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Synthesis and Biological Evaluation of Novel Thioapio Dideoxynucleosides

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This paper is dedicated to Prof. C. K. Chu (University of Georgia, USA) on the occasion of his 60th birthday.

Abstract—On the basis of the bioisosteric rationale to apio dideoxynucleosides, novel thioapio dideoxynucleosides have been synthesized, starting from 1,3-dihydroxyacetone via thioapio sugar acetate **6** as a key intermediate. The intermediate **6** was condensed with silylated pyrimidine bases such as N^4-benzoylcytosine, uracil or thymine in the presence of TMSOTf to give the β-anomers (**8a**, **11a**, and **12a**) and α-anomers (**8b**, **11b**, and **12b**), respectively. The intermediate **6** was also condensed with silylated 6-chloropurine to give the 6-chloropurine derivatives **14a** and **14b** which were converted to adenine derivatives **15a** and **15b**, N^6-methyladenine derivatives **16a** and **16b**, and hypoxanthine derivatives **17a** and **17b**, respectively. The guanine analogues **20a** and **20b** were also synthesized from the condensation of sugar acetate **6** with 2-acetamido-6-chloropurine. All synthesized final compounds were tested against HIV-1. Most of the synthesized compounds exhibited toxicity-dependent anti-HIV-1 activity, among which 6-chloropurine derivative **14b** was found to be the most cytotoxic and showed good cytotoxicity against colon cancer cell lines. Although we could not find good anti-HIV agents in this study, findings of some anticancer activity in this series will allow this class of nucleosides to be the new template for the development of new anticancer agents (Fig. 1).

Introduction

Apio dideoxynucleosides (apio-ddNs, **1**) are non-classical nucleosides in which 4′-hydroxymethyl group of the classical 2′,3′-dideoxynucleosides moves to the C3′ position.[1–4] Among this type of nucleosides, adenine analogue (apio-ddA) was reported to exhibit anti-HIV activity comparable to parent 2′,3′-dideoxyadenosine.[5,6] Apio-ddA also appears to possess metabolic resistance to adenosine deaminase or stabilization of the glycosyl bond.[3] Nevertheless, since systematic structure–activity relationship study in apio dideoxynucleosides has not been fulfilled so far, it is thought that much more effort should be made in this class of nucleosides to search for new antiviral agents.

Based on the bioisosteric rationale, we have recently reported the synthesis and structure–activity relationship study of apio dideoxydidehydro nucleosides **2a** among which several compounds exhibited significant antiviral activity against human cytomegalovirus (HCMV), but its thioapio analogue **2b** did not exhibit any antiviral activity.[7] Since substitution of oxygen with sulfur resulted in big differences in antiviral activity despite of their bioisosteric relationships, it was of great

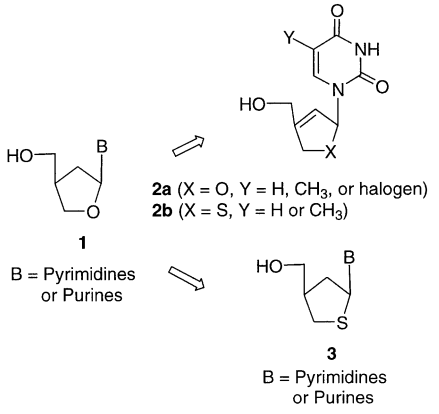

Figure 1.

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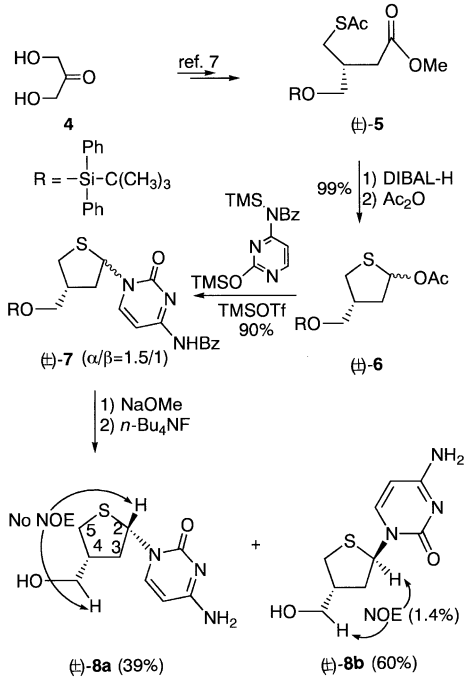


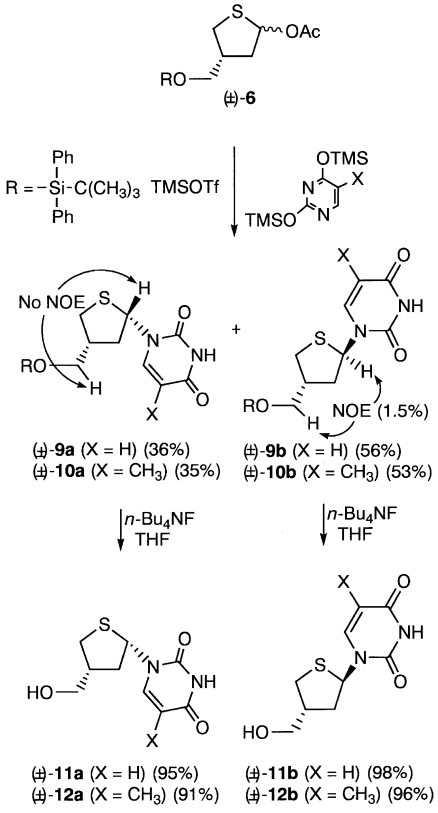
Scheme 1.

interest to synthesize the thioapio dideoxynucleoside analogues **3**, based on the apio dideoxynucleosides **1** and to compare their antiviral activities. In this study, we found that most of the thioapio dideoxynucleoside analogues **3** showed cytotoxicity instead of antiviral activity, among which some analogues exhibited significant antitumor activity against tumor cell lines tested. Thus, we wish to report the synthesis and antiviral and antitumor activities of the novel thioapio dideoxynucleosides, starting from 1,3-dihydroxyacetone.

Results and Discussion

Chemistry

Our synthetic strategy was to synthesize the thioapio sugar acetate as a glycosyl donor and then to condense with various pyrimidine and purine bases. Synthesis of the glycosyl donor **6** could be achieved, starting from 1,3-dihydroxyacetone and is shown in Scheme 1. 1,3-Dihydroxyacetone (**4**) was converted to the thioester (±)-**5** according to the known procedure developed by our laboratory.[7] Treatment of (±)-**5** with DIBAL-H at −78 °C followed by acetylation of the resulting thiolactol with acetic anhydride gave thioapio acetate (±)-**6** in 99% yield. Condensation of (±)-**6** with silylated N^4-benzoylcytosine in the presence of TMSOTf afforded the inseparable anomeric mixture of (±)-**7** (α/β = 1.5/1) in 90% yield. Removal of benzoyl group with sodium methoxide and silyl group with *n*-tetrabutylammonium fluoride gave the β-cytosine analogue (±)-**8a** and α-cytosine analogue (±)-**8b** after the purification by preparative thin layer chromatography (PTLC). Anomeric configurations of (±)-**8a** and (±)-**8b** were easily assigned by ^{1}H NOE experiment. Irradiation of 2-H in (±)-**8a** gave no NOE effect on the methylene proton of

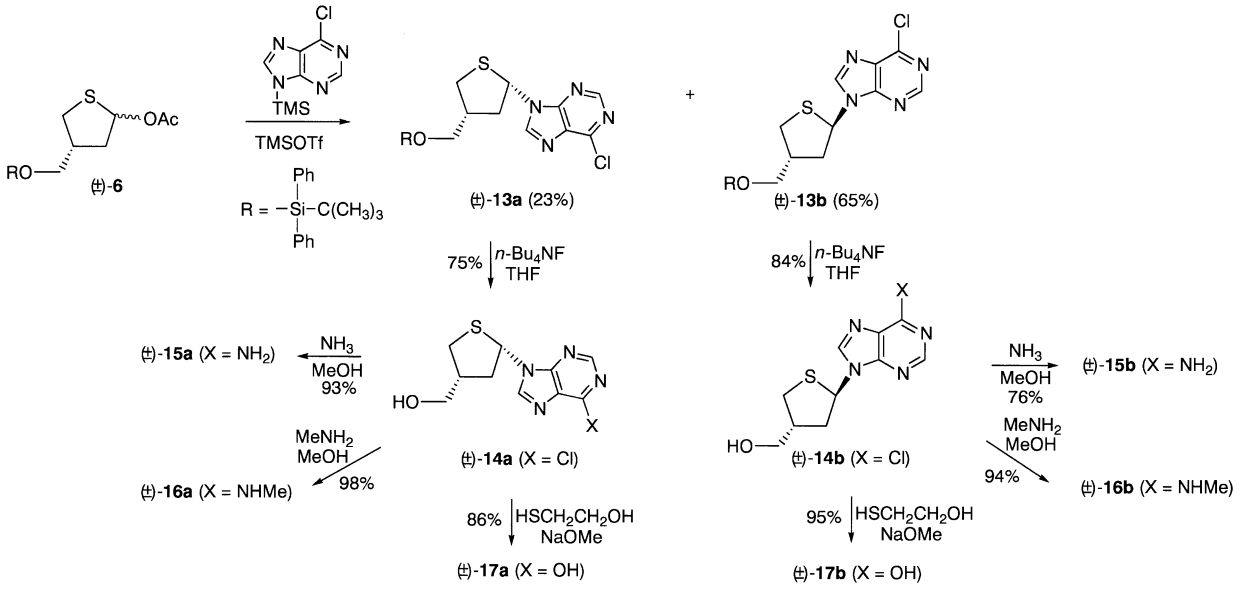


Scheme 2.

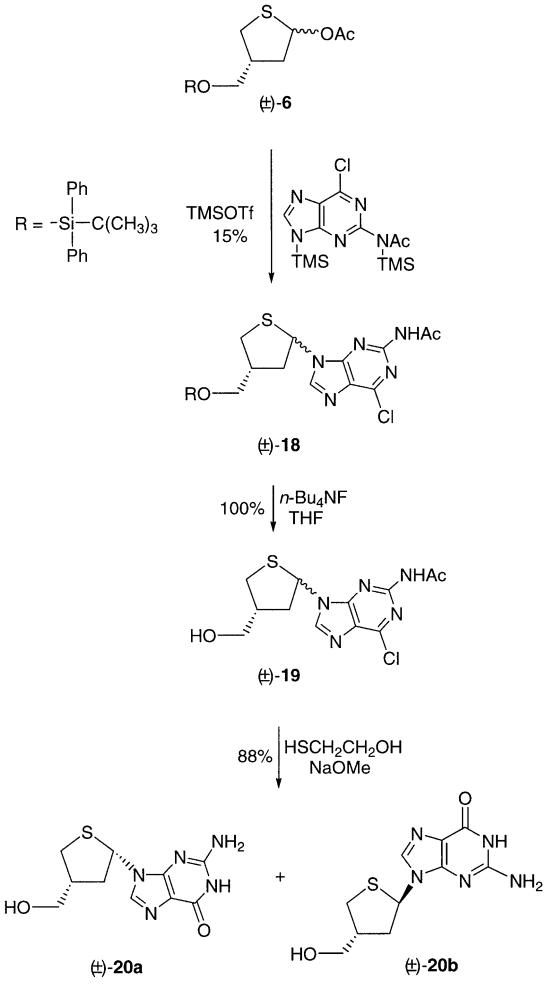
4-CH_2OH, indicating β-anomer, while NOE effect (1.4%) was observed on the same experiment about (±)-**8b**, showing α-anomer.

For the synthesis of uracil and thymine analogues as shown in Scheme 2, acetate (±)-**6** was first condensed with silylated uracil in the presence of TMSOTf to give β-anomer (±)-**9a** and α-anomer (±)-**9b** after separation by silica gel column chromatography. Anomeric configurations of two anomers were also assigned based on the same ^{1}H NOE experiment as the cytosine analogues, (±)-**8a** and (±)-**8b**. Desilylation of (±)-**9a** and (±)-**9b** with *n*-tetrabutylammonium fluoride afforded the final uracil derivatives (±)-**11a** and (±)-**11b**, respectively. The thymine analogues (±)-**12a** and (±)-**12b** were also synthesized according to the same procedure used in the preparation of (±)-**11a** and (±)-**11b**.

Synthesis of the mono-substituted purine nucleosides is depicted in Scheme 3. Lewis acid catalyzed condensation of (±)-**6** with silylated 6-chloropurine gave β-anomer (±)-**13a** and α-anomer (±)-**13b** in 1/3 ratio after separated on silica gel. The β-anomer (±)-**13a** was treated with *n*-tetrabutylammonium fluoride in THF to give (±)-**14a**. Compound (±)-**14a** was converted to the adenine derivative (±)-**15a** by heating with methanolic ammonia, to the *N*-methyladenine analogue (±)-**16a** by treating with methylamine, and to the hypoxanthine analogue (±)-**17a** by refluxing with mercaptoethanol and sodium methoxide, respectively. The α-anomers (±)-**15b** ∼(±)-**17b** were also obtained from (±)-**14b** according to the same procedure used for the synthesis of the corresponding β-anomers (±)-**15a** ∼(±)-**17a**.



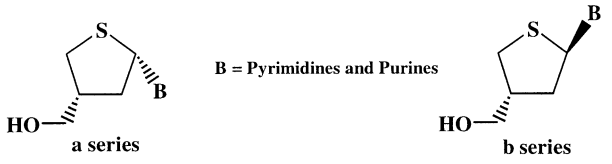
Scheme 3.



Scheme 4.

Finally, for the synthesis of the guanine derivative, (±)-**6** was condensed with silylated 2-acetamido-6-chloropurine to give the inseparable anomeric mixture of (±)-**18** in very poor yield (15%). Silyl protecting group of (±)-**18** was removed to give the inseparable anomeric mixture of (±)-**19** which was refluxed with mercaptoethanol and sodium methoxide to yield (±)-**20a** and (±)-**20b** (Scheme 4).

Table 1. Anti-HIV Activity of the Synthesized Thioapio Dideoxynucleosides



Compd	EC_{50} (μg/mL)[a]	CC_{50} (μg/mL)[b]
8a (B = cytosine)	> 100	> 100
8b (B = cytosine)	> 100	> 100
11a (B = uracil)	> 100	> 100
11b (B = uracil)	> 100	> 100
12a (B = thymine)	> 100	> 100
12b (B = thymine)	> 100	> 100
14a (B = 6-chloropurine)	2.31	< 2.31
14b (B = 6-chloropurine)	1.35	< 1.35
15a (B = adenine)	19.15	< 19.15
15b (B = adenine)	17.6	< 17.6
16a (B = N^6-methyladenine)	48.2	< 48.2
16b (B = N^6-methyladenine)	54.8	< 54.8
17a (B = hypoxanthine)	> 100	> 100
17b (B = hypoxanthine)	> 100	> 100
20a (B = guanine)	> 100	> 100
20b (B = guanine)	> 100	> 100
AZT	0.002	1.0

[a]Indicative of 50% cytopathic concentration in virus-infected MT-4 cells.
[b]Indicative of 50% survival concentration in virus-uninfected MT-4 cells.

Biological Evaluation

Antiviral assay against HIV-1 was performed for all the final nucleosides and its result is shown in Table 1. Uracil, thymine, cytosine, hypoxanthine, and guanine analogues showed neither anti-HIV activity nor

Table 2. The cytotoxic potential of **14a** and **14b** in cultured human cancer cells

Test samples	A549[a]	Col2[b]
14a	61.9[c]	59.2[c]
14b	60.8[c]	18.5[c] (3.8 μg/mL[d])

[a]A549: human lung carcinoma cells.
[b]Col2: human colon carcinoma cells.
[c]% of survival compared to control culture at test concentration of 50 μg/mL. IC_{50} values could not be calculated because their survival percents were more than 50%.
[d]IC_{50} value, indicative of 50% survival determined with serial dilutions of the test compound.

cytotoxicity in MT-4 cells and other derivatives exhibited toxicity-dependent anti-HIV activity because they showed less than 50% survival at the same concentration exhibiting anti-HIV-1 activity in virus-uninfected MT-4 cells. Among compounds tested, 6-chloropurine derivatives **14a** and **14b** were found to be highly cytotoxic. Therefore, based on the cytotoxicity of **14a** and **14b**, their cytotoxic potentials were evaluated in cultured human lung (A549) and colon (Col2) cancer cells. As shown in Table 2, **14a** showed the approximate 60% survival compared to control cultures at the test concentration of 50 μg/mL in either lung or colon cells. Compound **14b**, however, was found to be relatively high cytotoxic to colon cells; 18.5% survival of control in colon, 60.8% in lung. Further determination indicated that the IC_{50} value of **14b** in cultured colon cells was 3.8 μg/mL. In conclusion, the cytotoxic potential of **14a** was relatively low compared to **14b**. In addition, the cytotoxic susceptibility of **14b** was also shown in colon cells.

Conclusion

We have completed the synthesis and biological evaluation of novel thioapio dideoxynucleosides, starting from 1,3-dihydroxyacetone. Most of the synthesized compounds exhibited toxicity-dependent anti-HIV-1 activity, among which 6-chloropurine derivative **14b** was found to be the most cytotoxic and showed good cytotoxicity against colon cancer cell lines. Although we could not find good anti-HIV agents in this study, findings of some anticancer activity in this series will allow this class of nucleosides to be the new template for the development of new anticancer agents.

Experimental

Ultra violet (UV) spectra were recorded on a Beckman DU-68 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on Varian-400 spectrometer, using $CDCl_3$, DMSO-d_6 or CD_3OD and chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane as internal standard. FAB mass spectra were recorded on Jeol HX 110 spectrometer. Elemental analyses were performed by the general instrument laboratory of Ewha Womans University, Korea. TLC was performed on Merck precoated $60F_{254}$ plates. Column chromatography was performed using silica gel 60 (230–400 mesh, Merck). All the anhydrous solvents were distilled over CaH_2 or P_2O_5 or Na/benzophenone prior to use.

(±)-Acetic acid *cis* and *trans*- 4-(*tert*-butyl-diphenyl-silanyloxymethyl)-tetrahydro-thiophen-2-yl ester {(±)-6}. To a stirred solution of (±)-**5**[7] (2.884 g, 6.49 mmol) in anhydrous toluene (30 mL) was added DIBAL-H (18.2 mL, 18.2 mmol, 1.0 M solution in toluene) at −78 °C, and the reaction mixture was stirred for 30 min at the same temperature. The reaction mixture was quenched with methanol (4.8 mL) and allowed to warm to ambient temperature. After the reaction mixture was partitioned between saturated $NaHCO_3$ solution (10 mL) and ethyl acetate (60 mL), the organic layer was dried over $MgSO_4$, filtered, and evaporated to give the crude lactol as a syrup. The crude lactol was used for the next step without further purification.

^1H NMR ($CDCl_3$) δ 1.81 (td, 1H, $J=4.7$, 12.5 Hz, major 3-H_a), 1.98 (d, 1H, $J=5.4$ Hz, major OH), 2.05–2.13 (m, 1H, minor 3-H_a), 2.24 (dd, 1H, $J=4.0$, 12.5 Hz, major 3-H_b), 2.36–2.48 (m, 1H, minor 3-H_b), 2.65–2.89 (m, 4H, major 4-H and 5-H_a, and minor 4-H and 5-H_a), 3.04 (d, 1H, $J=6.5$ Hz, minor 5-H_b), 3.16 (dd, 1H, $J=5.7$, 9.0 Hz, major 5-H_b), 3.62 (d, 1H, $J=9.0$ Hz, minor OH), 3.68–3.85 (m, 4H, major TBDPSOCH_2, and minor TBDPSOCH_2), 5.54 (t, 1H, $J=4.7$ Hz, major 2-H), 5.64 (m, 1H, minor 2-H), 7.40–7.72 (m, 20H, major 2*Ar and minor 2*Ar).

To a stirred solution of the above crude lactol and DMAP (29 mg) in pyridine (2.3 mL) and methylene chloride (40 mL) was added acetic anhydride (1.4 mL, 14.84 mmol), and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was diluted with methylene chloride (200 mL), washed with water (50 mL), saturated $NaHCO_3$ (50 mL), and brine (50 mL), dried over $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 8:1) to give (±)-**6** (2.674 g, 99%) as a colorless syrup.

^1H NMR ($CDCl_3$) δ 1.06 (s, 18H, major and minor *t*-C_4H_9), 1.79–1.93 (m, 1H, major 3-H_a), 1.96 (s, 3H, minor OCOCH_3), 2.05 (s, 3H, major OCOCH_3), 2.05–2.15 (m, 1H, minor 3-H_a), 2.22–2.36 (m, 2H, major 3-H_b and minor 3-H_b), 2.60–2.81 (m, 3H, major 4-H and 5-H_a, and minor 5-H_a), 2.89–3.10 (m, 3H, major 5-H_b, and minor 4-H and 5-H_b), 3.67–3.79 (m, 4H, major TBDPSOCH_2 and minor TBDPSOCH_2), 6.11–6.14 (m, 2H, major 2-H and minor 2-H), 7.36–7.67 (m, 20H, major 2*Ar and minor 2*Ar). Anal. calcd for $C_{23}H_{30}O_3SSi$: C, 66.62; H, 7.29; S, 7.73. Found: C, 66.63; H, 7.45; S, 7.53.

(±)-α and β-*N*-{1-[4-(*tert*-Butyl-diphenyl-silanyloxymethyl)-tetrahydro-thiophen-2-yl]-2-oxo-1,2-dihydro-4-yl}-benzamide {(±)-7}. To a solution of silylated N^4-benzoylcytosine, prepared from refluxing N^4-benzoylcytosine (156 mg, 0.72 mmol) and ammonium sulfate (catalytic amount) in HMDS (5 mL), in anhydrous

$ClCH_2CH_2Cl$ (2 mL) was added a solution of (±)-**6** (200 mg, 0.48 mmol) in anhydrous $ClCH_2CH_2Cl$ (3 mL) followed by addition of TMSOTf (0.13 mL, 0.72 mmol) at 0 °C, and the reaction mixture was stirred at the same temperature for 30 min. After the reaction mixture was quenched with saturated $NaHCO_3$ solution (3 mL), the reaction mixture was filtered through a Celite pad and diluted with methylene chloride (60 mL) and water (20 mL). The organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 1:1) to give inseparable mixture, (±)-**7** (248 mg, 90%, α/β = 1.5:1 determined by ^1H NMR) as a white solid; UV (CH_2Cl_2) λ_{max} 258 nm; ^1H NMR ($CDCl_3$) δ 1.05 (s, 18H, major and minor *t*-C_4H_9), 1.58–1.68 (m, 1H, minor 3-H_a), 2.20–2.46 (m, 3H, major 3-H and 4-H), 2.52–2.67 (m, 1H, minor 4-H), 2.78–2.82 (m, 1H, minor 3-H_b), 2.89–3.13 (m, 3H, major 5-H_a and minor 5-H), 3.24 (dd, 1H, J = 6.5, 10.5 Hz, major 5-H_b), 3.65 (dd, 2H, J = 5.9, 10.3 Hz, major TBDPSOCH_a, and minor TBDPSOCH_a), 3.72 (dd, 2H, J = 4.9, 10.3 Hz, major TBDPSOCH_b and minor TBDPSOCH_b), 6.29 (dd, 1H, J = 2.6, 5.2 Hz, major 2-H), 6.52 (dd, 1H, J = 7.0, 8.6 Hz, minor 2-H), 7.36–7.92 (m, 32H, major 3*Ar and H-5, and minor 3*Ar and H-5), 8.25 (d, 1H, J = 7.5 Hz, minor H-6), 8.41 (d, 1H, J = 7.5 Hz, major H-6), 8.87 (br s, 2H, major N*H* and minor N*H*). Anal. calcd for $C_{32}H_{35}N_3O_3SSi$: C, 67.45; H, 6.19; N, 7.37. Found: C, 67.32; H, 6.35; N, 7.15.

(±)-α and β-4-Amino-1-(4-hydroxymethyl-tetrahydrothiophen-2-yl)-1*H*-pyrimidin-2-one {(±)-8a, 8b}. To a solution of the above α and β mixture (248 mg, 0.44 mmol) in MeOH (5 mL) and CH_2Cl_2 (2 mL) was added NaOMe (0.08 mL, 0.08 mmol, 1.0 M solution in methanol) at ambient temperature and the reaction mixture was stirred at the same temperature for 3 h. The mixture was neutralized with acetic acid and then evaporated. The resulting residue was purified by silica gel column chromatography (methylene chloride/methanol = 10:1) to give inseparable debenzoylated mixture (200 mg, 99%). To a solution of the above debenzoylated mixture (200 mg, 0.43 mmol) in THF (5 mL) was added tetrabutylammonium fluoride (0.47 mL, 0.47 mmol, 1.0 M solution in THF) at ambient temperature and the reaction mixture was stirred at the same temperature for 1 h. To the reaction mixture was added methanol (1 mL) and the volatiles were evaporated. The resulting residue was purified by silica gel column chromatography (methylene chloride/methanol = 7.5:1) to give inseparable mixture (±)-**8a** and **8b** (98 mg, 100%). The mixture was separated for analysis by preparative TLC. (±)-**8a**: FAB-LRMS m/z 228 ($M+H^+$); UV (MeOH) λ_{max} 270 nm; ^1H NMR (MeOH-d_4) δ 1.67 (td, 1H, J = 9.6, 12.0 Hz, 3-H_a), 2.52 (m, 2H, 3-H_a, 4-H), 2.98 (dd, 1H, J = 6.0, 10.0 Hz, 5-H_a), 3.04 (t, 1H, J = 10.0 Hz, 5-H_b), 3.60 (dd, 1H, J = 6.0, 10.8 Hz, HOCH_a), 3.67 (dd, 1H, J = 5.2, 10.8 Hz, HOCH_b), 5.96(d, 1H, J = 8.0 Hz, H-5), 6.43 (dd, 1H, J = 7.2, 9.6 Hz, 2-H), 8.01 (d, 1H, J = 7.2 Hz, H-6). Anal. calcd for $C_9H_{13}N_3O_2S$: C, 47.56; H, 5.77; N, 18.49. Found: C, 47.89; H, 5.90; N, 18.09. (±)-**8b**: FAB-LRMS m/z 228 ($M+H^+$); UV (MeOH) λ_{max} 270 nm; ^1H NMR (MeOH-d_4) δ 2.18–2.22 (m, 2H, 3-H), 2.54 (m, 1H, 4-H), 2.82 (dd, 1H, J = 8.0, 10.4 Hz, 5-H_a), 3.29 (dd, 1H, J = 7.2, 10.4 Hz, 5-H_b), 3.62 (d, 2H, J = 6.0 Hz, HOCH_2), 5.94 (d, 1H, J = 7.2 Hz, H-5), 6.23 (irregular t, 1H, J = 4.4, 5.2 Hz, 2-H), 8.13 (d, 1H, J = 7.6 Hz, H-6). Anal. calcd for $C_9H_{13}N_3O_2S$: C, 47.56; H, 5.77; N, 18.49. Found: C, 47.16; H, 5.38; N, 18.24.

(±)-α and β-1-[4-(*tert*-Butyl-diphenyl-silanyloxymethyl)-tetrahydro-thiophen-2-yl]-1-*H*-pyrimidine-2,4-dione {(±)-9a, 9b}. To a stirred solution of silylated uracil, prepared from refluxing uracil (61 mg, 0.54 mmol) and ammonium sulfate (catalytic amount) in HMDS (5 mL), in anhydrous $ClCH_2CH_2Cl$ (2 mL) was added a solution of (±)-**6** (150 mg, 0.36 mmol) in anhydrous $ClCH_2CH_2Cl$ (3 mL) followed by addition of TMSOTf (0.1 mL, 0.55 mmol) at 0 °C, and the reaction mixture was stirred at the same temperature for 1 h. After the reaction mixture was quenched with saturated $NaHCO_3$ solution (3 mL), the reaction mixture was filtered through a Celite pad and diluted with methylene chloride (60 mL) and water (20 mL). The organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 2:1) to give (±)-**9a** (61 mg, 36%) as a white solid and (±)-**9b** (95 mg, 56%) as a white solid.

(±)-**9a**: mp 157–158 °C; UV (CH_2Cl_2) λ_{max} 264 nm; ^1H NMR ($CDCl_3$) δ 1.06 (s, 9H, *t*-C_4H_9), 1.57–1.71 (m, 1H, 3-H_a), 2.48–2.63 (m, 2H, 3-H_b and 4-H), 2.93–3.08 (m, 2H, 5-H), 3.67 (dd, 1H, J = 5.2, 10.4 Hz, TBDPSOCH_a), 3.73 (dd, 1H, J = 4.9, 10.4 Hz, TBDPSOCH_b), 5.80 (dd, 1H, J = 2.1, 8.1 Hz, H-5), 6.39 (dd, 1H, J = 6.7, 9.1 Hz, 2-H), 7.36–7.63 (m, 10H, 2*Ar), 7.67 (d, 1H, J = 8.1 Hz, H-6), 8.59 (br s, 1H, imide H). Anal. calcd for $C_{25}H_{30}N_2O_3SSi$: C, 64.34; H, 6.48; N, 6.00. Found: C, 64.32; H, 6.44; N, 6.15.

(±)-**9b**: mp 153–154 °C; UV (CH_2Cl_2) λ_{max} 265 nm; ^1H NMR ($CDCl_3$) δ 1.06 (s, 9H, *t*-C_4H_9), 2.04–2.13 (m, 1H, 3-H_a), 2.18–2.30 (m, 1H, 3-H_b), 2.42–2.54 (m, 1H, 4-H), 2.88 (dd, 1H, J = 8.5, 10.6 Hz, 5-H_a), 3.21 (dd, 1H, J = 6.7, 10.6 Hz, 5-H_b), 3.65 (dd, 1H, J = 6.5, 10.3 Hz, TBDPSOCH_a), 3.70 (dd, 1H, J = 5.5, 10.3 Hz, TBDPSOCH_b), 5.75 (dd, 1H, J = 2.0, 8.1 Hz, H-5), 6.18 (dd, 1H, J = 3.0, 6.5 Hz, 2-H), 7.36–7.65 (m, 10H, 2*Ar), 7.82 (d, 1H, J = 8.1 Hz, H-6), 8.71 (br s, 1H, imide H). Anal. calcd for $C_{25}H_{30}N_2O_3SSi$: C, 64.34; H, 6.48; N, 6.00. Found: C, 64.74; H, 6.85; N, 5.80.

(±)-α and β-1-[4-(*tert*-Butyl-diphenyl-silanyloxymethyl)-tetrahydro-thiophen-2-yl]-5-methyl-1*H*-pyrimidine-2,4-dione {(±)-10a, 10b}. To a solution of silylated thymine, prepared from refluxing thymine (85 mg, 0.67 mmol) and ammonium sulfate (catalytic amount) in HMDS (5 mL), in anhydrous $ClCH_2CH_2Cl$ (2 mL) was added a solution of (±)-**6** (200 mg, 0.48 mmol) in anhydrous $ClCH_2CH_2Cl$ (3 mL) followed by addition of TMSOTf (0.12 mL, 0.66 mmol) at 0 °C, and the reaction mixture was stirred at the same temperature for 45 min. After the reaction mixture was quenched with saturated $NaHCO_3$ solution (3 mL), the reaction

mixture was filtered through a Celite pad and diluted with methylene chloride (60 mL) and water (20 mL). The organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 2:1) to give (±)-**10a** (82 mg, 35%) as a white solid and (±)-**10b** (122 mg, 53%) as a white solid.

(±)-**10a**: mp 160–161 °C; UV (CH_2Cl_2) λ_{max} 270 nm; ^{1}H NMR ($CDCl_3$) δ 1.06 (s, 9H, *t*-C_4H_9), 1.60–1.75 (m, 1H, 3-H_a), 1.96 (d, 3H, *J* = 1.1 Hz, CH_3), 2.49–2.61 (m, 2H, 3-H_b, 4-H), 2.96 (dd, 1H, *J* = 6.5, 10.4 Hz, 5-H_a), 3.07 (t, 1H, *J* = 10.4 Hz, 5-H_b), 3.69 (dd, 1H, *J* = 5.0, 10.4 Hz, TBDPSOCH_a), 3.74 (dd, 1H, *J* = 4.8, 10.4 Hz, TBDPSOCH_b), 6.42 (dd, 1H, *J* = 6.7, 9.4 Hz, 2-H), 7.36–7.65 (m, 11H, H-6, 2*Ar), 8.27 (br s, 1H, imide H). Anal. calcd for $C_{26}H_{32}N_2O_3SSi$: C, 64.96; H, 6.71; N, 5.83. Found: C, 64.67; H, 6.33; N, 5.76.

(±)-**10b**: mp 165–166 °C; UV (CH_2Cl_2) λ_{max} 270 nm; ^{1}H NMR ($CDCl_3$) δ 1.06 (s, 9H, *t*-C_4H_9), 1.96 (d, 3H, *J* = 1.0 Hz, CH_3), 2.02–2.11 (m, 1H, 3-H_a), 2.19–2.31 (m, 1H, 3-H_b), 2.48–2.60 (m, 1H, 4-H), 2.89 (dd, 1H, *J* = 7.8, 10.6 Hz, 5-H_a), 3.23 (dd, 1H, *J* = 6.6, 10.6 Hz, 5-H_b), 3.62–3.73 (m, 2H, TBDPSOCH_2), 6.22 (dd, 1H, *J* = 3.4, 6.8 Hz, 2-H), 7.39 (m, 11H, H-6, 2*Ar), 8.95 (br s, 1H, imide H). Anal. calcd for $C_{26}H_{32}N_2O_3SSi$: C, 64.96; H, 6.71; N, 5.83. Found: C, 64.73; H, 6.54; N, 5.88.

(±)-β-1-(4-Hydroxymethyl-tetrahydro-thiophen-2-yl)-1*H*-pyrimidine-2,4-dione {(±)-11a}. To a solution of (±)-**9a** (136 mg, 0.29 mmol) in THF (4 mL) was added TBAF (0.32 mL, 0.32 mmol, 1.0 M solution in THF) and the reaction mixture was stirred at ambient temperature for 1 h. The solvent was removed to give the residue, which was purified by silica gel column chromatography (methylene chloride/methanol = 15:1) to afford (±)-**11a** (62 mg, 95%) as a white solid; FAB-LRMS *m*/*z* 229 (M + H$^+$); mp 174–175 °C; UV (MeOH) λ_{max} 264 nm; ^{1}H NMR (MeOH-d_4) δ 1.73 (td, 1H, *J* = 9.2, 12.0 Hz, 3-H_a), 2.46–2.59 (m, 2H, 3-H_b, 4-H), 2.99 (dd, 1H, *J* = 6.4, 10.8 Hz, 5-H_a), 3.05 (t, 1H, *J* = 10.8 Hz, 5-H_b), 3.61 (dd, 1H, *J* = 6.0, 11.2 Hz, HOCH_a), 3.68 (dd, 1H, *J* = 5.6, 11.2 Hz, HOCH_b), 5.78 (d, 1H, *J* = 8.0 Hz, H-5), 6.36 (dd, 1H, *J* = 6.8, 8.8 Hz, 2-H), 7.98 (d, 1H, *J* = 8.0 Hz, H-6). Anal. calcd for $C_9H_{12}N_2O_3S$: C, 47.35; H, 5.30; N, 12.27. Found: C, 47.22; H, 5.31; N, 12.28.

(±)-α-1-(4-Hydroxymethyl-tetrahydro-thiophen-2-yl)-1-*H*-pyrimidine-2,4-dione {(±)-11b}. Compound (±)-**9b** (200 mg, 0.43 mmol) was subjected to the desilylation as described for the preparation of (±)-**11a**. Purification by silica gel column chromatography (methylene chloride/methanol = 15:1) gave (±)-**11b** (96 mg, 98%) as a sticky oil; FAB-LRMS *m*/*z* 229 (M + H$^+$); UV (MeOH) λ_{max} 264 nm; ^{1}H NMR (MeOH-d_4) δ 2.19–2.22 (m, 2H, 3-H_2), 2.58 (m, 1H, 4-H), 2.82 (dd, 1H, *J* = 8.0, 10.8 Hz, 5-H_a), 3.30 (dd, 1H, *J* = 6.8, 10.8 Hz, 5-H_b), 3.60 (d, 2H, *J* = 6.8 Hz, HOCH_2), 5.73 (d, 1H, *J* = 8.0 Hz, H-5), 6.18 (irregular t, 1H, *J* = 4.4, 5.2 Hz, 2-H), 8.08 (d, 1H, *J* = 8.4 Hz, H-6). Anal. calcd for $C_9H_{12}N_2O_3S$: C, 47.35; H, 5.30; N, 12.27. Found: C, 47.65; H, 5.70; N, 12.07.

(±)-β-1-(4-Hydroxymethyl-tetrahydro-thiophen-2-yl)-5-methyl-1-*H*-pyrimidine-2,4-dione {(±)-12a}. Compound (±)-**10a** (80 mg, 0.17 mmol) was subjected to the desilylation as described for the preparation of (±)-**11a**. Purification by silica gel column chromatography (methylene chloride/methanol = 15:1) gave (±)-**12a** (37 mg, 91%) as a white solid; FAB-LRMS *m*/*z* 243 (M + H$^+$); mp 216–217 °C; UV (MeOH) λ_{max} 270 nm; ^{1}H NMR (MeOH-d_4)?δ 1.76 (td, 1H, *J* = 9.6, 13.6 Hz, 3-H_a), 1.92 (d, 3H, *J* = 1.2 Hz, CH_3), 2.47–2.55 (m, 2H, 3-H_b, 4-CH), 2.98 (dd, 1H, *J* = 6.0, 10.0 Hz, 5-H_a), 3.08 (t, 1H, *J* = 10.4 Hz, 5-H_b), 3.62 (dd, 2H, *J* = 6.0, 11.2 Hz, HOCH_a), 3.69 (dd, 1H, *J* = 4.8, 11.2 Hz, HOCH_b), 6.37 (dd, 1H, *J* = 6.4, 9.6 Hz, 2-H), 7.70 (d, 1H, *J* = 0.8 Hz, H-6). Anal. calcd for $C_{10}H_{14}N_2O_3S$: C, 49.57; H, 5.82; N, 11.56. Found: C, 49.66; H, 5.86; N, 11.16.

(±)-α-1-(4-Hydroxymethyl-tetrahydro-thiophen-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione {(±)-12b}. Compound (±)-**10b** (104 mg, 0.22 mmol) was subjected to the desilylation as described for the preparation of (±)-**11a**. Purification by silica gel column chromatography (methylene chloride/methanol = 15:1) gave (±)-**12b** (50 mg, 96%) as a white solid; FAB-LRMS *m*/*z* 243 (M + H$^+$); mp 138–139 °C; UV (MeOH) λ_{max} 271 nm; ^{1}H NMR (MeOH-d_4) δ 1.92 (d, 3H, *J* = 0.8 Hz, CH_3), 2.18–2.20 (m, 2H, 3-H), 2.62 (m, 1H, 4-H), 2.82 (dd, 1H, *J* = 7.2, 10.4 Hz, 5-H_a), 3.33 (dd, 1H, *J* = 6.8, 10.4 Hz, 5-H_b), 3.58 (dd, 1H, *J* = 6.4, 10.8 Hz, HOCH_a), 3.62 (dd, 1H, *J* = 6.8, 10.8 Hz, HOCH_b), 6.20 (irregular t, 1H, *J* = 5.2, 6.0 Hz, 2-H), 7.83 (d, 1H, *J* = 1.2 Hz, H-6). Anal. calcd for $C_{10}H_{14}N_2O_3S$: C, 49.57; H, 5.82; N, 11.56. Found: C, 49.56; H, 5.88; N, 11.47.

(±)-α and β-9-[4-(*tert*-Butyl-diphenyl-silanyloxymethyl)-tetrahydro-thiophen-2-yl]-6-chloro-9*H*-purine {(±)-13a, 13b}. To a solution of silylated 6-chloropurine, prepared from refluxing 6-chloropurine (388 mg, 2.51 mmol) and ammonium sulfate (catalytic amount) in HMDS (5 mL), in anhydrous $ClCH_2CH_2Cl$ (5 mL) was added a solution of (±)-**6** (800 mg, 1.93 mmol) in anhydrous $ClCH_2CH_2Cl$ (8 mL) followed by addition of TMSOTf (0.46 mL, 2.54 mmol) at 0 °C, and the reaction mixture was stirred at the same temperature for 40 min, and then overnight at ambient temperature. After the reaction mixture was quenched with saturated $NaHCO_3$ solution (6 mL), the reaction mixture was filtered through a Celite pad and diluted with methylene chloride (100 mL) and water (20 mL). The organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 3.3:1) to give (±)-**13a** (227 mg, 23%) as a white solid and (±)-**13b** (637 mg, 65%) as a white solid.

(±)-**13a**: UV (CH_2Cl_2) λ_{max} 265 nm; ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H, *t*-C_4H_9), 2.18–2.31 (m, 1H, 3-H_a), 2.61–2.83 (m, 2H, 3-H_b, 4-H), 3.08 (dd, 1H, *J* = 6.2, 10.2 Hz, 5-H_a), 3.27 (t, 1H, *J* = 10.2 Hz, 5-H_b), 3.74 (d, 2H, *J* = 5.4 Hz, TBDPSOCH_2), 6.34 (dd, 1H, *J* = 6.8, 8.4 Hz,

2-H), 7.35–7.63 (m, 10H, 2*Ar), 8.45 (s, 1H, H-2), 8.74 (s, 1H, H-8). Anal. calcd for $C_{26}H_{29}ClN_4OSSi$: C, 61.33; H, 5.74; N, 11.00. Found: C, 61.58; H, 5.88; N, 11.35.

(±)-**13b**: mp 105–106 °C; UV (CH_2Cl_2) λ_{max} 266 nm; ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H, *t*-C_4H_9), 2.28–2.50 (m, 2H, 3-H), 2.53–2.68 (m, 1H, 4-H), 2.97 (t, 1H, J = 10.2 Hz, 5-H_a), 3.33 (dd, 1H, J = 6.7, 10.2 Hz, 5-H_b), 3.71 (dd, 1H, J = 5.8, 10.3 Hz, TBDPSOCH_a), 3.76 (dd, 1H, J = 5.3, 10.3 Hz, TBDPSOCH_b), 6.24 (dd, 1H, J = 2.1, 5.8 Hz, 2-H), 7.35–7.64 (m, 10H, 2*Ar), 8.53 (s, 1H, H-2), 8.75 (s, 1H, H-8). Anal. calcd for $C_{26}H_{29}ClN_4OSSi$: C, 61.33; H, 5.74; N, 11.00. Found: C, 61.46; H, 5.55; N, 10.85.

(±)-β-[5-(6-Chloro-purin-9-yl)-tetrahydro-thiophen-3-yl]-methanol {(±)-14a}. Compound (±)-**13a** (252 mg, 0.49 mmol) was subjected to the desilylation as described for the preparation of (±)-**11a**. Purification by silica gel column chromatography (methylene chloride/methanol = 23:1) gave (±)-**14a** (101 mg, 75%) as a white solid: FAB-LRMS m/z 271 ($M+H^+$); mp 110–111 °C; UV (MeOH) λ_{max} 264 nm; ^{1}H NMR (MeOH-d_4) δ 2.35 (td, 1H, J = 8.4, 12.4 Hz, 4-H_a), 2.67 (m, 1H, 3-H), 2.83 (td, 1H, J = 6.4, 12.8 Hz, 4-H_b), 3.10 (dd, 1H, J = 6.4, 10.4 Hz, 2-H_a), 3.27 (t, 1H, J = 10.4 Hz, 2-H_b), 3.69 (dd, 1H, J = 6.0, 11.2 Hz, HOCH_a), 3.71 (dd, 1H, J = 6.0, 10.8 Hz, HOCH_b), 6.47 (t, 1H, J = 8.4 Hz, 5-H), 8.75 (s, 1H, H-2), 8.85 (s, 1H, H-8). Anal. calcd for $C_{10}H_{11}ClN_4OS$: C, 44.36; H, 4.10; N, 20.69. Found: C, 44.76; H, 3.86; N, 20.98.

(±)-α-[5-(6-Chloro-purin-9-yl)-tetrahydro-thiophen-3-yl]-methanol {(±)-14b}. Compound (±)-**13b** (683 mg, 1.34 mmol) was subjected to the desilylation as described for the preparation of (±)-**11a**. Purification by silica gel column chromatography (methylene chloride/methanol = 23:1) gave (±)-**14b** (304 mg, 84%) as a white solid: FAB-LRMS m/z 271 ($M+H^+$); mp 136–137 °C; UV (MeOH) λ_{max} 265 nm; ^{1}H NMR (MeOH-d_4) δ 2.33 (m, 1H, 4-H_a), 2.60 (m, 1H, 4-H_b), 2.73 (m, 1H, 3-H), 2.91 (t, 1H, J = 9.6 Hz, 2-H_a), 3.41 (dd, 1H, J = 7.2, 10.0 Hz, 2-H_b), 3.67 (d, 2H, J = 6.0 Hz, HOCH_2), 6.38 (d, 1H, J = 6.4 Hz, 5-H), 8.74 (s, 1H, H-2), 8.88 (s, 1H, H-8). Anal. calcd for $C_{10}H_{11}ClN_4OS$: C, 44.36; H, 4.10; N, 20.69. Found: C, 44.67; H, 4.35; N, 20.29.

(±)-β-[5-(6-Amino-purin-9-yl)-tetrahydro-thiophen-3-yl]-methanol {(±)-15a}. A solution of (±)-**14a** (30 mg, 0.11 mmol) in methanolic ammonia (5 mL) was heated at 80 °C in a steel bomb for 15 h. After cooling, the solvent was removed and the resulting residue was purified by silica gel column chromatography (methylene chloride/methanol = 9:1) to give (±)-**15a** (26 mg, 93%) as a white solid: FAB-LRMS m/z 252 ($M+H^+$); mp 196–197 °C; UV (MeOH) λ_{max} 260 nm; ^{1}H NMR (DMSO-d_6) δ 2.36–2.65 (m, 2H, 4-H_a, 3-H), 2.67–2.77 (m, 1H, 4-H_b), 3.09 (dd, 1H, J = 5.8, 10.2 Hz, 2-H_a), 3.21 (t, 1H, J = 10.2 Hz, 2-H_b), 3.56–3.72 (m, 2H, HOCH_2), 4.91 (pseudo t, 1H, J = 5.2, 5.4 Hz, OH), 6.37 (dd, 1H, J = 6.9, 8.7 Hz, 5-H), 7.36 (s, 2H, NH_2), 8.24 (s, 1H, H-2), 8.50 (s, 1H, H-8). Anal. calcd for $C_{10}H_{13}N_5OS$: C, 47.79; H, 5.21; N, 27.87. Found: C, 47.75; H, 5.30; N, 27.88.

(±)-α-[5-(6-Amino-purin-9-yl)-tetrahydro-thiophen-3-yl]-methanol {(±)-15b}. Compound (±)-**14b** (34 mg, 0.13 mmol) was subjected to the amination as described for the preparation of (±)-**15a**. Purification by silica gel column chromatography (methylene chloride/methanol = 9:1) gave (±)-**15b** (24 mg 76%) as a white solid and recovered starting material (±)-**14b** (8 mg, 24%): FAB-LRMS m/z 252 ($M+H^+$); mp 202–203 °C; UV (MeOH) λ_{max} 260 nm; ^{1}H NMR (DMSO-d_6) δ 2.24–2.36 (m, 1H, 4-H_a), 2.51–2.62 (m, 1H, 4-H_b), 2.69–2.93 (m, 2H, 3-H, 2-H_a), 3.39 (dd, 1H, J = 6.0, 9.4 Hz, 2-H_b), 3.59 (d, 2H, J = 5.5 Hz, HOCH_2), 4.49 (br t, 1H, OH), 6.27 (dd, 1H, J = 2.9, 6.3 Hz, 5-H), 7.36 (br s, 2H, NH_2), 8.24 (s, 1H, H-2), 8.47 (s, 1H, H-8). Anal. calcd for $C_{10}H_{13}N_5OS$: C, 47.79; H, 5.21; N, 27.87. Found: C, 47.64; H, 5.08; N, 28.04.

(±)-β-[5-(6-Methylamino-purin-9-yl)-tetrahydro-thiophen-3-yl]-methanol {(±)-16a}. A solution of (±)-**14a** (28 mg, 0.10 mmol) in methylamine (3 mL, 40% solution in water) and MeOH (4 mL) was heated at 80 °C for 10 h. After cooling, the solvent was removed and the residue was purified by silica gel column chromatography (methylene chloride/methanol = 9:1) to give (±)-**16a** (27 mg, 98%) as a white solid: FAB-LRMS m/z 266 ($M+H^+$); mp 210–211 °C; UV (MeOH) λ_{max} 266 nm; ^{1}H NMR (MeOH-d_4) δ 2.26 (td, 1H, J = 8.8, 12.0 Hz, 4-H_a), 2.64 (m, 1H, 3-H), 2.77 (td, 1H, J = 6.4, 12.8 Hz, 4-H_b), 3.08 (dd, 1H, J = 6.0, 10.0 Hz, 2-H_a), 3.22 (t, 1H, J = 10.4 Hz, 2-H_b), 3.31 (s, 3H, CH_3), 3.66 (dd, 1H, J = 6.4, 11.2 Hz, HOCH_a), 3.71 (dd, 1H, J = 6.0, 10.8 Hz, HOCH_b), 6.32 (dd, 1H, J = 7.2, 8.4 Hz, 5-H), 8.26 (s, 1H, H-2), 8.38 (s, 1H, H-8). Anal. calcd for $C_{10}H_{15}N_5OS$: C, 49.79; H, 5.70; N, 26.39. Found: C, 49.39; H, 6.09; N, 25.99.

(±)-α-[5-(6-Methylamino-purin-9-yl)-tetrahydro-thiophen-3-yl]-methanol {(±)-16b}. Compound (±)-**14b** (36 mg, 0.13 mmol) was subjected to the methylamination as described for the preparation of (±)-**16a**. Purification by silica gel column chromatography (methylene chloride/methanol = 9:1) gave (±)-**16b** (33 mg, 94%) as a white solid: FAB-LRMS m/z 266 ($M+H^+$); mp 220–221 °C; UV (MeOH) λ_{max} 265 nm; ^{1}H NMR (MeOH-d_4) δ 2.28 (ddd, 1H, J = 6.0, 11.2, 13.6 Hz, 4-H_a), 2.50 (ddd, 1H, J = 2.4, 5.2, 14.0 Hz, 4-H_b), 2.67 (m, 1H, 3-H), 2.88 (irregular t, 1H, J = 8.8, 10.4 Hz, 2-H_a), 3.35 (s, 3H, CH_3), 3.37 (dd, 1H, J = 6.8, 10.0 Hz, 2-H_b), 3.66 (d, 2H, J = 6.4 Hz, HOCH_2), 6.23 (dd, 1H, J = 2.0, 6.0 Hz, 5-H), 8.27 (s, 1H, H-2), 8.34 (s, 1H, H-8). Anal. calcd for $C_{10}H_{15}N_5OS$: C, 49.79; H, 5.70; N, 26.39. Found: C, 49.98; H, 6.00; N, 26.08.

(±)-β-9-(4-Hydroxymethyl-tetrahydro-thiophen-2-yl)-9*H*-purin-6-ol {(±)-17a}. To a solution of (±)-**14a** (25 mg, 0.09 mmol) in MeOH (6 mL) were added 2-mercaptoethanol (0.03 mL, 0.43 mmol) and NaOMe (0.39 mL, 0.39 mmol, 1.0 M solution in MeOH) and the reaction mixture was refluxed for 6 h. After cooling, the reaction mixture was neutralized with glacial AcOH and evaporated. The resulting residue was purified by silica gel column chromatography (methylene chloride/methanol = 8:1) to give (±)-**17a** (20 mg, 86%) as a white

solid: FAB-LRMS m/z 253 ($M+H^+$); mp 222–223 °C; UV (MeOH) λ_{max} 246 nm; ^{1}H NMR (DMSO-d_6) δ 2.30–2.43 (m, 1H, 3-H_a), 2.52–2.65 (m, 1H, 4-H), 2.69–2.78 (m, 1H, 3-H_b), 3.09 (dd, 1H, $J=5.9$, 10.3 Hz, 5-H_a), 3.20 (t, 1H, $J=10.3$ Hz, 5-H_b), 3.59 (dd, 1H, $J=6.4$, 10.7 Hz, HOCH_a), 3.66 (dd, 1H, $J=5.7$, 10.7 Hz, HOCH_b), 4.93 (br s, 1H, OH), 6.33 (dd, 1H, $J=7.0$, 8.6 Hz, 2-H), 8.16 (s, 1H, H-2), 8.44 (s, 1H, H-8), 12.5 (br s, 1H, OH). Anal. calcd for $C_{10}H_{12}N_4O_2$: C, 47.61; H, 4.79; N, 22.21. Found: C, 47.93; H, 4.77; N, 22.02.

(±)-α-9-(4-Hydroxymethyl-tetrahydro-thiophen-2-yl)-9*H*-purin-6-ol {(±)-17b}. (±)-**17b** was synthesized starting from compound (±)-**14b** (34 mg, 0.12 mmol) in similar procedure as described for the preparation of (±)-**17a**. Purification by silica gel column chromatography (methylene chloride/methanol = 8:1) gave (±)-**17b** (30 mg, 95%) as a white solid: FAB-LRMS m/z 253 ($M+H^+$); mp 217–218 °C; UV (MeOH) λ_{max} 246 nm; ^{1}H NMR (DMSO-d_6) δ 2.25–2.37 (m, 1H, 3-H_a), 2.49–2.58 (m, 1H, 3-H_b), 2.71–2.93 (m, 2H, 4-CH, 5-H_a), 3.37 (dd, 1H, $J=6.4$, 9.8 Hz, 5-H_b), 3.59 (d, 2H, $J=5.8$ Hz, HOCH_2), 4.88 (br s, 1H, OH), 6.25 (dd, 1H, $J=2.7$, 6.6 Hz, 2-H), 8.16 (s, 1H, H-2), 8.42 (s, 1H, H-8), 12.5 (br s, 1H, OH). Anal. calcd for $C_{10}H_{12}N_4O_2$: C, 47.61; H, 4.79; N, 22.21. Found: C, 47.66; H, 4.82; N, 22.22.

(±)-α and β-*N*-{9-[4-(*tert*-Butyl-diphenyl-silanyloxymethyl)-tetrahydro-thiophen-2-yl]-6-chloro-9*H*-purin-2-yl}-acetamide {(±)-18}. To a stirred solution of silylated 2-acetamido-6-chloropurine, prepared from refluxing 2-acetamido-6-chloropurine (429 mg, 2.03 mmol) and ammonium sulfate (catalytic amount) in HMDS (8 mL), in anhydrous $ClCH_2CH_2Cl$ (5 mL) was added a solution of (±)-**6** (600 mg, 1.45 mmol) in anhydrous $ClCH_2CH_2Cl$ (7 mL) followed by addition of TMSOTf (0.37 mL, 2.04 mmol) at 0 °C, and the reaction mixture was stirred at ambient temperature for 10 h. After the reaction mixture was quenched with saturated $NaHCO_3$ solution (5 mL), the reaction mixture was filtered through a Celite pad and diluted with methylene chloride (80 mL) and water (20 mL). The organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 1.5:1) to give inseparable mixture, (±)-**18** (121 mg, 15%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9H, β-t-C$_4H_9$), 1.05 (s, 9H, α-t-C$_4H_9$), 2.21 (td, 1H, $J=8.4$, 11.6 Hz, β-3-H_a), 2.31 (ddd, 1H, $J=6.4$, 11.2, 13.2 Hz, α-3-H_a), 2.45 (ddd, 1H, $J=2.0$, 5.2, 13.2 Hz, α-3-H_b), 2.54 (s, 3H, β-COCH_3), 2.55 (s, 3H, α-COCH_3), 2.57–2.78 (m, 3H, β-3-H_b, α-4-H, β-4-H), 2.95 (t, 1H, $J=10.0$ Hz, α-5-H_a), 3.07 (dd, 1H, $J=6.0$, 10.8 Hz, β-5-H_a), 3.21 (t, 1H, $J=10.4$ Hz, β-5-H_b), 3.31 (dd, 1H, $J=6.8$, 10.4 Hz, α-5-H_b), 3.70–3.78 (m, 4H, α-TBDPSOCH_2, β-TBDPSOCH_2), 6.09 (dd, 1H, $J=2.0$ Hz, α-2-H), 6.22 (dd, 1H, $J=6.8$, 8.4 Hz, β-2-H), 7.36–7.63 (m, 20H, α-2*Ar, β-2*Ar), 8.03 (br s, 2H, α-NH, β-NH), 8.30 (s, 1H, β-H-8), 8.35 (s, 1H, α-H-8). Anal. calcd for $C_{28}H_{32}ClN_5O_2SSi$: C, 59.40; H, 5.70; N, 12.37. Found: C, 59.79; H, 5.88; N, 12.01.

(±)-α and β-*N*-[6-Chloro-9-(4-hydroxymethyl-tetrahydro-thiophen-2-yl)-9*H*-purin-2-yl]-acetamide {(±)-19}. To a stirred solution of (±)-**18** (116 mg, 0.21 mmol) in THF (3 mL) was added tetrabutylammonium fluoride (0.22 mL, 0.22 mmol, 1.0 M solution in THF) at ambient temperature for 30 min. The volatiles were evaporated and the residue was purified by silica gel column chromatography (methylene chloride/methanol = 13.5:1) to give inseparable mixture of (±)-**19** (68 mg, 100%) as a white solid.

(±)-α and β-2-Amino-9-(4-hydroxymethyl-tetrahydro-thiophen-2-yl)-1,9-dihydro-purin-6-one {(±)-20}. To a solution of (±)-**19** (46 mg, 0.14 mmol) in MeOH (5 mL) were added 2-mercaptoethanol (0.06 mL, 0.86 mmol) and NaOMe (0.84 mL, 0.84 mmol, 1.0 M solution in MeOH) and the reaction mixture was refluxed for 7 h. After cooling, the reaction mixture was neutralized with glacial AcOH and evaporated. The resulting residue was purified by silica gel column chromatography (methylene chloride/methanol = 6:1) to give inseparable mixture (±)-**20** (33 mg, 88%) as a white solid, and then reversed-phase (Octadecyl-C_{18}) chromatography (H_2O →20% methanol in H_2O) to give a small amount of each pure epimer, (±)-**20a** and (±)-**20b** for analysis, respectively.

(±)-**20a**: FAB-LRMS m/z 268 ($M+H^+$); UV (MeOH) λ_{max} 256 nm; ^{1}H NMR (MeOH-d_4) δ 2.17 (td, 1H, $J=8.8$, 12.0 Hz, 3-H_a), 2.58 (m, 1H, 4-H), 2.64–2.72 (m, 1H, 3-H_b), 3.03 (dd, 1H, $J=6.4$, 10.0 Hz, 5-H_a), 3.18 (t, 1H, $J=10.4$ Hz, 5-H_b), 3.63 (dd, 1H, $J=6.0$, 11.2 Hz, HOCH_a), 3.70 (dd, 1H, $J=6.0$, 11.2 Hz, HOCH_b), 6.15 (dd, 1H, $J=7.2$, 8.8 Hz, 2-H), 8.02 (s, 1H, H-8). Anal. calcd for $C_{10}H_{13}N_5O_2S$: C, 44.93; H, 4.90; N, 26.20. Found: C, 45.12; H, 4.76; N, 26.57.

(±)-**20b**: FAB-LRMS m/z 268 ($M+H^+$); UV (MeOH) λ_{max} 256 nm; ^{1}H NMR (MeOH-d_4) δ 2.20 (ddd, 1H, $J=6.4$, 10.8, 13.2 Hz, 3-H_a), 2.46 (ddd, 1H, $J=2.4$, 5.2, 13.6 Hz, 3-H_b), 2.68 (m, 1H, 4-H), 2.84 (t, 1H, $J=10.0$ Hz, 5-H_a), 3.33 (dd, 1H, $J=6.8$, 10.4 Hz, 5-H_b), 3.65 (d, 2H, $J=6.4$ Hz, HOCH_2), 6.05 (dd, 1H, $J=2.8$, 6.4 Hz, 2-H), 8.03 (s, 1H, H-8). Anal. calcd for $C_{10}H_{13}N_5O_2S$: C, 44.93; H, 4.90; N, 26.20. Found: C, 44.91; H, 4.85; N, 26.00.

Evaluation of anti-HIV activity and cytotoxicity

Anti-HIV activity and cytotoxicity were determined as described previously.[7,8]

Evaluation of cytotoxic potential against human cancer cell lines

Cytotoxic potential was determined as described previously.[9] Briefly, human cells (A549, lung carcinoma; Col2, colon carcinoma) in log growth phase were counted, diluted to 5×10^4 cells/mL with fresh medium, and added to 96-well microtiter plates (190 μL/well) containing test materials (10 μL in 10% aqueous DMSO). Test plates were incubated for 3 days at 37 °C in a CO_2 incubator. For zero day controls, cells were incubated for 30 min at 37 °C in a CO_2 incubator. All treatments were performed in triplicate. After the incubation periods,

cells were fixed by the addition of 50 μL of cold 50% aqueous TCA solution (4 °C for 30 min), washed 4-5 times with tap water, and air-dried. The fixed cells were stained with SRB (0.4% w/v in 1% aqueous acetic acid) for 30 min. Free SRB solution was then removed by rinsing with 1% acetic acid. The plates were then air-dried, the bound dye was solubilized with 200 μL of 10 mM tris-base (pH 10.0), and absorbance was determined at 515 nm using an ELISA plate reader. Finally, the absorbance values obtained with each of the treatment procedures were averaged, and the averaged value obtained with the zero day control was subtracted. These results were expressed as a percentage, relative to solvent-treated control incubations, and IC_{50} values were calculated using non-linear regression analyses (percent survival versus concentration).

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References and Notes

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