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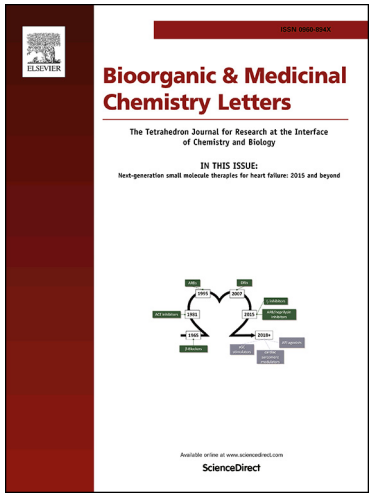

Xylo-C-nucleosides with a pyrrolo[2,1-f][1,2,4]triazin-4-amine heterocyclic base: Synthesis and antiproliferative properties

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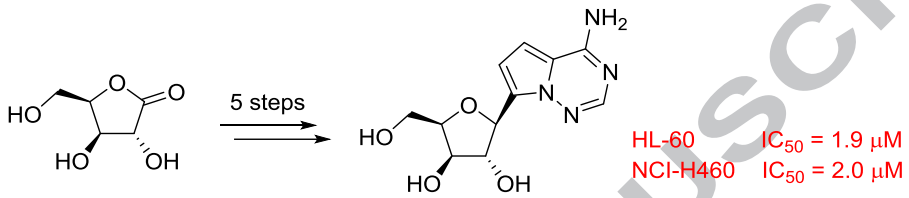

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Xylo-C-nucleosides with a pyrrolo[2,1-f][1,2,4]triazin-4-amine heterocyclic base: Synthesis and antiproliferative properties

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ABSTRACT

The synthesis of a xylo-C-nucleoside containing pyrrolo[2,1-f][1,2,4]triazin-4-amine as nucleobase along with that of its 1'-cyano analogue is described. Among different experimental conditions explored in order to optimize a key debenzylation step in the presented synthetic route, it was found that palladium catalyzed hydrogen transfer allowed for obtaining the target compounds in good yields. The resulting mixture of epimers was separated and each was characterized by NOESY NMR experiments. In vitro antiproliferative assays showed that the 1'-unsubstituted analogue was active against a panel of tumor cell lines such as the human leukemia HL-60 (IC_{50} = 1.9 µM) and lung cancer NCI-H460 (IC_{50} = 2.0 µM) cells.

C-Nucleosides are a unique class of nucleoside analogues in which a C-C bond is present between the sugar moiety and the nucleobase in place of the typical C-N linkage. Owing to this structural feature, C-nucleosides are more stable against chemical and enzymatical hydrolysis. Some C-nucleosides containing nucleobases that are structurally different from the canonical heteroaromatic DNA and RNA bases occur in nature. Examples include showdomycin and formycin A and B (Figure 1), which act as nucleoside antibiotics.[1,2] Pyrazomycin (Figure 1), another natural C-nucleoside isolated from *Streptomyces candidus*,[3] exhibits activity against several tumor cell lines, including Walker carcinosarcoma 256 and Gardner lymphosarcoma.[4]

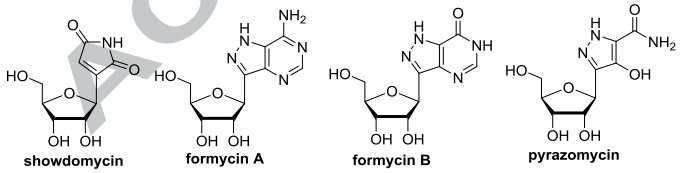

Figure 1. Structures of naturally occurring C-nucleosides.

Over the years, many C-nucleoside analogues have also been prepared by chemical synthesis. In some cases, interesting biologically properties have been found,[5-8] particularly when the heterocycle pyrrolo[2,1-f][1,2,4]triazin-4-amine[9] (or 4-aza-7,9-dideazaadenine) was introduced as nucleobase. For example, 4-aza-7,9-dideazaadenosine (compound **1**, Figure 2) has been found to be active against several cancer cell lines such as leukemic and pancreatic adenocarcinoma.[5,10] Another analogue, GS-5734, is currently being developed as a promising antiviral prodrug for the treatment of Ebola virus infections.[6]

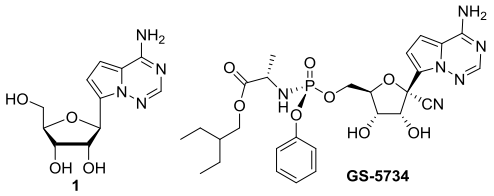

Figure 2. Structures of synthetic C-nucleosides.

On the other hand, nucleosides with a xylose-configured sugar ring, i.e., 3'-epimers of ribonucleosides, are an underexplored class of nucleoside analogues, although some of them have been reported to possess antiviral or antitumor properties (Figure 3).

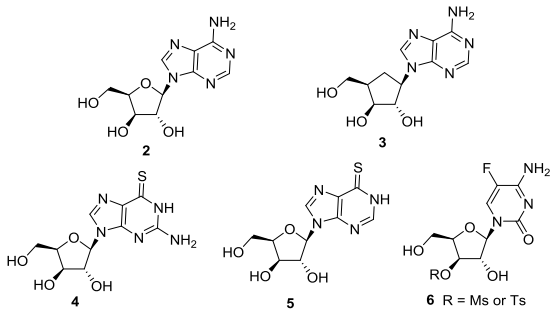

Figure 3. Examples of bioactive xylo-nucleoside analogues.

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In particular, 9-β-D-xylofuranosyladenine (**2**) is an antitumor agent active against the proliferation of ascites tumor cells,[11] while its carbocyclic congener **3** exhibits activity against leukemia cells.[12] After its synthesis in 1971,[13] 9-β-D-xylofuranosyl-6- thioguanine **4** was found to be able to significantly inhibit murine tumor cell growth.[14] The antitumor effects of 9-β-D-xylofuranosyl-6-mercaptopurine (**5**) on three murine tumors including sarcoma 180, Ehrlich, and TA3 ascites tumors were also reported.[15] 1-β-D-Xylofuranosyl-5-fluorocytosine derivatives **6** with different leaving groups at the 3'-position showed good inhibitory activities against mouse leukemia cells.[16] Nonetheless, the synthesis and biological properties of dually modified C-nucleosides comprising a xylosyl sugar moiety have not been investigated to date. Based on the above considerations, we identified xylo-C-nucleoside derivatives **7** and **8** featuring a pyrrolo[2,1-f][1,2,4]triazin-4-amine nucleobase as targets for this study (Figure 4).

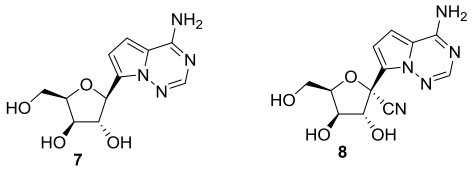
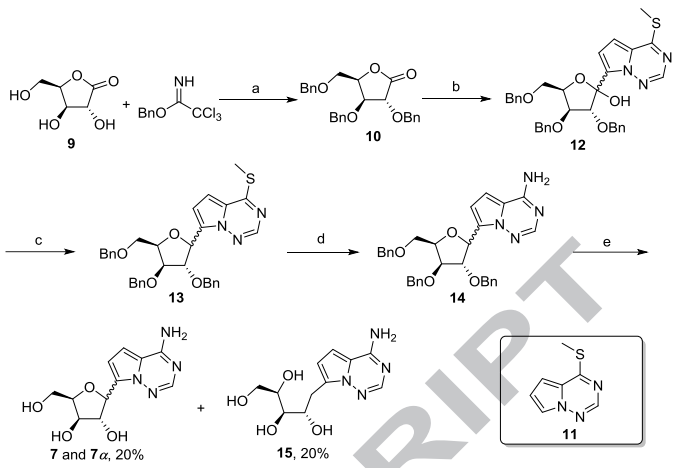


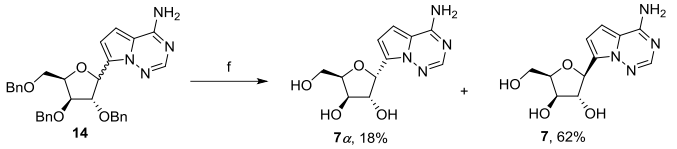
Figure 4. Xylo-C-nucleosides developed in this study.

At first, we focused on the development of a suitable synthetic route for the preparation of both **7** and **8**. To this end, commercially available D-xylono-1,4-lactone **9** was selected as starting material, as shown in Scheme 1. Initial protection of all free hydroxy groups of **9** as benzyl ethers was achieved by using benzyl trichloroacetimidate in the presence of triflic acid (TfOH) at 0 °C. Lactone **10** was thus obtained in 90% yield. Its coupling partner thiomethyl substituted nucleobase **11** was prepared according to a literature procedure.[17] In the presence of lithium diisopropylamide (LDA), compound **11** was deprotonated to generate a carbanion, which attacked the carbonyl group of **10** to afford coupling product **12** as a mixture of two epimers in 60% yield. Subsequent reduction of the 1'-hydroxy group was accomplished by treating hemiacetal **12** with triethylsilane and $BF_3{\cdot}OEt_2$, which led to the formation of **13** in good yield (85%). Although a good stereoselectivity was observed in the case of ribosyl-nucleoside analogues under the same reaction conditions (Et_3SiH, $BF_3{\cdot}OEt_2$),[18] compound **13** was identified as a mixture of two inseparable anomers (α:β = 2:3). The thiomethyl group was then replaced by an amino group using methanolic ammonia under heating, thus providing benzyl protected xylo-C-nucleoside **14**.



Scheme 1. Synthesis of target xylo-C-nucleoside **7**. *Reagents and conditions:* (a) TfOH, dioxane, 0 °C, 3 h, 90%; (b) **11**, LDA, THF, -78 °C, 3 h, 60%; (c) Et_3SiH, $BF_3{\cdot}OEt_2$, DCM, 0 °C, 1 h, 85%; (d) NH_3/MeOH, 100 °C, 12 h, 85%. (e) H_2, $Pd(OH)_2$/C, AcOH, rt, 48 h.

Subsequent removal of the benzyl protecting groups by $Pd(OH)_2$-catalyzed hydrogenolysis in acetic acid led to a mixture of xylo-C-nucleosides **7** and **7*α*** in poor yield (20%), along with 20% of the ring-opening product **15**. In an effort to increase the yield of the debenzylation reaction, several conditions were investigated. However, treatment with boron trichloride, a reagent commonly used for the removal of benzyl groups, only resulted in a complex mixture of products. Under oxidative debenzylation conditions in the presence of a combination of $NaBrO_3$ and $Na_2S_2O_4$,[19,20] no product was formed. Further attempts to convert the benzyl groups into benzoates by oxidation with ruthenium tetroxide also met with failure.[21,22]



Scheme 2. Optimization of the synthesis of target xylo-C-nucleoside **7**. (f) cyclohexene, $Pd(OH)_2$/C, EtOH, reflux, overnight.

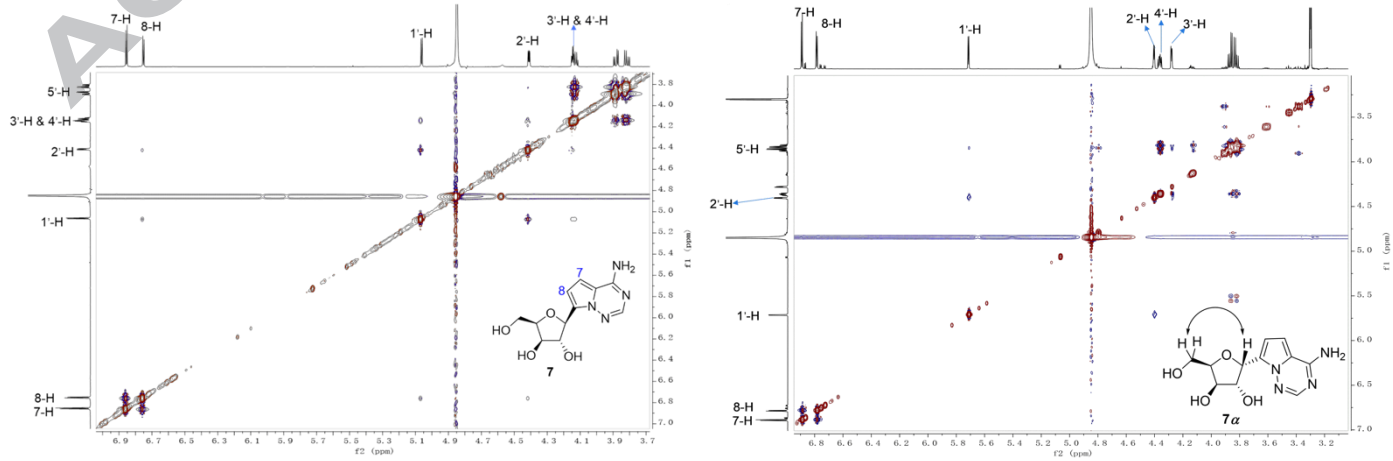


Figure 5. NOESY spectra of compounds **7** and **7*α***.

Table 1. Antiproliferative activity of xylo-C-nucleoside **7**.

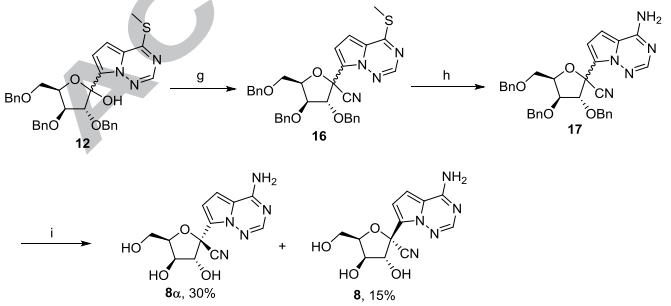
	IC_{50} (μM)[a]							
	Capan-1	Hap-1	HCT-116	NCI-H460	DND-41	HL-60	K-562	Z-138
Compound **7**	2.7±1.0	9.3±4.2	8.4±0.8	2.0±0.1	2.8±0.4	1.9±0.1	4.3±2.3	4.0±3.7
Docetaxel	0.019±0.014	0.018±0.009	0.004±0.0004	0.007±0.004	0.014±0.009	0.047±0.005	0.016±0.0004	0.009±0.0006
Staurosporine	0.048±0.002	0.039±0.017	0.037±0.016	0.024±0.002	0.023±0.014	0.019±0.009	0.083±0.013	0.018±0.010

[a]IC_{50} indicates the half maximal inhibitory concentration.

It has been reported that the Birch reduction can also be used for the cleavage of benzyl groups.[23,24] However, treatment of **14** with sodium in liquid NH_3 led only to the decomposition of the starting material. Nonetheless, deprotected C-nucleosides **7** and **7*α*** could be obtained in a pleasing 80% overall yield by using $Pd(OH)_2$ on carbon (Pearlman's catalyst) and cyclohexene as hydrogen donor under reflux (Scheme 2).[25,26] The two epimers were separated by silica chromatography and target xylo-C-nucleoside **7** was eventually isolated in 62% yield.

The structures of compound **7** and its epimer **7*α*** were determined by NOESY NMR experiments. The main difference between the two epimers is the direction of the nucleobase, which is pointed upward and downward in **7** and **7*α***, respectively, therefore resulting in different *H,H*-correlations. As shown in Figure 5 (spectrum on the left), correlations between 8-H and 2'-H as well as 1'-H and 4'-H were observed, which provided the evidences needed for the characterization of compound **7** as the *β* anomer. The existence of a NOESY correlation between 1'-H and 5'-H (Figure 5, spectrum on the right) together with the absence of any correlation between 8-H and other protons further confirmed the configuration of **7*α***.

Next, a cyano group was introduced at the 1'-position of compound **12** upon reaction with TMSCN in the presence of TMSOTf as promotor[27] to provide nitrile compound **16** in 80% yield (Scheme 3). Replacement of the thiomethyl group with an amino functionality at the 4-position of **16** led to compound **17**, which then underwent removal of the benzyl protecting groups by using BCl_3 in DCM to afford a mixture of **8** and **8*α*** (*α*:*β* = 2:1) in an overall 45% yield. Benzyl deprotection by palladium mediated hydrogenolysis was disappointing in this case, leading to compounds **8** and **8*α*** in poor yield. C-Nucleoside **8** was isolated by silica chromatography in 15% and then further purified by reverse phase HPLC chromatography.



Scheme 3. Synthesis of target xylo-C-nucleoside **8**. *Reagents and conditions:* (g) TMSOTf, TMSCN, DCM, 0 °C, 2.5 h, 80%; (h) NH_3/MeOH, 100 °C, overnight, 86%; (i) BCl_3, DCM, 0 °C to rt, 2.5 h.

In order to evaluate the biological properties of the synthesized xylo-C-nucleosides **7** and **8**, both compounds were screened against eight different tumor cell lines including pancreatic adenocarcinoma (Capan-1), Hap-1 (chronic myeloid leukemia), colorectal carcinoma (HCT-116), lung carcinoma (NCI-H460), DND-41 (acute lymphoblastic leukemia), HL-60 (acute myeloid leukemia), K-562 (chronic myeloid leukemia), and Z-138 (non-Hodgkin lymphoma). The inhibition of cell proliferation induced by the tested compounds was calculated as IC_{50}, which is the half maximal inhibitory concentration. The IC_{50} was calculated by interpolation based on the semi-log dose response. Compound **7** displayed antiproliferative activity against all above cell lines (Table 1), in particular exhibiting an IC_{50} = 1.9 and 2.0 μM against HL-60 and NCI-H460 cells, respectively. In contrast, no significant cytotoxicity was observed for 1'-cyano-xylo-C-nucleoside **8**.

In summary, a synthetic route was developed for the preparation of novel xylo-C-nucleosides **7** and **8** featuring a pyrrolo[2,1-f][1,2,4]triazin-4-amine base. Suitable reaction conditions were established to accomplish a key debenzylation step in the synthetic route. Furthermore, 2D NMR experiments were performed to analyze the structures of the different C-nucleoside epimers formed upon removal of the benzyl functionalities. Both xylo-C-nucleosides **7** and **8** were evaluated against different tumor cell lines. The results of this screening revealed that compound **7** possessed a micromolar antiproliferative activity against a variety of tumor cells such as HL-60 and NCI-H460, while compound **8** displayed no significant cytotoxicity.

Acknowledgments

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Supplementary Material

Supplementary material to this article can be found online at

Highlights

- The first examples of a new series of antitumor nucleoside analogues are described
- C-nucleosides with a xylose sugar ring were synthesized and characterized
- Compound **7** displayed a micromolar antiproliferative activity