Nucleosides and Nucleotides. 186. Synthesis and Biological Activities of Pyrimidine Carbocyclic Nucleosides with a Hydroxamino Group Instead of a Hydroxymethyl Group at the 4'-Position of the Sugar Moiety

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Pyrimidine carbocyclic nucleosides with a hydroxamino group instead of a hydroxymethyl group at the 4'-position of the sugar moiety were designed as potential antitumor and/or antiviral agents. Pd (O) catalyzed reactions of enantiomERICally pure (+)-(1R,4S)-4-{[[tert-butyldiphenylsilyl]oxy]-1-(ethoxycarbonyloxy)2-cyclopentene (9) with N3-benzoylthymine and -uracil gave carbocyclic nucleosides 10 and 11. Subsequent Pd (O) catalyzed reactions of N3-benzoyl-1-{[1R,4S]-4-{(ethoxycarbonyloxy)-2-cyclopenten-1-yl}thymine (14) and -uracil (15) with O-benzylhydroxylamine smoothly gave the hydroxamino-substituted carbocyclic nucleosides 16 and 17. From these nucleosides, the target compounds were prepared after deprotection or further reactions. The 2',3'-dideoxy-2',3'-dideoxythymidine (DT4) analogue 20 was the most effective compound, with IC_{50} values of 27.3 and 34.5 \mu M against KB and L1210 cells in vitro. Carboxylic analogues of uridine and cytidine (29 and 32) were less effective than 20 against both cell lines.

Key words: carbocyclic nucleoside; hydroxylamine; antitumor activity; antiviral activity

Hydroxylamine derivatives have interesting chemical properties. They can be readily reduced to amines and readily oxidized to nitrones. Additionally, the oxidation of hydroxylamine by ceric sulfate and OH-radicals produces NH_{2}O radicals. Therefore, if such a substitutant can be introduced into the sugar moiety of a nucleoside, the result may be a unique nucleoside with a variety of biological activities. Recently, we synthesized several nucleosides with a hydroxamino group instead of a hydroxymethyl group of the 2' or 3' position at the sugar moiety. Among them, 2'-deoxy-2',3'-hydroxyminothymidine (2'-DHAC) and 3'-deoxy-2',3'-hydroxyminothymidine (3'-DHAC) (Fig. 1) showed cytotoxicity against several tumor cell lines in vitro, and antileukemic activity against the mouse P388 model in vivo.\textsuperscript{2,5} We also detected 2'-NHO radicals of 2'-DHAC in neutral aqueous solution at room temperature by ESR.\textsuperscript{4}

A nucleoside with a hydroxamino group instead of a hydroxymethyl group at the 4'-position of the sugar moiety, such as 1, (Fig. 1) would be expected to be a substrate of certain nucleoside kinases, since the hydroxymino group may mimic to the hydroxymethyl group. However, substitution of the hydroxymethyl group of a d-ribose or 2-deoxy-d-ribose moiety by a hydroxamino group would be difficult because that such nucleosides are not sufficiently stable. Therefore, we designed and synthesized carbocyclic nucleoside analogues with a hydroxymino group at the carbocyclic moiety, such as 2—4 depicted in Fig. 1, and evaluated their cytotoxicity against tumor cells in vitro and their antiviral activities against human immunodeficiency virus type-1 (HIV-1) in vitro.

Chemistry

The synthetic route to the target compounds is outlined in Chart 1. The target compounds were straightforwardly synthesized using Pd-chemistry. EnantiomERICally pure (+)-(1R,4S)-1-acetoxy-4-hydroxy-2-cyclopentene (6) was obtained by hydrolysis of the corresponding racemic diacetate 5 with porcine liver esterase in 99% ee.\textsuperscript{6} Compound 5 was protected with a tert-butyldiphenylsilyl (TBDPS) group, followed by deacylation to give 8, which was converted into an ethoxycarbonyl derivative 9. Compound 9 was reacted with N3-benzoylthymine in the presence of Pd_{3}(dba)_{3}, CHCl_{3} and PPh_{3} to smoothly give the desired thymine-carbocycle 10 in 94% yield. The configuration at the 1'-position was confirmed by nuclear Overhauser effect (NOE) experiments. When the 5'α-proton in 10 was irradiated, NOE enhancements of 18% and 16% were observed at the 1' and 4'-protons, respectively. Therefore, N3-benzoylthymine was introduced via a double inversion, and 10 has the desired configuration at the 1'-position. To introduce nucleobases into similar carbocycles using Pd-chemistry, previous methods have used an acetox or benzoyloxy group as a leaving group.\textsuperscript{8,9} In our experiments, the ethoxycarbonyloxy group

\begin{figure}[h]
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\caption{Fig. 1}
\end{figure}

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was found to be a good choice as the leaving group, as expected.

Deprotection of the TBDPS group by tetrabutylammonium fluoride (TBAF), followed by ethoxycarbonylation of the resulting hydroxyl group, gave a substrate 14 for the next Pd-catalyzed reaction to introduce a hydroxylamino group at the 4'-position. Compound 14 was treated with O-benzylhydroxylamine under conditions similar to those described above to give 16 in 84% yield. Removal of the benzyl group at the N3-position using NaOMe and subsequently the O-benzyl group by BCl3 gave 20 in 80% yield in two steps. Compound 20 provides a 4'-hydroxyaminated-carbocyclic equivalent of the anti-HIV agent 2',3'-dideoxy-2',3'-dideoxynucleoside (D4T). After protection of the hydroxyl group in 20 with a TBDPS group, the resulting 22 was hydrogenated in the presence of Pd/C in AcOEt to give cyclopentyl derivative 24 in 89% yield. When the same reaction was performed in MeOH, the N-O bond cleavage was also detected along with 24. The TBDPS group in 24 was removed by treatment with HCl in a mixture of dioxane and MeOH to give 26 as a hydrochloride.

Uracil derivatives 21 and 27 were synthesized in a manner similar to that described for the synthesis of thymine analogues, as shown in Chart 1.

Compound 23 was cis-dihydroxylated using OsO4 in the presence of 4-methylmorpholine N-oxide (NMO) to give 28 in 67% yield. The stereochemistry of 28 was determined using NOE experiments. Upon irradiation of the 1'-proton of 28, 1.2% enhancement was observed at the 4'-proton, and 1.9% enhancement at the 2'-proton was detected upon irradiation of the 6-proton at the uracil moiety. Therefore, cis-dihydroxylation selectively occurred at the α-face of the carbocyclic ring. Deprotection of the TBDPS group in 28 with TBAF gave carbocyclic uridine derivative 29.

Protection of the cis-hydroxyl group in 28 with a tert-butylidemethylsilyl (TBS) group gave 30, which was further converted into a cytosine derivative 31 in a usual manner. Finally, 31 was deprotected with HCl in a mixture of dioxane and MeOH to give carbocyclic cytidine analogue 32 as a dihydrochloride.

**Biological Activity**

The cytotoxicities of 4'-hydroxyaminoo-substituted carbocyclic nucleosides 20, 21, 26, 27, 29 and 32 were investigated in vitro using mouse L1210 leukemia and human KB pharyngeal carcinoma cells.10 The results are summarized in Table 1. Among the nucleosides, D4T analogue 20 was the most effective, with IC50 values of 27.3 and 34.5 µM against KB and L1210 cells in vitro, while uracil analogue 21 was only effective against L1210 cells, with an IC50 value of 88 µM. Cyclopentyl analogues 26 and 27 were devoid of any activities against both cell lines. Carbocyclic ribo-nucleoside analogues 29 and 32 were less cytotoxic than 20, and cytosine analogue 32 was slightly more active than uracil analogue 29.

The antiviral activities of the nucleosides against HIV-1
Table 1. Cytotoxic Effects against L1210 and KB Cells and Anti-HIV-1 Activity<sup>a</sup> of Various 4'-Hydroxylamine-Substituted Uracil and Cytosine Carbonyl Nucleosides

<table>
<thead>
<tr>
<th>Compds</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>CC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
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<tbody>
<tr>
<td></td>
<td>L1210</td>
<td>KB</td>
<td>HIV</td>
</tr>
<tr>
<td>20</td>
<td>34.5</td>
<td>27.3</td>
<td>&gt;0.34</td>
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<tr>
<td>21</td>
<td>88</td>
<td>&gt;300</td>
<td>&gt;0.35</td>
</tr>
<tr>
<td>26</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>&gt;0.26</td>
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<tr>
<td>29</td>
<td>&gt;300</td>
<td>20</td>
<td>&gt;0.35</td>
</tr>
<tr>
<td>32</td>
<td>&gt;300</td>
<td>57</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AZT</td>
<td>71</td>
<td>33</td>
<td>ND</td>
</tr>
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</table>

<sup>a</sup> Tumor cell growth inhibitory activity assay in vitro was done following the method.<sup>11</sup> Each tumor cell <2x10<sup>6</sup> cells/well was incubated in the presence or absence of compounds for 72 h. (3,4,5-dimethyl-2-yl)-5,2,3-dimethylbenzene (MTT)-reagent was added to each well and plate was incubated for 4 h more, the resulting MTT-formazan was dissolved in DMSO and the OD (540 nm) was measured. Percent inhibition was calculated by the following equation: ([1-OD (540 nm) of sample/well/OD (540 nm) of control well] x 100). IC<sub>50</sub>, µg/ml was given as the concentration at 50% inhibition of cell growth. To evaluate anti-HIV activity, HIV-1 11b strain vs. MT-4 cells were used, respectively. Briefly, cells were infected with viruses at a multiplicity of infection (m.o.i.) of 0.02. Immediately after the virus infection, a cell suspension (100 µl) was placed into each well containing various concentrations of the compounds (100 µM). After 4 days of incubation at 36°C, the number of viable cells was determined by the MTT method.<sup>11</sup> c) Not determined.

were also examined in vitro (Table 1). However, 20, 21, and 26 were too cytotoxic to the host human T-leukemic MT-4 cells to measure their anti-HIV activities. Compound 27 showed no activity against HIV at up to 300 µM.

Although we do not have any direct evidence that these carbocyclic nucleosides became substrates of certain nucleoside kinases, based on the biological data shown in Table 1, there appears to be some nucleobase specificity for the cytotoxicity; thymine derivative 20 is more active than uracil derivative 21, and cytosine derivative 22 is more active than uracil derivative 29. These results could reflect the substrate specificities of thymidine kinase and uridine/ cytidine kinase. Moreover, 20, 21, and 26 were more active against human leukemic MT-4 cells than against human KB cells, which are derived from solid tumors. These results could reflect that kinase activity is usually higher in T-leukemic cells than in cells derived from solid tumors. Therefore, it is likely that the hydroxymino group may be a biosiose of the hydroxymethyl group in nucleosides, and accepts a phosphorus group by certain nucleoside kinases.

Experimental

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus (Yanagimoto, Japan) and are uncorrected. Fast atom bombardment mass spectrometry (FAB-MS) was done on a JEOL JMS-HX110 instrument at an ionizing voltage of 70 eV. The H-NMR spectra were recorded on a JEOL JNM-GX 270 (270 MHz) or Bruker ARX 500 (500 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet), or br (broad). All exchangeable protons were detected by disappearance in the addition of D<sub>2</sub>O. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. IR spectra were recorded with a JEOL A-102 spectrometer. TLC was done on Merck Kieselgel F<sub>254</sub> precoated plates (Merck, Germany). The silica gel used for column chromatography was YMC gel 60A (70–230 mesh) (YMC Co., Ltd., Japan).

(+)-(1R,4S)-1-Acetoxy-4-hydroxy-2-cyclopentene (6) Porcine liver esterase (45 g, purchased from Sigma) was added to a stirred solution of 5 (43.0 g, 233 mmol) in phosphate buffer (0.1 M, 200 ml, pH 7.0) at 37°C. The mixture was stirred at 37°C for 2 days and quenched by addition of Et<sub>2</sub>O (200 ml). The mixture was filtered through a Celite pad, which was washed with AcOEt. The combined filtrate and washings were concentrated in vacuo, and the residue was extracted with CHCl<sub>3</sub> (500 ml x 5). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt=10:1) to give 6 (11.2 g, 34% as a yellow oil); [α]<sub>23</sub><sup>19</sup> = +68° (c=1.02, CHCl<sub>3</sub>); [λ]<sup>19</sup> = +68° (c=1.02, CHCl<sub>3</sub>, 99% ee).

(1R,4S)-4-[(tert-Butyldiphenylsilyloxy)-1-(ethoxycarbonyloxy)-2-cyclopentene] (9) A mixture of 6 (5.60 g, 39.4 mmol), TBDPSCI (15.2 ml, 58.5 mmol), and imidazole (5.31 g, 78.0 mmol) in dimethylformamide (DMF, 100 ml) was stirred 24 h at 0°C and diluted with Et<sub>2</sub>O (500 ml). The mixture was washed with H<sub>2</sub>O (200 ml x 2) and HCl (1 M, 200 ml) x 2, then with H<sub>2</sub>O (200 ml x 2). The mixture was filtered through a Celite pad, which was washed with 5% HCl (200 ml). The mixture was filtered through a Celite pad, which was washed with 5% HCl (200 ml). The mixture was filtered through a Celite pad, which was washed with 5% HCl (200 ml). The mixture was filtered through a Celite pad, which was washed with 5% HCl (200 ml).
in THF (30 ml) was stirred at room temperature for 4 h. Work-up and purification were performed as described above to give 13 (1.09 g, 91% as a pale yellow foam). H-NMR (400 MHz, CDCl₃): 8.76 (1H, brs, 3-NH), 7.37—7.24 (6H, m, H-6, Ph), 6.13 (1H, dd, H-3'), J=2.0, 2.0, 5.6Hz), 5.78 (1H, dd, H-2', J=1.8, 1.8, 5.6 Hz), 5.69 (1H, brs, 4'-NH), 5.66 (1H, m, H-1'), 4.71 (2H, s, benzyl), 4.16 (1H, m, H-4'), 2.73 (1H, dd, H-5'a, J=8.5, 8.5, 14.7Hz), 1.74 (3H, d, 5-Me, J=1.2 Hz), 1.63 (1H, dd, H-5'b, J=4.6, 4.6, 14.5Hz). FAB-MS m/z: 314 (M+H⁺), 100%. FAB-HR-MS m/z: 314.1509 (Calcd for C₂₃H₂₅NO₂ (M+H⁺): 314.1504).

1-(14R,5S)-4-(N-Hydroxycamo-2-2-cyclopenten-1-yl)thymine (20) A mixture of 18 (1.2 g, 3.8 mmol) and BC13 (1.8 ml in CHCl₃), 20 ml) was stirred for 2 days at room temperature, and the reaction was quenched by addition of MeOH, and the solvent was removed in vacuo. The residue was coevaopulated several times with MeOH, and purified by silica gel column chromatography (CHCl₃/ MeOH=1:10) to give 20 (777 mg, 91% as a white foam). H-NMR (270 MHz, MeOH-d₄): 7.35 (1H, d, H-6, J=1.0Hz), 6.27 (1H, dd, H-3', J=2.0, 2.0, 5.6Hz), 6.20 (1H, ddd, H-2', J=2.0, 2.0, 5.6Hz), 5.54 (1H, m, H-1'), 4.57 (1H, m, H-4'), 2.74 (1H, ddd, H-5'a, J=8.5, 8.5, 14.7Hz), 1.74 (3H, d, 5-Me, J=1.2 Hz), 1.63 (1H, dd, H-5'b, J=4.6, 4.6, 14.5Hz). FAB-MS m/z: 314 (M+H⁺), 100%. FAB-HR-MS m/z: 314.1509 (Calcd for C₂₃H₂₅NO₂(M+H⁺): 314.1504).

N-Benyloxy-1-(14R,5S)-4-(N-Benzoylamino-2-2-cyclopenten-1-yl)uracil (19) A mixture of 17 (1.6 g, 3.97 mmol) in MeOH (30 ml) containing NaOMe (28%, 1 ml) was stirred for 24 h at room temperature, and neutralized by addition of aqueous HCl (1s), and the solvent was removed in vacuo. The residue was coevaopulated several times with MeOH, and purified by silica gel column chromatography (CHCl₃/MeOH=1:10) to give 19 (1.1 g, 1.17 mmol, 93% as a pale yellow foam). H-NMR (500 MHz, CDCl₃): 8.95 (1H, brs, 3-NH), 7.82—7.31 (6H, m, H-6, Ph), 6.14 (1H, m, H-3'), 5.77 (1H, m, H-2'), 5.69 (1H, brs, 4'-NH), 5.65 (1H, m, H-1'), 5.40 (1H, d, H-5, J=8.0Hz), 4.70, 4.67 (each 1H, each d, benzyl), each J=11.3Hz), 4.16 (1H, m, H-4'), 2.71 (1H, ddd, H-5'a, J=8.6, 8.7, 14.9Hz), 1.64 (1H, dd, H-5'b, J=4.1, 4.1, 14.8Hz). FAB-MS m/z: 300 (M+H⁺), 100%. FAB-HR-MS m/z: 300.1344 (Calcd for C₂₃H₂₅NO₂(M+H⁺): 300.1347).

1-(14R,5S)-4-(N-Hydroxycamo-2-2-cyclopenten-1-yl)uracil (21) A mixture of 19 (50 mg, 0.17 mmol) in MeOH (30 ml) containing AcOEt (28%, 1 ml) was stirred for 24 h at room temperature, and neutralized by addition of aqueous HCl (1s), and the solvent was removed in vacuo. The residue was coevaopulated several times with MeOH, and purified by silica gel column chromatography (CHCl₃/MeOH=1:10) to give 21 (19.2 mg, 52% as a white foam). H-NMR (270 MHz, CDCl₃): 8.25 (1H, brs, 3-NH), 7.68—7.31 (10H, m, Ph), 6.99 (1H, s, H-6), 6.02 (1H, d, H-3', J=5.6Hz), 5.67 (1H, d, H-1', J=5.6Hz), 5.54 (1H, m, H-1'), 5.22 (1H, brs, 4'-NH), 4.10 (1H, m, H-4'), 2.60 (1H, ddd, H-5'a, J=8.6, 8.6, 14.2Hz), 1.66 (3H, s, 5-Me), 1.52 (1H, ddd, H-5'b, J=5.6, 5.6, 13.9Hz), 1.08 (9H, s, t-Bu). FAB-MS m/z: 462 (M+H⁺), 100%. FAB-HR-MS m/z: 462.2190 (Calcd for C₂₃H₂₅NO₂Si(M+H⁺): 462.2191).

1-(14R,5S)-4-(N-Benzoylamino-2-2-cyclopenten-1-yl)uracil (22) A mixture of 20 (48.8 mg, 2.19 mmol) TBPDSI (779, 3.00 mmol), and imidazole (272.4 mg, 4.01 mmol) in DMF (10 ml) was stirred for 2 days at room temperature and diluted with AcOEt (15 ml), which was washed with H₂O (10 ml)x3 and brine (0.1 ml), dried (Na₂SO₄), and concentrated in vacuo. The residue was purified by silica gel column chromatography (CHCl₃/MeOH=1:10) to give 22 (1.2 g, 88% as a white solid). H-NMR (400 MHz, CDCl₃): 8.25 (1H, brs, 3-NH), 7.17—7.17 (10H, m, Ph), 6.99 (1H, s, H-6), 6.02 (1H, d, H-3', J=5.6Hz), 5.67 (1H, d, H-1', J=5.6Hz), 5.54 (1H, m, H-1'), 5.22 (1H, brs, 4'-NH), 4.10 (1H, m, H-4'), 2.60 (1H, ddd, H-5'a, J=8.6, 8.6, 14.2Hz), 1.66 (3H, s, 5-Me), 1.52 (1H, ddd, H-5'b, J=5.6, 5.6, 13.9Hz), 1.08 (9H, s, t-Bu). FAB-MS m/z: 462 (M+H⁺), 100%. FAB-HR-MS m/z: 462.2190 (Calcd for C₂₃H₂₅NO₂Si(M+H⁺): 462.2191).
1-[1R,2S,3R,4S]-4-[N-(tert-Butylidihydroxyisoxazolyl)hydrocyclopen-1-yl]thymine (24) A mixture of 22 (138 mg, 0.299 mmol) and 10% Pd/C (20 mg) in AcOEt (3 ml) was stirred for 24 h under H₂ atmosphere at room temperature and filtered through a Celite pad, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt=1:1) to give 25 (117 mg, 89% as a white foam).

1-[1R,2S,3R,4S]-4-[N-(tert-Butylidihydroxyisoxazolyl)hydrocyclopen-1-yl]thymine (24) A mixture of 22 (138 mg, 0.299 mmol) and 10% Pd/C (20 mg) in AcOEt (3 ml) was stirred for 24 h under H₂ atmosphere at room temperature and filtered through a Celite pad, and the filtrate was concentrated in vacuo.

1-[1R,2S,3R,4S]-4-[N-(tert-Butylidihydroxyisoxazolyl)hydrocyclopen-1-yl]uracil (25) A mixture of 23 (89 mg, 0.20 mmol) and 10% Pd/C (10 mg) in AcOEt (2 ml) was stirred for 20 h under H₂ atmosphere at room temperature and filtrated through a Celite pad, and the filtrate was concentrated in vacuo.

1-[1R,2S,3R,4S]-4-[N-(tert-Butylidihydroxyisoxazolyl)hydrocyclopen-1-yl]thymine (24) A mixture of 22 (138 mg, 0.299 mmol) and 10% Pd/C (20 mg) in AcOEt (3 ml) was stirred for 24 h under H₂ atmosphere at room temperature, and the solvent was removed in vacuo. The residue was dissolved in H₂O (15 ml), which was washed with CHCl₃ (10 ml×3). The aqueous layer was concentrated in vacuo to give 26 (48 mg, 94% as a white foam).

1-[1R,2S,3R,4S]-4-[N-(tert-Butylidihydroxyisoxazolyl)hydrocyclopen-1-yl]uracil (25) A mixture of 23 (61 mg, 0.14 mmol) in MeOH (1 ml) and HCl (4 ml in 1,4-dioxane, 1.5 ml) was stirred for 24 h at room temperature, and the solvent was removed in vacuo. The residue was dissolved in H₂O (15 ml), which was washed with CHCl₃ (10 ml×3). The aqueous layer was concentrated in vacuo to give 27 (33 mg, 98% as a white foam).

1-[1R,2S,3R,4S]-4-[N-(tert-Butylidihydroxyisoxazolyl)hydrocyclopen-1-yl]uracil (25) A mixture of 23 (400 mg, 0.89 mmol), NMO (210 mg, 1.79 mmol) and OsO₄ (3 mg in tert-ButOH containing 1% tert-ButOOH, 6 ml, 0.12 mmol) in THF (2 ml) and H₂O (2 ml) was stirred for 2 h at room temperature and further amounts of NMO (50 mg, 0.852 mmol), and OsO₄ (3 ml, 0.06 mmol) were added to the mixture at 0°C. After the mixture was stirred for further 2 h at room temperature, the reaction was quenched by addition of saturated aqueous Na₂SO₄, and the mixture was extracted with AcOEt (30 ml). The organic layer was washed with H₂O (30 ml), saturated aqueous NaHCO₃ (20 ml), saturated aqueous Na₂SO₃ (30 ml), and brine (30 ml), dried (Na₂SO₄), and concentrated in vacuo. The residue was purified by silica gel column chromatography (CHCl₃, MeOH=50:1) to give 30 (110 mg, 95% as a white foam).

1-[1R,2S,3R,4S]-2,3-Dihydroxy-4-[N-(tert-Butylidihydroxyisoxazolyl)hydrocyclopen-1-yl]uracil (26) A mixture of 22 (400 mg, 0.89 mmol), NMO (210 mg, 1.79 mmol) and OsO₄ (3 mg in tert-ButOH containing 1% tert-ButOOH, 6 ml, 0.12 mmol) in THF (2 ml) and H₂O (2 ml) was stirred for 2 h at room temperature and further amounts of NMO (50 mg, 0.852 mmol), and OsO₄ (3 ml, 0.06 mmol) were added to the mixture at 0°C. After the mixture was stirred for further 2 h at room temperature, the reaction was quenched by addition of saturated aqueous Na₂SO₄, and the mixture was extracted with AcOEt (30 ml). The organic layer was washed with H₂O (30 ml), saturated aqueous NaHCO₃ (20 ml), saturated aqueous Na₂SO₃ (30 ml), and brine (30 ml), dried (Na₂SO₄), and concentrated in vacuo. The residue was purified by silica gel column chromatography (CHCl₃, MeOH=50:1) to give 30 (110 mg, 95% as a white foam).

1-[1R,2S,3R,4S]-2,3-Dihydroxy-4-[N-(tert-Butylidihydroxyisoxazolyl)hydrocyclopen-1-yl]uracil Hydrochloride (28) A solution of 29 (29 mg, 0.066 mmol) in MeOH (0.5 ml) and HCl (4 ml in 1,4-dioxane, 0.5 ml) was stirred for 24 h at room temperature, and the solvent was removed in vacuo. The residue was evaporated several times with dioxane to give 29 (16 mg, 95% as a white powder).

1-[1R,2S,3R,4S]-2,3-Dihydroxy-4-[N-(tert-Butylidihydroxyisoxazolyl)hydrocyclopen-1-yl]uracil (26) A mixture of 22 (400 mg, 0.89 mmol), NMO (210 mg, 1.79 mmol) and OsO₄ (3 mg in tert-ButOH containing 1% tert-ButOOH, 6 ml, 0.12 mmol) in THF (2 ml) and H₂O (2 ml) was stirred for 2 h at room temperature and further amounts of NMO (50 mg, 0.852 mmol), and OsO₄ (3 ml, 0.06 mmol) were added to the mixture at 0°C. After the mixture was stirred for further 2 h at room temperature, the reaction was quenched by addition of saturated aqueous Na₂SO₄, and the mixture was extracted with AcOEt (30 ml). The organic layer was washed with H₂O (30 ml), saturated aqueous NaHCO₃ (20 ml), saturated aqueous Na₂SO₃ (30 ml), and brine (30 ml), dried (Na₂SO₄), and concentrated in vacuo. The residue was purified by silica gel column chromatography (CHCl₃, MeOH=50:1) to give 30 (110 mg, 95% as a white foam).

1-[1R,2S,3R,4S]-2,3-Dihydroxy-4-[N-(tert-Butylidihydroxyisoxazolyl)hydrocyclopen-1-yl]thymine (24) A mixture of 22 (138 mg, 0.299 mmol) and 10% Pd/C (20 mg) in AcOEt (3 ml) was stirred for 24 h under H₂ atmosphere at room temperature, and the solvent was removed in vacuo. The residue was evaporated several times with dioxane to give 29 (16 mg, 95% as a white powder).

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References and Notes


