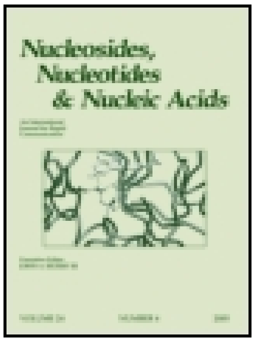

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Merck Research Laboratories, Kenilworth, NJ , USA

ABSTRACT
Novel 2′-modified guanosine nucleosides were synthesized from inexpensive starting materials in 7–10 steps via hydroazidation or hydrocyanation reactions of the corresponding 2′-olefin. The antiviral effectiveness of the guanosine nucleosides was evaluated by converting them to the corresponding 5′-O-triphosphates (compounds **38–44**) and testing their biochemical inhibitory activity against the wild-type NS5B polymerase.

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Introduction

Nucleoside analogues have found widespread therapeutic application for more than half a century for the treatment of both DNA and RNA viral infections as well as a variety of cancers.[1] Nucleosides are prodrugs that inhibit viral replication or cancer cell division by being metabolized intracellularly into the corresponding 5′-O-nucleoside triphosphates (NTPs) which are subsequently incorporated into the growing DNA or RNA chain.[2,3] Compound **1** (Figure 1) was synthesized in 1966 and it was later determined that the introduction of a 2′-beta methyl group leads to the inhibition of the HCV NS5b polymerase.[4] Since then numerous inhibitors with the 2′-beta methyl scaffold such as NM-107[5a] (**2**, Figure 1), PSI-938[5a] (**3**, Figure 1) and most recently sofosbuvir[5b] (**4**, Figure 1) have provided proof of the concept that inhibition of the viral polymerase can be an effective method of HCV treatment.

Most known effective inhibitors in the literature possessed a 2′-alpha OH or isosteres such as 2′-alpha F, but there was very little known about other modifications. Therefore, our goal was to probe the tolerance for steric and electronic modifications at the 2′-alpha position as well as the need for hydrogen bond

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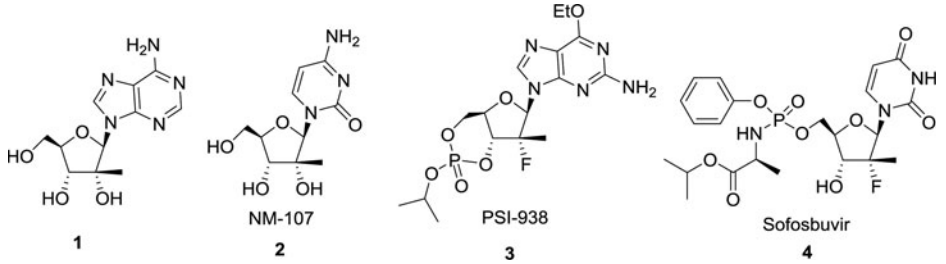

Figure 1. Examples of base and sugar modified nucleosides active intracellularly as inhibitors of RNA viruses.

donors/acceptors. Functionalities such as the alpha substituted azide, amino and cyano groups (**5**, Figure 2) fit this criteria and also have the potential to be readily converted to additional functionalities to further elaborate the SAR.

Due to the challenges of nucleoside chemistry it was important to develop robust methodologies that would allow us to incorporate functionality in an efficient manner. Our group had previously demonstrated that the hydroazidation/hydrocyanation chemistry developed in the Carreira group could be performed on complex systems.[6] Thus, we envisioned that a variety of novel substituents could be obtained from a common intermediate such as the exocyclic alkene (**6**, Figure 2).[7]

The other differentiating factor for novel analogs was the nucleoside base. Since it was unknown how the potency of analogs would compare to 2′-OH/2′-F analogs, it was important to use a base that was highly active in cellular/biochemical assays which have been used to benchmark compounds in the literature.[5] Even though guanosine, uridine and cytidine are all found in clinical candidates, literature suggested that guanosines generally have the best intrinsic potency, therefore, they were the initial focus of our attention.[5]

Synthesis

The chemistry to synthesize the 2′-alkene was initiated from the readily available compound **7** which was peracetylated to provide **8** (Scheme 1). Protection of the guanosine was necessary to avoid functional group incompatibilities during later parts of the synthesis, therefore, we chose to use both the 6-OMe and 6-OEt groups that have been shown to be viable guanosine prodrugs motifs in the clinic.[5] Protection as the 6-OEt/OMe was accomplished using Mitsunobu conditions with either methanol or ethanol followed by removal of the hydroxy protecting groups to

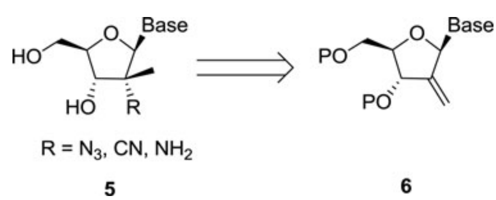

Figure 2. 2′alpha position modified nucleosides via the 2′-olefin.

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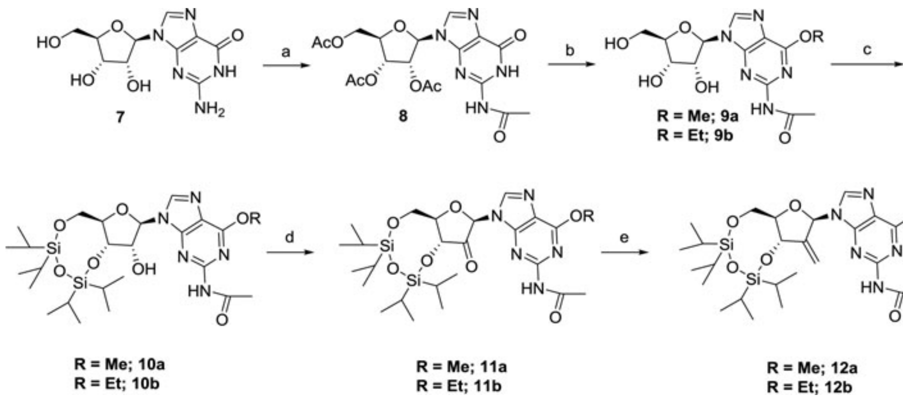
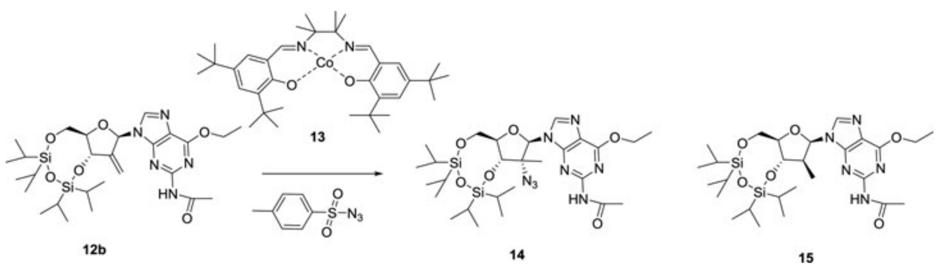


Scheme 1. Reagents and conditions. (a) acetic anhydride, pyridine, 70°C; 92%; (b) *i*. DIAD, EtOH or MeOH, dioxane. *ii*. Ammonium hydroxide, methanol; **9a**: 93% over two steps, **9b**: 86% over two steps.; (c) tetraisopropyldisiloxanedichloride pyridine; **10a**: 97%, **10b**: 82%; (d) Dess-Martin periodinane, CH_2Cl_2; **11a**: 65%, **11b**: 78%; (e) methyltriphenylphosphonium bromide, potassium hexamethyldisilazide, THF; **12a**: 52%, **12b**: 66%.

provide **9a** and **9b**. The 3′- and 5′- alcohols were protected as the 3′-5′-bis-silyl ether using 1,3-dichloro-1,1,3,3-tetrakis(2-methylethyl)-disiloxane to provide **10a** and **10b**. TEMPO mediated oxidation of the 2′-alcohols to the ketones provided compounds **11a** and **11b**. These compounds could be converted to the exocyclic alkenes **12a** and **12b** by treatment with an excess of the potassium salt of methylphosphonium bromide in good yield and on a multigram scale.

With compound **12b** in hand, it was possible to test the hydroazidation reaction (Scheme 2).[8] First attempts of the hydroazidation chemistry (Table 1, Entries 1 and 2) were performed by dissolving a catalytic amount of Co catalyst **13** and 1.5 or 3 eq



Scheme 2. Hydroazidation reaction of **12b**.

Table 1. Reagents and conditions.

Entry	Co (eq)	TsN_3 (eq)	$PhSiH_3$ (eq)	TsN_3/EtOH[a]	Yield%
1	0.02	1.5	1.5	1/99	<5
2	0.02	3	1.5	3/97	<5
3	0.02	10	1.5	10/90	15
4	0.02	15	1.5	50/50	42
5	0.02	30	1.5	100/0	45
6	0.02	30	1.5[b]	100/0	65

[a]Ratio by volume of TsN_3 and ethanol at start of reaction. [b]Phenylsilane was added dropwise in EtOH (equal to volume of TsN_3).

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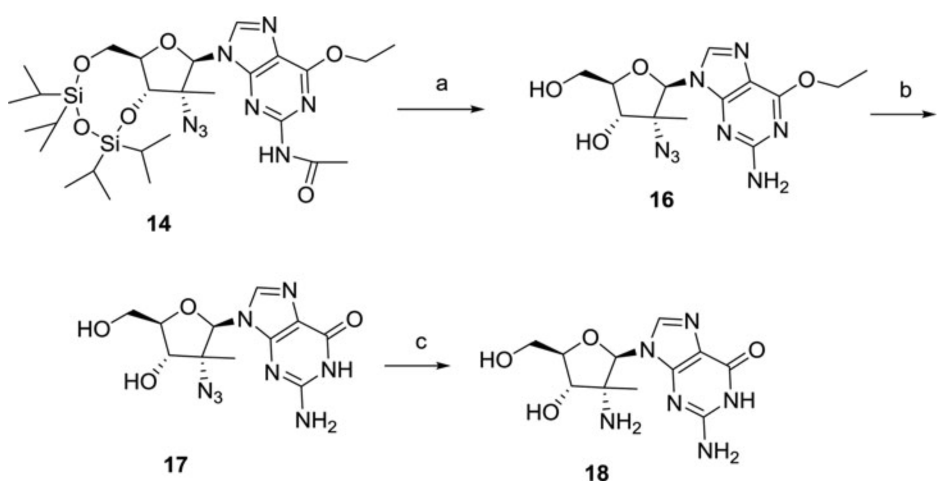
of TsN_3 in ethanol and adding neat phenylsilane. This provided very small amounts of the desired product **14** and large amounts of side product **15**. Even though the yield was very low, we isolated only the desired alpha-isomer of the 2′-azide most likely due to the steric congestion of the beta face of the ribose. Several attempts were made to improve the yield of the reaction including modifications to the protecting groups on the substrate, catalyst (various ligands/alternative metals), solvent, azide reagent and silane but none of these modifications improved the reaction. Therefore, it was essential to better understand the reaction by making incremental changes to solvent and equivalents of reagents. From entries 1 and 2, it was observed that the reaction was extremely fast (all starting olefin was consumed upon the complete addition of Ph_3SiH), therefore, it was hypothesized that the product ratio could be improved by having a larger effective concentration of TsN_3. Incremental increases in the equivalents of TsN_3 increased the yield of compound **14** by reducing the amount of side product **15** (entries 3 and 4). The best yield of 45% was achieved by removing the solvent and using 30 equivalents of TsN_3 (entry 5). Due to the fast reaction rate upon addition of the phenylsilane, it was hypothesized that the addition rate may also impact the yield of the reaction. Addition of the phenylsilane dropwise in ethanol further improved the yield of the conversion to 65% which was sufficient to access the desired targets (entry 6).

With the yield optimized to be able to provide gram quantities of **14**, we were able to obtain the desired 6-OEt nucleoside **16** by deprotection using TBAF followed by treatment with methanolic ammonia (Scheme 3). The 6-OEt protecting group was removed using 1M HCl at 50°C to provide the desired 2′-azido guanosine analog **17**. The 2′-alpha amino analog **18** was synthesized via hydrogenation of the azide **17** in the presence of $Pd(OH)_2$.



Scheme 3. Synthesis of 2′-amino modified nucleosides.

Reagents and conditions. (a) *i*. TBAF, THF, room temperature, *ii*. 7M ammonia in methanol, (98% over two steps); (b) 1M HCl, THF, 50°C, 64%; (c) H_2, $Pd(OH)_2$, MeOH, 91%.

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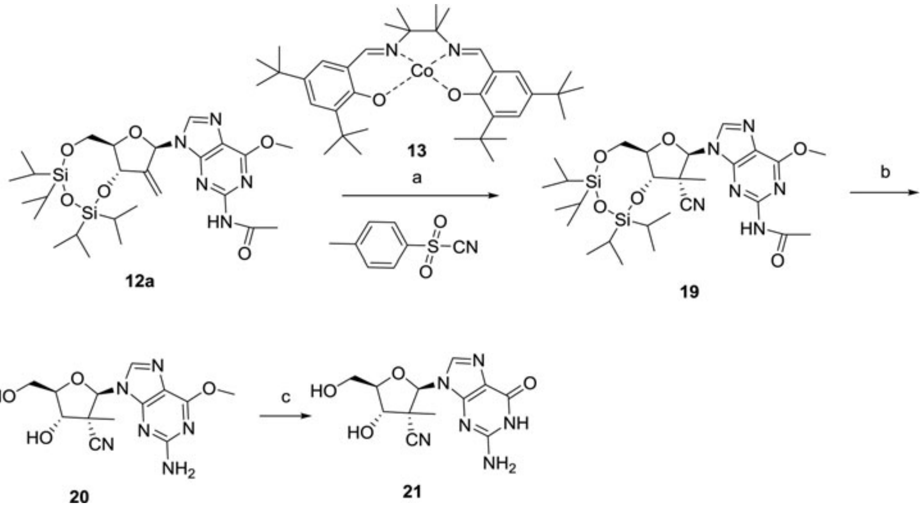
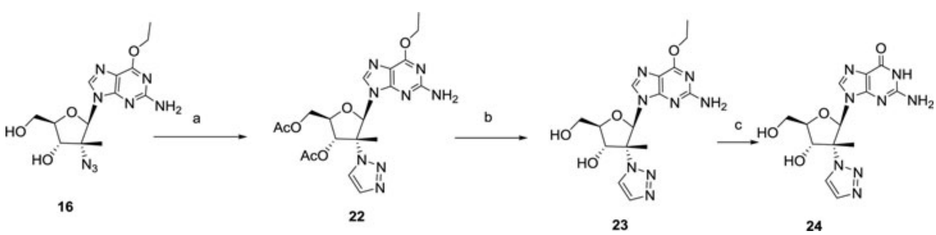
The hydrocyanation reaction[9] (Scheme 4) proved to require additional optimization since the optimized hydroazidation reaction was run solvent-free and TsCN is a solid. Therefore, it was important to identify an appropriate solvent for the reaction that could solubilize the TsCN while maintaining high effective concentrations. After screening numerous solvents it was found that the use of 20 equivalents of TsCN dissolved in a minimal amount of dioxane provided a reasonable yield of desired compound **19** as a single diastereomer. Once again, it was important to add the phenylsilane very slowly in ethanol in order to minimize production of the reduced side product. The target 6-OMe nucleoside **20** was reached via global deprotection of **19** using TBAF followed by sodium methoxide. Treatment of the 6-OMe nucleoside **20** with 1M HCl at 50°C provided the desired 2′-CN guanosine nucleoside **21**.



Scheme 4. Synthesis of 2′-CN guanosine analog.

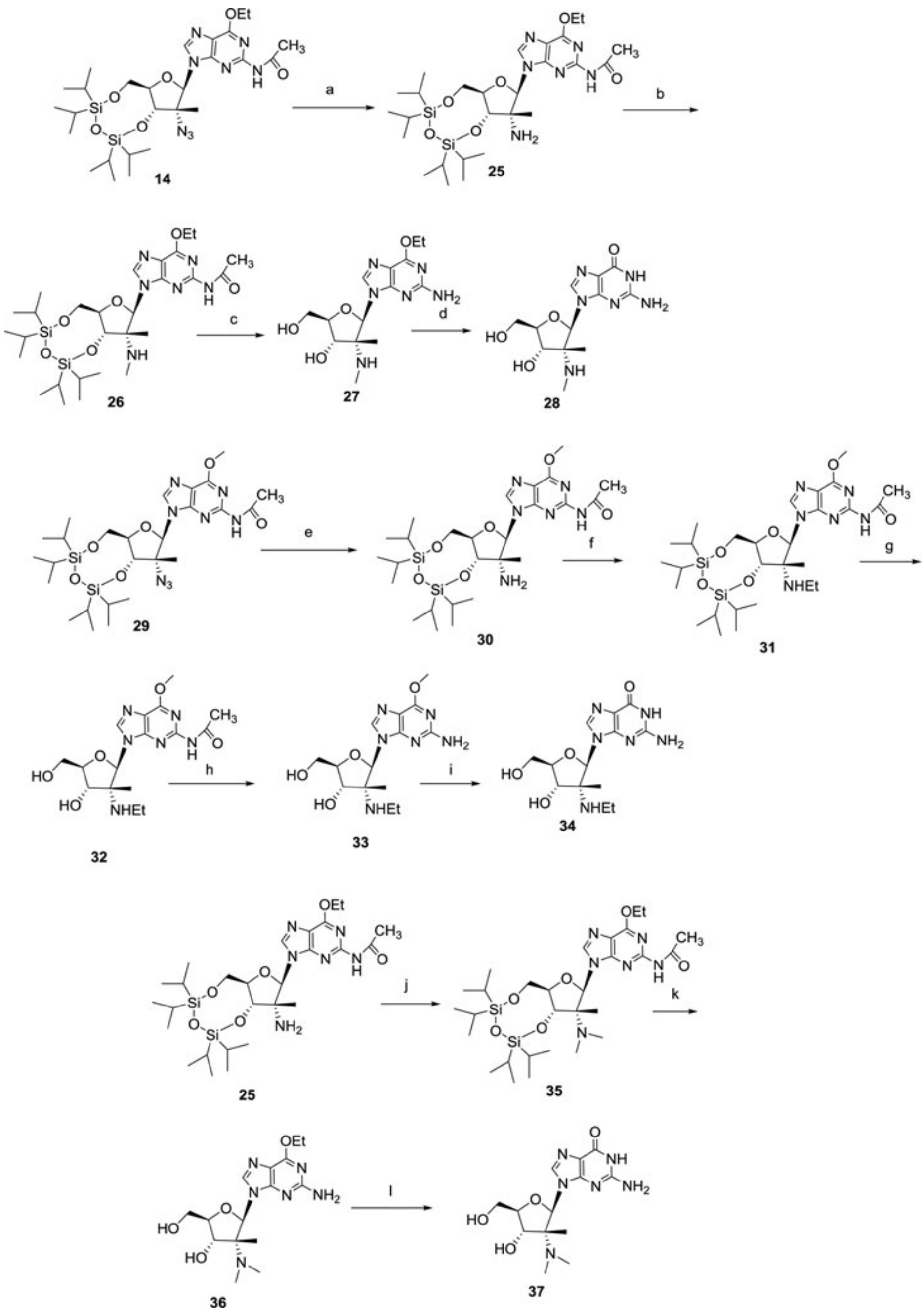
Reagents and conditions. (a) **13**, tosyl cyanide, phenylsilane, dioxane, ethanol, 25%; (b) *i*. TBAF, THF, 98%, *ii*. NaOMe, MeOH, 67%; (c) 1M HCl, THF, 50°C, 68%.



Scheme 5. Synthesis of 2′-triazolo guanosine nucleoside.

Reagents and conditions. (a) *i*. Acetic anhydride, DMAP, triethylamine, 66%, *ii*. vinyl acetate, 140°C, 90%; (b) 7M ammonia in MeOH, 90%; (c) 1M HCl, THF, 60°C, 76%.

The versatility of the 2′-azido/amino functionality enabled us to investigate additional functionality at this position that would probe the steric/electronic environment. As shown in Scheme 5, it was possible to convert the azido guanosine intermediate **16** to the corresponding triazole **22** by protecting it as the *bis*-acetyl followed by stirring at high temperature with vinyl acetate.[10] Subsequent deprotection using methanolic ammonia provided the 6-OEt guanosine analog **23**. The 2′-triazolo guanosine nucleoside **24** was accessed by treatment of **23** with 1M HCl at 50°C.



Scheme 6. Synthesis of 2′-NHMe, NHEt, and NMe_2 guanosine analogs

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Reagents and conditions. (a) H_2, $Pd(OH)_2$, methanol 61%; (b) formaldehyde, sodium triacetoxyborohydride, triethylamine; 43%; (c) *i*. TBAF, THF, *ii*. 7M ammonia, methanol, (58% over two steps); (d) 1M HCl, dioxane, 74%; (e) H_2, 10% Pd/C, methanol, 81%; (f) acetaldehyde, sodium triacetoxyborohydride, triethylamine, 100%; (g) TBAF, THF, 99%; (h) 7M ammonia, methanol, 99%; (i) 1M HCl, dioxane; 77%; (j) formaldehyde, sodium triacetoxyborohydride, triethylamine, 34%; (k) *i*. TBAF, THF, *ii*. 7M ammonia, methanol, (78% over 2 steps); (l) 1M HCl, dioxane, 82%.

The 2′-amino guanosine was an excellent starting point for the synthesis of N-alkyl analogs. The azido intermediate **14** could be reduced to the corresponding amine **25** (Scheme 6). Mono alkylation of the amine to the corresponding NHMe (**26**) was accomplished using reductive amination chemistry. The corresponding nucleoside **27** was synthesized via global deprotection using TBAF followed by treatment with methanolic ammonia. Treatment of **27** with 1M HCl at 50°C provided the desired guanosine analog **28**. The corresponding NHEt compound **34** was formed using similar chemistry from compound **29** (**29** was synthesized from **12a** in 60% yield). The synthesis of the NMe_2 guanosine analog was initiated by the reductive amination of compound **25** in the presence of excess formaldehyde to provide compound **35**. Global deprotection using TBAF followed by methanolic ammonia gave access to compound **36**. Treatment of the 6-OMe nucleoside **36** with 1M HCl at 50°C provided the desired NMe_2 guanosine analog **37**.

Results and discussion

With these 2′-diversified nucleosides in hand, the next step was to evaluate their HCV inhibitory activity in the 1b genotype replicon assay.[11] If the nucleosides were successfully converted to the nucleoside triphosphate within the replicon cell, there could be viral inhibition. While none of the nucleosides demonstrated potency in the cellular assay (Table 2), it was encouraging that none of the them were cytotoxic. The lack of potency in the replicon assay can be explained by the fact that the

Table 2. Anti-HCV and cytotoxicity of nucleosides.

Nuc.	Base	R	EC_{50} (μM)	CC_{50} (μM)
16	6-OEt guanosine	N_3	>100	>100
20	6-OMe guanosine	CN	>100	>100
17	guanosine	N_3	>100	>100
18	guanosine	NH_2	>100	>100
21	guanosine	CN	>100	>100
27	6-OEt guanosine	NHMe	>100	>100
33	6-OMe guanosine	NHEt	>100	>100
36	6-OEt guanosine	NMe_2	>100	>100
23	6-OEt guanosine	triazole	>100	>100
28	guanosine	NHMe	>100	>100
34	guanosine	NHEt	>100	>100
37	guanosine	NMe_2	>100	>100
24	guanosine	triazole	>100	>100

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Table 3. Activities of the corresponding NTPs against HCV NS5B polymerase.

Compound	NTP[a]	R	IC_{50} (μM)
38	guanosine	N_3	0.5
39	guanosine	NH_2	0.6
40	guanosine	CN	0.1
41	guanosine	NHMe	0.6
42	guanosine	NHEt	38
43	guanosine	NMe_2	>100
44	guanosine	triazole	110

[a]Prepared and tested as triethylamine salts.

initial phosphorylation during the step-wise transformation to the NTP is rate limiting as observed with previous 2′-F guanosines analogs.[12] Therefore, the antiviral effectiveness of the guanosine nucleosides was evaluated by converting them to the corresponding NTPs (compounds **38–44**) and testing their biochemical inhibitory activity against the wild-type NS5B polymerase (Table 3).

Several of the guanosine NTPs demonstrated excellent activity suggesting that there was indeed a rate limiting phosphorylation step. It was also apparent from these data that sterics and electronics both play a role in the tolerance of substitution at the 2′-alpha position of HCV nucleosides. The NTP potency of the azido and CN analogs (**38, 40**) suggest that hydrogen bond acceptors/donors are not necessary at the 2′-alpha position for inhibitory effect. The NTP data for the amines (**39, 41–43**) and triazole (**44**) also suggest that the success of an inhibitor is driven by sterics. The amine (**39**) and NHMe (**41**) appear to be tolerated but dialkylation (**43**) or conversion to NHEt (**42**) leads to a significant loss in potency. The data trend for this series of analogs will assist in the discovery of novel HCV nucleoside inhibitors.

Conclusions

In summary, a series of nucleosides bearing novel substitutions at the 2′-alpha position were synthesized from the 2′-exocyclic alkene of the ribose. These nucleosides demonstrate that a variety of sterically unhindered functionalities with significantly different electronic properties as well as hydrogen bonding potential are tolerated at the 2′-alpha position. The 2′-azido, amino,cyano and NHMe guanosine nucleosides (**17, 18, 21** and **28**) are of particular interest due to the intrinsic biochemical potency of their corresponding NTPs. Although the parent nucleosides did not demonstrate antiviral activity in the cellular assays, it is possible that monophosphate prodrugs such as the one demonstrated in sofosbuvir can be used to bypass the inefficient initial kinase step. Application of this as well as the use of alternative pyrimidine and purine bases and other novel 2′-modifications will be described elsewhere.

Experimental section

^{1}H-NMR spectra were obtained on 400 MHz Bruker spectrometers. MS analyses were performed on a Waters micromass ZQ instrument equipped with a Waters

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Acquity UPLC system. The solvent system used was MeCN/H2O with 0.1% formic acid. All reagents and solvents were obtained from commercial sources.

Synthesis of compound 13

6,6′-((1E,1′E)-((2,3-dimethylbutane-2,3-diyl)bis(azanylylidene)) bis(methanylylidene))bis(2,4-di-tert-butylphenol) (733 mg, 1.33 mmol) was taken up in ethanol (10 mL) and the resulting suspension was heated to 80°C and allowed to stir at this temperature for 5 minutes. Cobalt (II) acetate (236 mg, 1.33 mmol) was then added and the resulting reaction was allowed to stir at 80°C for an additional 2 hours. The reaction was cooled to room temperature using an ice bath and was filtered. The collected red solid was dried under vacuum to provide compound **13** (579 mg, 72%). MS (ES+) $C_{36}H_{54}CoN_2O_2$ requires: 605.3, found: 606.2 $(M+H)^+$. See the supplementary information section of reference 9 for additional characterization information.

Synthesis of compound 8

Guanosine hydrate (15 g, 53 mmol) was dissolved in pyridine (120 mL) and acetic anhydride (60 mL). To the resulting solution was added 4-dimethylamino pyridine (6.46 g, 53 mmol) and the reaction was heated to 70°C and allowed to stir at this temperature for 3 hours. The reaction was cooled in an ice bath and treated dropwise with methanol (60 mL). Half of the reaction volume was removed *in vacuo* and the reaction was diluted with dichloromethane and washed with 0.2 M potassium dihydrogensulfate, water, and saturated sodium bicarbonate. The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo* and the residue obtained was purified using flash column chromatography on silica gel (5% methanol in dichloromethane) to provide 22 g of compound **8** (92%). MS (ES+) $C_{18}H_{21}N_5O_9$ requires: 451.1, found: 452.2 $(M+H)^+$

Synthesis of compound 9b

Compound **8** (3.2 g, 7.09 mmol) was dissolved in dioxane (50 mL) and treated with diisopropylazodicarboxylate (1.65 mL, 8.5 mmol) and ethanol (391 mg, 8.5 mmol). The resulting reaction was allowed to stir for fifteen minutes and then concentrated *in vacuo*. The residue obtained was dissolved in methanol (15 mL) and ammonium hydroxide (15 mL) and stirred for 3 hours to provide a suspension, which was filtered to provide 1.4 g of compound **9b**. The remaining solution was concentrated *in vacuo* and the residue obtained was triturated with chloroform to provide an additional 0.8 g of compound **9b** (86%). MS (ES+) $C_{14}H_{19}N_5O_6$ requires: 353.1, found: 354.2 $(M+H)^+$

Synthesis of compound 10b

Compound **9b** (1.46 g, 4.13 mmol) was azeotroped with pyridine (2 × 20 mL) and then suspended in pyridine (30 mL). The resulting solution was treated with

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tetraisopropyldisiloxanedichloride (1.43 g, 4.43 mmol) dropwise over fifteen minutes and allowed to stir at room temperature for 3 hours. The reaction was diluted with water (1 mL) and concentrated *in vacuo*. The residue obtained was diluted with water (50 mL) and ethyl acetate (50 mL). The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue obtained was azeotroped with toluene (2 × 50 mL) and purified using flash column chromatography on silica gel (5% methanol in dichloromethane) to provide 2.02 g of product **10b** as a white solid (82%). MS (ES+) $C_{26}H_{45}N_5O_7Si_2$ requires: 595.3, found: 596.2 $(M+H)^+$.

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Synthesis of compound 11b

Compound **10b** (0.4 g, 0.671 mmol) was dissolved in dichloromethane (100 mL) and the resulting solution was cooled in an ice bath and treated with Dess-Martin Periodinane (0.569 g, 1.34 mmol). The reaction was stirred for 15 hours and then filtered through a pad of silica and sodium sulfate. The solution was diluted with diethyl ether (400 mL) and washed with a mixture of saturated sodium bicarbonate and 10% sodium thiosulfate (1:1). The organic layer was dried over sodium sulfate, filtered and concentrated *in vacuo*. The resulting residue was purified using flash column chromatography on silica gel (hexanes/ethyl acetate 0% →100%) to provide 310 mg of **11b** (78%). MS (ES+) $C_{26}H_{43}N_5O_7Si_2$ requires: 593.3, found: 594.2 $(M+H)^+$.

Synthesis of compound 12b

Methyltriphenylphosphonium bromide (626 mg, 1.75 mmol) was dissolved in tetrahydrofuran (15 mL) and the resulting suspension was treated with KHMDS (0.5 M in tetrahydrofuran, 3.5 mL, 1.75 mmol). The reaction was allowed to stir at room temperature for 20 minutes and then was cooled in an ice bath. A solution of compound **11b** (260 mg, 0.438 mmol) in tetrahydrofuran (5 mL) was added dropwise and the reaction was allowed to warm to room temperature over 4 hours. Saturated ammonium chloride (30 mL) was added and resulting solution was extracted with ethyl acetate (3 × 20 mL). The organic extracts were washed with brine, dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue obtained was purified using flash column chromatography on silica gel (2:1 hexanes/ethyl acetate) to provide 170 mg of compound **12b** (66%). MS (ES+) $C_{27}H_{45}N_5O_6Si_2$ requires: 591.3, found: 592.2 $(M+H)^+$.

Synthesis of compound 14

Compound **12b** (637 mg, 1.07 mmol) and Compound **13** (19 mg, 0.032 mmol) were dissolved in tosyl azide (6.0 g, 30.4 mmol) and stirred for 5 minutes at room temperature. A solution of phenylsilane (151 mg, 1.39 mmol) in ethanol (3 mL) was added dropwise over 20 minutes and the reaction was stirred for an additional 30 minutes. The reaction was quenched with brine (50 mL) and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with brine, dried over sodium sulfate and concentrated *in vacuo*. Silica gel column chromatography (3:1 hexanes/ethyl acetate) provided 440 mg of compound **14** (65%). MS (ES+) $C_{27}H_{46}N_8O_6Si_2$ requires: 634.3, found: 635.2 $(M+H)^+$.

Synthesis of compound 16

To a solution of compound **14** (80 mg, 0.126 mmol) in tetrahydrofuran (1 mL) was added tetrabutylammonium fluoride (1.0 M, 0.252 mL). The reaction was allowed to stir for 2 hours and was then concentrated *in vacuo*. The resulting residue was dissolved in 7M ammonia in methanol (3 mL) and allowed to stir at 100°C in a pressure tube for 15 hours. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The resulting residue was purified using column chromatography (dichloromethane/methanol 0% to 5%) to provide 43 mg of compound **16** (98%). MS (ES+) $C_{13}H_{18}N_8O_4$ requires: 350.1, found: 373.2 (M+Na). ^{1}H-NMR (400 MHz, CD_3OD): δ:8.3 (s, 1H), 5.86 (s, 1H), 4.52 (q, 2H, J = 7.1 Hz), 4.49 (d, 1H, J = 9.21 Hz), 4.0 (m, 2H), 3.84 (m, 1H), 1.42 (t, 3H, J = 7.09 Hz), 1.15 (s, 3H).

Synthesis of compound 17

A solution of compound **16** (5 mg, 10.4 µmol) and 1M HCl (0.5 mL) in tetrahydrofuran (0.5 mL) was heated to 50°C and allowed to stir at this temperature for 24 hours. The reaction mixture was then concentrated *in vacuo* and the residue obtained was purified using flash column chromatography on silica gel (0 to 20% dichloromethane/methanol) to provide 2.1 mg of compound **17** (64%). MS (ES+) $C_{11}H_{14}N_8O_4$ requires: 322.1, found: 345.2 $(M+Na)^+$. ^{1}H-NMR (400 MHz, CD_3OD): δ:8.22 (s, 1H), 5.81 (s, 1H), 4.34 (m, 1H), 4.0 (m, 2H), 3.81 (m, 1H), 1.17 (s, 3H).

Synthesis of compound 18

The azide **17** (30 mg, 0.09 mmol) was dissolved in MeOH (2 mL) and a small portion of $Pd(OH)_2$ (~15 mg) was added. To the flask was affixed a balloon of H_2 and the flask was filled and purged 5×, then allowed to stir under H_2 for 1 hour. The solution was filtered over celite and washed with MeOH. The combined MeOH solution was concentrated *in vacuo* to give the pure amine product **18** as a white solid (24 mg, 91%). MS (ES+) $C_{11}H_{16}N_6O_4$ requires: 296.1, found: 319.2 $(M+Na)^+$. ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (s, 1H), 6.03 (s, 1H), 4.30 (d, 1H, J = 7.2 Hz), 3.99 (ddd, 1H, J = 7.2, 3.3, 2.7 Hz), 3.92 (dd, 1H, J = 12.5, 2.5 Hz), 3.77 (dd, 1H, J = 12.7, 3.5 Hz), 1.09 (s, 3H).

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Synthesis of compound 19

Tosyl cyanide (20.3 g, 112 mmol) was added to a solution of compound **12a** (2.16 g, 3.74 mmol) and compound **13** (113 mg, 0.19 mmol) in dioxane (7 mL) and the reaction was stirred for 5 minutes at 20°C. A solution of phenylsilane (485 mg, 4.49 mmol) in ethanol (14 mL) was added dropwise over 30 minutes. The reaction was stirred for an additional 1 hour and then quenched with brine (100 mL) and extracted with ethyl acetate (3 × 100 mL). The solids were filtered and ethyl acetate (200 mL) was added. The combined organic layers were washed with water, brine, dried over sodium sulfate and concentrated *in vacuo*. Silica gel column chromatography (4:1 hexanes/ethyl acetate → 1:1 hexanes ethyl acetate) provided 560 mg of compound **19** (25%). MS (ES+) $C_{27}H_{44}N_6O_6Si_2$ requires: 604.3, found: 605.2 $(M+H)^+$.

Synthesis of compound 20

To a stirred solution of compound **19** (495 mg, 0.82 mmol) in THF (5 mL) was added 1M TBAF in THF (2.5 mL). The reaction was stirred for 1 hour and then concentrated under reduced pressure to provide a yellow oil. Silica gel column chromatography (5% methanol/DCM → 10% methanol/DCM) provided 290 mg of the free diol (98%). The material (290 mg, 0.8 mmol) was dissolved in MeOH (5 mL) and treated with sodium methoxide (0.43 g, 0.8 mmol). The reaction was stirred at 65 ºC for 1 hour and then concentrated *in vacuo*. The residue was dissolved in water (20 mL) and ethyl acetate (20 mL). The aqueous layer was extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were dried over sodium sulfate and concentrated to provide 172 mg of compound **20** (67%). MS (ES+) $C_{13}H_{16}N_6O_4$ requires: 320.1, found: 321.1 $(M+H)^+$. ^{1}H-NMR (400 MHz, CD_3OD): δ:8.32 (s, 1H), 6.46(s, 1H), 4.40 (m, 1H), 4.05 (s, 3H), 4.04 (m, 2H), 3.87 (m, 1H), 1.14 (s, 3H).

Synthesis of compound 21

To a stirred solution of compound **20** (110 mg, 0.343 mmol) in THF (6 mL) was added 1N HCl (6 mL). The solution was heated at 50 ºC for 48 hours and then the solvent was removed *in vacuo*. The residue was purified by HPLC (5% water/acetonitrile) to provide compound **21**. (71 mg, 68%). MS (ES+) $C_{12}H_{14}N_6O_4$ requires: 306.1, found: 307.2 $(M+H)^+$. ^{1}H-NMR (400 MHz, CD_3OD): δ:8.48 (s, 1H), 6.41(s, 1H), 4.33 (m, 1H), 4.05–4.0 (m, 2H), 3.85 (m, 1H), 1.19 (s, 3H).

Synthesis of compound 22

Compound **16** (150 mg, 0.428 mmol), DMAP (5.2 mg, 0.043 mmol), triethylamine (0.13 mL, 0.94 mmol), and acetic anhydride (0.089 mL, 0.94 mmol) were all dissolved in acetonitrile (2 mL) and stirred for 12 hours at room temperature. The reaction was quenched with water (20 mL) and extracted with ethyl acetate (3 × 20 mL).

The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0–5% methylene chloride methanol) to provide the di-acetate (171 mg, 66%). The intermediate was dissolved in vinyl acetate (0.5 mL) and the reaction was stirred at 140°C in a microwave reactor for 4 hours. The solvent was removed *in vacuo* and the residue was purified by silica gel chromatography (methylene chloride/methanol 0–10%) to provide compound **22** (117 mg, 90%). MS (ES+) $C_{19}H_{24}N_8O_6$ requires: 460.2, found: 461.2 $(M+H)^+$.

Synthesis of compound 23

Compound **22** (38 mg, 0.083 mmol) was dissolved in 7M ammonia in methanol (2 mL) and stirred at room temperature for 2 hours. The solvent was removed *in vacuo* and the residue was purified by silica gel chromatography (0–15% methanol/DCM) to provide compound **23** (28 mg, 90%). MS (ES+) $C_{15}H_{20}N_8O_4$ requires: 376.1, found: 376.98 $(M+H)^+$. ^{1}H-NMR (400 MHz, CD_3OD): δ:8.48 (s, 1H), 8.24 (s, 1H), 7.85 (s, 1H), 7.05 (s, 1H), 4.64 (q, 2H, J = 7.0 Hz), 4.52 (d, 1H, J = 9.1 Hz), 4.0 (m, 2H), 3.84 (m, 1H), 1.42 (t, 3H, J = 7.15 Hz), 1.2 (s, 3H).

Synthesis of compound 24

Compound **23** (50 mg, 0.13 mmol) was dissolved in THF (2 mL) and 1 M HCl (2 mL) and stirred at 60°C for 24 hours. The solvent was removed *in vacuo* and the residue was purified by silica gel chromatography (0–25% methanol/methylene chloride) to provide compound **24** as a white solid (35 mg, 76%). MS (ES+) $C_{13}H_{16}N_8O_4$ requires: 348.1, found: 349.2 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO): δ:10.65 (s, 1H), 8.24 (s, 1H), 7.9 (s, 1H), 7.05 (s, 1H), 6.8 (s, 1H). 6.5 (bs, 1H), 5.9 (bs, 1H), 4.44 (m, 1H), 3.85 (m, 1H), 3.71 (m, 1H). 3.65 (m, 1H), 1.2 (s, 3H).

Synthesis of compound 25

Compound **14** (1.2 g, 1.89 mmol) was dissolved in MeOH (10 mL) and $Pd(OH)_2$(2.0 g) was added. The reaction was stirred for 2.5 hours under an atmosphere of hydrogen. The reaction was filtered over celite and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 to 10% to 15% MeOH/DCM) to provide the desired compound **25** (700 mg, 61%). MS (ES+) $C_{27}H_{48}N_6O_6Si_2$ requires: 608.3, found: 609.2 $(M+H)^+$.

Synthesis of compounds 26 and 35

Compound **25** (121 mg, 0.2 mmol), triethyl amine (0.11 mL, 0.8 mmol), and paraformaldehyde (179 mg, 5.96 mmol) were dissolved in dichloroethane (3 mL) and stirred for 30 minutes. Sodium triacetoxyborohydride (211 mg, 0.99 mmol) was added and the reaction was stirred for 12 hours. The reaction was quenched

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with methanol (0.5 mL) and was filtered over celite and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 → 100% hexanes/ethyl acetate) to provide compound **26** (53 mg, 43%) and compound **35** (43 mg, 34%). Compound **26**: MS (ES+) $C_{28}H_{50}N_6O_6Si_2$ requires: 622.3, found: 623.4 $(M+H)^+$. Compound **35**: MS (ES+) $C_{29}H_{52}N_6O_6Si_2$ requires: 636.3, found: 637.5 $(M+H)^+$.

Synthesis of compound 27

Compound **26** (53.6 mg, 0.086 mmol) was dissolved in THF (1.5 mL) and treated with 1M TBAF (0.17 mL, 0.17 mmol) and the reaction was stirred for 2 hours. The solvent was removed *in vacuo* and the crude residue was treated with 7M ammonia in methanol (2 mL) and stirred at 80°C in a sealed tube reactor. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (0 15% methanol in DCM) to provide compound **27** as a white solid (17 mg, 58%). MS (ES+) $C_{14}H_{22}N_6O_4$ requires: 338.3, found: 339.2 $(M+H)^+$. ^{1}H-NMR (400 MHz, CD_3OD): δ:8.32 (s, 1H), 6.08 (s, 1H), 4.55 (q, 2H, J = 7.0 Hz), 4.25 (d, 1H, J = 9.1 Hz), 4.0 (m, 2H), 3.84 (m, 1H), 2.5 (s, 3H). 1.42 (t, 3H, J = 7.15 Hz), 0.85 (s, 3H).

Synthesis of compound 28

Compound **27** (15.2 mg, 0.045 mmol) was dissolved in THF (0.5 mL) and 1M HCl (0.5 mL) and stirred at 60°C for 24 hours. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (0 → 35% methanol in DCM) to provide compound **28** as a white solid (10.3 mg, 74%). MS (ES+) $C_{12}H_{18}N_6O_4$ requires: 310.3, found: 311.3 $(M+H)^+$. ^{1}H-NMR (400 MHz, CD_3OD): δ:8.22 (s, 1H), 6.02 (s, 1H), 4.2 (m, 1H), 4.05–3.95 (m, 2 H), 3.84 (m, 1H), 2.5 (s, 3H). 0.85 (s, 3H).

Synthesis of compound 29

Compound **29** was synthesized from **12a** in 60% yield using the same chemistry outlined for compound **14**. The 6-OMe compound **12a** was synthesized using the chemistry outlined for compound **12b** except MeOH was used in the Mitsunobu reaction rather than EtOH.

Synthesis of compound 30

Compound **29** (2.05 g, 3.31 mmol) and 10% Pd/C (410 mg) were suspended in MeOH (33 mL) and stirred under a hydrogen atmosphere for 12 hours. The reaction mixture was filtered through Celite and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc) to provide compound **30** (1.59 g, 81%). MS (ES+) $C_{26}H_{46}N_6O_6Si_2$ requires: 594.3, found: 595.5 $(M+H)^+$.

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Synthesis of compound 31

Compound **30** (100 mg, 0.168 mmol), triethyl amine (0.023 mL, 0.168 mmol), and acetaldehyde (0.014 mL, 0.252 mmol) were dissolved in dichloroethane (3 mL) and stirred for 30 minutes. Sodium triacetoxyborohydride (43 mg, 0.2 mmol) was added the reaction was stirred for 12 hours. The reaction was quenched with methanol (0.5 mL) and was filtered over celite and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0 → 100% hexanes/ethyl acetate) to provide compound **31** (105 mg, 100%). MS (ES+) $C_{28}H_{50}N_6O_6Si_2$ requires: 622.4, found: 623.6 $(M+H)^+$.

Synthesis of compound 32

Compound **31** (105 mg, 0.169 mmol) was dissolved in THF (3 mL) and treated with 1M TBAF (0.17 mL, 0.17 mmol). The reaction was stirred for 4 hours and then the solvent was removed *in vacuo* and the crude residue was purified by silica gel column chromatography (0 → 15% methanol in DCM) to provide compound **32** as a white solid (64 mg, 99%). MS (ES+) $C_{16}H_{24}N_6O_5$ requires: 380.3, found: 381.3 $(M+H)^+$.

Synthesis of compound 33

Compound **32** (64 mg, 0.168 mmol) was treated with 7M ammonia in methanol (2 mL) and stirred at 80°C in a sealed tube reactor. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (0 → 15% methanol in DCM) to provide compound **33** as a white solid (56.4 mg, 99%). MS (ES+) $C_{14}H_{22}N_6O_4$ requires: 338.3, found: 339.2 $(M+H)^+$. ^{1}H-NMR (400 MHz, CD_3OD): δ:8.32 (s, 1H), 6.1 (s, 1H), 4.23 (m, 1H), 4.1 (m, 1H), 4.05 (s, 3H), 4.0 (m, 1H), 3.84 (m, 1H), 2.9–2.8 (m, 2 H), 1.2 (t, 3H, J = 7.0 Hz), 0.85 (s, 3H).

Synthesis of compound 34

Compound **33** (53.3 mg, 0.158 mmol) was dissolved in THF (2 mL) and 1M HCl (2 mL) and stirred at 60°C for 24 hours. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (0 → 35% 2N ammonia methanol in DCM) to provide compound **34** as a white solid (39.3 mg, 77%). MS (ES+) $C_{13}H_{20}N_6O_4$ requires: 324.3, found: 325.2 $(M+H)^+$. ^{1}H-NMR (400 MHz, CD_3OD): δ:8.2 (s, 1H), 6.1 (s, 1H), 4.23 (m, 1H), 4.1 (m, 1 H), 3.95 (m, 1H), 3.8 (m, 1H), 3.05–2.95 (m, 2H). 1.25 (t, 3H, J = 7.0 Hz), 1.0 (s, 3H).

Synthesis of compound 36

Compound **35** (43.6 mg, 0.068 mmol) was dissolved in THF (1.5 mL) and treated with 1M TBAF (0.17 mL, 0.17 mmol) and the reaction was stirred for 2 hours. The solvent was removed *in vacuo* and the crude residue was treated with 7M ammonia in methanol (2 mL) and stirred at 80°C in a sealed tube reactor. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (0 → 15% methanol in DCM) to provide compound **36** as a white solid (18.8 mg, 77%). MS (ES+) $C_{15}H_{24}N_6O_4$ requires: 352.3, found: 353.2 $(M+H)^+$. ^{1}H-NMR (400 MHz, CD_3OD): δ:8.2 (s, 1H), 6.22 (s, 1H), 4.55 (q, 2H, J = 7.2 Hz), 4.15 (d, 1H, J = 9.1 Hz), 4.05 (m, 1H), 3.9 (m, 1H), 3.8 (m, 1H), 2.5 (s, 6H), 1.2 (t, 3H, J = 7.0 Hz), 0.85 (s, 3H).

Synthesis of compound 37

Compound **36** (16.3 mg, 0.046 mmol) was dissolved in THF (0.5 mL) and 1M HCl (0.5 mL) and stirred at 60°C for 24 hours. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (0 → 35% 2N ammonia methanol in DCM) to provide compound **37** as a white solid (12.3 mg, 82%). MS (ES+) $C_{13}H_{20}N_6O_4$ requires: 324.3, found: 325.3 $(M+H)^+$. ^{1}H-NMR (400 MHz, CD_3OD): δ:8.15 (s, 1H), 6.25 (s, 1H), 4.25 (m, 1H), 4.08 (m, 1 H), 3.95 (m, 1H), 3.82 (m, 1H), 2.8 (s, 6H), 1.4 (s, 3H).

Synthesis of nucleoside triphosphates 38–44

Nucleoside triphosphates **38–44** were synthesized as triethylamine salts by Trilink Biotechnologies using general synthetic methods.[13] All compounds were isolated as 10 mM solution in water and purity of >95% was confirmed with MS and AX-HPLC.

Cell-based anti-HCV activity

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.

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

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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; 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.

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