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2’-Modified Guanosine Analogos for the Treatment of HCV

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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.

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
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 1](image1.png)

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

![Figure 2](image2.png)

**Figure 2.** 2′alpha position modified nucleosides via the 2′-olefin.
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, CH₂Cl₂; 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.](image)

**Table 1.** Reagents and conditions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Co (eq)</th>
<th>TsN₃ (eq)</th>
<th>PhSiH₃ (eq)</th>
<th>TsN₃/ETOHa</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02</td>
<td>1.5</td>
<td>1.5</td>
<td>1/99</td>
<td>&lt;5</td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
<td>3</td>
<td>1.5</td>
<td>3/97</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3</td>
<td>0.02</td>
<td>10</td>
<td>1.5</td>
<td>10/90</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>0.02</td>
<td>15</td>
<td>1.5</td>
<td>50/50</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>0.02</td>
<td>30</td>
<td>1.5</td>
<td>100/0</td>
<td>45</td>
</tr>
<tr>
<td>6</td>
<td>0.02</td>
<td>30</td>
<td>1.5b</td>
<td>100/0</td>
<td>65</td>
</tr>
</tbody>
</table>

*a* Ratio by volume of TsN₃ and ethanol at start of reaction. *b* Phenylsilane was added dropwise in EtOH (equal to volume of TsN₃).
of TsN₂ 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 Ph₃SiH), therefore, it was hypothesized that the product ratio could be improved by having a larger effective concentration of TsN₂. Incremental increases in the equivalents of TsN₂ 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 TsN₂ (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.


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) H₂, Pd(OH)₂, MeOH, 91%.
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.\textsuperscript{[10]} Subsequent deprotection using methanolic ammonia provided the 6-OEt guanosine analog 23. The 2′-triazo1 guanosine nucleoside 24 was accessed by treatment of 23 with 1M HCl at 50°C.

\textbf{Scheme 6.} Synthesis of 2′-NHMe, NHet, and NMe$_2$ guanosine analogs
Reagents and conditions. (a) H₂, 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) H₂, 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 NMe₂ 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 NMe₂ 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. 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>
<thead>
<tr>
<th>Nuc.</th>
<th>Base</th>
<th>R</th>
<th>EC₅₀ (µM)</th>
<th>CC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>6-OEt guanosine</td>
<td>Nₓ</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>20</td>
<td>6-OMe guanosine</td>
<td>CN</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>18</td>
<td>guanosine</td>
<td>Nₓ</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>17</td>
<td>guanosine</td>
<td>NH₂</td>
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<td>&gt;100</td>
</tr>
<tr>
<td>21</td>
<td>guanosine</td>
<td>CN</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>27</td>
<td>6-OEt guanosine</td>
<td>NHMe</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>33</td>
<td>6-OMe guanosine</td>
<td>NHEt</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
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<td>6-OEt guanosine</td>
<td>NMe₂</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>23</td>
<td>6-OEt guanosine</td>
<td>triazole</td>
<td>&gt;100</td>
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<tr>
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<td>NHEt</td>
<td>&gt;100</td>
<td>&gt;100</td>
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<tr>
<td>37</td>
<td>guanosine</td>
<td>NMe₂</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>24</td>
<td>guanosine</td>
<td>triazole</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>
Table 3. Activities of the corresponding NTPs against HCV NSSB polymerase.

<table>
<thead>
<tr>
<th>Compound</th>
<th>NTP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>R</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
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</thead>
<tbody>
<tr>
<td>38</td>
<td>guanosine</td>
<td>N&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.5</td>
</tr>
<tr>
<td>39</td>
<td>guanosine</td>
<td>NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.6</td>
</tr>
<tr>
<td>40</td>
<td>guanosine</td>
<td>CN&lt;sup&gt;-&lt;/sup&gt;</td>
<td>0.1</td>
</tr>
<tr>
<td>41</td>
<td>guanosine</td>
<td>NHMe</td>
<td>0.6</td>
</tr>
<tr>
<td>42</td>
<td>guanosine</td>
<td>NHEt</td>
<td>38</td>
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<tr>
<td>43</td>
<td>guanosine</td>
<td>NMe&lt;sub&gt;2&lt;/sub&gt;</td>
<td>&gt;100</td>
</tr>
<tr>
<td>44</td>
<td>guanosine</td>
<td>triazole</td>
<td>110</td>
</tr>
</tbody>
</table>

<sup>a</sup>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. 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 steric and electronic effects 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 steric effects. 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

<sup>1</sup>H-NMR spectra were obtained on 400 MHz Bruker spectrometers. MS analyses were performed on a Waters micromass ZQ instrument equipped with a Waters
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_{2}O_{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_{5}O_{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_{5}O_{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
tetraisopropylsiloxanedichloride (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_{5}O_{7}Si_{2} requires: 595.3, found: 596.2 (M+H)^{+}.

**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_{5}O_{7}Si_{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 (hexanes/ethyl acetate 2:1) to provide 170 mg of compound 12b (66%). MS (ES+) C_{27}H_{45}N_{5}O_{6}Si_{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_{8}O_{6}Si_{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_{8}O_{4} requires: 350.1, found: 373.2 (M+Na). ^1H-NMR (400 MHz, CD_{3}OD): 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 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_{8}O_{4} requires: 322.1, found: 345.2 (M+Na)^+. ^1H-NMR (400 MHz, CD_{3}OD): 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_{6}O_{4} requires: 296.1, found: 319.2 (M+Na)^+. ^1H NMR (400 MHz, CD$_3$OD) 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).
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_{6}O_{6}Si_{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°C 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_{6}O_{4} requires: 320.1, found: 321.1 (M+H)^+. ¹H-NMR (400 MHz, CD_{3}OD): 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°C 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_{6}O_{4} requires: 306.1, found: 307.2 (M+H)^+. ¹H-NMR (400 MHz, CD_{3}OD): δ:8.48 (s, 1H), 6.41 (s, 1H), 4.33 (m, 1H), 4.05–4.0 (m, 2H), 3.85 (m, 1H), 4.04 (m, 2H), 3.87 (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)^+.

^1H-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)^+.

^1H-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), 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
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_{6}O_{8}Si_{2} requires: 622.3, found: 623.4 (M+H)^+. Compound 35: MS (ES+) C_{29}H_{52}N_{6}O_{6}Si_{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_{6}O_{4} requires: 338.3, found: 339.2 (M+H)^+. 1H-NMR (400 MHz, CD_{3}OD): 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). 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_{6}O_{4} requires: 310.3, found: 311.3 (M+H)^+. 1H-NMR (400 MHz, CD_{3}OD): δ: 8.22 (s, 1H), 6.02 (s, 1H), 4.2 (m, 1H), 4.05–3.95 (m, 2H), 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_{6}O_{6}Si_{2} requires: 594.3, found: 595.5 (M+H)^+. 
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 and the reaction was stirred for 12 hours. The reaction was quenched with methanol (0.5 mL) and was filtered over celite and concentrated \textit{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+) \( 	ext{C}_{28}	ext{H}_{50}	ext{N}_{6}	ext{O}_{6}	ext{Si}_{2} \) 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 \textit{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+) \( 	ext{C}_{16}	ext{H}_{24}	ext{N}_{6}	ext{O}_{5} \) 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 \textit{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+) \( 	ext{C}_{14}	ext{H}_{22}	ext{N}_{6}	ext{O}_{4} \) requires: 338.3, found: 339.2 (M+H)+. \(^1\text{H}-\text{NMR} (400 \text{ MHz}, \text{CD}_3\text{OD}): \delta: 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 \textit{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+) \( 	ext{C}_{13}	ext{H}_{20}	ext{N}_{6}	ext{O}_{4} \) requires: 324.3, found: 325.2 (M+H)+. \(^1\text{H}-\text{NMR} (400 \text{ MHz}, \text{CD}_3\text{OD}): \delta: 8.2 (s, 1H), 6.1 (s, 1H), 4.23 (m, 1H), 4.1 (m, 1H), 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 \textit{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_{6}O_{4} requires: 352.3, found: 353.2 (M+H)^+. ^{1}H-NMR (400 MHz, CD_{3}OD): 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_{6}O_{4} requires: 324.3, found: 325.3 (M+H)^+. ^{1}H-NMR (400 MHz, CD_{3}OD): δ: 8.15 (s, 1H), 6.25 (s, 1H), 4.25 (m, 1H), 4.08 (m, 1H), 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 [33P]-CTP; 10 mM MgCl₂, 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 IC₅₀ values can then be calculated from experiments with 10 serial 3-fold dilutions of the inhibitor in duplicate.

References


