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Synthesis of 5-thiodidehydropyranylcytosine derivatives as potential anti-HIV agents

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ABSTRACT

As a part of our ongoing efforts to identify new anti-HIV agents, a 5′-thiopyrano-nucleoside derivative **4**, designed based on 4′-thioD4C **1** and cyclohexenylnucleoside **3**, was synthesized. The dihydrothiopyran skeleton of **4** was constructed by the ring closing metathesis of **21** which was synthesized from but-2-yne-1,4-diol. After converting the protecting group from MOM to TBS followed by oxidation, a Pummerer-type thioglycosylation reaction of **24** with persilylated uracil gave the desired 5-thiodihydrothiopyranyluracils **25** and **26** as a mixture of anomers. The conversion of **25** to a cytosine derivative and subsequent deprotection gave a 5-thiodidehydropyranosylcytosine derivative **4** in good yield. The anti-HIV activity of **4** was also evaluated.

HAART (Highly Active Anti-Retroviral Therapy) is the most successful AIDS treatment to date for controlling HIV replication and has greatly improved the lifespan of AIDS patients.[1] However, the emergence of drug resistant viruses during the course of chemotherapy for AIDS continues to be a serious problem even after the establishment of HAART.[2] The HAART protocol involves the use of a cocktail of anti-HIV drugs including nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs).[1] Since NRTIs play an important role in HAART,[2] the development of an effective NRTI for HIV, which is also effective for a drug-resistant AIDS virus, would be highly desirable. As a result, a number of nucleoside analogues that are effective for treating HIV have been reported to date.[3] Our efforts in this area have involved attempts to prepare new antiviral nucleoside derivatives that have the potential for use in HIV therapy. Our strategy involved the construction of a novel pseudosugar skeleton that could serve as a structurally novel NRTI. Such a compound could then be used in place of currently used NRTIs that are ineffective against viruses that are resistant to the NRTIs now in use.[4] We previously reported on the synthesis of dihydrothiopyrano-nucleoside **2**[4c,e] which was designed based on the known anti-HIV L-4′-thioD4C **1**.[5] Unfortunately, dihydrothiopyrano-nucleoside **2** did not show anti-HIV activity. Cyclohexenylcytosine **3**, however, was found to have weak anti-HIV activity.[4d] Although these results were insufficient to obtain antiviral nucleosides, we hypothesized that the transformation of the carbocyclic derivative **3** into its 5′-thio counterpart might potentiate anti-HIV activity. To confirm this, we envisioned synthesizing a dihydrothiopyranocytosine derivative **4** as a potential HIV-nucleoside. In this Letter, we report on the synthesis of a racemic mixture of 5-thio-2,3-didehydropyranosylcytosine **4** starting

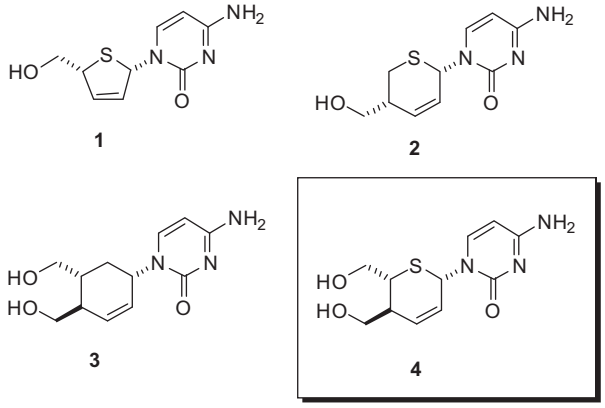

Chart 1.

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from but-2-yne-1,4-diol and a preliminary evaluation of its anti-HIV activity (Chart 1).

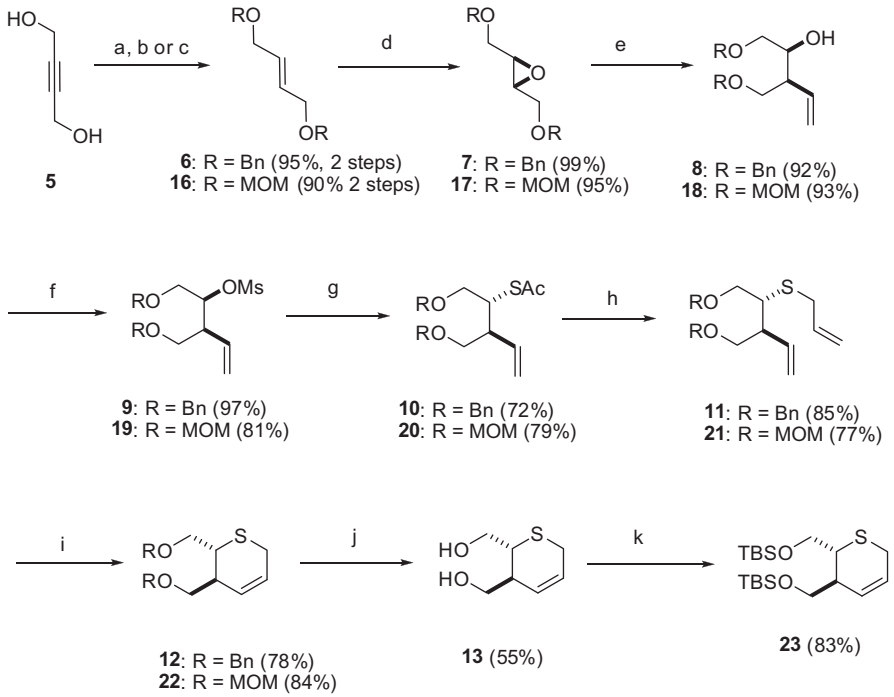
As discussed above, we previously reported on the synthesis of dihydrothiopyrano-nucleoside **2**,[4c,e] which involves two key reactions: (1) the construction of a dihydrothiopyran skeleton by ring-closing metathesis (RCM) and (2) a Pummerer-type thioglycosylation reaction. It should be possible to apply this basic synthetic concept to the synthesis of 5-thio-2,3-didehydropyranosylcytosine **4**. Thus, we started the synthesis from but-2-yne-1,4-diol to construct a dihydrothiopyran skeleton as a pseudosugar. The synthesis of the homoallyl alcohol derivative **8** was accomplished in four steps: (1) the conversion of but-2-yne-1,4-diol **5** to *trans*-but-2-ene-1,4-diol by hydroaluminamtion, (2) benzylation of the hydroxyl groups, (3) epoxidation by *m*CPBA treatment, and (4) cleavage of the resulting oxirane ring by an organocopper reagent.[6] To obtain the intermediate for the RCM reaction, it is necessary to introduce an allyl sulfide unit at the 2-position of **8**. This was achieved as follows. After mesylation of the secondary hydroxyl group of **8**, a nucleophilic substitution reaction by treatment with potassium thioacetate in the presence of crown ether gave the thioacetate **10** in good yield. The acetyl group of **10** was removed by treatment with NaOMe and the resulting thiol derivative was subsequently treated with allyl bromide to give the diene **11** in 85% yield. The diene **11** was then subjected to the RCM reaction. From our previous results, it appeared possible that the use of the Grubbs 1st catalyst might not be efficient in RCM reactions for compounds containing a sulfur atom in their structure.[4c,e] Therefore, the Grubbs 2nd catalyst was used for the RCM reaction of **11**. As expected, when **11** was reacted in the presence of 10 mol % of 2nd Grubbs catalyst in refluxing benzene, the dihydrothiopyran **12** was produced in 78% yield (Scheme 1).

Prior to carrying out the Pummerer-type thioglycosylation reaction,[4c,e,7] the dihydrothiopyran derivative **12** was converted into the corresponding sulfoxide by treatment with $NaIO_4$. The reaction gave sulfoxide **14** in excellent yield as an inseparable 1:1 mixture of diastereomers. The resulting sulfoxide **14**, in hand, was treated with bis(TMS)uracil in the presence of TMSOTf and excess iPr$_2$NEt to give the desired 5′-thionucleoside **15** in 55% yield as an inseparable mixture of α- and β-anomers (Scheme 2).

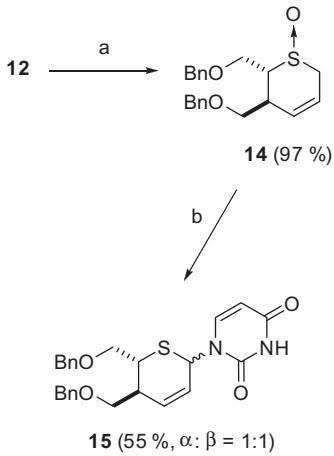
Although attempts were made to remove benzyl groups of **15**, the free nucleosides could not be obtained.[8] Alternatively, deprotection of the dihydrothiopyran derivative **12** was also attempted, which gave the deprotected products, but in poor yields. Failure to obtain free nucleosides or pseudosugar derivatives can be attributed to the instability of **15** and **12** under the reductive or Lewis acid conditions used (data not shown). These results prompted us to synthesize the target compound using other protecting groups.

After serious consideration, we re-examed the entire synthesis starting from the MOM protected derivative **16**. As can be seen in Scheme 1, all reactions starting from **5** to **12** were reproducible using a series of the MOM protected compounds and gave the desired products **16**–**22** in good yields. It was necessary to convert the MOM group of **22** into another protecting group that tolerates the conditions for the Pummerer-type thioglycosylation reaction.[4c,e,7] To accomplish this, we attempted to convert **22** into a TBS-protected derivative. Since the pseudosugar skeleton, as described above, was assumed to be unstable under acidic conditions, we used Fujioka's method[9] to deprotect the MOM group, since conditions close to neutral are used for this method. Compound **22** was reacted with TMSOTf in the presence of 2,2′-bipyridyl and was subsequently treated with H_2O to give the desired diol **13** in 55% yield.[9] Protection of **13** with a TBS group using standard methodology (TBSCl, imidazole) resulted in the predominant formation of the monosilylated product (data not shown). Instead of TBSCl and imidazole, the use of TBSOTf and 2,6-lutidine resulted in an improved yield, giving the bis(TBS) derivative **23** in 83% yield (Scheme 1).

By the method described above, compound **23** was oxidized to the corresponding sulfoxide **24**, which was subjected to the



Scheme 1. Reagents and conditions: (a) $LiAlH_4$, THF, reflux; (b) BnCl, NaH, THF, DMF, rt; or (c) MOMCl, iPr$_2$NEt, CH_2Cl_2, 40 °C; (d) *m*CPBA, CH_2Cl_2, rt; (e) H_2C=CHMgCl, CuI, Et_2O, −40 °C; (f) MsCl, Et_3N, CH_2Cl_2, rt; (g) KSAc, 18-crown-6, CH_3CN, reflux; (h) NaOMe, allyl bromide, CH_3CN, rt; (i) 2nd Grubbs cat., benzene, reflux; (j) TMSOTf, 2,2′-bipyridyl, CH_2Cl_2, 0 °C, then H_2O, Et_2O, rt; (k) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt.



Scheme 2. Reagents and conditions: (a) $NaIO_4$, CH_3CN, rt; (b) bis(TMS)uracil, TMSOTf, iPr$_2$NEt, $PhCH_3/CH_2Cl_2$.

Pummerer-type thioglycosylation reaction.[4c,e,7] The reaction of **24** with bis(TMS)uracil, TMSOTf and iPr$_2$NEt afforded an anomeric mixture of the 5′-thionucleosides **25** and **26** in 63% yields. After careful separation by silica gel column chromatography, the stereochemistry at the 1′-position of the isolated **25** and **26** was determined in NOE experiments as depicted in Figure 1.[10]

The uracil ring of the β-5′-thionucleoside **25** was converted into cytosine by treatment with triisopropylbenzenesulfonyl chloride (TPSCl) and triethylamine in the presence of DMAP followed by treatment with ammonium hydroxide[11] to give **27** which contained inseparable impurities. Finally, the TBS groups of **27** were deprotected by treatment with TBAF to furnish the desired 5-thiodidehydropyranosylcytosine **4**[12] in 72% yield from **25**. The α-anomer of **29**[13] was prepared from **26** following the same sequence of reactions (Scheme 3).

Anti-HIV-1 activities of 5-thiodidehydropyranosylcytosine **4** and its anomer **29** were evaluated by the inhibition of HIV replication in peripheral blood mononuclear cells (PBMC), which was determined by a p24 HIV antigen capture assay.[14] The results are summarized in Figure 2. As expected, the β-5-thiodidehydropyranosylcytosine derivative **4** showed significant anti-HIV-1 activity at concentrations of 10 and 100 nM. It is noteworthy that the inhibitory activity of **4** at a concentration of 10 nM suppressed HIV replication to a level of 65% of the control while Lamivudine suppressed it to 37% at the same concentration. Unexpectedly, the suppression of HIV replication for **4** at a concentration of 100 nM was reduced to the level of 75% of the control. Although the reason for this is not clear at this time, the assay was performed using a racemic mixture of the 5′-thiopyrano-nucleoside and the inactive enantiomer of **4** might have had an influence on the anti-HIV activity of the mixture.[15] The α-anomer **29**, on the other hand, showed only weak activity compared with **4**. This result is consistent with the fact that the nucleosides having antiviral and antitumor activities generally possess β-configurations, analogous to naturally occurring nucleosides.

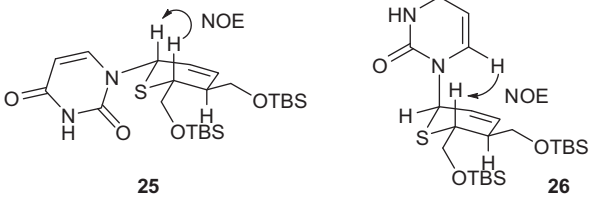
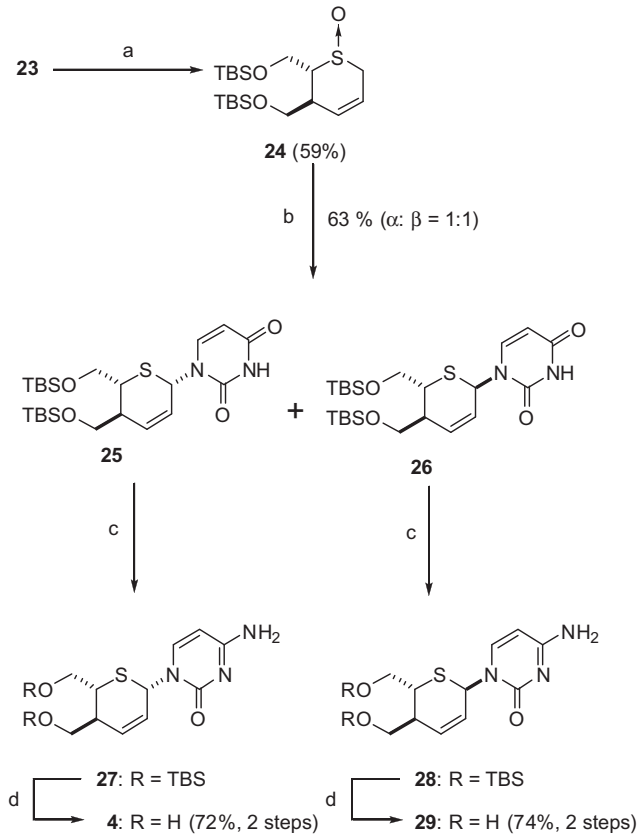


Figure 1. NOE experiments of **25** and **26**.



Scheme 3. Reagents and conditions: (a) $NaIO_4$, CH_3CN, rt (recovery of **23**: 20%); (b) bis(TMS)uracil, TMSOTf, iPr$_2$NEt, $PhCH_3/CH_2Cl_2$; (c) TPSCl, DMAP Et_3N, CH_3CN, rt, then NH_4OH; (d) TBAF, THF.

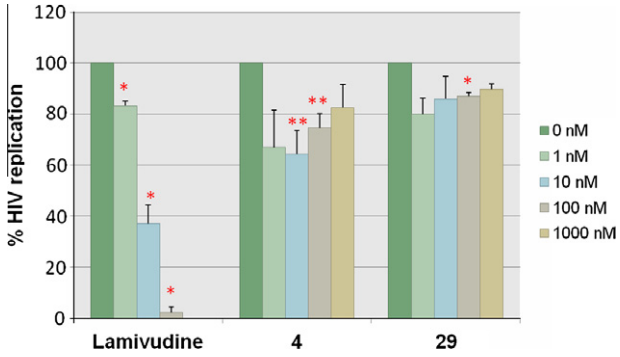


Figure 2. Summary of anti-HIV-1 activities of **4** and **29**. Anti-HIV-1 activity was evaluated by the inhibition of HIV replication in peripheral blood mononuclear cells (PBMC) from healthy volunteers, which was determined by a p24 HIV antigen capture assay (Perkin–Elmer)[14] using Lamivudine as a positive control ($^{*}p$ <0.01, $^{**}p$ <0.05). Statistical analyses were performed using the Student's t-test.

In conclusion, we report on the design and synthesis of the novel 5′-thiodidehydropyrano-nucleosides **4** and **29**. The findings indicate that **4** has significant anti-HIV activity. To obtain more potent analogues for inhibiting HIV replication, investigations on structure–activity relationships including the enantioselective synthesis of both enantiomers of **4** will be needed. These studies are currently underway and the results will be reported in future reports.

Acknowledgments

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12. Data for **4**: ^{1}H NMR (400 MHz, CD_3OD) δ 2.69–2.73 (m, 1H), 2.98–3.04 (m, 1H), 3.45–3.59 (m, 4H), 5.78 (ddd, J = 1.4, 3.4, 11.1 Hz, 1H), 5.81 (d, J = 7.7 Hz, 1H), 5.89–5.90 (m, 1H), 6.14 (ddd, J = 2.4, 5.8, 11.1 Hz, 1H), 7.52 (d, J = 7.7 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 38.8, 41.3, 52.1, 63.6, 66.2, 96.6, 124.8, 134.9, 143.5, 158.3, 167.5. EI-MS m/z 269 (M^+). HRMS Calcd for $C_{11}H_{15}N_3O_3S$: 269.0834, Found 269.0829.
13. Data for **29**: ^{1}H NMR (400 MHz, CD_3OD) δ 2.25–2.42 (m, 1H), 3.00 (dd, J = 5.8, 13.0 Hz, 1H), 3.65–3.75 (m, 4H), 5.75 (ddd, J = 1.9, 4.3, 10.6 Hz, 1H), 5.79 (d, J = 7.7 Hz, 1H), 6.09–6.14 (m, 2H), 7.61 (d, J = 7.7 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 40.5, 41.1, 52.9, 63.8, 63.9, 96.5, 125.3, 137.6, 143.8, 158.2, 167.5. EI-MS: m/z 269 (M^+). HRMS Calcd for $C_{11}H_{15}N_3O_3S$: 269.0834, Found 269.0830.
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15. To clarify an active enantiomer of **4** and influence to anti-HIV activity by the inactive enantiomer, the study on the enantioselective synthesis of **4** is currently under way.