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Tricyclic 2′-C-Modified Nucleosides as Potential Anti-HCV Therapeutics

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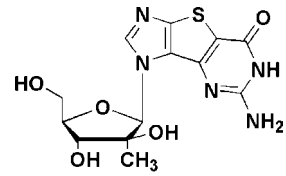
ABSTRACT



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Promising biological activity in a number of therapeutic areas has been reported for both tricyclic nucleosides and 2′-modified nucleosides. In particular, disubstitution at the C-2′ position of nucleosides has resulted in significant activity against the hepatitis C virus (HCV). Combining this with the observation that tricyclic nucleosides developed in our laboratory have been shown to inhibit the RNA-dependent RNA polymerase NS5B led to the design of a series of 2′-modified tricyclic nucleosides. Details of the synthesis, structural characterization, and preliminary biological results are reported.

The hepatitis C virus (HCV) is a blood borne virus that has affected more than 5 million people in the United States and nearly 200 million people worldwide.[1] HCV is a leading cause of long-term liver cirrhosis, resulting in liver transplants, liver failure, and, in many cases, death. These complications are particularly life-threatening for patients that are coinfected with the human immunodeficiency virus (HIV). HCV consists of several genotypes, with genotype 1 (HCV-GT1) being the most common. The primary treatment for HCV-GT1 currently consists of a combination of pegylated interferon administered with the nucleoside analogue Ribavirin, however the success rate of this treatment is reported to be less than 50%. Although several promising nucleoside analogues are currently in clinical trials, most have failed to make it to market.[2,3]

The process by which HCV replicates relies on the use of an RNA-dependent RNA-polymerase (RdRp), in particular, NS5B. The NS5B polymerase is known as a "right hand" polymerase made up of a palm (the active site), fingers and a thumb domain.[4] To date, NS5B is the only known site in which elongation of new virus occurs, designating it as a prime target for new drug development.

To date a number of 2′-modified nucleosides (Figure 1) have shown potent biological activity against HCV.[5,6] Moreover, a thiophene-expanded tricyclic purine nucleoside

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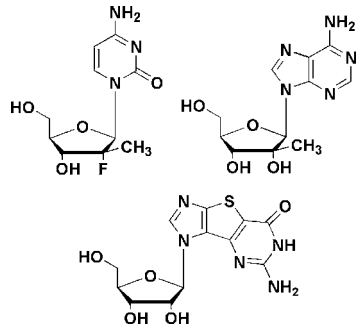

Figure 1. Modified nucleoside analogues exhibiting anti-HCV properties.

analogue developed in our laboratories has also shown interesting HCV inhibition (Figure 1).[7]

In an effort to further explore the potential for increasing the HCV activity, the tricyclic nucleoside was modified at the C-2′ position of the sugar (Figure 2). It was hoped that

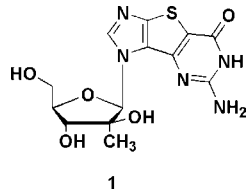

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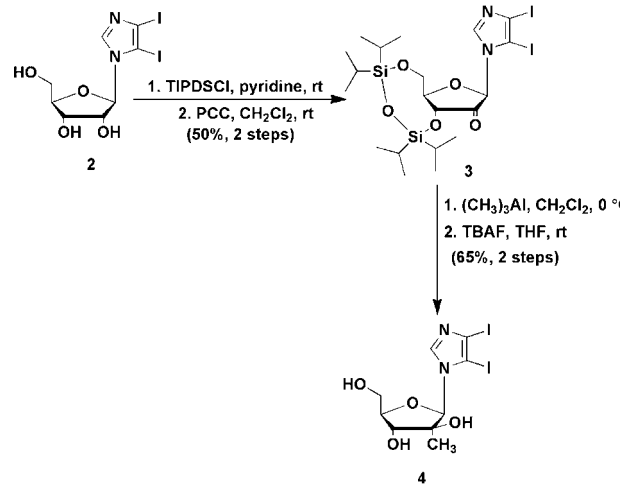
Figure 2. Tricyclic C-2′ modified target.

by combining these two leads, a synergistic increase in inhibition properties against HCV would be realized.

Two synthetic routes were considered in order to realize the target (Figure 2); adding the methyl group to the preconstructed tricyclic nucleoside at the last step or alternatively, adding the methyl group prior to construction of the tricyclic scaffold. Both routes proceed by way of a key intermediate (**2**, Scheme 1), which could be obtained through a 3-step process using the Vorbrüggen[8] coupling method as previously reported from our laboratory.[9]

The first pathway was quickly abandoned due to the need for additional protection and deprotection steps and very low yields. Turning to the second route, intermediate **2** was bis-protected at the 3′- and 5′-hydroxyls using tetraisoproyldisiloxane chloride (TIPDSCl) in preparation for selective oxidation of the 2′−OH with pyridinium chlorochromate (PCC) (Scheme 1).[10]

Scheme 1. Initial Route to the C-2′-Me Intermediate



Intermediate **3** was then treated with trimethylaluminum[11] to afford the C-2′ methyl substituted TIPS-protected intermediate, which was then immediately deblocked using TBAF to provide **4**. Trimethylaluminum was used instead of the more common Grignard and lithium reagents due to reports of higher yields and the stereoselective addition of the methyl to the α-face of the sugar.[12,13] This approach was desired since the ultimate goal was to replace the hydroxyl group

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(10) 2-(4,5-Diiodo-imidazol-1-yl)-5,5,7,7-tetraisopropyl-1-β-D-ribofuranose (**3**): Compound **2** (10.0 g, 22.1 mmol), previously dried under high vacuum, was treated with anhydrous pyridine (200 mL), and the solution was stirred for 15 min, at which point 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (8.5 mL, 26.5 mmol) was added and the reaction was stirred for 14 h. The solvent was removed under reduced pressure, and the crude compound was purified by column chromatography eluting with 6:1 hexanes/ethyl acetate to provide **3** as a sticky white foam (12.5 g, 17.3 mmol, 81.4%). The intermediate (9.0 g, 13.0 mmol) was stirred with PCC (7.0 g, 32.4 mmol) in anhydrous CH_2Cl_2 (200 mL) over oven 4 Å dried molecular sieves. The reaction turned from orange to a dark red-brown color within the first 30 min. The reaction was stirred for 12 h at rt at which point TLC analysis confirmed the absence of starting material. The crude reaction mixture was filtered over a pad of celite, and the solvent was removed under reduced pressure. The crude compound was purified by column chromatography eluting with 6:1 hexanes/ethyl acetate to provide **3** as a light-yellow foam (6.0 g, 8.7 mmol, 66.9%). ^{1}H NMR ($CDCl_3$) δ 0.99 (m, 28H), 3.75 (dd, 1H), 4.00 (dd, 1H), 4.09 (d, 2H), 4.77 (d, 1H), 5.59 (s, 1H), 7.82 (s, 1H). 13C NMR ($CDCl_3$) δ12.3 (multiple peaks), 17.5 (multiple peaks), 60.3, 72.4, 78.7, 84.4, 92.5, 99.2, 139.8, 204.5. HRMS calculated for $C_{20}H_{34}I_2N_2O_5Si_2$ $[MH]^+$ 693.0175; Found, 693.0175.

(11) 2-(4,5-Diiodo-imidazol-1-yl)-5,5,7,7-tetraisopropyl-3-methyl- 1-β-D-ribofuranose (**4**): Intermediate **3** (4.0 g, 5.8 mmol) was added to anhydrous CH_2Cl_2 (100 mL) and cooled to 0 °C. Trimethylaluminum (3 M in hexanes, 6.7 mL, 20.2 mmol) was added, and the mixture was vigorously stirred at rt for 2 h. The solution was then cooled in an ice bath, quenched with saturated $NaHCO_3$ solution, extracted with CH_2Cl_2 (3 × 150 mL), the organic layers were combined and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude compound was then purified by column chromatography eluting with 6:1 hexanes/EtOAc to afford **4** as a white solid (3.0 g, 4.2 mmol, 73.2%). The intermediate (2.0 g, 2.8 mmol) was dissolved in anhydrous THF (75 mL) and tetrabutylammonium fluoride (1M in THF, 11.3 mL, 11.3 mmol) was added. The mixture was allowed to stir at rt for 5 h before the solvent was removed under reduced pressure. The crude compound was purified by column chromatography eluting with 10:1 EtOAc/MeOH to give **26** as an off white hygroscopic solid (1 g, 2.15 mmol, 76.3%). ^{1}H NMR (CD_3OD) δ 1.28 (s, 3H), 3.82 (dd, 1H), 3.95 (d, 2H), 4.08 (d, 1H), 5.67 (s, 1H), 8.12 (s, 1H). ^{13}C NMR (CD_3OD) δ 17.6, 61.4, 75.4, 79.4, 81.2, 85.3, 93.8, 96.5, 142.4. HRMS calculated for $C_9H_{12}I_2N_2O_4$ $[MH]^+$ 466.8966; Found, 466.8966.

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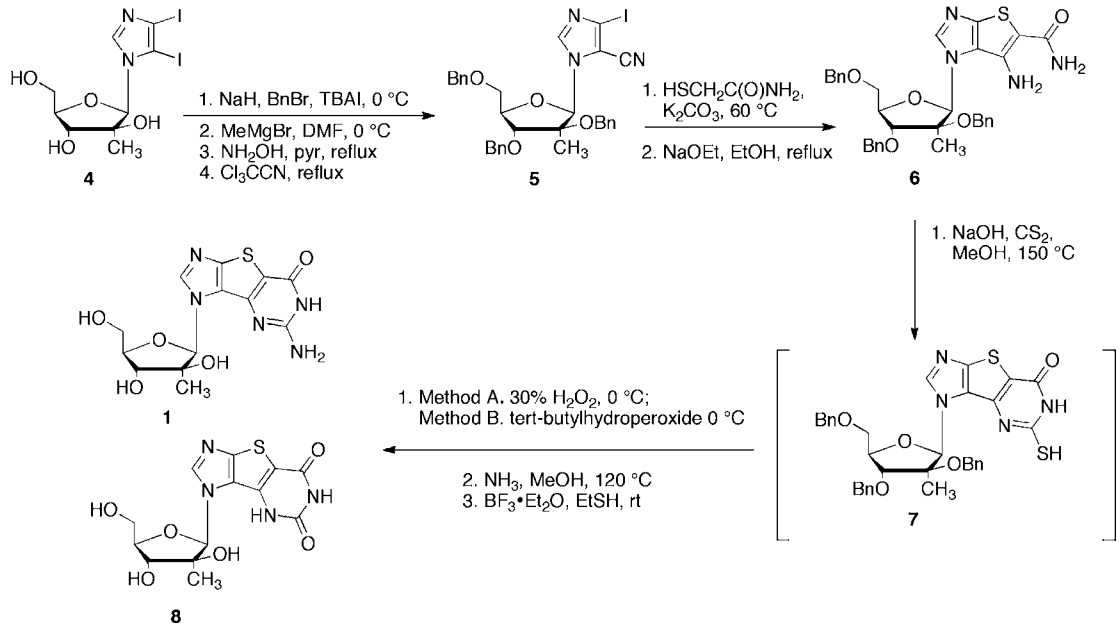
Scheme 2. Synthetic Route to **1**



functionality with a fluorine, which is known to proceed through inversion of configuration by the replacement of the "up" OH.[6]

While the use of trimethylaluminum was reported to give stereoselective yields, to our knowledge, no spectroscopic proof for this selective addition has been reported. In an effort to confirm the correct isomer was obtained, a series of 1D-NOE NMR experiments were run. Irradiations of the anomeric proton, 2′-Me, 3′-H, and the 4′-H showed similar correlations thus providing insight as to the stereochemistry of the sugar. Specifically, the anomeric proton was found to have the strongest correlation to the 2′-Me group. This signal was normalized to 1 as a reference for measuring the strength of other correlations. The anomeric proton showed a relative abundance of 100% (normalized), the 4′-H showed a relative abundance of 38%, while the 3′-H showed relatively no correlation to the 2′-Me (less than 1%). These correlations strongly suggest that the Me addition occurred to the α-face of the C-2′-position as expected.

Proceeding with the construction of the tricyclic base, blocking the hydroxyl groups was undertaken next. Employing the benzyl (Bn) group, the protected intermediate was obtained in a 65% yield as shown in Scheme 2. The remaining steps proceeded as previously reported for the guanosine ribose analogue.[9] Replacement of the C-5 iodo with the formyl group followed by conversion to the oxime, which was then dehydrated to give the cyano modified **5**.

Derivitization of C-4 with thioglycolamide then afforded an intermediate which was then treated with base to give the bicyclic analogue **6**. Closure of the third ring with standard conditions is known to lead to a competitive formation of the desired tricyclic guanosine analogue (**1**) and the undesired tricyclic xanthosine analogue (**8**), a problem also encountered with the formation of guanosine. Previously we reported[14] a mechanistic study that favors the preferential formation of the guanosine analogue when the sugar is a ribose,[9] however this did not prove fruitful with the C-2′-modification and the xanthosine was the major product in all cases.

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(15) New procedure for 1-[(5-Aminoimidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl-7-one)]-*β*-D-ribofuranose 2,3,5-Triol (**1**) and 1-[(5-hydroxylimidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl-7-one)]-*β*-D-ribofuranose (**8**): In a steel reaction vessel, compound **6** (2.62 g, 4.38 mmol) was dissolved in 40 mL anhydrous MeOH, NaOH (1.76 g, 44 mmol) was added, and the mixture was stirred at room temperature for 30 min until a clear solution was obtained. CS_2 (1.6 mL, 26.3 mmol) was added, the vessel was sealed, and the reaction was heated in an oil bath at 145 °C for 18 h. The vessel was cooled, and the solvent was removed under reduced pressure to give an orange solid to which THF (80 mL) was added. The mixture was cooled to 0 °C and 11.4 mL tert-butyl hydroperoxide (5 M in decane) was added. This mixture was stirred at 0 °C for 4 h until TLC analysis confirmed the disappearance of the starting material. The reaction mixture was then transferred to a steel reaction vessel, cooled to −78 °C, and NH_3 was bubbled into the solution for 15 min. The reaction vessel was sealed and heated in an oil bath at 120 °C for 18 h. The vessel was cooled, and the reaction mixture was evaporated to dryness under vacuum to give a yellow solid. The crude mixture was purified by column chromatography eluting with hexanes/EtOAc (3:1) to afford the guanosine and xanthosine analogues as colorless oils. The crude compounds were then dried before anhydrous CH_2Cl_2 (15 mL) was added. At this point, 12 molar equiv of BF_3•Et_2O and 12 molar equiv of EtSH were added to the solution dropwise. The reaction was allowed to proceed for 48 h at room temperature before TLC analysis confirmed complete product formation. The solvent was removed under reduced pressure to give a crude solid. The crude compound was purified via column chromatography using a solvent system of 10:1 MeOH/EtOAc. This provided the guanosine analogue **1** and the xanthosine analogue **8** in a 1.2:1 ratio. Each final compound was rinsed 3 times with CH_2Cl_2, rinsed 3 times with EtOAc, and finally rinsed 3 times with a 5:1 mixture of EtOAc/MeOH. Guanosine **1**: ^{1}H NMR (DMSO-d_6) δ 1.44 (s, 3H), 3.65 (dd, 1H), 4.20 (d, 2H), 4.39 (d, 1H), 5.43 (broad m, 3H), 5.72 (s, 1H), 6.51 (s, 2H), 8.35 (s, 1H), 10.98 (broad s, 1H). Xanthosine **8**: ^{1}H NMR (DMSO-d_6) δ 1.12 (s, 3H), 3.82−3.93 (m, 1H), 4.01−4.11 (m, 1H), 5.40 (broad m, 2H), 5.83 (s, 1H), 8.45 (s, 1H), 10.68 (s, 1H), 11.42 (s, 1H).

In examining the reaction more closely, it appeared that the water present in the 30% aqueous hydrogen peroxide solution used was displacing the intermediate sulfenic (SOH) or sulfinic (SO_2H) acid group on **7** (Scheme 2) more rapidly than the ammonia, thereby resulting in Bn-protected xanthosine **8** as the major product. In an effort to overcome this obstacle, use of a non-nucleophilic peroxide was tried and, indeed, proved highly successful. Thus, by substituting tert-butyl hydrogen peroxide in the ring closure reaction, the ratio of the tricyclic guanosine to xanthosine was increased significantly from 1:20 to 1.2:1.[15] Deblocking as before provided **1** and **8**, with the desired guanosine **1** being the major product.

Compounds **1** and **8** were then tested for anti-HCV activity (genotypes 1a and 1b) and cytotoxicity in two separate HCV replicon-containing cell lines using an established[16] standardized three day assay. Unfortunately, neither compound showed any significant activity at concentrations up to 10 μM. It should also be noted that neither compound exhibited cytotoxicity at concentrations of 100 μM. While initial results against HCV were disappointing, the compounds will be tested against other viruses as soon as sufficient amounts can be obtained.

In summary, two novel tricyclic C-2′ modified nucleosides were successfully obtained and screened for potential activity against HCV. Although the compounds did not show activity, possibly due to their poor solubility in the assay system, the new ring closure protocol significantly increased the ratio of the desired guanosine analogue so that scaleup can now be accomplished, however it still appears that more anhydrous conditions may be necessary in order to completely eliminate the formation of the xanthosine side product. Future efforts are focused on pursuit of the analogous 2′-fluoro, 2′-methyl compounds. The results of those studies will be reported elsewhere as they become available.

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Supporting Information Available: Experimental details, ^1H, ^{13}C NMR and HRMS data provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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