Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.8b01300 • Publication Date (Web): 17 Jan 2019

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Discovery of a Series of 2'-α-Fluoro,2'-β-Bromo-Ribonucleosides and their Phosphoramidate Prodrugs as Potent Pan-Genotypic Inhibitors of Hepatitis C Virus

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KEYWORDS: 2'-modifications, anti-HCV drug, phosphoramidate prodrug, ribo nucleoside, NS5B inhibitor

ABSTRACT: HCV nucleoside inhibitors display pan-genotypic activity, a high barrier to selection of resistant virus and are some of the most potent direct-acting agents with durable sustained virologic response in humans. Herein, we report, the discovery of β-D-2′-Br,2′-F-uridine phosphoramidate diastereomers **27** and **28**, as nontoxic pan-genotypic anti-HCV agents. Extensive profiling of these two phosphorous diastereomers was performed to select one for in depth preclinical profiling. The 5′-triphosphate formed from these phosphoramidates selectively inhibited HCV NS5B polymerase with no inhibition of human polymerases and cellular mitochondrial RNA polymerase up to 100 μM. Both are non-toxic by a variety of measures and display good stability in human blood and favorable metabolism in human intestinal microsomes and liver microsomes. Ultimately, a preliminary oral pharmacokinetics study in male beagles showed that **28** is superior to **27** and is an attractive candidate for further studies to establish its potential value as a new clinical anti-HCV agent.

INTRODUCTION

An estimated 180 million people worldwide are infected with hepatitis C virus (HCV) and about 71 million people have chronic HCV infection. Among those with chronic infection about 15-30% will develop cirrhosis within 20 years and have a significant increased risk for developing an end-stage chronic liver disease, such as hepatocellular carcinoma (HCC).[1] About four hundred thousand people die each year as a result of HCV infection, mostly from cirrhosis and hepatocellular carcinoma. Just a few years ago, the combination therapy with pegylated interferon (Peg-IFN)-alpha plus ribavirin (RBV) was used to treat chronically infected patients.[2] However, the combination of a long treatment

course, variable efficacy across genotypes, a poor safely profile and low patient tolerability has resulted in very limited use of this therapy.

Advances in understanding HCV viral replication cycle revealed multiple proteins or steps in viral life cycle that could be pursued as potential targets for antiviral therapy.[3] Among them, NS3/4A protease, NS5A and NS5B RNA dependent RNA (RdRp) polymerase were extensively studied and as a result, direct acting antivirals (DAA) targeting these viral proteins now forms the mainstay of current HCV treatment.[4] Early NS3/4A protease inhibitors (telaprevir and boceprevir) and NS5A inhibitor (daclatasvir) exhibited high potency, but generally showed a low barrier to resistance. Moreover their activity profile was limited to specific genotypes and associated with significantly high rate of side effects.[5] On the other hand, sofosbuvir (SOF), a nucleoside inhibitor of NS5B RdRp, quickly became the backbone of interferon free HCV treatment[6] due to its high potency, pan-genotypic activity, high barrier to resistance selection and low incidence of side effects.[7] Thus, SOF, in combination with velpatasvir or voxilaprevir (QD) is now part of the most prescribed single tablet regimens for the treatment of HCV genotypes 1-6.[8] Another pan-genotypic combination regimen comprised of glecaprevir and pibrentasvir with a 8-week (BID) treatment course has also been approved for adults who have been previously treated with an NS5A or NS3/4A protease inhibitor and also for new subjects without cirrhosis and preferably low viral load. Although DAAs have improved the HCV treatment prospects significantly, the treatment course remains long at anywhere from eight to twelve weeks. Furthermore, treatment has been dampened due to high cost of currently available DAA. One company, Gilead, markets the only nucleoside (SOF) and does not yet permit other competing companies to use SOF as part of their fixed dose combination with

their DAAs. Therefore, there remains a need for additional safe, pan-genotypic nucleoside analogs with a high barrier to resistance that could be formulated with other non-Gilead DAAs. These should be cost effective and when combined with potent pangenotypic DAAs could lead to a shorter duration of treatment while maintaining very high cure rates.

NS5B RdRp is responsible for replication of HCV RNA and is conserved across all the genotypes and, as such, is considered a target of choice for HCV treatment.[3] Nucleoside inhibitors, such as SOF, are anabolized intracellularly to their 5'-triphosphate form and compete as alternative substrates of the NS5B polymerase. Once incorporated into the growing viral RNA chain, elongation is blocked and RNA replication is stopped.[9] During the last decade, various structural modifications have been made to natural nucleosides with the aim of developing potent and selective anti-HCV nucleoside analogs.[6,10,11] Among those structural modifications, 2'-variations showed higher selectivity rates toward the HCV polymerase *versus* human polymerases, thus crossing the first big hurdle for nucleoside analogs. But so far, SOF (**1**) is the only nucleoside inhibitor approved by the FDA despite a large number of nucleoside analogs that reached human clinical trials (Figure 1). Many of these analogs along with other known preclinical HCV nucleoside inhibitors possess a 2'-α-OH or isosteric group such as a fluorine atom and many also contain a 2'-β–*C*-methyl.[11,12,13] Molecular modeling studies suggest that the 2'-*C*-methyl group of the incorporated nucleoside analog might exerts steric clash to the next incoming nucleotide substrate.[14] We theorized that replacement of the methyl group with other groups of similar size, such as halogens, may exert similar steric effects in the 2'-β-position resulting in new HCV selective inhibitors.

Recently, we reported β-D-2'-deoxy-2'-β-chloro-2'-α–fluoro uridine prodrug, **2**, as non-toxic, potent, pan-genotypic anti-HCV NS5B inhibitor (Figure 1).[15] Pinho et al. reported that the monophosphate prodrug of β-D-2'-deoxy-2'-dichloro-uridine showed activity against HCV NS5B (G1b) while in our hands β-D-2'-deoxy-2'-dibromo uridine nucleotide prodrug showed micromolar potency.[16] Herein, we describe the synthesis of β-D-2'-deoxy-2'-β-bromo-2'-α-fluoro nucleosides and their phosphoramidate prodrugs, **3**, as potent, pan-genotypic anti-HCV agents and select the uridine analog as a potential clinical development candidate (Figure 1).[17]

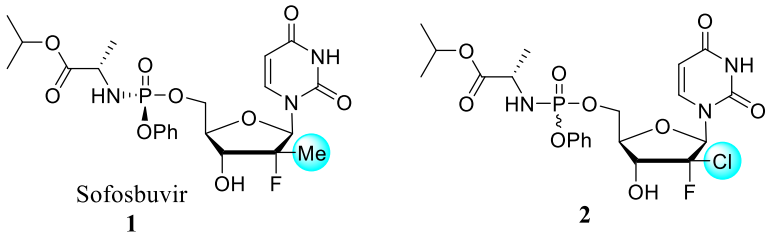


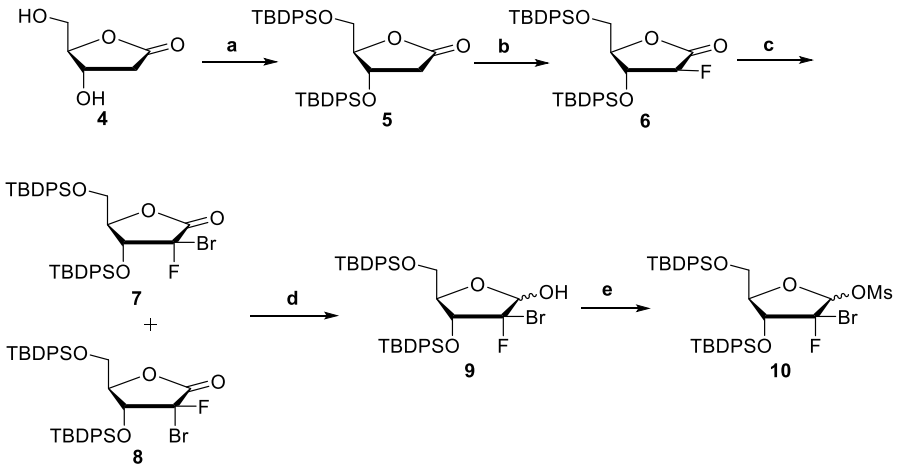
Figure 1. Selected potent HCV antiviral agents and targeted β-D-2'-deoxy-2'-β-bromo-2'-α-fluoro nucleoside prodrugs.

RESULTS AND DISCUSSION

Chemistry. For the preparation of the 2'-bromo-2'-fluoro nucleoside analogs, we chose to capitalize on our group's recently developed protocol[15] for the unsymmetrical synthesis of 2-dihalogenated compounds. Deoxyribonolactone **4** served as the starting material for

synthesis of purine and pyrimidine nucleosides and was readily obtained by oxidation of commercially available 2-deoxy-D-ribose (Scheme 1).[18] Protection of **4** with *tert*-butyldiphenylsilyl groups provided lactone **5** in 85% yield. Fluorination of lactone **5** with *N*-fluorodibenzenesulfonimide (NFSI) in presence of LiHMDS furnished 2-deoxy-2-fluoroarabinolactone **6** in 29% yield.[18] Lower reaction yields were obtained in the fluorination reaction due to a competing beta-elimination of *tert*-butyldiphenylsilyl alkoxide from the enolate along with formation of the 2-gem-difluoro (~5%) compound and a 25% recovery of starting material, **5**. Bromination of compound **6** using *N*-bromosuccinimide (NBS) in presence of LiHMDS afforded the diastereomeric mixture of 2-bromo-2-fluoro lactones **7** and **8** in approximately 1:1 ratio which were separated by flash chromatography on silica gel. The stereochemistry of these 2-dihalogenated compounds were determined at a later stage in the synthesis. Subsequent reduction of **7** with lithium-*tert*-butoxyaluminium hydride (LiAl(*t*-OBu)$_3$H) gave lactol **9** as a mixture of anomers (α/β ratio 1.2/1) in a 90% yield. The lactol **9** was converted to 1-mesylate **10** as an anomeric mixture by treatment with methanesulfonyl chloride and triethylamine in quantitative yield.

Scheme 1. Synthesis of 2-Br,2-F Lactone and Mesylate 10[a]



[a]Reagents and conditions: a) TBDPSCl, imidazole, DMF, rt, 24 h, 85%; b) NFSI, LiHMDS, THF, -78 °C, 1 h, 29%; c) NBS, LiHMDS, THF, -78 °C, 40 min, β-anomer 29% and α-anomer 31%; d) Li(*t*-BuO)$_3$AlH, THF, 2 h, 0 °C to rt, 90%; e) MsCl, Et$_3$N, DCM, 0 °C to rt, 1 h, 99%.

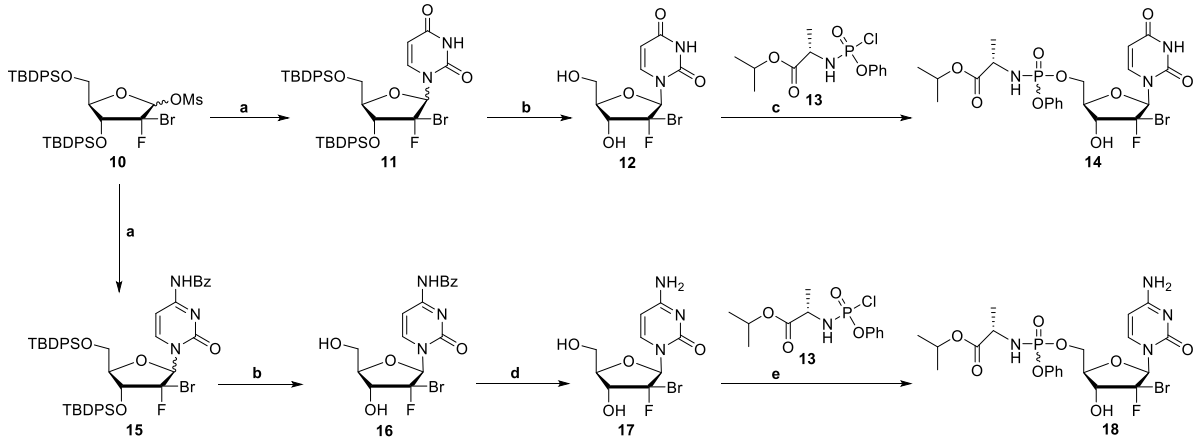
Coupling of 1-mesylate **10** with silylated uracil in presence of TMSOTf afforded the *N*-glycosylated product **11** as an inseparable mixture of α/β (ratio 2/1). Removal of the silyl groups and flash silica gel chromatographic separation gave β-anomer **12** in 21% and α-anomer in 46% yield. Phosphorylation of nucleoside **12** using phenyl-L-isopropylalaninyl phosphorochloridate **13**[19] gave phosphoramidate prodrug **14** as mixture of R_p/S_p isomers (7:3 ratio, assignment unknown). Following a similar protocol for the coupling and deprotection, we separated both α/β isomer of *N*-4-benzoyl protected cytosine nucleoside **16**.

The lower yields and unfavorable anomer ratios we observed in these coupling reactions deserves some further comments. We explored the sugar protecting groups, solvents and Lewis acids quite extensively[20,21] and found the above conditions, while not fully optimized, to provide the best yields and anomer ratios. It is worth noting that our initial attempts to

use Mitsunobu conditions using key lactol **9** and N^3-benzyluracil yielded a mixture of major *O*-glycosylated and minor *N*-glycosylated α and β products.

Deprotection of the *N*-4-amino group of β-**16** with methanolic ammonia furnished fully deprotected nucleoside **17** in 93% yield. Phosphoramidate prodrug **18** was obtained as a mixture of R_p/S_p isomers (ratio 6:4, assignment unknown) by reaction of nucleoside **17** with phenyl-L-isopropylalaninyl phosphorochloridate, **13** in presence of *t*-BuMgCl.

Scheme 2. Synthesis of 2'-α-F,2'-β-Br Pyrimidine Nucleosides and Their Corresponding Prodrugs[a]

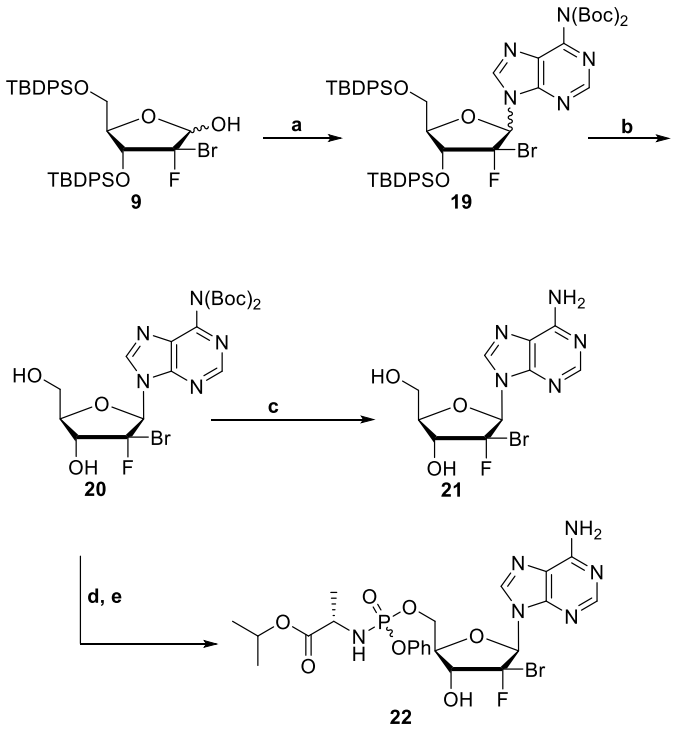


[a]Reagents and conditions: a) i) Uracil or N^4-benzoylcytosine, BSA, DCE, 60 °C, 30 min; ii) TMSOTf, DCE, 80 °C, 5 h, 55-57%; b) 1M TBAF in THF, THF, 0 °C to rt, 1 h, U analog: β-anomer 21%, α-anomer 46%, C analog: β-anomer 24%, α-anomer: 46%; c) NMI, THF, rt, 4 h, 45%; d) NH_3, MeOH, overnight, 93%; e) **13**, *t*-BuMgCl, THF, 0 °C to rt, 3 h, 21%.

Mitsunobu conditions were found to provide the best yields for coupling of purine bases to the 2-Br, 2-F sugar. Hence, coupling of lactol **9** with bis-Boc-adenine gave a mixture of α/β isomers **19** (α/β ratio 3/7) in 39% isolated yield (Scheme 3). Removal of silyl groups and chromatographic separation of α/β isomers gave β-anomer **20** in 44% yield. Further

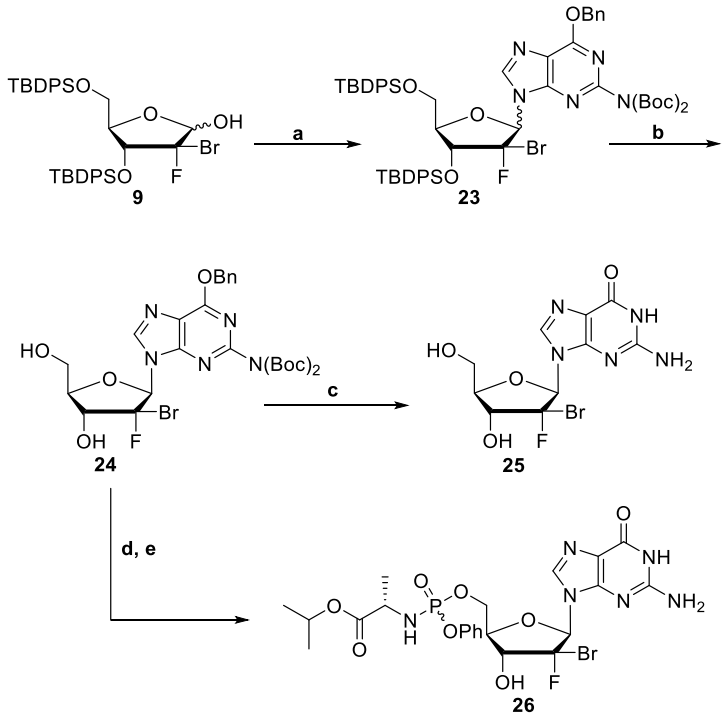
treatment of **20** with BCl_3 afforded adenine nucleoside **21** in 69% yield. Reaction of **20** with phenyl L-isopropylalaninyl phosphorochloridate **13** in presence of NMI followed by removal of the Boc groups using 50% TFA in DCM afforded prodrug **22** as a mixture of R_p/S_p isomers (ratio 7:3, assignment unknown). Likewise, the coupling of lactol **9** with bis-Boc-2-amino-6-benzyloxypurine afforded a mixture of α/β isomers **23** (α/β ratio 3/7) in 40% yield (Scheme 4). Again, removal of silyl groups and chromatographic separation of α/β isomers gave β isomer **24** in 44% yield. The benzyl and Boc protection where removed in one step using BCl_3 to furnish guanine nucleoside **25** in 65% yield. Reaction of compound **24** with phenyl L-isopropylalaninyl phosphorochloridate **13** in presence of NMI followed by Boc group removal using BCl_3 afforded prodrug **26** as a mixture of R_p/S_p (ratio ~ 1:1) isomers in 20% over two steps.

Scheme 3. Synthesis of 2'-α-F,2'-β-Br Adenosine Nucleoside 21 and its Prodrug 22[a]



[a]Reagents and conditions: a) bis-*N*-Boc adenine, DIAD, PPh_3, THF, rt, 24 h, 39%; b) 1M TBAF in THF, THF, 0 °C to rt, 1 h, β-anomer 44%, α-anomer: 25%; c) 1M BCl_3, DCM, -78 °C to rt, 1 h, 69%; d) (i) **13**, NMI, THF, 0 °C to rt, 3 h; (ii) 50% TFA-H_2O, 0 °C to rt, overnight, 40% over two steps.

Scheme 4. Synthesis of 2'-α-F,2'-β-Br Guanine Nucleoside 25 and its Prodrug 26[a]



[a]Reagents and conditions: a) 6-*O*-Bn-bis-*N*-Boc guanine, DIAD, PPh_3, THF, 0 °C to rt, 24 h, 40%; b) 1M TBAF in THF, THF, 0 °C to rt, 1 h, β-anomer 60%, α-anomer: 19%; c) 1M BCl_3, DCM, -20 °C to rt, 2 h, 65% d) (i) **13**, NMI, THF, 0 °C to rt, 3 h; (ii) 1M BCl_3, DCM, , -78 °C to rt, 3 h, 20% over two steps.

Identification of both α and β anomers was determined by 2D-NOE experiments (Figure 2). In all cases, NOEs between H1' and H4' in the β-nucleoside and H1' with H3'/H5' in α-nucleoside were observed.

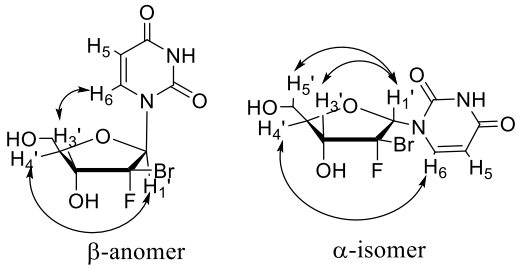


Figure 2. α/β Anomer assignment for nucleoside **12**.

Single crystal of **12** were grown from ethanol by slow evaporation of the solvent. The crystal structure showed a β orientation of both the uracil group and bromine atom and an α orientation of fluorine atom and thus confirming S-configuration at 2'-position and correct anomer selection from the glycosylation reaction (Figure 3).

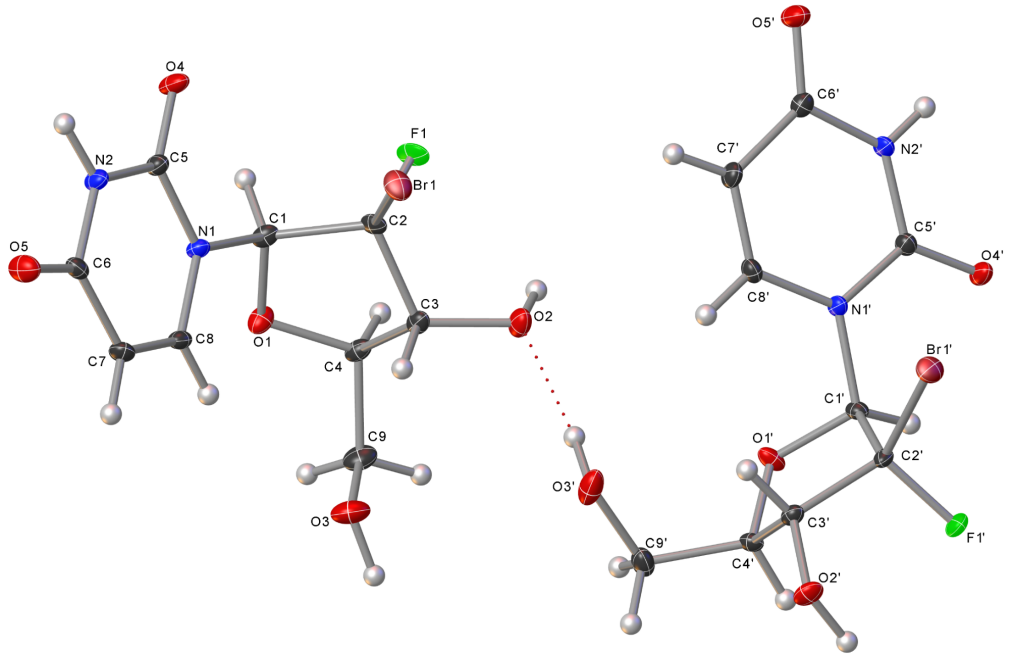


Figure 3. The ORTEP drawing of nucleoside **12** from X-ray crystal analysis.

Antiviral Profile. Parent nucleosides **12**, **17**, **21** and **25** and their corresponding phosphoramidate prodrugs **14**, **18**, **22** and **26** were evaluated for inhibition of HCV genotype 1b RNA replication in Huh-7 cells using a subgenomic HCV replicon system.[22] Cytotoxicity in Huh-7 cells was determined simultaneously by extraction and amplification of both HCV RNA and cellular ribosomal RNA (rRNA).[23] In addition, cytotoxicity was determined in primary human peripheral blood mononuclear (PBM) cells, human lymphoblastoid cells (CEM), and African Green monkey Vero cells.[24,25] The results are summarized in Table 1. The uracil, adenine, and guanine nucleoside analogs **12**, **21**, and

25 were devoid of anti-HCV activity up to 10 μM, while the corresponding monophosphate prodrugs of the uridine and guanine analogs **14** and **26** were active in the submicromolar range ($EC_{50's}$ of 0.4 and 0.6 μM, respectively). Only the cytosine nucleoside analog **17** displayed activity (EC_{50} = 3.9 μM) without the aid of a phosphoramidate prodrug. However, formation of its monophosphate prodrug, **18** allowed for a 5-fold increase of potency. This pattern of activity among the parent nucleoside analogs and their corresponding prodrugs is quite similar to what was observed with our recently reported 2'-Cl,2-F series[15] and the 2'Me,2'-F series[10,21,26] to which SOF belongs. In addition, none of the compounds tested, except for reference compound 2'-*C*-Me-cytidine (NM-107), displayed any cytotoxicity *versus* our panel of cell lines.

Uridine is the only RNA nucleoside in which the *de novo* synthesis of its monophosphate form does not proceed through the parent nucleoside. Indeed, the biosynthesis of uridine monophosphate in humans occurs via decarboxylation of 6-carboxy uridine monophosphate (orotidylate) which is catalyzed by orotidylate decarboxylase. This intricate pathway explains why uridine nucleoside analogs are generally not converted to their triphosphate forms and therefore need to be administered as monophosphate prodrugs to display activity. This also gives uridine derived nucleoside monophosphate prodrugs an inherent safety advantage in fighting liver diseases since it is well established that phosphoramidate prodrugs are largely absorbed and metabolized to their monophosphate forms in the liver. The polarity of the mono-, di-, and tri-nucleotide phosphates traps them in liver tissue until they are ultimately released to systemic circulation as the parent nucleoside analog and eliminated from the body.[27] These properties, unique to uridine analogs, can effectively reduce systemic exposure to uridine analog phosphates and

potential unwanted off target effects. With these considerations combined with the superior potency and lack of cellular toxicity in our panel of cell lines, the uridine phosphoramidate, **14**, was chosen for further study.

Table 1. HCV Genotype 1b Replicon Activity and Cytotoxicity of Synthesized Nucleosides and Their Phosphoramidate Prodrugs

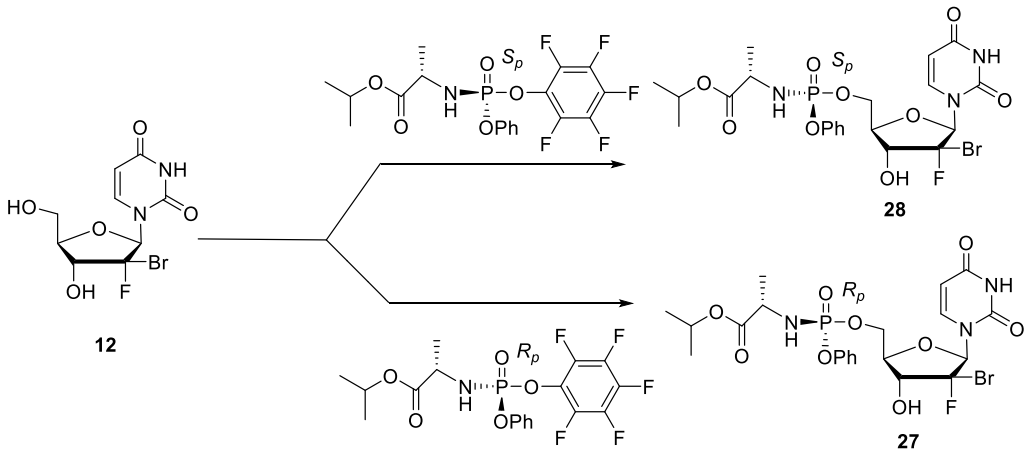
Compound	Anti-HCV EC_{50} μM (SD[a])		Cytotoxicity, CC_{50} μM (SD[a])			
	EC_{50}	EC_{90}	Huh-7	PBM	CEM	Vero
12	> 10	> 10	> 10	> 100	> 100	> 100
14	0.5 (0.05)	0.9 (0.01)	> 10	> 100	> 100	> 100
27	0.7 (0.1)	2.4 (0.1)	> 10	> 100	> 100	> 100
28	0.16 (0.002)	0.28 (0.003)	> 10	> 100	> 100	> 100
17	4.0 (1.6)	9.5 (0.3)	> 10	> 100	> 100	> 100
18	0.6 (0.1)	1.8 (0.2)	> 10	> 100	> 100	> 100
21	> 10	> 10	> 10	> 100	> 100	> 100
22	7.1 (0.2)	> 10	>10	> 100	> 100	> 100
25	> 10	> 10	> 10	> 100	> 100	> 100

26	0.6 (0.1)	1.8 (0.3)	> 10	> 100	> 100	> 100
2'-*C*-Me-C	2.8 (0.2)	9.9 (0.5)	> 100	60 (20)	22 (15)	> 100
SOF	0.5 (0.1)	0.9 (0.03)	> 100	> 100	> 100	> 100

[a]The SD were determined in Excel using the STDEV function (calculated using the "n-1" method) based on the average of three independent EC_{50} and EC_{90} values.

As phosphoramidate **14** is a diastereomeric mixture at the phosphorous center, each stereoisomer was studied independently to determine which may be the most promising to select for human clinical development. The S_P and R_P phosphorous diastereomers were initially separated by column chromatography providing compounds **27** and **28** (Figure 4). When larger amounts of phosphorous single diastereomers were needed the S_P and R_P diastereomers were synthesized by the known literature protocol utilizing isopropyl ((*R* or *S*)-(perfluorophenoxy)-(phenoxy)phosphoryl)-*L*-alaninate (Scheme 5) [28] Use of this synthetic approach also allowed us to assign the absolute stereochemistry to **27** and **28**.

Scheme 5. Synthesis of Diastereomers **27** and **28**[a]



[a]Reagents and conditions: a) *t*-BuMgCl, THF, -5 to 4 °C, 16 h, 68%.

The single diastereomers **27** and **28** were first evaluated for inhibition of HCV genotype 1b RNA replication in Huh-7 cells using a subgenomic HCV replicon system. As noted with SOF, the two diastereomers have different potencies, but again no cytotoxicity was seen in any of the four cell lines tested (Table 1). It is interesting to note that in the case of SOF, clone A cells were used and an 18-fold difference was seen for the diastereomers[9] whereas in our case the difference was only 2.7-fold and only 1.7-fold at the EC_{90}.

Next **27**, **28** and SOF were tested *versus* chimeric replicons GT3a, GT4a, GT5a and polymerase mutation S282T associated with drug resistance (built on a GT1b backbone), GT1a and GT1b stably transfected replicons systems in Huh-7 cells and finally in a GT2a infectious assay (Table 2). While both diastereomers were pan genotypic *versus* all genotypes tested, **28** was more potent than **27** against all genotypes tested. In this assay a bigger difference with **27** and **28** was noted versus GT1b with a 3.8-fold difference at the EC_{50} and a 7.2-fold at the EC_{90}. Similar to SOF, compound **28** displayed an 8 to 9-fold decrease in potency *versus* the S282T mutant while isomer **27** was 38 times less potent. On

a highly positive note, we found that in these assays, phosphoramidate **28** was equipotent or more potent versus SOF at both the EC_{50} and EC_{90} levels.

Table 2. Potency of **27**, **28** and SOF in Huh7 Cells Against Various HCV Genotypes and the Mutant S282T Virus.

Cmpd		EC_{50} and EC_{90} (μM) and (fold increase versus GT1b-WT)[a] (Chimeric replicons, 1a/1b replicons and 2a infectious assay)						
		GT1a	GT2a	GT1b-WT	GT1b/3a	GT1b/4a	GT1b/5a	GT1b/S282T
27	EC_{50}	0.12 ± 0.07 (1.3)	0.16 ± 0.0 (1.7)	0.092 ± 0.0 (1)	0.23 ± 0.01 (2.5)	0.17 ± 0.04 (1.8)	0.21 ± 0.09 (2.3)	3.6 ± 0.12 (38)
	EC_{90}	0.48 ± 0.004 (0.9)	0.83 ± 0.01 (1.6)	0.53 ± 0.7 (1)	1.6 ± 0.02 (3.1)	0.87 ± 0.23 (1.6)	0.80 ± 1.5 (1.5)	21 ± 0.3 (40)
28	EC_{50}	0.055 ± 0.0 (2.3)	0.046 ± 0.15 (1.9)	0.024 ± 0.0 (1)	0.038 ± 0.0 (1.5)	0.021 ± 0.0 (0.9)	0.030 ± 0.01 (1.3)	0.18 ± 0.01 (8)
	EC_{90}	0.19 ± 0.017 (2.6)	0.077 ± 0.0 (1.0)	0.074 ± 0.002 (1)	0.20 ± 0.001 (2.7)	0.13 ± 0.003 (1.7)	0.083 ± 0.02 (1.1)	1.2 ± 0.1 (16)
SOF	EC_{50}	0.061 ± 0.0 (1.8)	0.043 ± 0.01 (1.3)	0.034 ± 0.0 (1)	0.064 ± 0.0 (1.8)	0.058 ± 0.0 (1.7)	0.044 ± 0.01 (1.3)	0.30 ± 0.02 (9)
	EC_{90}	0.32 ±0.06 (2.1)	0.13 ± 0.02 (0.9)	0.15 ± 0.03 (1)	0.37 ± 0.01 (2.5)	0.15 ± 0.02 (1.0)	0.32 ± 0.15 (2.2)	4.4 ± 0.62 (29)

[a]The SD were determined in Excel using the STDEV function (calculated using the "n-1" method) based on the average of three independent EC_{50} and EC_{90} values.

Next, we looked at the functional strength of inhibitors **27** and **28** by evaluating the corresponding 5'-triphosphate, **12**-TP, *versus* a panel of HCV NS5B polymerases (Table 3). While the three inhibitors tested were all pan genotypic versus GT1-6 the $IC_{50's}$ were somewhat surprising in that NM-107-TP was by far the most potent when compared to **12**-TP and 2'-Me,2'-F UTP; which is not the case in cell culture nor in humans (in the case of NM-107-TP). These observations underscore the inherent obstacles in predicting clinical efficacy based on *in vitro* data. Also **12**-TP was noticeably less potent versus 2'-Me, 2'-F

UTP which one would not predict based on the cell culture data presented above. All three nucleoside 5'-triphosphate analogs had a loss of activity *versus* S282T with NM-107-TP having a 570-fold loss in activity *versus* this mutation. Interestingly, although **12**-TP had a 70-fold loss in activity *versus* the S282T mutation we have been unable to select this mutation in cell culture after multiple attempts.

Table 3. 12-TP IC_{50} (μM) *versus* Various Genotypes the NS5B Mutant S282T

	IC_{50} μM (SD)							
Cmpd	GT1b	GT1a	GT2a	GT3a	GT4a	GT5a	GT6a	GT1b S282T
12-TP	0.64 (0.018)	0.38 (0.0065)	1.2 (0.17)	1.8 (0.078)	1.8 (0.048)	1.2 (0.061)	0.59 (0.025)	45 (2.3)
IC_{50} fold change	1	0.59	1.9	2.8	2.8	1.9	0.92	70
2'-Me,2'-F-UTP	0.18 (0.0012)	0.096 (0.0015)	0.29 (0.035)	0.38 (0.013)	0.57 (0.016)	0.43 (0.012)	0.13 (0.0022)	7.3 (0.41)
IC_{50} fold change	1	0.53	1.6	2.1	3.2	2.4	0.72	41
2'-Me-UTP (NM-107-TP)	0.063 (0.0015)	0.025 (0.00018)	0.19 (0.020)	0.047 (0.0021)	0.07 (0.0013)	0.097 (0.0046)	0.047 (0.00089)	36 (1.7)
IC_{50}	1	0.40	3.0	0.75	1.1	1.5	0.75	570

fold change

Cellular Pharmacology. To better understand the difference in cell culture potency of the two diastereomers **27** and **28**, and also to explain the discrepancy between the cell culture data for **28** and SOF *versus* the enzymology data for their TPs, we undertook a study on the cellular uptake and egress profile of **27**, **28** and SOF in Huh-7 cells, and determined the T_{max} and half-lives of nucleoside 5'-triphosphate metabolites. Uptake incubations were performed at 10 μM for 2, 4, 8, 12, 24, 36 and 48 h (Figure 5). Compound **28** provided higher levels of NTP *versus* **27** at every time point studied except 48 h and also more rapidly produces NTP (T_{max} = 12 h and 24 h, respectively), thus explaining the difference in HCV replicon potency for the two diastereomers. Also, at every time point (except 48 h) **28** delivers substantially more NTP intracellularly *versus* SOF, again explaining the discrepancy observed above between the cell culture and enzymology data.

Next, to understand if the difference on potency and NTP formation for **27** and **28** was an artifact of the Huh-7 replicon system or if it might behave this way in other cell systems and ultimately in humans, we repeated the above uptake study but this time with primary human hepatocytes (Figure 5). The observed NTP levels for all phosphoramidates derivatives was substantially higher than that observed in Huh-7 cells and more importantly, **27** and **28** gave virtually identical NTP levels and $T_{max's}$ (8 h). On the other hand, SOF was slower to deliver its NTP with a T_{max} of 12 h, but it ultimately delivered NTP levels that were higher versus **27** and **28**.

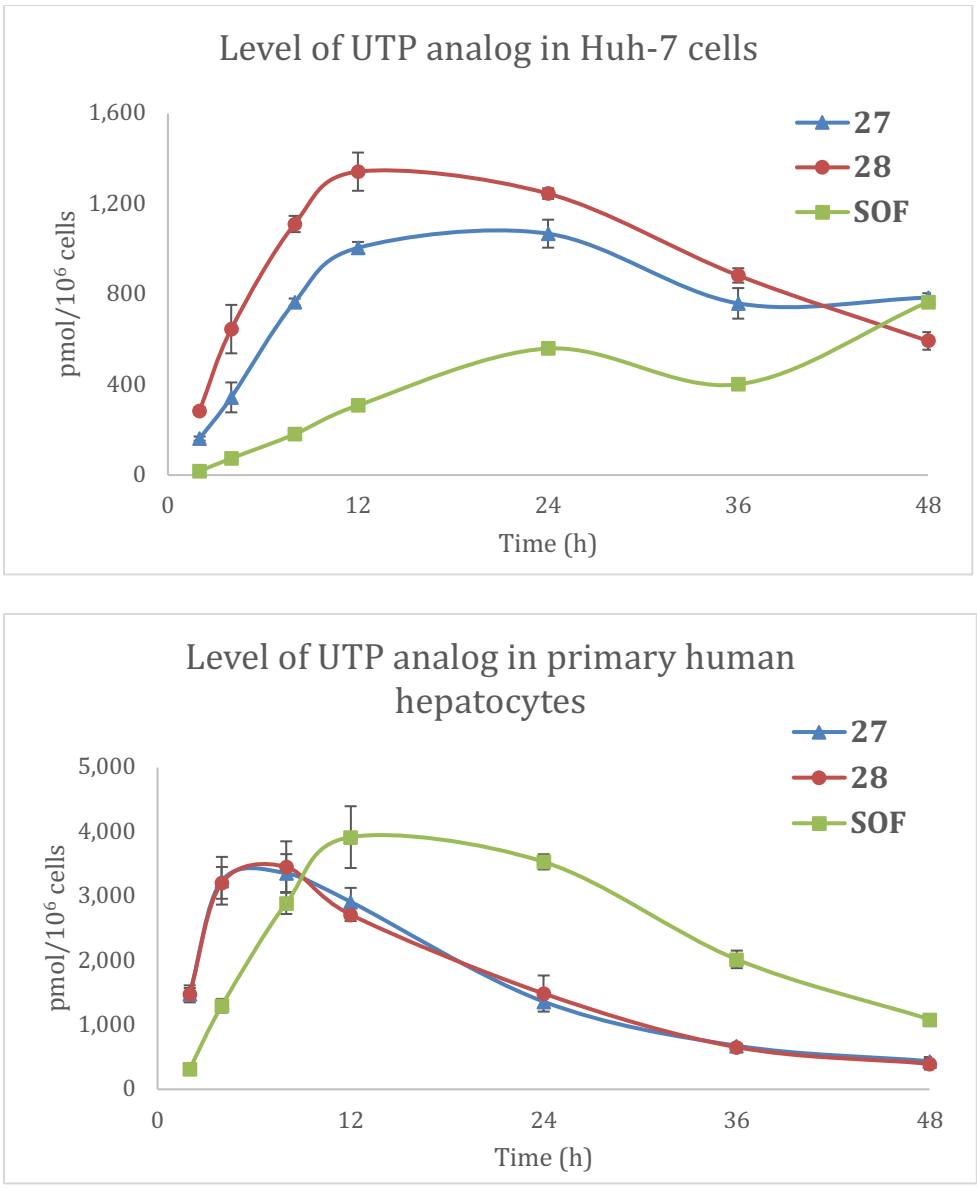
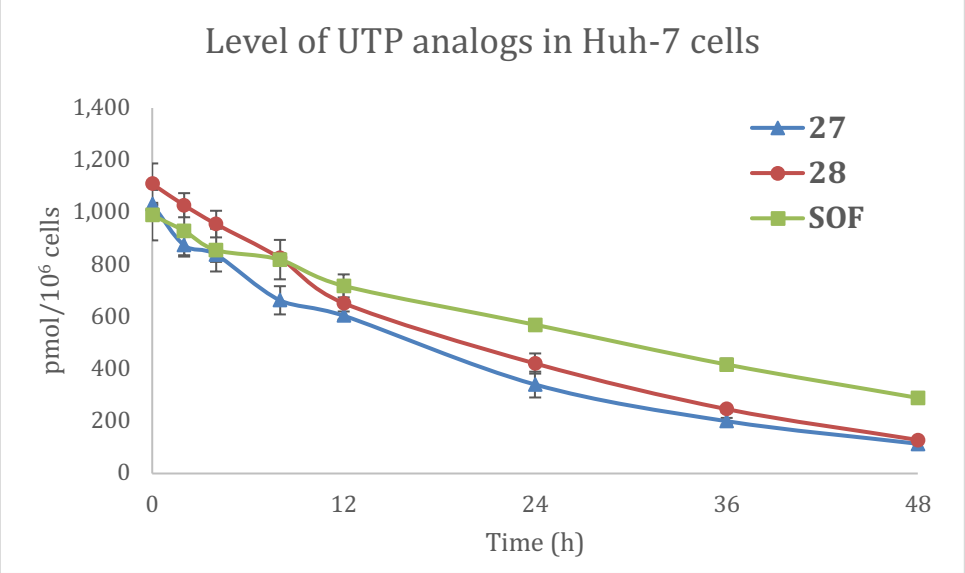


Figure 5. Cellular uptake of **27**, **28** and SOF in Huh-7 cells and primary human hepatocytes (pmol/10^6 cells).

The egress profile of **27**, **28** and SOF were next studied in Huh-7 cells to determine their $T_{1/2}$. Egress incubation was done in triplicate at 10 μM with pre-treatment for 24 h based on the above uptake studies, then a change to new media followed by harvesting cells at 0, 2,

4, 8, 12, 24, 36 and 48 h (Figure 6). As one might predict **27** and **28** did not show much difference in egress profile ($T_{1/2}$ = 15.4 h and 15.7 h, respectively) but both had shorter half-lives versus SOF ($T_{1/2}$ = 28.2 h). We also did a similar egress study in primary human hepatocytes in triplicate at 10 μM with pre-treatment for 12 h and **27**, **28** and SOF show similar egress profiles with $T_{1/2}$ of 15.3 h, 15.0 h and 15.3 h, respectively. Armed with this information, selection of one diastereomer to move forward could not be achieved based solely on cell culture potency and enzymology data as was done with SOF.[9]



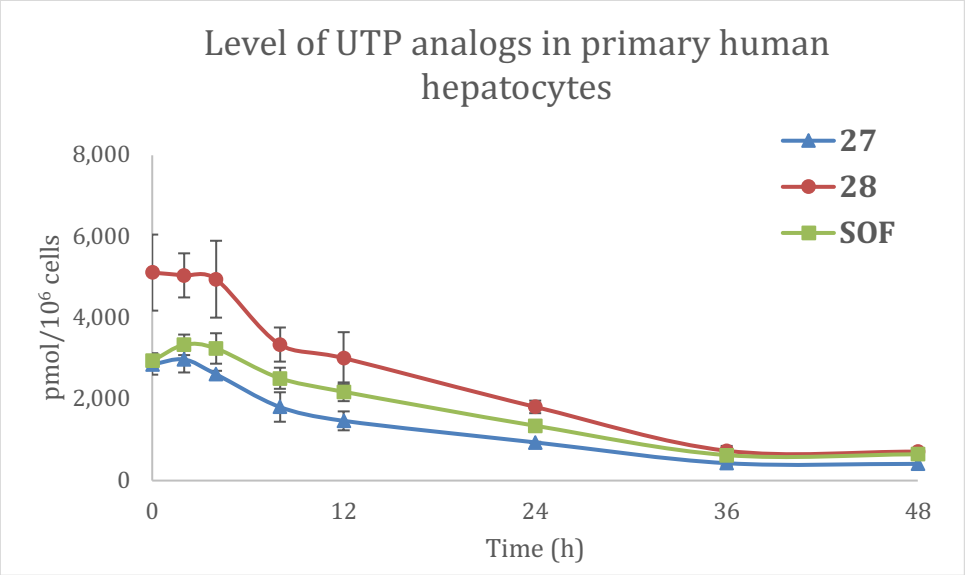


Figure 6. Cellular egress profiles of prodrug **27**, **28** and SOF in Huh-7 cells and primary human hepatocytes (pmol/10^6 cells).

Cytotoxicity Profile. Nucleoside analogs are known to potentially exhibit mitochondrial toxicity.[29] Mitochondrial dysfunction commonly involve the peripheral nervous system and brain, the retina, cardiac and other muscles; and the endocrine, renal, gastrointestinal, hematologic, and hepatic systems which can ultimately result in stroke, pancreatitis, lactic acidosis with liver failure, hepatic steatosis, and myopathy.[30] Therefore, the potential liability for the phosphoramidates **14**, **27**, **28** and the parent nucleoside **12** were evaluated and compared to SOF 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 ribosomal DNA using real-time PCR. Lamivudine (3TC) and β-D-2′,3′-dideoxycytidine (ddC) (at 10 μM) were used as negative and positive controls, respectively (Table 6). At the end of the 14-day assay, neither the parent nucleoside, **12** nor the prodrug diastereomer mixture, **14** showed

measurable mitochondrial toxicity up to 50 μM in HepG2 cell line (Table 6) whereas ddC, as anticipated, was highly toxic at 10 μM. Similar suppression of nuclear DNA for **28** and SOF at 50 μM was noted, with **28** having more impact on mitochondrial DNA levels *versus* SOF. Lactic acid levels were also measured in the culture supernatant after 14 days of incubation with each drug. Increased production of lactic acid (generally above 100% when normalized to ribosomal DNA control) is a marker for the HepG2 cells being under stress and associated with mitochondrial toxicity.[31] In this study, we did not observe increased lactic acid production with the parent nucleoside **12** nor its phosphoramidate diastereomer mixture **14** up to 50 μM. Conversely, at 50 μM an increased lactic acid production was observed for **28**, SOF and the positive control, ddC (at 10 μM) (Table 6). However, no mitochondrial toxicity was noted with **28** when evaluated at 10 μM.

HepG2 cells are highly proliferative immortalized cells that derive a significant proportion of their energy from glycolysis rather than mitochondrial oxidative phosphorylation. The use of glucose containing media with this cell line can mask the effects of potential mitochondrial toxicants. This is referred to as the Crabtree effect and can be evaluated by comparing the cytotoxic effect of drug candidates in glucose versus galactose supplemented media. In galactose containing media the HepG2 cells are forced to generate energy by mitochondrial oxidative phosphorylation so toxic effects of a drug on mitochondrial function is more pronounced. Therefore, **14** was also tested in HepG2 cells for 3 days in glucose and galactose supplemented media up to 100 μM and no toxicity signal was observed in either media. Extending this assay to 14 days for **27** and **28** showed no significant difference between using glucose or galactose supplemented assay media and again both **27** and **28** were not toxic up to 100 μM.

Pluripotent hematopoietic stem cells are found in the primary bone marrow of healthy adults. These cells proliferate and differentiate into all mature hematopoietic cells. When cultured in methylcellulose, individual progenitors called colony-forming cells proliferate and differentiate to form colonies of identifiable progeny. Inhibition of the burst forming unit-erythroid (BFU-E) lineage will present *in-vivo* as red blood cell loss and anemia. Inhibition of colony-forming unit-granulocyte, macrophage (CFU-GM) lineage will present *in-vivo* as neutropenia.[32]

While **12**, **14** and **27** were devoid of BFU-E and CFU-GM bone marrow toxicity up to 100 μM *versus* three separate donors, when **28** was tested we found an IC_{50} = 72 μM for BFU-E and an IC_{50} = 76 μM for CFU-GM. However, no marked cytotoxicity was noted at more physiologic concentrations of 10 μM. In all of these bone marrow toxicity assays we found the positive control AZT exhibited the expected high toxicity in both erythroid and myelomonocytic progenitors at the concentration tested, and 3TC exhibited no toxicity with both progenitors with an $IC_{50's}$ of > 100 μM.

Table 6. Effects of Compounds 12, 27, 28, and SOF 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 (Range)	Lactic acid production (% of control)
12	50	14 ± 3.7 / < 1 ± 0.004	> 50 / > 50	72 (63-83)	69 ± 14

14	50	39 ± 5.8 / 40 ± 3.9	> 50 / > 50	100 (99 - 104)	140 ± 17
27	50	31 ± 2.8 / < 1 ± 0.002	> 50 / > 50	65 (65 - 65)	100 ± 6.2
28	12.5	23 ± 7.9 / 11 ± 6.5		86 (79 - 93)	90 ± 14.6
	25	31 ± 5.6 / 39 ± 2.9	46 ± 8.0 / 32 ± 6.0	110 (110 - 110)	120 ± 1.1
	50	58 ± 7.7 / 72 ± 9.0		150 (150 - 150)	220 ± 6.5
SOF	12.5	6.7 ± 5.1 / 3.1 ± 6.4	> 50 / 45 ± 2.5	96 (90 - 100)	79 ± 17
	50	44 ± 2.8 / 58 ± 3.0		130 (120 - 150)	180 ± 12.5
3TC	10[b]	<10 / <10	> 10 / > 10	140	83 ± 35
ddC	10[b]	84 ± 9.0 / 50 ± 4.5	< 10 / < 10	7.3	200 ± 11
Untreated control	10[b]	0 / 0	N/A	100 (72 - 140)	100 ± 5.0

Cmpd, compound; Conc., concentration; MtDNA, mitochondrial DNA; nDNA, nuclear DNA, N/A, not applicable

Compound **27** and **28** were also evaluated for cytotoxicity in HepaRG cells in a 14-day assay and the CC_{50} values were 7.2 μM and 35 μM, respectively (Table 7). While there is a mild toxicity signal in this HepaRG cell line, we found SOF to have a CC_{50} of 44 μM, very similar to what we found with **28**. The cytotoxicity potential of **27** and **28** was also evaluated in primary human hepatocytes up to 200 μM and found $IC_{50's}$ of 140 μM and 46 μM, respectively. We tested *in vitro* for nephrotoxicity in HK-2 cells in a 3-day assay and found that **28**, **27** and SOF all had $CC_{50's} > 300$ μM. We evaluated the potential for cardiac toxicity by incubating **28**, **27** and SOF with human embryonic stem cell-induced ventricular cardiomyocytes and all three compounds had $CC_{50's} > 100$ μM (Table 7).

Next, **27** and **28** were evaluated for potential effects on hERG (human ether-à-go-go-related gene) potassium channels using CHO cells stably expressing hERG potassium channels at room temperature utilizing the whole-cell patch clamp technique. Both compounds were determined to have IC_{50} values greater than 30 μM while the positive control, amitriptyline had an IC_{50} of 3 μM (Table 7). For comparison, SOF has been reported to be free from hERG channel inhibition,[33,34] although symptomatic bradycardia has been reported when co-administered with amiodarone.[35] However, these effects were found to be non-hERG related.[36]

Table 7. Cytotoxicity (IC_{50}; μM) of Compounds 27 and 28 in Various Cell Lines

Cmpd	HepG2 3 day glucose / galactose	HepG2 14 day glucose / galactose	Bone marrow BFU-E	Bone marrow CFU-GM	HepRG 14 day glucose versus galactose	Primary human hepatocytes	HK-2 cells 3 day	Cardio-myocytes	hERG
27	> 100	> 100	> 100	> 100	7.2	140	> 300	> 100	> 30
28	> 100	> 100	72*	76**	35	46	> 300	> 100	> 30

*Donor specific data 15.8, >100, >100 μM

**Donor specific data 26.5, >100, >100 μM

A Mini Ames study was conducted to evaluate **27** and **28** ability to induce reverse mutations both in the presence and absence of S9 mix at the histidine locus in the genome of four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and at the tryptophan locus in the genome of *Escherichia coli* WP2 *uvr*A (*p*KM101). For **27** and **28** cytotoxicity or precipitate was not observed under any of the test conditions. Both test articles did not induce more than 2-fold increase in strain TA98, TA100, and WP2 *uvr*A (pKM101) nor 3-fold increase in strain TA1535 and TA1537 in the mean number of revertant colonies at any dose level relative to the concurrent negative/solvent control, either in the presence or absence of the S9 mix and no dose response was observed with any strain.

In contrast, for all the bacterial tested strains used in this study, the mean number of *his*$^+$ and *trp*$^+$ revertant colonies observed for the negative/solvent control was comparable to the laboratory historical negative control data. All positive controls induced the expected increase more than three-fold in the mean number of revertant colonies, in the presence and absence of S9 mix, when compared to the concurrent negative/solvent control. This

study concluded that, like SOF,[33] both **27** and **28** were negative for mutagenicity under the conditions of this study.

Selectivity for cellular RNA polymerase. We also tested whether nucleoside 5'-triphosphate analog of **14** was a substrate for the human mitochondrial RNA polymerase (POLRMT). Each nucleoside triphosphate was incubated at 100 μM with POLRMT enzyme and the appropriate DNA/RNA primer/template hybrid and incorporation was evaluated at 2 h. NTP analog incorporation was normalized to that of natural rNTP substrates. As shown in Table 8, **12**-TP was incorporated 7.4% as compared to natural UTP. This value was comparable to the active metabolite of SOF (3.1%) and 2'-*C*-Me-UTP (9.8%). Similar to 2'-*C*-methyl-2'-F-UTP, the active metabolite of SOF, **12**-TP was an exceedingly poor substrate for POLRMT.

Table 8. Incorporation of 12-TP into the Mitochondrial RNA Polymerase (POLRMT)

Inhibitor	POLRMT % incorporation
12-TP	7.4 ± 1.3
2'-F,2'-*C*-Me-UTP	3.1 ± 1.4
2'-*C*-Me-UTP	9.8 ± 4.7
UTP	100

The effect of **12**-TP with human DNA polymerases was evaluated (Table 9). Inhibition of cellular DNA polymerases α, β and γ was determined *in vitro* using commercially available enzymes and appropriate DNA primers and templates and incubated with

increasing concentrations of compound from 0 to 100 μM in a tris-buffered reaction at 37 ºC. Aphidicolin was used as a positive control for DNA polymerase α and ddTTP for DNA polymerases β and γ. Both **12**-TP and 2'-F,2'-*C*-Me-UTP were found to have no inhibitory potency against DNA polymerases α, β and γ (IC_{50} > 100 μM). The positive controls, aphidicolin and ddTTP, inhibited as expected verifying the validity of the test system.

Table 9. Impact of 12-TP on Human DNA Polymerase Activity

Compound	IC_{50} (μM)		
	DNA pol α	DNA pol β	DNA pol γ
12-TP	> 100	> 100	> 100
2'-F,2'-*C*-Me-UTP	> 100	> 100	> 100
Aphidicolin	4.3 ± 0.006	NA	NA
ddTTP	NA	8.4 ± 0.11	0.10 ± 0.07

NA: Not available

Stability in human liver microsomes and gastric fluid. Compounds **27** and **28** were both evaluated for their stability to human liver microsomes. Both compounds were rapidly metabolized with **27** having 27% remaining after 60 min, while **28** was even more extensively metabolized with only 3% remaining after 60 min (Table 10). Incubations of both **27** and **28** with human liver microsomes lacking the NADPD regeneration system

demonstrated that these compounds are metabolized by a non-NADPH dependent metabolism path. Furthermore, these two diastereomers were tested for stability to human intestinal microsomes and found to be quite stable with **27** having a $T_{1/2}$ = 132 min while **28** had a $T_{1/2}$ > 145 min (Table 11). Both **27** and **28** were stable in simulated intestinal fluid at 24 h and had about a 2% loss at 24 h in simulated gastric fluid, both at 37 °C. These data combined with good stability in human plasma (**27** 72%; **28** 90% remaining at 120 min @ 37 °C) indicated that, upon absorption in the GI tract, the compounds could pass to the liver *via* the portal vein largely intact and rapidly convert to the monophosphate form once in liver tissue and thus minimizing systemic exposure to the prodrug form.[27,37]

The presence of an aliphatic bromide in this series of nucleoside analogs warrants some discussion of its stability and potential as an alkylation agent in biological systems. Typically, primary and secondary aliphatic bromides are suitable targets for alkylation by proteins containing nucleophilic amino acids such as arginine, lysine or cysteine *via* substitution reactions while tertiary bromides are more prone to elimination reactions and carbocation or free radical formation and subsequent reaction. It has long been understood that electron withdrawing groups attached to tertiary bromides reduces their reactivity.[38] In the series presented herein, the powerful electron withdrawing effects of the 2'-fluorine atoms suppress both carbocation and free radical formation and, as such, provide a suitably stable tertiary alkyl bromide for *in vivo* utilization.

Table 10. Stability of Compounds 27 and 28 in Human Liver Microsomes

Cmpd	Human Liver Microsomes (0.5 mg protein/mL)

	R^2	$T_{1/2}$ (min)	$Cl_{int(mic)}$ (μL/min/mg)	$Cl_{int(liver)}$ (mL/min/kg)	Remaining (T = 60 min)	Remaining (*NCF = 60 min)
27	0.9827	18.4	75.3	67.8	8.9%	26.6%
28	0.8767	19.7	70.2	63.2	8.0%	3.7%
Testosterone	0.9921	15.5	89.4	80.4	6.5%	86.1%
Diclofenac	0.9935	10.7	129.3	116.3	2.0%	88.1%
Propafenone	0.9638	6.1	226.4	203.8	0.1%	95.6%

*NCF: no co-factor. No NADPH regenerating system is added into NCF sample (replaced by buffer) during the 60 min incubation, if the NCF remaining is less than 60%, then non-NADPH dependent metabolism occurred

R^2: correlation coefficient of the linear regression for the determination of kinetic constant

$T_{1/2}$: half life

Cl_{int} (Mic): the intrinsic clearance; Cl_{int} (Mic) = 0.693/half-life/mg microsome protein per mL

Cl_{int}(liver) = Cl_{int} (Mic) * mg microsomal protein/g liver weight * g liver weight/kg body weight

Table 11. Stability of Compounds 27 and 28 in Biorelevant Fluids at 37 °C

Cmpd	Human intestinal microsomes $T_{1/2}$ (min)	Simulated intestinal fluid (24 h)	Simulated gastric fluid (24 h)	Human plasma (remaining at 120 min)
27	132	stable	~2 % loss	72%
28	> 145	stable	~2 % loss	90%

Compound **14** was found to be 100% stable in male beagle plasma up to 2 h, but highly unstable to rat plasma (0.2% at 2 h). Thus, we choose a preliminary animal study in male

beagles to determine if there was a significant difference in the liver pharmacokinetics of **27** *versus* **28**. We utilized a partially optimized formulation that was administered by oral gavage tube. After a single dose at 10 mg/kg in portal vein cannulated fasted male beagles (2/group; > 6 months old), portal vein and peripheral vein plasma were collected at 0.5 h, 1 h, 2 h and 4 h; in addition, livers were collected at 4 h. We analyzed plasma and liver tissue for prodrugs **27** and **28**, parent nucleoside **12** and, in addition, we looked for **12**-TP in liver tissue only. Not surprisingly the level of **27** and **28** observed in liver tissue was quite low to below the level of detection and the level of **12** in the liver was ten to twenty times lower than the level of **12**-TP. Portal vein levels observed for prodrug **28** were higher than that observed for **27** at every time point except 4 h. For example, at 30 min the level of **28** (average 3,635 ng/mL) was 2.7 times higher than that observed for **27**. There was no marked difference in systemic exposure with **27** and **28** based on low levels of both parent nucleoside and prodrug levels observed in peripheral blood. Despite the small sample size and some variability among the phosphoramidates, the group of dogs that received **28** had **12**-TP levels (average 20,130 ng/mL) in the liver that were 2 times higher than that seen with **27**; clearly indicating the superior absorption and liver uptake of phosphoramidate **28**.

CONCLUSION

Herein, we disclose the synthesis and biological evaluation of a unique series of 2′-bromo,2′-fluoro nucleosides. Among the synthesized compounds, **27** and **28** were potent and specific inhibitors of HCV in culture, but based on all of the data presented and weighted on preliminary liver pharmacokinetic data in dogs, which showed **28** to be better absorbed and produce two times the level of **12**-TP in liver tissue, compound **28** was chosen

to further evaluate as an HCV clinical candidate. Compound **28** had excellent pan-genotypic anti-HCV replicon activity similar to that of SOF. Its NTP (**12**-TP) was a specific inhibitor of HCV NS5B polymerase GT1-6 with no inhibition of human α, β, γ DNA polymerase and showed low incorporation by human mitochondrial RNA polymerase (POLRMT). No marked mitochondrial (MtDNA, nuclear DNA) including lactic acid and bone marrow toxicities were observed up to 10 μM and only mild toxicities were observed for both **28** and SOF at 50 μM. At physiologically concentrations, no increase in lactic acid was noted for **28** and SOF. No toxicities were observed in a large number of cell lines, a Mini Ames was negative *versus* five strains and there was no *in vitro* hERG liability. Compound **28** was highly stable in human blood for up to 2 h, was rapidly metabolized in human hepatocytes, and showed low metabolism in human intestinal microsomes. The novel nucleotide analog **28** has an excellent preclinical profile, suggesting further development to establish its potential value as a clinical anti-HCV nucleoside analog.

EXPERIMENTAL SECTION

General Procedures. Anhydrous solvents were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). All commercially available reagents were used without further purifications. Reagents were purchased from commercial sources. All the reactions were carried out under nitrogen in oven-dried glassware unless otherwise noted. Thin layer chromatography was performed on Analtech GHLF silica gel plates. Column chromatography was accomplished on Combiflash Rf200 or via reverse-phase high performance liquid chromatography. ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra were recorded on a Bruker Ascend 400 spectrometer at 25 °C (400 MHz, 101 MHz, 377 MHz and 162 MHz)

as noted and residual proton solvent signals were used as internal standards. Deuterium exchange and decoupling experiments were utilized to confirm proton assignments. NMR processing was performed with MestReNova version 10.0.2-15465. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), br (broad), bs (broad singlet), m (multiplet). Coupling constants (*J*) are in hertz (Hz). Mass spectra were determined on a Micromass Platform LC spectrometer using electrospray ionization. Purity of final compounds was determined to be >95%, using UPLC analyses performed on a Waters Acquity UPLC System with a Kinetex LC column (2.1 mm Å, 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. Photodiode array detection was from 190 to 360 nm.

2-Deoxy-2-bromo-2-fluoro-3,5-di-*O*-(*tert*-butyldiphenylsilyl)-D-ribonolactone (7, 8).

To a solution of **6** (5.6 g, 8.94 mmol) and NBS (3.18 g, 17.9 mmol) in THF (45 mL) was added LiHMDS in THF (1 M in THF, 14.31 mL, 14.31 mmol), at -78 °C under N_2, dropwise over a period of 15 min. The suspension was stirred at -78 °C for 40 minutes and then quenched with a saturated aqueous solution of NH_4Cl (30 mL). The mixture was allowed to warm to rt and extracted with hexanes (3 x 50 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (30 mL), water (30 mL) and brine (30 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated.

The residue was purified two times by flash column chromatography using 0-1% EtOAC/hexane gradient to give **7** (1.82 g, 29%) and **8** (1.94 g, 31%) as a colorless liquid.

Compound **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72-7.66 (m, 4H), 7.54-7.35 (m, 16H), 4.68-4.65 (m, 2H), 3.68-3.66 (m, 2H), 1.15 (s, 9H), 0.97 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1 (d, *J* = 26.1 Hz), 136.1, 135.8, 135.6, 135.5, 132.4, 132.3, 132.1, 131.4, 130.5, 130.5, 130.0, 130.0, 128.1, 128.0, 127.9, 127.8, 93.0 (d, *J* = 276.1 Hz), 85.4, 75.7 (d, *J* = 15.0 Hz), 61.7, 26.8, 26.7, 19.4, 19.1; ^{19}F NMR (377 MHz, $CDCl_3$) δ -136.0. HRMS (ESI): m/z $[M+Na]^+$ calcd. for $C_{37}H_{42}BrFNaO_4Si_2$: 727.1687, found: 727.1672.

Compound **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63-7.68 (m, 4H), 7.29-7.49 (m, *J* = 78.6 Hz, 16H), 4.56 (dd, *J* = 14.6, 7.9 Hz, 1H), 4.26-4.22 (m, 1H), 3.70-3.72 (dd, *J* = 12.5 Hz, 3.4 Hz, 1H), 3.46 (dd, *J* = 12.5, 3.5 Hz, 1H), 1.13 (s, 9H), 0.86 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.4 (d, *J* = 27.7 Hz), 136.1, 135.8, 135.7, 135.4, 132.7, 132.2, 132.0, 130.7, 130.6, 130.4, 129.9, 129.8, 128.1, 128.0, 127.8, 127.7, 99.3 (d, *J* = 279.1 Hz), 81.2, 81.1, 73.2 (d, *J* = 20.9 Hz), 60.2, 26.7, 26.6, 19.6, 19.1. ^{19}F NMR (377 MHz, $CDCl_3$) δ -128.0 (d, *J* = 14.9 Hz). HRMS (ESI): m/z $[M+Na]^+$ calcd. for $C_{37}H_{42}BrFNaO_4Si_2$: 727.1687, found: 727.1667.

(3*S*,4*R*,5*R*)-3-Bromo-4-((*tert*-butyldiphenylsilyl)oxy)-5-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-fluorotetrahydrofuran-2-ol (9).

To a suspension of **7** (1.81g, 2.57 mmol) in anhydrous THF (15 mL) was added 1 M solution of $LiAl(O^tBu)_3H$ in THF (5.14 mL, 5.14 mmol) at 0 °C. After being stirred at rt for 2 h, the reaction was quenched with saturated NH_4Cl (25 mL) at 0 °C. The mixture was allowed to warm slowly to rt and stirred for 2 h. The reaction mixture was filtered through

a pad of celite and washed with ethyl acetate (30 mL). The aqueous layer was extracted with ethyl acetate (30 mL), and the combined organic layer was washed with saturated $NaHCO_3$ (25 mL), water (20 mL), and brine (20 mL). The solution was dried over Na_2SO_4, filtered and concentrated in *vacuo* to give crude product **9** (1.63 g, 90%) as a mixture of anomers (α/β ratio 1.2:1). The crude product was used as such in the next step.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (m, 4H), 7.67-7.61 (m, 4H), 7.55 – 7.29 (m, 37H), 5.39 (dd, *J* = 11.5, 5.3 Hz, 1H), 5.23 (dd, *J* = 9.1, 6.0 Hz, 1.2H), 4.76 (dd, *J* = 16.4, 6.0 Hz, 1.2H), 4.60 (dd, *J* = 10.8, 5.3 Hz, 1H), 4.33 (m, 1H), 4.18 (s, 0H), 3.69 (d, *J* = 9.1 Hz, 1H), 3.60 (td, *J* = 12.0, 11.5, 3.0 Hz, 2H), 3.51-3.41 (m, 2H), 3.25 (dd, *J* = 11.5, 2.5 Hz, 1H), 1.10 (d, *J* = 10.1 Hz, 20H), 0.92 (d, *J* = 2.3 Hz, 20H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.2, 135.9, 135.9, 135.7, 135.6, 135.6, 135.5, 133.0, 132.9, 132.6, 132.5, 132.1, 132.0, 131.9, 131.8, 130.3, 130.2, 130.2, 130.2, 130.1, 129.0, 129.7, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 108.4 (d, *J* = 265.5 Hz), 103.2 (d, *J* = 273.9 Hz), 101.4 (d, *J* = 19.2 Hz), 99.3 (d, *J* = 31.5 Hz), 83.8, 83.4, 77.6 (d, *J* = 15.7 Hz), 76.7 (d, *J* = 15.1 Hz), 63.1, 62.5, 26.9, 26.9, 26.8, 26.8, 19.44, 19.11, 19.01. ^{19}F NMR (377 MHz, $CDCl_3$) δ -131.45 (s), -139.68 (d, *J* = 10.9 Hz). HRMS (ESI): m/z $[M+Na]^+$ calcd. for $C_{37}H_{44}BrFNaO_4Si_2$: 729.1843, found: 729.1831.

(3*S*,4*R*,5*R*)-3-Bromo-4-((*tert*-butyldiphenylsilyl)oxy)-5-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-fluorotetrahydrofuran-2-yl methanesulfonate (10).

To a solution of **9** (1.6 g, 2.26 mmol) in CH_2Cl_2 (15 mL) were added Et_3N (0.62 mL, 4.5 mmol) and MsCl (0.26 mL, 3.4 mmol) 0 °C. After stirring 1 h at 0 °C, the mixture was allowed to warm up to rt and stirred for 1 h. The reaction mixture was then diluted with

CH_2Cl_2 (100 mL), washed with 1N HCl (25 mL) followed by 5% $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give crude product **10** (1.72 g, quantitative) as a mixture of anomers (α/β ratio 2.5:1). This anomeric mixture was dried under high vacuum and used as described in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75-7.34 (m, 54H), 6.25 (s, 1.7H), 6.06 (d, *J* = 7.4 Hz, 1H), 4.61-4.59 (m, 1.7H), 4.53-4.51 (m, 2.7H), 4.32 (dd, *J*=19.5, 7.7 Hz, 1H), 3.72-3.54 (m, 3.7H), 3.41 (dd, *J* = 11.9, 6.5 Hz, 1H), 3.17 (s, 5.4H), 2.78 (s, 3H), 1.15 (2s merged, 24.3H), 1.01 (2s merged, 24.3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.2, 136.0, 135.8, 135.8, 135.6, 135.6, 135.5, 135.5, 132.9, 132.7, 132.7, 132.6, 132.5, 132.2, 131.9, 131.3, 130.4, 130.3, 130.3, 130.3, 129.9, 129.8, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 104.9 (d, *J* = 19.6 Hz), 104.7 (d, *J* = 260.1 Hz), 102.8 (d, *J* = 36.5 Hz), 101.7 (d, *J* = 288.7 Hz), 87.9, 84.9, 77.0 (d, *J* = 15.8 Hz), 76.7 (d, *J* = 15.4 Hz), 63.6, 62.5, 40.2, 39.9, 26.8, 26.8, 26.7, 19.47, 19.42, 19.17, 19.13. ^{19}F NMR (377 MHz, $CDCl_3$) δ -131.1, -133.4 (dd, *J* = 20.4, 6.7 Hz).

1-((3*S*,4*R*,5*R*)-3-Bromo-4-((*tert*-butyldiphenylsilyl)oxy)-5-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-fluorotetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (11).

To a suspension of uracil (0.187 g, 1.67 mmol) in DCE (1 mL) was added BSA (0.817 mL, 3.34 mmol). The reaction mixture was stirred at 60 °C for 30 min and then allowed to cool to rt. To the resulting homogeneous solution, **10** (0.655 g, 0.835 mmol) in DCE (2 mL) and TMSOTf (0.604 mL, 3.34 mmol) were added. The reaction mixture was then stirred at 80 ° C for 5 h. The reaction was quenched by addition of 5% aqueous solution of $NaHCO_3$ (15 mL) at 0 °C, filtered through Celite and washed with ethyl acetate (25 mL).

The aqueous layer was extracted with ethyl acetate (25 mL), and the combined organic layers were washed with a saturated solution of $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL). The solution was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane gradient to afford **11** (0.385 g, 57%) as a 2:1 (α:β) mixture.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (2s merged, 1.5H), 7.79-7.12 (m, 31.5 H), 6.46 – 6.38 (2d merged, 1.5H), 5.80-5.77 (m, 1.5H), 4.80 – 4.72 (m, 1H), 4.57 (dd, *J* = 15.1, 8.2 Hz, 0.5H), 4.33-4.29 (m, 1H), 4.17 – 4.07 (m, 0.5H), 3.98 (dd, *J* = 12.1, 2.4 Hz, 0.5H), 3.82 (dd, *J* = 12.1, 2.4 Hz, 0.5H), 3.69 (dd, *J* = 11.8, 2.7 Hz, 1H), 3.45 (dd, *J* = 11.8, 3.6 Hz, 1H), 1.10 -1.08 (2s merged, 13.5H), 13.5 (s, 13.5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.0, 162.7, 150.2, 150.1, 140.7, 140.6, 138.9, 136.3, 136.1, 135.9, 135.6, 135.5, 135.5, 135.2, 133.1, 132.7, 132.5, 132.4, 132.1, 131.8, 131.6, 131.5, 130.3, 130.1, 129.9, 129.8, 129.8, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 108.3 (d, *J* = 268.1 Hz), 102.6, 102.5 (d, *J* = 273.2 Hz), 102.5, 88.1 (d, *J* = 39.3 Hz), 87.7 (d, *J* = 15.4 Hz), 84.1, 82.5, 77.7 (d, *J* = 15.4 Hz), 76.8 (d, *J* = 16.2 Hz), 62.7, 61.0, 26.9, 26.8, 26.7, 19.4, 19.4, 19.3, 19.0. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{41}H_{47}BrFN_2O_5Si_2$: 801.2191, found: 801.2186.

1-((2*R*,3*S*,4*R*,5*R*)-3-Bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (β-12) and 1-((2S,3*S*,4*R*,5*R*)-3-Bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (α-12).

To a stirred solution of **11** (0.385g, 0.48 mmol) in anhydrous THF (2.5 mL), was added 1 M solution of TBAF in THF (0.962 mL, 0.962 mmol) at 0 °C. The reaction mixture was stirred for 1 h at rt. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using 0-6% MeOH/CH_2Cl_2 gradient to afford **12** (β-isomer, 34 mg, 21%) and α-isomer (72 mg, 46%).

β-Anomer (**12**)- ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.96 (d, J = 8.1 Hz, 1H), 6.36 (d, J = 16.5 Hz, 1H), 5.76 (d, J = 8.2 Hz, 1H), 4.44 (dd, J = 19.8, 9.2 Hz, 1H), 4.01 (dd, J = 12.7, 2.2 Hz, 1H), 3.98 – 3.92 (m, 1H), 3.80 (dd, J = 12.7, 2.7 Hz, 1H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 164.2, 150.6, 139.9, 109.3 (d, J = 261.5 Hz), 101.8, 88.4 (d, J = 39.7 Hz), 81.4, 75.2 (d, J = 17.2 Hz), 58.5. ^{19}F NMR ((400 MHz, MeOD-d_4) δ -122.60 (s). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_9H_{11}BrFN_2O$: 324.9835, found: 324.9833. α -anomer: ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.62 (dd, J = 8.2, 3.4 Hz, 1H), 6.59 (d, J = 18.1 Hz, 1H), 5.75 (d, J = 8.2 Hz, 1H), 4.62 (dd, J = 20.6, 8.8 Hz, 1H), 4.22-4.17 (m, 1H), 3.89 (dd, J = 12.7, 2.5 Hz, 1H), 3.70 (dd, J = 12.7, 3.4 Hz, 1H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 164.3, 150.7, 141.4 (d, J = 6.1 Hz), 103.5 (d, J = 268.2 Hz), 101.5, 87.3 (d, J = 14.8 Hz), 82.6, 76.0 (d, J = 16.5 Hz)], 59.8. ^{19}F NMR (377 MHz, MeOD-d_4) δ -139.05 (s). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_9H_{11}BrFN_2O$: 324.9835, found: 324.9834.

(2S)-Isopropyl ((((2*R*,3*R*,4*S*,5*R*)-4-bromo-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-propanoate (14).

To a stirred solution of high vacuum dried **12** (21 mg, 0.064 mmol) and (2*S*)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate, **13** (39 mg, 0.13 mmol) in 1 mL of

anhydrous THF under nitrogen atmosphere, was added NMI (10 μL, 0.13 mmol) slowly. After stirring for 2 h at 0 °C, the reaction was warmed slowly to warmed to rt and stirred for 2 h. The reaction was quenched with isopropyl alcohol (0.2 mL). The solvent was removed under reduced pressure and the residue was purified by flash chromatography using 0-6% MeOH/CH_2Cl_2 to afford **14** (17 mg, 45%) as a diastereomeric (R_p/S_p ~ratio 7:3) mixture.

^{1}H NMR (400 MHz, MeOD-d_4) δ 7.57 (2d merged, each J = 8.1 Hz, 1H), 7.42-7.38 (m, 2H), 7.32–7.15 (m, 3H), 6.39-6.32 (m, merged, 1H), 5.70 (2d, each J = 8.1 Hz, 1H), 5.04-4.96 (m, 1H), 4.65–4.33 (m, 4H), 4.23 – 4.08 (m, 1H), 3.95-3.90 (m, 1H), 1.38 – 1.32 (m, 3H), 1.36–1.23 (m, 6H). ^{19}F NMR (377 MHz, MeOD-d_4) δ -121.77, -122.10. ^{13}C NMR (101 MHz, MeOD-d_4) δ 173.2 (d, J = 4.5 Hz), 172.9 (d, J = 5.4 Hz), 164.1, 150.7, 150.4, 139.9, 139.6, 129.5, 124.9, 124.9, 120.0, 119.9, 119.9, 109.9, 107.3, 102.2, 102.1, 79.2, 79.2, 76.1 (d, J = 17.2 Hz), 75.8 (d, J = 17.3 Hz), 68.8, 68.8, 64.1, 63.7, 50.4, 50.3, 20.6, 20.5, 19.1 (d, J = 6.4 Hz), 18.9 (d, J = 7.4 Hz). ^{31}P NMR (162 MHz, MeOD-d_4) δ 3.64, 3.54. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{21}H_{27}BrFN_3O_9P$: 594.0652, found: 594.0642.

_N_-(1-((3_S_,4_R_,5_R_)-3-Bromo-4-((*tert*-butyldiphenylsilyl)oxy)-5-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-fluorotetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (15).

To a suspension of *N*-benzoyl cytosine (0.502 g, 2.33 mmol) in DCE (1 mL) was added BSA (1.142 mL, 4.67 mmol). The reaction mixture was stirred at 60 °C for 30 min and then allowed to cool to rt. To the resulting homogeneous solution, **10** (0.916 g, 1.16 mmol) in DCE (2 mL) and TMSOTf (0.846 mL, 4.67 mmol) were added. The reaction mixture

was then stirred at 80 ° C for 5 h. The reaction was quenched by addition of 5% aqueous solution of $NaHCO_3$ (15 mL) at 0 °C. The aqueous layer was extracted with ethyl acetate (50 mL), and the combined organic layers were washed with a saturated solution of $NaHCO_3$ (25 mL), water (25 mL), and brine (25 mL). The solution was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography using EtOAc/hexane gradient to afford **15** (0.576 g, 55%) as a 2:1 (α:β) mixture.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 – 7.25 (m, 38H), 6.68 (d, *J* = 15.0 Hz, 1H), 6.59 (d, *J* = 14.2 Hz, 0.4H), 4.77 (dd, *J* = 15.3, 6.7 Hz, 1H), 4.62 (dd, *J* = 12.5, 7.7 Hz, 0.4H), 4.49 – 4.39 (m, 1H), 4.24 – 4.16 (m, 0.4H), 3.95 (dd, *J* = 12.1, 2.4 Hz, 0.4H), 3.80-3.71 (m, 1.4H), 3.54 (dd, *J* = 11.8, 4.0 Hz, 1H), 1.13 (s, 3.6H), 1.11 (s, 9H), 0.99 (s, 3.6H), 0.96 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9, 162.7, 162.4, 154.5, 145.4, 144.0, 136.8, 136.3, 136.2, 136.1, 136.0, 136.0, 135.9, 135.7, 135.7, 135.7, 135.6, 135.6, 135.4, 135.3, 133.7, 133.5, 133.2, 133.1, 132.8, 132.7, 132.5, 132.1, 132.0, 131.6, 131.5, 130.3, 130.3, 130.3, 130.1, 130.0, 129.8, 129.7, 129.5, 129.4, 129.3, 128.9, 128.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 127.3, 127.2, 108.3 (d, *J* = 271.8 Hz), 102.0 (d, *J* = 273.9 Hz), 88.8 (d, *J* = 38.0 Hz), 88.6 (d, *J* = 15.6 Hz), 84.4, 82.6, 78.2 (d, *J* = 15.5 Hz), 77.3 (d, *J* = 16.2 Hz), 62.7, 61.3, 26.9, 26.8, 26.8, 26.7, 19.4, 19.0. ^{19}F NMR (377 MHz, $CDCl_3$) δ -118.98 (t, *J* = 13.3 Hz), -136.16. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{48}H_{52}BrFN_3O_5Si_2$: 904.2613, found: 904.2605.

***N*-(1-((2*R*,3*S*,4*R*,5*R*)-3-Bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)benzamide (16).**

To a stirred solution of **15** (0.560 g, 0.62 mmol) in anhydrous THF (2.5 mL), was added 1 M solution of TBAF in THF (0.930 mL, 0.930 mmol) at 0 °C. The reaction mixture was stirred for 1 h at rt. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using 0-5% MeOH/CH_2Cl_2 gradient to afford β-**16** (β-isomer, 62 mg, 24%) and α-isomer (122 mg in 46%).

β-Anomer: ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.47 (d, J = 7.6 Hz, 1H), 8.01-7.99 (m, 2H), 7.69-7.64 (m, 2H), 7.58-7.54 (m, 2H), 6.52 (d, J = 16.0 Hz, 1H), 4.51 (dd, J = 19.2, 9.0 Hz, 1H), 4.13 – 3.97 (m, 1H), 3.85 (dd, J = 12.5, 2.5 Hz, 1H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 167.8, 163.9, 156.4, 144.4, 133.2, 132.8, 128.4, 127.8, 108.9 (d, J = 262.9 Hz), 97.5, 89.4 (d, J = 40.5 Hz), 81.7, 75.3 (d, J = 17.1 Hz) 58.5. ^{19}F NMR (377 MHz, MeOD-d_4) δ -123.31 (s). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{16}H_{16}BrFN_3O_5$: 428.0257, found: 428.0251.

α-Anomer: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.39 (s, 1H), 8.16 (d, J = 5.9 Hz, 1H), 8.02 (d, J = 7.3 Hz, 2H), 7.65 (t, J = 7.4 Hz, 1H), 7.53 (t, J = 7.7 Hz, 2H), 7.42 (d, J = 7.6 Hz, 1H), 6.69 (d, J = 17.6 Hz, 1H), 6.60 (d, J = 6.9 Hz, 1H), 5.13 (dd, J = 6.5, 4.9 Hz, 1H), 4.57 (dt, J = 21.2, 7.8 Hz, 1H), 4.22 (d, J = 8.4 Hz, 1H), 3.77-3.71 (m, 1H), 3.65 – 3.52 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.9, 164.2, 154.9, 146.9 (d, J = 5.0 Hz), 133.5, 133.3, 129.0, 128.9, 104.2 (d, J = 269.5 Hz), 97.2, 87.9 (d, J = 14.8 Hz), 83.3, 76.4 (d, J=16.2 Hz), 60.3. ^{19}F NMR (377 MHz, DMSO-d_6) δ -135.76 (t, J = 19.3 Hz). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{16}H_{16}BrFN_3O_5$: 428.0257, found: 428.0250.

4-Amino-1-((2*R*,3*S*,4*R*,5*R*)-3-bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1*H*)-one (17).

Compound **16** (60 mg, 0.14 mmol) was dissolved in 20% NH_3/MeOH (5 mL) and the reaction mixture was stirred overnight at rt. After the solvent was removed under reduced pressure, the crude product was purified by flash chromatography using 0-10% MeOH/CH_2Cl_2 gradient to afford nucleoside **17** (42 mg, 93%).

^{1}H NMR (400 MHz, MeOD-d_4) δ 7.93 (d, J = 7.5 Hz, 1H), 6.44 (d, J = 16.8 Hz, 1H), 5.95 (d, J = 7.6 Hz, 1H), 4.42 (dd, J = 19.5, 9.3 Hz, 1H), 4.01 (dd, J = 12.7, 2.2 Hz, 1H), 3.96 – 3.90 (m, 1H), 3.81 (dd, J = 12.7, 2.7 Hz, 1H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 166.2, 156.6, 140.5, 109.5 (d, J = 261.9 Hz), 95.2, 89.1 (d, J = 38.1 Hz), 81.1, 75.4 (d, J = 17.2 Hz), 58.6. ^{19}F NMR (377 MHz, MeOD-d_4) δ -122.31. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_9H_{12}BrFN_3O_4$: 323.9995, found: 323.9992.

Isopropyl ((((2R,3R,4S,5R)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-4-bromo-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (18).

To vacuum dried **17** (20 mg, 0.062 mmol) in 1 mL anhydrous THF under nitrogen atmosphere was added *t*-BuMgCl (0.093 mL, 0.093 mmol, 1.5 eq) at 0 °C. After stirring at 0 °C for 30 min, (2*S*)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate, **13** (18 mg, 0.062 mmol) in 1 mL of anhydrous THF was added. The reaction mixture was allowed to attain rt and stirred for 3 h. The reaction was quenched with isopropyl alcohol (0.2 mL). The solvent was removed under reduced pressure and the residue was purified by flash chromatography using 0-6% MeOH/CH_2Cl_2 to afford **18** (7.7 mg, 21%) as a diastereomeric (R_p/S_p ~ratio 6:4) mixture.

^{1}H NMR (400 MHz, MeOD-d_4) δ 7.55 (2d merged, 1H), 7.42-7.37 (m, 2H), 7.29 – 7.20 (m, 3H), 6.48-6.40 (m, 1H), 5.92-5.88 (2d merged, 1H), 5.04-4.97 (m, 1H), 4.61 –4.34 (m, 4H), 4.14-4.09(m, 1H), 3.95-3.90 (m, 1H), 1.37-1.31 (m, 3H), 1.26-1.23 (m, 6H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 173.2 (d, J = 4.5 Hz), 172.9 (d, J = 5.3 Hz), 166.2, 166.1, 156.4, 156.4, 150.7, 150.7, 150.7, 150.6, 140.4, 140.2, 129.5, 124.9, 120.0, 120.0, 119.9, 119.9, 108.9 (d, J = 262.8 Hz), 95.5, 95.5, 79.0, 78.9, 76.2 (d, J = 17.4 Hz), 75.9 (d, J = 17.4 Hz)], 68.8, 68.8, 64.1, 63.8, 50.4, 50.3, 20.6, 20.5, 19.1 (d, J = 6.5 Hz), 18.9 (d, J = 7.5 Hz). ^{19}F NMR (377 MHz, MeOD-d_4) δ -121.75, -122.03. ^{31}P NMR (162 MHz, MeOD-d_4) δ 3.59, 3.49. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{21}H_{28}BrFN_4O_8P$: 593.0812, found: 593.0813.

3',5'-bis-*O*-*tert*-Butyldiphenylsilyl-2'-β-bromo,2'-α-fluoro-N^6-bis-*tert*-butylcarbonate-2'-deoxyadenosine (19).

To a solution of lactol **9** (0.42 g. 0.6 mmol) triphenyl phosphine (0.283 g, 1.08 mmol) and *N*-Boc_2 adenine (0.3 g, 1.08 mmol) in THF (5 mL) under nitrogen atmosphere was added DIAD (0.212 mL, 1.08) dropwise at 0 °C. The resulting solution was stirred for 24 h at rt and then evaporated under reduced pressure to give a yellow syrup. This syrup was purified by silica gel column chromatography with a mixture of hexane and ethyl acetate (15% EA) to give mixture of α:β product **19** (1:0.4) as white solid (0.242 g, 39%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 0.4H), 8.67 (s, 1H), 8.40 (d, J = 3.5 Hz, 0.4H), 8.12 (s, 1H), 7.76 – 7.66 (m, 28H), 6.67 (d, J = 16.9 Hz, 0.4H), 6.51 (d, J = 13.1 Hz, 1H), 5.00 (dd, J = 17.1, 7.0 Hz, 0.4H), 4.81 (dd, J = 12.6, 7.2 Hz, 1H), 4.49 – 4.30 (m, 1.4 H), 3.79 – 3.68 (m, 2.4H), 3.50 (dd, J = 11.9, 2.9 Hz, 0.4H), 1.49 (s, 7H), 1.42 (s, 18H), 1.12-0.97 (m, 25H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -122.77, -136.83 (t, J = 17.2 Hz). ^{13}C NMR

(101 MHz, $CDCl_3$) δ 153.5, 152.5, 152.5, 152.2, 150.5, 150.4, 150.3, 150.1, 143.6, 143.5, 142.5, 136.1, 135.9, 135.9, 135.5, 135.5, 135.5, 135.4, 132.7, 132.7, 132.4, 132.3, 132.2, 131.5, 130.2, 130.3, 130.3, 130.3, 129.8, 129.8, 129.7, 128.8, 128.3, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 108.2 (d, J = 269.6 Hz), 102.3 (d, J = 272.4 Hz), 88.4 (d, J = 37.2 Hz), 87.6 (d, J = 16.4 Hz), 83.9, 83.8, 83.7, 77.9 (d, J = 15.3 Hz), 77.6 (d, J = 15.5 Hz). 62.6, 27.8, 27.8, 27.7, 26.8, 26.8, 19.4, 19.4, 19.1, 19.0. ^{19}F NMR (377 MHz, $CDCl_3$) δ -122.77, -136.83 (t, J = 17.2 Hz). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{52}H_{64}BrFN_5O_7Si_2$: 1024.3512, found: 1024.3505.

2'-β-Bromo-2'-α-fluoro-*N*^{6}-bis-*tert*-butylcarbonate 2'-deoxyadenosine (20).

To a stirred solution of **19** (0.242 g, 0.24 mmol) in THF (3 mL), was added 1M solution of TBAF in THF (0.519 mL, 0.516 mmol) at 0 °C. The reaction mixture was allowed to stir for 1 h at the same temperature. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using 0-6% MeOH/CH_2Cl_2 gradient to afford **20** (β-isomer, 58 mg, 44%) and α-isomer (25 mg, 19%).

α-Anomer: ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.90 (s, 1H), 8.71 (s, 1H), 6.91 (d, J = 17.2 Hz, 2H), 4.83-4.75 (m, 1H), 4.43 (d, J = 10.1 Hz, 1H), 3.96 (dd, J = 12.7, 2.4 Hz, 1H), 3.77 (dd, J = 12.7, 3.3 Hz, 1H), 1.39 (s, 18H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 154.9, 153.6, 151.4, 151.3, 146.6, 146.5, 129.8, 104.5 (d, J = 268.0 Hz), 89.5 (d, J = 15.9 Hz), 85.4, 84.6, 77.6 (d, J = 16.6 Hz), 61.2, 27.9. ^{19}F NMR (377 MHz, MeOD-d_4) δ -136.09. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{20}H_{28}BrFN_5O$: 548.1156, found: 548.1146

β-Anomer: ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.96 (s, 1H), 8.91 (s, 1H), 6.71 (d, J = 14.7 Hz, 1H), 4.92-4.82 (m, 1H), 4.14 (d, J = 10.4 Hz, 1H), 4.07 (dd, J = 12.7, 2.2 Hz, 1H), 3.93

(dd, J = 12.7, 3.3 Hz, 1H), 1.37 (s, 18H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 154.4, 153.5, 151.4, 151.2, 145.7, 130.3, 110.5 (d, J = 261.3 Hz), 89.9 (d, J = 38.8 Hz), 85.4, 83.6, 76.3 (d, J = 16.9 Hz), 60.6, 27.9. ^{19}F NMR (377 MHz, MeOD-d_4) δ -123.63. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{20}H_{28}BrFN_5O$: 548.1156, found: 548.1147.

(2*R*,3*R*,4*S*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-bromo-4-fluoro-2-(hydroxymethyl)tetrahydrofuran-3-ol (21).

To a solution of beta product **20** (0.040 g, 0.072 mmol) in dry DCM (1 mL) at -78 °C was added dropwise a 1 M solution BCl_3 in DCM (0.145 mL, 0.145 mmol) and reaction mixture was allowed to warm to rt and stirred for 1 h. The reaction was quenched by careful addition of MeOH and volatiles were evaporated under reduced pressure. The residue was purified by silica gel column chromatography using 0-10% MeOH/CH_2Cl_2 gradient to afford the nucleoside derivative **21** (17.3 mg, 69%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 8.18 (s, 1H), 7.43 (bs, 2H, NH_2), 6.54 (d, J = 7.2 Hz, 1H), 6.47 (d, J = 15.8 Hz, 1H), 5.37 (t, J = 5.1 Hz, 1H, OH), 4.79 (dt, J = 22.1, 8.3 Hz, 1H), 3.99-3.97 (m, 1H), 3.89-3.84 (m, 1H), 3.79-3.73 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.6, 153.4, 149.5, 139.0, 119.2, 110.4 (d, J = 258.9 Hz), 88.1 (d, J = 38.1 Hz), 82.2, 75.0 (d, J = 16.7 Hz), 59.9. ^{19}F NMR (377 MHz, DMSO-d_6) δ -120.95. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{10}H_{12}BrFN_5O_3$: 348.0108, found: 348.0105.

Isopropyl ((((2*R*,3*R*,4*S*,5*R*)-5-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-4-bromo-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (22).

To a stirred solution of **20** (60 mg, 0.11 mmol) in anhydrous THF (1 mL) were added (2*S*)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate (66 mg, 0.21 mmol) in 1 mL of anhydrous THF and NMI (34 μL, 0.44 mmol) slowly at 0 °C under nitrogen atmosphere. The reaction mixture allowed to warm slowly to rt and stirred for 3 h. The reaction was quenched with isopropyl alcohol (0.5 mL). The solvents were evaporated under reduced pressure. A 50% TFA in DCM (3 mL) solution was added to the crude product at 0 °C and the stirred at rt overnight and then concentrated *in vacuo*. A 5% aqueous solution of $NaHCO_3$ (2 mL) was slowly added and the water was evaporated. The residue was purified by flash chromatography using 0-10% MeOH/CH_2Cl_2 to afford **22** (26 mg, 40%) as a diastereomeric (R_p/S_p ~ratio 7:3) mixture over two steps.

^{1}H NMR (400 MHz, MeOD-d_4) δ 8.26-8.24 (m, 2H), 7.37-7.33 (m, 2H), 7.26-7.18 (m, 3H), 6.56-6.50 (m, 1H), 5.07-4.96 (m, 2H), 4.62-4.57 (m, 2H), 4.31-4.26 (m, 1H), 3.93-3.86 (m, 1H), 1.32-1.29 (m, 3H), 1.22-1.13 (m, 6H). ^{9}F NMR (377 MHz, MeOD-d_4) δ -124.58 (dd, J = 19.4, 16.1 Hz), -124.71 (dd, J = 19.4, 16.2 Hz). ^{13}C NMR (101 MHz, MeOD-d_4) δ 173.1 (d, J = 4.6 Hz), 172.9 (d, J = 5.6 Hz), 156.1, 156.1, 152.8, 152.8, 150.7, 150.6, 149.1, 149.0, 139.3, 139.0, 129.4, 129.4, 124.8, 120.0, 119.9, 119.0, 118.9, 108.4 (d, J = 260.2 Hz), 108.4 (d, J = 260.3 Hz), 88.9 (d, J = 39.1 Hz), 88.7 (d, J = 39.2 Hz), 80.0 (d, J = 8.3 Hz), 79.9 (d, J = 8.7 Hz), 75.9 (d, J = 17.1Hz), 75.6 (d, J = 17.2 Hz), 68.8, 68.7, 65.2, 64.6, 50.4, 50.2, 20.5, 20.5, 20.5, 20.4, 19.1 (d, J = 6.5Hz), 18.9 (d, J = 7.1Hz). ^{31}P NMR (162 MHz, MeOD-d_4) δ 3.63, 3.51. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{22}H_{28}BrFN_6O_7P$: 617.0924, found: 617.0925.

3',5'-bis-*O-tert*-Butyldiphenylsilyl-2'-deoxy-2'-β-bromo-2'-α-fluoro-6-benzyloxy-N^2-bis-*tert*-butylcarbonatepurine ribonucleoside (23).

To a solution of lactol **9** (0.5 g. 0.71 mmol) triphenyl phosphine (0.427 g, 1.27 mmol) and O^6-benzyl-*N*-Boc_2 guanine (0.57 g, 1.27 mmol) in THF (5 mL) under nitrogen atmosphere was added DIAD (0.25 mL, 1.27mmol) dropwise at 0 °C. The resulting solution was stirred for 24 h at rt and then evaporated under reduced pressure to give yellow syrup. This syrup was purified by silica gel column chromatography with a mixture of hexane and ethyl acetate (15%) to give mixture of α/β product (ratio ~1:0.4) **23** as white solid (0.320 g, 40%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 3.6 Hz, 0.3H), 7.95 (s, 1H), 7.69-7.29 (m, 32.5 H), 6.56-6.47 (m, 1.3 H), 5.66-5.62 (m, 2.6H), 4.91 (dd, *J* = 16.5, 6.8 Hz, 0.3H), 4.68-4.64 (m, 1H), 4.42-4.40 (m, 0.3H), 4.26-4.24 (m, 0.3H), 3.90 – 3.58 (m, 2.6H), 3.47-3.42 (m, 0.3H), 1.40-1.28 (m, 23.5H), 1.09- 0.96 (m, 23.5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.1, 160.9, 153.3, 152.7, 152.2, 152.1, 150.7, 150.7, 150.4, 142.1, 142.1, 140.7, 140.5, 136.1, 135.9, 135.8, 135.8, 135.8, 135.6, 135.5, 135.5, 135.4, 135.4, 132.7, 132.7, 132.6, 132.5, 132.4, 132.4, 132.1, 131.6, 131.6, 130.4, 130.3, 130.3, 130.3, 130.1, 130.1, 129.9, 129.9, 129.8, 129.8, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 127.9, 127.8, 127.8, 127.7, 127.6, 119.7, 119.6, 119.3, 108.4 (d, *J* = 272.8 Hz), 102.4 (d, *J* = 273.7 Hz), 87.7 (d, *J* = 16.4 Hz), 87.5 (d, *J* = 36.4 Hz) 84.0, 83.1, 83.0, 82.8, 78.0 (d, *J* = 15.4 Hz), 77.6 (d, *J* = 15.5 Hz), 72.2, 68.9, 68.8, 62.7, 62.3, 27.8, 27.8, 26.8, 26.6, 21.6, 19.4, 19.4, 19.1, 19.00. ^{19}F NMR (377 MHz, $CDCl_3$) δ -122.03 (t, 9.8 Hz), -134.67 (td, *J* = 16.5, 3.9 Hz). HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{59}H_{70}BrFN_5O_8Si_2$: 1130.3930, found: 1130.3928.

3',5'-bis-*O-tert*-Butyldiphenylsilyl-2'-deoxy-2'-β-bromo-2'-α-fluoro-6-benzyloxy-N^2-bis-*tert*-butylcarbonatepurine ribonucleoside (24).

To a stirred solution of **23** (0.300 g, 0.24 mmol) in THF (5 mL), was added 1 M solution of TBAF in THF (0.58 mL, 0.58 mmol) at 0 °C. The reaction mixture was allowed to stir for 1 h at the same temperature. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using 0-5% MeOH/CH_2Cl_2 gradient to afford **24** (β-isomer, 104 mg, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.40-7.34 (m, 3H), 6.42 (d, *J* = 14.8 Hz, 1H), 5.61 (s, 2H), 4.90-4.93 (m, 1H), 4.12-4.07 (m, 2H), 3.86 (d, *J* = 4.4 Hz, 1H), 1.39 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.0, 152.1, 152.1, 150.6, 141.6, 135.5, 128.6, 128.4, 128.4, 119.8, 108.8 (d, *J* = 261.2 Hz), 89.1 (d, *J* = 38.5 Hz), 83.6, 82.0, 77.3, 74.3 (d, *J* = 16.7 Hz), 69.1, 59.1, 27.9. ^{19}F NMR (377 MHz, $CDCl_3$) δ -122.41. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{27}H_{34}BrFN_5O_8$: 654.1575, found: 654.1566.

2-Amino-9-((2*R*,3*S*,4*R*,5*R*)-3-bromo-3-fluoro-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,9-dihydro-6*H*-purin-6-one (25).

To a solution of beta product **24** (0.058 g, 0.088 mmol) in dry DCM (1 mL) at -78 °C was added 1M solution BCl_3 in DCM (0.177 mL, 0.177 mmol) dropwise and reaction mixture was allowed to warm to -20 °C and stirred for 1 h. The reaction was next stirred at rt for 2 h. The reaction was quenched by careful addition of MeOH (0.3 mL) and volatiles

were evaporated under reduced pressure. The residue was purified by silica gel column chromatography using 0-10% MeOH/CH_2Cl_2 gradient to afford the nucleoside derivative **25** (21 mg, 65%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (bs, 1H), 7.99 (s, 1H), 6.81 (bs, 2H), 6.54 (d, J = 7.2 Hz, 1H), 6.17 (d, J = 15.8 Hz, 1H), 5.38 (t, J = 5.2 Hz, 1H), 4.66 – 4.55 (m, 1H), 3.92 (d, J = 9.2 Hz, 1H), 3.85-3.78 (m, 1H), 3.73-3.68 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.1, 154.6, 151.5, 134.8, 116.8, 110.4 (d, J = 258.8 Hz), 87.3 (d, J = 37.7 Hz), 82.0, 74.7 (d, J = 16.5 Hz), 59.5. ^{19}F NMR (377 MHz, DMSO- d_6) δ -122.73. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{10}H_{12}BrFN_5O_4$: 364.0057, found: 364.0054.

Isopropyl ((((2*R*,3*R*,4*S*,5*R*)-5-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-4-bromo-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (26).

To a stirred solution of **24** (52 mg, 0.08 mmol) in anhydrous THF (1.5 mL) were added (2*S*)-isopropyl 2-((chloro(phenoxy)phosphoryl)amino)propanoate (48 mg, 0.16mmol) in 1 mL of anhydrous THF and NMI (25 μL, 0.32 mmol) slowly at 0 °C under a nitrogen atmosphere. The reaction mixture was allowed to warm slowly to rt and stirred for 3 h. The reaction was quenched with isopropyl alcohol (0.5 mL). The solvents were evaporated under reduced pressure. The residue was purified by column using 0-10% MeOH/CH_2Cl_2 gradient to obtain crude the 6-*O*-Bn-2-*N*-Boc_2 prodrug intermediate (25 mg, 0.027mmol). This crude product was dissolved in anhydrous DCM (3 mL) and cooled at -78 °C. A 1M solution of BCl_3 (32 μL, 0.032 mmol) in DCM was added dropwise and the reaction mixture was allowed to warm to -20 °C then stirred for 1 h. The reaction was next stirred

at rt for 2 h. The reaction was quenched by careful addition of MeOH (0.3 mL) and the volatiles were evaporated under reduced pressure. The residue was first purified by silica gel column chromatography using 0-10% MeOH/CH_2Cl_2 gradient and secondly by C18 reverse phase column chromatography using water:acetonitrile (95:5 to 80:20) to afford the guanine nucleoside prodrug **26** (10 mg, 20% over two steps) as a diastereomeric (R_p/S_p ~ratio 1:1).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 and 7.79 (2S, 1H), 7.37-7.33 (m, 2H), 7.15-7.23 (m, 3H), 6.86 (bs, 2H), 6.28-6.22 (m, 1H), 6.09 (b, 1H), 4.87-4.77 (m, 2H), 4.43-4.39 (m, 2H), 4.18-4.07 (m, 1H), 3.84-3.72 (m, 1H), 1.22-1.20 (m, 3H), 1.12-1.10 (m, 1H). ^{13}C NMR (101 MHz, MeOD -d_4) δ 174.6, 174.5, 174.4, 174.3, 159.3, 155.5, 155.4, 152.9, 152.7, 152.1, 152.0, 137.7, 137.3, 130.8, 130.3, 126.2, 121.4, 121.4, 121.3, 118.1, 117.9, 111.2, 108.6, 90.4, 90.1, 90.0, 89.7, 81.3, 81.2, 81.1, 81.0, 77.5, 77.3, 77.2, 77.1, 70.2, 70.1, 66.7, 66.1, 66.1, 51.8, 51.6, 21.9, 21.9, 21.8, 20.5, 20.5, 20.4, 20.3. ^{19}F NMR (377 MHz, DMSO-d_6) δ -121.50, -121.80. ^{31}P NMR (162 MHz, DMSO-d_6) δ 3.61, 3.40. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{22}H_{28}BrFN_6O_8P$: 633.0874.1575, found: 633.0864.

Isopropyl ((*R*)-(((2*R*,3*R*,4*S*,5*R*)-4-bromo-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (27).

The nucleoside **12** (600 mg, 1.8 mmol) was dried at 50 °C under high vacuum for 1 h before adding dry THF (6 mL) at 25 °C. The mixture was cooled to -5 °C and *tert*-butylmagnesium chloride (3.6 mL, 3.6 mmol, 1M in THF) was introduced. The reaction mixture was stirred at -5 °C for 15 min then warmed to 25 °C and stirred for an additional

15 min. A solution of isopropyl ((*R*)-(perfluorophenoxy)-(phenoxy)phosphoryl)-*L*-alaninate (800 mg, 1.8 mmol, dried 2 h under high vacuum) in THF (6 mL) was added dropwise to the white suspension at 0 °C. After 16 h stirring at 4 °C, the white suspension was treated with 1M HCl (10 mL). The mixture was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with water (10 mL), brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH 1% to 5%) to afford **27** (747 mg, 68%) as white foam along with 104 mg (17%) of the unreacted nucleoside **12**.

^{1}H NMR (400 MHz, MeOD -d_4) δ 7.57 (d, J = 8.2, 1H), 7.42-7.38 (m, 2H), 7.27 – 7.20 (m, 3H), 6.37 (d, J = 16.9 Hz, 1H), 5.73 (d, J = 8.2, 1H), 5.04 – 4.97 (m, 1H), 4.59 (d, 1H), 4.47-4.37 (m, 2H), 4.16-4.13 (m, 1H), 4.01 – 3.82 (m, 1H), 1.37-1.32 (m, 3H), 1.27-1.24 (m, 6H). ^{13}C NMR (101 MHz, MeOD -d_4) δ 173.2 (d, J = 4.4 Hz), 164.1, 150.7, 150.5, 139.6, 129.5, 124.9, 119.9, 119.9, 108.6 (d, J = 262.5 Hz), 102.2, 79.2 (d, J = 8.7 Hz), 75.8 (d, J = 17.2 Hz), 68.8, 63.8, 50.4, 20.6, 20.5, 18.9 (d, J = 7.4 Hz). ^{19}F NMR (377 MHz, MeOD -d_4) δ -122.08. ^{31}P NMR (162 MHz, MeOD-d_4) δ 3.64. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{21}H_{27}BrFN_3O_9P$: 594.0652, found: 594.0657.

Isopropyl ((*S*)-(((2*R*,3*R*,4*S*,5*R*)-4-bromo-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-*L*-alaninate (28).

Compound **12** (600 mg, 1.8 mmol) was dried at 50 °C under high vacuum for 1 h before adding dry THF (6 mL) at 25 °C. The mixture was cooled to -5 °C and *tert*-butylmagnesium chloride (3.6 mL, 3.6 mmol, 1M in THF) was introduced. The reaction mixture was stirred

at -5 °C for 15 min then warmed to 25 °C and stirred for an additional 15 min. A solution of isopropyl ((*S*)-(perfluorophenoxy)-(phenoxy)phosphoryl)-*L*-alaninate (750 mg, 1.7 mmol, dried 2 h under high vacuum) in THF (6 mL) was added dropwise to the white suspension at 0 °C. After 16 h stirring at 4 °C, the clear solution was quenched by addition of 1M HCl (10 mL). The mixture was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with water (10 mL), brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2 then CH_2Cl_2/MeOH 1% to 5%) to afford **28** (744 mg, 68%) as white foam along with 92 mg (15%) of the unreacted nucleoside **12**.

^{1}H NMR (400 MHz, MeOD-d_4) δ 7.56 (d, J = 8.2 Hz, 1H), 7.42 – 7.37 (m, 2H), 7.31 – 7.17 (m, 3H), 6.34 (d, J = 16.9 Hz, 2H), 5.68 (d, J = 8.1 Hz, 1H), 5.05 – 4.95 (m, 1H), 4.59 – 4.36 (m, 3H), 4.20 – 4.08 (m, 1H), 3.97-3.88 (m, 1H), 1.37 (d, J = 7.1 Hz, 3H), 1.25-1,23 (m, 6H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 172.9 (d, J = 5.4 Hz), 164.1, 150.7(d, J = 6.8 Hz), 150.4, 139.9, 129.5, 124.9, 120.0, 119.9, 108.6 (d, J = 262.6 Hz), 102.1, 79.3 (d, J = 7.8 Hz), 76.1 (d, J = 17.3 Hz), 68.8, 64.1, 50.3, 20.6, 20.5, 19.1(d, J = 6.4 Hz). ^{19}F NMR (377 MHz, MeOD-d_4) δ -121.77. ^{31}P NMR (162 MHz, MeOD-d_4) δ 3.55. HRMS (ESI): m/z $[M+H]^+$ calcd. for $C_{21}H_{27}BrFN_3O_9P$: 594.0652, found: 594.0651.

Crystallography. A suitable crystal (0.83 × 0.57 × 0.51 mm) was selected and mounted on a loop with paratone oil on a Brucker APEX-II CCD 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 minimization.

Results from X-ray structure determination of **12** are the following. Crystal data for $C_9H_{10}BrFN_2O_5$ (M =325.10 g/mol): tetragonal, space group $P4_12_12$ (no. 92), a = 17.6568(3) Å, b = 17.6568(3) Å, c = 14.3690(3) Å, α = 90°, β = 90°, γ = 90°, V = 4479.69(18) Å^3, Z = 16, T = 100(2) K, μ(MoKα) = 3.700 mm^{-1}, $Dcalc$ = 1.928 g/cm^3. Intensity data were collected on a Bruker APEX II CCD diffractometer with monochromated MoKα radiation (λ = 0.7103 Å) at 100(2) K in the 2θ range 3.654 – 61.012°. The user interface Olex2 was used for the crystallographic calculations and crystal structure visualization. (Dolomanov et al., 2009). The structure was solved with Superflip by charge flipping and refined by least-squares minimization using SHELXL (Sheldrick, 2008 and Sheldrick, 2015). All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were refined using a "riding" model. A total of 40560 reflections were measured ($3.654 \leq 2\theta \leq 61.012$), while 6,853 unique data (R_{int} = 0.0456, R_{sigma} = 0.0264), which were used in all calculations. The final R_1 was 0.0284 ($I > 2\sigma(I)$) and wR_2 was 0.0665 (all data). GOF = 1.046. The maximum and the minimum peak on the final difference Fourier map corresponded to 0.59 and −0.47 e/Å^3, respectively. The Flack absolute structure parameter was refined to −0.003(4), thus corroborating the stereochemistry of the title compound.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI. Supporting Information include all experimental procedures and in vitro experimental protocols along with Molecular Formula Strings for compounds **2**, **4**-**28**.

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ACKNOWLEDGMENTS

This work was supported in part by NIH grant 5P30-AI-50409 (CFAR). Dr. Schinazi is the Chairman and a major shareholder of Cocrystal Pharma, Inc. Emory received no funding from Cocrystal Pharma, Inc. to perform this work and vice versa. We thank Dr. John Bacsa, Emory X-ray Crystallography Facility for the X-ray structural analysis. We also acknowledge the use of the Rigaku SYNERGY diffractometer, supported by the National Science Foundation under grant CHE-1626172.

ABBREVIATIONS USED

BSA, *N*,*O*-bis(trimethylsilyl)acetamide; DAA, direct acting antivirals; DCE, 1,2-dichloroethane; DCM, dichloromethane; DIAD, diisopropyl azodicarboxylate; DMF, dimethyl formamide; GT, genotype; LiHMDS, lithium bis(trimethylsilyl)amide; MsCl, trimethylsulfonyl chloride; NBS, *N*-bromosuccinimide; NFSI, *N*-fluorobenzenesulfonimide; NMI, *N*-methylimidazole; NOE, nuclear overhauser effect; rt, room temperature; SD, standard deviation; SOF, sofosbuvir; TBDPS, *t*-butyldiphenylsilyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMSOTf, trimethysilyl

trifluoromethanesulfonate; TP, triphosphate; NTP, nucleoside triphosphate; RdRp, RNA dependent RNA polymerase.

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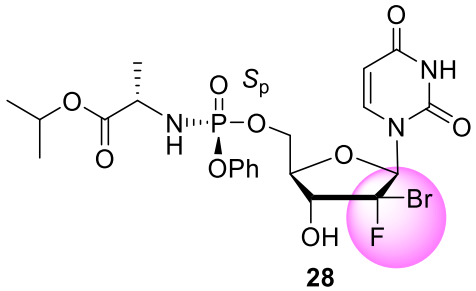

28

HCV genotype	1a	1b	2a	3a	4a	5a	1b/S282T
IC_{50} (nM)	55	46	24	38	30	21	180

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

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