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Synthesis and Anti-HIV Activity of D- and L-Thietanose Nucleosides

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Abstract

Various D- and L-thietanose nucleosides (**22-27**, **33-35**, **50-52**, **58-60**) were synthesized from D- and L-xylose. The four-membered thietane ring was efficiently synthesized by the cyclization of 1-thioacetyl-3-mesylate (**4/38**) under basic conditions. Condensation with various heterocyclic bases was conducted via Pummerer-type rearrangement to afford various nucleoside derivatives. Among the synthesized nucleosides, D-uridine (**23**), D-cytidine (**24**), D-5-fluorocytidine (**25**) and L-cytidine (**52**) analogs showed moderate anti-HIV activity with EC₅₀ of 6.9, 1.3, 5.8 and 14.1 μM, respectively. However, these four nucleoside analogs are cytotoxic in PBM and CEM cells. The other nucleosides are neither active nor cytotoxic. Interestingly, the oxetanocin A analog **33** was not active. Comparison of the minimized RTs complexed with the corresponding triphosphates of the cytidine analog **24** and the adenosine analog **33** by molecular modeling studies showed that there is no difference in the binding mode of the triphosphate of the cytidine analog **24** to the active site of HIV-1 RT from that of the triphosphate of the adenosine analog **33**. Modeling studies on the initial monophosphorylation step by deoxycytidine kinase showed that the catalytic efficiency of phosphorylation through a nucleophilic attack of the 4'-hydroxyl group of thietanose on the γ-phosphate of ATP is diminished in the case of L-cytidine analog (**52**) due to the increased distance between the 4'-hydroxyl group and the γ-phosphate.

Introduction

Nucleoside analogs have been well established as one of the richest source for antiviral agents despite of their toxicity¹ and drug resistance.² Various nucleosides have been reported as potent inhibitors against viruses such as human immunodeficiency virus (HIV),³ herpes simplex virus (HSV),⁴ hepatitis B virus (HBV),⁵ varicella zoster virus (VZV)⁶ and human cytomegalovirus (HCMV).⁷ In the search for new antiviral nucleosides with favorable antiviral and toxicological profiles, numerous modifications have been conducted on naturally occurring nucleosides. A natural nucleoside, oxetanocin A, which has a four-membered oxetane ring instead of a five-membered ribose ring, showed antiviral and antitumor activity (Figure 1).⁸ The unique structure as well as the interesting biological activity of oxetanocin A have prompted nucleoside chemists to explore the antiviral and antitumor activities of the nucleosides with four-member ring sugar-like moieties.⁹ And the study of structure-activity relationships of oxetanocin A resulted in several bioactive analogs.⁹ Oxetanocin T, in which the heterocyclic base is thymine, showed activity against VZV, HSV-1 and HSV-2,¹⁰ oxetanocin G with guanine as the heterocyclic base, inhibited HBV replication in cell culture with an EC₅₀ of 1.5 μM.¹¹ The carbocyclic analog of oxetanocin A was also found to be active

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against HBV and HSV,¹² and the carbocyclic oxetanocin G was also active against HBV and HSV.¹² Additionally thietanose nucleosides which have a four-membered thietane ring are of interest because the replacement of oxygen in the sugar ring with a sulfur usually increased the nucleoside's stability towards acidic hydrolysis as well as against phosphorylases which cleave the glycosyl bond, thus, resulting in the inactivation of nucleosides.¹³ However, only two synthetic methodologies for the synthesis of thietanose nucleosides have been reported in the literature, presumably due to the synthetic difficulties.¹⁴ Formulation of the thietane ring as well as the condensation reaction with heterocyclic bases occurred in poor to moderate yield, which was not sufficient enough to conduct the structure-activity relationship studies of thietanose nucleosides. For the synthesis of various thietanose nucleosides, an efficient method of synthesis of a common intermediate is required, from which various analogs can be prepared for the structure-activity relationships of the thietanose nucleosides. Herein, we report the synthesis and anti-HIV activity of various D- and L-thietanose nucleosides including D- and L-3'-thio analogs of oxetanocin A.

Results and discussion

Chemistry

β -3-Hydroxy thietane such as compound **7** can serve as the key intermediate for the synthesis of various 2'-substituted thietanose nucleosides. The β -3-hydroxy group of **7** in the thietane ring can be derivatized to other desired moieties such as methylene **9**, or hydroxymethyl group **10** etc. under mild reaction conditions. To synthesize the key intermediate compound **7**, the substitution reaction of 1-thioacetyl-3-mesylate **4**, reported by several groups,¹⁵ was adapted with some modifications. To synthesize the D-3'-thio analogs with various heterocyclic bases (**22-27**, **33-35**), D-xylose **1** was converted to the diol **2** in 5 steps by using the reported method (Scheme 1).¹⁶ The diol **2** was mesylated with MsCl, TEA and DMAP to give the 1,3-dimesylate **3** in 90% yield, which was selectively converted to the mono-thioacetate **4** by the treatment with KSac in DMF at room temperature in 80% yield. By employing the similar substitution reaction as previously reported,¹⁵ a solution of the mono-thioacetate **4** and NaHCO₃ in deoxygenated 95% aqueous EtOH was refluxed under nitrogen atmosphere for 6 h to give compound **5** in 92% yield. The compound **5** was deprotected by the treatment with 4% TFA followed by neutralization with Amberlite® (OH⁻) resin to give the triol **6** in 90% yield. The compound **6** was oxidatively cleaved to give the key intermediate **7** in 70% yield, from which a various 2'-substituted thietanose nucleosides were synthesized. For optimum yield of the 1,2-diol cleavage, several reaction conditions such as the amount of NaIO₄ (1.2 eq.), vigorous stirring, reaction time (1 h), and temperature (0°C) turned out to be critical.

The selective protection of the primary alcohol in compound **7** with TBDPSCl was explored with a few base catalysts, from which 2,4,6-collidine was found to give the highest selectivity between the primary and secondary hydroxyl groups. Thus, treatment of **7** with triethylamine and a catalytic amount of 2,4,6-collidine in CH₂Cl₂ gave the mono-protected compound **8** in 85% yield with no di-protected compound. Compound **8** was then oxidized under Moffatt-type conditions using DMSO and acetic anhydride to give a ketone as the intermediate, which was converted to the methylene derivative with the Petasis' reagent (Cp₂TiMe₂) to give compound **9** in 40% yield. Compound **9** underwent hydroboration by the treatment with BH₃·SMe₂ followed by oxidation with 3% H₂O₂ and 1N NaOH to give the undesired 2'- β -hydroxymethyl derivative **10** as the major product in 70% yield, along with the desired 2'- α -hydroxymethyl compound **11** in 12% yield. The sterically hindered nature of the β -side of compound **9** caused by the bulky silyl protecting group was probably unfavorable for the approach of BH₃ from the β -side, resulting in the formation of the undesired β -configuration of the 2'-hydroxymethyl group. This hypothesis can be supported by the similar result obtained for the synthesis of (-)-cyclobut-A.¹⁷ In order to obtain the intermediate with the correct configuration at the 2'-

position, the major product **10** was oxidized to an aldehyde and equilibrated under basic conditions. Thus, Swern oxidation of compound **10** followed by epimerization with NaOMe and reduction with NaBH₄ gave the desired product **11** in 47% overall yield in three steps. Compound **11** was protected by treating with TBDPSCl and imidazole to give compound **12** in 90% yield.

Condensation of the thietanose derivative **12** with heterocyclic bases was conducted by the Pummerer-type rearrangement after formation of a sulfoxide with mCPBA (Scheme 2).¹⁸ Silylated heterocyclic bases, such as thymine, uracil, 5-fluorouracil, 5-chlorouracil, 5-bromouracil, 6-chloropurine and 6-chloro-2-fluoropurine, were obtained under reflux conditions using HMDS and acetonitrile, which were condensed with the corresponding sulfoxide of **12** in the presence of TMSOTf, triethylamine and catalytic amount of ZnI₂ to provide the protected nucleoside derivatives (**13**, **14**, **16**, **17**, **18**, **28** and **29**) as α/β mixtures in 28-46% yield. For the pyrimidine nucleosides α and β anomers cannot be separated from each other, while for the purine derivatives, it was possible to separate the desired β anomers from their α anomers by using silica gel column chromatography. Condensation of compound **12** with thymine, uracil, 5-fluorouracil, 5-chlorouracil and 5-bromouracil gave anomeric mixtures of protected nucleosides **13**, **14**, **16**, **17** and **18** with the β/α ratio of 2:1 in 46%, 40%, 37%, 32% and 28% yield, respectively. The uracil, 5-fluorouracil, 5-chlorouracil and 5-bromouracil derivatives **14**, **16**, **17** and **18** were aminated by the treatment with 2,4,6-triisopropylbenzenesulfonyl chloride, triethylamine and DMAP in acetonitrile followed by aqueous ammonia solution to give the cytosine, 5-fluorocytosine, 5-chlorocytosine and 5-bromocytosine derivatives **15**, **19**, **20** and **21**, respectively. The nucleoside analogs **13-15**, **19-21** were treated with TBAF in THF at room temperature to afford pyrimidine thietanose nucleosides **22-27**.

Compound **12** was condensed with 6-chloropurine and 6-chloro-2-fluoropurine to give compounds **28** and **29** as well as their α -isomers in 40-45% yield with a β/α ratio of 5:4 (Scheme 2). Compound **28** was converted to the adenine derivative **30** in 72% yield by using NaN₃ in DMF followed by hydrogenation with 10 % Pd (0) on carbon in methanol. Compound **28** was also converted to the inosine derivative **31** in 85% yield by treatment with NaOMe and 2-mercaptoethanol in methanol. On the other hand, the guanosine analog **32** was obtained in 74 % yield by treatment of **29** with NaOMe and 2-mercaptoethanol in methanol followed by amination in ethanolic ammonia at 60°C. Deprotection of compounds **30-32** by TBAF gave the purine thietanose nucleosides **33-35** in 89-94 % yield.

The L-3'-thio analogs of oxetanocin A were also synthesized under similar reaction conditions starting from the opposite enantiomer, L-xylose (Scheme 3). L-Xylose was converted to the key intermediate **46**, the enantiomer of compound **12**, which was obtained through the thietane diol **41**, the enantiomer of compound **7**. After coupling of **46** with the appropriate silylated pyrimidine and purine bases, the L-3'-thio analogs (**50-52**, **58-60**) of oxetanocin A were obtained by using the same procedures employed for the synthesis of D-isomers (Scheme 4).

Anti-HIV activity

The antiviral activity of the synthesized D- and L-thietanose nucleosides (**22-27**, **33-35**, **50-52**, **58-60**) was evaluated against HIV-1 in human PBM cells *in vitro* using AZT as a positive control and the results are summarized in Table 1. Among the synthesized nucleosides, D-uridine (**23**), D-cytidine (**24**), D-5-fluorocytidine and L-cytidine (**52**) analogs show moderate anti-HIV activity with an EC₅₀ value of 6.9, 1.3, 5.8 and 14.1 μ M, respectively. However, these four nucleoside analogs are also cytotoxic in PBM cells and CEM cells.

We synthesized D-cytidine analog at the early stages of this project, when some antiviral activity for D-cytidine analog (**24**, Table 1) is observed, then we decided to explore the SAR at the 5-

position of the pyrimidine ring. The addition of halogen atom at the 5-position of the pyrimidine ring has been found to alter the kinetic parameters as well as antiviral potency of the corresponding nucleoside analogues.¹⁹ It is well known that the modification of 5-position of the cytosine ring with fluorine may result in reduced toxicity and in some cases increase the antiviral potency.²⁰ However, introduction of different halogens at the 5-position in this thietanose series (**25**, **26** & **27**), resulted in the decrease in antiviral potency (Table 1). The complete inactivity of **26** was unexpected. The other nucleosides showed neither anti-HIV activity nor cytotoxicity up to 100 μ M. It is well known that nucleosides/nucleotides analogs can inhibit human DNA polymerases which may cause side effects such as cytotoxicity, mitochondrial toxicity, etc.²¹ Consequently, if compounds **24**, **25** **27** and **52** can be phosphorylated to triphosphates, the low selectivity of these nucleotides between the HIV RT and cellular polymerase may explain the observed antiviral activity as well as the cytotoxicity. Viral inhibition may also be due to the deleterious effect of the nucleosides on the cellular viability, caused by their metabolites to other cellular machineries other than polymerase (e.g. ribonucleotide reductase, CTP synthase etc.²²). To confirm the hypothesis, however, more detail experiments have to be conducted. As all the purine analogs including the 3'-thio-oxetanocin A analog **33** showed no detectable anti-HIV activity while the compound **33** was found to have similar binding characteristics with the HIV-RT by molecular modeling studies (vide infra), the purine thietanose nucleosides might have a low level of phosphorylation by the initial nucleoside kinase, which probably results in a low, or if any, levels of the triphosphate.

Molecular modeling studies

The molecular modeling studies of the synthesized thietanose adenosine derivative **33** as well as oxetanocin A were conducted to compare the conformations of these two nucleosides with that of thymidine triphosphate in the crystal structure of HIV-1 reverse transcriptase (RT) (PDB code 1RTD).²³ The most stable conformation of thietanose adenosine derivative **33** was obtained by a Monte Carlo conformational search using the molecular graphics and simulation program, MacroModel version 7.0 (Schrödinger, Inc.).²⁴ The crystal structure of oxetanocin A was obtained from the Cambridge Structural Database. The three conformations of thietanose adenosine derivative **33**, oxetanocin A and TTP were superimposed using Sybyl 6.7 (Figure 2). The C3'-C4' bond (in blue) in thietanose adenosine derivative **33** is displaced by 0.6 Å from that of the C4'-C5' bond (in orange) in TTP, and the 3'-sulfur atom in thietanose adenosine derivative **33** extrudes from that of the 3'-oxygen in oxetanocin A and the 4'-oxygen in TTP. Another noticeable feature is that 2'-hydroxymethyl groups of thietanose adenosine derivative **33** and oxetanocin A are completely overlapped with the 3'-hydroxy group of TTP. It is well known that the 3'-hydroxy pocket, composed of Asp113, Ala114, Tyr115 and Gln151, strengthens the binding of natural substrates to the active site of HIV-1 RT through hydrogen bonding, particularly with Tyr 115.²¹ This 3'-hydroxy pocket also plays an important role in binding of AZTTP to HIV RT, which was demonstrated in our previous molecular modeling studies.²⁵ Thus, the 2'-hydroxymethyl groups of thietanose adenosine derivative **33** could be nicely accommodated at the 3'-OH pocket, which would favorably contribute to their binding affinity to RT.

While the *D*-cytidine analog **24** is moderately active against HIV, which has the same sugar conformation as *D*-adenosine analog **33** based on the Monte Carlo conformational search, the *D*-adenosine analog **33** does not show any anti-HIV activity. To understand the difference of anti-HIV activity between the cytidine analog **24** and adenosine analog **33**, molecular modeling studies using the crystal structure of HIV-1 RT (PDB code 1RTD) were conducted replacing TTP with the corresponding triphosphates of the nucleoside analogs **24** and **33**.²³ Each triphosphate was docked to the active site of HIV-1 RT and the resulting complex was minimized using Kollman-All Atom force field. In the minimized structures, both nucleoside

triphosphates are nicely accommodated to the active site of HIV-1 RT without any steric hindrance with amino acid residues of the active site of RT. As expected, each hydroxy moiety of 2'-hydroxymethyl group (-CH₂OH) of the two thietanose nucleoside triphosphates is hydrogen-bonded to Tyr 115 of 3'-hydroxy pocket in the active site of HIV RT as well as their own β -phosphate (Figure 3). From the molecular modeling studies, it is suggested that there would be no difference in the ability of both the nucleosides triphosphates to bind to the active site of HIV RT. Thus, the lack of anti-HIV activity of the adenosine analog **33** might have originated from the lack of phosphorylation by the initial kinase.

To investigate this possibility, we conducted molecular modeling studies of D- and L-thietanose cytidine analog (**24**, **52**) with deoxycytidine kinase (dCK, PDB code 1P60).²⁶ Detailed analysis of the reported crystal structure (1P60.pdb) revealed that Glu53 and Arg128 are the acid-base pair which abstracts the proton and initiates the catalysis (Figure 4).²⁶ The distance between the nucleoside 5'-OH and Glu53 is critical for the phosphorylation. The 3'-OH is critical for activity, thus if 3'-OH is missing, then the catalytic efficiency decreases.²⁷ The final minimized structures of the **24** and **52** revealed that there is considerable preference for D-thietanose analog (**24**) over L-thietanose analog (**52**). Catalytic efficiency of phosphorylation through a nucleophilic attack of the 4'-hydroxyl group of thietanose on the γ -phosphate of ATP is diminished in the case of compound (**52**) due to the increased distance between the 4'-hydroxyl group and the γ -phosphate. Slight steric clashes are also observed between Leu82 and the 2'-hydroxymethyl group of **52** (Figure 5), which is due to the distinct binding mode of L-thietanose analogs. Thus, it can be concluded from the studies that the increased distance for nucleophilic attack on the γ -phosphate coupled with the steric clashes observed for compound **52**, makes it less likely to get phosphorylated as efficiently as compound **24**, which may partially explain the lower anti-HIV activity.

In summary, various thietanose nucleosides such as D- and L-3'-thio analogs of oxetanocin A and 2'- α - and 2'- β -hydroxythietanose nucleosides were efficiently synthesized from D- and L-xylose. Among the synthesized nucleosides, D-uridine (**23**), D-cytidine (**24**), 5-fluorocytidine (**25**) and L-cytidine (**52**) analogs show moderate anti-HIV activity as well as cytotoxicity. Molecular modeling studies show that the triphosphates of D-cytidine analog **24** and adenosine analog **33** are nicely accommodated to the active site of HIV RT through hydrogen bonding interactions, but the initial kinase (dCK) may not efficiently phosphorylate the compound **33**, resulting in low level of its triphosphate, which, in turn, provides no anti-HIV activities.

Experimental section

Melting points were determined on a Mel-temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Inova 500 spectrometer at 500 MHz for ¹H NMR. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), quin. (quintet), m (multiplet), or br s (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220-440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

1,2-O-Isopropylidene-3,5-di-O-mesyl- α -D-ribofuranoside (**3**)

A solution of compound **2** (33.2g, 175 mmol) and pyridine (56.6 mL, 700 mmol) in CH₂Cl₂ (500 mL) was treated with MsCl (33.8 mL, 437 mmol) and DMAP (4.3g, 35 mmol) at 0°C for 18 h. The reaction mixture was diluted with CH₂Cl₂ (500 mL), washed with 1N HCl, aq NaHCO₃, and brine, and dried over MgSO₄. The filtrate was concentrated and crystallized

with a 1:10 mixture of CH_2Cl_2 and hexanes to give the product **3** (29.3g, 0.0816 mol, 90%) as a white solid: mp 115~116°C; $[\alpha]^{22}_{\text{D}}$ 77.8 ° (c 0.43, CHCl_3); ^1H NMR (CDCl_3) δ 5.84 (d, J = 3.4 Hz, 1H), 4.82~4.74 (m, 2H), 4.55 (dd, J = 11.8, 1.9 Hz, 1H), 4.41~4.30 (m, 2H), 3.16 (s, 3H), 3.08 (s, 3H), 1.58 (s, 3H), 1.38 (s, 3H); Anal. ($\text{C}_{10}\text{H}_{18}\text{O}_9\text{S}_2$) C, H.

5-S-Acetyl-1,2-O-isopropylidene-3-O-mesyl- α -D-ribofuranoside (**4**)

A solution of compound **3** (81.5g, 235 mmol) in DMF (400 mL) was treated with KSAc (29.5g, 258 mmol) at room temperature. After 16 h, the reaction mixture was diluted with EtOAc, washed with water (2 X 1 L), and dried over MgSO_4 . The filtrate was concentrated and purified with 20% EtOAc in hexanes by silica gel column chromatography to give product **4** (61.4g, 188 mmol, 80%) as a brownish solid: mp 112~113°C; $[\alpha]^{23}_{\text{D}}$ 68.5 ° (c 0.35, CHCl_3); ^1H NMR (CDCl_3) δ 5.77 (d, J = 3.7 Hz, 1H), 4.75 (t, J = 4.2 Hz, 1H), 4.48 (dd, J = 8.8, 4.6 Hz, 1H), 4.31~4.25 (m, 1H), 3.35 (dd, J = 14.5, 4.1 Hz, 1H), 3.23 (J = 14.5, 5.1 Hz, 1H), 3.16 (s, 3H), 2.36 (s, 3H), 1.55 (s, 3H), 1.34 (s, 3H); Anal. ($\text{C}_{11}\text{H}_{18}\text{O}_7\text{S}_2$) C, H, S.

3,5-Thioanhydro-1,2-O-isopropylidene- α -D-xylofuranoside (**5**)

A mixture of EtOH (100 mL) and water (5 mL) was refluxed under Ar for 2 h and cooled. Compound **4** (2.12g, 6.50 mmol) and solid NaHCO_3 (1.09g, 13.0 mmol) was added to the solvent and the resulting mixture was refluxed for 8 h. The reaction mixture was cooled, diluted with Et_2O , filtered, and purified with 5% Et_2O in hexanes by silica gel column chromatography to give the product **5** (1.12g, 5.98 mmol, 92%) as oil: $[\alpha]^{22}_{\text{D}}$ 78.7 ° (c 2.1, CHCl_3); ^1H NMR (CDCl_3) δ 6.41 (d, J = 3.4 Hz, 1H), 5.26 (t, J = 5.3 Hz, 1H), 4.71 (d, J = 3.4 Hz, 1H), 4.08 (d, J = 5.3 Hz, 1H), 3.49 (dd, J = 10.6, 5.4 Hz, 1H), 2.84 (d, J = 10.6 Hz, 1H), 1.43 (s, 3H), 1.36 (s, 3H); Anal. ($\text{C}_8\text{H}_{12}\text{O}_3\text{S}$) C, H, S.

3,5-Thioanhydro- α -D-xylofuranose (**6**)

Compound **5** (1.10g, 5.84 mmol) was with 4% TFA solution in water at room temperature for 8 h, the reaction mixture was neutralized with Amberlite (OH) resin which was pre-washed with water (2X100 mL). After filtration, the filtrate was concentrated and purified with 50% EtOAc in hexanes by silica gel column chromatography to give the product **6** (0.75g, 5.06 mmol, 87%): ^1H NMR (CDCl_3) for major δ 6.11 (d, J = 2.3 Hz, 1H), 5.16~5.12 (m, 1H), 4.17 (d, J = 2.5 Hz, 1H), 3.95 (d, J = 5.9 Hz, 1H), 3.50 (dd, J = 10.9, 6.3 Hz, 1H), 3.00 (d, J = 10.9 Hz, 1H), for minor δ 5.56 (s, 1H), 5.29~5.24 (m, 1H), 4.37 (s, 1H), 3.95 (d, J = 5.9 Hz, 1H), 3.64 (dd, J = 10.9, 6.1 Hz, 1H), 3.12 (d, J = 10.9 Hz, 1H); Anal. ($\text{C}_5\text{H}_8\text{O}_3\text{S}$) C, H, S.

(2R,3S)-2-Hydroxymethyl-thietan-3-ol (**7**)

A solution of compound **6** (1.92g, 12.9 mmol) in MeOH (50 mL) was treated with an aqueous solution of NaIO_4 (3.04g, 14.2 mmol) in water (25 mL) drop wise at 0°C for 10 min with vigorous stirring. After 20 min, well-grounded NaBH_4 was added portion wise to the reaction mixture at 0°C. After 30 min, the resulting mixture was filtered and the filtrate was neutralized with 1N HCl and concentrated. The residue was dissolved in a mixture of CH_2Cl_2 and MeOH (1:1) and the white solid was filtered off. The filtrate was concentrated and purified with 3~5% MeOH in CH_2Cl_2 by silica gel column chromatography to give the products **7** (1.08g, 9.0 mmol, 70%): $[\alpha]^{24}_{\text{D}}$ 166.2 ° (c 0.63, MeOH); ^1H NMR (CDCl_3) δ 5.00 (quin, J = 8.1 Hz, 1H), 4.09~3.98 (m, 2H), 3.85 (d, J = 8.9 Hz, 1H), 3.76~3.71 (m, 1H), 3.37~3.24 (m, 3H); Anal. ($\text{C}_4\text{H}_8\text{O}_2\text{S} \cdot 0.35\text{H}_2\text{O}$) C, H, S.

(2R,3S)-2-(tert-Butyl-diphenyl-silanyloxymethyl)-thietan-3-ol (**8**)

A solution of compound **7** (5.00g, 41.6 mmol), triethylamine (11.6 mL, 83.2 mmol), and 2,4,6-collidine (0.46 mL, 4.2 mmol) in CH_2Cl_2 was treated with TBDPSCI (11.45 mL, 43.7 mmol) at 0°C for 2 h. The resulting mixture was concentrated and purified with 5% EtOAc in hexanes

by silica gel column chromatography to give the product **8** (11.9g, 33.2 mmol, 82%) as oil: $[\alpha]^{20}_D$ 74.1 ° (c 0.91, CHCl₃); ¹H NMR (CDCl₃) δ 7.84~7.38 (m, 10H), 5.06 (quin, *J* = 8.1 Hz, 1H), 4.19~4.10 (m, 2H), 3.88 (d, *J* = 9.7 Hz, 1H), 3.73~3.67 (m, 1H), 3.54 (t, *J* = 8.3 Hz, 1H), 3.33 (t, *J* = 8.3 Hz, 1H), 1.12 (s, 9H); Anal. (C₂₀H₂₆O₂SSi) C, H, S.

***tert*-Butyl-[(2*S*)-3-methylene-thietan-2-ylmethoxy]-diphenylsilane (**9**)**

Compound **8** (10.9g, 30.4 mmol) was treated with DMSO (45 mL) and Ac₂O (30 mL) at room temperature for overnight. The reaction mixture was quenched with ice, diluted with Et₂O (500 mL), washed with water. The organic layer was dried over anhydrous Na₂SO₄, and concentrated. The residue was purified with 3% EtOAc in hexanes by silica gel column chromatography to give the intermediate ketone (10.0g, 28.1 mmol, 92%) as oil. A mixture of titanocene dichloride (38.7g, 115 mmol) in Et₂O (1 L) was treated with 1.6 M MeLi solution (214 mL) in Et₂O at room temperature for 2 h. The reaction mixture was quenched with ice and extracted with Et₂O. The organic layer was dried over anhydrous Na₂SO₄ and concentrated to dryness. This reagent (Petasis reagent) was dissolved in THF (80 mL). A solution of the intermediate ketone (10.0g, 28.1 mmol) in Et₂O (50 mL) was treated with the freshly prepared Petasis reagent solution. The resulting mixture was refluxed at 60°C bath temperature. After 48 h, the reaction mixture was cooled to room temperature, diluted with hexanes, and filtered through the silica gel pad. The filtrate was concentrated and purified with 2% EtOAc in hexanes by silica gel column chromatography to give the product **9** (4.12g, 11.6 mmol, 41%) as oil: $[\alpha]^{22}_D$ 73.6 ° (c 0.80, CH₂Cl₂); ¹H NMR (CDCl₃) δ 7.72~7.65 (m, 4H), 7.47~7.37 (m, 6H), 4.86 (d, *J* = 2.1 Hz, 1H), 4.71 (d, *J* = 2.1 Hz, 1H), 4.44~4.37 (m, 1H), 4.00 (dd, *J* = 10.3, 6.8 Hz, 1H), 3.87 (dd, *J* = 10.3, 6.8 Hz, 1H), 3.84~3.74 (m, 2H), 1.06 (s, 9H); Anal. (C₂₁H₂₆OSSi) C, H, S.

[(2*S*,3*R*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-thietan-3-yl]-methanol (10**)**

A solution of compound **9** (4.12g, 11.6 mmol) in THF (200 mL) was treated with 2M BH₃SMe₂ solution (11.5 mL) in THF at 0°C for 30 min. The reaction mixture was warmed up to room temperature and stirred for 2 h. The reaction mixture was quenched with 3% H₂O₂ (26 mL) and 1N NaOH (23 mL). After 2 h, the reaction mixture was treated with sat. Na₂S₂O₃, and extracted with EtOAc. The organic layer was washed with brine and dried over Na₂SO₄. The filtrate was concentrated and purified with 10% EtOAc in hexanes by silica gel column chromatography to give the product **10** (3.35g, 8.13 mmol, 70% yield) as well as compound **11** (0.51g, 1.37 mmol, 12% yield): $[\alpha]^{22}_D$ 43.4 ° (c 1.18, CHCl₃); ¹H NMR (CDCl₃) δ 7.78~7.32 (m, 10H), 4.28 (t, *J* = 10.1 Hz, 1H), 4.01 (t, *J* = 9.9 Hz, 1H), 3.80~3.42 (m, 5H), 3.02~2.83 (m, 2H), 1.07 (s, 9H); Anal. (C₂₁H₂₈O₂SSi) C, H, S.

[(2*R*,3*S*)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-thietan-3-yl]-methanol (11**)**

Oxalyl chloride (1.56 mL, 17.9 mmol) was added drop wise to a solution of DMSO (1.90 mL, 26.8 mmol) in CH₂Cl₂ (30 mL) at -78°C. After 10 min, a solution of compound **10** (3.33g, 8.94 mmol) in CH₂Cl₂ (20 mL) was added to the reaction mixture at -78°C. After 20 min, TEA (5.0 mL, 36 mmol) was added at -78°C. After 10 min, the reaction mixture was warmed up to room temperature for 1 h. The resulting mixture was diluted with CH₂Cl₂, and washed with water. The organic layer was dried over anhydrous Na₂SO₄ and concentrated to dryness. The aldehyde was dissolved in MeOH (30 mL), and treated with NaOMe (1.45g, 26.8 mmol) for 16 h. The resulting mixture was treated with NaBH₄ (1.01g, 26.7 mmol) for 2 h. The reaction mixture was neutralized with 1N HCl, concentrated to a tenth volume of the solution, extracted with CH₂Cl₂. The organic layer was dried over anhydrous Na₂SO₄, concentrated, filtered and purified by silica gel column chromatography with 10% EtOAc in hexanes to give the product **11** (1.58g, 4.24 mmol, 47% yield): $[\alpha]^{24}_D$ 40.7 ° (c 0.81, CH₂Cl₂); ¹H NMR (CDCl₃) δ

7.70~7.36 (m, 10H), 3.82~3.58 (m, 5H), 3.22~3.10 (m, 1H), 3.01~2.93 (m, 2H), 1.06 (s, 9H); Anal. (C₂₁H₂₈O₂SSi) C, H, S.

(2R,3S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietane (12)

A solution of compound **11** (2.00g, 5.37 mmol) and imidazole (0.77g, 11 mmol) in CH₂Cl₂ was treated with TBDPSCl (1.54 mL, 5.92 mmol) at room temperature for 3 h. The reaction mixture was concentrated and purified with 2% EtOAc in hexanes to give the product **12** (2.95g, 4.83 mmol, 90%) as oil: [α]_D²⁵ 45.0 ° (c 0.38, CH₂Cl₂); ¹H NMR (CDCl₃) δ 7.70~7.33 (m, 20H), 3.90 (dd, *J* = 9.7, 6.1 Hz, 1H), 3.77 (dd, *J* = 10.2, 6.6 Hz, 1H), 3.73~3.65 (m, 3H), 3.16~2.95 (m, 3H), 1.05 (s, 9H), 1.04 (s, 9H); Anal. (C₃₇H₄₆O₂SSi₂) C, H, S.

1-[(2R,3S,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-thymine (13)

A solution of compound **12** (0.250g, 0.409 mmol) in CH₂Cl₂ was treated with a solution of 77% mCPBA (0.092g, 0.411 mmol) in CH₂Cl₂ drop wise at -78°C. The mixture was stirred at -78°C for 30 min. The reaction was quenched with aq. Na₂S₂O₃, diluted with CH₂Cl₂, washed with aq Na₂S₂O₃, aq NaHCO₃, and brine, and dried over MgSO₄. The filtrate was concentrated to dryness. The crude sulfoxide was used for the next reaction without further purification. A mixture of thymine (0.155g, 1.23 mmol) in acetonitrile (10 mL) and HMDS (10 mL) was refluxed for 3 h until the solution became clear. After evaporation, the residue was treated successively with a solution of the crude sulfoxide in toluene (10 mL), triethylamine (0.11 mL, 0.79 mmol), TMSOTf (0.16 mL, 0.88 mmol), and ZnI₂ (0.039g, 0.12 mmol) at 0°C. After 24 h, the reaction was quenched with water at 0°C and the reaction mixture was diluted with CH₂Cl₂. The organic layer was dried over Na₂SO₄, filtered, concentrated and purified with 30% EtOAc in hexane by preparative TLC to give the product **13** (0.138g, 0.188 mmol, 46%) as an α/β mixture (1:3): UV(CH₂Cl₂) λ_{max} 270.0 nm; ¹H NMR (CDCl₃) δ 9.03, 8.99 (2br s, 1H), 7.98, 7.80 (2s, 1H), 7.72~7.30 (m, 20H), 6.30 (d, *J* = 6.8 Hz, 0.75H), 6.20 (d, *J* = 7.7 Hz, 0.25H), 3.88 (dd, *J* = 9.8, 5.9 Hz, 0.25H), 3.81~3.45 (m, 5H), 3.26~3.19 (m, 0.75H), 1.87, 1.83 (2s, 3H), 1.07, 1.044, 1.036, 0.98 (4s, 18H); Anal. (C₄₂H₅₀N₂O₄SSi₂) C, H, N, S.

1-[(2R,3S,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-uracil (14)

See the procedure of compound **13** for condensation reaction with thymine. The title compound **14** was obtained on 0.301-mmol scale in 40% yield: UV(CH₂Cl₂) λ_{max} 265.0 nm; ¹H NMR (CDCl₃) δ 8.58, 8.53 (2br s, 1H), 8.19 (d, *J* = 7.8 Hz, 0.3H), 8.16 (d, *J* = 7.8 Hz, 0.7H), 7.70~7.33 (m, 20H), 6.24 (d, *J* = 6.8 Hz, 0.7H), 6.20 (d, *J* = 7.8 Hz, 0.3H), 5.69 (d, *J* = 8.8 Hz, 0.3H), 5.60 (d, *J* = 7.8 Hz, 0.7H), 3.85 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.77~3.46 (m, 5H), 3.26~3.20 (m, 0.7H), 1.07, 1.05, 1.03, 0.98 (4s, 18H); Anal. (C₄₁H₄₈N₂O₄SSi₂) C, H, N, S.

1-[(2R,3S,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-cytosine (15)

A solution of compound **14** (0.120g, 0.166 mmol) in acetonitrile was treated with triethylamine (0.07 mL, 0.50 mmol), 2,4,6-triisopropylbenzenesulfonyl chloride (0.151g, 0.499 mmol), and DMAP (0.061 g, 0.50 mmol) at room temperature. After 16 h, ammonia water was added to the resulting mixture. After 3h, the mixture was diluted with CH₂Cl₂ (50 mL), and the organic layer was washed with brine, dried over Na₂SO₄, concentrated and purified by column chromatography with 5% MeOH in CH₂Cl₂ to give a α/β mixture of compound **15** (0.090g, 0.125 mmol, 75% yield): UV(CH₂Cl₂) λ_{max} 285.5 nm; ¹H NMR (CDCl₃) δ 8.31 (d, *J* = 7.8 Hz, 0.3H), 8.25 (d, *J* = 7.8 Hz, 0.7H), 7.69~7.28 (m, 20H), 6.26 (d, *J* = 6.8 Hz, 0.7H), 6.24 (d, *J* = 7.8 Hz, 0.3H), 5.62 (d, *J* = 6.8 Hz, 0.3H), 5.58 (d, *J* = 6.8 Hz, 0.7H), 3.92 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.83~3.46 (m, 5H), 3.18~3.11 (m, 0.7H), 1.07, 1.03, 1.00, (3s, 18H); Anal. (C₄₁H₄₉N₃O₃SSi₂) C, H, N, S.

1-[(2R,3S,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-5-fluorouracil (16)

See the procedure of compound **13** for condensation reaction with thymine. The title compound **16** was obtained on 0.389-mmol scale in 37% yield: UV(CH₂Cl₂) λ_{max} 271 nm; ¹H NMR (CDCl₃) δ 8.58, 8.53 (2br s, 1H), 8.19 (d, *J* = 7.8 Hz, 0.3H), 8.16 (d, *J* = 7.8 Hz, 0.7H), 7.70~7.33 (m, 20H), 6.24 (d, *J* = 6.8 Hz, 0.7H), 6.20 (d, *J* = 7.8 Hz, 0.3H), 5.69 (d, *J* = 8.8 Hz, 0.3H), 5.60 (d, *J* = 7.8 Hz, 0.7H), 3.85 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.77~3.46 (m, 5H), 3.26~3.20 (m, 0.7H), 1.07, 1.05, 1.03, 0.98 (4s, 18H); HRMS (ESI, M+Na⁺) Calcd for C₄₁H₄₇FN₂O₄SSi₂Na 761.2677, found 761.2671.

1-[(2R,3S,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-5-chlorouracil (17)

See the procedure of compound **13** for condensation reaction with thymine. The title compound **17** was obtained on 0.291-mmol scale in 32% yield: UV(CH₂Cl₂) λ_{max} 273.0 nm; ¹H NMR (CDCl₃) δ 8.58, 8.53 (2br s, 1H), 8.19 (d, *J* = 7.8 Hz, 0.3H), 8.16 (d, *J* = 7.8 Hz, 0.7H), 7.70~7.33 (m, 20H), 6.24 (d, *J* = 6.8 Hz, 0.7H), 6.20 (d, *J* = 7.8 Hz, 0.3H), 5.69 (d, *J* = 8.8 Hz, 0.3H), 5.60 (d, *J* = 7.8 Hz, 0.7H), 3.85 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.77~3.46 (m, 5H), 3.26~3.20 (m, 0.7H), 1.07, 1.05, 1.03, 0.98 (4s, 18H); HRMS (ESI, M+Na⁺) Calcd for C₄₁H₄₇ClN₂O₄SSi₂Na 777.2382, found 777.2378.

1-[(2R,3S,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-5-bromouracil (18)

See the procedure of compound **13** for condensation reaction with thymine. The title compound **18** was obtained on 0.375-mmol scale in 28% yield: UV(CH₂Cl₂) λ_{max} 278.0 nm; ¹H NMR (CDCl₃) δ 8.58, 8.53 (2br s, 1H), 8.19 (d, *J* = 7.8 Hz, 0.3H), 8.16 (d, *J* = 7.8 Hz, 0.7H), 7.70~7.33 (m, 20H), 6.24 (d, *J* = 6.8 Hz, 0.7H), 6.20 (d, *J* = 7.8 Hz, 0.3H), 5.69 (d, *J* = 8.8 Hz, 0.3H), 5.60 (d, *J* = 7.8 Hz, 0.7H), 3.85 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.77~3.46 (m, 5H), 3.26~3.20 (m, 0.7H), 1.07, 1.05, 1.03, 0.98 (4s, 18H); HRMS (ESI, M+Na⁺) Calcd for C₄₁H₄₇BrN₂O₄SSi₂Na 821.1876, found 821.1867.

1-[(2R,3S,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-5-fluorocytosine (19)

See the procedure of compound **14** for the amination reaction with uridine analog. The title compound **19** was obtained on 0.284-mmol scale in 73% yield: UV(CH₂Cl₂) λ_{max} 285 nm; ¹H NMR (CDCl₃) δ 8.31 (d, *J* = 7.8 Hz, 0.3H), 8.25 (d, *J* = 7.8 Hz, 0.7H), 7.69~7.28 (m, 20H), 6.26 (d, *J* = 6.8 Hz, 0.7H), 6.24 (d, *J* = 7.8 Hz, 0.3H), 5.62 (d, *J* = 6.8 Hz, 0.3H), 5.58 (d, *J* = 6.8 Hz, 0.7H), 3.92 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.83~3.46 (m, 5H), 3.18~3.11 (m, 0.7H), 1.07, 1.03, 1.00, (3s, 18H); HRMS (ESI, M+Na⁺) Calcd for C₄₁H₄₈FN₃O₃SSi₂Na 760.2837, found 760.2845.

1-[(2R,3S,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-5-chlorocytosine (20)

See the procedure of compound **14** for the amination reaction with uridine analog. The title compound **20** was obtained on 0.318-mmol scale in 68% yield: UV(CH₂Cl₂) λ_{max} 277 nm; ¹H NMR (CDCl₃) δ 8.31 (d, *J* = 7.8 Hz, 0.3H), 8.25 (d, *J* = 7.8 Hz, 0.7H), 7.69~7.28 (m, 20H), 6.26 (d, *J* = 6.8 Hz, 0.7H), 6.24 (d, *J* = 7.8 Hz, 0.3H), 5.62 (d, *J* = 6.8 Hz, 0.3H), 5.58 (d, *J* = 6.8 Hz, 0.7H), 3.92 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.83~3.46 (m, 5H), 3.18~3.11 (m, 0.7H), 1.07, 1.03, 1.00, (3s, 18H); HRMS (ESI, M+Na⁺) Calcd for C₄₁H₄₈FN₃O₃SSi₂Na 776.2541, found 776.2533.

1-[(2R,3S,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-5-bromocytosine (21)

See the procedure of compound **14** for the amination reaction with uridine analog. The title compound **21** was obtained on 0.338-mmol scale in 63% yield: UV(CH₂Cl₂) λ_{max} 292 nm; ¹H NMR (CDCl₃) δ 8.31 (d, *J* = 7.8 Hz, 0.3H), 8.25 (d, *J* = 7.8 Hz, 0.7H), 7.69~7.28 (m, 20H), 6.26 (d, *J* = 6.8 Hz, 0.7H), 6.24 (d, *J* = 7.8 Hz, 0.3H), 5.62 (d, *J* = 6.8 Hz, 0.3H), 5.58 (d, *J* = 6.8 Hz, 0.7H), 3.92 (dd, *J* = 10.7, 5.8 Hz, 0.3H), 3.83~3.46 (m, 5H), 3.18~3.11 (m, 0.7H), 1.07, 1.03, 1.00, (3s, 18H); HRMS (ESI, M+Na⁺) Calcd for C₄₁H₄₈BrN₃O₃SSi₂Na 820.2036, found 820.2027.

1-[(2R,3S,4R)-2,3-dihydroxymethyl-thietan-4-yl]-thymine (22)

A solution of compound **13** (0.130g, 0.177 mmol) in THF (10 mL) was treated with 0.35 mL of 1M TBAF solution in acetonitrile at 0°C. After 3 h, the reaction mixture was concentrated and purified by prep TLC with 7% MeOH in CH₂Cl₂ to give compound **22** (0.029g, 0.112 mmol, 63% yield) and α -isomer (0.011g, 0.043 mmol, 24% yield): mp >200°C (dec.); [α]_D²⁶ -86.8° (c 0.075, DMSO); UV(H₂O) λ_{max} 264.0 nm (ϵ 9,400, pH 2), 262.0 nm (ϵ 9,900, pH 7), 265.5 nm (ϵ 7,100, pH 11); ¹H NMR (MeOH) δ 8.30 (s, 1H), 6.08 (d, *J* = 6.8 Hz, 1H), 3.76 (dd, *J* = 11.7, 3.9 Hz, 1H), 3.72 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.58 (d, *J* = 3.9 Hz, 2H), 3.54~3.49 (m, 1H), 3.32~3.26 (m, 1H), 1.95 (s, 3H); Anal. (C₁₀H₁₄N₂O₄S) C, H, N, S.

1-[(2R,3S,4R)-2,3-dihydroxymethyl-thietan-4-yl]-uracil (23)

See the procedure of compound **22** for deprotection reaction with 1M TBAF solution. The title compound **23** was obtained on 0.083-mmol scale in 59% yield: mp >200°C (dec.); [α]_D²² -35.4° (c 0.17, MeOH); UV(H₂O) λ_{max} 265.5 nm (ϵ 9,200, pH 2), 265.5 nm (ϵ 8,500, pH 7), 265.5 nm (ϵ 6,900, pH 11); ¹H NMR (MeOH) δ 8.46 (d, *J* = 7.8 Hz, 1H), 6.07 (d, *J* = 6.8 Hz, 1H), 5.81 (d, *J* = 7.8 Hz, 1H), 3.76 (dd, *J* = Hz, 1H), 3.71 (dd, *J* = Hz, 1H), 3.60 (d, *J* = Hz, 2H), 3.54~3.49 (m, 1H), 3.28~3.21 (m, 1H); Anal. (C₉H₁₂N₂O₄S) C, H, N, S.

1-[(2R,3S,4R)-2,3-dihydroxymethyl-thietan-4-yl]-cytosine (24)

See the procedure of compound **22** for deprotection reaction with 1M TBAF solution. The title compound **24** was obtained on 0.118-mmol scale in 59% yield: mp 125~126°C; [α]_D²³ -59.2° (c 0.14, MeOH); UV(H₂O) λ_{max} 282.5 nm (ϵ 12,500, pH 2), 274.5 nm (ϵ 9,700, pH 7), 275.5 nm (ϵ 9,600, pH 11); ¹H NMR (MeOH) δ 8.43 (d, *J* = 6.8 Hz, 1H), 6.04 (s, *J* = 6.8 Hz, 1H), 6.00 (d, *J* = 7.8 Hz, 1H), 3.75 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.69 (dd, *J* = 11.7, 5.6 Hz, 1H), 3.66~3.58 (m, 1H), 3.51~3.47 (m, 1H), 3.14 (quin, *J* = 6.8 Hz, 1H); Anal. (C₉H₁₂N₂O₄S) C, H, N, S.

1-[(2R,3S,4R)-2,3-dihydroxymethyl-thietan-4-yl]-5-fluorocytosine (25)

See the procedure of compound **22** for deprotection reaction with 1M TBAF solution. The title compound **25** was obtained on 0.109-mmol scale in 51% yield: mp 125~126°C; [α]_D²⁶ -53.3° (c 0.30, MeOH); UV(H₂O) λ_{max} 272 nm (ϵ 9,226, pH 2), 288 nm (ϵ 14,146, pH 7), 278nm (ϵ 12,588, pH 11); ¹H NMR (MeOH) δ 8.65 (d, *J* = 6.8 Hz, 1H), 6.04 (dd, *J* = 6.6, 2.2 Hz, 1H), 3.76 (dd, *J* = 9.0, 4.4 Hz, 2H), 3.67 (dd, *J* = 5.6, 3.9 Hz, 2H), 3.55~3.51 (m, 1H), 3.24~3.20 (m, 1H); Anal. (C₉H₁₂N₂O₄S) C, H, N, S.

1-[(2R,3S,4R)-2,3-dihydroxymethyl-thietan-4-yl]-5-chlorocytosine (26)

See the procedure of compound **22** for deprotection reaction with 1M TBAF solution. The title compound **26** was obtained on 0.112-mmol scale in 46% yield: mp 125~126°C; [α]_D²⁵ -43.4° (c 0.22, MeOH); UV(H₂O) λ_{max} 297 nm (ϵ 5,486, pH 2), 291 nm (ϵ 4,567, pH 7), 291 nm (ϵ 6,903, pH 11); ¹H NMR (MeOH) δ 8.61 (s, 1H), 5.87 (d, *J* = 6.3 Hz, 1H), 3.62 (dd, *J* = 8.5,

4.1 Hz, 2H), 3.55 (dd, $J = 5.6, 2.9$ Hz, 1H), 3.42~3.38 (m, 1H), 3.13~3.07 (m, 1H); Anal. ($C_9H_{12}N_2O_4S$) C, H, N, S.

1-[(2*R*,3*S*,4*R*)-2,3-dihydroxymethyl-thietan-4-yl]-5-bromocytosine (27)

See the procedure of compound **22** for deprotection reaction with 1M TBAF solution. The title compound **27** was obtained on 0.105-mmol scale in 37% yield: mp 125~126°C; $[\alpha]^{23}_D -25.3^\circ$ (c 0.14, MeOH); UV(H_2O) λ_{max} 302 nm (ϵ 8,311, pH 2), 293 nm (ϵ 6,585, pH 7), 293 nm (ϵ 7,005, pH 11); 1H NMR (MeOH) δ 8.83 (s, 1H), 6.00 (s, $J = 6.4$ Hz, 1H), 3.74 (dd, $J = 8.1, 4.1$ Hz, 2H), 3.68 (dd, $J = 5.6, 3.2$ Hz, 2H), 3.56~3.52 (m, 1H), 3.33~3.28 (m, 1H); Anal. ($C_9H_{12}N_2O_4S$) C, H, N, S.

9-[(2*R*,3*S*,4*R*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-6-chloropurine (28)

See the procedure of compound **13** for condensation reaction with thymine. The title compound **28** as well as α -isomer were obtained on 0.622-mmol scale in 45% yield as a 5:4 mixture of compound **28** and α -isomer: $[\alpha]^{23}_D 16.8^\circ$ (c 0.65, $CHCl_3$); UV(CH_2Cl_2) λ_{max} 264.5 nm; 1H NMR ($CDCl_3$) δ 8.87 (s, 1H), 8.86 (s, 1H), 7.66~7.27 (m, 20H), 6.56 (d, $J = 7.1$ Hz, 1H), 3.82~3.62 (m, 5H), 3.39~3.31 (m, 1H), 1.06 (s, 9H), 1.02 (s, 9H); Anal. ($C_{42}H_{47}ClN_4O_2SSi_2$) C, H, N, S.

9-[(2*R*,3*S*,4*R*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-6-chloro-2-fluoropurine (29)

See the procedure of compound **13** for condensation reaction with thymine. The title compound **29** as well as α -isomer were obtained on 0.797-mmol scale in 40% yield as a 5:4 mixture of compound **29** and α -isomer: $[\alpha]^{24}_D 9.3^\circ$ (c 0.21, $CHCl_3$); UV(CH_2Cl_2) λ_{max} 254.5 nm; 1H NMR ($CDCl_3$) δ 8.85 (s, 1H), 7.64~7.24 (m, 20H), 6.47 (d, $J = 6.8$ Hz, 1H), 3.83~3.65 (m, 5H), 3.36~3.28 (m, 1H), 1.06 (s, 9H), 1.03 (s, 9H); Anal. ($C_{42}H_{46}ClFN_4O_2SSi_2$) C, H, N, S.

9-[(2*R*,3*S*,4*R*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-adenine (30)

A solution of compound **28** (0.202g, 0.265 mmol) in DMF (2 mL) was treated with NaN_3 (0.172g, 2.65 mol) at room temperature for 16h. The resulting mixture was directly filtered through silica gel pad using eluent as a 1:1 mixture of hexanes and EtOAc. The filtrate was concentrated to dryness. A solution of the crude azide in MeOH (15 mL) was treated with 0.030g of Pd(0)/C and H_2 balloon for 48 h. The resulting mixture was filtered through celite pad and the filtrate was concentrated and purified by column chromatography with 2% MeOH in CH_2Cl_2 to give the product **30** (0.143g, 0.192 mmol, 72% yield): $[\alpha]^{23}_D 41.5^\circ$ (c 0.17, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 272.0 nm; 1H NMR ($CDCl_3$) δ 8.44 (s, 1H), 7.85 (s, 1H), 7.66~7.30 (m, 20H), 6.06 (br s, 2H), 6.03 (d, $J = 8.6$ Hz, 1H), 3.91~3.83 (m, 2H), 3.74~3.54 (m, 3H), 3.43~3.34 (m, 1H), 1.09 (s, 9H), 1.05 (s, 9H); Anal. ($C_{42}H_{49}N_5O_2SSi_2$) C, H, N, S.

9-[(2*R*,3*S*,4*R*)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-hypoxanthine (31)

A solution of compound **28** (0.134g, 0.176 mmol), 2-mercaptoethanol (0.049 mL, 0.70 mmol) and NaOMe (0.038g, 0.70 mmol) in MeOH was refluxed for 6 h. The mixture was cooled to room temperature, neutralized with AcOH, concentrated, and purified by column chromatography with 2% MeOH in CH_2Cl_2 to give the product **31** (0.112g, 0.150 mmol, 85% yield): $[\alpha]^{24}_D 27.9^\circ$ (c 0.21, CH_2Cl_2); UV(CH_2Cl_2) λ_{max} 262.5 nm; 1H NMR ($CDCl_3$) δ 8.45 (s, 1H), 7.72 (s, 1H), 7.68~7.12 (m, 20H), 6.56 (d, $J = 5.9$ Hz, 1H), 3.89 (dd $J = 10.7, 5.9$ Hz, 1H), 3.85~3.68 (m, 4H), 3.39~3.32 (m, 1H), 1.05 (s, 9H), 1.01 (s, 9H); Anal. ($C_{42}H_{48}N_4O_3SSi_2$) C, H, N, S.

9-[(2R,3S,4R)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-guanine (32)

A solution of compound **29** (0.138g, 0.177 mmol), 2-mercaptoethanol (0.049 mL, 0.70 mmol) and NaOMe (0.019g, 0.35 mmol) in MeOH was refluxed for 4 h. The mixture was cooled to room temperature, neutralized with AcOH, concentrated, and filtered through silica gel pad. The filtrate was concentrated to dryness. The residue was treated with ethanolic ammonia at 60°C for 6 h. The reaction mixture was concentrated and purified by column chromatography with 2% MeOH in CH₂Cl₂ to give the product **32** (0.115g, 0.151 mmol, 74% yield): [α]_D²⁴ 17.8 ° (c 0.12, CHCl₃); UV(CH₂Cl₂) $\lambda_{\max}$ 264.0 nm; ¹H NMR (CDCl₃) δ 8.36 (s, 1H), 7.75~7.24 (m, 20H), 6.36 (d, *J* = 6.8 Hz, 1H), 3.95~3.63 (m, 5H), 3.37~3.30 (m, 1H), 1.04 (s, 9H), 0.99 (s, 9H); Anal. (C₄₂H₄₉N₅O₃SSi₂) C, H, N, S.

9-[(2R,3S,4R)-2,3-dihydroxymethyl-thietan-4-yl]-adenine (33)

See the procedure of compound **22** for deprotection reaction with 1M TBAF solution. The title compound **33** was obtained on 0.188-mmol scale in 94% yield: mp >200°C; [α]_D²⁵ 95.2 ° (c 0.057, MeOH); UV(H₂O) $\lambda_{\max}$ 272.5 nm (ϵ 15,000, pH 2), 270.5 nm (ϵ 10,000, pH 7), 271.5 nm (ϵ 10,000, pH 11); ¹H NMR (MeOH) δ 8.84 (s, 1H), 8.24 (s, 1H), 6.17 (d, *J* = 6.6 Hz, 1H), 3.80 (d, *J* = 4.6 Hz, 2H), 3.73 (dd, *J* = 11.7, 6.6 Hz, 1H), 3.69 (dd, *J* = 11.7, 5.1 Hz, 1H), 3.62~3.56 (m, 1H), 3.55~3.48 (m, 1H); Anal. (C₁₀H₁₃N₅O₂S) C, H, N, S.

9-[(2R,3S,4R)-2,3-dihydroxymethyl-thietan-4-yl]-hypoxanthine (34)

See the procedure of compound **22** for deprotection reaction with 1M TBAF solution. The title compound **34** was obtained on 0.150-mmol scale in 89% yield: mp 213~214°C; [α]_D²⁶ 22.0 ° (c 0.13, MeOH); UV(H₂O) $\lambda_{\max}$ 256.5 nm (ϵ 7,600, pH 2), 258.0 nm (ϵ 7,200, pH 7), 263.0 nm (ϵ 7,600, pH 11); ¹H NMR (MeOH-*d*₄) δ 8.91 (s, 1H), 8.02 (s, 1H), 6.36 (d, *J* = 5.9 Hz, 1H), 3.83 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.78 (dd, *J* = 11.7, 4.9 Hz, 1H), 3.77~3.70 (m, 2H), 3.60 (q, *J* = 4.9 Hz, 1H), 3.48 (quin, *J* = 5.9 Hz, 1H); Anal. (C₁₀H₁₂N₄O₃S) C, H, N, S.

9-[(2R,3S,4R)-2,3-dihydroxymethyl-thietan-4-yl]-guanine (35)

See the procedure of compound **22** for deprotection reaction with 1M TBAF solution. The title compound **35** was obtained on 0.132-mmol scale in 91% yield: mp >200°C (dec.); [α]_D²³ -73.7 ° (c 0.11, DMSO); UV(H₂O) $\lambda_{\max}$ 251.5 nm (ϵ 7,300, pH 2), 266.0 nm (ϵ 6,900, pH 7), 266.0 nm (ϵ 7,100, pH 11); ¹H NMR (DMSO-*d*₆) δ 13.48 (br s, 1H), 8.79 (s, 1H), 6.23 (d, *J* = 6.8 Hz, 1H), 5.07 (t, *J* = 4.9 Hz, 1H), 4.99 (br s, 1H), 3.79~3.46 (m, 5H), 3.30~2.26 (m, 1H); Anal. (C₁₀H₁₃N₅O₃S) C, H, N, S.

(-)-[2R,3R,4S,5S]-Methanesulfonic acid 5-methanesulfonyloxymethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yl ester (37)

Using the same procedure described for the preparation of compound **3**, the title compound was prepared on a 81.6 mmol scale in 90% yield as white solid: mp 115~116°C; [α]_D²² -80.2 ° (c 0.57, CHCl₃); ¹H NMR (CDCl₃) δ 5.84 (d, *J* = 3.4 Hz, 1H), 4.82~4.74 (m, 2H), 4.55 (dd, *J* = 11.8, 1.9 Hz, 1H), 4.41~4.30 (m, 2H), 3.16 (s, 3H), 3.08 (s, 3H), 1.58 (s, 3H), 1.38 (s, 3H); Anal. (C₁₀H₁₈O₉S₂) C, H

(-)-[2R,3R,4S,5S]-Thioacetic acid S-(6-methanesulfonyloxy-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-ylmethyl) ester (38)

Was prepared from compound **37** on a 14.0 mmol scale in 80 % yield as brownish solid by the same procedure described for **4**: mp 112~113°C; [α]_D²³ -70.0 ° (c 0.53, CHCl₃); ¹H NMR (CDCl₃) δ 5.77 (d, *J* = 3.7 Hz, 1H), 4.75 (t, *J* = 4.2 Hz, 1H), 4.48 (dd, *J* = 8.8, 4.6 Hz, 1H), 4.31~4.25 (m, 1H), 3.35 (dd, *J* = 14.5, 4.1 Hz, 1H), 3.23 (*J* = 14.5, 5.1 Hz, 1H), 3.16 (s, 3H), 2.36 (s, 3H), 1.55 (s, 3H), 1.34 (s, 3H); Anal. (C₁₁H₁₈O₇S₂) C, H, S.

(-)-4,4-Dimethyl-tetrahydro-3,5,6-trioxa-2-thia-cyclobuta[a]pentalene (39)

Was prepared from compound **38** on a 30.85 mmol scale in 95 % yield as yellowish oil by the same procedure described for **5**: $[\alpha]^{23}_{\text{D}} -89.4^{\circ}$ (c 1.76, CH_2Cl_2); ^1H NMR (CDCl_3) δ 6.41 (d, $J = 3.4$ Hz, 1H), 5.26 (t, $J = 5.3$ Hz, 1H), 4.71 (d, $J = 3.4$ Hz, 1H), 4.08 (d, $J = 5.3$ Hz, 1H), 3.49 (dd, $J = 10.6$, 5.4 Hz, 1H), 2.84 (d, $J = 10.6$ Hz, 1H), 1.43 (s, 3H), 1.36 (s, 3H); Anal. ($\text{C}_8\text{H}_{12}\text{O}_3\text{S}$) C, H, S.

(-)-2-Oxa-6-thia-bicyclo[3.2.0]heptane-3,4-diol (40)

Was prepared from compound **39** on a 27.77 mmol scale in 90 % yield as yellowish oil (anomeric mixture) by the same procedure described for **6**.

(-)-[2R,3S]-2-Hydroxymethyl-thietan-3-ol (41)

Was prepared from compound **40** on a 10.11 mmol scale in 70 % yield as yellowish oil by the same procedure described for **7**: $[\alpha]^{24}_{\text{D}} -173.5^{\circ}$ (c 0.84, MeOH); ^1H NMR (CDCl_3) δ 5.00 (quin, $J = 8.1$ Hz, 1H), 4.09~3.98 (m, 2H), 3.85 (d, $J = 8.9$ Hz, 1H), 3.76~3.71 (m, 1H), 3.37~3.24 (m, 3H); Anal. ($\text{C}_4\text{H}_8\text{O}_2\text{S} \cdot 0.35\text{H}_2\text{O}$) C, H, S.

(-)-[2R,3R]-2-(tert-Butyl-diphenyl-silanyloxymethyl)-thietan-3-ol (42)

Was prepared from compound **41** on a 17.10 mmol scale in 85 % yield as yellowish oil by the same procedure described for **8**: $[\alpha]^{22}_{\text{D}} -78.6^{\circ}$ (c 0.68, CHCl_3); ^1H NMR (CDCl_3) δ 7.84~7.38 (m, 10H), 5.06 (quin, $J = 8.1$ Hz, 1H), 4.19~4.10 (m, 2H), 3.88 (d, $J = 9.7$ Hz, 1H), 3.73~3.67 (m, 1H), 3.54 (t, $J = 8.3$ Hz, 1H), 3.33 (t, $J = 8.3$ Hz, 1H), 1.12 (s, 9H); Anal. ($\text{C}_{20}\text{H}_{26}\text{O}_2\text{SSi}$) C, H, S.

(-)-[2R]-tert-Butyl-(3-methylene-thietan-2-ylmethoxy)-diphenyl-silane (43)

Was prepared from compound **42** on a 5.32 mmol scale in 40 % yield as yellowish oil by the same procedure described for **9**: $[\alpha]^{23}_{\text{D}} -71.8^{\circ}$ (c 1.60, CH_2Cl_2); ^1H NMR (CDCl_3) δ 7.72~7.65 (m, 4H), 7.47~7.37 (m, 6H), 4.86 (d, $J = 2.1$ Hz, 1H), 4.71 (d, $J = 2.1$ Hz, 1H), 4.44~4.37 (m, 1H), 4.00 (dd, $J = 10.3$, 6.8 Hz, 1H), 3.87 (dd, $J = 10.3$, 6.8 Hz, 1H), 3.84~3.74 (m, 2H), 1.06 (s, 9H); Anal. ($\text{C}_{21}\text{H}_{26}\text{OSSi}$) C, H, S.

(-)-[2R,3R]-[2-(tert-Butyl-diphenyl-silanyloxymethyl)-thietan-3-yl]-methanol (44)

Was prepared from compound **43** on a 4.43 mmol scale in 64 % yield as yellowish oil by the same procedure described for **10**: $[\alpha]^{22}_{\text{D}} -32.1^{\circ}$ (c 0.82, CHCl_3); ^1H NMR (CDCl_3) δ 7.78~7.32 (m, 10H), 4.28 (t, $J = 10.1$ Hz, 1H), 4.01 (t, $J = 9.9$ Hz, 1H), 3.80~3.42 (m, 5H), 3.02~2.83 (m, 2H), 1.07 (s, 9H); Anal. ($\text{C}_{21}\text{H}_{28}\text{O}_2\text{SSi}$) C, H, S.

(-)-[2R,3S]-[2-(tert-Butyl-diphenyl-silanyloxymethyl)-thietan-3-yl]-methanol (45)

Was prepared from compound **44** on a 2.27 mmol scale in 45 % yield as yellowish oil by the same procedure described for **11**: $[\alpha]^{22}_{\text{D}} -39.5^{\circ}$ (c 0.91, CH_2Cl_2); ^1H NMR (CDCl_3) δ 7.70~7.36 (m, 10H), 3.82~3.58 (m, 5H), 3.22~3.10 (m, 1H), 3.01~2.93 (m, 2H), 1.06 (s, 9H); Anal. ($\text{C}_{21}\text{H}_{28}\text{O}_2\text{SSi}$) C, H, S.

(-)-[2R,3S]-2,3-Bis-(tert-butyl-diphenyl-silanyloxymethyl)-thietane (46)

Was prepared from compound **45** on a 2.16 mmol scale in 92 % yield as yellowish oil by the same procedure described for **12**: $[\alpha]^{24}_{\text{D}} -44.7^{\circ}$ (c 0.43, CH_2Cl_2); ^1H NMR (CDCl_3) δ 7.70~7.33 (m, 20H), 3.90 (dd, $J = 9.7$, 6.1 Hz, 1H), 3.77 (dd, $J = 10.2$, 6.6 Hz, 1H), 3.73~3.65 (m, 3H), 3.16~2.95 (m, 3H), 1.05 (s, 9H), 1.04 (s, 9H); Anal. ($\text{C}_{37}\text{H}_{46}\text{O}_2\text{SSi}_2$) C, H, S.

1-[(2S,3R,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-thymine (47)

Was prepared from the coupling of sulfoxide which was obtained from the oxidation of compound **46** with silylated thymine on a 0.18 mmol scale in 44 % yield (α/β mixture, $\alpha:\beta = 1:3$ as determined by NMR) as by the same procedure described for **13**: UV(CH₂Cl₂) $\lambda_{\max}$ 270.0 nm; Anal. (C₄₂H₅₀N₂O₄SSi₂) C, H, N, S.

1-[(2S,3R,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-uracil (48)

Using the similar condensation procedure for the preparation of compound **13**. The title compound **48** was obtained on 0.325-mmol scale in 40% yield as a α/β mixture ($\alpha:\beta = 1:2$ as determined by NMR): UV(CH₂Cl₂) $\lambda_{\max}$ 265.0 nm; Anal. (C₄₁H₄₈N₂O₄SSi₂) C, H, N, S.

1-[(2S,3R,4R/S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-cytosine (49)

Using the similar condensation procedure for the preparation of compound **15**. The title compound **49** was obtained on 0.16-mmol scale in 75% yield as a α/β mixture: UV(CH₂Cl₂) $\lambda_{\max}$ 285.5 nm; Anal. (C₄₁H₄₉N₃O₃SSi₂) C, H, N, S.

1-[(2S,3R,4S)-2,3-dihydroxymethyl-thietan-4-yl]-thymine (50)

Was prepared from compound **47** on a 0.043 mmol scale in 63 % yield as white solid by the same procedure described for **22**: mp >200°C (dec.); [α]_D²⁶ 82.8 ° (c 0.13, DMSO); UV(H₂O) $\lambda_{\max}$ 264.0 nm (ϵ 9,400, pH 2), 262.0 nm (ϵ 9,900, pH 7), 265.5 nm (ϵ 7,100, pH 11); ¹H NMR (MeOH) δ 8.30 (s, 1H), 6.08 (d, $J = 6.8$ Hz, 1H), 3.76 (dd, $J = 11.7, 3.9$ Hz, 1H), 3.72 (dd, $J = 11.7, 4.9$ Hz, 1H), 3.58 (d, $J = 3.9$ Hz, 2H), 3.54~3.49 (m, 1H), 3.32~3.26 (m, 1H), 1.95 (s, 3H); Anal. (C₁₀H₁₄N₂O₄S) C, H, N, S.

1-[(2S,3R,4S)-2,3-dihydroxymethyl-thietan-4-yl]-uracil (51)

Was prepared from compound **48** on a 0.043 mmol scale in 59 % yield as white solid by the same procedure described for **23**: mp >200°C (dec.); [α]_D²⁴ 33.2 ° (c 0.23, MeOH); UV(H₂O) $\lambda_{\max}$ 265.5 nm (ϵ 9,200, pH 2), 265.5 nm (ϵ 8,500, pH 7), 265.5 nm (ϵ 6,900, pH 11); ¹H NMR (MeOH) δ 8.46 (d, $J = 7.8$ Hz, 1H), 6.07 (d, $J = 6.8$ Hz, 1H), 5.81 (d, $J = 7.8$ Hz, 1H), 3.76 (dd, $J = \text{Hz}, 1\text{H}$), 3.71 (dd, $J = \text{Hz}, 1\text{H}$), 3.60 (d, $J = \text{Hz}, 2\text{H}$), 3.54~3.49 (m, 1H), 3.28~3.21 (m, 1H); Anal. (C₉H₁₂N₂O₄S) C, H, N, S.

1-[(2S,3R,4S)-2,3-dihydroxymethyl-thietan-4-yl]-cytosine (52)

Was prepared from compound **49** on a 0.043 mmol scale in 56 % yield as white solid by the same procedure described for **24**: mp 126~127°C; [α]_D²⁵ 61.7 ° (c 0.11, MeOH); UV(H₂O) $\lambda_{\max}$ 282.5 nm (ϵ 12,500, pH 2), 274.5 nm (ϵ 9,700, pH 7), 275.5 nm (ϵ 9,600, pH 11); ¹H NMR (MeOH) δ 8.43 (d, $J = 6.8$ Hz, 1H), 6.04 (s, $J = 6.8$ Hz, 1H), 6.00 (d, $J = 7.8$ Hz, 1H), 3.75 (dd, $J = 11.7, 4.9$ Hz, 1H), 3.69 (dd, $J = 11.7, 5.6$ Hz, 1H), 3.66~3.58 (m, 1H), 3.51~3.47 (m, 1H), 3.14 (quin, $J = 6.8$ Hz, 1H); Anal. (C₉H₁₂N₂O₄S) C, H, N, S.

9-[(2S,3R,4S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-6-chloropurine (53)

Using the same condensation procedure for the preparation of compound **28**, the title compounds **53** was obtained on 0.370-mmol scale in 40% yield as well as its α anomer with the ratio of $\alpha:\beta = 4:5$. [α]_D²² -16.8 ° (c 0.58, CHCl₃); UV(CH₂Cl₂) $\lambda_{\max}$ 264.5 nm; ¹H NMR (CDCl₃) δ 8.87 (s, 1H), 8.86 (s, 1H), 7.66~7.27 (m, 20H), 6.56 (d, $J = 7.1$ Hz, 1H), 3.82~3.62 (m, 5H), 3.39~3.31 (m, 1H), 1.06 (s, 9H), 1.02 (s, 9H); Anal. (C₄₂H₄₇ClN₄O₂SSi₂) C, H, N, S.

9-[(2S,3R,4S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-6-chloro-2-fluoropurine (54)

Using the same condensation procedure of compound **29**, the title compound **54** was obtained on 0.797-mmol scale in 23% yield together with its α anomer (α : β = 4:5): $[\alpha]^{24}_D$ -10.1 ° (c 0.54, CHCl₃); UV(CH₂Cl₂) $\lambda_{\max}$ 254 nm; ¹H NMR (CDCl₃) δ 8.85 (s, 1H), 7.64~7.24 (m, 20H), 6.47 (d, J = 6.8 Hz, 1H), 3.83~3.65 (m, 5H), 3.36~3.28 (m, 1H), 1.06 (s, 9H), 1.03 (s, 9H); Anal. (C₄₂H₄₆ClFN₄O₂SSi₂) C, H, N, S.

9-[(2S,3R,4S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-adenine (55)

Was prepared from compound **53** on a 0.078 mmol scale in 74 % yield as white solid by the same procedure described for **30**: $[\alpha]^{23}_D$ -40.7 ° (c 0.15, CH₂Cl₂); UV(CH₂Cl₂) $\lambda_{\max}$ 272.0 nm; ¹H NMR (CDCl₃) δ 8.44 (s, 1H), 7.85 (s, 1H), 7.66~7.30 (m, 20H), 6.06 (br s, 2H), 6.03 (d, J = 8.6 Hz, 1H), 3.91~3.83 (m, 2H), 3.74~3.54 (m, 3H), 3.43~3.34 (m, 1H), 1.09 (s, 9H), 1.05 (s, 9H); Anal. (C₄₂H₄₉N₅O₂SSi₂) C, H, N, S.

9-[(2S,3R,4S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-hypoxanthine (56)

Was prepared from compound **53** on a 0.11 mmol scale in 84 % yield as white solid by the same procedure described for **31**: $[\alpha]^{24}_D$ 26.9 ° (c 0.13, CH₂Cl₂); UV(CH₂Cl₂) $\lambda_{\max}$ 262.5 nm; ¹H NMR (CDCl₃) δ 8.45 (s, 1H), 7.72 (s, 1H), 7.68~7.12 (m, 20H), 6.56 (d, J = 5.9 Hz, 1H), 3.89 (dd J = 10.7, 5.9 Hz, 1H), 3.85~3.68 (m, 4H), 3.39~3.32 (m, 1H), 1.05 (s, 9H), 1.01 (s, 9H); Anal. (C₄₂H₄₈N₄O₃SSi₂) C, H, N, S.

9-[(2S,3R,4S)-2,3-Bis-(*tert*-butyl-diphenyl-silanyloxymethyl)-thietan-4-yl]-guanine (57)

Was prepared from compound **54** on a 0.11 mmol scale in 84 % yield as white solid by the same procedure described for **32**: $[\alpha]^{22}_D$ 17.8 ° (c 0.29, CHCl₃); UV(CH₂Cl₂) $\lambda_{\max}$ 264.0 nm; ¹H NMR (CDCl₃) δ 8.36 (s, 1H), 7.75~7.24 (m, 20H), 6.36 (d, J = 6.8 Hz, 1H), 3.95~3.63 (m, 5H), 3.37~3.30 (m, 1H), 1.04 (s, 9H), 0.99 (s, 9H); Anal. (C₄₂H₄₉N₅O₃SSi₂) C, H, N, S.

9-[(2S,3R,4S)-2,3-dihydroxymethyl-thietan-4-yl]-adenine (58)

Using the same procedure for deprotection of compound **33**, the title compound **58** was obtained on 0.094-mmol scale in 84% yield: mp >200°C; $[\alpha]^{25}_D$ -93.3 ° (c 0.11, MeOH); UV (H₂O) $\lambda_{\max}$ 272.5 nm (ϵ 15,000, pH 2), 270.5 nm (ϵ 10,000, pH 7), 271.5 nm (ϵ 10,000, pH 11); ¹H NMR (MeOH) δ 8.84 (s, 1H), 8.24 (s, 1H), 6.17 (d, J = 6.6 Hz, 1H), 3.80 (d, J = 4.6 Hz, 2H), 3.73 (dd, J = 11.7, 6.6 Hz, 1H), 3.69 (dd, J = 11.7, 5.1 Hz, 1H), 3.62~3.56 (m, 1H), 3.55~3.48 (m, 1H); Anal. (C₁₀H₁₃N₅O₂S) C, H, N, S.

9-[(2S,3R,4S)-2,3-dihydroxymethyl-thietan-4-yl]-hypoxanthine (59)

Using the same procedure for the deprotection of compound **33**, the title compound **59** was obtained on 0.145-mmol scale in 86% yield: mp 213~214°C; $[\alpha]^{27}_D$ -23.9 ° (c 0.15, MeOH); UV(H₂O) $\lambda_{\max}$ 256.5 nm (ϵ 7,600, pH 2), 258.0 nm (ϵ 7,200, pH 7), 263.0 nm (ϵ 7,600, pH 11); ¹H NMR (MeOH-*d*₄) δ 8.91 (s, 1H), 8.02 (s, 1H), 6.36 (d, J = 5.9 Hz, 1H), 3.83 (dd, J = 11.7, 4.9 Hz, 1H), 3.78 (dd, J = 11.7, 4.9 Hz, 1H), 3.77~3.70 (m, 2H), 3.60 (q, J = 4.9 Hz, 1H), 3.48 (quin, J = 5.9 Hz, 1H); Anal. (C₁₀H₁₂N₄O₃S) C, H, N, S.

9-[(2S,3R,4S)-2,3-dihydroxymethyl-thietan-4-yl]-guanine (60)

Using the same procedure for the deprotection of compound **25**, the title compound **35** was obtained on 0.124-mmol scale in 86% yield: mp >200°C (dec.); $[\alpha]^{23}_D$ 70.7 ° (c 0.12, DMSO); UV(H₂O) $\lambda_{\max}$ 251.5 nm (ϵ 7,300, pH 2), 266.0 nm (ϵ 6,900, pH 7), 266.0 nm (ϵ 7,100, pH 11); ¹H NMR (DMSO-*d*₆) δ 13.48 (br s, 1H), 8.79 (s, 1H), 6.23 (d, J = 6.8 Hz, 1H), 5.07 (t, J

= 4.9 Hz, 1H), 4.99 (br s, 1H), 3.79~3.46 (m, 5H), 3.30~2.26 (m, 1H); Anal. (C₁₀H₁₃N₅O₃S) C, H, N, S.

Antiviral Assay

Human peripheral blood mononuclear (PBM) cells (obtained from Atlanta Red Cross) were isolated by Ficoll-Hypaque discontinuous gradient centrifugation from healthy seronegative donors. Cells were stimulated with phytohemagglutinin A (Difco, Sparks, MD) for 2-3 days prior to use. HIV-1_{LAI} obtained from the Centers for Disease Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays. The molecular infectious clones HIV-1_{xxB_{RU}} was obtained from Dr. John Mellors (University of Pittsburgh). Infections were done in bulk for one hour, either with 100 TCID₅₀ / 1 × 10⁷ cells for a flask (T25) assay or with 200 TCID₅₀ / 6 × 10⁵ cells/well for a 24 well plate assay. Cells were added to a plate or flask containing a ten-fold serial dilution of the test compound. Assay medium was RPMI-1640 supplemented with heat inactivated 16% fetal bovine serum, 1.6 mM L-glutamine, 80 IU/ml penicillin, 80 µg/ml streptomycin, 0.0008% DEAE-Dextran, 0.045% sodium bicarbonate, and 26 IU/ml recombinant interleukin-2 (Chiron Corp, Emeryville, CA). AZT was used as a positive control for the assay. Untreated and uninfected PBM cells were grown in parallel at equivalent cell concentrations as controls. The cell cultures were maintained in a humidified 5% CO₂-air at 37°C for 5 days and supernatants were collected for reverse transcriptase (RT) activity.

Supernatants were centrifuged at 12,000 rpm for 2 h to pellet the virus. The pellet was solubilized with vortexing in 100 µl virus solubilization buffer (VSB) containing 0.5% Triton X-100, 0.8 M NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 20% glycerol, and 0.05 M Tris, pH 7.8. Ten µl of each sample were added to 75 µl RT reaction mixture (0.06 M Tris, pH 7.8, 0.012 M MgCl₂, 0.006 M dithiothreitol, 0.006 mg/ml poly (rA)_n oligo (dT)₁₂₋₁₈, 96 µg/ml dATP, and 1 µM of 0.08 mCi/ml ³H-thymidine triphosphate (Moravek Biochemicals, Brea, CA)) and incubated at 37°C for 2 h. The reaction was stopped by the addition of 100 µl 10% trichloroacetic acid containing 0.05% sodium pyrophosphate. The acid insoluble product was harvested onto filter paper using a Packard Harvester (Meriden, CT), and the RT activity was read on a Packard Direct Beta Counter (Meriden, CT). The RT results were expressed in counts per minute (CPM) per milliliter. The antiviral 50% effective concentration (EC₅₀) and 90% effective concentration (EC₉₀) were determined from the concentration-response curve using the median effect method.²⁸

Cytotoxicity assays

The compounds were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells, in CEM (T-lymphoblastoid cell line obtained from American Type Culture Collection, Rockville, MD.) and Vero (African green monkey kidney) cells. PBM cells were obtained from whole blood of healthy seronegative donors (HIV-1 and hepatitis B virus) by single-step Ficoll-Hypaque discontinuous gradient centrifugation. Log phase Vero, CEM and PHA-stimulated human PBM cells were seeded at a density of 5 × 10³, 2.5 × 10³ and 5 × 10⁴ cells/well respectively. All of the cells were plated in 96-well cell culture plates containing ten-fold serial dilutions of the test drug. The cultures were incubated for 3, 4, and 5 days for Vero, CEM, and PBM cells, respectively in a humidified 5% CO₂-air at 37°C. At the end of incubation, MTT tetrazolium dye solution (Cell titer 96®, Promega, Madison, WI) was added to each well and incubated overnight. The reaction was stopped with stop solubilization solution (Promega, Madison, WI). The plates were incubated for 5 h to ensure that the formazan crystals were dissolved. The plates were read at a wavelength of 570 nm using an ELISA plate reader (Bio-tek instruments, Inc., Winooski, VT, Model # EL 312e). The 50% inhibition concentration (IC₅₀) was determined from the concentration-response curve using the median effect method.²⁸

Molecular modeling studies

(a) Conformational analysis: The initial conformations of D-cytidine analog **24** and D-adenosine analog **33** were constructed and geometrically-optimized through a Monte Carlo conformational search using MMFF94s force field in Macromodel, version 7.0 (Schrödinger, Inc.). (b) Binding affinity study to HIV-1 reverse transcriptase: All molecular modeling of the enzyme-substrate complexes was carried out using Sybyl 6.7 (Tripos Associates, St. Louis, MO) on a Silicon Graphics Octane2 workstation. The enzyme site of the enzyme-ligand complex was constructed based on the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer-template duplex (PDB entry 1rtd). A model of the NRTI binding site was constructed which consisted of residues between Lys1 and Pro243 in the p66 subunit, and a 7:4 (template-primer) duplex. The geometrically-optimized structures of each inhibitor, obtained from the geometry optimization study, were used as the initial Cartesian coordinates. The heterocyclic moiety of n+1th nucleotide in template overhang was modified to the base complementary to the incoming NRTIs. Thus, the adenine moiety which was in the original X-ray structure (1rtd) was modified to guanine. The inhibitor triphosphates were manually docked to the active site of the enzyme by adjusting the torsional angles to those found in the X-ray structure. Gasteiger-Hückel charge was given to the enzyme-ligand complex with formal charges (+2) to two Mg atoms in the active site. Then, Kollman-All-Atom charges were loaded to enzyme site from the biopolymer module in Sybyl. In order to eliminate local strains resulting from merging inhibitors and/or point mutations, residues inside 6Å from the merged inhibitors and mutated residues were annealed until energy change from one iteration to the next was less than 0.05 kcal/mol. The annealed enzyme-inhibitor complexes were minimized by using Kollman-All-Atom Force Field until iteration number reached 5,000. (c) Binding affinity study to Deoxycytidine Kinase (dCK): All molecular modeling of the enzyme-substrate complexes was carried out using Sybyl 6.7 (Tripos Associates, St. Louis, MO) on a Silicon Graphics Octane2 workstation. The enzyme site of the enzyme-ligand complex was constructed based on the X-ray structure of the catalytic complex of dCK with dC and adenosine diphosphate (ADP) (PDB entry: 1P60).²⁶ Terminal residues were capped with acetyl and methyl group, Mg²⁺ atom was added and γ-phosphate was added. Substrate coordinates of **24** and **52** were constructed and geometrically-optimized through a Monte Carlo conformational search using MMFF94s force field in Macromodel, version 7.0 (Schrödinger, Inc.). Substrates were docked into the binding pocket based on the coordinates of dC. Gasteiger-Hückel charges and Kollman-All-Atom charge were loaded to the ligands and dCK, respectively, with formal charges (+2) to Mg atom in the active site. And the residues inside 6Å (hot region: 6Å, interesting region: 12 Å) from substrate were annealed until energy change from one iteration to the next was less than 0.05 kcal/mol. The annealed dCK-substrate complex was fully minimized by using the Kollman-All-Atom force field for 5000 iterations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Oxetanocin A, T & G and carbocyclic analogs oxetanocin A & G

Figure 2.

Superimposed structures of adenosine analog **33**, oxetanocin A and TTP where the C3'-C4' bond in adenosine analog **33** is in blue, the C3'-C4' bond in oxetanocin A is in red, and the C4'-C5' bond in TTP is in orange.

Figure 3.

The minimized structures of HIV reverse transcriptase complexed with (a) the triphosphate of cytidine analog **24** and (b) the triphosphate of adenosine analog **33** show that both triphosphates are bound to the active site of HIV RT, interacting with Tyr115 and their own β -phosphate through hydrogen bonding.

Figure 4.

The minimized structures of deoxycytidine kinase (dCK) complexed with (a) the D-cytidine analog (**24**) and (b) the L-cytidine analog (**52**) revealed the increased distance (1.2 Å) between the 4'-hydroxyl group and the γ -phosphate in the case of compound (**52**) which may be responsible for the lower catalytic efficiency of phosphorylation through the nucleophilic attack.

Figure 5.

Steric clashes are observed between compound **52** and Leu82, but are absent in the case of compound **24**.

Scheme 1.**Synthesis of the key intermediate 12**

^a*Reagents and conditions:* a) MsCl, pyr. DMAP, CH₂Cl₂; b) KSAc, DMF; c) NaHCO₃, EtOH/H₂O, reflux; d) 4% TFA, OH⁻ resin; e) NaIO₄, NaBH₄, MeOH; f) TBDPSCl, TEA, DMAP, CH₂Cl₂; g) i. Ac₂O, DMSO, ii. Petasis reagent, THF/Et₂O (1:1), reflux; h) BH₃SMe₂, THF, H₂O₂, 1N NaOH; i) Swern Oxidation, NaOMe, MeOH, NaBH₄; j) TBDPSCl, imidazole, CH₂Cl₂

Scheme 2.

Synthesis of D-thietanose nucleosides

Reagents and conditions: a) mCPBA, CH₂Cl₂; b) pyrimidine or purine, HMDS, CH₃CN, TMSOTf, TEA, ZnI₂, toluene; c) 2,4,6-triisopropylbenzenesulfonyl chloride, TEA, DMAP, CH₃CN, NH₄OH; d) TBAF, THF; e) i. NaN₃, DMF, ii. H₂, Pd(0), MeOH; f) NaOMe, 2-mercaptoethanol, MeOH; g) i. NaOMe, 2-mercaptoethanol, MeOH, ii. NH₃, EtOH, 60°C,

Scheme 3.

Synthesis of L-3'-thio analogs of oxetanocin A

^aReagents and conditions: a) MsCl, pyr. DMAP, CH₂Cl₂; b) KSac, DMF; c) NaHCO₃, EtOH/H₂O, reflux; d) 4% TFA, OH⁻ resin; e) NaIO₄, NaBH₄, MeOH; f) TBDPSCl, TEA, 2,4,6-Collidine, CH₂Cl₂; g) Ac₂O, DMSO; h) Petasis reagent; i) BH₃SMe₂, THF; j) (COCl)₂, DMSO, NaOMe, MeOH; k) NaBH₄, MeOH; l) TBDPSCl, imidazole, CH₂Cl₂.

Scheme 4.

Synthesis of L-thietanose nucleosides

Reagents and conditions: a) mCPBA, CH₂Cl₂; b) pyrimidine or purine, HMDS, CH₃CN, TMSOTf, TEA, ZnI₂, toluene; c) 2,4,6-triisopropylbenzenesulfonyl chloride, TEA, DMAP, CH₃CN, NH₄OH; d) TBAF, THF; e) i. NaN₃, DMF, ii. H₂, Pd(0), MeOH; f) NaOMe, 2-mecaptoethanol, MeOH; g) i. NaOMe, 2-mecaptoethanol, MeOH, ii. NH₃, EtOH, 60°C,

Table 1
Anti-HIV activity of D- and L-3'-thio thietanose nucleoside analogs

Compd	Conf.	Base	HIV (PBM)				Toxicity (IC ₅₀ , μM)		
			EC ₅₀ (μM)	EC ₉₀ (μM)	PBM	CEM	Vero		
22	D	Thymine	>100	>100	>100	>100	>100	>100	>100
23	D	Uracil	6.9	23.1	8.5	4.9	4.3	54.8	>100
24	D	Cytosine	1.3	5.3	≤1.0	19.0	>100	>100	>100
25	D	5-Fluorocytosine	5.8	13.9	<1.0	>100	>100	>100	>100
26	D	5-Chlorocytosine	>100	>100	>100	>100	>100	>100	>100
27	D	5-Bromocytosine	11.5	41.9	>100	1.8	>100	>100	>100
33	D	Adenine	>100	>100	>100	>100	>100	>100	>100
34	D	Hypoxanthine	>100	>100	>100	>100	>100	>100	>100
35	D	Guanine	>100	>100	>100	>100	>100	>100	>100
50	L	Thymine	>100	>100	>100	>100	>100	>100	>100
51	L	Uracil	>100	>100	>100	>100	>100	>100	>100
52	L	Cytosine	14.1	47.6	13.1	45.6	>100	>100	>100
58	L	Adenine	>100	>100	>100	>100	>100	>100	>100
59	L	Hypoxanthine	>100	>100	>100	>100	>100	>100	>100
60	L	Guanine	>100	>100	>100	>100	>100	>100	>100
AZT			0.004	0.01	>100	14.3		29.0	