HHS Public Access

Author manuscript

Bioorg Med Chem Lett. Author manuscript; available in PMC 2018 December 01.

Published in final edited form as:

Bioorg Med Chem Lett. 2017 December 01; 27(23): 5296–5299. doi:10.1016/j.bmcl.2017.10.024.

Synthesis and anti-HCV activity of a series of β-D-2′-deoxy-2′-dibromo nucleosides and their corresponding phosphoramidate prodrugs

Zhe Chen[a], **Bryan D. Cox**[a], **Ethel C. Garnier-Amblard**[b], **Tamara R. McBrayer**[b], **Steven J. Coats**[b], **Raymond F. Schinazi**[a,*], and **Franck Amblard**[a,*]

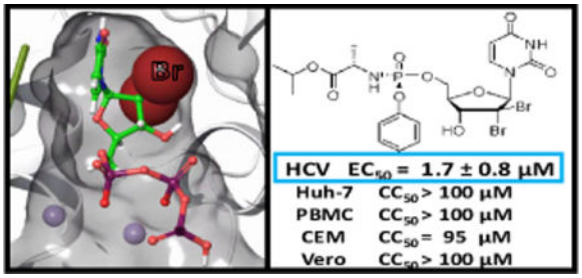
[a]Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University, School of Medicine, Atlanta, Georgia 30322, United States

[b]Cocrystal Pharma, Inc., Tucker, Georgia 30084, United States

Abstract

Several β-D-2′-deoxy-2′-substituted nucleoside analogs have displayed potent and selective anti-HCV activities and some of them have reached human clinical trials. In that regard, we report herein the synthesis of a series of 2′-deoxy, 2′-dibromo substituted U, C, G and A nucleosides **10a–d** and their corresponding phosphoramidate prodrugs **13a–d**. The synthesized nucleosides **10a–d** and prodrugs **13a–d** were evaluated for their inhibitory activity against HCV as well as cellular toxicity. The results showed that the most potent compound was prodrug **13a**, which exhibited micromolar inhibitory activity (EC_{50} = 1.5 ± 0.8 μM) with no observed toxicity. In addition, molecular modeling and free energy perturbation calculations for the 5′-triphosphate formed from **13a** and related 2′-modified nucleotides are discussed.

Graphical abstract



Keywords

Synthesis; Nucleoside; Prodrug; Hepatitis C virus

*Corresponding authors (RFS and FA) Tel.: +1-404-727-1414; rschina@emory.edu and famblar@emory.edu.

Supplementary Material

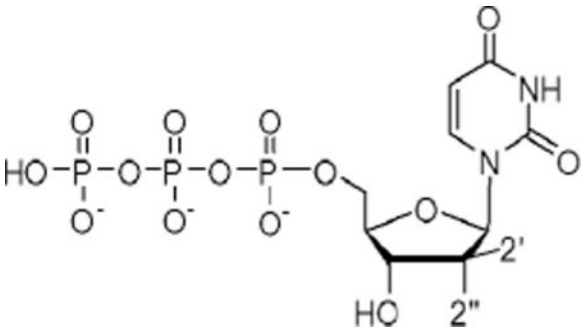
Supplementary data associated with this article can be found, in the online version, at http://xxxx.

Hepatitis C virus (HCV) is a blood-borne pathogen infecting an estimated 180 million subjects worldwide with 55–85% of infected individuals progressing to chronic HCV infection.[1] More concerning is that approximately 30% of those chronically infected persons will develop liver cirrhosis and 1–7% of these will go on to develop hepatocellular carcinoma.[2,3] Just a few years ago, the standard of care for HCV patients was pegylated interferon-α (PEG-IFN) and ribavirin (RBV) in combination with boceprevir, telaprevir, (two first-generation NS3/4A protease inhibitors) and simeprevir (a second-generation NS3/4A protease inhibitor), which demonstrated limited efficacy and often intolerable side effiects.[4] Since their initial discovery, nucleoside analogs, as inhibitors of HCV NS5B polymerase, have been favored due to their high genetic barrier to drug resistance and pan-genotypic activity.[5] To date, sofosbuvir is the only approved nucleoside analog for HCV treatment and remains the backbone of several combination therapies. Compared with previous treatments, sofosbuvir-based regimens provide a durable cure rate along with fewer side effects. However, the duration of approved sofosbuvir treatment regimens remains at 8–12 weeks.[6] Therefore, there is still an unmet need to develop novel pan-genotypic and more efficacious nucleoside analogs, which could lead to new short to ultra-short combination therapies with improved safety profiles and higher barrier to resistance.[7]

Inspired by the success of 2′-halogenated nucleoside analogs such as sofosbuvir **1**[8] or gemcitabine **2**,[9] several groups, including ours, studied extensively 2′-dihalogenated nucleosides as potential anti-HCV agents. Thus, Pinho et al. reported the discovery of a β-D-2′-deoxy-2′-dichlorouridine nucleotide prodrug **3** inhibiting HCV replication[10] while we recently reported a new β-D-2′-Cl, 2′-F-uridine phosphoramidate nucleotide **4** as a non-toxic, pan-genotypic, potent and specific anti-HCV NS5B polymerase agent.[11] As part of our continuing efforts in this area, we report herein the synthesis and antiviral evaluation of a series of 2′-dibromo nucleosides **10a–d** and their corresponding phosphoramidate prodrugs **13a–d**.

To determine if the HCV NS5 RdRp active site can accommodate 2′-dibromo substitutions, molecular modeling studies were initiated. Crystal structures of the HCV RdRP complexed to the RNA primer/template reveal dynamic rearrangement of residues contacting the 2′-OH versus 2′-F moieties[12] and based on the β-D-2′-Me, 2′-F-uridine diphosphate HCV RdRp structure (PDBID 4WTG), we predicted that β-D-2′-di-Br-uridine triphosphate in the active site would incur only minimal steric clash (Figure 2). However, because reliable energy calculations require the use of methods that account for the flexibility in the polymerase active site we next performed alchemical free energy perturbation (FEP) calculations. FEP calculates relative binding free energy between two ligands by mutating one to the other over a series of molecular dynamics simulations in solution and bound to the protein.[13] FEP calculations yield accurate ΔΔG as the change of binding energy between the first and second ligands.[14] A negative ΔΔG value suggests a gain in binding energy. To evaluate this approach for 2′-modified HCV nucleotide analogs, FEP calculations were performed on known inhibitors relative to the UTP-bound complex (PDBID 4WTA) using Desmond MD with the OPLS-2005 force field on a GPU-enabled EXXACT MD server. It must be noted that the FEP ΔΔG results do not reflect the overall antiviral activity as many other pharmacologic steps are not included (cell penetration, prodrug processing, phosphorylation

by host kinases and etc.), nor do these calculations necessarily recapture the enzymatic IC_{50} as they do not account for the chain-terminating chemical steps. However, these calculations predict how well the 5′-triphosphate analog binds to the active site; a critical step for antiviral activity. The calculations were performed in the physiologically relevant tri-anion and tetra-anion protonation states.[15] The FEP ΔΔG for β-D-2′-Me, 2′-F-uridine triphosphate (the active form of sofosbuvir) was −2.52 ± 0.18 kcal/mol relative to UTP (Table 1), which agrees with the potent inhibitory activity of the drug. Two other 2′-halogenated analogs recently reported as HCV inhibitors, β-D-2′-Cl-2″-F and β-D-2′-Cl-2″-Cl-uridine, display negative FEP ΔΔG relative to UTP in both protonation states in agreement with their experimental antiviral activity. Interestingly, the FEP ΔΔG for the proposed 2′-dibromo UTP analog was large and negative for both tri- and tetra-anions (−2.87 ± 0.26 and −4.21 ± 0.18 kcal/mol, respectively) suggesting this analog can favorably bind to the active site relative to UTP thereby meriting synthesis and testing.



Synthesis of nucleosides **10a–d** is described in Scheme 1. Oxidation of commercially available 2-deoxy-D-ribose **5** with Br_2 followed by silylation with tert-butyldimethylchorosilane (TBDMSCl) yielded the di-silyl protected lactone **6** in 70% yield.[16] Reaction of **6** with 2.2 equivalent of *N*-bromosuccinimide (NBS) in presence of LiHMDS gave the lactone **7** in 80% yield. Reduction of lactone **7** with lithium diisobutylaluminium hydride (DIBAL-H) provided the desired lactol intermediate, which was subjected to benzoylation with BzCl in presence of Et_3N yielding benzoylate **8** in 81% yield. Initial glycosylation of **8** with silylated protected nucleobases (Uracil, N^4-Bz-cytosine[17], N^6-diBoc-adenine or N^4-diBoc-O^6-Bn-Guanine[18]) to obtain nucleoside intermediates **9a–d** was attempted using standard Vorbruggen conditions in the presence of TMSOTf. However, these coupling reactions, especially for C and G nucleosides (**9b** and **9d**), needed long reaction times (> 24 h) and elevated temperatures (> 100 °C), which resulted in low yields probably due to the instability of these nucleosides to the reaction conditions. In order to improve the yields for formation of nucleosides **9a–d**, a set of experiments were carried out under microwave irradiation (MW) using CH_3CN as the solvent. After screening several temperatures and reaction times, it was found that the coupling reactions did not proceed at 80 °C, while a messy mixture was obtained when the temperature was increased to 140 °C. Best results were obtained when reactions were irradiated at 120 °C for 10 min with a maximum power of 200W. Under these conditions, compounds **9a–d** were obtained as inseparable mixtures of α/β anomers, in yields ranging from 40% to 60%. It is noteworthy that the silylated purine nucleobases need to be Boc

protected (N^6-diBoc-adenine or N^2-diBoc-O^6-Bn-guanine) in order for the glycosylation step to work however, these group fall off either during the reaction or during workup.

Finally, deprotection of compounds **9a–d** was performed and separation of each α and β isomers was achieved with the unprotected nucleoside analog using preparative reverse phase HPLC. Thus, treatment of compounds of **9a** and **9c** with TBAF in THF gave, after purification, β isomers **10a** and **10c** and α isomers **11a** and **11c** as anomer mixtures in 40–50% yield (**10a**:**11a** and **10c**:**11c** ratio: 3:5 and 1:2 respectively). Debenzoylation of **9b** in a saturated solution of NH_3 in MeOH, followed by treatment with TBAF in THF yielded β isomer **10b** and isomer **11b** in 30–35% (ratio: 5:7). Desilylation of **9d** with TBAF, followed by debenzylation in TFA afforded β isomer **10d** and α isomer **11d** in 30–35% (ratio = 1:1.2).

α and β anomers were identified using 1H 2D-NOESY experiments. For example, clear NOE enhancements were observed between H5 of the uracil nucleobase and H3′ of the sugar as well as between H1′ and H4′ for β-isomer **10a**. While obvious NOE interactions between the H5 and H4′, H1′ and H3′ were observed for its α-isomer **11a** (Figure 2).

In order to be a substrate for HCV NS5B, nucleosides analogs need to be activated to their triphosphate form. This multistep process is often limited by the first phosphorylation step which led to the development of nucleoside monophosphate prodrugs.[19] These prodrugs allow for the intracellularly delivery of monophosphate species after enzymatic and/or chemical cleavage of different masking groups. Among all these prodrugs, the phosphoramidate/phosphonamidate approach is now, by far, the most popular and lead to the approval of compounds like sofosbuvir (HCV) and tenofovir alafenamide (HIV).[20] As both of these compounds are *R*p isomers, we have decided to prepare pure monophosphate prodrugs of nucleosides **10a–d** using chiral pentafluorophenyl reagent **12** synthesized according to Ross *et al*' procedure.[21] Thus, reaction of nucleosides **10a–d** with **12** in the presence of tert-butyl magnesium chloride provided the desired *R*p prodrugs **13a–d** in 18–25% yield.

Compounds **10a–d** and their corresponding phosphoramidate prodrugs **13a–d** 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 CEM, and African Green monkey Vero cells.[24,25] None of the nucleosides synthesized showed antiviral activity at concentration up to 10 μM (**10b–d**) or 33 μM (**10a**). However, while prodrugs **13b–d** did not display any anti-HCV activity, uracil prodrug **13a** was found weakly active (EC_{50} = 1.5 μM) and non-toxic versus all four tested cell lines. The discrepancy, in term of activity, between nucleoside **10a** and its monophosphate prodrug **13a** can most probably be attributed to a lack of phosphorylation by the cellular kinases.

In addition, all of the nucleosides **10a–d** and prodrugs **13a–d** were evaluated for inhibitory activities against West Nile, Dengue, Chikungunya, RSV, Influenza, Ebola, Norovirus and

HIV, respectively. However, none of them exhibited obvious activities when tested up to 10 μM (Data not shown).

In summary, a series of β-D-2′-deoxy-2′-dibromo substituted U, C A and G nucleosides **10a–d** and their corresponding phosphoramidate prodrugs **13a–d** were synthesized and evaluated against HCV along with other viruses. Molecular modeling studies and FEP calculations indicated that the 2′-deoxy-2′-dibromo sugar ring modification binds favorably to the HCV RdRp active site. However, all four nucleosides **10a–d** and three prodrugs **13b–d** displayed no apparent inhibitory activities against HCV. Notably, the β-D-2′-Br_2-uridine phosphoramidate prodrug analog **13a** exhibited moderate inhibitory activity against HCV (EC_{50}= 1.5 ± 0.8 μM). Since FEP calculations suggest that this agent may be a potent substrate to the HCV RdRp, the modest activity is likely due to other limiting factors unaccounted for by the model (such as prodrug processing or low host kinase phosphorylation). Despite the weak potency of prodrug **13a** as HCV inhibitor, further modifications of these 2′-dihalogenonucleosides are currently being investigated and will be subject of future publications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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.

References and notes

1. Hepatitis, C. Global Hepatitis Report. World Health Organization; Geneva: 2017. www.who.int
2. Lavanchy D. Liver Int. 2009; 29:74–81.
3. Goossens N, Hoshida Y. Clin Mol Hepatol. 2015; 21:105–114. [PubMed: 26157746]
4. Yau AH, Yoshida EM, Can J. Gastroenterol Hepatol. 2014; 28:445–451.
5. Coats SJ, Garnier-Amblard EC, Amblard F, Ehteshami M, Amiralaei S, Zhang H, Zhou L, Boucle SR, Lu X, Bondada L, Shelton JR, Li H, Liu P, Li C, Cho JH, Chavre SN, Zhou S, Mathew J, Schinazi RF. Antiviral Res. 2014; 102:119–147. [PubMed: 24275341]
6. Curry MP, Tapper EB, Bacon B, Dieterich D, Flamm SL, Guest L, Kowdley KV, Lee Y, Milligan S, Tsai N, Younossi Z, Afdhal NH. Aliment Pharmacol Ther. 2017; 46:540–548. [PubMed: 28691377]
7. Lau G, Benhamou Y, Chen G, Li J, Shao Q, Ji D, Li F, Li B, Liu J, Hou J, Sun J, Wang C, Chen J, Wu V, Wong A, Wong C, Tsang S, Want Y, Bassit L, Tao S, Jiang Y, Hsiao H, Ke R, Perelson AS, Schinazi RF. Lancet Gastroenterol Hepatol. 2016; 1:97–104. [PubMed: 27917405]
8. Gentile I, Maraolo AE, Buonomo AR, Zappulo E, Borgia G, the discovery of sofosbuvir: a revolution for therapy of chronic hepatitis C. Expert Opin Drug Discov. 2015; 10:1363–1377. [PubMed: 26563720]
9. Shelton J, Lu X, Hollenbaugh J, Cho JH, Amblard F, Schinazi RF. Chem Rev. 2016:14379–14455. [PubMed: 27960273]
10. Pinho P, Kalayanov G, Westerlind H, Rosenquist Å, Wähling H, Sund C, Almeida M, Ayesa S, Tejbrant J, Targett-Adams P, Eneroth A, Lindqvist A. Bioorg Med Chem Lett. 2017; 27:3468–3471. [PubMed: 28622881]

11. Zhou S, Mahmoud S, Liu P, Zhou L, Ehteshami M, Bassit L, Tao S, Domaoal RA, Sari O, Schutter CD, Amiralaei S, Khalil A, Ollinger Russell O, McBrayer T, Whitaker T, Abou-Taleb N, Amblard F, Coats SJ, Schinazi RF. J Med Chem. 2017; 60:5424–5437. [PubMed: 28595015]
12. Appleby TC, Perry JK, Murakami E, Barauskas O, Feng J, Cho A, Fox D, Wetmore DR, McGrath ME, Ray AS, Sofia MJ. Science. 2015; 347:771–775. [PubMed: 25678663]
13. Christ CD, Mark AE, van Gunsteren WF. J Comput Chem. 2010; 31:1569–1582. [PubMed: 20033914]
14. Wang L, Wu Y, Deng Y, Kim B, Pierce L, Krilov G, Lupyan D, Robinson S, Dahlgren MK, Greenwood J, Romero DL. J Am Chem Soc. 2015; 137:2695–2703. [PubMed: 25625324]
15. Corfu NA, Sigel H. Eur J Biochem. 1991; 199:659–669. [PubMed: 1868851]
16. Cen Y, Sauve AA. J Org Chem. 2009; 74:5779–5789. [PubMed: 19958035]
17. Porcheddu A, Giacomelli G, Piredda I, Carta M, Nieddu G. Eur J Org Chem. 2008:5786–5797.
18. Kramer RA, Bleicher KH, Wennemers H. Helvetica Chimica Acta. 2012; 95:2621–2634.
19. Pradere U, Garnier-Amblard EC, Coats SJ, Amblard F, Schinazi RF. Chem Rev. 2014; 114:9154–9218. [PubMed: 25144792]
20. Ray AS, Fordyce MW, Hitchcock MJM. Antiviral Research. 2016; 125:63–70. [PubMed: 26640223]
21. Ross BS, Reddy PG, Zhang HR, Rachakonda S, Sofia M. J Org Chem. 2011; 76:8311–8319. [PubMed: 21916475]
22. Rondla R, Coats SJ, McBrayer TR, Grier J, Johns M, Tharnish PM, Whitaker T, Zhou L-H, Schinazi RF. Antivir Chem Chemother. 2009; 20:99–106. [PubMed: 19843980]
23. Stuyver LJ, Whitaker T, McBrayer TR, Hernandez-Santiago BI, Lostia S, Tharnish PM, Ramesh M, Chu CK, Jordan R, Shi J, Rachakonda S, Watanabe KA, Otto MJ, Schinazi RF. Antimicrob Agents Chemother. 2003; 47:244–254. [PubMed: 12499198]
24. Schinazi RF, Sommadossi JP, Saalmann V, Cannon DL, Xie M-W, Hart GC, Smith GA, Hahn EF. Antimicrob Agents Chemother. 1990; 34:1061–1067. [PubMed: 2393266]
25. Stuyver LJ, Lostia S, Adams M, Mathew J, Pai BS, Grier J, Tharnish P, Choi Y, Chong Y, Choo H, Chu CK, Otto MJ, Schinazi RF. Antimicrob Agents Chemother. 2002; 46:3854–3860. [PubMed: 12435688]

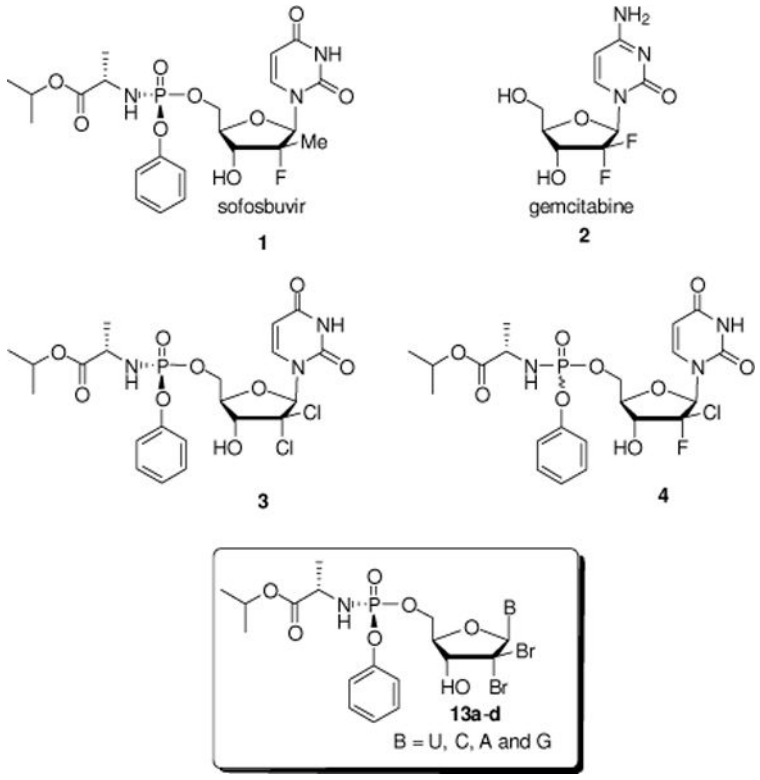


Figure 1.

Selected β-D-2′-deoxy-2′-disubstituted nucleoside and nucleotide analogs and targeted 2′-dibromo nucleotide analogs **13a–d**.

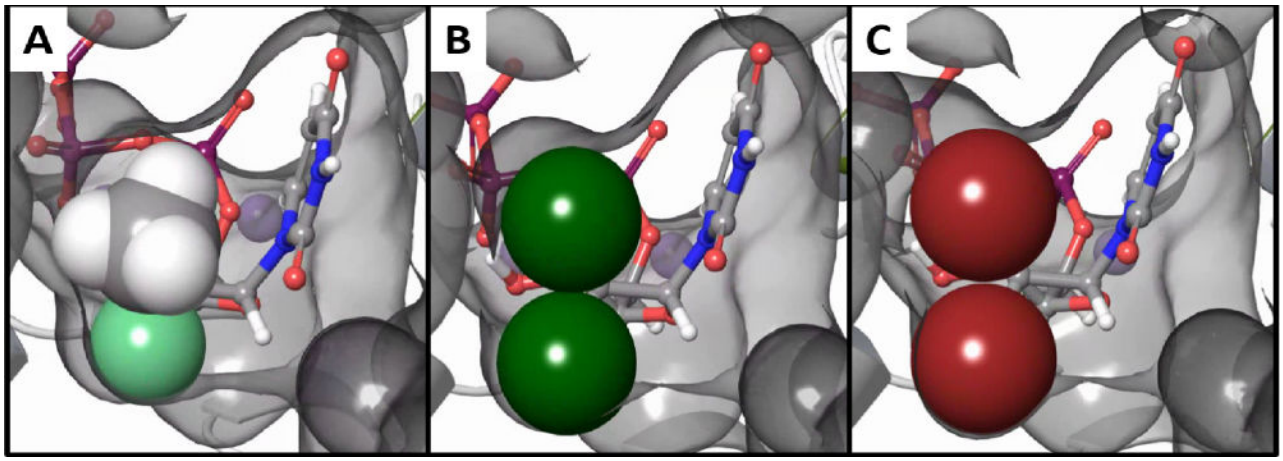


Figure 2.

Models of 2′-modified uridine triphosphate analogs in the HCV NS5 active site (PDBID 4WTG) refined using Prime MMGBSA (5 Å cut-off). The active site surface is rendered in grey, and the Van der Waals radii of the 2′ substitutions are shown as spheres. A) β-D-2′-Me,2′-F-uridine 5′-triphosphate, the active form of sofosbuvir (Prime MMGBSA ΔGbind = −25.3 kcal/mol), B) β-D-2′-diCl-uridine 5′-triphosphate (−29.9 kcal/mol), and C) β-D-2′-diBr-uridine 5′-triphosphate (−30.7 kcal/mol).

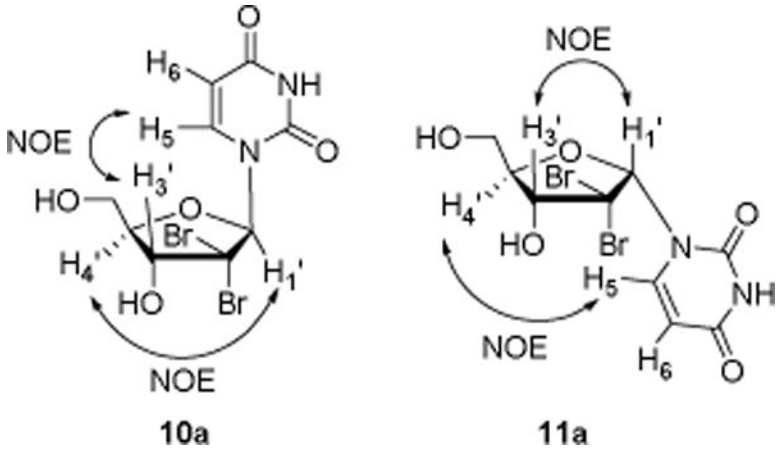
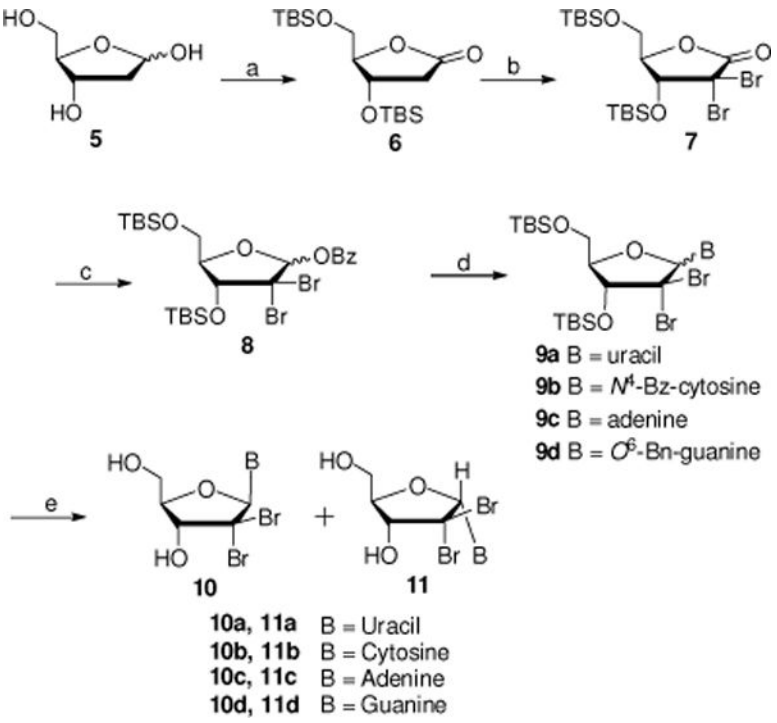


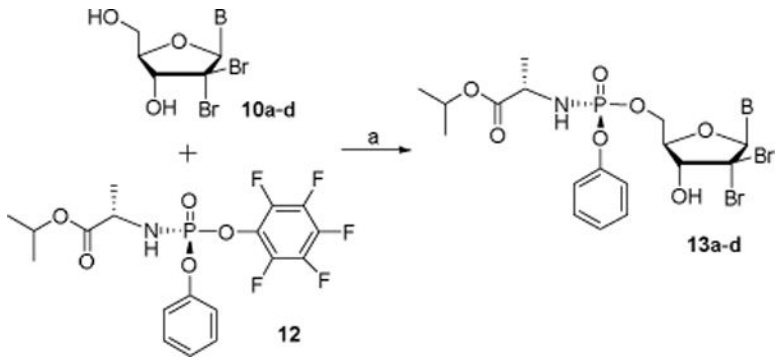
Figure 3.

Anomer assignment for nucleosides **10a** and **11a** *via* NOE experiments.



Scheme 1.

Reagents and conditions: (a) (i) Br2, H2O, rt, 5 d; (ii) TBDMCl, imidazole, DMF, rt, 24 h, 70% over two steps; (b) NBS, LiHMDS, THF, −78 °C to −10 °C, 4–6 h, 80%; (c) (i) DIBAL-H, toluene, −78 °C, 2 h, rt; (ii) BzCl, Et_3N, DCM, 0 °C to rt, 12 h, 81%; (d) (i) nucleobase (Uracil, N^4-Bz-cytosine, N^6-diBoc-adenine or N^4-diBoc-O^6-Bn-Guanine), BSA, CH3CN, 60 °C, 30 min; (ii) TMSOTf, CH_3CN, MW, 120 °C, 10 min; (e) for **9a** and **9c**: TBAF, THF, 0 °C, 1 h, 40–50% yield; for **9b**: (i) NH_3/MeOH, rt, 16–24 h; (ii) TBAF, THF, 0 °C, 1 h, 33% yield; for **9d**: (i) TBAF, THF, 0 °C, 1 h; (ii) TFA, rt, 48–72 h, 33% yield.



Scheme 2.

Synthesis of nucleoside phosphoramidates **13a–d**. Reagents and conditions: (a) ^{t}BuMgCl, THF, 0 °C to rt, 24 h, 18–25%.

Table 1

Results of FEP calculations on 2′-modified nucleotide triphosphates in the HCV NS5 RdRp active site.

2′ Group	2″ Group	FEP ΔΔG (kcal/mol)			
		Tri-anion		Tetra-anion	
−CH3	−F	−2.52	± 0.18	0.44	± 0.13
−Cl	−F	−0.45	± 0.25	−0.67	± 0.23
−Cl	−Cl	−1.89	± 0.27	−3.16	± 0.25
−Br	−Br	−2.87	± 0.26	−4.21	± 0.18

Table 2

HCV genotype 1b replicon activity and cytotoxicity of nucleosides **10a–d** and their phosphoramidate prodrugs **13a–d**

Compound	Anti HCV activity (μM)		Cytotoxicity, CC_{50} (μM)			
	EC_{50}	EC_{90}	Huh-7	PBM	CEM	Vero
10a	> 33	> 33	> 33	> 100	> 100	> 100
10b	> 10	> 10	> 10	> 100	82	> 100
10c	> 10	> 10	> 10	> 100	> 100	> 100
10d	> 10	> 10	> 10	> 100	> 100	> 100
13a	1.5 ± 0.8	2.7 ± 0.3	> 10	> 100	95	> 100
13b	> 10	> 10	> 10	> 100	73	> 100
13c	> 10	> 10	> 10	57	33	60
13d	> 10	> 10	> 10	> 100	34	> 100
2′*C*-Me C	1.7 ± 0.8	6.5 ± 1.9	> 10	> 100	> 100	> 100
sofosbuvir	0.2 ± 0.1	0.5 ± 0.3	> 10	> 100	> 100	> 100

All assays were performed in replicates. Only means + SD of three replicates are shown

Bioorg Med Chem Lett. Author manuscript; available in PMC 2018 December 01.