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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 2825–2827

# Synthesis of 5′,9-anhydro-3-(β-D-ribofuranosyl)xanthine, and 3,5′-anhydro-xanthosine as potential anti-hepatitis C virus agents

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Received 20 January 2005; revised 21 February 2005; accepted 22 February 2005

**Abstract**—5′,9-Anhydro-3-(β-D-ribofuranosyl)xanthine and 3,5′-anhydro-xanthosine were prepared as potential anti-hepatitis C virus (HCV) agents from uridine and xanthosine, respectively.


Anhydronucleosides or cyclonucleosides, in which the nucleic base is additionally linked to the sugar moiety usually with loss of a water molecule, have drawn attention largely for the conformational studies or as intermediates for further modification of nucleosides. Although many 5′,6-*O*- or 5′,6-*N*-anhydro-pyrimidine nucleosides,[1,2] and 3,5′-anhydro-purine nucleosides[3,4] have been reported, to our knowledge, there have been very few precedent publications[5] on 5′,9-anhydro-3-(β-D-ribofuranosyl)purines (5′,9-anhydro-purine-*iso*-nucleosides). In this communication, we report the synthesis of 5′,9-anhydro-*iso* xanthosine (**2**) and its positional isomer, 3,5′-anhydro-xanthosine (**3**), as potential anti-hepatitis C virus (HCV) agents.

Hepatitis C virus (HCV) is one of the most important flavivirus infections in humans and is responsible for the second most common cause of viral hepatitis. Currently, over 2% of the US population, and an estimated 170 million people worldwide are HCV carriers.[6] The only approved therapies for chronic hepatitis C are interferon-α (INF-α), or pegylated-interferon-α either alone or in combination with ribavirin. However, their therapeutic value is compromised largely due to adverse effects,[7,8] which necessitates additional options for the treatment of hepatitis C. In this context, we launched a search for novel anti-HCV agents and have discovered anti-hepatitis C-virus (HCV) activity of 3,5′-anhydro-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (**1**).[9] Based on this lead structure, anhydroxanthine derivatives, **2** and **3** were prepared (Fig. 1).

For the synthesis of the target compound **2**, a synthetic pathway was designed so that numerous modified purine 5′,9-anhydro-analogues could be prepared for SAR studies (Scheme 1). The key intermediate, 6-amino-5′,6-*N*-anhydrouridine (**5**)[10] was prepared in 72% yield by sequential treatment of 5′-azido-5-bromo-5′-deoxy-2′,3′-*O*-isopropylidene-uridine (**4**) with triphenylphosphine and ammonium hydroxide in one-pot. This is an improved modification of the known method for the synthesis of **5** that required two separate reactions: reduction of azide by catalytic hydrogenation, followed by base catalyzed cyclization.[11] **5** was treated with $NaNO_2$/HCl in THF–$H_2O$ (2:1) to give a 5-nitroso intermediate, which, without purification, was directly

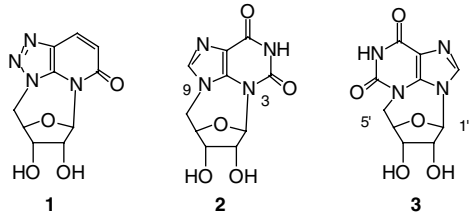


**Figure 1.**

*Keywords*: 5′,9-Anhydro-3-(β-D-ribofuranosyl)xanthine; 3,5′-Anhydro-xanthosine; 5′,9-Anhydro-3-(β-D-ribofuranosyl)purines, 5′,9-Anhydro-purine-isonucleosides; Anti-hepatitis C virus agent; HCV.
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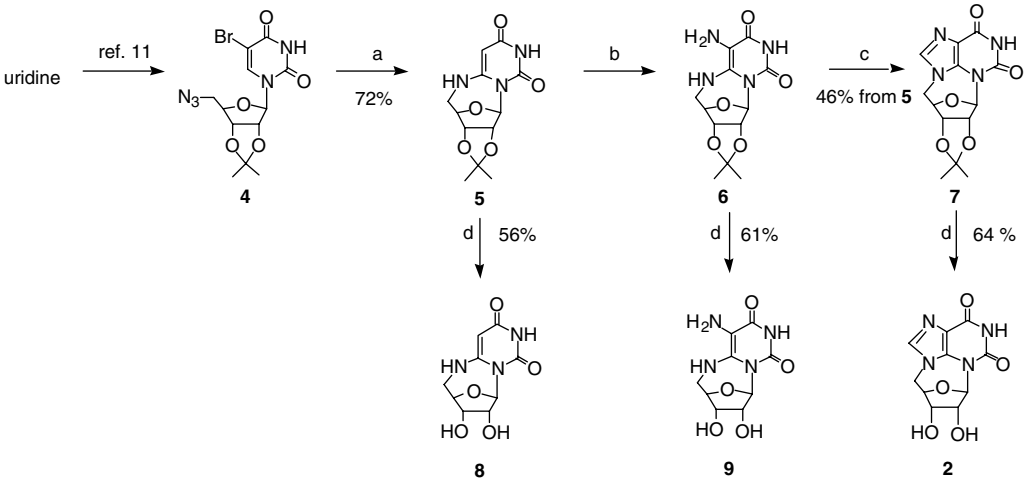
doi:10.1016/j.tetlet.2005.02.120



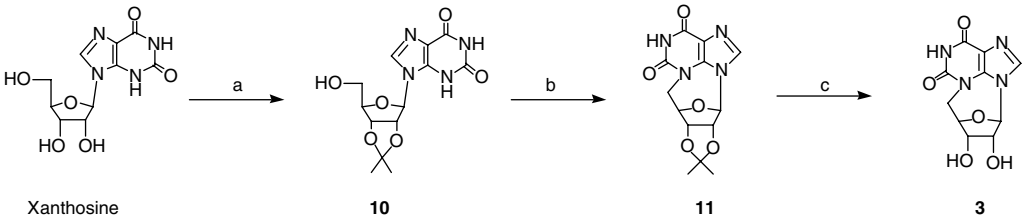

**Scheme 1.** Reagents and conditions: (a) $PPh_3$, THF, rt, 6 h, then $NH_4OH$, rt, 6 h; (b) 1. $NaNO_2$, HCl, THF–$H_2O$, 0 °C, 15 min, 2. $NaHCO_3$, 3. Zn, HCl, MeOH, 90 °C, 30 min, 4. $NaHCO_3$; (c) diethoxymethyl acetate, DMF, 90 °C, 30 min; (d) HCl, $H_2O$.

reduced with Zn/HCl in methanol to give the 5-amino intermediate **6**.[12] Treatment of **6** with diethoxymethyl acetate in DMF at 90 °C afforded 5′,9-anhydro-2′,3′-*O*-isopropylidene-*iso* xanthosine (**7**)[13] in 46% yield from **5**.[14] It is noteworthy that the original synthesis of **7**, in which uridine was converted to a 5′-*O*-tosyl-*iso* xanthosine, and then cyclized to **7**, suffered low yield in our hands.[5] Hydrolysis of **7** with 0.5 M HCl gave the first target compound **2**.[15]

A positional isomer of **2**, 3,5′-anhydroxanthosine (**3**),[16] was prepared from xanthosine by 2′,3′-*O*-isopropylidenation with 2,2-dimethoxypropane/acetone/TsOH, followed by cyclization using Mitsunobu reaction, and subsequent acidic removal of the 2′,3′-*O*-isopropylidene group (Scheme 2).

The structures of these target compounds were determined by 1D-, 2D-$^1$H NMR, $^{13}$C NMR and high-resolution mass spectroscopy. Usually the chemical shifts of protons of the sugar moiety in ribonucleosides are down fielded in the order of 1′ > 2′ > 3′ > 4′ > 5′. However, chemical shifts of those protons of **2** are reversed except for 1′-proton where the order is 5′ ($\delta$ 4.65) > 4′ ($\delta$ 4.54) > 5″ ($\delta$ 4.40) > 3′ ($\delta$ 4.14) > 2′ ($\delta$ 4.03). The same trend is also found in the case of compounds **1** and **3**. These changes in chemical shifts indicate that the deshielding heterocycle moiety is linked to 5′ carbon, which results in lower field resonances for 5′-, 4′- and 3′-protons due to their relative proximity to the base compared with those of regular ribonucleosides. Another feature of $^1$H NMR is the large difference in chemical shifts of the 5′- and 5″-protons of the target compounds. For example, the chemical shifts of H-5′ and H-5″ of **3** are $\delta$ 4.62–4.55 and $\delta$ 3.72, respectively, reflecting the rigid structures of the anhydro-nucleosides due to the additional linkage between the base and sugar moiety. 5′,9-Anhydro-*iso* xanthosine (**2**) exhibited an improved anti-HCV activity ($EC_{90}$ 13 M) in a replicon system[17,18] compared to that of the lead compound **1** ($EC_{90}$ 70 μM). However, the positional isomer **3** showed no anti-HCV activity in the same cell culture system. In order to find whether the imidazole function is essential for the anti-HCV activity, we prepared **8** and **9** from the intermediates, **5** and **6**, respectively, by acidic deprotection (Scheme 1). The diamine **9**[19] showed moderate activity ($EC_{90}$ 85 μM), which is less potent than **2**, but is comparable to **1**, while compound **8**[20] was devoid of any activity, which shows that nitrogen at 5-position of the pyrimidine moiety is essential. Furthermore, the imidazole moiety did increase the potency. The detailed biological studies of **2** and its derivatives will be published elsewhere.

In summary, we have developed a new and efficient synthetic procedure for the novel compound, 5′,9-anhydro-*iso* xanthosine (**2**), and its positional analogue **3**. Compound **2** was found to possess more potent anti-HCV activity in a replicon system than the lead compound, **1**.




**Scheme 2.** Reagents and conditions: (a) acetone, 2,2-dimethoxypropane, TsOH; (b) $Ph_3P$, DEAD, DMF; (c) HCl, $H_2O$.

## Acknowledgements

This work was supported in parts by the NIH grants 1R43 AI-52868 (biology) and 1 R43 AI-056720 (chemistry).

Dr. Raymond F. Schinazi is a founder and director of Pharmasset Inc., and his protocols have been reviewed by Emory University's Conflict of Interest Committee. Dr. Schinazi's group is supported by the Department of Veterans Affairs and he received no funding from Pharmasset, Inc.

## Supplementary data

NMR spectra of compounds, **2**, **3**, **5**, **7**, **8** and **9** are available online as supplementary data with the paper in ScienceDirect. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.02.120.

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10. Compound **5**: UV $\lambda_{max}$ 278 nm (MeOH); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.85 (s, 1H, H–*N*3), 6.86 (d, 1H, $J$ = 6.8 Hz, H–$N^6$), 6.37 (s, 1H, H-1′), 4.91 (s, 1H, H-5), 4.74 and 4.72 (2d, 2H, $J$ = 5.6, 8.0 Hz, H-2′,3′), 4.45 (s, 1H, H-4′), 3.34 (ddd, $J$ = 2.8, 6.4, 13.2 Hz, 1H, H-5′), 3.01 (d, 1H, $J$ = 13.2 Hz, H-5″), 1.41 (s, 3H, $H_3C$), 1.26 (s, 3H, $H_3C$); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 162.35, 157.65, 151.05, 111.23, 89.59, 84.51, 83.45, 82.50, 82.48, 49.59, 26.09, 24.32. HRFABMS calcd 280.0933 for $C_{12}H_{14}N_3O_5$ $(M-H)^-$, found 280.0946.
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13. Compound **7**: UV $\lambda_{max}$ 236, 265 nm (MeOH); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.27 (s, 1H, NH), 7.72 (s, 1H, H-8), 6.33 (s, 1H, H-1′), 4.87–4.81 (m, 3H, H-2′,3′,4′), 4.74 (d, 1H, $J$ = 14.0 Hz, H-5′), 4.20 (dd, 1H, $J$ = 4.0, 14.0 Hz, H-5″), 1.45 (s, 3H, $CH_3$), 1.24 (s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 157.15, 149.03, 139.86, 138.90, 117.81, 111.95, 90.63, 84.57, 83.77, 80.96, 51.83, 25.84, 24.27; HRFABMS calcd 313.1124 for $C_{13}H_{14}N_4O_5$ $(M+Li)^+$, found 313.1130.
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15. Compound **2**: UV $\lambda_{max}$ 238, 264 nm (MeOH); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.26 (s, 1H, NH), 7.84 (s, 1H, H-8), 6.04 (s, 1H, H-1′), 4.65 (d, 1H, $J$ = 12.8 Hz, H-5′), 4.54 (t, 1H, $J$ = 3.2 Hz, H-4′), 4.40 (dd, 1H, $J$ = 3.20, 13.6 Hz, H-5″), 4.14 (t, 1H, $J$ = 5.2 Hz, H-3′), 4.03 (d, 1H, $J$ = 4.8 Hz, H-2′); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 157.12, 148.67, 139.28, 138.28, 116.90, 93.15, 82.58, 74.42, 71.01, 52.21; HRFABMS calcd. 265.0573 for $C_{10}H_9N_4O_5$ $(M-H)^-$, found 265.0574.
16. Compound **3**: UV $\lambda_{max}$ 236, 265 nm (MeOH); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.39 (s, 1H, NH), 8.13 (s, 1H, H-8), 6.20 (s, 1H, H-1′), 4.62–4.55 (m, 2H, H-4′, 5′), 4.22 (dd, 1H, $J$ = 4.0, 5.6 Hz, H-3′), 3.91 (d, 1H, $J$ = 6.0 Hz, H-2′), 3.72 (dd, 1H, $J$ = 3.20, 14.8 Hz, H-5″); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 157.22, 150.98, 140.61, 134.45, 117.31, 92.68, 83.70, 75.82, 70.09, 51.35; HRFABMS calcd. 265.0573 for $C_{10}H_9N_4O_5$ $(M-H)^-$, found 265.0580.
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19. Compound **9**: UV $\lambda_{max}$ 288 nm (MeOH); $^1$H NMR of HCl salt form of **9** (400 MHz, DMSO-$d_6$) $\delta$ 11.60 (br s, 1H, H–*N* 3), 8.95 (br s, 1H, $D_2O$ exchangable, not assigned), 8.45 and 7.90 (2s, 1H, $D_2O$ exchangable, not assigned), 7.13 (d, 1H, $J$ = 6.4 Hz, H–$N^6$), 6.21 (s, 1H, H-1′), 4.30 (s, 1H, H-4′), 4.21 and 4.04 (2d, 2H, $J$ = 6.4 Hz each, H-2′,3′), 3.10–3.04 (m, 1H, H-5′, H-5″ may be buried by water peak); HRFABMS calcd 255.0729 for $C_9H_{11}N_4O_5$ $(M-H)^-$, found 255.0736.
20. Compound **8**: UV $\lambda_{max}$ 279 nm (MeOH); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.89 (s, 1H, NH), 7.00 (d, 1H, $J$ = 6.0 Hz, H–$N^6$), 6.28 (s, 1H, H-1′), 4.93 (s, 1H, H-5), 4.27 (s, 1H, H-4′), 4.18 (d, 1H, $J$ = 6.0 Hz, H-3′), 4.04 (d, 1H, $J$ = 6.0 Hz, H-2′), 3.27 (m, 1H, H-5′), 2.97 (d, 1H, $J$ = 12.4 Hz, H-5″).