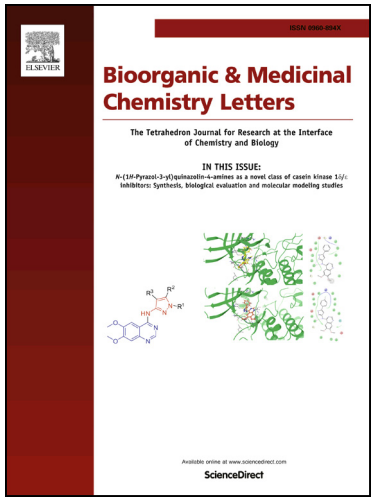

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Concise syntheses and HCV NS5B polymerase inhibition of (2'*R*)-3 and (2'*S*)-2'-ethynyluridine-10 and related nucleosides

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ABSTRACT

(2'*R*)-Ethynyl uridine **3**, and its (2'*S*)-diastereomer **10**, are synthesised in a divergent fashion from the inexpensive parent nucleoside. Both nucleoside analogues are obtained from a total of 5 simple synthetic steps and 3 trivial column chromatography purifications. To evaluate their effectiveness against HCV NS5B polymerase, the nucleosides were converted to their respective 5'-O-triphosphates. Subsequently, this lead to the discovery of the 2'-β-ethynyl **18** and -propynyl **20** nucleotides having significantly improved potency over Sofosbuvir triphosphate **24**.

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For more than half a century nucleoside analogues have found widespread therapeutic application.[1] During this period, base and sugar modified derivatives of the natural products remain the foundation for the counteraction of both DNA and RNA virus infections as well as in the treatment of a variety of cancers. In most cases, these analogues mimic their physiological counterparts, being metabolised into the corresponding 5'-O-triphosphates, subsequently incorporated into the growing DNA or RNA chain and, as a result, inhibit viral replication or cancer cell division. In his seminal work, in 1959, Prusoff described the synthesis and biological activities of 5-iodo-2'-deoxyuridine (a thymidine analogue; IDU, **1**, Figure 1), ultimately to become the first marketed antiviral agent against Herpes Simplex Virus (HSV).[2] From these ground breaking activities, nucleoside analogues have been designed to combat a variety of viral diseases[3] such as hepatitis B (HBV),[4] human immunodeficiency virus (HIV)[5] and other herpes viruses such as human cytomegalovirus (CMV).[3] Most recently, following more than a decade of investigation of β-D-2'-β-methylribose derivatives by a variety of groups,[6] Sofosbuvir, a monophosphate prodrug that incorporates β-D-2'-deoxy-2'-α-fluoro-2'-β-*C*-methyluridine (**2, Figure 1**) was approved by the FDA in late 2013 for the treatment of hepatitis C (HCV).[7]

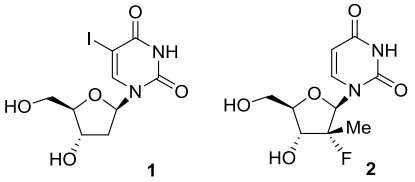


Figure 1. Examples of base and sugar modified nucleosides used in the treatment of DNA and RNA viruses..

Towards a similar goal, we wished to effectively replace the methyl group in nucleosides such as **2** with an ethynyl functionality (**3** and **4**, Figure 2) and subsequently investigate their antiviral properties.

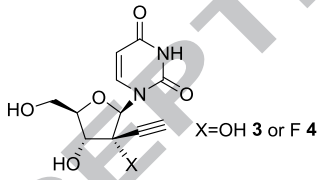
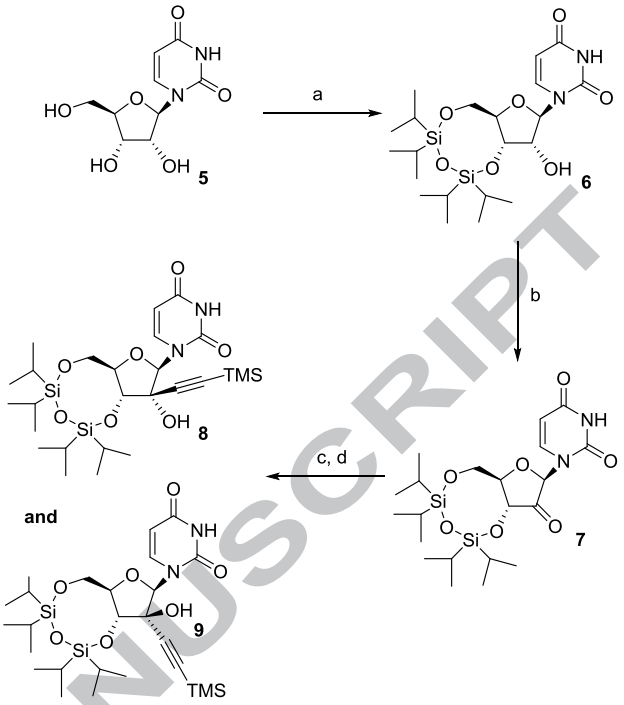


Figure 2. Ethynyl analogues **3** and **4** of nucleoside **2**..

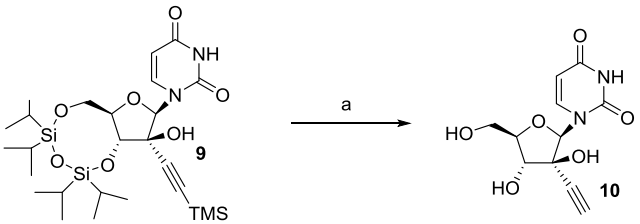
It is commonly known the syntheses of base and ribose modified nucleosides is a challenging problem, often resulting in severe restrictions to potential applications and alternative strategies have been and are currently being explored.[8] Our syntheses began with the readily available and inexpensive parent nucleoside **5 (**Scheme 1**)**. On exposure to 1,3-dichloro-1,1,3,3-tetrakis(2-methylethyl)-disiloxane in anhydous pyridine, the natural product generated the 3', 5'- diprotected bis-silyl ether **6**.[9] Although the intermediate **6** is commercially available, it was convenient, at least on larger scales, to prepare and use this material in crude form. Subsequent TEMPO-mediated oxidation provided the ketone **7**, again, used in the next step without purification. On treatment with an excess of freshly prepared lithium trimethylsilylacetylide at -78°C, in THF, the ketone **7** was converted to the (2'*R*)-tertiary-alcohol **8**, along with the expected (2'*S*)-epimer **9**, identified by detailed NMR experiments.[10] Fortunately, both diastereomers can easily be separated by silica gel column chromatography, providing gram quantities of each component for further manipulation.[11]



Scheme 1. Reagents and conditions: a) 1,3-dichloro-1,1,3,3-tetrakis(2-methylethyl)-disiloxane, pyridine, room temperature, 100%; b) TEMPO, tBuOCl, KBr, $NaHCO_3$, ice bath to room temperature, 83%; c) Trimethylsilylacetylene, *n*-BuLi, THF, Et_3N, -70°C; d) Silica gel column chromatography, compound **9**, 69%, followed by compound **8**, 6%.

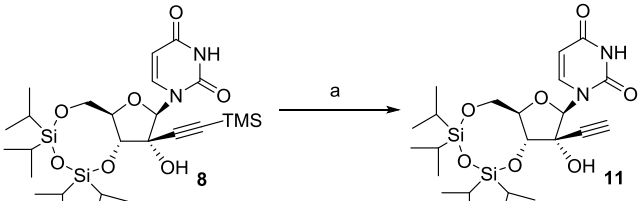
The intermediates **8** and **9** offer a variety of opportunities for further modification. The simplest, of course, is complete desilylation, achieved with fluoride. to the titled compounds **3** and **10**. For example, on exposure to tetra-n-butylammonium fluoride, the (2'*R*)-tri-silyl diastereomer **8**, provides the ribose modified nucleoside **3** in 64% yield, following purification by silica gel column chromatography (Scheme 2). Using the same protocol, the trisilyl protected nucleoside **9**, provided the titled triol **10**.

The nucleoside **3** had been previously prepared in 5 steps from 1,3,5-tri-*O*-benzoyl-α-ribofuranose)[12] Although the overall yield in the previous route is higher, the present methodology employs an alternative using inexpensive reagents in a robust fashion.



Scheme 2. Reagents and conditions: a) $Et_4NF{:}H_2O$, THF, room temperature followed by silica gel column chromatography, (64% for **3** from **8**; 57% for **10** from **9**).

In addition, the present route provides intermediates such as **8** and **9** that are more amenable for further modification (Scheme 3). For example, selective deprotection of the trimethylsilyl group in compound **8** was achieved with methanolic potassium carbonate, providing the terminal acetylene **11**.



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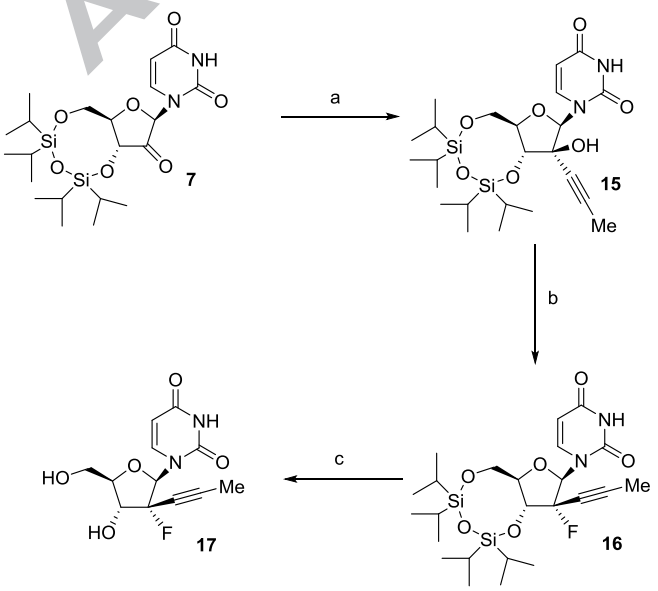
Scheme 3. Reagents and conditions: a) K_2CO_3, MeOH, room temperature, 40%; b) Boc_2O, DMAP, room temperature, 67%; c) *n*-BuLi, HMPA MeI, -70°C to room temperature, 53%; d) 20% TFA in CH_2Cl_2, room temperature, 83%; e) TBAF, room temperature, 71%.

The exposed hydroxyl and base imide are masked as their *tert*-butyl carbonate and carbamates **12**, prior to alkylation with iodomethane in the presence of *n*-butyllithium and HMPA to the protected nucleoside **13**. Finally, removal of the protecting groups provides the C2'-3-carbon uridine analogue **14**.

As mentioned above, an advantage of the current methodology, at least from a medicinal chemistry perspective, is the availability of the 2'-(S)-intermediates, such as **9**, for further modification. In a separate approach, treatment of the ketone **7** with propynylmagnesium chloride provided the alcohol **15**, as the exclusive product (Scheme 4).



Scheme 4. Reagents and conditions: a) Propynylmagnesium bromide, THF, ice bath, 54%; b) DAST, toluene, -20C, 35%; c) $Et_4NF{:}H_2O$, THF, room temperature, 63%.

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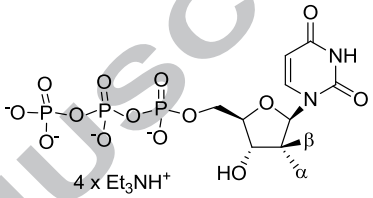
Subsequent exposure to DAST proceeds with inversion, delivering the fluoride **16**. Removal of the silicon protecting groups provides the nucleoside **17**, a close derivative of **3.**

As a potential measure of their effectiveness as antiviral agents, the aforementioned nucleosides were converted to the corresponding 5'-O-triphosphates **18-24**[13] and subsequent inhibitory activity against the wild-type NS5B polymerase evaluated (Table 1).[14]



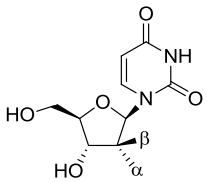
NTP	α	β	IC_{50} (μM)
18	—OH	—≡	0.09
19	—≡	—OH	~54
20	—OH	—≡—Me	0.16
21	—F	—≡	3.6
22	—F	—≡—Me	9.9
23	—OH	—Me	0.43[a]
24	—F	—Me	1.1[a,15]

[a]Prepared and tested as dimethylhexylammonium salts.

Table 1 Activities of the nucleoside triphosphates against NS5B polymerase.

As expected, the *ribo*-analogue **18** was considerably more potent than its *xylo*-counterpart **19,** and approximately 4-fold more active than the C2'-β-Me analogue **23**. Introduction of a methyl group at the terminii of the acetylene was tolerated. The propynyl derivative **20** was slightly less potent than **18**, while in the fluoride series the 3-carbon C2' analogue **22** was 2-3 times less active than the ethynyl **21**. Importantly, the triphosphate derived from Sofosbuvir **24** is only 3-fold more potent than than it's 2'-acetylene analogue **21**, while being an order of magnitude less effective than the corresponding hydroxy analogue **18**.

The anti-HCV activities, evaluated in the 1b genotype replicon assay and cytotoxicity of the most promising nucleosides were evaluated and are shown in Table 2.[16]



Nuc.	α	β	EC_{50} (μM)	CC_{50} (μM)
3	—OH	—≡	>100	>100
14	—OH	—≡—Me	>100	>100
4	—F	—≡	N.T.[a]	>100
17	—F	—≡—Me	>100	>100

[a]Not Tested.

Table 2. Anti-HCV and Cytotoxicity of Nucleosides.

Of the nucleosides analyzed (**3**, **14** and **17**) none displayed any appreciable activity up to a concentration of 100μM. To exert anti-HCV activity, the nucleosides must first be metabolized by kinases to the 5'-O-triphosphates. Based on experience with other C2'-uridine nucleosides (including **2**, the key component of Sofosbuvir), the rate-limiting process in this step-wise transformation, is the initial phosphorylation, and hence the lack of potency displayed in Table 2 is not surprising.[17] Importantly, none of the nucleosides tested demonstrated cytotoxicity.

In summary, a series of nucleosides bearing an ethynyl or propynyl substituent at the C2'-β-position and a hydroxyl or fluoro as the α-substituent were prepared. The synthetic route provides rapid access to both C2'-epimeric intermediates **8** and **9** and hence allowing flexibility in the design of novel nucleosides. To evaluate their potential as anti-HCV agents, the corresponding 5'-*O*-triphosphates were prepared and their inhibition of wild type HCV NS5B polymerase was evaluated and compared to **24**, the active triphosphate of sofosbuvir. While the fluoro derivative **21** was approximately 3 times less potent, the β-substituted ribose analogues **18** and **20** were 7- and 10-fold more potent than sofosbuvir triphosphate respectively. Although the nucleosides did not display anti-viral activity, monophosphate prodrugs, such as the one demonstrated in Sofosbuvir, have been developed to by-pass the inefficient initial kinase step. Application of this and other studies related to the nucleosides described in this paper will be presented elsewhere.

References and Notes:

[1] Jordheim, L. P.; Durantel, D.; Zoulim, F.; Doumontet, C;. *Nature Rev. Drug Discov*, **2013**, *12*, 447.

[2] Prusoff, W. H.; *Biochim. Biophys. Acta*, **1959**, *32*, 295.

[3] De Clerq, E.; *Med. Res. Rev.*, **2013**, *33*, 1215.

[4] (a) Ohishi, W.; Chayama, K.; *Hepatol. Res*, **2012**, *42*, 219. (b) Fung, J., Lai, C-L.; Seto, W-K.; Yuen, M-F.; *J. Antimicrob. Chemother*., **2011**, *66*, 2715. (c) Kim, S. S.; Cheong, J. Y.; Cho, S. W.; *Gut Liver*, **2011**, *5*, 278.

[5] Kukhanova, W. H.; *Mol. Biol.* **2012**, *46*, 768.

[6] Sofia, M. J.; *Adv. Pharmacol.* **2013**, *57*, 39.

[7] (a) Sovaldi (sofosbuvir): FDA press release dated December 6, 2013. http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm377888.htm. (b) Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H-R.; Bansal, S.; Espiritu, C.; Keilmam, M.; Lam, A. M.; Micolochick Steuer, H. M.; Niu, C.; Otto, M. J.; Furman, P. A.; *J. Med. Chem*., **2010**, *53*, 7202.

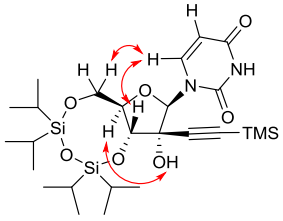
[8] Mikhailopulo, I. A; Miroshnikov, A. I.; *Mendeleev Commun.* **2011**, *21*, 57.

[9] ***Robust preparation of intermediates 8 and 9***,. Uridine (**5**; 20.00g; 82mmol) was azeotroped with pyridine (approx. 50ml) and then dissolved in anhydrous pyridine (180ml). 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (30ml; 82ml) was added dropwise to this solution, while cooled in an ice bath, under an atmosphere of nitrogen. The resulting reaction mixture was allowed to warm to room temperature overnight and patitioned between EtOAc and 10% aq. HCl. The organic phase was separated, washed with a further portion of 10% aq. HCl, sat. aq. sodium bicarbonate, brine, dried ($MgSO_4$). The volatiles were removed under reduced pressure to provide the 1',3'-diprotected uridine intermediate (**6**; 39.9g; 100%). LCMS: MH+, 487.48. This material was used in the next step (below) without purification.

Sodium bicarbonate (20.67g; 246mmol) was dissolved in water (80ml) and added to a stirred solution of the crude alcohol (**6**; 39.9g; 82mmol), TEMPO (1.92g; 12.3mmol) and potassium bromide (1.46g; 12.3mmol) in dichloromethane (120ml). The resulting mixture was cooled in an ice bath and stirred vigorously before the dropwise addition of sodium hypochlorite (189ml of a 5.75% aq. solution; 176mmol). The reaction was stirred until LCMS indicated that the reaction was complete (approx. 1h.) then partitioned between 10% aq. sodium thiosulfate and dichloromethane. The organic phase was separated, washed with an additional portion of sodium thiosulfate, brine, dried ($MgSO_4$), and the volatiles removed under reduced pressure to give the crude ketone (**7**; 33.0g; 83%) that co-exists with its hydrate form, consistent with a previous observation in a similar system..[11] LCMS: MH^+, 485.50 and $(M+H_2O+H)^+$, 503.27. This material was used in the next step (below) without purification.

n-Butyllithium (77ml of a 1.6M solution in hexane; 124mmol) was added dropwise to a stirred solution of trimethylsilylacetylene (12.16g; 124mmol) in anhydrous THF (125ml) at -78°C, under an atmosphere of nitrogen. When the addition was complete, the solution was stirred for a further 30min. and the crude ketone (**7**; 20.00g; 41.3mmol) in THF (40ml) was added. After stirring for a further 3h., the reaction was quenched by the addition of sat. aq. ammonium chloride followed by EtOAc. The organic layer was separated, dried (MgSO4) and the volatiles removed under reduced pressure. The residue was purified by silica gel column chromatography using 0 to 20% EtOAc in hexanes as eluent to give (2'-*S*)-intermediate (**9**; 16.60g; 69.0%).: ^{1}H NMR ($CDCl_3$): δ 8.18 (br. s, 1H), 7.87 (d, *J*=8.2Hz, 1H), 6.05 (s, 1H), 5.70 (dd, *J*=8.2 and 2.3Hz, 1H), 4.16 (dd, *J*=13.4 and 1.1Hz, 1H), 4.11 (d, *J*=9.3Hz, 1H), 4.01 (dd, *J*=13.4 and 2.5Hz, 1H), 3.96 (ddd, *J*=9.3, 2.5 and 1.1Hz, 1H), 2.85 (s, 1H), 0.93-1.13 (m, 28H), 0.20 (s, 9H). ^{13}C NMR ($CDCl_3$) -0.29 (3C), 12.68, 12.91, 13.07, 13.48, 16.67, 16.83, 17.00, 17.02, 17.22, 17.36, 17.39, 17.53, 59.86, 72.95, 76.60, 81.41, 88.91, 94.73, 100.92, 101.67, 139.75, 151.00, 162.70. LCMS: MH^+, 583.36. Followed by the (2'-*R*)-isomer (**8**; 1.46g; 6.1%): 1H NMR (CDCl3): 8.15 (br. s, 1H), 7.60 (d, *J*=8.1Hz, 1H), 6.08 (s, 1H), 5.70 (dd, *J*=8.1 and 2.3Hz, 1H), 4.35 (d, J=8.9Hz, 1H), 4.21 (d, *J*=12.5Hz, 1H), (H6, 1H), (H4, 1H), (s, 2OH, 1H), 0.98-1.18 (m, 28H), 0.16 (s, 9H). ^{13}C NMR ($CDCl_3$): -0.65 (3C), 12.40, 12.47, 12.94, 13.81, 16.94, 16.97, 17.07, 17.17, 17.19, 17.20, 17.21, 17.34, 59.56, 74.64, 76.15, 81.26, 90.50, 93.16, 101.39, 101.84, 139.53, 149.73, 162.53. LCMS: MH^+, 583.36.

[10] Key NOE interactions (red arrows) of compound **8**.



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[11] For a similar synthetic approach see: Wang, G, Beigelman, L.; WO2013/096680

[12](a) Harry-O'kuru,; R. E.; Kryjak, E. A.; Wolfe, M. S..; *Nucleosides Nucleotides* **1997**, *16*, 1457. (b) Harry-O'kuru, R. E.; Smith, J. M.; Wolfe, M. S.; *J. Org. Chem.*, **1997**, *62*, 1754. [11] (a) Harry-O'kuru,; R. E.; Kryjak, E. A.; Wolfe, M. S..; *Nucleosides Nucleotides* **1997**, *16*, 1457. (b) Harry-O'kuru, R. E.; Smith, J. M.; Wolfe, M. S.; *J. Org. Chem.*, **1997**, *62*, 1754.

[13] ***General Preparation of Nucleoside 5'-Triphosphates*** The preparation 5'-triphosphates.(**18-22**) were carried under contractual agreement with TriLink Biotechnologies, San Diego, CA.and analysed as the triethylammonium salts using general synthetic methods: (a) Ludwig, J.; *Acta Biochim. Biophys. Acad. Sci. Hung.***1981**, *16*, 131. (b) Mishra, N. C.; Broom, A. D.; *J. Chem. Soc., Chem. Commun*. **1991**, *18*, 1276. Triphosphates **23** and **24** were prepared in a similar manner and examined as the dimethylhexylammonium salts.

[14] ***Inhibition of HCV NS5B Polymerase by Nucleoside Triphosphate Analogs.*** This assay is a modified version of the assay described in International Publication No. WO2002/057287. Briefly, 50 μL reactions containing 20 mM HEPES (pH 7.3); 7.5 mM DTT; 20 units/ml RNasIN; 1 μM each of ATP, GTP, UTP and CTP; 20 μCi/mL [^{33}P]-CTP; 10 mM $MgCl_2$

60 mM NaCl; 100 µg/ml BSA; 0.021 µM DCoH heteropolymer RNA template; and 5 nM NS5B (1b-BKΔ55) enzyme are incubated at room temperature for 1 hour. The assay is then terminated by the addition of 500 mM EDTA (50 µL). The reaction mixture is transferred to a Millipore DE81 filter plate and the incorporation of labeled CTP is determined using Packard TopCount. Compound IC_{50} values can then be calculated from experiments with 10 serial 3-fold dilutions of the inhibitor in duplicate. The intrinsic potency (Ki) of an NTP inhibitor is derived from its NS5B IC_{50} using the Cheng-Prusoff equation for a competitive inhibitor, as described in Cheng *et al*., *Biochem Pharmacol* 22:3099-3108 (1973): $Ki = IC_{50} / (1+[S]/K_m)$, where [S] = 1 µM, and K_m is the concentration of cognate NTP yielding half-maximal enzyme activity in the assay absent exogenous inhibitors.

[15] Murakami, E.; Niu, C.; Bao, H.; Micolochick Steuer, H. M.; Whitaker, T.; Nachman, T.; Sofia, M. A.; Wang, P.; Otto, M. J.; Furman, P. A.; *J. Antimicrob. Chemother*., **2007**, *52*, 458.

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[16] ***Cell-Based anti-HCV Activity and Cytotoxicity***. Replicon cells (1b-Con1) are seeded at 5000 cells/well in 96-well plates one day prior to inhibitor treatment. Various concentrations of an inhibitor in DMSO are added to the replicon cells, with the final concentration of DMSO at 0.5% and fetal bovine serum at 10% in the assay media. Cells are harvested three days post dosing. The replicon RNA level is determined using real-time RT-PCR (Taqman assay) with GAPDH RNA as endogenous control. EC_{50} values are calculated from experiments with 10 serial twofold dilutions of the inhibitor in triplicate. To measure cytotoxicity in replicon cells of an inhibitor, an MTS assay is performed according to the manufacturer's protocol for CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Cat # G3580) three days post dosing on cells treated identically as in replicon activity assays. CC_{50} is the concentration of inhibitor that yields 50% inhibition compared to vehicle-treated cells. Effect of an inhibitor on cellular DNA synthesis is determined by a scintillation proximity assay. Replicon cells are seeded in 96-well Cytostar-T Scintillating Microplates (PerkinElmer, Cat # RPNQ0163) one day prior to inhibitor treatment. Various concentrations of an inhibitor in triplicate are added with [methyl-^{14}C]-thymidine (PerkinElmer, Cat # NEC568050UC, final concentration 0.5 µCi/mL media) and incubated for three days. DNA CC_{50} is the inhibitor concentration that yields 50% inhibition of labeled thymidine incorporation as measured by Packard TopCount compared to vehicle-treated cells.

[17] Furman,; P. A.; Muramami, E.; Niu, C..; Lam, A. M.; Espiritu, C.; Bansal, S.; Bao, H.; Tolstykh, T.; Sreuer, H. M.; Keilman, M.; Zennou, V.; Bourne, N.; Veselenak, R. L.; Chang, W.; Ross, B. S.; Du, J.; Otto, M. J.; Sofia, M. J.; *Antiviral Res.* **2011**, *91*, 120