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Synthesis of Novel D-2′-Deoxy-2′-*C*-difluoromethylene-4′-thiocytidine as a Potential Antitumor Agent

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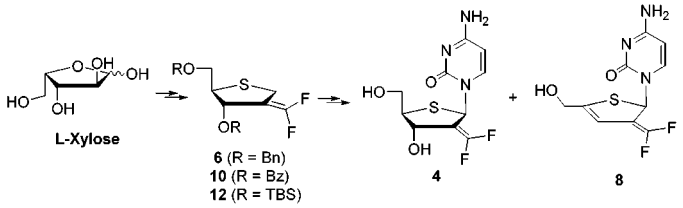
ABSTRACT



2′-Deoxy-2′-*C*-difluoromethylene-4′-thiocytidine (4) as a potential antitumor agent was synthesized starting from L-xylose via 2-deoxy-2-*C*-difluoromethylene-4-thiosugar as a key intermediate. An elimination product, 8, was always formed as the major product during removal of the protecting groups under acidic or basic conditions. However, utilizing neutral reaction conditions to remove the protecting groups afforded the desired product 4 exclusively.

Promising biological activities such as antiviral and antitumor are generally exhibited by 2′-modified nucleosides, among which 2′-difluoro-modified gemcitabine (**1**)[1] exhibits potent antitumor activity and is being clinically used for the treatment of various solid tumors. Recently, on the basis of the antitumor activity of **1**, Yoshimura and co-workers synthesized two of its corresponding 4′-thio analogues **2** and **3** of which the former was found to be potent against human T-cell leukemia CCRF-HSB-2 cells[2] and the latter much more potent than compound **2** and 1-β-D-arabinofuranosyl cytosine (ara-C) in the same cells.[2]

Therefore, on the basis of the bioisosteric rationale, it was of interest to design and synthesize compound **4** (Figure 1),

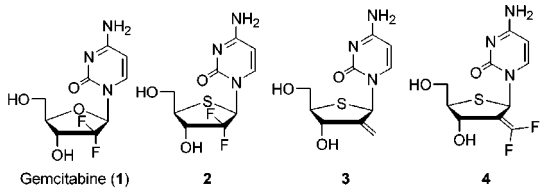


Figure 1. Rationale for the design of the target nucleoside **4**.

which combines the characteristics of compounds **2** and **3**, and evaluate its antitumor activity. During preparation of our target compound **4** (2′-*C*-difluoromethylene), especially during removal of the protecting groups, we have encountered the occurrence of unique chemistry in its sugar moiety in sharp contrast to the preparation of **3** (2′-*C*-methylene).[3]

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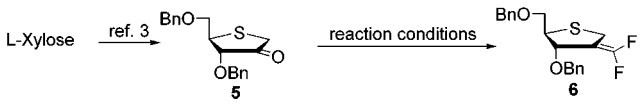
Herein, we report the synthesis of a novel D-2′-deoxy-2′-*C*-difluoromethylene-4′-thiocytidine and its related chemistry combined with biological activity.

Our strategy toward the target nucleoside **4** involved preparation of a glycosyl donor, 2-deoxy-2-*C*-difluoromethylene-4-thiosugar, and then condensation with silylated cytosine. The key step for the synthesis of the glycosyl donor **6** (Scheme 1) was to introduce the difluoromethylene group

Scheme 1



reaction conditions	yield
Ph_3P, Zn-dust, CF_2Br_2 MeCN, rt to reflux, 5 h	2%
Ph_3P, Zn-dust, CF_2Br_2 DMF, rt and then 60 °C, 15 h	decomposed
Ph_3P, Zn-dust, CF_2Br_2 CH_2Cl_2, rt, 60 h	no reaction
Zn-dust, CF_2Br_2, HMPT THF, reflux, 1 h	**47%**

at the C2 position. Thus, L-xylose was converted to the known ketone **5**[3] according to an efficient procedure developed in our laboratory. Initial attempts to synthesize the difluoromethylene compound **6** from ketone **5**, utilizing dibromodifluoromethane/Zn-dust/triphenylphosphine in various solvents such as CH_3CN, DMF, or CH_2Cl_2 either failed to produce the desired compound **6** or resulted in disappointing yields (2%).[4] However, using HMPT[5] instead of triphenylphosphine under refluxing conditions gave the key intermediate **6** in 47% yield.

The key intermediate **6** obtained was oxidized to the sulfoxide, which was condensed with silylated N^4-acetylcytosine to give the protected nucleosides **7a** and **7b** (Scheme 2). The anomeric configurations of **7a** and **7b** were readily assigned by 2D NOESY experiments. Treatment of **7a** with boron tribromide in methylene chloride followed by quenching with methanol did not give the desired nucleoside **4** but afforded the elimination product **8** exclusively. Employing boron trichloride in methylene chloride followed by quenching with methanol also gave the same result. Since this phenomenon never occurred in the case of 2′-deoxy-2′-*C*-methylene-4′-thiocytidine,[3] it is believed that strong electronegative difluoro substituents played a major role in forming the elimination product under strongly acidic conditions.

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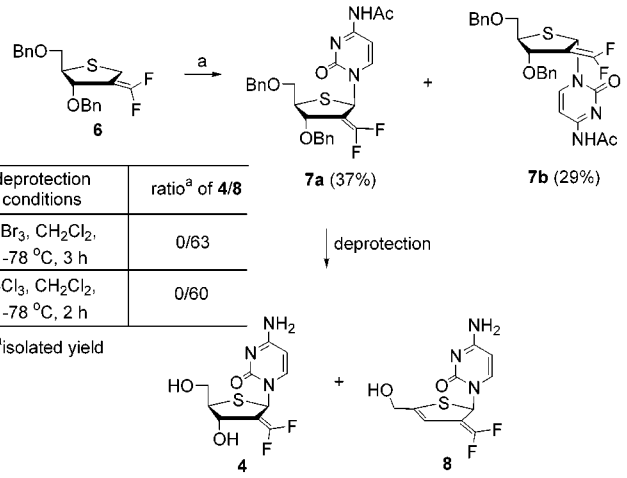
Scheme 2[a]



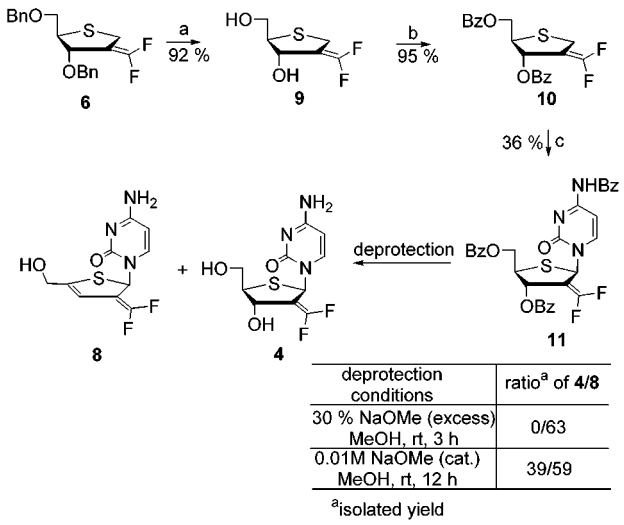
deprotection conditions	ratio[a] of **4/8**
BBr_3, CH_2Cl_2, -78 °C, 3 h	0/63
BCl_3, CH_2Cl_2, -78 °C, 2 h	0/60

[a]isolated yield

[a] Reagents: (a) i. *m*CPBA, CH_2Cl_2, −78 °C; ii. silylated N^4-acetylcytosine, TMSOTf, $ClCH_2CH_2Cl$, 0−50 °C, 1 h.

To avoid the formation of the elimination product **8** obtained under acidic conditions, we decided to change the protecting group to a benzoyl group (Scheme 3). Treatment

Scheme 3[a]



deprotection conditions	ratio[a] of **4/8**
30 % NaOMe (excess) MeOH, rt, 3 h	0/63
0.01M NaOMe (cat.) MeOH, rt, 12 h	39/59

[a]isolated yield

[a] Reagents (a) BBr_3, CH_2Cl_2, −78 °C, 3 h; (b) BzCl, pyridine, rt, 1 h; (c) i. *m*CPBA, CH_2Cl_2, −78 °C; ii. silylated N^4-benzoyl cytosine, TMSOTf, $ClCH_2CH_2Cl$, 0−50 °C, 1 h.

of **6** with boron tribromide at −78 °C gave only the diol **9** in 92% yield. Unlike the case of **7a**, the elimination product was not detected, probably due to the absence of an electron-withdrawing nucleobase. The diol **9** was reacted with benzoyl chloride in pyridine to give dibenzoate **10**, which was converted to the protected nucleoside **11** according to a procedure similar to that used in Scheme 2. However, it is interesting to note that condensation with dibenzoate **10** only afforded the β-isomer **11**, unlike condensation with the

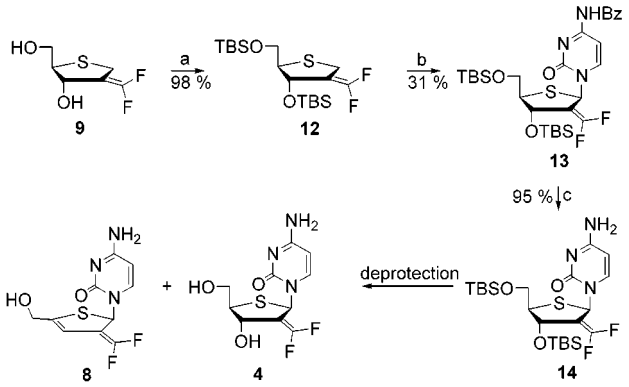
dibenzyl derivative **6**. It is likely that these results are related to the stability of condensation products; the α-isomer might be thermodynamically unstable under the reaction conditions, presumably due to the electron-withdrawing benzoyl groups. To remove the benzoyl protecting groups of **11**, we first used more than 3 equiv of sodium methoxide, in which only elimination product **8** was produced in 63% yield after silica gel column chromatography. However, using a catalytic amount of diluted 0.01 M sodium methoxide gave the desired nucleoside **4**, but elimination product **8** was still obtained as a major product. As removal of the protecting groups under basic conditions failed to give the desired nucleoside **4** as a major product, we decided to remove the protecting groups under neutral or almost neutral conditions (Scheme 4).

The diol **9** was converted to *tert*-butyldimethylsilyl (TBS) protected sugar **12**. After *m*CPBA oxidation of **12**, the resulting sulfoxide was condensed with N^4-benzoylcytosine to give **13** (31%) as the main product as in the case of dibenzoate sugar **10**. Treatment of **13** with sodium methoxide gave **14** in 95% yield without forming any elimination product. Since *n*-tetrabutylammonium fluoride as desilylating agent was too basic, we used the neutralized (acetic acid treated) desilylating agent for the removal of the TBS groups in **14**, resulting in the exclusive formation of the desired nucleoside **4** in 99% yield. Similarly, using neutral triethylamine-trihydrofluoride[6] as desilylating agent also produced the same results. However, use of a weak acid, 70% acetic acid, as desilylating agent yielded the elimination product **8** as the main product (85%).

The final nucleosides **4** and **8** were tested against solid tumor cell lines such as lung cancer (A549) and colon cancer (Col2), but they did not exhibit any cytotoxicity in this system. Biological evaluation against leukemia cells is in progress in our laboratory and it will be reported elsewhere.

In summary, on the basis of the bioisosteric rationale we have synthesized a novel 2′-deoxy-2′-*C*-difluoromethylene-4′-thiocytidine (**4**) along with the unexpected elimination product **8** and evaluated their cytotoxicity against tumor cell lines. These studies not only helped generate novel 2′-modified nucleosides for biological evaluation but also show useful pointers for designing potential anticancer agents.

Scheme 4[a]



deprotection conditions	ratio[a] of **4**/**8**
AcOH, *n*-Bu_4NF THF, rt, 2 d	99/0
NEt_3.3HF CH_2Cl_2, rt, 3 d	99/0
70 % AcOH rt, 6 d	0/85

[a]isolated yield

[a] Reagents: (a) TBSOTf, NEt_3, CH_2Cl_2, 0 °C, 30 min; (b) i. *m*CPBA, CH_2Cl_2, −78 °C; ii. silylated N^4-benzoylcytosine, TMSOTf, $ClCH_2CH_2Cl$, 0−50 °C, 1 h; (c) 30% NaOMe, MeOH, rt, 3 h.

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Supporting Information Available: Complete experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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