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Synthesis of enantiomerically pure D- and L-bicyclo[3.1.0]hexenyl carbanucleosides and their antiviral evaluation

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ABSTRACT

Based upon the fact that L-nucleosides have been generally known to be less cytotoxic than D-counterparts, L-bicyclo[3.1.0]hexenyl carbanucleoside derivatives with a fixed *north* conformation were designed and synthesized by employing a novel synthetic strategy starting from (*R*)-epichlorohydrin in order to search for new anti-HIV agents with high potency and less cytotoxicity. A tandem alkylation, γ-lactonization, a chemoselective reduction of ester in the presence of γ-lactone functional group, a RCM reaction, and a Mitsunobu coupling reaction were used as key reactions. D-Counterpart nucleosides were also prepared according to the same synthetic method. Among the synthesized carbanucleosides, D-thymine nucleoside, D-**2** and L-thymine nucleoside, L-**2** exhibited excellent anti-HIV-1 and -2 activities, in MT-4 cells, which were higher than those of ddI, an anti-AIDS drug. Whereas D-**2** exhibited high cytotoxicity in MT-4 cell lines, L-**2** did not show any discernible cytotoxicity in all cell lines tested, reflecting that L-**2** may be a good candidate for an anti-AIDS drug. L-**2** also showed weak anti-HSV-2 activity without cytotoxicity. However, none of the synthesized nucleosides exhibited antiviral activities against RNA viruses including coxsakie, influenza, corona and polio viruses, maybe due to their 2′,3′-dideoxy structure. Potent antiviral effects of D-**2** and L-**2** indicate that nucleosides belonging to a class of D4Ns can be an excellent candidate for anti-DNA virus agents. This research strongly supports L-nucleosides of a class of D4Ns to be a very promising candidate for antiviral agents due to its low cytotoxicity and a good antiviral activity.

1. Introduction

In the 25 years since the initial discovery that acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV), the disease has grown into a global infectious disease.[1] HIV is a pathogenic retrovirus and requires reverse transcriptase (RT) to copy its single-stranded RNA genome into a double-stranded DNA copy for integration in the host cell.[2] Therefore, RT of AIDS virus has been the most important candidate for the treatment of AIDS. In the search for an effective chemotherapeutic agent of HIV infections for targeting RT, initial attempts were focused on the development of 2′,3′-dideoxynucleosides (ddNs) and 2′,3′-didehydro-2′,3′-dideoxynucleosides (d4Ns).[3,4] Among them, the nucleoside RT inhibitors (NRTIs)[5] such as 3′-azido-3′-deoxythymidine (AZT),[6] 2′,3′-dideoxyinosine (ddI),[7] 2′,3′-dideoxycytidine (ddC),[8] L-2′,3′-dideoxy-3′-thiacytidine (3TC)[9] and 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T)[10] have been widely used to treat AIDS patients (Fig. 1).

Particularly, an important structural characteristic of d4T is the presence of a 2′,3′-double bond, which renders the pseudosugar ring to be nearly planar and highly rigid. Other potent NRTIs, such as abacavir (ABC),[11] and 2′,3′-didehydro-2′,3′-dideoxy-2′-fluorocytidine (d4FC),[12] have also a double bond on their sugar ring. In this respect, the 2′,3′-double bond is considered to be crucial for plays in enhancing anti-HIV activity (Fig. 2).

However, oxanucleosides belonging to ddNs and d4Ns, have intrinsic weakness that their glycosidic bond is unstable to acidic conditions involving a gastric environment and to enzymes such as pyrimidine and purine phosphorylases.[13] In spite of the defect, ddNs and d4Ns derivatives have been the most promising candidates for AIDS treatment. To overcome easy cleavage of the glycosidic bond of oxanucleosides, carbocyclic nucleosides,[14] which have a methylene group instead of the ring oxygen atom of tetrahydrofuran in oxanucleosides, have been synthesized with an anticipation of searching for better antiviral profile and lower cytotoxicity with improved stability.

Conformationally rigid methanocarba (MC) nucleosides built on a bicyclo[3.1.0]hexane template have a *south* (*S*, $_3E$)[15] or *north* (*N*, $_2E$)[16] conformation in the pseudorotational cycle,[17] which is a conformation normal nucleosides take for the most time (Fig. 3). When nucleosides bind to the active site of enzymes, each conformer exhibits different affinity. Therefore, rigid MC nucleosides have been used to investigate the conformational preferences of

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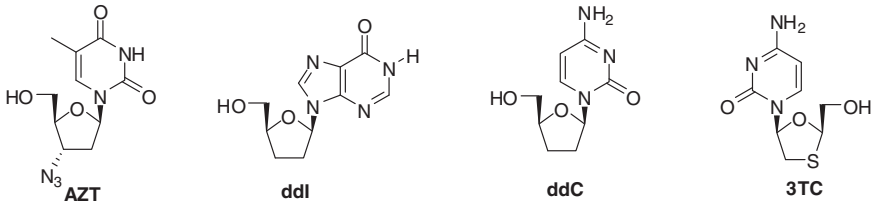


Figure 1. Anti-HIV NRTIs.

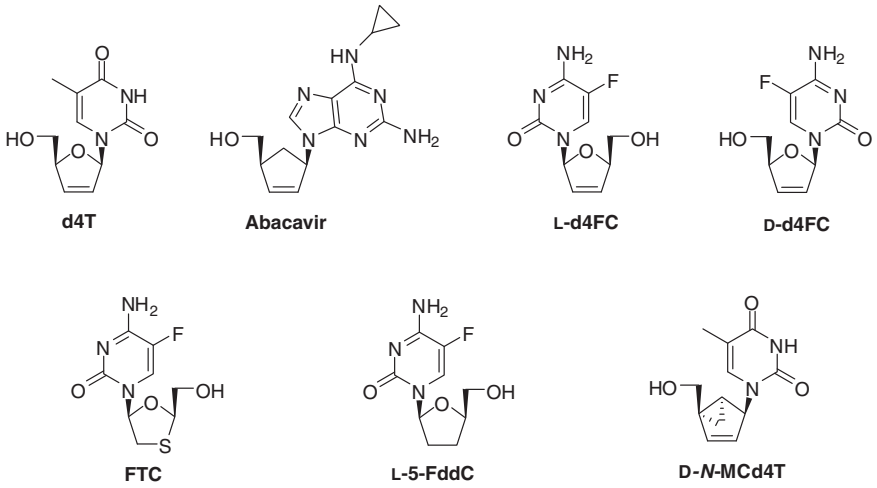


Figure 2. Several potent anti-HIV NRTIs with 2′,3′-double bond or L-type.

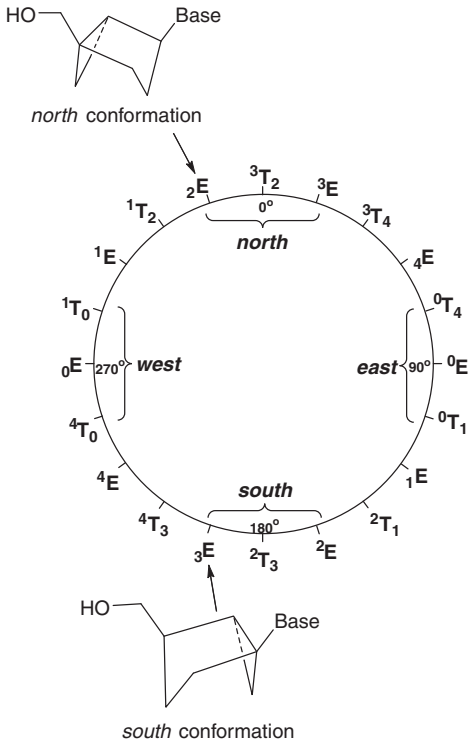


Figure 3. Conformationally locked bicyclo[3.1.0]hexenyl nucleosides in the pseudorotational cycle.

various enzymes associated with nucleoside metabolism and nucleotide polymerization.[18,19]

On the other hand, a number of L-nucleosides such as 3TC, FTC,[20] L-d4FC and L-5-FddC[21] have also been reported to show highly potent anti-HIV activity (Figs. 1 and 2). L-Nucleosides have been generally known to be less cytotoxic than D-counterparts. D-*N*-MCd4T,[22] bearing a locked *north* conformation and a 2′,3′-double bond in a single structure has been synthesized and its anti-HIV activity was compared with that of d4T (Fig. 2). Although D-*N*-MCd4T was less potent than d4T, it showed significant antiviral activity against HIV-1 and HIV-2 with less cytotoxicity in varied cell types, and was stable under conditions that would cleave d4T.

On the basis of these findings, as a part of our ongoing efforts to search for antiviral agents with better potency, less cytotoxicity and improved stability, it was of interest to synthesize conformationally *north*-locked and enantiopure D- and L-bicyclo[3.1.0]hexenyl carbanucleoside derivatives involving L-*N*-MCd4T (L-**2**),[23] and compare antiviral activity of D-carbanucleosides with that of L-carbanucleosides. D- and L-Bicyclo[3.1.0]hexenyl carbanucleoside derivatives with a natural nucleobase were prepared using (*S*)- and (*R*)-epichlorohydrin, respectively, as a starting material and their antiviral activities against HIV-1 and -2, coxsackie B1 and B3 and HSV-1 and -2 were evaluated.

A new strategy for the synthesis of L-bicyclo[3.1.0]hexenyl carbanucleoside analogs (L-**1**–L-**5**) and its D-counterpart were successfully accomplished by using cyclopropanation via a tandem alkylation, γ-lactonization, a chemoselective reduction of ester in the presence of lactone functional group, a Grignard reaction, a ring-closing metathesis (RCM) reaction and a Mitsunobu reaction as the key steps, starting from (*R*)- and (*S*)-epichlorohydrin, respectively.

2. Results and discussion

2.1. Chemistry

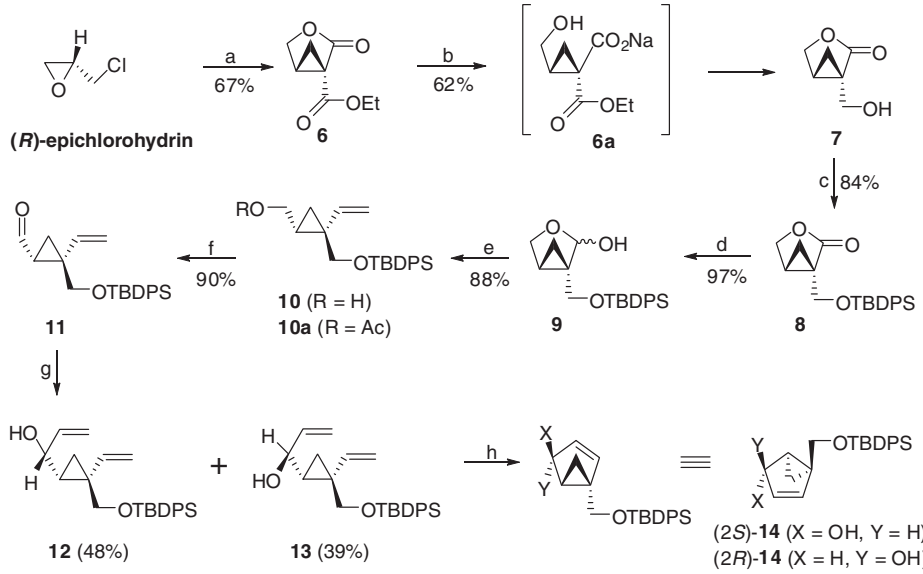
Synthesis of a glycosyl donor with a locked conformation, bicyclo[3.1.0]hexenol (2*S*)-**14** is outlined in Scheme 1. Compound **6**

was synthesized in two steps according to the procedure reported by Tsuji and co-workers.[24,25] Reaction of (*R*)-epichlorohydrin with diethyl malonate and sodium metal gave cyclopropane-fused γ-lactone **6** via tandem alkylation followed by lactonization. The lactone moiety of **6** might be more susceptible to hydrolysis than the ester, maybe due to the structural constraint caused by a fused cyclopropane ring. Treatment of **6** with 1 equiv of sodium hydroxide in EtOH afforded monocarboxylate sodium salt **6a**, the ester of which was selectively reduced by sodium borohydride under reflux and then recyclized back to γ-lactone **7** under acidic conditions. Protection of hydroxyl group of **7** with *tert*-butyldiphenylsilyl chloride produced silyl ether **8**, which was reduced with Dibal-H at −78 °C to afford the corresponding lactol **9** in 97% yield. Wittig reaction of lactol **9** with methyltriphenylphosphonium bromide in the presence of potassium *tert*-butoxide afforded hydroxy olefin **10** in 88% yield after quenching with saturated aqueous NH_4Cl solution. However, when the reaction mixture was partitioned between EtOAc and water without quenching with saturated NH_4Cl aqueous solution after the Wittig reaction, a new byproduct appeared at a higher R_f value than that of the desired compound **10** in 10–20% yield. It turned out to be an acetylated compound **10a** by ^1H, ^{13}C, COSY (Correlation spectroscopy) and HMBC (Heteronuclear multiple bond correlation) NMR experiments. The acetyl group might be derived from ethyl acetate used for extraction due to basic reaction conditions. Swern oxidation of **10** with oxalyl chloride and dimethyl sulfoxide at −78 °C smoothly proceeded to produce aldehyde **11**, which was subjected to a Grignard reaction with vinylmagnesium bromide to afford allylic alcohols **12** (48%) and **13** (39%), easily separated by normal silica gel column chromatography. The stereochemistry of **12** and **13** was not determined until the corresponding bicyclo[3.1.0]hexenol scaffold was formed via a RCM (ring-closure metathesis) reaction in the next step. RCM reaction[26] of dienes **12** and **13** with a second generation Grubbs catalyst produced the bicyclo[3.1.0]hex-3-en-2-ol analogs, (2*R*)-**14** and (2*S*)-**14**, respectively. The stereochemistry of them was confirmed by comparing their ^1H NMR spectra with that of the authentic enantio-counterpart, an enantiomer of (2*S*)-**14**, prepared from a chiral bicyclo[3.1.0]hexane template,[27] indicating that the RCM product (2*S*)-**14** derived from compound **13** has the desired (2*S*)-stereochemistry for a Mitsunobu reaction with nucleobases to yield various conformationally locked nucleosides. (2*R*)-**14** can be also potential starting material for a palladium-mediated coupling reaction with nucleobases to produce the desired β-anomer.

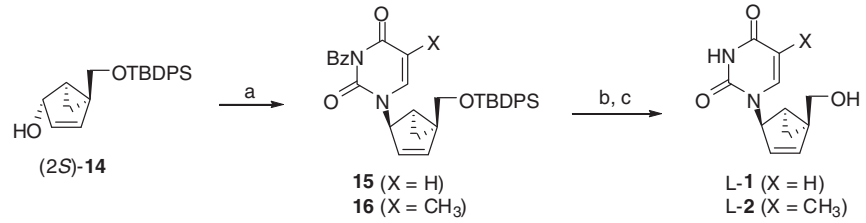
Synthesis of uracil and thymine L-nucleoside derivatives, L-**1** and L-**2** is shown in Scheme 2. Condensation of the glycosyl donor, bicyclo[3.1.0]hexenol (2*S*)-**14** with N^3-benzoyluracil and N^3-benzoylthymine under Mitsunobu conditions[28] smoothly proceeded to give protected N^3-benzoyluracil nucleoside **15** and protected N^3-benzoylthymine nucleoside **16**, respectively, in 21% and 40% yield. Finally, deprotection of N^3-benzoyl group of compounds **15** and **16** with ammonium hydroxide aqueous solution in methanol followed by desilylation with TBAF (*n*-tetrabutylammonium fluoride) gave the desired uracil and thymine L-nucleosides, L-**1** and L-**2**, respectively, in 85% and 91% yield. On the other hand, the corresponding cytosine analog L-**3** was synthesized starting from the uracil nucleoside analog L-**1**, via acetylation of the free hydroxyl group followed by successive three conventional reactions ((i) 1,2,4-triazole, phosphorus oxychloride, pyridine; (ii) ammonium hydroxide, 1,4-dioxane; (iii) methanolic ammonia),[29] in overall 42% yield (Scheme 3).

Synthesis of adenine L-nucleoside L-**4** is depicted in Scheme 4. Under Mitsunobu conditions, coupling of (2*S*)-**14** with 6-chloropurine generated protected 6-chloropurine nucleoside **18** in 21% yield. Amination with methanolic ammonia at 80 °C to adenine nucleoside followed by desilylation of TBDPS group with TBAF gave the final adenine L-nucleoside L-**4** in 44% yield from **18**. For the synthesis of guanine nucleoside L-**5**, condensation of (2*S*)-**14** with 2-amino-6-chloropurine under Mitsunobu conditions afforded protected 2-amino-6-chloropurine nucleoside **19** (Scheme 4), which was subjected to desilylation with 1 M TBAF and conversion of 2-amino-6-chloropurine nucleobase into guanine with 2-mercaptoethanol and 1 N NaOMe under reflux to produce guanine L-nucleoside L-**5**. The yields of each Mitsunobu condensation are generally low, probably because the fixed conformation induced by the fused cyclopropyl group and the double bond causes the backside attack of nucleobases to be disfavorable. In case of the locked conformation, increased steric hindrance between a methinyl hydrogen or a 4′-methylene group and nucleobase is expected.

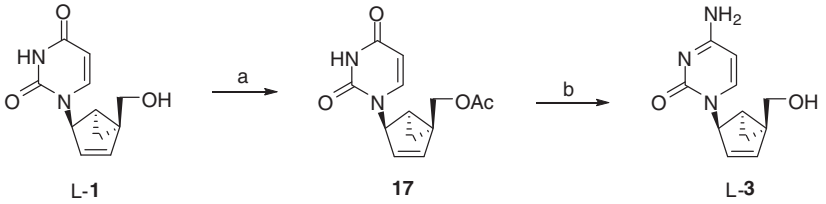
D-Bicyclo[3.1.0]hexenyl carbanucleoside derivatives D-**1**–D-**4** were also synthesized starting from (*S*)-epichlorohydrin (Scheme 5) instead of (*R*)-epichlorohydrin used for the synthesis of



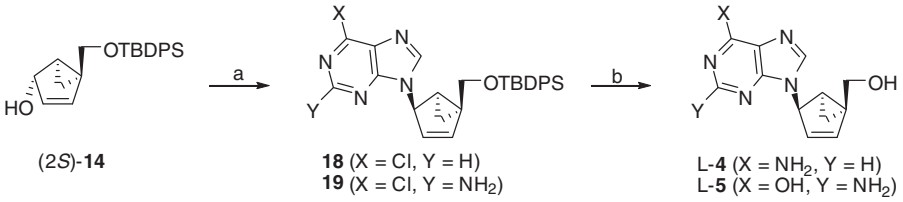
Scheme 1. Reagents and conditions: (a) $(EtO_2C)_2CH_2$, Na, EtOH, 80 °C, 20 h; (b) (i) 1 equiv NaOH, EtOH, rt, 16 h; (ii) $NaBH_4$, reflux, 3 h, then 2 N HCl, rt, 18 h; (c) TBDPSCl, imidazole, CH_2Cl_2, rt, overnight; (d) Dibal-H, CH_2Cl_2, −78 °C, 30 min; (e) CH_3PPh_3Br, *t*-BuOK, THF, rt, 3 h; (f) oxalyl chloride, DMSO, CH_2Cl_2, −78 °C, 1 h, then Et_3N, rt, 1 h; (g) vinylmagnesium bromide, THF, −78 °C, 1 h; (h) 2nd generation Grubbs catalyst, CH_2Cl_2, rt, 1.5 h, 85% for (2*S*)-**14** from **13**, 84% for (2*R*)-**14** from **12**.



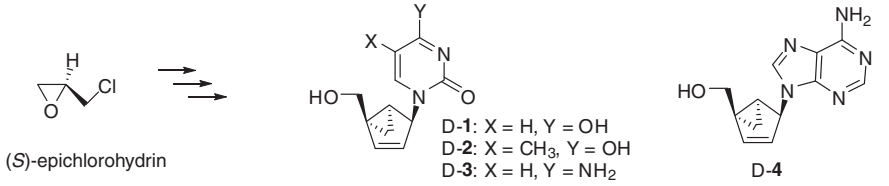
Scheme 2. Reagents and conditions: (a) PPh_3, DEAD, *N*-benzoyluracil or *N*-benzoylthymine, THF, 0 °C, 1 h, 21% for **15**, 28% for **16**; (b) 28% NH_4OH/MeOH, rt, 6 h and then TBAF, THF, rt, 1 h, 82% for **1**, 76% for **2**.



Scheme 3. Reagents and conditions: (a) (i) Ac_2O, pyridine, rt, overnight, 100% from L-**1**; (b) (i) 1,2,4-triazole, $POCl_3$, pyridine, rt, overnight; (ii) 28% NH_4OH, 1,4-dioxane, MeOH, rt, overnight; (iii) NH_3/MeOH, rt, overnight, 42%.



Scheme 4. Reagents and conditions: (a) PPh_3, DEAD, 6-chloropurine or 2-amino-6-chloropurine, THF, 0 °C, 1 h, 21% for **17**, 17% for **18**; (b) NH_3/MeOH, 80 °C, 4 h and then TBAF, THF, rt, 1 h, 44% for L-**4**; TBAF, THF, rt, 1.5 h and then 2-mercaptoethanol, 1 N NaOMe, MeOH, 80 °C, 7 h, 97% for L-**5**.



Scheme 5.

L-nucleosides by employing the same methodology used for the synthesis of L-bicyclo[3.1.0]hexenyl carbanucleosides.

The structures of the synthesized compounds were determined by ^1H, ^{13}C, HMBC and COSY NMR, mass and UV spectrometric analyses.

Antiviral activities with the final conformation-locked D- and L-carbanucleosides were evaluated against HIV-1 (IIIB) and -2 (ROD), coxsackie B1 and B3 viruses, and HSV-1 and -2. First, when tested in MT-4 (HTLV-1 and -2-infected human T lymphocyte) cells, among the final nucleosides thymine nucleoside analogs D-**2** and L-**2** showed the most potent anti-HIV-1 and -2 activity and their IC_{50} values against HIV-1 and -2 were 0.033 and 0.112 μg/mL for D-**2**, and 1.175 and 2.270 μg/mL for L-**2**, respectively (Table 1). The bigger difference in the two strains (IIIB and ROD) in the activity of D-**2** than in that of L-**2** reflects that L-nucleosides might fit almost equally at both the active sites of the two strains, whereas D-nucleosides might fit better at the active site for IIIB strain than that for ROD strain. Comparison of IC_{50} values of D-**2** and L-**2** against HIV-1 and -2 with those (2.31 and 2.76 μg/mL) of ddI, which is clinically used for the treatment of AIDS patients indicates that both thymine nucleosides D-**2** and L-**2** exhibit more potent anti-HIV-1 and -2 activities than ddI used as a positive control. Although anti-HIV activity of D-**2** exceeds that of L-**2**, L-**2** (CC_{50} >100 μg mL^{-1}) is less cytotoxic than D-**2** (CC_{50} = 8.558 μg mL^{-1}), which corresponds to the fact that L-nucleosides have been generally known to be less toxic than D-counterpart. Selective indexes (CC_{50}/EC_{50}) toward HIV-1 and -2 showed 263 and 76, respectively, for D-**2** and more than 85.09 and more than 44.05, respectively, for L-**2**. D-Adenine nucleoside, D-**4** exhibited weak anti-HIV-1 activity (IC_{50} = 40.47 μg mL^{-1}) but does not show anti-HIV-2 activity. D-Cytosine nucleoside, D-**3** showed cytotoxicity-derived anti-HIV-1 and -2 activities. The other nucleoside derivatives showed neither anti-HIV-1 and -2 activities nor cytotoxicity. According to the literature,[16a] bicyclo[3.1.0]hexyl nucleosides with no double bond showed no or weak anti-HIV activity, indicating that the internal double bond is essential for high anti-HIV activity. Other antiviral effects for the final nucleoside derivatives were evaluated against coxsackie B1 and B3 viruses, and poliovirus 3. However, all nucleosides neither exhibited antiviral activities nor cytotoxicity up to 100 μg mL^{-1}. The final nucleosides belong to 2′,3′-didehy-

Table 1
Anti-HIV-1 and -2 activities of D- and L-bicyclo[3.1.0]hexenyl carbanucleoside derivatives

Compound	Toxicity (CC_{50}[a], μg/mL)	HIV (IC_{50}[b], μg/mL)		Selective index[e]	
		IIIB[c]	ROD[d]	IIIB	ROD
L-**1**	>100.00	>100.00	>100.00	ND	ND
L-**2**	>100.00	1.175	2.270	>85.09	>44.05
L-**3**	>100.00	>100.00	>100.00	ND	ND
L-**4**	>100.00	>100.00	>100.00	ND	ND
L-**5**	>100.00	>100.00	>100.00	ND	ND
D-**1**	>100.00	>100.00	>100.00	ND	ND
D-**2**	8.558	0.033	0.112	263	76
D-**3**	61.80	>61.80	>61.80	<1	<1
D-**4**	>100.00	40.47	>100.00	>2.47	ND
ddC	10.66	0.043	0.040	248.73	266.70
ddI	>100.00	2.31	5.76	>43.29	>17.38

[a] 50% cytotoxic concentration.
[b] 50% inhibitory concentration.
[c] HIV-1 strain.
[d] HIV-2 strain.
[e] CC_{50}/EC_{50}.

dro-2′,3′-dideoxynucleosides whereas coxsakie viruses and poliovirus belong to RNA viruses. This point might result in lack of antiviral activities against coxsakie viruses and poliovirus. Antiviral evaluation against HSV-1 and -2 was conducted and only L-thymine nucleoside, L-**2** showed weak anti-HSV-2 activity (IC_{50} = 59.23 μg mL^{-1}) without discernible cytotoxicity. Antiviral inhibition of L-thymine nucleoside, L-**2** was also performed against FluA (influenza virus type A) (H1N1) strain Taiwan, FluA (H3N2) strain Johannesburg, FluB (influenza virus type B) strain Panama, FCV (feline coronavirus) strain WSU, and FIP (feline infectious peritonitis virus) strain WSU, but it neither showed antiviral activity nor cytotoxicity up to 100 μg mL^{-1} (not shown), maybe because both influenza and corona viruses are viruses with a RNA genome. All L-nucleosides did not show any cytotoxicity up to 100 μg mL^{-1} in all cell lines tested.

According to the obtained antiviral and cytotoxic results, D- and L-bicyclo[3.1.0]hexenyl carbanucleosides synthesized exerts their antiviral activity for DNA virus such as HSV-2 and AIDS virus requiring a RNA-dependent DNA polymerization process during its life-cycle and L-nucleoside is less cytotoxic than D-nucleoside (e.g., L-**2** vs D-**2** and L-**4** vs D-**4**).

3. Conclusions

In order to search for new anti-HIV agents with high potency and less cytotoxicity, D- and L-bicyclo[3.1.0]hexenyl carbanucleoside derivatives were designed and synthesized by a novel synthetic strategy starting from (*S*)- and (*R*)-epichlorohydrin, respectively. A tandem alkylation, γ-lactonization, a chemoselective reduction of ester in the presence of γ-lactone functional group, a Grignard reaction, a RCM reaction, and a Mitsunobu coupling reaction were used as key reactions to achieve the synthesis of *north* conformation-locked D- and L-carbanucleoside analogs. The synthesized bicyclic carbanucleosides were characterized by ^1H, ^{13}C, HMBC, HSQC and COSY NMR, mass and UV spectra, melting point and optical rotation.

D-Thymine nucleoside, D-**2** and L-thymine nucleoside, L-2 were found to show more potent anti-HIV-1 and -2 activities than ddI in MT-4 cells. Whereas D-**2** exhibited cytotoxicity (CC_{50} = 8.558 μg mL^{-1}), L-**2** did not show any discernible cytotoxicity, suggesting that L-**2** may be a better candidate for the development of novel anti-AIDS drug than D-**2**. L-**2** was also found to exhibit weak anti-HSV-2 activity without cytotoxicity. In addition, antiviral activities against RNA viruses such as coxsakie viruses, and poliovirus were tested, but all compounds did not show any significant antiviral activities, maybe due to a 2′,3′-dideoxy structure of the compounds. L-**2** was also evaluated against other RNA viruses such as influenza and corona viruses, but showed neither antiviral activity nor cytotoxicity. It is interesting to note that all L-nucleosides did not show cytotoxicity up to 100 μg mL^{-1} in all cells tested. Probably, the low cytotoxicity of L-nucleosides may be closely associated with a L-type, an unnatural type. Potent antiviral activities of D- and L-thymine carbanucleosides, D-**2** and L-**2** indicate that a nucleoside belonging to a class of D4Ns can be an excellent candidate for anti-AIDS drug and anti-DNA virus agents. This research strongly supports L-nucleosides of a class of D4Ns to be a very promising candidate for antiviral agents due to its low cytotoxicity.

4. Experimental section

4.1. General methods

All chemical reagents were commercially available. Melting points are uncorrected. Ultraviolet (UV) spectra were recorded in methylene chloride or MeOH on a JASCO V-530 UV–vis spectrophotometer. Optical rotations were measured in chloroform, MeOH or DMF on a JASCO DIP-370 digital polarimeter. Nuclear Magnetic Resonance (NMR) data were recorded on a Bruker AC 200, Varian Unity INOVA 400 spectrometer and Varian Unity AS 500 spectrometer, using $CDCl_3$ or CD_3OD and chemical shifts were reported in parts per million (ppm) with reference to the respective residual solvent or deuterated peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 7.26 and δ_C 77.0 for $CDCl_3$). Coupling constants are reported in hertz. The abbreviations used are as follows: s (singlet), d (doublet), q (quartet), qd (quartet of doublets), m (multiplet), dd (doublet of doublets), br s (broad singlet), or br d (broad doublet). All the reactions described below were performed under nitrogen or argon atmosphere and monitored by thin-layer chromatography (TLC). TLC was performed on Merck precoated 60 F_{254} plates. Column chromatography was performed using Silica Gel 60 (230–400 mesh, Merck). All anhydrous solvents were distilled over CaH_2 or Na/benzophenone prior to use.

4.1.1. (−)-(1*S*,5*R*)-Ethyl 2-oxo-3-oxabicyclo[3.1.0]hexane-1-carboxylate (6)

Sodium (2.42 g, 105 mmol) was carefully dissolved in EtOH (195 mL) at 0 °C, and diethyl malonate (16.7 mL, 110 mmol) was added at 0 °C over 5 min to the solution. (*R*)-(−)-Epichlorohydrin

(7.8 mL, 100 mmol) in EtOH (5 mL) was added dropwise to the solution at room temperature over 1 h, and the reaction mixture was refluxed for 20 h. The mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was dissolved in methylene chloride and washed with H_2O. The organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexanes and ethyl acetate (3:1) as the eluent to give cyclopropane-fused lactone **6** (11.40 g, 67%) as a colorless oil: $[\alpha]_D^{25}$ −121.44 (*c* 1.32, $CHCl_3$), (lit.[24] $[\alpha]_D^{25}$ −146.58 (*c* 1.22, EtOH)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.32 (dd, 1H, *J* = 4.8, 9.2 Hz, 4-*H*H), 4.22 (qd, 2H, *J* = 2.4, 6.8 Hz, CH_2), 4.15 (d, 1H, *J* = 9.2 Hz, 4-H*H*), 2.70 (m, 1H, 5-H), 2.04 (dd, 1H, *J* = 4.8, 8.4 Hz, 6-*H*H), 1.34 (t, 1H, *J* = 4.8 Hz, 6-H*H*), 1.27 (t, 3H, *J* = 6.8 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.76, 166.90, 67.21, 62.22, 29.56, 28.12, 20.93, 14.28; *m*/*z* (FAB+) 170 (M^+).

4.1.2. (−)-(1*S*,5*R*)-1-(Hydroxymethyl)-3-oxa-bicyclo[3.1.0] hexan-2-one (7)

A solution of **6** (10.56 g, 62.04 mmol) in EtOH (204 mL) was treated with sodium hydroxide (2.48 g, 62.04 mmol) in EtOH (204 mL). After stirring for 16 h at room temperature, the reaction mixture was treated with sodium borohydride (11.74 g, 310.21 mmol) and refluxed for 3 h. After cooling to room temperature, 2 N HCl (186 mL) was added slowly at 0 °C. EtOH in the reaction mixture was evaporated off under reduced pressure and to the resulting solution was added 2 N HCl (408 mL). After being stirred for 18 h at room temperature, the aqueous layer was extracted with methylene chloride several times. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography using methylene chloride and MeOH (20:1) as the eluent to give lactone **7** (4.97 g, 62%) as a colorless oil: $[\alpha]_D^{25}$ −71.81 (*c* 2.07, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.30 (dd, 1H, *J* = 4.8, 9.2 Hz, 4-*H*H), 4.13 (d, 1H, *J* = 9.6 Hz, 4-H*H*), 4.06 (d, 1H, *J* = 12.0 Hz, C*H*HOH), 3.57 (d, 1H, *J* = 12.4 Hz, CH*H*OH), 3.41 (br s, 1H, O*H*), 2.28 (m, 1H, 5-H), 1.28 (dd, 1H, *J* = 4.8, 7.6 Hz, 6-*H*H), 0.94 (t, 1H, *J* = 5.2 Hz, 6-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.72, 69.13, 60.65, 30.78, 22.01, 16.45.

4.1.3. (1*S*,5*R*)-(−)-1-(*tert*-Butyl-diphenyl-silanyloxymethyl)-3-oxa-bicyclo[3.1.0]hexan-2-one (8)

To a solution of **7** (4.70 g, 36.66 mmol) and imidazole (4.99 g, 73.31 mmol) in anhydrous methylene chloride (70 mL) was added *tert*-butyldiphenylsilyl chloride (9.38 mL, 36.66 mmol) dropwise at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was extracted with methylene chloride. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using hexanes and ethyl acetate (7:1) as the eluent to give silyl ether **8** (11.35 g, 84%) as a white solid: mp 61.5–63.3 °C; $[\alpha]_D^{25}$ −29.09 (*c* 1.39, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.68–7.38 (m, 10H, 2 × Ph), 4.34 (d, 1H, *J* = 10.8 Hz, TBDPSOC*H*H), 4.25 (dd, 1H, *J* = 4.4, 9.2 Hz, TBDPSOCH*H*), 4.13 (d, 1H, *J* = 9.2 Hz, 4-*H*H), 3.68 (d, 1H, *J* = 11.0 Hz, 4-H*H*), 2.10 (m, 1H, 5-H), 1.32 (dd, 1H, *J* = 4.8, 7.6 Hz, 6-*H*H), 1.04 (s, 9H, *tert*-butyl), 0.87 (t, 1H, *J* = 4.8 Hz, 6-H*H*); ^{13}C NMR (50 MHz, $CDCl_3$) δ 176.28, 135.70, 135.62, 133.36, 133.06, 129.97, 129.95, 127.88, 68.60, 60.57, 30.37, 26.90, 21.22, 19.33, 15.28; *m*/*z* (ESI) 389 (M+Na)$^+$.

4.1.4. (1*S*,5*R*)-1-(*tert*-Butyl-diphenyl-silanyloxymethyl)-3-oxa-bicyclo[3.1.0]hexan-2-ol (9)

To a stirred solution of **8** (14.02 g, 38.24 mmol) in anhydrous methylene chloride (200 mL) was added diisobutylaluminum hydride (Dibal-H, 42.06 mL, 42.06 mmol, 1.0 M solution in hexanes) at −78 °C, and the reaction mixture was stirred for 30 min at the same temperature. MeOH (42.06 mL), hexanes (84.12 mL) and ethyl acetate (84.12 mL) were added successively and the resulting mixture was stirred overnight, allowing it to reach room temperature. The generated gel was filtered off through a pad of Celite, and the filtrate collected was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using hexanes and ethyl acetate (4:1) as the eluent to give lactol **9** (13.67 g, 97%) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.72–7.37 (m, 10H, 2 × Ph), 5.32 (s, 1H, 2-H), 4.18 (d, 1H, *J* = 11.2 Hz, TBDPSOC*H*H), 4.12 (dd, 1H, *J* = 3.2, 8.4 Hz, 4-*H*H), 3.77 (d, 1H, *J* = 8.4 Hz, 4-H*H*), 3.67 (d, 1H, *J* = 11.2 Hz, TBDPSOCH*H*), 1.47 (m, 1H, 5-H), 1.09 (s, 9H, *tert*-butyl), 0.60–0.50 (m, 2H, 6-H_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 135.95, 133.25, 133.18, 130.32, 130.27, 128.18, 100.09, 67.79, 64.59, 34.21, 27.18, 20.51, 19.50, 13.04; *m*/*z* (ESI) 391 (M+Na)$^+$.

4.1.5. (1*R*,2*S*)-(−)-2-[(*tert*-Butyl-diphenyl-silanyloxymethyl)-2-vinylcyclopropyl]methanol (10)

To a stirred suspension of methyltriphenylphosphonium bromide (8.92 g, 24.96 mmol) in anhydrous THF (100 mL) was added potassium *tert*-butoxide (2.80 g, 24.96 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h to give a yellow suspension. The lactol **9** (4.60 g, 12.48 mmol) in anhydrous THF (30 mL) was added dropwise at 0 °C, and the reaction mixture was allowed to reach room temperature. After being stirred for an additional 3 h, it was treated with saturated aqueous NH_4Cl solution (30 mL). The aqueous layer was extracted with methylene chloride and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by silica gel column chromatography using hexanes and ethyl acetate (3:1) as the eluent to give hydroxyl olefin **10** (4.03 g, 88%) as a colorless oil: $[\alpha]_D^{25}$ −37.24 (*c* 1.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68–7.39 (m, 10H, 2 × Ph), 5.95 (dd, 1H, *J* = 11.0, 17.5 Hz, –C*H*=CH_2), 5.20 (d, 1H, *J* = 11.0 Hz, –CH=C*H*H), 5.17 (d, 1H, *J* = 17.5 Hz, –CH=CH*H*), 3.75 (d, 1H, *J* = 10.0 Hz, TBDPSOC*H*H), 3.72 (dd, 1H, *J* = 6.0, 11.0 Hz, C*H*HOH), 3.57 (d, 1H, *J* = 10.5 Hz, TBDPSOCH*H*), 3.44 (dd, 1H, *J* = 9.0, 12.0 Hz, CH*H*OH), 1.33 (m, 1H, 1-H), 1.07 (s, 9H, *tert*-butyl), 0.84 (dd, 1H, *J* = 5.0, 8.5 Hz, 3-*H*H), 0.68 (t, 1H, *J* = 5.5 Hz, 3-H*H*); ^{13}C NMR (50 MHz, $CDCl_3$) δ 136.85, 135.73, 133.74, 133.71, 129.72, 129.70, 127.71, 115.99, 68.62, 62.67, 29.44, 26.97, 25.53, 19.40, 13.70; *m*/*z* (FAB+) 349 (M−H_2O)$^+$.

4.1.6. (1*R*,2*S*)-2-[(*tert*-Buthyl-diphenyl-silanyloxymethyl)-2-vinylcyclopropyl]methylacetate (10a)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68–7.39 (m, 10H, 2 × Ph), 5.96 (dd, 1H, *J* = 10.5, 17.5 Hz, –C*H*=CH_2), 5.19 (dd, 1H, *J* = 1.5, 10.5 Hz, –CH=C*H*H), 5.13 (dd, 1H, *J* = 1.5, 17.5 Hz, –CH=CH*H*), 4.17 (dd, 1H, *J* = 7.0, 12.0 Hz, AcOC*H*H), 3.85 (dd, 1H, *J* = 9.0, 11.0 Hz, AcOCH*H*), 3.73 (d, 1H, *J* = 10.0 Hz, TBDPSOC*H*H), 3.55 (d, 1H, *J* = 10.0 Hz, TBDPSOCH*H*), 2.06 (s, 3H, $COCH_3$), 1.35 (m, 1H, 1-H), 1.07 (s, 9H, *tert*-butyl), 0.83 (dd, 1H, *J* = 5.5, 8.0 Hz, 3-*H*H), 0.70 (t, 1H, *J* = 5.0 Hz, 3-H*H*); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.10, 136.25, 135.64, 133.67, 129.62, 127.62, 116.37, 68.56, 64.67, 29.88, 26.84, 21.09, 21.00, 19.35, 12.83.

4.1.7. (1*R*,2*S*)-(−)-2-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2-vinylcyclopropanecarbaldehyde (11)

To a stirred solution of oxalyl chloride (1.63 mL, 18.68 mmol) in anhydrous methylene chloride (100 mL) was added dimethyl sulfoxide (2.81 mL, 39.60 mmol) in anhydrous methylene chloride (10 mL) at −78 °C, and the mixture was stirred at the same temperature for 20 min. To this mixture was added a solution of **10** (4.03 g, 11.00 mmol) in anhydrous methylene chloride (50 mL), and the reaction mixture was stirred at −78 °C for 1 h. After the addition of triethylamine (10.58 mL, 75.91 mmol) at −78 °C, the mixture was gradually warmed to room temperature and stirred for 1 h.

The reaction mixture was quenched with saturated aqueous NH_4Cl solution (50 mL) and then extracted with methylene chloride. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using hexanes and ethyl acetate (15:1) as the eluent to give aldehyde **11** (3.60 g, 90%) as a colorless oil: $[\alpha]_D^{25}$ −36.81 (*c* 1.06, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.12 (d, 1H, *J* = 6.0 Hz, CHO), 7.67–7.35 (m, 10H, 2 × Ph), 5.91 (dd, 1H, *J* = 10.4, 17.5 Hz, –C*H*=CH_2), 5.26 (d, 1H, *J* = 2.8 Hz, –CH=C*H*H), 5.19 (dd, 1H, *J* = 1.2, 3.2 Hz, –CH=CH*H*), 3.81 (d, 1H, *J* = 10.4 Hz, TBDPSOC*H*H), 3.65 (d, 1H, *J* = 10.4 Hz, TBDPSOCH*H*), 2.13 (m, 1H, 1-H), 1.65 (t, 1H, *J* = 5.2 Hz, 3-*H*H), 1.46 (dd, 1H, *J* = 5.2, 8.4 Hz, 3-H*H*), 1.05 (s, 9H, *tert*-butyl); ^{13}C NMR (50 MHz, $CDCl_3$) δ 135.97, 134.95, 133.47, 133.45, 130.22, 128.16, 118.07, 66.27, 36.40, 33.44, 27.21, 19.69, 16.53; *m*/*z* (ESI) 365 $(M+H)^+$.

4.1.8. (1*R*)-(−)-1-[(1*R*,2*S*)-2-(*tert*-Butyl-diphenyl-silanyloxy methyl)-2-vinylcyclopropyl]prop-2-en-1-ol (12) and (1*S*)-(−)-1-[(1*R*,2*S*)-2-(*tert*-butyl-diphenyl-silanyloxymethyl)-2-vinylcyclo propyl]prop-2-en-1-ol (13)

To a stirred solution of **11** (3.60 g, 9.88 mmol) in anhydrous THF (40 mL) was added dropwise vinylmagnesium bromide (14.81 mL, 14.81 mmol, 1.0 M solution in THF) at −78 °C, and the reaction mixture was stirred at the same temperature for 1 h. After the mixture was quenched with saturated aqueous NH_4Cl solution (20 mL), the mixture was allowed to warm to room temperature and then extracted with ether. The organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting oil was purified by silica gel column chromatography using hexanes and ethyl acetate (18:1→5:1) as the eluent to give allylic alcohols **12** (1.86 g, 48%) and **13** (1.51 g, 39%) as a colorless oil, respectively. Compound **12** (more polar material): $[\alpha]_D^{25}$ −22.80 (*c* 1.06, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.69–7.35 (m, 10H, 2 × Ph), 6.06–5.87 (m, 2H, 2 × –C*H*=CH_2), 5.37–5.09 (m, 4H, 2 × –CH=CH_2), 3.75 (d, 1H, *J* = 10.4 Hz, TBDPSOC*H*H), 3.75–3.66 (m, 1H, C*H*OH), 3.50 (d, 1H, *J* = 10.4 Hz, TBDPSOCH*H*), 1.25–1.13 (m, 1H, 1-H), 1.07 (s, 9H, *tert*-butyl), 0.89 (s, 1H, 3-*H*H), 0.85 (s, 1H, 3-H*H*); ^{13}C NMR (50 MHz, $CDCl_3$) δ 140.53, 137.33, 136.03, 133.95, 130.02, 130.01, 128.01, 116.32, 114.54, 73.48, 68.93, 30.11, 29.23, 27.23, 19.71, 13.62; *m*/*z* (ESI) 415 (83, $M+Na)^+$. Compound **13** (less polar material): $[\alpha]_D^{25}$ −55.94 (*c* 1.84, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.71–7.36 (m, 10H, 2 × Ph), 6.13–5.90 (m, 2H, 2 × –C*H*=CH_2), 5.33–5.09 (m, 4H, 2 × –CH=CH_2), 3.76 (d, 1H, *J* = 10.4 Hz, TBDPSOC*H*H), 3.67–3.65 (m, 1H, C*H*OH), 3.58 (d, 1H, *J* = 10.4 Hz, TBDPSOCH*H*), 1.23–1.11 (m, 1H, 1-H), 1.09 (s, 9H, *tert*-butyl), 0.86 (dd, 1H, *J* = 5.2, 8.8 Hz, 3-*H*H), 0.72 (t, 1H, *J* = 5.2 Hz, 3-H*H*); ^{13}C NMR (50 MHz, $CDCl_3$) δ 140.06, 137.28, 136.07, 136.05, 134.02, 133.96, 130.05, 130.02, 128.03, 128.02, 116.63, 114.67, 73.45, 69.00, 30.35, 30.20, 27.27, 19.70, 14.19; *m*/*z* (ESI) 415 (60, $M+Na)^+$.

4.1.9. (1*R*,2*S*,5*S*)-(+)-5-(*tert*-Butyl-diphenyl-silanyloxymethyl)-bicyclo[3.1.0]hex-3-en-2-ol ((2*S*)-14) and (1*R*,2*R*,5*S*)-(−)-5-(*tert*-butyl-diphenyl-silanyloxymethyl)-bicyclo[3.1.0]-hex-3-en-2-ol ((2*R*)-14)

Compound (2*S*)-**14** : To a stirred solution of **13** (1.51 g, 3.85 mmol) in anhydrous methylene chloride (40 mL) was added Grubbs catalyst 2nd generation (229 mg, 0.07 mmol) at 0 °C, and the reaction mixture was stirred for 1.5 h at room temperature. After the volatiles were removed, the resulting residue was purified by column chromatography using hexanes and ethyl acetate (4:1) as the eluent to give bicyclo[3.1.0]hexenol (2*S*)-**14** (1.19 g, 85%) as a colorless oil: $[\alpha]_D^{25}$ +10.30 (*c* 1.13, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68–7.38 (m, 10H, 2 × Ph), 6.03 (d, 1H, *J* = 5.5 Hz, 3-H), 5.30 (d, 1H, *J* = 5.5 Hz, 4-H), 5.22 (d, 1H, *J* = 7.0 Hz, 2-H), 3.85 (d, 1H, *J* = 10.5 Hz, TBDPSOC*H*H), 3.76 (d, 1H, *J* = 10.5 Hz, TBDPSOCH*H*), 1.58 (m, 1H, 1-H), 1.06 (s, 9H, *tert*-butyl), 0.73 (dd, 1H, *J* = 3.5, 7.5 Hz, 6-*H*H), 0.65 (t, 1H, *J* = 4.0 Hz, 6-H*H*); ^{13}C NMR (50 MHz, $CDCl_3$) δ 133.62, 131.24, 131.22, 129.56, 129.51, 126.78, 125.62, 123.74, 77.06, 64.82, 40.30, 29.26, 25.45, 22.99, 22.19; *m*/*z* (EI) 346 (7, $M-H_2O)^+$. Compound (2*R*)-**14** : (2*R*)-**14** was synthesized in 84% yield using similar procedure to the synthesis of (2*S*)-**14**: $[\alpha]_D^{25}$ −30.90 (*c* 1.05, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70–7.39 (m, 10H, 2 × Ph), 6.21 (d, 1H, *J* = 5.5 Hz, 3-H), 5.57 (br d, 1H, *J* = 5.5 Hz, 4-H), 4.39 (d, 1H, *J* = 1.5 Hz, 2-H), 3.97 (d, 1H, *J* = 11.0 Hz, TBDPSOC*H*H), 3.74 (d, 1H, *J* = 11.5 Hz, TBDPSOCH*H*), 1.64 (dd, 1H, *J* = 4.0, 8.5 Hz, 1-H), 1.08 (s, 9H, *tert*-butyl), 1.00 (dd, 1H, *J* = 4.0, 8.0 Hz, 6-*H*H), 0.20 (t, 1H, *J* = 4.0 Hz, 6-H*H*); ^{13}C NMR (50 MHz, $CDCl_3$) δ 140.37, 135.81, 135.76, 133.98, 133.87, 130.11, 129.83, 127.82, 127.81, 77.58, 65.11, 37.16, 30.05, 27.04, 25.53, 19.46; *m*/*z* (EI) 346 (12, $M-H_2O)^+$.

4.1.10. (−)-3-Benzoyl-1-[(1*R*,2*R*,5*S*)-5-(*tert*-butyl-diphenyl-silanyloxymethyl)bicyclo[3.1.0]hex-3-en-2-yl]pyrimidine-2,4(1*H*,3*H*)-dione (15)

To a stirred solution of (2*S*)-**14** (163.7 mg, 0.44 mmol), triphenyl phosphine (294.5 mg, 1.12 mmol), and N^3-benzoyluracil (145.6 mg, 0.67 mmol) in anhydrous THF (5 mL) was added DEAD (diethyl azodicarboxylate, 0.18 mL, 1.12 mmol) dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. After the volatiles were removed in vacuo, the resulting residue was purified by silica gel column chromatography using hexanes and ethyl acetate (6:1) as the eluent to give N^3-benzoyluracil nucleoside **15** (52.5 mg, 21%) as a colorless sticky oil: UV (CH_2Cl_2) λ_{max} 254 nm; $[\alpha]_D^{25}$ −55.22 (*c* 1.13, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00–7.42 (m, 15H, 3 × Ph), 7.55 (d, 1H, *J* = 8.0 Hz, H-6), 6.43 (d, 1H, *J* = 5.5 Hz, 4-H), 5.59 (d, 1H, *J* = 8.5 Hz, H-5), 5.53 (br s, 1H, 2-H), 5.45 (m, 1H, 3-H), 4.38 (d, 1H, *J* = 11.5 Hz, TBDPSOC*H*H), 3.43 (d, 1H, *J* = 11.0 Hz, TBDPSOCH*H*), 1.68 (m, 1H, 1-H), 1.12 (s, 9H, *tert*-butyl), 1.07 (dd, 1H, *J* = 4.5, 8.0 Hz, 6-*H*H), 0.46 (t, 1H, *J* = 4.5 Hz, 6-H*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.37, 162.53, 150.02, 143.62, 141.93, 135.88, 135.71, 135.28, 133.38, 133.16, 131.88, 130.77, 130.34, 130.27, 129.39, 128.17, 128.14, 126.25, 102.80, 65.72, 60.76, 38.74, 28.15, 27.19, 24.18, 19.58; LRMS (FAB+) *m*/*z* 563 (20, $M+H)^+$, 585 (10, $M+Na)^+$; HRMS (FAB+) calcd for $C_{34}H_{35}N_2O_4Si$ $(M+H)^+$: 563.2366, found: 563.2386.

4.1.11. (−)-1-[(1*R*,2*R*,5*S*)-5-(Hydroxymethyl)bicyclo[3.1.0]hex-3-en-2-yl]pyrimidine-2,4(1*H*,3*H*)-dione (ι-1)

A solution of **15** (68.7 mg, 0.12 mmol) in MeOH (4 mL) was treated with concentrated aqueous ammonium hydroxide solution (28%, 0.5 mL) and stirred at room temperature for 6 h. After evaporation under reduced pressure, the residue was purified by silica gel chromatography using hexanes and ethyl acetate (2:1) as the eluent to give TBDPS-protected uracil nucleoside (54.4 mg, 97%) as a sticky oil: UV (CH_2Cl_2) λ_{max} 267 nm; $[\alpha]_D^{25}$ −69.51 (*c* 1.45, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (br s, 1H, NH), 7.64–7.35 (m, 10H, 2 × Ph), 7.47 (d, 1H, *J* = 8.4 Hz, H-6), 6.34 (d, 1H, *J* = 5.2 Hz, 4-H), 5.52 (br s, 1H, 2-H), 5.47 (dd, 1H, *J* = 1.6, 8.0 Hz, H-5), 5.36 (m, 1H, 3-H), 4.30 (d, 1H, *J* = 11.2 Hz, TBDPSOC*H*H), 3.36 (d, 1H, *J* = 11.2 Hz, TBDPSOCH*H*), 1.57 (m, 1H, 1-H), 1.05 (s, 9H, *tert*-butyl), 1.01 (dd, 1H, *J* = 4.8, 8.8 Hz, 6-*H*H), 0.40 (t, 1H, *J* = 4.4 Hz, 6-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.95, 151.25, 143.16, 142.14, 135.82, 135.65, 133.35, 133.14, 130.24, 130.17, 128.08, 128.04, 126.42, 102.88, 65.64, 60.39, 38.60, 28.14, 27.10, 24.06, 19.50; LRMS (FAB+) *m*/*z* 459 (7, $M+H)^+$, 481 (3, $M+Na)^+$; HRMS (FAB+) calcd for $C_{27}H_{31}N_2O_3Si$ $(M+H)^+$: 459.2104, found: 459.2098. To a stirred solution of TBDPS-protected uracil nucleoside (45 mg, 0.10 mmol) in THF (2 mL) was added TBAF (*n*-tetrabutylammonium fluoride, 0.12 mL, 0.12 mmol, 1 M solution in THF). And the reaction mixture was stirred at room temperature for 1 h. After the reaction mixture was concentrated in vacuo, the

resulting residue was purified by silica gel column chromatography using methylene chloride and MeOH (15:1) as the eluent to give the final uracil nucleoside L-**1** (18.2 mg, 85%) as a white solid: mp 151.2–152.2 °C; UV (MeOH) λ_{max} 266 nm; $[\alpha]_D^{25}$ −125.08 (*c* 1.17, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (d, 1H, *J* = 8.0 Hz, H-6), 6.46 (d, 1H, *J* = 4.4 Hz, 4-H), 5.64 (d, 1H, *J* = 7.6 Hz, H-5), 5.39–5.37 (m, 2H, 2-H, 3-H), 4.04 (d, 1H, *J* = 12.0 Hz, C*H*HOH), 3.43 (d, 1H, *J* = 11.6 Hz, CH*H*OH), 1.70 (m, 1H, 1-H), 1.15 (dd, 1H, *J* = 4.4, 8.8 Hz, 6-*H*H), 0.44 (t, 1H, *J* = 4.4 Hz, 6-H*H*); ^{13}C NMR (400 MHz, CD_3OD) δ 165.17, 151.40, 143.24, 142.66, 125.68, 101.43, 62.95, 60.90, 38.82, 27.62, 23.81; LRMS (FAB+) *m*/*z* 221 (5, M+H)$^+$; HRMS (FAB+) calcd for $C_{11}H_{13}N_2O_3$ (M+H)$^+$: 221.0926, found: 221.0916.

4.1.12. (−)-4-Amino-1-[(1*R*,2*R*,5*S*)-5-(hydroxymethyl)bicyclo[3.1.0]hex-3-en-2-yl]pyrimidin-2(1*H*)-one (L-3)

To a stirred solution of uracil nucleoside **1** (18.7 mg, 0.08 mmol) in anhydrous pyridine (2 mL) was added acetic anhydride (0.016 mL, 0.17 mmol) at room temperature, and the reaction mixture was stirred at the same temperature overnight. The reaction mixture was evaporated in vacuo and partitioned between ethyl acetate and dilute aqueous HCl solution. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using methylene chloride and MeOH (30:1) as the eluent to give the acetylated nucleoside **17** (22.3 mg, 100%) as a sticky oil. To a stirred suspension of the acetylated nucleoside **17** (22.3 mg, 0.09 mmol) and 1,2,4-triazole (93.6 mg, 1.28 mmol) in anhydrous pyridine (2 mL) was added phosphorus oxychloride (0.084 mL, 8.50 mmol) at 0 °C, and the reaction mixture was stirred at room temperature overnight. After water (0.02 mL) was added slowly to the reaction mixture to destroy the excess phosphorus oxychloride the volatiles were evaporated in vacuo. 1,4-Dioxane (3 mL) and concentrated aqueous ammonium hydroxide solution (28%, 1.5 mL) were added to the reaction mixture at 0 °C and the reaction mixture was stirred at room temperature overnight. After the volatiles were evaporated under reduced pressure, methanol (1.5 mL) and methanolic ammonia (1.5 mL) was added to the resulting residue, and the reaction mixture was stirred at room temperature overnight. After the volatiles were removed in vacuo, the resulting residue was purified by silica gel column chromatography using methylene chloride and MeOH (7:1) as the eluent to give the final cytosine nucleoside L-**3** (7.9 mg, 42%) as a light brownish solid: mp 194.3–195.2 °C dec.; UV (MeOH) λ_{max} 274 nm; $[\alpha]_D^{25}$ −105.27 (*c* 0.46, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.50 (d, 1H, *J* = 7.2 Hz, H-6), 6.43 (d, 1H, *J* = 5.6 Hz, 4-H), 5.85 (d, 1H, *J* = 7.2 Hz, H-5), 5.48 (br s, 1H, 2-H), 5.39–5.37 (m, 1H, 3-H), 4.04 (d, 1H, *J* = 12.0 Hz, C*H*HOH), 3.42 (d, 1H, *J* = 12.4 Hz, CH*H*OH), 1.66 (m, 1H, 1-H), 1.14 (dd, 1H, *J* = 4.4, 8.8 Hz, 6-*H*H), 0.44 (t, 1H, *J* = 4.0 Hz, 6-H*H*); ^{13}C NMR (100 MHz, CD_3OD) δ 166.15, 157.47, 142.98, 142.66, 126.34, 95.00, 63.08, 61.77, 38.72, 28.23, 23.83; LRMS (FAB+) *m*/*z* 220 (5, M+H)$^+$, 242 (4, M+Na)$^+$; HRMS (FAB+) calcd for $C_{11}H_{14}N_3O_2$ (M+H)$^+$: 220.1086, found: 220.1095.

4.1.13. (−)-3-Benzoyl-1-[(1*R*,2*R*,5*S*)-5-(*tert*-butyl-diphenylsilanyloxymethyl) bicyclo[3.1.0]hex-3-en-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (16)

A solution of triphenylphosphine (427.7 mg, 1.63 mmol) in anhydrous THF (5 mL) was treated with DEAD (0.28 mL, 1.61 mmol) dropwise at 0 °C. After the mixture was stirred for 30 min at 0 °C, a suspension of (2*S*)-**14** (237.8 mg, 0.65 mmol) and N^3-benzoylthymine (225.3 mg, 0.98 mmol) in THF (15 mL) was added. The reaction mixture was stirred at 0 °C for 1 h. After the volatiles were removed in vacuo, the resulting residue was purified by silica gel column chromatography using hexanes and ethyl acetate (6:1) as the eluent to give N^3-benzoylthymine nucleoside **16** (151.0 mg, 40%) as a sticky oil: UV (CH_2Cl_2) λ_{max} 254 nm; $[\alpha]_D^{25}$ −42.37 (*c* 0.77, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97–7.39 (m, 15H, 3 × Ph), 7.19 (d, 1H, *J* = 1.5 Hz, H-6), 6.46 (d, 1H, *J* = 5.0 Hz, 4-H), 5.49 (br s, 1H, 2-H), 5.41 (m, 1H, 3-H), 4.28 (d, 1H, *J* = 11.0 Hz, TBDPSOC*H*H), 3.55 (d, 1H, *J* = 11.5 Hz, TBDPSOCH*H*), 1.71 (m, 1H, 1-H), 1.68 (d, 3H, *J* = 1.5 Hz, CH_3), 1.12 (s, 9H, *tert*-butyl), 1.07 (dd, 1H, *J* = 4.5, 8.5 Hz, 6-*H*H), 0.42 (t, 1H, *J* = 4.5 Hz, 6-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.56, 163.19, 150.06, 143.52, 137.14, 135.75, 135.64, 135.11, 133.65, 133.46, 132.01, 130.69, 130.23, 130.14, 129.32, 128.09, 128.06, 125.86, 111.18, 65.44, 61.04, 38.75, 28.70, 27.27, 24.00, 19.68, 12.41; LRMS (FAB+) *m*/*z* 577 (17, M+H)$^+$, 599 (12, M+Na)$^+$; HRMS (FAB+) calcd for $C_{35}H_{37}N_2O_4Si$ (M+H)$^+$: 577.2523, found: 577.2525.

4.1.14. (−)-1-[(1*R*,2*R*,5*S*)-5-(Hydroxymethyl)bicyclo[3.1.0]hex-3-en-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (L-2)

A solution of **16** (151.0 mg, 0.26 mmol) in MeOH (12.5 mL) was treated with concentrated aqueous ammonium hydroxide solution (28%, 0.9 mL) and stirred at room temperature for 4 h. After evaporating under reduced pressure, the residue was purified by silica gel column chromatography using hexanes and ethyl acetate (2:1) as the eluent to give TBDPS-protected thymine nucleoside (109.4 mg, 88%) as a white foam: UV (CH_2Cl_2) λ_{max} 270 nm; $[\alpha]_D^{25}$ −64.15 (*c* 1.30, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67–7.36 (m, 10H, 2 × Ph), 7.08 (d, 1H, *J* = 1.0 Hz, H-6), 6.42 (dd, 1H, *J* = 1.0, 5.0 Hz, 4-H), 5.51 (d, 1H, *J* = 1.5 Hz, 2-H), 5.37 (m, 1H, 3-H), 4.23 (d, 1H, *J* = 11.5 Hz, TBDPSOC*H*H), 3.52 (d, 1H, *J* = 11.0 Hz, TBDPSOCH*H*), 1.66 (s, 3H, CH_3), 1.65 (m, 1H, 1-H), 1.09 (s, 9H, *tert*-butyl), 1.05 (dd, 1H, *J* = 4.5, 8.5 Hz, 6-*H*H), 0.42 (t, 1H, *J* = 4.5 Hz, 6-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.22, 151.21, 143.12, 137.35, 135.75, 135.62, 133.64, 133.46, 130.17, 130.08, 128.04, 128.01, 126.05, 111.14, 65.41, 60.79, 38.66, 28.70, 27.22, 23.94, 19.63, 12.39; LRMS (FAB+) *m*/*z* 473 (36, M+H)$^+$, 495 (20, M+Na)$^+$; HRMS (FAB+) calcd for $C_{28}H_{33}N_2O_3Si$ (M+H)$^+$: 473.2260, found: 473.2278. To a stirred solution of TBDPS-protected thymine nucleoside (105.6 mg, 0.22 mmol) in THF (7 mL) was added TBAF (0.26 mL, 0.26 mmol, 1 M solution in THF) and the reaction mixture was stirred at room temperature for 1 h. After the reaction mixture was concentrated in vacuo, the resulting residue was purified by silica gel column chromatography using methylene chloride and MeOH (15:1) as the eluent to give the final thymine nucleoside L-**2** (47.8 mg, 91%) as a white solid: mp 183.3–184.2 °C; UV (MeOH) λ_{max} 270 nm; $[\alpha]_D^{25}$ −165.60 (*c* 1.05, MeOH); ^{1}H NMR (CD_3OD) δ 7.38 (q, 1H, *J* = 1.5 Hz, H-6), 6.46 (m, 1H, 4-H), 5.41–5.39 (m, 2H, 2-H, 3-H), 4.14 (d, 1H, *J* = 12.5 Hz, C*H*HOH), 3.41 (d, 1H, *J* = 12.5 Hz, CH*H*OH), 1.83 (d, 3H, *J* = 1.5 Hz, CH_3), 1.70 (m, 1H, 1-H), 1.16 (dd, 1H, *J* = 4.5, 8.5 Hz, 6-*H*H), 0.45 (t, 1H, *J* = 4.0 Hz, 6-H*H*); ^{13}C NMR (100 MHz, CD_3OD) δ 165.31, 151.52, 143.00, 138.39, 126.02, 110.23, 63.10, 60.58, 38.83, 27.56, 23.80, 11.22; LRMS (FAB+) *m*/*z* 235 (3, M+H)$^+$; HRMS (FAB+) calcd for $C_{12}H_{15}N_2O_3$ (M+H)$^+$: 235.1083, found: 235.1092.

4.1.15. (−)-9-[(1*R*,2*R*,5*S*)-5-(*tert*-Butyl-diphenylsilanyloxymethyl)bicyclo[3.1.0]hex-3-en-2-yl]-6-chloro-9*H*-purine (18)

To a stirred solution of (2*S*)-**14** (102.7 mg, 0.28 mmol), triphenyl phosphine (184.7 mg, 0.70 mmol), and 6-chloropurine (65.3 mg, 0.42 mmol) in anhydrous THF (5 mL) was added DEAD (0.11 mL, 0.70 mmol) dropwise at 0 °C and the reaction mixture was stirred at 0 °C for 1 h. After the volatiles were removed in vacuo, the resulting residue was purified by silica gel column chromatography using hexanes and ethyl acetate (7:1) as the eluent to give 6-chloropurine nucleoside **18** (29.4 mg, 21%) as a colorless sticky oil: UV (CH_2Cl_2) λ_{max} 266 nm; $[\alpha]_D^{25}$ −57.34 (*c* 0.97, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H, H-8), 8.27 (s, 1H, H-2), 7.67–7.32 (m, 10H, 2 × Ph), 6.50 (d, 1H, *J* = 5.0 Hz, 4-H), 5.60–

5.57 (m, 2H, 2-H, 3-H), 4.20 (d, 1H, J = 11.0 Hz, TBDPSOC*H*H), 3.62 (d, 1H, J = 11.5 Hz, TBDPSOCH*H*), 1.73 (m, 1H, 1-H), 1.19 (dd, 1H, J = 4.5, 8.5 Hz, 6-*H*H), 1.09 (s, 9H, *tert*-butyl), 0.56 (t, 1H, J = 4.5 Hz, 6-H*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.05, 151.38, 151.15, 144.36, 143.71, 135.87, 135.76, 133.41, 133.40, 132.22, 130.15, 130.08, 128.04, 128.00, 125.19, 64.83, 60.40, 38.81, 28.66, 27.16, 24.55, 19.43; LRMS (FAB+) m/z 501 (30, M+H)$^+$; HRMS (FAB+) calcd for $C_{28}H_{30}ClN_4OSi$ (M+H)$^+$: 501.1877, found: 501.1857.

4.1.16. (−)-[(1*S*,4*R*,5*R*)-4-(6-amino-9*H*-purin-9-yl)bicyclo[3.1.0] hex-2-en-1-yl]methanol (L-4)

A solution of **18** (41.8 mg, 0.08 mmol) in methanolic ammonia (5 mL) was heated to 80 °C in a glass bomb for 4 h. After cooling to room temperature, the volatiles were removed in vacuo. The mixture was filtered and washed with methylene chloride and MeOH (15:1), and the resulting filtrate was evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using hexanes and ethyl acetate (1:2) as the eluent to give TBDPS-protected adenine nucleoside (37.6 mg), which was contaminated by triphenylphosphine oxide, as a colorless sticky oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H, H-8), 7.93 (s, 1H, H-2), 7.63–7.27 (m, 10H, 2 × Ph), 6.40 (d, 1H, J = 7.0 Hz, 4-H), 5.54 (m, 1H, 3-H), 5.47 (br s, 1H, 2-H), 4.14 (d, 1H, J = 11.2 Hz, TBDPSOC*H*H), 3.55 (d, 1H, J = 11.2 Hz, TBDPSOCH*H*), 1.68 (m, 1H, 1-H), 1.10 (dd, 1H, J = 4.8, 8.0 Hz, 6-*H*H), 1.05 (s, 9H, *tert*-butyl), 0.48 (t, 1H, J = 4.4 Hz, 6-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.83, 152.94, 149.45, 142.78, 139.49, 135.82, 135.72, 133.45, 130.05, 130.00, 127.97, 127.93, 125.81, 119.81, 65.05, 59.71, 38.58, 29.90, 27.10, 24.51, 19.39; LRMS (FAB+) m/z 482 (55, M+H)$^+$, 504 (5, M+Na)$^+$; HRMS (FAB+) calcd for $C_{28}H_{32}N_5OSi$ (M+H)$^+$: 482.2376, found: 482.2375. To a stirred solution of impure TBDPS-protected adenine nucleoside (37.6 mg, 0.08 mmol) in THF (2 mL) was added TBAF (0.09 mL, 0.09 mmol, 1 M solution in THF) and the reaction mixture was stirred at room temperature for 1 h. After the reaction mixture was concentrated in vacuo, the resulting residue was purified by silica gel column chromatography using methylene chloride and MeOH (15:1) as the eluent to give the final adenine nucleoside L-**4** (8.9 mg, 44% from **18**) as a white solid: mp 199.8–201.5 °C (dec.); UV (MeOH) λ_{max} 262 nm; $[\alpha]_D^{25}$ −152.02 (c 0.34, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (s, 1H, H-8), 8.08 (s, 1H, H-2), 6.49 (dd, 1H, J = 1.2, 5.2 Hz, 2-H), 5.56 (m, 1H, 3-H), 5.41 (br s, 1H, 4-H), 4.11 (d, 1H, J = 11.6 Hz, C*H*HOH), 3.48 (d, 1H, J = 11.6 Hz, CH*H*OH), 1.90 (m, 1H, 5-H), 1.25 (dd, 1H, J = 4.4, 8.4 Hz, 6-*H*H), 0.56 (t, 1H, J = 4.4 Hz, 6-H*H*); ^{13}C NMR (100 MHz, CD_3OD) δ 156.17, 152.23, 148.66, 142.53, 140.18, 125.77, 119.21, 63.37, 60.99, 38.85, 28.11, 24.25; LRMS (FAB+) m/z 244 (12, M+H)$^+$; HRMS (FAB+) calcd for $C_{12}H_{14}N_5O$ (M+H)$^+$: 244.1198, found: 244.1210.

4.1.17. 9-[(1*R*,2*R*,5*S*)-5-(*tert*-Butyl-diphenyl-silanyloxymethyl) bicyclo[3.1.0] hex-3-en-2-yl]-6-chloro-9*H*-purin-2-amine (19) and (−)-2-amino-9-[(1*R*,2*R*,5*S*)-5-(hydroxymethyl)bicyclo [3.1.0]hex-3-en-2-yl]-1*H*-purin-6(9*H*)-one (L-5)

To a stirred suspension of (2*S*)-**14** (147.5 mg, 0.40 mmol), triphenyl phosphine (265.3 mg, 1.01 mmol), and 2-amino-6-chloropurine (102.9 mg, 0.61 mmol) in anhydrous THF (7 mL) was added DEAD (0.16 mL, 1.01 mmol) dropwise at 0 °C and the reaction mixture was stirred at 0 °C for 1 h. After the volatiles were removed in vacuo, the mixture was filtered and washed with methylene chloride and MeOH (15:1) and the resulting filtrate was evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using hexanes and ethyl acetate (3:1) as the eluent to give 2-amino-6-chloropurine nucleoside **19** (116.9 mg), which was contaminated by triphenylphosphine oxide, as a colorless sticky oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H, H-8), 7.60–7.22 (m, 10H, 2 × Ph), 6.92 (br s, 2H, NH_2), 6.36 (d, 1H, J = 5.6 Hz, 4-H), 5.45 (br s, 1H, 2-H), 5.25 (m, 1H, 3-H), 4.04 (d, 1H, J = 10.8 Hz, TBDPSOC*H*H), 3.56 (d, 1H, J = 11.2 Hz, TBDPSOCH*H*), 1.62 (m, 1H, 1-H), 1.07 (dd, 1H, J = 4.4, 8.4 Hz, 6-*H*H), 1.00 (s, 9H, *tert*-butyl), 0.42 (t, 1H, J = 4.4 Hz, 6-H*H*). To a stirred solution of impure **19** (86.5 mg, 0.17 mmol) in THF (5 mL) was added TBAF (0.18 mL, 0.18 mmol, 1 M solution in THF) and the reaction mixture was stirred at room temperature for 1.5 h. After the reaction mixture was concentrated in vacuo, the resulting residue was purified by silica gel column chromatography using methylene chloride and MeOH (20:1) as the eluent to give 2-amino-6-chloropurine nucleoside (14.1 mg, 17% from (2*S*)-**14**) as a white solid: UV (MeOH) λ_{max} 310, 248 nm; ^{1}H NMR (500 MHz, CD_3OD) δ 8.06 (s, 1H, H-8), 6.53 (dd, 1H, J = 1.0, 5.5 Hz, 2-H), 5.59 (m, 1H, 3-H), 5.38 (br s, 1H, 4-H), 4.10 (d, 1H, J = 12.5 Hz, C*H*HOH), 3.52 (d, 1H, J = 11.5 Hz, CH*H*OH), 1.90 (dd, 1H, J = 3.5, 7.5 Hz, 5-H), 1.26 (dd, 1H, J = 4.5, 8.5 Hz, 6-*H*H), 0.57 (t, 1H, J = 4.5 Hz, 6-H*H*); ^{13}C NMR (100 MHz, CD_3OD) δ 160.30, 153.21, 150.32, 142.76, 141.81, 125.51, 123.98, 63.08, 60.30, 38.86, 27.82, 24.19. To a solution of 2-amino-6-chloropurine nucleoside (15.2 mg, 0.05 mmol) in MeOH (4 mL) were added 2-mercaptoethanol (0.03 mL, 0.44 mmol) and 1 N sodium methoxide (0.5 mL, 0.49 mmol) and the mixture was stirred under reflux for 7 h. After cooling to room temperature, the reaction mixture was neutralized with glacial acetic acid and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using methylene chloride and MeOH (7:1) as the eluent to give the final guanine nucleoside L-**5** (14.1 mg, 99%) as a white solid: mp 202.4–204.0 °C dec.; UV (MEOH) λ_{max} 254 nm; $[\alpha]_D^{25}$ −100.08 (c 0.29, DMF); ^{1}H NMR (500 MHz, CD_3OD) δ 7.72 (s, 1H, H-8), 6.49 (d, 1H, J = 5.0 Hz, 4-H), 5.57 (m, 1H, 3-H), 5.28 (br s, 1H, 2-H), 4.06 (d, 1H, J = 12.0 Hz, C*H*HOH), 3.53 (d, 1H, J = 12.0 Hz, CH*H*OH), 1.86 (dd, 1H, J = 4.5, 8.5 Hz, 1-H), 1.25 (dd, 1H, J = 4.5, 8.5 Hz, 6-*H*H), 0.54 (t, 1H, J = 4.0 Hz, 6-H*H*); ^{13}C NMR (125 MHz, CD_3OD) δ 158.59, 154.29, 150.97, 142.36, 137.06, 125.87, 116.47, 63.17, 60.08, 38.78, 28.22, 24.29; LRMS (FAB+) m/z 260 (12, M+H)$^+$, 282 (43, M+Na)$^+$.

4.1.18. Synthesis of D-1, D-2, D-3 and D-4

Synthesis of D-**1**, D-**2**, D-**3** and D-**4** was accomplished by the same procedures used for the synthesis of L-**1**, L-**2**, L-**3** and L-**4**. All spectral data were identical to those of L-nucleosides, except the values of optical rotation, which revealed same values as those of the corresponding L-nucleosides with an opposite sign.

4.1.19. Cells and medium

HIV-infected and uninfected MT-4 cells, a human T4-positive cell line carrying human T-lymphotropic virus type 1 (HTLV-1), were obtained through the Pharmaceutical Screening Center of the Korea Research Institute of Chemical Technology in Korea. The cell line, having an RPMI-1640 medium supplemented with 10% (w/v) fetal bovine serum (FBS), gentamycin (40 μg/mL), and 2 mM L-glutamine (growth medium), was incubated at 37 °C in a humidified atmosphere containing 5% CO_2. The viability of the cells was investigated by the trypan blue dye exclusion method. For an assay, the cells were seeded at a density of 1.0×10^5 cells/well.

4.1.20. Virus

HIV-$1_{HTLV\text{-}IIIB}$ and HIV-2_{ROD} were used in the anti-HIV assay. HIV-1 was obtained from the culture supernatant of H9 cells persistently infected with HTLV-IIIB (derived from a pool of American patients with AIDS). To harvest HIV-1, the cells were pelleted by centrifugation, and the supernatant containing infectious HIV-1 was aliquoted and stored at −70 °C in a refrigerator (deep freezer; ULT-1685, REVOC).

4.1.21. Antiviral assay on an MT-4 cell line

The MT-4 cells[30] (1.0×10^5 cells/mL) were exposed to cell-free HIV-$1_{HTLV\text{-}IIIB}$ or HIV-2_{ROD} at a dose of 100 $TCID_{50}$/mL (50% tissue culture infectious dose) and cultured at 37 °C for 1.5 h. The compounds were tested and compared to ddI and ddC obtained from Sigma for cytotoxicity and for their ability to inhibit HIV replication. The compounds were first dissolved in 100% DMSO and then diluted with RPMI-1640/10% FBS just before use. The maximum final concentration of DMSO added to the cell cultures was 0.5% at the highest concentration of the compound. We have determined that at that concentration DMSO does not interfere with cell growth. The HIV-1-induced cytopathic effects were monitored by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay. In the microplate tests (96 wells), 50 μL of each compound diluted or phosphate-buffered 0.85% (w/v) sodium chloride (PBS) alone was distributed in triplicate. The cells were adjusted to 1.0×10^5 cells/mL and then were plated in each well at the rate of 200 μL per well. A virus suspension (200 μL) was added to the cells with or without drugs and cultured for 6 days.

4.1.22. Cytotoxicity by MTT assay[31,32]

Infected cultures were carried out in parallel to determine the cytotoxicity of the compound. Briefly, 100 μL of a cell suspension was collected and mixed with 10 μL of a solution of MTT at 7.5 mg/mL in PBS. After 1.5 h of incubation at 37 °C, most of the supernatant was removed, and the formazan precipitate was dissolved in 100 μL of 0.04 N HCl in 2-propanol. The absorbance at 540 and 690 nm was measured on a multiwall scanning spectrophotometer (enzyme-linked immunosorbent assay plate reader; ERA-400, SLT). The percentage of toxicity was defined with uninfected and untreated control cells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration required to reduce the viability of uninfected cells at 5 days of incubation in the presence of the compounds. The concentration achieving 50% protection or the concentration required to inhibit HIV-induced destruction of MT-4 cells by 50% was defined as the 50% inhibitory concentration (IC_{50}). HIV-infected or uninfected MT-4 cells (1.0×10^5 cells/mL) as target cells were suspended with various concentrations of the samples and cultured for 5 days in a CO_2 incubator at 37 °C. The anti-AIDS activities of the samples were evaluated after 5 days of HIV infection.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.05.026.

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