97 (m, 2H), 3.87 (dd, J = 12.9, 3.4 Hz, 1H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 157.9, 154.2, 151.6, 135.9, 116.1, 114.1 (d, J = 250.7 Hz), 87.2 (d, J = 39.9 Hz), 81.7, 73.6 (d, J = 17.7 Hz), 59.1. ^{19}F NMR (377 MHz, MeOD-d_4) δ -126.58 (t, J = 16.6 Hz). HRMS (ESI): m/z [M+H]$^+$ calcd. For $C_{10}H_{12}ClFN_5O_4$: 320.0562, Found: 320.0551.

(2S)-Isopropyl

2-(((((2R,3R,4S,5R)-5-(2-amino-6-chloro-9H-purin-9-yl)-4-chloro-4-fluoro-3-hydroxy

tetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (28)

To a solution of compound **26** (30 mg, 0.06 mmol) in THF (0.4 mL) was added NMI (20 mg, 0.24 mmol) at 0 ^{o}C. The ice-bath was removed and the reaction mixture was stirred at rt for 30 min. The reaction mixture was treated with the phenyl *L*-isopropylalaninyl phosphorochloridate **15** (1 M in THF, 0.2 mL, 0.2 mmol) and stirred at rt for 3.5 h. The reaction was quenched by addition of *i*PrOH (0.1mL), and concentrated *in vacuo*. After purification of the residue by preparative TLC (DCM/MeOH = 20: 1), the 6-Cl-2-*N*-Boc$_2$-purine prodrug intermediate was added to a mixture of trifluoroacetic acid (2 mL) and H_2O (0.5 mL) at 0 ^{o}C. The solution was then stirred at rt for 34 h. The volatiles were removed *in vacuo*, and the residue was purified by preparative TLC (DCM/MeOH = 15:1) to afford product **28** (10 mg, 47%, *R*p/*S*p 2.3/1.0) as a white solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.87 (s, 0.3H), 7.85 (s, 0.7H), 7.41-7.28 (m, 2H), 7.28-7.22 (m, 2H), 7.22-7.16 (m, 1H), 6.34 (d, *J* = 16 Hz, 0.7H), 6.32 (d, *J* = 16 Hz, 0.3H), 5.03-4.92 (m, 1H), 4.75 (dd, *J* = 18.1, 8.9 Hz, 1H), 4.64-4.55 (m, 1H), 4.55-4.45 (m, 1H), 4.28-4.15 (m, 1H), 3.98-3.84 (m, 1H), 1.37-1.26 (m, 3H), 1.27-1.11 (m, 6H). ^{19}F NMR (377 MHz, MeOD-d_4) δ -125.44 (t, *J* = 17.2 Hz), -125.77 (t, *J* = 17.1 Hz). ^{31}P NMR (162 MHz, MeOD-d_4) δ 3.74 (s), 3.69 (s). HRMS (ESI): m/z $[M+H]^+$ calcd. For $C_{22}H_{28}ClFN_6O_8P$: 589.1379, Found: 589.1362.

7-((2R,3S,4R,5R)-4-((tert-Butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)meth yl)-3-chloro-3-fluorotetrahydrofuran-2-yl)-4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidin

***e*(32a) and *7-((2S,3S,4R,5R)-4-((tert-butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)-4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidine (32b)**

To a solution of compound **10** (318 mg, 0.48 mmol), 4-chloro-7-iodo-1H-pyrrolo[2,3-d]pyrimidine **29** (134 mg, 0.48 mmol) and triphenylphosphine (252 mg, 0.96 mmol) in anhydrous THF was added DIAD (0.19 mL, 0.96 mmol). After 2 days at rt, the volatiles were removed *in vacuo* and the residue was purified on silica gel (DCM/MeOH 10:1 to 9:1) to afford **32a** (β-isomer, 150 mg, 34 %) and **32b** (α-isomer, 122 mg, 27%).

Compound **32a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.72-7.29 (m, 21H), 6.72 (d, *J* = 13.1 Hz, 1H), 4.58 (dd, *J* = 11.9, 7.3 Hz, 1H), 4.26-4.28 (m 1H), 3.83 (dd, *J* = 11.9, 2.6 Hz, 1H), 3.66 (dd, *J* = 11.9, 4.6 Hz, 1H), 1.11 (s, 9H), 0.99 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -123.55 (t, *J* = 12.5 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.8, 151.1, 150.9, 136.8 – 127.1 (m), 87.5 (d, *J* = 38.4 Hz), 82.9, 76.3 (d, *J* = 16.5 Hz), 62.3, 52.8, 26.8 (d, *J* = 5.8 Hz), 19.3 (d, *J* = 22.7 Hz). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{43}H_{46}Cl_2FIN_3O_3Si_2$: 924.1484, found: 924.1467.

Compound **32b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.79-7.20 (m, 21H), 6.75 (d, *J* = 14.8 Hz, 1H), 4.85 (dd, *J* = 14.8, 6.7 Hz, 1H), 4.42-4.43 (m 1H), 3.76 (dd, *J* = 11.8, 2.6 Hz, 1H), 3.53 (dd, *J* = 11.8, 3.1 Hz, 1H), 1.12 (s, 9H), 0.96 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -136.22 (t, *J* = 14.6 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.7, 151.2,

136.0 – 127.6 (m), 86.5 (d, *J* = 17.2 Hz), 84.1, 62.7, 53.3, 26.8 (d, *J* = 7.3 Hz), 19.4, 19.1.

7-((2R,3S,4R,5R)-4-((tert-Butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)-4-chloro-5-fluoro-7H-pyrrolo[2,3-d]pyrimidine(33a) and 7-((2S,3S,4R,5R)-4-((tert-butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)-4-chloro-5-fluoro-7H-pyrrolo[2,3-d]pyrimidine (33b)

Compound **33a-b** were obtained from **10** using the same procedure as for compounds **32a-b** using 4-chloro-7-fluoro-1H-pyrrolo[2,3-d]pyrimidine **30** instead 4-chloro-7-iodo-1H-pyrrolo[2,3-d]pyrimidine **29**.

Compound **33a**: Yield 34%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 7.78-7.27 (m, 20H), 7.05 (d, *J* = 2.4 Hz, 1H), 6.78 (dd, *J* = 13.4, 1.5 Hz, 1H), 4.61 (dd, *J* = 13.5, 7.7 Hz, 1H), 4.25-4.27 (m, 1H), 3.91 (dd, *J* = 12.0, 2.5 Hz, 1H), 3.74 (dd, *J* = 12.0, 3.7 Hz, 1H), 1.11 (s, 9H), 0.99 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -123.54 (t, *J* = 13.3 Hz, F-2'), -167.99 (F-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.8, 150.6 (d, *J* = 3.9 Hz), 147.2, 141.4 (d, *J* = 254.4 Hz), 137.5 – 125.9 (m), 113.2 (d, *J* = 257.7 Hz), 109.5 (d, *J* = 27.4 Hz), 107.7 (d, *J* = 14.5 Hz), 87.0 (d, *J* = 39.0 Hz), 82.6, 75.7 (d, *J* = 16.6 Hz), 61.9, 26.8 (d, *J* = 6.5 Hz), 19.3 (d, *J* = 27.2 Hz). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{43}H_{46}Cl_2F_2N_3O_3Si_2$: 816.2423, found: 816.2405.

Compound **33b**: Yield 23 %, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.76-7.21 (m,

21H), 6.79 (dd, *J* = 15.7, 1.7 Hz, 1H), 4.86 (dd, *J* = 15.7, 7.0 Hz, 1H), 4.38-4.39 (m, 1H), 3.77 (dd, *J* = 11.8, 2.4 Hz, 1H), 3.51 (dd, *J* = 11.9, 2.9 Hz, 1H), 1.12 (s, 9H), 0.96 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -137.56 (t, *J* = 15.4 Hz, F-2'), -168.51 (F-7). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.8, 150.6 (d, *J* = 3.9 Hz), 148.1, 141.8 (d, *J* = 253.6 Hz), 136.3-127.3 (m), 110.8 (dd, *J* = 27.6, 5.9 Hz), 109.9 (d, *J* = 262.8 Hz), 107.7 (d, *J* = 14.5 Hz), 85.9 (d, *J* = 16.9 Hz), 83.74 62.7, 26.8 (d, *J* = 5.4 Hz), 19.3 (d, *J* = 37.0 Hz).

7-((2R,3S,4R,5R)-4-((tert-Butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (34a) and

7-((2S,3S,4R,5R)-4-((tert-butyldiphenylsilyl)oxy)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)-4-chloro-7H-pyrrolo[2,3-d]pyrimidine (34b)

Compound **34a-b** was obtained from **10** using the same procedure as for compound **32a-b** using 4-chloro-1H-pyrrolo[2,3-d]pyrimidine **31** instead of 4-chloro-7-iodo-1H-pyrrolo[2,3-d]pyrimidine **29**.

Compound **34a**: Yield 36%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.79-7.23 (m, 21H), 6.73 (d, *J* = 13.8 Hz, 1H), 6.24 (d, *J* = 3.8 Hz, 1H), 4.71 (dd, *J* = 14.1, 7.8 Hz, 1H), 4.30 (d, *J* = 7.8 Hz, 1H), 3.93 (dd, *J* = 12.0, 2.3 Hz, 1H), 3.79 (dd, *J* = 12.0, 3.7 Hz, 1H), 1.12 (s, 9H), 0.99 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -123.32 (t, *J* = 14.0 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.1, 151.4, 150.9, 136.7-127.3 (m), 126.9, 118.0, 113.4 (d, *J* = 257.3 Hz), 100.6, 87.6 (d, *J* = 38.7 Hz), 82.5, 75.8 (d, *J* = 16.6 Hz), 61.9, 26.8 (d, *J* =

7.0 Hz), 19.3 (d, *J* = 19.6 Hz). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{43}H_{47}Cl_2FN_3O_3Si_2$: 798.2517, found: 798.2496.

Compound **34b**: Yield 23%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.78-7.16 (m, 21H), 6.75 (d, 1H), 6.24 (d, *J* = 3.8 Hz, 1H), 4.87 (dd, *J* = 15.8, 7.0 Hz, 1H), 4.43 (dd, *J* = 6.5, 3.1 Hz, 1H), 3.78 (dd, *J* = 11.8, 2.4 Hz, 1H), 3.54 (dd, *J* = 11.8, 3.0 Hz, 1H), 1.12 (s, 9H), 0.97 (s, 9H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -137.26 (t, *J* = 16.0 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.2, 151.9, 151.1, 136.7-127.0 (m), 117.85, 101.14, 86.3 (d, *J* = 16.7 Hz), 83.6, 62.7, 26.8 (d, *J* = 6.1 Hz), 19.3 (d, *J* = 37.3 Hz).

(2R,3R,4S,5R)-5-(4-Amino-5-iodo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-chloro-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (35)

To a solution of compound **32a** (114 mg, 0.12 mmol) in 1,4-dioxane (6 ml) and NH_4OH (30%, 12 mL) was placed in a steel vessel which was then sealed and heated at 120 ^{o}C for 16 h. After cooling at rt, the solvent was evaporated and the residue was purified by flash chromatography on silica gel (DCM/MeOH 10:1 to 9:1) to afford compound **35** (29 mg, 56 %). ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.15 (d, *J* = 1.7 Hz, 1H), 7.75 (d, *J* = 1.8 Hz, 1H), 6.58 (d, *J* = 14.9 Hz, 1H), 4.59 (dd, *J* = 19.4, 9.2 Hz, 1H), 4.11-3.98 (m, 2H), 3.85 (dd, *J* = 12.9, 2.9 Hz, 1H). ^{19}F NMR (377 MHz, MeOD-d_4) δ -125.73 (t, *J* = 17.5 Hz). ^{13}C NMR (101 MHz, MeOD-d_4) δ 157.5, 151.7 (C-2), 150.0, 126.7, 114.4 (d, *J* = 250.3 Hz), 103.7, 87.4 (d, *J* = 39.8 Hz), 81.3, 73.5 (d, *J* = 18.1 Hz), 58.9, 51.2. HRMS (ESI): m/z

$[M+H]^+$ calcd. for $C_{11}H_{13}ClFIN_4O_3$: 428.9627, found: 428.9619.

(2R,3R,4S,5R)-5-(4-Amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-chloro-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (36)

Compound **36** was obtained from **33a** using the same procedure as for compound **35**. Yield 62%. ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.13 (s, 1H), 7.39 (d, J = 2.1 Hz, 1H), 6.62 (dd, J = 15.2, 1.8 Hz, 1H), 4.56 (dd, J = 19.7, 9.0 Hz, 1H), 4.07-3.97 (m, 2H), 3.85 (dd, J = 12.5, 2.6 Hz, 1H).^{19}F NMR (377 MHz, MeOD-d_4) δ -125.45- -125.59 (m,), -170.50. ^{13}C NMR (101 MHz, MeOD-d_4) δ 156.2 (d, J = 2.6 Hz), 152.5, 146.2, 143.9 (d, J = 246.7 Hz), 114.6 (d, J = 250.3 Hz), 103.9 (d, J= 28.3 Hz), 87.1 (d, J= 39.7 Hz), 81.2, 73.4 (d, J = 17.7 Hz), 58.9. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{11}H_{12}ClF_2N_4O_3$: 321.0566, found: 321.0558.

(2R,3R,4S,5R)-5-(4-Amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-chloro-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (37)

Compound **37** was obtained from **34a** using the same procedure as for compound **35**. Yield 40%. ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.12 (s, 1H), 7.49 (d, J = 3.8 Hz, 1H), 6.66 (d, J = 3.8 Hz, 1H), 6.57 (d, J = 15.7 Hz, 1H, H-1'), 4.63 (dd, J = 19.3, 9.3 Hz, 1H, H-3'), 4.11-3.99 (m, 2H, H-4'), 3.87 (dd, J = 12.7, 3.1 Hz, 1H, H-5').^{19}F NMR (377 MHz, MeOD-d_4) δ -124.76- -125.06 (m).^{13}C NMR (101 MHz, MeOD-d_4) δ 157.7, 151.1, 149.9, 121.8, 114.7 (d, J = 250.2 Hz), 103.3, 100.1, 87.8 (d, J = 39.0 Hz), 81.1, 73.7 (d, J = 17.8

Hz), 59.2. HR-MS (ESI): m/z $[M+H]^+$ calcd. for $C_{11}H_{13}ClFN_4O_3$: 303.0660, found: 303.0651.

Isopropyl (((((2R,3R,4S,5R)-5-(4-amino-5-iodo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-chloro-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (38)

To a solution of compound **35** (30 mg, 0.07 mmol) in THF (2 mL) at 0 ^oC was added NMI (23 µl, 0.28 mmol). The reaction was stirred at 0 ^oC for 15 min. Then, phenyl *L*-isopropylalaninyl phosphorochloridate **15** (44 mg, 0.14 mmol) was slowly added. The mixture was stirred for 2 h at rt and quenched with EtOAc (4 mL). The organic layer was washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (DCM/MeOH 96/4) to afford prodrug **38** (19 mg, 38%). ^1H NMR (400 MHz, DMSO-d_6) δ 8.16 (d, J = 1.2 Hz, 1H), 7.57 (d, J = 9.2 Hz, 1H), 7.43-7.11 (m, 5H), 6.77-6.63 (m, 2H), 6.57 (dd, J = 16.4, 10.5 Hz, 1H), 6.10 (m, 1H), 4.82 (m, 1H), 4.57 (s, 1H), 4.47-4.29 (m, 2H), 4.22-4.05 (m, 1H), 3.75-3.80 (m, 1H), 1.22-1.18 (m, 3H), 1.18-1.05 (m, 6H).^{19}F NMR (377 MHz, DMSO-d_6) δ -121.78 (t, J = 18.4 Hz), -122.03 (t, J = 18.1 Hz).^{31}P NMR (162 MHz, DMSO-d_6) δ 3.65, 3.56. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{23}H_{28}ClFIN_5O_7P$: 698.0444, found: 698.0434.

Isopropyl

((((2R,3R,4S,5R)-5-(4-Amino-5-fluoro-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-chloro-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (39)

Compound **39** was obtained from **36** using the same procedure as for compound **38**. Yield: 43%. ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.13 (s, 1H), 7.39 (d, *J* = 2.0 Hz, 1H), 7.31-7.17 (m, 5H), 7.03 (t, *J* = 7.2 Hz, 1H), 6.62 (dd, *J* = 15.3, 1.7 Hz, 1H), 4.56 (dd, *J* = 19.7, 9.0 Hz, 1H), 4.06-3.97 (m, 2H), 3.83-3.91 (m, 2H), 1.31-1.15 (m, 9H).^{19}F NMR (377 MHz, MeOD-d_4) δ -125.53 (t, *J* = 17.3 Hz), -170.48.^{31}P NMR (162 MHz, MeOD-d_4) δ -0.84. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{23}H_{28}ClF_2N_5O_7P$: 590.1383, found: 590.1373.

Isopropyl

((((2R,3R,4S,5R)-5-(4-amino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-4-chloro-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (40)

Compound **40** was obtained from **37** using the same procedure as for compound **38**. Yield: 38%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, *J* = 3.2 Hz, 1H), 7.40-7.18 (m, 6H), 6.68 (dd, *J* = 15.8, 8.5 Hz, 1H), 6.37 (dd, *J* = 22.1, 3.8 Hz, 1H), 5.34 (d, *J* = 12.6 Hz, 2H), 4.98-5.06 (m, 1H), 4.75-4.46 (m, 3H), 4.26-3.97 (m, 3H), 1.38 (dd, *J* = 7.0, 3.3 Hz, 3H), 1.26-1.19 (m, 6H).^{19}F NMR (377 MHz, $CDCl_3$) δ -123.88 (t, *J* = 17.5 Hz), -124.46 (t, *J* = 17.0 Hz).^{31}P NMR (162 MHz, $CDCl_3$) δ 3.68, 3.0. LC-MS (ESI): m/z $[M+H]^+$ calcd. for $C_{23}H_{29}ClFN_5O_7P$: 571.14, found: 572.14

1-((2S,3S,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-chloro-3-fluorotetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione (41)

To a solution of compound **14** (280 mg, 1.0 mmol) in DMF (5mL) were added imidazole (340 mg, 5 mmol) and TBDMSCl (375 mg, 2.5 mmol) at 0 ^{o}C. The reaction mixture was stirred overnight at rt. The reaction was quenched with water (50 mL) and extracted with EtOAc. The organic layer was washed with water and dried over Na_2SO_4. Solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography eluting with hexanes/EtOAc (5:1 to 3:1) to afford **41** (421 mg, 83%). Single colorless prism-shaped crystals of (**41**) were obtained from *i*-PrOAc.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 7.39 (dd, *J* = 8.3, 2.9 Hz, 1H), 6.44 (d, *J* = 14.7 Hz, 1H), 5.91-5.69 (m, 1H), 4.67 (dd, *J* = 15.6, 7.0 Hz, 1H), 4.15 (dt, *J* = 7.6, 2.5 Hz, 1H), 3.92 (dd, *J* = 11.8, 3.1 Hz, 1H), 3.76 (dd, *J* = 11.8, 2.7 Hz, 1H), 0.93 (s, 18H), 0.21 and 0.17 (2s, 6H), 0.1 (s, 6H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -138.28 (t, *J* = 15.4 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.1, 150.4, 140.5, 140.4, 109.9 (d, *J* = 262.6 Hz), 102.4, 86.9 (d, *J* = 16.4 Hz), 84.2, 75.6 (d, *J* = 16.5 Hz), 61.0, 25.8, 25.5, 18.2, 18.0, -4.3, -5.0, -5.3, -5.5. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{21}H_{39}ClFN_2O_5Si_2$: 509.2070, Found: 509.2057.

Crystallography: A suitable crystal (0.58 × 0.42 × 0.16 mm) was selected and mounted on a loop with paratone oil on a APEXII diffractometer. The crystal was cooled to *T* = 100(2) K during data collection. The structure was solved with the **XT** (Sheldrick, 2015)

structure solution program using combined Patterson and dual-space recycling methods and by using **Olex2** (Dolomanov et al., 2009) as the graphical interface. The crystal structure was refined with version 2014/7 of **XL** (Sheldrick, 2008) using Least Squares minimisation.

Experimental Extended. A colorless prism-shaped crystal with dimensions 0.58×0.42×0.16 mm was mounted on a loop with paratone oil. X-ray diffraction data were collected using a APEXII diffractometer equipped with an Oxford Cryosystems low-temperature apparatus operating at T = 100(2) K.

Data were measured using ω scans of 1.00° per frame for 30.00 s using MoK$_\alpha$ radiation (sealed tube, 45 kV, 35 mA). The total number of runs and images was based on the strategy calculation from the program **APEX2** (Bruker). The maximum resolution achieved was Θ = 30.643°.

Unit cell indexing was performed by using the **APEX2** (Bruker) software and refined using **SAINT** (Bruker, V8.34A, 2013) on 9881 reflections, 48% of the observed reflections. Data reduction, scaling and absorption corrections were performed using **SAINT** (Bruker, V8.34A, 2013) and **SADABS-2014/5** (Bruker,2014/5). The value of wR_2(int) was 0.0749 before and 0.0513 after correction. The ratio of minimum to maximum transmission is 0.8851. The λ/2 correction factor is 0.00150. The final completeness is 99.8% out to 30.643° in Θ. The absorption coefficient μ of this material is 0.263 mm^{-1} at this wavelength (λ = 0.71073 Å) and the minimum and maximum transmissions are 0.6604 and 0.7461.

The structure was solved in the space group P1 with the XT (Sheldrick, 2015) structure solution program using combined Patterson and dual-space recycling methods. The space group $P2_12_12_1$ (# 19) was determined by the XT (Sheldrick, 2015) structure solution program. The crystal structure was refined by Least Squares using version 2014/7 of **XL** (Sheldrick, 2008). All non-hydrogen atoms were refined anisotropically. Hydrogen atom positions were calculated geometrically and refined using the riding model. Flack parameter was refined to -0.02(3), confirming the absolute stereochemistry. Determination of absolute structure using Bayesian statistics on Bijvoet differences using the program within **PLATON** (Spek, 2003) also report that we have the correct enantiomer based on this comparison. Note: The Flack parameter is used to determine chirality of the crystal studied, the value should be near 0, a value of 1 mans that the stereochemistry is wrong and the model should be inverted. A value of 0.5 means that the crystal consists of a racemic mixture of the two enantiomers.

Cellular uptake and egress studies: For cellular uptake study, compounds were incubated in triplicate with the Huh-7 cells at 10 μM for up to 72 h. The incubation was stopped and samples collected for intracellular metabolites determination at 1, 2, 4, 8, 12, 24, 48 and 72 hours. For cellular egress study, each compound was incubated in triplicate with Huh-7 cells at 10 μM for 24 h. Afterwards, compound-containing medium was removed and replaced with medium free of compound for continued incubation for a further 48 hours. From the point of medium replacement, incubation was stopped and

samples collected at 0, 1, 2, 4, 8, 12, 24 and 48 hours to determine the decay of **16**-TP and sofosbuvir-TP.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Experimental procedures for all biological assays

Molecular Formula Strings.

Author Information

Corresponding Author

*(R.F.S.) Telephone: +1-404-727-1414. Email: rschina@emory.edu

[+] current address: Zagazig University, Chemistry Department, Zagazig, Egypt

Abbreviations used

LiHMDS, lithium bis(trimethylsilyl)amide;

DMF, dimethyl formamide;

THF, tetrahydrofuran;

TMSOTf, trimethysilyl trifluoromethanesulfonate;

$Et_3N.3HF$, triethylamine trihydrofluoride;

NMI, N-methylimidazole;

MsCl, trimethylsulfonyl chloride;

DCM, dichloromethane;

BSA, N,O-bis(trimethylsilyl)acetamide;

DIAD, diisopropyl azodicarboxylate;

PPh_3, triphenyl phosphine;

TFA, trifluoroacetic acid;

NOE, nuclear overhauser effect;

TBDMSCl, *t*-butyldimethylsilylchloride;

i-PrOAc, isopropylacetate;

i-PrOH, isopropylalcohol;

SOF, sofosbuvir;

GT, genotype;

EtOAc, ethyl acetate;

NH_4Cl, ammonium chloride;

$KMnO_4$, potassium permanganate;

Na_2SO_4, sodium sulfate;

$NaHCO_3$, sodium bicarbonate;

rt, room temperature.

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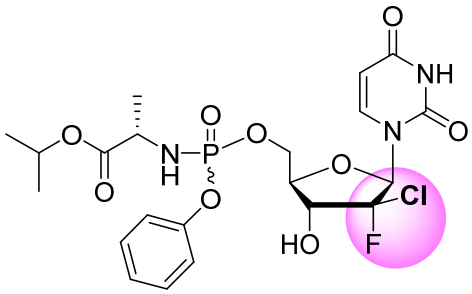
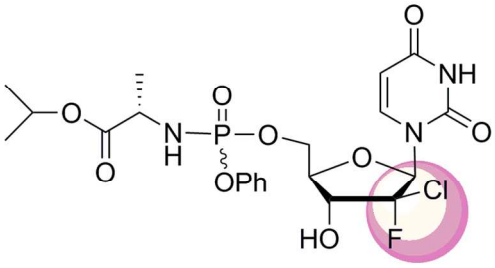
Table of Contents graphic



HCV genotype	1a	1b	2a/k	2b	3a	4
EC_{50} (μM)	0.13	0.15	0.05	0.09	0.15	0.17

Tox in	Huh7	PBM	CEM	Vero
CC_{50} (μM)	>10	>100	>100	>100



Anti-HCV activity (EC_{50}, μM)					
GT1a	GT1b	GT2a/k	GT2b	GT3a	GT4
0.13	0.15	0.05	0.09	0.15	0.17

Cytotoxicity (CC_{50}, μM)			
Huh-7	PBM	CEM	Vero
>100	>100	>100	>100

129x35mm (300 x 300 DPI)