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Synthesis and anti-HCV activity of 1-(1′,3′-*O*-anhydro-3′-*C*-methyl-β-D-psicofuranosyl)uracil

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ABSTRACT

Synthesis of a novel 1′,2′-oxetane-uridine bearing a 2′-*C*-methyl substituent, [1-(1′,3′-*O*-anhydro-3′-*C*-methyl-β-D-psicofuranosyl)uracil], is described. Key to its construction was the use of 6-*O*-(*p*-toluoyl)-1,2:3,4-di-*O*-isopropylidene-3-*C*-methyl-D-psicofuranose as a nucleosidation substrate, which itself was derived from D-fructose. Anti-HCV activity was examined for the corresponding triphosphate which was not found to be an inhibitor of HCV NS5B 1b wild type polymerase in vitro. The 1′,2′-oxetane uridine triphosphate without 2′-*C*-methyl substitution was similarly inactive, however, the guanosine analog displayed modest inhibition (IC_{50} = 10 μM).

Conformationally restricted nucleosides have been investigated extensively in the context of antisense oligonucleotides and for their therapeutic potential as antiviral agents.[1] The C3′-*endo* (*North*) sugar ring conformational preference exhibited by ribonucleosides on introduction of a 2′-*C*-methyl substituent has yielded a class of nucleoside triphosphates (NTPs) which are highly potent inhibitors of hepatitis C virus (HCV) NS5B polymerase; 2′-*C*-methylcytidine **1**,[2] 2′-*C*-methylguanosine **2**,[3] and 2′-F-2′-*C*-methyluridine **3**[4] being the primary examples (Fig. 1). In the case of **3**, the corresponding prodrug sofosbuvir[5] has been granted regulatory approval, significantly augmenting the existing standard of care for the treatment of chronic HCV, a global disease burden affecting over 170 million people.

Conformationally restricted C3′-*endo* (*North*) bicyclic ribonucleosides bearing a 2′-*O*,4′-*C*-methylene bridge, **4**, have been explored in a wide range of applications,[1] however the corresponding NTPs have only relatively recently been demonstrated to be inhibitors of HCV NS5B polymerase in vitro (Fig. 2).[7]

1′,2′-Oxetane bicyclic nucleosides are similarly forced to adopt a *North* sugar conformation,[8] however the inhibitory potential of the respective uridine **5** and guanosine **6** NTPs against HCV NS5B polymerase has not been established to date. Accordingly, these systems were evaluated along with the novel 2′-*C*-methyl-containing 1′,2′-oxetane-uridine **7** [1-(1′,3′-*O*-anhydro-3′-*C*-methyl-β-D-psicofuranosyl)uracil], the synthesis of which demanded the construction of two adjacent stereospecific quaternary centers.

1′,2′-Oxetane nucleosides **5** and **6** were synthesized according to the literature procedures.[9,10] The uridine derivative **5** was obtained via the direct nucleosidation of diacetonide **8**, which resulted in 45% yield and a disappointing 0.8:1.0 β:α anomeric

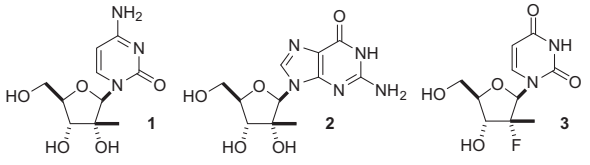

Figure 1. Examples of nucleosides bearing a 2′-*C*-methyl substituent.

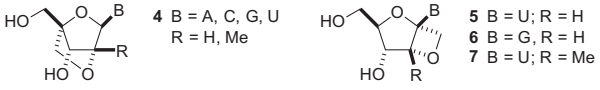

Figure 2. 2′-*O*,4′-*C*-methylene and 1′,2′-oxetane bicyclic nucleosides.

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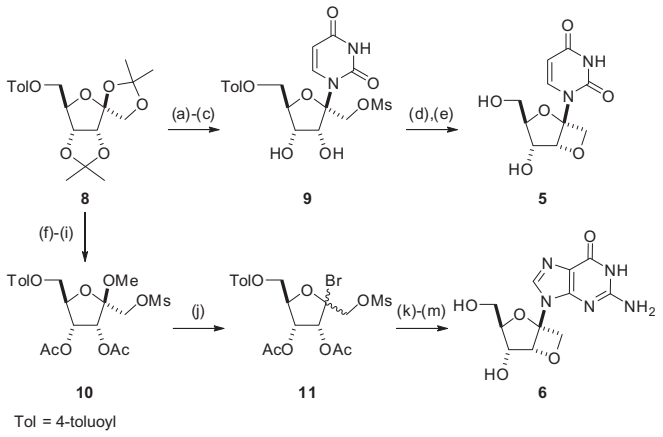
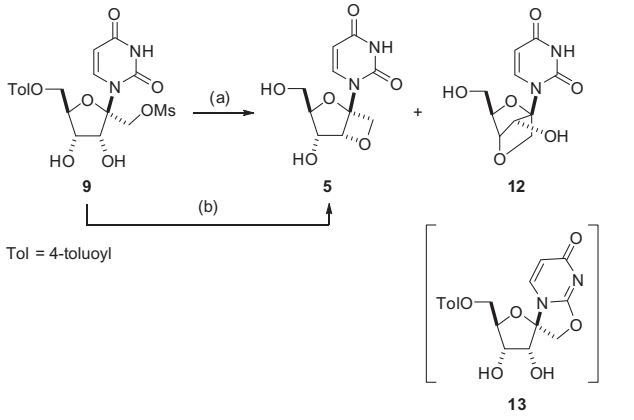
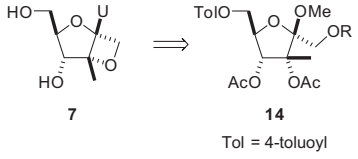


Scheme 1. Reagents and conditions: (a) silylated uracil, TMSOTf, CH_3CN, 0 °C to rt, 16 h, 23% α and 20% β; (b) MsCl, py, rt, 16 h, 95%; (c) TFA/H_2O (9:1), rt, 2 h, 87%; (d) NaHMDS, THF, 0 °C to rt, 3 h; (e) NH_3, MeOH, rt, 48 h, 60% (over 2 steps); (f) HCl, MeOH, rt, 24 h, 65%; (g) MsCl, py, rt, 2 h; (h) TFA/H_2O (9:1), rt, 1 h; (i) Ac_2O, py, rt, 2 h, 69% (over 3 steps); (j) HBr, AcOH, CH_2Cl_2, rt, 3 h; (k) silylated N^2-acetyl-O^6-diphenylcarbamoylguanine, $SnCl_4$, CH_3CN, 80 °C, 1 h; (l) TFA/H_2O (9:1), rt, 0.5 h 30% (over 3 steps); (m) NaOMe, MeOH, rt, 64 h, 81%.



Scheme 2. Reagents and conditions: (a) NaOMe, MeOH, rt, 64 h; (b) (i) NaHMDS, THF, 0 °C to rt, 3 h, (ii) NaOMe, MeOH, rt.



Scheme 3. Retrosynthetic analysis of oxetane **7**.

ratio. The analogous 1,2-oxetane guanosine **6** was obtained via the nucleosidation of the bromide **11** with N^2-acetyl-O^6-diphenylcarbamoylguanine, giving predominantly the β anomer (30% yield over 3 steps following the removal of the diphenylcarbamoyl protection), due to the neighboring group participation of the acetate. The methyl glycoside **10** was synthesized in four steps from the diacetonide **8** in 45% overall yield (Scheme 1).

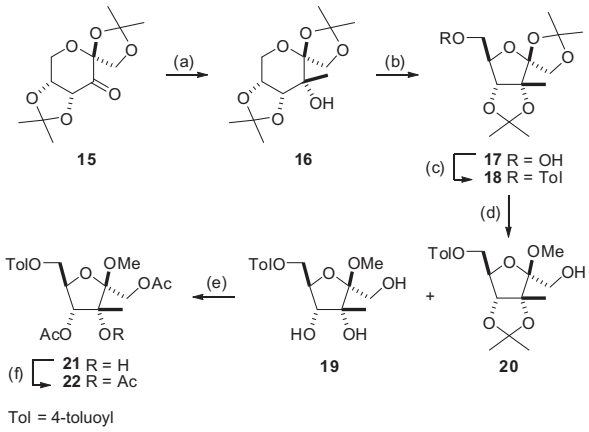
Interestingly, during the cyclization of **9** into the desired oxetane uridine **5**, the regioisomeric 1′-*C*,3′-*O*-methylene bridged bicyclic nucleoside **12**[11] was also obtained. When NaHMDS in THF was used, the [2.1.2]-bicyclic product was formed only in trace amounts (<5% yield) (Scheme 2). However, upon treating the mesylate **9** with NaOMe in MeOH, the synthesis of **12** was favored, with a 1.5:1 ratio of **12**:**5** observed. The analogous structure was not formed in the case of the corresponding guanosine derivative; the expected oxetane structure **6** was obtained following treatment with either NaHMDS/THF or NaOMe/MeOH. Formation of the [2.1.2]-bicycle **12** may potentially proceed via the protected 1′,2′-*O*-anhydro-1-β-D-psicofuranosyluracil intermediate **13**.[10–13]

The initial strategy to construct the 2′-*C*-methyl-containing 1′,2′-oxetane-uridine **7** utilized an analogous procedure to that used for the synthesis of the guanosine **6** (Scheme 3). It was anticipated that acetate protection at OH-3 in methyl psicofuranose **14** would facilitate a favorable β:α ratio due to neighboring group participation.

The synthesis of the methyl glycoside **22** started with D-fructose, which was converted into diacetonide and then oxidized to give ketone **15**[14] following literature conditions. Stereoselective addition of MeLi to the ketone moiety of **15** afforded **16** as a single diastereoisomer in 82% yield (Scheme 4). Isomerization to the furanose form was achieved using a catalytic amount of sulfuric acid in acetone, to produce the 3-*C*-methyl diacetonide **17** in moderate yield.[15] Stereospecific introduction of the methyl group was confirmed by single crystal X-ray analysis (Fig. 3). Protection of the free hydroxyl in **17** using *p*-toluoyl chloride in pyridine gave the ester **18**. Removal of both acetonide groups and concomitant Fischer glycosylation was achieved by treatment with methanolic HCl, which furnished the methyl glycoside **19** in 39% yield. In addition, the 2,3-acetonide **20** was also isolated from the reaction in 11% yield. Peracetylation was achieved in two steps by the treatment of **19** with Ac_2O in pyridine to give initially the 1,4-di-*O*-acetate **21**. Subsequent acetylation of the less reactive tertiary hydroxyl, by heating in neat Ac_2O with NaOAc at 120 °C, gave the 1,3,4-tri-*O*-acetate **22** in excellent yield.

In order to obtain a reactive nucleosidation donor, the bromination of **22** was investigated. Bromination attempts using either HBr in AcOH or $TiBr_4$ proved unsuccessful, with only the unstable elimination product **23** being isolated in both cases in ~50% yield (Scheme 5). Formation of the nucleoside directly from the methyl glycoside **22** using the persilylated nucleobase with either TMSOTf in toluene or $SnCl_4$ in CH_3CN was also unsuccessful. Elimination product **23** was again isolated as the major component of the reaction performed in toluene, while $SnCl_4$/MeCN conditions resulted in formation of the oxazoline **24** (Scheme 5).

As the methyl psicofuranoside **22** proved to be unsuitable, alternative nucleosidation substrates were investigated. Based on a reported synthesis of [1-(1′,3′-*O*-anhydro-β-D-psicofuranosyl)thymine], oxetane **27** was identified as an analogous potential intermediate for



Scheme 4. Reagents and conditions: (a) MeLi, LiBr, Et_2O, rt, 0.5 h, 82%; (b) H_2SO_4, 2,2-dimethoxypropane, acetone, rt, 20 h, 58%; (c) *p*-toluoyl chloride, py, rt, 3 h, 94%; (d) HCl, MeOH, rt to 40 °C, 18 h, **19** (39%) and **20** (11%); (e) Ac_2O, py, rt, 18 h, 98%; (f) Ac_2O, NaOAc, 120 °C, 20 h, 91%.

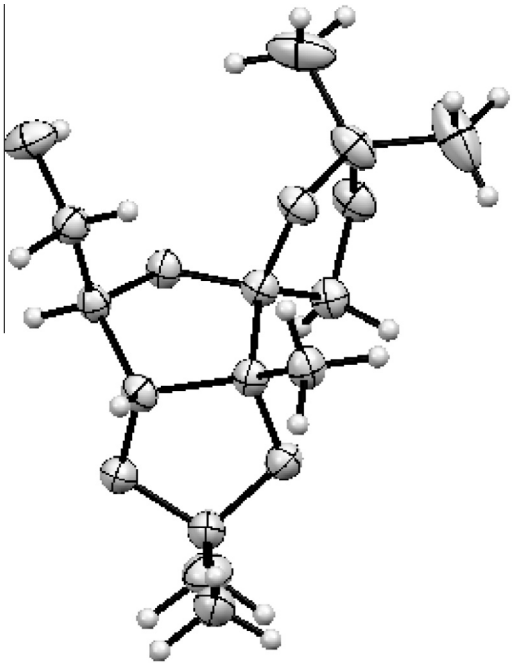
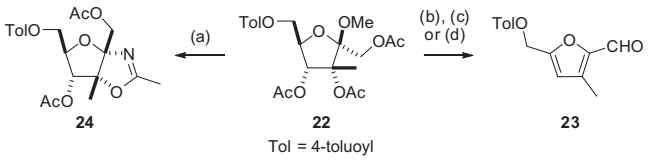
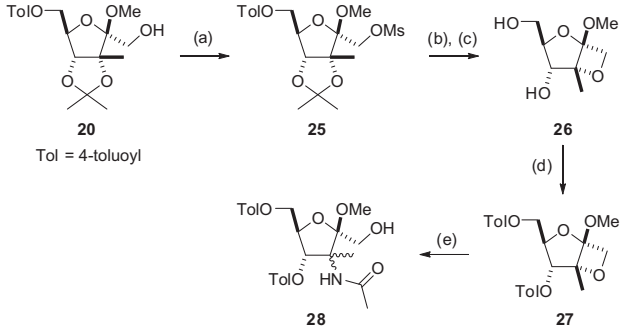


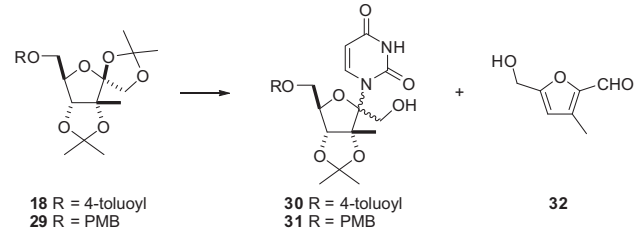
Figure 3. ORTEP drawing of the X-ray crystallographic structure of **17** (CCDC 1006958).



Scheme 5. Reagents and conditions: (a) persilylated nucleobase, CH_3CN, $SnCl_4$, 80 °C, 2 h; (b) HBr in AcOH, CH_2Cl_2, 0 °C to rt, 1.5 h; (c) $TiBr_4$, EtOAc, CH_2Cl_2, rt, 1 h; (d) persilylated nucleobase, toluene, TMSOTf, 80 °C to reflux, 6 h.



Scheme 6. Reagents and conditions: (a) MsCl, py, rt, 18 h, 88%; (b) TFA/H_2O (9:1), rt, 0.5 h; (c) NaOMe, MeOH, rt, 18 h; (d) *p*-toluoyl chloride, py, rt, 19 h, 46% (over 3 steps); (e) persilylated uracil, MeCN, $SnCl_4$, 85 °C, 20 h, 30% (**28**).



Scheme 7. See Table 1 for conditions.

nucleosidation (Scheme 6).[12] The formation of **27** was achieved in four steps starting from the 2,3-acetonide-protected **20** (Scheme 6). Treatment of **20** with MsCl and pyridine gave mesylate **25** from which the acetonide protection was removed using TFA/H_2O. Subsequent oxetane ring closure was effected using NaOMe in MeOH, with concomitant removal of the *p*-toluoyl ester. Reprotection with *p*-toluoyl chloride furnished the key ditoluoyl ester protected 1,3-anhydro-3-*C*-methyl intermediate **27**. Treatment of **27** with persilylated uracil and $SnCl_4$, however, resulted in the opening of the oxetane ring and in the incorporation of acetonitrile giving **28** in 30% yield; no nucleosidation products were observed.

1′,2′-Oxetane nucleosides have alternatively been synthesized by the nucleosidation of related analogs of **18** bearing 1,2:3,4-di-*O*-isopropylidene protection (Scheme 7).[8a,10] Various conditions were screened using **18** as the nucleosidation substrate (Table 1) in order to both effect base coupling and form the desired β-anomer. In this instance, the use of silylated uracil in CH_3CN with TMSOTf as the promoter (entry 1, Table 1) yielded the required nucleoside **30** in 30% yield with a 3.5:1 α:β anomer ratio. One third of the diacetonide starting material **18** was recovered, however, neither elevation of the reaction temperature, nor using $BF_3{\cdot}OEt$ as the catalyst was beneficial, resulting instead in substrate degradation (entries 2 and 3). Silylation of uracil using BSA resulted in an improved α:β ratio but at the expense of the coupling yield, with 60% starting material recovered (entry 4). Increasing the reaction time did not lead to any improvement in conversion and only further increased the α-selectivity (entry 5). The use of other less polar solvent systems and/or $SnCl_4$ as a promoter was also investigated (entries 6–9), however, no improvement was observed over the original conditions.

To rule out the possibility that, through participation, the *p*-toluoyl protecting group was influencing the stereochemical outcome of the nucleosidation, the reaction with *p*-methoxy benzyl protected **29** was attempted using the preferred conditions for **18**. In this case (entry 10), none of the desired nucleoside was formed and elimination of the starting material was accompanied by loss of the PMB protection to give furan **32** (Scheme 7).[16]

Opening of diacetonide **18** with azide was also explored due to the potential for subsequent construction of the nucleobase. *N*-Glycosidation with azide nucleophiles has been reported for related psicofuranosyl systems, in which the β-azide was obtained preferentially in ratios of up to 18:1.[17] As with the Vorbrüggen conditions[18] using uracil, the reaction of **18** with $TMSN_3$ in the presence of TMSOTf produced the anomeric azide in 30% yield, with the undesired α isomer again preferentially formed in a 6:1 ratio.

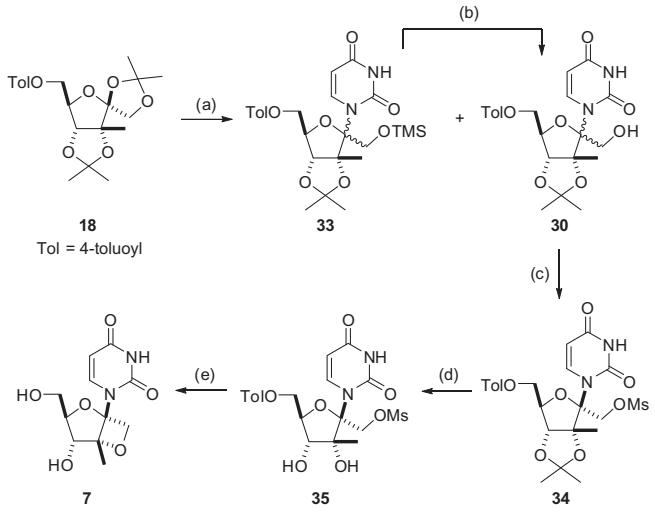
Utilizing the preferred nucleosidation conditions for **18**, (6 equiv silylated uracil, CH_3CN, 2 equiv TMSOTf, rt, 16 h), the uridines α-**30** and β-**30** were isolated, along with an inseparable α:β anomer mixture of the TMS protected **33** (Scheme 8). Conversion of **33** to generate additional **30** was achieved cleanly using AcOH and H_2O. Thus the desired 3′-*C*-methyl-psicofuranosyl uracil β-**30** was isolated in 8% overall yield from **18** along with the α anomer in 22% yield. Synthesis of the target 1′,2′-oxetane uridine **7** was achieved by the treatment of β-**30** with mesyl chloride and pyridine to give **34**, followed by cleavage of the acetonide protecting group with TFA/H_2O. Treatment of the diol **35** with NaHMDS in THF effected closure of the oxetane ring and was accompanied by loss of the toluoyl ester to give the desired 2′-*C*-methyl-containing 1′,2′-oxetane-uridine **7** in good yield. In contrast to **6**, performing the reaction with NaOMe in MeOH furnished only the oxetane **7** and no [2.1.2]-bicyclic derivative was isolated.

The three oxetane nucleosides **5**, **6,** and **7** were evaluated in a whole cell-based HCV replicon assay and none was found to possess inhibitory activity (EC_{50} >45 μM) nor cytotoxicity (CC_{50} >100 μM). Against the purified HCV NS5B 1b wild type polymerase,

Table 1
Solvent, temperature, and promoter screening for the synthesis of **30**

Entry[a]	Sugar	Solvent	Promoter[c]	Temp (°C)	Time (h)	Ratio β:α	Yield of **30** (%)
1	**18**	CH_3CN	TMSOTf	rt	16	1:3.5	30
2	**18**	CH_3CN	TMSOTf	90	16	—	—
3	**18**	CH_3CN	BF_3·OEt	rt	16	—	—
4[b]	**18**	CH_3CN	TMSOTf	rt	18	1:2	10
5[b]	**18**	CH_3CN	TMSOTf	rt	64	1:4	<10
6	**18**	$CHCl_3$	TMSOTf	rt to 50	2	1:5	10
7	**18**	$CHCl_3$	$SnCl_4$	rt to 50	2	—	—
8	**18**	TBME/CH_2Cl_2	TMSOTf	rt	16	—	Trace
9	**18**	TBME/CH_2Cl_2	$SnCl_4$	rt	16	—	Trace
10	**29**	CH_3CN	TMSOTf	rt	16	—	—

[a] Persilylated uracil was used in all examples. Uracil was treated with $(NH_4)_2SO_4$ and HMDS at 130 °C. Sugar was added as a solution, in the indicated solvent, to the crude silylated uracil.
[b] Persilylated uracil was generated in situ by treatment of the sugar and uracil with BSA in CH_3CN at 90 °C.
[c] Promoters were added to a solution of the base and sugar at 0 °C and then warmed to the indicated temperature.



Scheme 8. Reagents and conditions: (a) persilylated uracil, TMSOTf, CH_3CN, 0 °C to rt, 16 h; (b) AcOH, H_2O, THF, rt to 40 °C, 0.5 h; (c) MsCl, py, rt, 16 h; (d) TFA/H_2O (9:1), 40 °C, 1 h, 68% (over 2 steps); (e) NaHMDS, THF, 0 °C to rt, 2 h, 61%.

the corresponding oxetane uridine triphosphate **5-TP** and 2′-*C*-methyl oxetane uridine triphosphate **7-TP** were both inactive (IC_{50} >100 μM).[19] The oxetane guanosine triphosphate **6-TP**, however, was observed to be a modest inhibitor of HCV NS5B polymerase (IC_{50} = 10 μM). In the case of oxetane guanosine **6**, inefficient processing of the free nucleoside to the respective nucleoside triphosphate by cellular kinases may explain the absence of activity observed in the replicon assay. Although subsequent phosphorylations could potentially be compromised, this issue might be circumvented by incorporation of a monophosphate prodrug kinase by-pass strategy.

In summary, 2′-*C*-methyl-containing 1′,2′-oxetane-uridine **7** [1-(1′,3′-*O*-anhydro-3′-*C*-methyl-β-D-psicofuranosyl)uracil], was synthesized from 6-*O*-(*p*-toluoyl)-1,2:3,4-di-*O*-isopropylidene-3-*C*-methyl-D-psicofuranose and its anti-HCV activity was examined in vitro along with 1′,2′-oxetane uridine and 1′,2′-oxetane guanosine. The respective triphosphate of 1′,2′-oxetane guanosine **6-TP** was found to possess modest activity against the NS5B 1b wild type polymerase.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures, characterization data and ^1H and ^{13}C NMR spectra for all new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.09.069.

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