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

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Pyrimidine carbocyclic nucleosides with a hydroxyamino 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-butylidiphenyloxy)-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 tert-benzylhydroxylamine smoothly gave the hydroxyamino-substituted carbocyclic nucleosides 16 and 17. From these nucleosides, the target compounds were prepared after deprotection or further reactions. The 2’,3’-didehydro-2’,3’-dideoxythymidine (D4T) analogue 20 was the most effective compound, with IC50 values of 27.3 and 34.5 μM against KB and L1210 cells in vitro. Carbocyclic 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 sulfate2) and OH-radicals3) produces NH2O· radicals. Therefore, if such a substituent 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 hydroxyamino group instead of a hydroxymethyl group at the 4’-position of the sugar moiety. Among them, 2’-deoxy-2’-hydroxymethylaminocytidine (2’-DHAC) and 3’-deoxy-3’-hydroxymethylaminocytidine (3’-DHAC) (Fig. 1) showed cytotoxicity against several tumor cell lines in vitro, and antileukemic activity against the mouse P388 model in vivo.4,5) We also detected 2’-NHO· radicals of 2’-DHAC in neutral aqueous solution at room temperature by ESR.5)

A nucleoside with a hydroxyamino 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 hydroxymethyl 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 hydroxymino group would be difficult because such nucleosides are not sufficiently stable. Therefore, we designed and synthesized carbocyclic nucleoside analogues with a hydroxyamino 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.6) Compound 5 was protected with a tert-butyldiphenyloxy (TBDDS) group, followed by deacetylation to give 8, which was converted into an ethoxycarbonyl derivative 9. Compound 9 was reacted with N3-benzoylthymine7) in the presence of Pd2(dba)3·C6H6, CHCl3 and Ph3P 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 acetoxyl or benzoxylxy group as a leaving group.5,9) In our experiments, the ethoxycarbonylxy group

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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 benzoyl 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-didehydro-2',3'-dideoxythymidine (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-butyldimethylsilyl (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'-hydroxyamino-substituted carbocyclic nucleosides 20, 21, 26, 27, 29 and 32 were investigated in vitro using mouse L1210 leukemia and human KB pharyngeal carcinoma cells. 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...
were detected by disappearance on the addition of D2O. UV absorption spec-
trum was determined by the MTT method.11) Not determined.

**Table 1. Cytotoxic Effects against L1210 and KB Cells**

<table>
<thead>
<tr>
<th>Compds</th>
<th>IC50 (μM)</th>
<th>EC50 (μM)</th>
<th>CC50 (μM)</th>
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<tbody>
<tr>
<td>L1210</td>
<td>KB</td>
<td>HIV</td>
<td>MT-4</td>
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<tr>
<td>20</td>
<td>34.5</td>
<td>27.3</td>
<td>&gt;0.34</td>
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<tr>
<td>26</td>
<td>&gt;80</td>
<td>&gt;300</td>
<td>&gt;0.35</td>
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<tr>
<td>27</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>&gt;0.26</td>
</tr>
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<td>29</td>
<td>&gt;300</td>
<td>57</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>32</td>
<td>71</td>
<td>33</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AZT</td>
<td>ND</td>
<td>ND</td>
<td>&lt;0.0011</td>
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</tbody>
</table>

<sup>a</sup> Tumor cell growth inhibitory activity assay in vitro was done following the method.10) Each tumor cell (2 x 10<sup>5</sup> cells/well) was incubated in the presence or absence of compounds for 72 h. 3-(4,5-dimethylthiazol-2-yl)-2,5-dihydrotetrazolium bromide (MTT)-reagent was added to each well and plate and incubated for 4 h more, the resulting MTT-formazan was dissolved in DMSO and the OD (540 nm) was measured. Percent inhibition was calculated as follows: % inhibition = [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. b) To evaluate anti-HIV-1 activity, HIV-1 Hbl strain vs. MT-4 cells were used, respectively. Briefly, cells were infected with viruses at a mul-
tiplicity of infection (m.o.i.) of 0.02. Immediately after the virus infection, a cell sus-
pension (100 μl) was placed into each well containing various concentrations of the compounds (100 μl). After 4 days of incubation at 36 °C, the number of viable cells was determined by the MTT method.<sup>1)</sup> c) Not determined.

were also examined in vitro (Table 1).<sup>11</sup>) 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 nucleo-
sidases, based on the biological data shown in Table 1, there appears to be some nucleobase specificity for the cyto-
xicity; thymine derivative 20 is more active than uracil derivative 21, and cytosine derivative 32 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. Therefore, it is likely that the hydroxy group may be a biososete of the hydroxymethyl group in nucleosides, and accepts a phosphate 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 1H-NMR spectra were recorded on a JEOL JNM-GX 270 (270 MHz) or Bruker ARX 500 (500 MHz) spectrome-
ter with tetramethylsilane as an internal standard. Chemical shifts are re-
ported 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 on the addition of D2O. UV absorption spec-
tra were recorded with a Shimadzu UV-240 spectrophotometer. IR spectra were recorded with a JEOI 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).

(+)-1(4R,5S)-1-Acetoxy-4-hydroxy-2-cyclopentenone (6) Porcine liver es-
terase (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 EtOH

(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 puri-
ified by silica gel column chromatography (hexane: AcOEt = 2:1) to give 6 (11.2 g, 34% as a yellow oil): δ<sup>1H</sup> 6.88 (c = 1.02, CH<sub>3</sub>); δ<sup>13C</sup> 68.08 (c = 1.02, CH<sub>3</sub>), 99% ee.)

1H-NMR (500 MHz, CDCl<sub>3</sub>): δ<sup>1H</sup> 7.69—7.34 (10H, m, Ph), 5.91 (1H, d, δ = 2, 7.9 Hz), 5.87 (1H, d, δ = 3, 5.6 Hz), 5.29 (1H, d, δ = 3, 6.3 Hz), 4.67 (1H, d, δ = 4, 5.9 Hz), 2.04 (2H, s, OCH<sub>2</sub>CH<sub>3</sub>).

FAB-MS: m/z: 411 (M+H<sup>+</sup>), 11%, FAB-HR-MS m/z: 411.1978 (Calcd for C<sub>25</sub>H<sub>20</sub>N<sub>4</sub>O<sub>5</sub>Si·H<sub>2</sub>O: M<sup>+</sup>·H<sup>+</sup>: 411.1970).

**N3-Benzoyl-1-(4R,5S)-4-[(3-tert-butyldiphenylsilyloxy)-2-cyclopenten-
1-yl]thymine (10)** A mixture of N3-benzoylthymine (3.5 g, 15.2 mmol), 9 (4.48 g, 4.48 mmol), Pd(dba)<sub>2</sub>·CHCl<sub>3</sub> (1.33 g, 1.48 mmol), and PPh<sub>3</sub> (2.14 g, 1.68 mmol) in toluene (200 ml) was stirred at room temperature for 1 day, the mixture was filtered and the filtrate was concentrated in vacuo.

**N3-Benzoyl-1-(4R,5S)-4-[(3-tert-butyldiphenylsilyloxy)-2-cyclopenten-
1-yl]uracil (11)** A mixture of 6 (7.67, 16.5 mmol), N3-benzoyluracil (4.60 g, 21.3 mmol), Pd(dba)<sub>2</sub>·CHCl<sub>3</sub> (735 mg, 0.71 mmol), and PPh<sub>3</sub> (1.46 g, 1.46 mmol) in toluene (200 ml) was stirred at room temperature for 1 day, the mixture was filtered and the filtrate was concentrated in vacuo.

**N3-Benzoyl-1-(4R,5S)-4-[(3-tert-butyldiphenylsilyloxy)-2-cyclopenten-
1-yl]carboxylic acid (12)** A mixture of 6 (50.3 g, 96.62 mmol) and TBAF (1 M in THF, 11.6 ml, 11.6 mmol) in THF (50 ml) was stirred at room temperature for 30 min, and the solvent was removed in vacuo. The residue was coevaporated with MeOH and purified by silica gel column chromatography (CHCl<sub>3</sub> : MeOH = 99:1).

**N3-Benzoyl-1-(4R,5S)-4-[(3-tert-butyldiphenylsilyloxy)-2-cyclopenten-
1-yl]carboxylic acid (12)** A mixture of 6 (7.67, 16.5 mmol), N3-benzoyluracil (4.60 g, 21.3 mmol), Pd(dba)<sub>2</sub>·CHCl<sub>3</sub> (735 mg, 0.71 mmol), and PPh<sub>3</sub> (1.46 g, 1.46 mmol) in toluene (200 ml) was stirred at room temperature for 1 day, the mixture was filtered and the filtrate was concentrated in vacuo.

**N3-Benzoyl-1-(4R,5S)-4-[(3-tert-butyldiphenylsilyloxy)-2-cyclopenten-
1-yl]carboxylic acid (12)** A mixture of 6 (7.67, 16.5 mmol), N3-benzoyluracil (4.60 g, 21.3 mmol), Pd(dba)<sub>2</sub>·CHCl<sub>3</sub> (735 mg, 0.71 mmol), and PPh<sub>3</sub> (1.46 g, 1.46 mmol) in toluene (200 ml) was stirred at room temperature for 1 day, the mixture was filtered and the filtrate was concentrated in vacuo.
in THF (30 ml) was stirred at room temperature for 4 h. Work-up and purifica-
tion were performed as described above to give 13 (1.09 g, 91% as a pale yellow foam).

1-H-NMR (400 MHz, CDCl3) 8.76 (1H, brs, 3-NH), 7.37—7.24 (6H, m, H-6, H-Ph), 6.13 (1H, ddd, H-3'), J = 2.0, 2.0, 5.6 Hz), 5.78 (1H, ddd, 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, benzilic), 4.16 (1H, m, H-4'), 2.73 (1H, ddd, H-5'a, J = 8.5, 8.5, 14.7 Hz), 1.74 (3H, d, 5-Me, J = 1.2 Hz), 1.63 (1H, ddd, H-5'b, J = 4.6, 4.6, 14.5 Hz). FAB-LR-MS m/z: 314 (M+H′), 100%. FAB-HR-MS m/z: 314.1509 (Calcd for C16H17N2O4Si (M+H′): 314.1504).

1-[(1R,4S)-4-(4-Hydroxyamino)-2-cyclopenten-1-yl]thymine (20) A mixture of 18 (1.2 g, 3.83 mmol) and BCl3 (1 x in CH2Cl2, 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 coevaporated several times with MeOH, and the residue dissolved in AcOEt (30 ml) was washed with H2O (150 ml)x2 and brine (150 ml). The organic phase was dried (Na2SO4) and evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt=1:1) to give 19 (1.11 g, 3.71 mmol, 93% as a pale yellow foam).

1-H-NMR (500 MHz, CDCl3) 8.95 (1H, brs, 3-NH), 7.82—7.31 (6H, m, H-6, H-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'), 5.60 (1H, d, H-5', J = 8.0 Hz), 4.70 (2H, d, each benzylic, each other, J = 11.3 Hz), 4.16 (1H, m, H-4'), 2.73 (1H, ddd, H-5'a, J = 8.6, 8.7, 14.9 Hz), 1.64 (4H, brs, H-5'b, J = 4.1, 4.2, 14.8 Hz). FAB-LR-MS m/z: 300 (M+H′), 100%. FAB-HR-MS m/z: 300.1344 (Calcd for C16H17N2O4Si (M+H′): 300.1347).

1-[(1R,4S)-4-(4-Hydroxyamino)-2-cyclopenten-1-yl]uracil (21) A mixture of 19 (50 mg, 0.17 mmol) and BCl3 (1 x in CH2Cl2, 2 ml) in CH2Cl2 (1 ml) was stirred for 24 h at room temperature at room temperature, work-up and purification were performed as described above to give 21 (30 mg, 86% as a white solid), mp 176—178°C. 1-H-NMR (400 MHz, CDCl3) 11.22 (1H, brs, 3-NH), 7.57 (1H, d, H-6, J = 7.8 Hz), 7.28 (1H, brs, 4'-NH), 6.08 (1H, ddd, H-3', J = 2.0, 2.0, 5.3 Hz), 5.93 (1H, brs, 4'-NH), 5.78 (1H, ddd, H-2', J = 1.7, 1.7, 5.6 Hz), 5.57 (1H, d, H-5', J = 8.1 Hz), 4.67 (2H, d, each benzilic, each other, J = 11.3 Hz), 3.73 (2H, s, benzilic), 3.72 (2H, s, benzilic), 3.60 (2H, d, each benzylic, each other, J = 11.3 Hz). 13C-NMR (100 MHz, CDCl3) 168.48, 161.52, 153.99, 149.49, 140.11, 135.90, 134.90, 134.05, 131.11, 130.21, 128.84, 79.81, 63.44, 58.86, 37.21, 14.29. FAB-LR-MS m/z: 371 (M+H′), 55%. FAB-HR-MS m/z: 371.1262 (Calcd for C16H17N2O4Si (M+H′): 371.1242).

1-N-Benzyl-1-(1R,4S)-4-(5-Benzoylamino)-2-cyclopenten-1-yl]thymine (16) Pd(dba), CHCl3 (456 mg, 1.74 mmol) was added to a stirred solution of 14 (1.67 g, 4.34 mmol), PPh3 (456 mg, 1.74 mmol), NaOMe (267 mg, 6.53 mmol), and NH2OBn-HCl (1.04 g, 6.53 mmol) in THF (20 ml) and H2O (3 ml) at room temperature. After the mixture was stirred for 12 h at room temperature, the solvent was removed in vacuo. The residue was coevaporated several times with EtOH and purified by silica gel column chromatography (hexane:AcOEt=1:1) to give 16 (1.21 g, 87% as a yellow foam). 1-H-NMR (CDCl3) 7.93—7.36 (11H, m, Ph), 6.15 (1H, ddd, H-3', J = 2.0, 2.0, 5.6 Hz), 5.80 (1H, ddd, H-2', J = 2.0, 2.0, 5.6 Hz), 5.71 (1H, brs, 4'-NH), 5.65 (1H, m, H-1'), 4.73 (2H, s, benzilic), 4.17 (1H, m, H-4'), 2.74 (1H, ddd, H-5'a, J = 8.3, 8.3, 14.9 Hz), 1.75 (3H, d, 5-Me, J = 1.0 Hz), 1.70 (1H, ddd, H-5'b, J = 4.6, 4.6, 14.5 Hz). 13C-NMR (100 MHz, CDCl3) 169.37, 162.98, 149.38, 135.71, 131.27, 137.12, 138.82, 132.16, 131.58, 130.34, 129.00, 128.46, 128.44, 128.12, 110.90, 77.21, 76.88, 65.26, 59.29, 34.35, 12.38. FAB-LR-MS m/z: 418 (M+H′), 100%. FAB-HR-MS m/z: 418.1760 (Calcd for C16H17N2O4Si (M+H′): 418.1767).

1-N-Benzyl-1-(1R,4S)-4-(5-Benzoylamino)-2-cyclopenten-1-yl]uracil (17) A mixture of 15 (1.10 g, 2.97 mmol), Pd(dba), CHCl3 (154 mg, 0.19 mmol), and PPh3 (311 mg, 1.19 mmol) was added to a stirred solution of aqueous NaOH (2 x, 2.33 ml, 4.46 mmol) and NH2OBn-HCl (711 mg, 4.46 mmol) in THF (20 ml) at room temperature. After the mixture was stirred for 7 h at room temperature, work-up and purification were performed as described above to give 17 (1.09 g, 84% as a pale yellow foam).

1-H-NMR (400 MHz, CDCl3) 7.94—7.33 (11H, m, H-6, H-Ph), 6.18 (1H, ddd, H-3', J = 2.0, 2.0, 5.6 Hz), 5.82 (1H, ddd, H-2', J = 2.2, 2.2, 5.4 Hz), 5.71 (1H, brs, 4'-NH), 5.64 (1H, m, H-1'), 5.47 (1H, d, H-5', J = 8.1 Hz), 4.73, 4.69 (each 1H, each d, benzilic, each J = 11.2 Hz), 4.19 (1H, m, H-4'), 2.75 (1H, ddd, H-5'a, J = 9.3, 9.3, 14.7 Hz), 1.71 (1H, ddd, H-5'b, J = 4.2, 4.2, 14.9 Hz). FAB-LR-MS m/z: 404 (M+H′), 25%. FAB-HR-MS m/z: 404.1609 (Calcd for C16H17N2O4Si (M+H′): 404.1609).
1-[[(IS,AR)-4-[N-(tert-Butyldiphenylsilyloxy)amino]cyclopent-1-yl]-thymine (24) A mixture of 223 (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 24 (117 mg, 94% as a white foam).

1-H-NMR (400 MHz, CDCl₃, 4 ml) 6.16 (1H, s, 3'-H), 7.72 (1H, ddd, 4'-H), 7.45 (1H, ddd, 5'-H), 4.34 (1H, dd, 2'-H), 3.87 (1H, ddd, 3'-H), 2.60 (2H, m, H-2, H-3), 1.30 (9H, s, tert-Bu), 1.16 (9H, t, J=7.2 Hz), 0.88 (6H, m, 4,4,4,4,4-trisopropyl-1,1-dimethylethyl).

1C-NMR (100 MHz, CDCl₃, 4 ml) 179.61, 146.81, 139.01, 135.82, 130.16, 127.62, 126.29, 78.59, 77.30, 76.95, 75.65, 74.27, 73.45, 61.39, 58.17, 34.43, 33.31, 25.47, 22.49, 22.08, 21.82, 21.61, 14.44, 12.85, 11.64.

FAB-HR-MS m/z: 540.2205 (Calcd for C₂₆H₂₄N₃O₅Si (M⁺)+ 540.2286).

1-[[(IS,AR)-4-[N-(tert-Butyldiphenylsilyloxy)amino]cyclopent-1-yl]-thymine Hydrochloride (26) A mixture of 24 (90 mg, 0.19 mmol) in AcOEt (1.5 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 to 26 (48 mg, 94% as a white foam).

1H-NMR (400 MHz, MeOH-d₄, 0.5 ml) 7.72—7.37 (10H, m, Ph), 7.12—7.11 (2H, m, H-3, H-4), 3.91 (1H, m, H-4), 2.53 (1H, d, 5-Me, J=7.7 Hz), 2.12 (3H, s, H-2, H-3, H-5), 1.09 (9H, s, tert-Bu).

1C-NMR (100 MHz, MeOH-d₄, 0.5 ml) 175.90, 160.15, 152.41, 131.48, 130.13, 129.95, 128.41, 126.34, 77.38, 75.64, 73.59, 73.57, 65.77, 63.34, 32.66, 31.08, 29.59, 28.83, 27.67, 23.89, 22.56, 20.26, 14.22, 13.90, 12.85, 11.80.

FAB-HR-MS m/z: 540.2205 (Calcd for C₂₆H₂₄N₃O₅Si·HCl (M⁺) 540.2286).


