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Synthesis of 2′-*O*,4′-*C*-alkylene-bridged ribonucleosides and their evaluation as inhibitors of HCV NS5B polymerase

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ABSTRACT

The synthesis of 2′-*O*,4′-*C*-methylene-bridged bicyclic guanine ribonucleosides bearing 2′-*C*-methyl or 5′-*C*-methyl modifications is described. Key to the successful installation of the methyl functionality in both cases was the use of a one-pot oxidation–Grignard procedure to avoid formation of the respective unreactive hydrates prior to alkylation. The 2′-*C*-methyl- and 5′-*C*-methyl-modified bicyclic guanosines were evaluated, along with the known uracil-, cytosine-, adenine-, guanine-LNA and guanine-ENA nucleosides, as potential antiviral agents and found to be inactive in the hepatitis C virus (HCV) cell-based replicon assay. Examination of the corresponding nucleoside triphosphates, however, against the purified HCV NS5B polymerase indicated that LNA-G and 2′-*C*-methyl-LNA-G are potent inhibitors of both 1b wild type and S282T mutant enzymes in vitro. Activity was further demonstrated for the LNA-G-triphosphate against HCV NS5B polymerase genotypes 1a, 2a, 3a and 4a. A phosphorylation by-pass prodrug strategy may be required to promote anti-HCV activity in the replicon assay.

Bicyclic ribonucleosides bearing a 2′-*O*,4′-*C*-methylene-bridge have been extensively investigated since their introduction by Imanishi and Wengel at the end of the twentieth century.[1] Oligonucleotides containing these locked nucleic acid (LNA) nucleosides have subsequently found numerous therapeutic and biotechnology applications.[2] In particular, recent clinical evaluation of the LNA-containing antisense oligonucleotide, miravirsen, in patients with chronic hepatitis C virus (HCV) genotype 1 infection resulted in significant virologic suppression by targeting a noncoding host microRNA.[3] To date, however, there have been no reports of the evaluation of the LNA ribonucleosides themselves in terms of their activity against the HCV NS5B viral polymerase.

HCV is a blood-borne pathogen infecting an estimated 2.8% of the world's population.[4] The existing standard of care for patients presenting with genotype 1 virus includes pegylated interferon-α and ribavirin in combination with one of the four currently approved direct acting antivirals, boceprevir, telaprevir, simeprevir and sofosbuvir. Of these, the HCV NS3/4A protease inhibitors boceprevir, telaprevir and simeprevir offer a new therapeutic approach but their utility can be limited by drug–drug interactions and the emergence of resistant mutants (e.g. R155K, D168V).[5] In contrast, nucleosides, such as sofosbuvir, have attracted much attention over the past decade as they have the potential to be selective inhibitors of the HCV NS5B polymerase with a higher barrier to resistance.[6] The major class of variously prodrugged nucleosides displaying clinical efficacy has incorporated the 2′-*C*-methyl modification; for example, as 2′-*C*-methylcytidine **1**,[7] 2′-*C*-methylguanosine **2**[8] and 2′-F-2′-*C*-methyluridine, for which regulatory approval has recently been granted in its phosphoramidate guise as sofosbuvir **3**[9] (Fig. 1).

2′-*C*-Methyl nucleoside systems are known to preferentially adopt the $C_{3'}$-*endo* (*N*) sugar ring conformation.[10] Post phosphorylation by host cell kinases the corresponding active metabolite 2′-*C*-methyl nucleoside triphosphates behave as terminators of the nascent HCV RNA polymerase RNA chain. This $C_{3'}$-*endo* conformational restriction is also well described for 2′-*O*,4′-*C*-methylene- and ethylene-bridged ribonucleosides (LNA[11] and ENA[12] nucleosides, respectively), however, the activity of this class of nucleosides and their analogs as inhibitors of HCV RNA polymerase has not been established. Accordingly, the synthesis of the known uracil-, cytosine-, adenine-, guanine-LNA nucleosides **4**–**7**

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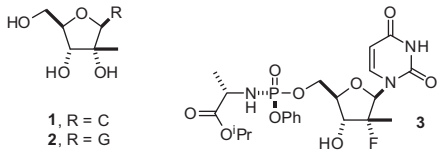


Figure 1. Examples of nucleosides bearing a 2′-*C*-methyl substituent.

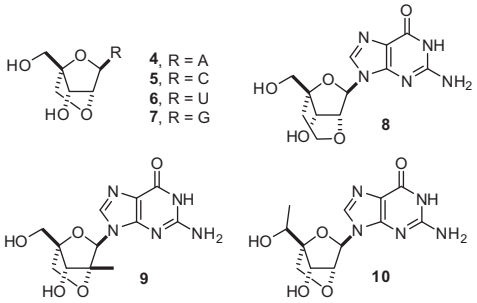


Figure 2. Bicyclic nucleosides under investigation.

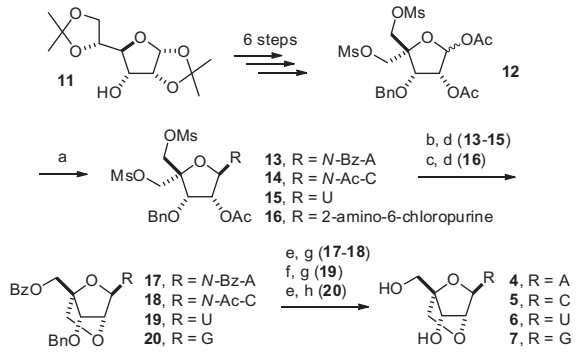
and the known guanine-ENA nucleoside **8** was undertaken, along with the previously unknown guanosine analogs bearing 2′-*C*-methyl and 5′-*C*-methyl modifications **9** and **10** (Fig. 2).

Access to LNA-A **4**, LNA-C **5**, LNA-U **6** and LNA-G **7** was conveniently achieved via common intermediate **12** as described in various synthetic reports (Scheme 1).[13] The synthesis of common intermediate **12** was performed on 500 g scale in six steps starting from commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose **11**.[14] Condensation of diacetate **12** with the four nucleobases, *N*-benzoyladenine, *N*-acetylcytosine, 2-amino-6-chloropurine and uracil, under Vorbrüggen conditions,[15] generated the corresponding nucleosides **13**–**16** in good yield (62–96%). Nucleosides **13**–**15** were converted into the 2′-*O*,4′-*C*-methylene-bridged ribonucleosides **17**–**19** by stirring with aqueous sodium or lithium hydroxide in THF followed by 5′-O-substitution with sodium benzoate.

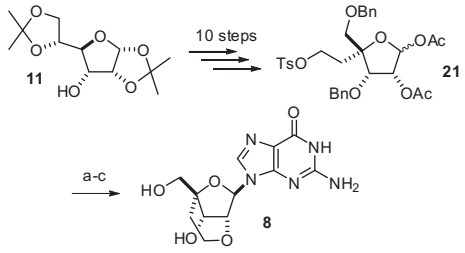
Chloropurine nucleoside **16** was converted into guanine bicyclic nucleoside **20** by treatment with 3-hydroxypropionitrile and sodium hydride followed by 5′-O-substitution with sodium benzoate. Finally, the bicyclic ribonucleosides **4**–**7** were obtained by consecutive 5′-*O*-benzoyl deprotection and 3′-*O*-debenzylation.[16]

Known guanine-ENA nucleoside **8** was prepared in three steps from tosylate intermediate **21**, itself prepared in ten steps from the same starting material, 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose **11**, using the method of Koizumi (Scheme 2).[17]

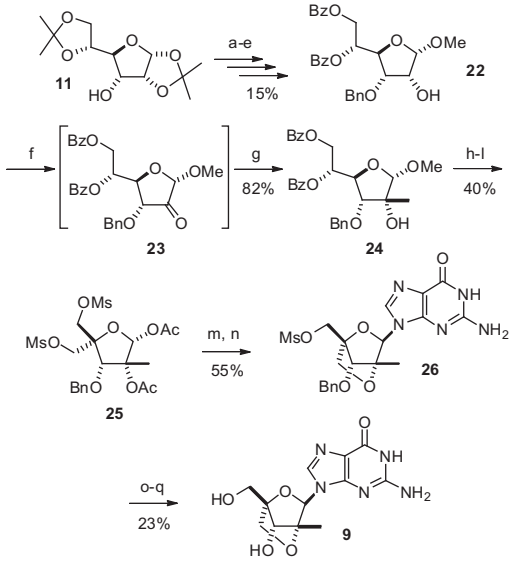
The 2′-*C*-methyl-modified guanine-LNA nucleoside **9** was also derived from the versatile 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose **11** via Grignard addition to the corresponding C-2 ketone using a modification of the procedure described by Eldrup et al. who stereospecifically introduced a 2-*C*-methyl substituent into a 3,5-di-*O*-protected methyl α-ribofuranose (Scheme 3).[18] Protecting group manipulation provided access to 5,6-di-*O*-benzoyl-3-*O*-benzyl-1-*O*-methyl-α-D-allofuranose **22** in 15% yield over five steps from **11**. In contrast to Eldrup's two step oxidation–Grignard procedure, a one-pot protocol was utilized in which initial Swern oxidation of **22** gave 2-keto-allofuranose **23**.[19] The reaction mixture containing ketone **23** was then added to a solution of methyl magnesium bromide at −78 °C to give the desired 2′-*C*-methyl-allofuranose **24** as a single diastereoisomer in 82% yield on 30 g scale. After periodate cleavage and introduction of the 4′-*C*-hydroxylmethyl group, **24** was converted into dimesylate **25** in 40% yield over five steps. Coupling of the diacetate **25** with 2-amino-6-chloropurine under Vorbrüggen conditions was followed by treatment



Scheme 1. Reagents and conditions: (a) Nucleobase, *N*,*O*-bis(trimethylsilyl)acetamide, TMSOTf, MeCN or 1,2-dichloroethane, reflux; (b) NaOH or LiOH·H_2O, THF–H_2O; (c) 3-hydroxypropionitrile, NaH, THF, 0 °C; (d) NaOBz, DMSO, 100 °C; (e) NH_3, MeOH; (f) NaOH or LiOH·H_2O, THF–H_2O; (g) 20% $Pd(OH)_2$/C, HCO_2H or HCO_2NH_4, THF–MeOH, reflux; (h) 10% Pd/C, HCO_2NH_4, MeOH, reflux.



Scheme 2. Reagents and conditions: (a) 2-amino-6-chloropurine, *N*,*O*-bis(trimethylsilyl)acetamide, TMSOTf, 1,2-dichloroethane, reflux; (b) 3-hydroxypropionitrile, NaH, THF, 0 °C; (c) 20% $Pd(OH)_2$/C, HCO_2H, THF–MeOH, reflux.



Scheme 3. Reagents and conditions: (a) NaH, BnBr, MeCN; (b) AcOH–H_2O; (c) BzCl, pyridine, MeCN, 60 °C; (d) HCl, 1,4-dioxane-MeOH; (e) $SnCl_4$, DCM, 35 °C; (f) trifluoroacetic anhydride, DMSO, Et_3N, THF, −78 °C to rt; (g) MeMgBr, THF, −78 °C; (h) NH_3, MeOH; (i) $NaIO_4$, H_2O; (j) CH_2O, NaOH, 1,4-dioxane–H_2O; (k) MsCl, pyridine; (l) Ac_2O, AcOH, H_2SO_4, 0 °C to rt; (m) 2-amino-6-chloropurine, *N*,*O*-bis(trimethylsilyl)acetamide, TMSOTf, 1,2-dichloroethane, reflux; (n) 3-hydroxypropionitrile, NaH, THF, 0 °C; (o) NaOBz, DMSO, 100 °C; (p) NH_3, MeOH; (q) 10% Pd/C, HCO_2NH_4, THF–MeOH, reflux.

of the resulting 6-chloro-nucleoside with 3-hydroxypropionitrile and sodium hydride. Simultaneously, the guanine base was