Syntheses and Structure−Activity Relationships of Novel Apio and Thioapio Dideoxydidehydronucleosides as Anti-HCMV Agents⊥

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On the basis of the fact that apio dideoxynucleosides, in which the furanose oxygen and the C2 of the 2,3-dideoxyribose are transposed, exhibited potent anti-HIV activity and 2′,3′-dideoxy-2′,3′-didehydronucleosides also showed potent anti-HIV activity, we synthesized apio dideoxydidehydronucleosides in which the oxygen atom and the double bond of the 2,3-dideoxy-2,3-didehydroribose are exchanged. The thioapio dideoxydidehydronucleosides were also synthesized since sulfur serves as a bioisostere of oxygen. Apio dideoxydidehydronucleosides **13a**−**f** were synthesized starting from 1,3-dihydroxyacetone, utilizing phenylselenenyl chemistry as a key step. The ratio of the anomeric mixture was variable from 1:1 to 5:1 during the condensation of nucleosidic bases with the phenylselenyl acetate **11** in the presence of a Lewis acid. This is in contrast with other glycosyl donors such as 5-*O*-(*tert*-butyldiphenylsilyl)-2-phenylselenenyl-2,3-dideoxyribosyl acetate which shows excellent neighboring group effect (α:β = 1:99). Thioapio dideoxydidehydronucleosides **22a**,**b** were synthesized from the lactone **9** via thiolactone **17** as a key intermediate which was synthesized from dicyclohexylcarbodiimide coupling of the mercapto acid produced from the basic hydrolysis of thioacetate **16**. The majority of apio analogues synthesized in this study exhibited moderate to potent anti-HCMV activity, among which the 5-fluorouracil derivative **13c** was found to be the most potent against HCMV, while thioapio analogues showed no activity against HCMV. However, all synthesized compounds did not exhibit any significant activities against HIV-1, HSV-1, and HSV-2. The fact that apio dideoxydidehydronucleosides were active against HCMV suggests that the apio dideoxydidehydro sugar moiety can serve as a novel template for the development of new antiviral agents.

Introduction

A number of 2′,3′-dideoxynucleosides (ddNs) and 2′,3′-dideoxy-2′,3′-didehydronucleosides (d4Ns) have been synthesized and evaluated for antiviral activities against human immunodeficiency virus (HIV)-1[1] and hepatitis B virus (HBV).[2] These classes of compounds exhibit their antiviral activities by inhibiting the reverse transcription process of the viral replicative cycle and/or by being incorporated into the viral DNA chain, resulting in viral DNA chain termination.[3−5] 2′,3′-Dideoxycytidine (**1**, ddC, Zalcitabine)[1] (Figure 1) is a potent anti-HIV agent being clinically used for the treatment of AIDS patients. However, this class of compounds show dose-dependent toxicity like peripheral neuropathy by inhibiting DNA polymerase γ.[6] 2′,3′-Dideoxy-2′,3′-didehydrothymidine (**2**, d4T, Starvudine) is the only nucleoside approved by the Food and Drug Administration (FDA) among d4Ns for the treatment of AIDS and AIDS-related complexes (ARC), but it also suffers from adverse effects such as peripheral neuropathy and the appearance of resistant strains.[7,8]

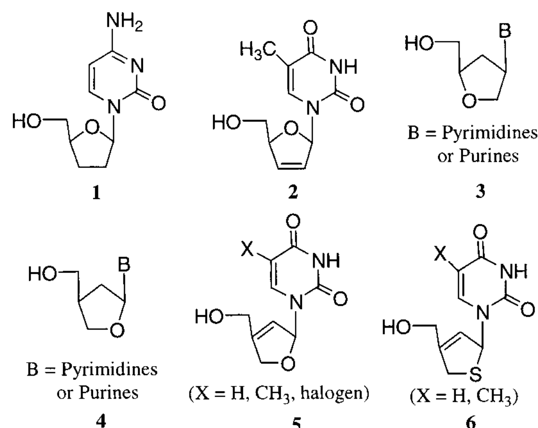

Figure 1. Rationale to the design of apio and thioapio d4Ns.

Iso dideoxynucleosides (iso ddNs, **3**)[9] and apio dideoxynucleosides (apio ddNs, **4**)[10] belong to a novel class of nucleosides in that the oxygen atom of the furanose ring moves to the C3 or C2 position, respectively. These compounds not only showed antiviral activities comparable to those of parent ddNs but also have metabolic advantages such as resistance to adenosine deaminase and glycosyl bond hydrolysis.

Based on the potent antiviral activity of apio ddNs and d4Ns, it was interesting to transpose the double

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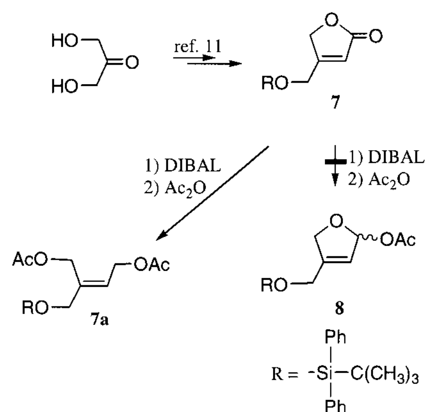
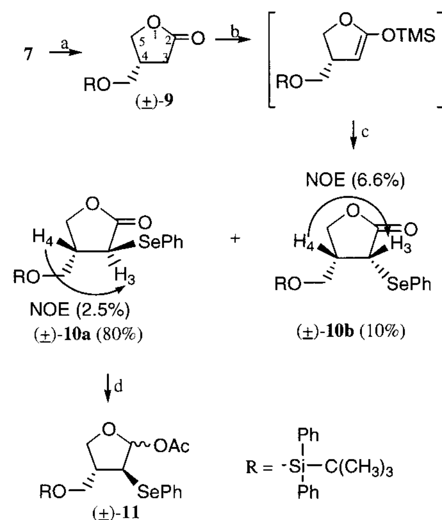
Scheme 1



Scheme 2[a]



[a] Reagents: (a) H_2, Pd/C, 99%; (b) LiHMDS, THF, −78 °C, then TMSCl, rt; (c) PhSeBr, −78 °C; (d) DIBAL, −78 °C, then A_2O, pyridine, rt, 85%.

bond and furanose ring oxygen of d4Ns for the design of apio dideoxydidehydronucleosides (**5**, apio d4Ns). We also designed thioapio analogues (**6**) of apio d4Ns since the sulfur atom serves as a bioisostere of the oxygen. Here, we report the first syntheses and structure−activity relationship (SAR) study of novel apio d4Ns **5** and thioapio d4Ns **6** as antiviral agents.

Results and Discussion

Chemistry. Our original synthetic plan was to synthesize a cyclic allylic acetate **8** and then condense with nucleosidic bases as seen in Scheme 1. However, when α,β-unsaturated lactone **7**,[11] which was easily synthesized from 1,3-dihydroxyacetone, was reduced with DIBAL at −78 °C followed by acetylation with acetic anhydride in pyridine, it did not give the desired cyclic allylic acetate **8** but afforded the linear allylic acetate **7a** as a major product. With the assumption that no formation of **8** occurred due to the rigidity of the double bond, we decided to insert a double bond at the nucleoside stage using phenylselenenyl chemistry as shown in Scheme 2.

α,β-Unsaturated lactone **7** was reduced to the saturated lactone (±)-**9** by catalytic hydrogenation in quantitative yield. The proton signals of (±)-**9** were assigned based on the known TBDMS-protected lactone,[12] in which two protons (δ 3.64) of $CH_2OTBDMS$ moved more upfield than those of 5-CH_2 (δ 4.20 and 4.39). In compound (±)-**9**, two protons (δ 3.70) of $CH_2OTBDPS$ also shifted more upfield than those of 5-CH_2 (δ 4.26 and 4.42). Treatment of (±)-**9** with LiHMDS at −78 °C followed by trapping of the enloate with trimethylsilyl chloride at room temperature gave the silyl enol ether, which without isolation was treated with phenylselenenyl bromide at −78 °C to give the desired (±)-**10a** as a major product (80%) and the undesired (±)-**10b** as a minor product (10%) after silica gel column chromatography.[13] The major formation of (±)-**10a** was achieved because the phenylselenenyl group was added from the opposite side to the bulky TBDPS group as reported by Campbell[12] and Chu.[13] In addition to the explanation of the stereochemistry of the phenylselenenyl group by steric effect, a ^{1}H NOE experiment was employed to confirm the configuration of the phenylselenenyl group in (±)-**10a** and (±)-**10b**. It was found that a NOE effect (2.5%) between 3-H and 4-H of the 3,4-*trans* isomer (±)-**10a** was smaller than that (6.6%) of the 3,4-*cis* isomer (±)-**10b**. To explain the fact that the 3,4-*trans* compound (±)-**10a** has a smaller ^3J between 3-H and 4-H (5.3 Hz) than the 3,4-*cis* compound (±)-**10b** (8.0 Hz), the preferred conformations of (±)-**10a** and (±)-**10b** were obtained from MM2 energy minimization. The energy-minimized structure of 3,4-*trans* (±)-**10a** appears to adopt predominantly the N (3-*exo*/4-*endo*) conformation in which the dihedral angle between H_3−C_3−C_4−H_4 is close to 110°, giving a small coupling constant (5.3 Hz) from the vicinal Karplus correlation. However, 3,4-*cis* (±)-**10b** seems to take the S (3-*endo*/4-*exo*) conformation in which the dihedral angle between H_3−C_3−C_4−H_4 is about 25°, giving a large coupling constant (8.0 Hz). This conformational difference between (±)-**10a** and (±)-**10b** appears to be due to the gauche effect between the furan oxygen and the electron-withdrawing selenium.[14] The desired (±)-**10a** was reacted with DIBAL at −78 °C to give the lactol, which without purification was treated with acetic anhydride in pyridine to afford the key intermediate (±)-**11** which is ready for condensation with nucleosidic bases.

Synthesis of the target apio d4Ns (±)-**13a**−(±)-**13f** from (±)-**11** is illustrated in Scheme 3. The glycosyl donor (±)-**11** was condensed with silylated uracil, thymine, and 5-halo-substituted uracils in the presence of TMSOTf as a Lewis acid in anhydrous 1,2-dichloroethane to give the protected nucleosides (±)-**12a**−(±)-**12f** as inseparable anomeric mixtures, respectively whose ratios were variable from 1:1 to 5:1, indicating that neighboring group effect by the phenylselenenyl group was zero or not as great in this ring system. This result contrasts with another glycosyl donor, 5-*O*-(*tert*-butyldiphenylsilyl)-2-phenylselenenyl-2,3-dideoxyribosyl acetate, which showed excellent neighboring group effect (α:β = 1:99) through the participation of the C2-phenylselenenyl group to the oxonium ion generated at the anomeric position during the condensation with silylated thymine.[13] It is presumed that the lack of a neighboring group effect by the phenylselenenyl group in this ring system might be due to the repulsive effect between incoming nucleosidic bases and the pseudoaxial bulky hydroxymethyl substituent as illustrated in Figure 2, which drove the reaction equlibrium to the

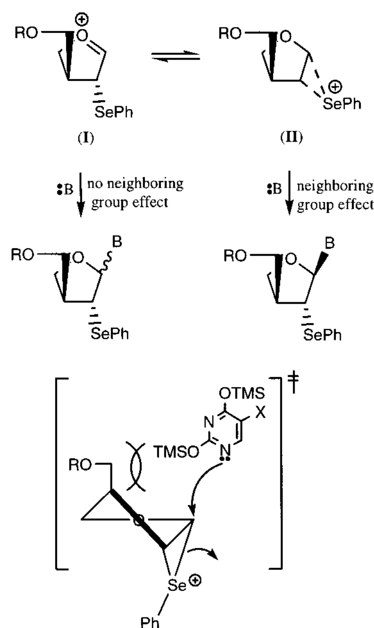
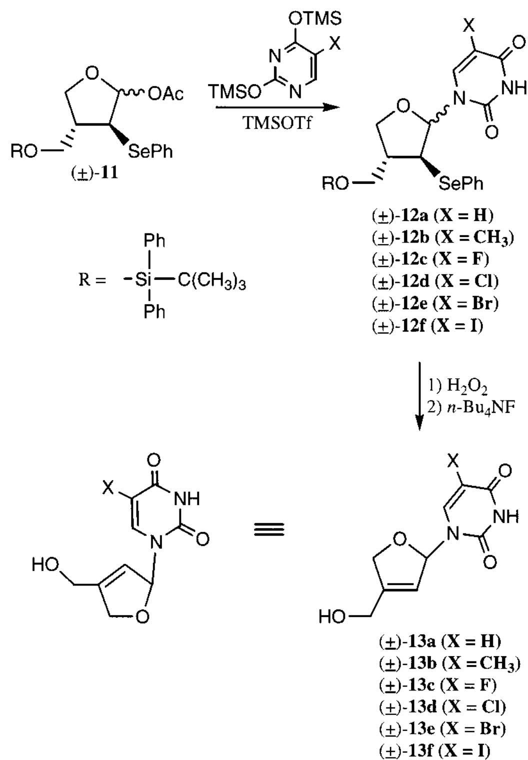


Figure 2. Neighboring group effect by the phenylselenenyl group is affected by the bulky TBDPS substituent as shown in the transition state.

Scheme 3



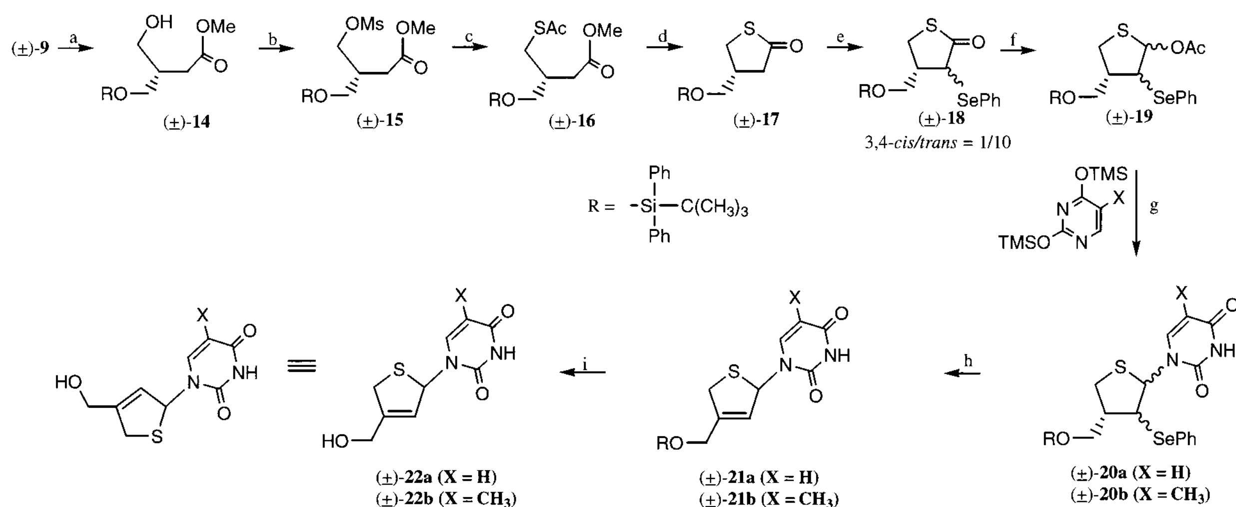
oxonium ion intermediate (**I**), not the episelenonium ion intermediate (**II**).

The protected nucleosides (±)-**12a**−(±)-**12f** were treated with hydrogen peroxide with a catalytic amount of pyridine to give the *syn*-eliminated products, which were deprotected with tetra-*n*-butylammonium fluoride in THF to afford the final apio d4Ns, (±)-**13a**−(±)-**13f**, respectively. From the ^{1}H NMR analysis of the synthesized nucleosides, two methylene protons of CH_2-OTBDPS or CH_2OH in (±)-**12a**−(±)-**12f** or (±)-**13a**−(±)-**13f** were found to always shift more upfield than the 5-H's, as in the case of compound (±)-**9**.

Attempts to synthesize cytosine and 6-substituted purine derivatives were made, but we could not obtain the final products. Although the condensation of the acetate (±)-**11** with silylated N^4-benzoylcytosine under TMSOTf followed by *syn* elimination by hydrogen peroxide produced the protected cytosine nucleoside in very good yield, deprotection of the TBDPS group resulted in the extensive decomposition on TLC. Condensation of (±)-**11** with silylated 6-chloropurine or adenine also did not afford the desired product as in the case of cytosine.

Synthesis of bioisosteric nucleosides, (±)-**22a** and (±)-**22b**, of apio d4Ns in which the oxygen atom was replaced by a sulfur atom is shown in Scheme 4. The lactone (±)-**9** was treated with 1 N NaOH followed by addition of dimethyl sulfate to yield the hydroxy ester (±)-**14** (78%). The primary hydroxyl group was converted to the mesylate (±)-**15** by reacting with mesyl chloride in pyridine. To synthesize the desired thiolactone (±)-**17**, we first treated (±)-**15** with sodium disulfide in DMF at 110 °C, but only small amounts of the desired product (±)-**15** were produced under this conditions. Therefore, we obtained thiolactone (±)-**17** by a three-step procedure in good yield from (±)-**15**: treatment of (±)-**15** with potassium thioacetate in DMF to give the thioacetyl ester (±)-**16**, which was reacted with aqueous NaOH in ethanol, followed by cyclization of the resulting mercapto acid with DCC. When the ^{1}H NMR of thiolactone (±)-**17** was compared to that of lactone (±)-**9**, their coupling patterns and chemical shifts were almost identical except for the different chemical shifts of the 5-H's. Two protons (δ 3.35 and 3.47) of the 5-H's in (±)-**17** were more shielded than those (δ 4.26 and 4.42) of the 5-H's in (±)-**9** because sulfur is less electronegative than oxygen; thus, in compound (±)-**17**, two protons of CH_2OTBDPS moved more downfield (δ 3.69) than those of 5-CH_2 (δ 3.35 and 3.47) in contrast to compound (±)-**9**. Introduction of the phenylselenenyl group at the α position of the thiolactone (±)-**17** was achieved using the same procedure shown in Scheme 2 to give the inseparable diastereomeric mixture of (±)-**18** (*cis/trans* = 1/10 as determined by ^{1}H NMR). Reduction of (±)-**18** with DIBAL in THF gave the lactol that was treated with acetic anhydride to afford the key intermediate (±)-**19**. Condensation of (±)-**19** with silylated uracil and thymine in the presence of TMSOTf produced (±)-**20a** and (±)-**20b**, respectively. Smooth conversion of the phenylselenenyl group in (±)-**20a** and (±)-**20b** to the double bond under the presence of the sulfur atom was achieved using *m*CPBA and pyridine at −30 °C to give (±)-**21a** and (±)-**21b**, respectively.[15] In this reaction condition, only the 3,4-*trans* isomer underwent *syn* elimination, while 3,4-*cis* isomer remained intact as selene oxide due to the absence of a *syn* hydrogen. TBDPS group was deprotected under the standard conditions to give the final thioapio analogues (±)-**22a** and (±)-**22b**, respectively. As in the case of apio d4Ns, attempts to synthesize the thioapio cytosine analogue were not successful. When ^{1}H NMR of thioapio nucleosides was compared with that of apio nucleosides, their coupling patterns and chemical shifts were very similar except for the chemical shifts of the 5-H's. It is interesting to note that only one proton of 5-H's in all

Scheme 4[a]



[a] Reagents: (a) 1 N NaOH, 1 h then $(CH_3)_2SO_4$, DMSO, 1 h, 89%; (b) MsCl, pyridine, rt; (c) KSAc, DMF, rt, 7 h, 86% from (±)-**14**; (d) NaOH, H_2O, EtOH, 50 °C, 2.5 h, then DCC, DMAP, CH_2Cl_2, 30 min, 70%; (e) i. LiHMDS, THF, −78 °C, 1 h, then TMSCl, 30 min, ii. PhSeBr, −78 °C, 30 mim, 99%; (f) i. DIBAL, THF, −78 °C, ii. Ac_2O, pyridine, 84%; (g) TMSOTf, $ClCH_2CH_2Cl$, rt, 74% for (±)-**20a**, 72% for (±)-**20b**; (h) *m*CPBA, CH_2Cl_2, −30 °C, 71% for (±)-**21a**, 75% for (±)-**21b**; (i) *n*-Bu_4NF, THF, 0 °C, 1 h, 76% for (±)-**22a**, 71% for (±)-**22b**.

Table 1. Anti-HCMV Activity[16,17] of Synthesized Apio d4Ns and Thioapio d4Ns

compd	EC_{50} (μg/mL) AD-169	EC_{50} (μg/mL) Davis	CC_{50} (μg/mL)
13a (X = O, Y = H)	11.7	>33.3	33.3
13b (X = O, Y = CH_3)	33.3	16.6	>100
13c (X = O, Y = F)	6.9	2.81	>100
13d (X = O, Y = Cl)	41.7	25.1	63.3
13e (X = O, Y = Br)	33.7	25.4	>100
13f (X = O, Y = I)	44.1	27.2	>100
22a (X = S, Y = H)	>100	>100	>100
22b (X = S, Y = CH_3)	>100	>100	>100
foscarnet (PFA)	64.9	38.7	>300
ganciclovir (GCV)	1.09	0.61	>10

of the apio and thioapio nucleosides showed allylic coupling ($J \cong 2$ Hz) probably because of the rigid sugar moiety.

Antiviral Activity. All synthesized compounds were tested against several viruses such as HIV-1 (MT-4 cells), HSV-1 (CCL81 cells), HSV-2 (CCL81 cells), and HCMV (AD-169 and Davis cells). All compounds exhibited neither antiviral activity nor cytotoxicity when tested up to 100 μg/mL against HIV-1, HSV-1, and HSV-2. However, it is interesting to note that apio d4Ns are only active against HCMV (Table 1), indicating this virus may allow the conformationally rigid sugar moiety for phosphorylation as well as for DNA polymerase unlike other viruses although the hydroxymethyl group orientation of the apio sugar is greatly different from that of the normal dideoxyribo sugar. For the evaluation of anti-HCMV activity, HCMV strains AD-169 (ATCC VR-538) and Davis (ATCC VR-807) were used and standard CPE inhibition assay was used.[14] HEL 299 (human embryonic lung fibroblast) cells were used for the cytotoxic assay. As shown in Table 1, most apio d4Ns showed moderate to potent anti-HCMV activity without cytotoxicity, among which the 5-fluorouracil derivative (±)-**13c** was found to be the most potent and other analogues showed moderate anti-HCMV activity. All apio d4Ns were more active than forscarnet (PFA) but less potent than ganciclovir (GCV) which was used for the control in both AD-169- and Davis-infected cells. Thioapio analogues (±)-**22a** and (±)-**22b** did not exhibit any significant antiviral activities. This result indicates that although the oxygen is similar to the sulfur in valence electrons, differences in size and electronegativity may play a key role in phosphorylation by kinases or in binding to the viral polymerases.

In summary, we accomplished the first syntheses of apio and thioapio d4Ns starting from 1,3-dihydroxyacetone using phenylselenenyl chemistry for the insertion of a double bond into the sugar moiety. When the synthesized final compounds were tested against several viruses such as HIV-1, HSV-1, HSV-2, and HCMV, they were only active against HCMV, indicating this virus allows the rigid sugar moiety unlike other viruses. These results suggest that the apio dideoxydidehydro sugar moiety can serve as a novel template for the development of new antiviral agents.

Experimental Section

Ultraviolet (UV) spectra were recorded on a Beckman DU-68 spectrophotometer; ^1H and ^{13}C NMR spectra were recorded on a Varian-400 spectrometer, using $CDCl_3$ or DMSO-d_6, and chemical shifts are 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, 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.

(±)-4-(*tert*-Butyldiphenylsilyloxymethyl)-4*H*,5*H*-dihydrofuran-2-one (9). To a solution of **7** (2.0 g, 5.7 mmol) in ethyl acetate (10 mL) was added 10% Pd/C (10 mg) and the mixture was stirred at ambient temperature for 48 h under H_2 atmosphere. The mixture was filtered through a Celite pad

and washed with ethyl acetate and methanol successively. Solvents were evaporated in vacuo and the residue was dried under high vacuum to give **9** (2.0 g, 99%): R_f = 0.54 (hexanes: ethyl acetate = 4:1); ^{1}H NMR ($CDCl_3$) δ 1.02 (s, 9 H, *tert*-butyl), 2.35 (dd, 1 H, J = 6.3 and 17.5 Hz, 3-H$_a$), 2.61 (dd, 1 H, J = 8.7 and 17.5 Hz, 3-H$_b$), 2.72−2.81 (m, 1 H, 4-H), 3.70 (d, 2 H, J = 5.8 Hz, SiO−CH_2), 4.26 (dd, 1 H, J = 5.4 and 9.1 Hz, 5-H$_a$), 4.42 (dd, 1 H, J = 6.6 and 9.1 Hz, 5-H$_b$), 7.41−7.68 (m, 10 H, 2xPh). Anal. ($C_{21}H_{26}O_3Si$) C, H.

(±)-3,4-*trans*-4-(*tert*-Butyldiphenylsilyloxymethyl)-3-phenylselenyl-4*H*,5*H*-dihydrofuran-2-one (10a) and Its *Cis* Isomer (10b). To a solution of **9** (1.72 g, 4.9 mmol) in anhydrous THF (5 mL) was added LiHMDS (1 M solution in THF, 5.93 mL, 5.93 mmol) at −78 °C and the mixture was stirred at the same temperature for 1 h. Chlorotrimethylsilane (1.43 mL, 5.9 mmol) was added to the reaction mixture and the mixture was allowed to stir at ambient temperature for 30 min. The mixture was again cooled to −78 °C and a solution of phenylselenenyl bromide (1.9 g, 7.35 mmol) in THF (2 mL) was added dropwise to the mixture. When dark brown color disappeared, reaction was stopped. The mixture was poured into ether (100 mL) and the ether layer was washed with H_2O (20 mL), dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate= 5:1) to give **10a** (2.13 g, 77%) as a colorless syrup and **10b** (0.21 g, 7.7%) as a colorless syrup.

10a: R_f = 0.67 (hexanes:ethyl acetate = 5:1); ^{1}H NMR ($CDCl_3$) δ 1.03 (s, 9 H, *tert*-butyl), 2.54−2.61 (m, 1 H, 4-H), 3.61 (dd, 1 H, J = 4.8 and 10.5 Hz, SiO−CH_a), 3.70 (dd, 1 H, J = 5.3 and 10.5 Hz, SiO−CH_b), 3.86 (d, 1 H, J = 5.3 Hz, 3-H), 4.10 (d, 2 H, J = 6.2 Hz, 5-H), 7.25−7.64 (m, 15 H, 3xPh). Anal. ($C_{27}H_{30}O_3SeSi$) C, H.

10b: R_f = 0.45 (hexanes:ethyl acetate = 5:1); ^{1}H NMR ($CDCl_3$) δ 1.03 (s, 9 H, *tert*-butyl), 2.81−2.90 (m, 1 H, 4-H), 3.86 (dd, 1 H, J = 4.8 and 10.4 Hz, SiO−CH_a), 3.89 (dd, 1 H, J = 4.0 and 10.4 Hz, SiO−CH_b), 4.01 (d, 1 H, J = 8.0 Hz, 3-H), 4.06 (dd, 1 H, J = 6.4 and 9.2 Hz, 5-H$_a$), 4.32 (dd, 1 H, J = 7.2 and 9.2 Hz, 5-H$_b$), 7.21−7.79 (m, 15 H, 3xPh). Anal. ($C_{27}H_{30}O_3SeSi$) C, H.

(±)-2-*O*-Acetyl-3,4-*trans*-4-(*tert*-butyldiphenylsilyloxymethyl)-3-phenylselenyltetrahydrofuran (11). To a solution of **10a** (2.05 g, 3.68 mmol) in anhydrous toluene (15 mL) at −78 °C was added DIBAL (1 M solution in toluene, 5.52 mL, 5.52 mmol) and the mixture was stirred at −78 °C for 2 h. The reaction mixture was quenched with MeOH (2 mL) and $CHCl_3$ (5 mL) and stirred for 10 min at −78 °C. To the reaction mixture were added 10% Na,K tartarate solution (20 mL) and $CHCl_3$ (100 mL). The organic layer was washed with brine (20 mL), dried ($MgSO_4$), filtered and evaporated. The crude lactol, without further purification, was dried under high vacuum for 1 h and acetylated by treating with acetic anhydride (0.69 mL, 7.36 mmol) and pyridine (10 mL) at room temperature overnight. After removal of the solvent under reduced pressure, the residue was dissolved in CH_2Cl_2 (50 mL), washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by flash silica gel column chromatography (hexanes:ethyl acetate = 8:1) to give an anomeric mixture (1:1) of **11** (1.7 g, 85%) as a pale yellow syrup: R_f = 0.68 (hexanes:ethyl acetate = 3:1); ^{1}H NMR ($CDCl_3$) δ 1.00 (s, 4.5 H, *tert*-butyl), 1.05 (s, 4.5 H, *tert*-butyl), 1.92 (s, 1.5 H, COCH_3), 2.09 (s, 1.5 H, COCH_3), 2.35−2.64 (m, 1 H, 4-H), 3.55−3.61 (m, 2 H, SiO−CH_2), 3.72−3.77 (m, 1 H, 3-H), 3.86−4.14 (m, 2 H, 5-H), 6.28 (d, 0.5 H, J = 1.9 Hz, 2-H), 6.43 (d, 0.5 H, J = 4.4 Hz, 2-H), 7.21−7.63 (m, 15 H, 3xPh). Anal. ($C_{29}H_{34}O_4SeSi$) C, H.

General Procedure for the Synthesis of 12a−f: Condensation. A suspension of nucleosidic base (0.75 mmol) and ammonium sulfate (catalytic amount) in hexadimethyldisilazane (HMDS; 6 mL) was heated at 140−150 °C until a clear solution was obtained. The reaction mixture was cooled to room temperature and HMDS was removed under reduced pressure with exclusion of moisture. To this residue was added a solution of **11** (0.5 mmol) in anhydrous 1,2-dichloroethane under nitrogen and the reaction mixture was cooled to 5 °C. TMSOTf (0.75 mmol) was added and the mixture was allowed to stir at ambient temperature for 1 h. The reaction mixture was poured into CH_2Cl_2, neutralized with saturated $NaHCO_3$ solution and stirred for 10 min. The organic layers were separated, washed once with saturated $NaHCO_3$ solution and brine, dried ($MgSO_4$), filtered and evaporated. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 2:1) to give the protected nucleosides **12a−f**, respectively.

(±)-1-[4-(*tert*-Butyldiphenylsilyloxymethyl)-3-phenylselenyltetrahydrofuran-2-yl]-1*H*-pyrimidine-2,4-dione (12a): yield 90% (3:1 anomeric mixture); R_f = 0.58 (hexanes: ethyl acetate = 1:1); UV (MeOH) λ_{max} 260 nm; ^{1}H NMR ($CDCl_3$) δ 1.06 (s, 2.25 H, *tert*-butyl), 1.09 (s, 0.75 H, *tert*-butyl), 2.40−2.48 (m, 0.75 H, 4-H), 2.50−2.60 (m, 0.25 H, 4-H), 3.56−3.95 (m, 2 H, SiO−CH_2), 3.97−4.08 (m, 2 H, 5-H), 4.21−4.39 (m, 1 H, 3-H), 5.54 (d, 0.75 H, J = 8.0 Hz, H-5), 5.69 (d, 0.25 H, J = 8.0 Hz, H-5), 6.10 (d, 0.75 H, J = 8.0 Hz, 2-H), 6.21 (d, 0.25 H, J = 4.9 Hz, 2-H), 7.07−7.66 (m, 16 H, 3xPh and H-6), 8.21 (br s, 0.25 H, NH), 8.52 (br s, 0.75 H, NH). Anal. ($C_{31}H_{34}N_2O_4SeSi$) C, H, N.

(±)-1-[4-(*tert*-Butyldiphenylsilyloxymethyl)-3-phenylselenyltetrahydrofuran-2-yl]-5-methyl-1*H*-pyrimidine-2,4-dione (12b): yield 63% (5:1 anomeric mixture); R_f = 0.45 (hexanes:ethyl acetate = 2:1); UV (MeOH) λ_{max} 265 nm; ^{1}H NMR ($CDCl_3$) δ 1.09 (s, 1.5 H, *tert*-butyl), 1.14 (s, 7.5 H, *tert*-butyl), 1.98 (s, 0.5 H, CH_3), 2.08 (s, 2.5 H, CH_3), 2.39−2.80 (m, 1 H, 4-H), 3.55−3.85 (m, 2 H, SiO−CH_2), 3.98−4.26 (m, 3 H, 3-H and 5-H), 6.13 (d, 0.83 H, J = 8.5 Hz, 2-H), 6.24 (d, 0.17 H, J = 5.4 Hz, 2-H), 7.25−7.70 (m, 16 H, 3xPh and H-6), 7.75 (br s, 1 H, NH). Anal. ($C_{32}H_{36}N_2O_4SeSi$) C, H, N.

(±)-1-[4-(*tert*-Butyldiphenylsilyloxymethyl)-3-phenylselenyltetrahydrofuran-2-yl]-5-fluoro-1*H*-pyrimidine-2,4-dione (12c): yield 62% (1:1 anomeric mixture); R_f = 0.56 (hexanes:ethyl acetate = 1:1); UV (MeOH) λ_{max} 268 nm; ^{1}H NMR ($CDCl_3$) δ 1.06 (s, 4.5 H, *tert*-butyl), 1.08 (s, 4.5 H, *tert*-butyl), 2.35−2.60 (m, 1 H, 4-H), 3.55−3.80 (m, 2 H, SiO−CH_2), 3.88−4.12 (m, 2 H, 5-H), 4.23−4.39 (m, 1 H, 3-H), 6.07 (dd, 0.5 H, J = 1.7 and 6.4 Hz, 2-H), 6.17 (dd, 0.5 H, J = 1.6 and 3.9 Hz, 2-H), 7.26−7.66 (m, 17 H, 3xPh, NH and H-6). Anal. ($C_{32}H_{36}FN_2O_4SeSi$) C, H, N.

(±)-1-[4-(*tert*-Butyldiphenylsilyloxymethyl)-3-phenylselenyltetrahydrofuran-2-yl]-5-chloro-1*H*-pyrimidine-2,4-dione (12d): yield 69% (1:1 anomeric mixture); R_f = 0.58 (hexanes:ethyl acetate = 1:1); UV (MeOH) λ_{max} 272 nm; ^{1}H NMR ($CDCl_3$) δ 1.07 (s, 4.5 H, *tert*-butyl), 1.09 (s, 4.5 H, *tert*-butyl), 2.35−2.60 (m, 1 H, 4-H), 3.61−3.80 (m, 2 H, SiO−CH_2), 3.86−4.04 (m, 2 H, 5-H), 4.12−4.35 (m, 1 H, 3-H), 6.07 (d, 0.5 H, J = 8.2 Hz, 2-H), 6.19 (d, 0.5 H, J = 5.3 Hz, 2-H), 7.18−7.68 (m, 16 H, 3xPh and H-6), 7.95 (br s, 1 H, NH). Anal. ($C_{31}H_{33}ClN_2O_4SeSi$) C, H, N.

(±)-1-[4-(*tert*-Butyldiphenylsilyloxymethyl)-3-phenylselenyltetrahydrofuran-2-yl]-5-bromo-1*H*-pyrimidine-2,4-dione (12e): yield 91% (1:1 anomeric mixture); R_f = 0.60 (hexanes:ethyl acetate = 1:1); UV (MeOH) λ_{max} 278 nm; ^{1}H NMR ($CDCl_3$) δ 1.07 (s, 4.5 H, *tert*-butyl), 1.10 (s, 4.5 H, *tert*-butyl), 2.35−2.58 (m, 1 H, 4-H), 3.62−3.79 (m, 2 H, SiO−CH_2), 3.90−4.13 (m, 2 H, 5-H), 4.29−4.35 (m, 1 H, 3-H), 6.07 (d, 0.5 H, J = 8.3 Hz, 2-H), 6.17 (d, 0.5 H, J = 5.3 Hz, 2-H), 7.18−7.66 (m, 16 H, 3xPh and H-6), 8.17 (br s, 1 H, NH). Anal. ($C_{31}H_{33}BrN_2O_4SeSi$) C, H, N.

(±)-1-[4-(*tert*-Butyldiphenylsilyloxymethyl)-3-phenylselenyltetrahydrofuran-2-yl]-5-iodo-1*H*-pyrimidine-2,4-dione (12f): yield 68% (3:1 anomeric mixture); R_f = 0.63 (hexanes:ethyl acetate = 1:1); UV (MeOH) λ_{max} 283 nm; ^{1}H NMR ($CDCl_3$) δ 1.07 (s, 2.25 H, *tert*-butyl), 1.12 (s, 6.75 H, *tert*-butyl), 2.39−2.46 (m, 1 H, 4-H), 3.61−3.79 (m, 2 H, SiO−CH_2), 3.89−4.17 (m, 2 H, 5-H), 4.31−4.38 (m, 1 H, 3-H), 6.05 (d, 0.75 H, J = 8.3 Hz, 2-H), 6.16 (d, 0.25 H, J = 5.3 Hz, 2-H), 7.18−7.66 (m, 16 H, 3xPh and H-6), 7.85 (br s, 1 H, NH). Anal. ($C_{31}H_{33}IN_2O_4SeSi$) C, H, N.

General Procedure for the Synthesis of 13a−f: *Syn* Elimination and Deprotection. To a solution of protected nucleosides **12a−f** (0.15 mmol) in CH_2Cl_2 (3 mL) were added

30% aqueous hydrogen peroxide (1.9 mmol) and 1 drop of pyridine and the mixture was stirred at ambient temperature for 1 h, respectively. The mixture was poured into saturated $NaHCO_3$ solution (10 mL) and CH_2Cl_2 (30 mL) and the organic layer was separated, washed with brine (20 mL), dried ($MgSO_4$), filtered and evaporated. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 2:1) to give the *syn*-eliminated nucleosides, respectively. A solution of the *syn*-eliminated nucleosides (0.17 mmol) in THF (5 mL) was treated with tetra-*n*-butylammonium fluoride (1 M solution in THF, 0.23 mL, 0.23 mmol) at 0 °C and the mixture was stirred at the same temperature for 30 min. The mixture was evaporated and the residue was purified by silica gel column chromatography (chloroform:methanol = 10:1) to give the final nucleosides **13a**−**f**, respectively.

(±)-1-(4-Hydroxymethyl-2,5-dihydrofuran-2-yl)-1*H*-pyrimidine-2,4-dione (13a): yield 84%; R_f = 0.38 (chloroform: methanol = 10:1); mp 326 °C (methanol/ether); UV (H_2O) λ_{max} 259 nm (ϵ 7850); ^{1}H NMR (DMSO-d_6) δ 4.17 (br s, 2 H, HOCH_2), 4.52 (d, 1 H, J = 13.2 Hz, 5-H_a), 4.75 (ddd, 1 H, J = 2.0, 5.2 and 13.2 Hz, 5-H_b), 5.12 (br s, 1 H, OH, D_2O exchangeable), 5.61 (d, 1 H, J = 8.0 Hz, H-5), 5.64 (br d, 1 H, J = 1.6 Hz, 3-H), 6.80 (m, 1 H, 2-H), 7.35 (d, 1 H, J = 8.0 Hz, H-6), 11.1 (br s, 1 H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 56.6, 74.8, 90.1, 101.9, 116.9, 140.6, 149.8, 150.5, 163.1. Anal. ($C_9H_{10}N_2O_4$) C, H, N.

(±)-1-(4-Hydroxymethyl-2,5-dihydrofuran-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione (13b): yield 44%; R_f = 0.50 (chloroform:methanol = 10:1); mp 320 °C (methanol/ether); UV (H_2O) λ_{max} 264 nm (ϵ 10760); ^{1}H NMR (DMSO-d_6) δ 1.77 (s, 3 H, CH_3), 4.18 (br s, 2 H, HOCH_2), 4.51 (d, 1 H, J = 13.2 Hz, 5-H_a), 4.78 (ddd, 1 H, J = 2.0, 4.0 and 13.2 Hz, 5-H_b), 5.12 (t, 1 H, J = 5.6 Hz, OH, D_2O exchangeable), 5.61 (br d, 1 H, J = 1.6 Hz, 3-H), 6.82 (m, 1 H, 2-H), 7.16 (s, 1 H, H-6), 11.3 (br s, 1 H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 12.0, 56.6, 74.7, 89.0, 109.7, 117.0, 135.9, 149.5, 150.5, 163.8. Anal. ($C_{10}H_{12}N_2O_4$) C, H, N.

(±)-5-Fluoro-1-(4-hydroxymethyl-2,5-dihydrofuran-2-yl)-1*H*-pyrimidine-2,4-dione (13c): yield 44%; R_f = 0.45 (chloroform:methanol = 10:1); mp 260 °C (methanol/ether); UV (H_2O) λ_{max} 268 nm (ϵ 7980); ^{1}H NMR (DMSO-d_6) δ 4.17 (br s, 2 H, OH−CH_2), 4.50 (d, 1 H, J = 13.2 Hz, 5-H_a), 4.80 (ddd, 1 H, J = 2.4, 5.2 and 13.2 Hz, 5-H_b), 5.10 (t, 1 H, J = 5.6 Hz, OH, D_2O exchangeable), 5.61 (d, 1 H, J = 1.6 Hz, 3-H), 6.79 (m, 1 H, 2-H), 7.65 (d, 1 H, J = 6.8 Hz, H-6), 11.8 (br s, 1 H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 56.6, 56.7, 74.9, 90.6, 116.5, 124.7, 141.3, 150.2, 159.8. Anal. ($C_9H_9FN_2O_4$) C, H, N.

(±)-5-Chloro-1-(4-hydroxymethyl-2,5-dihydrofuran-2-yl)-1*H*-pyrimidine-2,4-dione (13d): yield 90%; R_f = 0.44 (chloroform:methanol = 15:1); mp 333 °C dec (methanol/ether); UV (H_2O) λ_{max} 277 nm (ϵ 7750); ^{1}H NMR (DMSO-d_6) δ 4.17 (br s, 2 H, HOCH_2), 4.51 (d, 1 H, J = 13.2 Hz, 5-H_a), 4.81 (ddd, 1 H, J = 2.1, 5.1 and 13.2 Hz, 5-H_b), 5.14 (t, 1 H, J = 5.6 Hz, OH, D_2O exchangeable), 5.63 (d, 1 H, J = 1.5 Hz, 3-H), 6.78 (m, 1 H, 2-H), 7.66 (s, 1 H, H-6), 11.9 (br s, 1 H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 56.6, 56.7, 75.0, 90.8, 107.6, 116.5, 137.7, 150.2, 159.0. Anal. ($C_9H_9ClN_2O_4$) C, H, N.

(±)-5-Bromo-1-(4-hydroxymethyl-2,5-dihydrofuran-2-yl)-1*H*-pyrimidine-2,4-dione (13e): yield 90%; R_f = 0.45 (chloroform:methanol = 15:1); mp 292 °C (methanol/ether); UV (H_2O) λ_{max} 278 nm (ϵ 9530); ^{1}H NMR (DMSO-d_6) δ 4.18 (br s, 2 H, OHCH_2), 4.51 (d, 1 H, J = 13.2 Hz, 5-H_a), 4.81 (ddd, 1 H, J = 2.1, 5.1 and 13.2 Hz, 5-H_b), 5.15 (t, 1 H, J = 5.6 Hz, OH, D_2O exchangeable), 5.63 (d, 1 H, J = 1.6 Hz, 3-H), 6.78 (m, 1 H, 2-H), 7.70 (s, 1 H, H-6), 11.9 (br s, 1 H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 56.6, 56.7, 75.0, 90.8, 96.2, 116.5, 140.0, 150.4, 159.1. Anal. ($C_9H_9BrN_2O_4$) C, H, N.

(±)-1-(4-Hydroxymethyl-2,5-dihydrofuran-2-yl)-5-iodo-1*H*-pyrimidine-2,4-dione (13f): yield 45%; R_f = 0.46 (chloroform:methanol = 15:1); mp 241 °C (methanol/ether); UV (H_2O) λ_{max} 283 nm (ϵ 6810); ^{1}H NMR (DMSO-d_6) δ 4.18 (br d, 2 H, HOCH_2), 4.52 (d, 1 H, J = 13.2 Hz, 5-H_a), 4.81 (ddd, 1 H, J = 2.2, 5.1 and 13.2 Hz, 5-H_b), 5.16 (t, 1 H, J = 5.6 Hz, OH, D_2O exchangeable), 5.63 (d, 1 H, J = 1.5 Hz, 3-H), 6.76 (m, 1 H, 2-H), 7.77 (s, 1 H, H-6), 11.7 (br s, 1 H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 56.6, 56.7, 70.1, 74.9, 90.7, 116.6, 144.6, 150.1, 160.4. Anal. ($C_9H_9IN_2O_4$) C, H, N.

(±)-3-(*tert*-Butyldiphenylsilyloxymethyl)-4-hydroxymethylbutyric Acid Methyl Ester (14). To a solution of **9** (11.9 g, 33.4 mmol) in H_2O (36 mL) and ethanol (140 mL) was added NaOH (1.4 g, 35 mmol) and the reaction mixture was stirred at ambient temperature for 1 h and concentrated in vacuo. Without further purification, the resulting residue was dissolved in DMSO (40 mL) and toluene (10 mL). To this mixture was added dimethyl sulfate (3.8 mL, 40.2 mmol) slowly and stirred at ambient temperature for another 1 h. The mixture was evaporated and the residue was poured into ethyl acetate (200 mL) and water (50 mL). The organic layer was separated, washed with brine (50 mL) and dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 4:1) to give **14** (11.5 g, 89%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9 H, *tert*-butyl), 2.42 (dd, 1 H, J = 6.8 and 17.6 Hz, 2-H_a), 2.44 (br s, 1 H, OH), 2.56 (dd, 1 H, J = 8.8 and 17.6 Hz, 2-H_b), 2.73 (m, 1 H, 3-H), 3.65 (s, 3 H, OCH_3), 3.67−3.78 (m, 2 H, SiO−CH_2), 4.23 (dd, 1 H, J = 5.6 and 9.6 Hz, HO−CH_a), 4.39 (dd, 1 H, J = 7.2 and 9.6 Hz, HO−CH_b), 7.38−7.66 (m, 10 H, 2xPh). Anal. ($C_{22}H_{30}O_4Si$) C, H.

(±)-4-Acetylsulfanyl-3-(*tert*-butyldiphenylsilyloxymethyl)butyric Acid Methyl Ester (16). To a solution of **14** (7.7 g, 19.9 mmol) in anhydrous pyridine (30 mL) was added mesyl chloride (2.32 mL, 29.9 mmol) at 0 °C and the reaction mixture was stirred at ambient temperature for 3 h. The solvent was removed under reduced pressure and the residue was extracted with ethyl acetate. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 7:1) to give the mesylate **15** as a colorless oil, which without further purification, was treated with potassium thioacetate (2.3 g, 20.5 mmol) in anhydrous DMF (50 mL). The resulting mixture was stirred at ambient temperature for 7 h and the reaction mixture was partitioned between ether (200 mL x 3) and water (100 mL). The organic layer was washed with brine (50 mL) and dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 7.5:1) to give **16** (7.6 g, 86% in two steps) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 9 H, *tert*-butyl), 2.31 (s, 3 H, SCOCH_3), 2.30−2.42 (m, 2 H, 2-H_a and 3-H), 2.53 (dd, 1 H, J = 7.4 and 15.6 Hz, 2-H_b), 3.02 (dd, 1 H, J = 6.1 and 13.6 Hz, 4-H_a), 3.11 (dd, 1 H, J = 6.5 and 13.6, 4-H_b), 3.59 (d, 2 H, J = 5.7 Hz, SiO−CH_2), 3.64 (s, 3 H, OCH_3), 7.25−7.65 (m, 10 H, 2xAr). Anal. ($C_{24}H_{32}O_4SSi$) C, H.

(±)-4-(*tert*-Butyldiphenylsilyloxymethyl)-4*H*,5*H*-dihydrothiophen-2-one (17). To a solution of **16** (4.4 g, 10.0 mmol) in H_2O (100 mL) and EtOH (300 mL) was added NaOH (1.9 g, 50 mmol) and the reaction mixture was stirred at 50 °C for 2.5 h. After complete removal of the volatile materials, the residue was acidified to pH = 3 by adding HCl solution, which then was poured into methylene chloride (300 mL) and water (100 mL). The organic layer was washed with brine (50 mL) and dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexanes: ethyl acetate = 6:1) to give the crude mercapto acid derivative as an oil, which without further purification, was treated with dicyclohexylcarbodiimide (2.1 g, 10.1 mmol) and dimethylaminopyridine (6.1 g, 50.1 mmol) in anhydrous methylene chloride (250 mL). The reaction mixture was stirred at ambient temperature for 30 min. The resulting solid was filtered and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 16:1) to give **17** (2.6 g, 70% in two steps) as a white solid: MS m/z 371 (M + H^+); ^{1}H NMR ($CDCl_3$) δ 1.06 (s, 9 H, *tert*-butyl), 2.45 (dd, 1 H, J = 9.2 and 16.8 Hz, 3-H_a), 2.58 (dd, 1 H, J = 6.8 and 16.8 Hz, 3-H_b), 2.68−2.83 (m, 1 H, 4-H), 3.35 (dd, 1 H, J = 7.7 and 11.2 Hz, 5-H_a), 3.47 (dd, 1 H, J = 6.6 and 11.2 Hz, 5-H_b), 3.69 (d, 2 H, J = 5.9 Hz, SiO−CH_2), 7.36−7.64 (m, 10 H, 2xPh). Anal. ($C_{21}H_{26}O_2SSi$) C, H.

(±)-4-(2-*tert*-Butyldiphenylsilyloxymethyl)-3-phenyl-

selenyl-4*H*,5*H*-dihydrothiophen-2-one (18). To a solution of **17** (0.7 g, 1.89 mmol) in anhydrous THF (10 mL) was added LiHMDS (1 M solution in THF, 2.27 mL, 2.27 mmol) dropwise at −78 °C and the mixture was stirred for 1 h at the same temperature. To this mixture was added TMSCl (0.36 mL, 2.8 mmol) slowly and stirred for another 10 min at −78 °C. The reaction mixture was elevated to ambient temperature and stirred for 30 min. The reaction mixture was again cooled to −78 °C and a solution of PhSeBr (670 mg, 2.8 mmol) in THF (5 mL) was added quickly. The reaction mixture was stirred for 30 min and quenched by adding a few drops of ethyl acetate. The reaction mixture was evaporated under reduced pressure and the residue was diluted with ethyl acetate (150 mL) and water (50 mL). The organic layer was washed with brine and dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexanes: ethyl acetate = 15:1) to give an inseparable mixture of **18** (989 mg, 99%, 3,4-*trans*/3,4-*cis* = 10/1 determined by ^{1}H NMR) as a colorless oil. Analytical samples were obtained by preparative TLC to give pure **(±)-3,4-*trans*-18** and **(±)-3,4-*cis*-18**.

(±)-3,4-*trans*-18: ^{1}H NMR ($CDCl_3$) δ 1.03 (s, 9 H, *tert*-butyl), 2.47−2.59 (m, 1 H, 4-H), 3.32 (br d, 2 H, *J* = 6.4 Hz, 5-H), 3.85 (d, 1 H, *J* = 7.0 Hz, 3-H), 3.89 (dd, 1 H, *J* = 4.9 and 10.4 Hz, SiO−CH_a), 4.07 (dd, 1 H, *J* = 6.0 and 10.4 Hz, SiO−CH_b), 7.25−7.63 (m, 15 H, 3xPh). Anal. ($C_{27}H_{30}O_2SSeSi$) C, H.

(±)-3,4-*cis*-18: ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 9 H, *tert*-butyl), 2.40−2.43 (m, 1 H, 4-H), 3.05 (dd, 1 H, *J* = 2.4 and 10.4 Hz, 5-H_a), 3.25 (dd, 1 H, *J* = 4.4 and 10.4 Hz, 5-H_b), 3.86 (m, 3 H, 3-H and SiO−CH_2), 7.19−7.72 (m, 15 H, 3xPh). Anal. ($C_{27}H_{30}O_2SSeSi$) C, H.

(±)-2-*O*-Acetoxy-4-(*tert*-butyldiphenylsilyloxymethyl)-3-phenylselenyltetrahydrothiophene (19). An inseparable mixture of **18** (990 mg, 1.83 mmol) was converted to compound **19** (899 mg, 84% with small amounts of *cis* isomer) as an anomeric mixture (3:2 determined by ^{1}H NMR) according to the similar procedure used for the preparation of **11**. **(±)-3,4-*trans*-19:** ^{1}H NMR ($CDCl_3$) δ 1.01 (s, 5.4 H, *tert*-butyl), 1.05 (s, 3.6 H, *tert*-butyl), 1.92 (s, 1.2 H, CH_3), 2.09 (s, 1.8 H, CH_3), 2.44−2.57 (m, 0.4 H, H-4), 2.58−2.98 (m, 0.6 H, H-4), 2.98−3.07 (m, 2 H, H-5), 3.61 (dd, 0.6 H, *J* = 4.2 and 12.5 Hz, 3-H), 3.58−3.94 (m, 2.4 H, SiO−CH_2 and 3-H), 6.14 (d, 0.4 H, *J* = 4.8 Hz, 2-H), 6.22 (d, 0.6 H, *J* = 4.2 Hz, 2-H), 7.20−7.62 (m, 15 H, 3xPh). Anal. ($C_{29}H_{34}O_3SSeSi$) C, H.

(±)-1-[4-(*tert*-Butyldiphenylsilyloxymethyl)-3-phenylselenyltetrahydrothiophen-2-yl]-1*H*-pyrimidine-2,4-dione (20a). The suspension of uracil (118 mg, 1.05 mmol), HMDS (10 mL), and ammonium sulfate (catalytic amount) was refluxed under nitrogen atmosphere for 4 h and excess HMDS was removed under high vacuum. To the residue were added dry dichloroethane (5 mL), a solution of **19** (300 mg, 0.52 mmol) in dry 1,2-dichloroethane (15 mL), and TMSOTf (0.2 mL, 0.96 mmol) at ambient temperature and the resulting reaction mixture was stirred at ambient temperature for 1 h. Saturated $NaHCO_3$ (2 mL) solution was added to the reaction mixture and the mixture was stirred for another 30 min and diluted with methylene chloride (30 mL). The organic layer was washed with brine (10 mL) and dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 2:1) to give **20a** (243 mg, 74% with small amounts of *cis* isomer) as an anomeric mixture (4:1 ratio determined by ^{1}H NMR): UV (MeOH) λ_{max} 266 nm; ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 1.8 H, *tert*-butyl), 1.10 (s, 7.2 H, *tert*-butyl), 2.11−2.24 (m, 0.8 H, 4-H), 2.48−2.53 (m, 0.2 H, 4-H), 2.97 (dd, 0.8 H, *J* = 6.8 and 10.4 Hz, 5-H_a), 3.23 (dd, 0.2 H, *J* = 4.3 and 10.4 Hz, 5-H_a), 3.35 (dd, 0.2 H, *J* = 4.5 and 10.4 Hz, 5-H_b), 3.41 (t, 0.8 H, *J* = 10.4 Hz, 5-H_b), 3.55 (m, 1 H, 3-H), 3.90−4.06 (m, 2 H, SiO−CH_2), 6.36 (d, 0.2 H, *J* = 3.6 Hz, 2-H), 6.55 (d, 0.8 H, *J* = 9.6 Hz, 2-H), 7.17−7.71 (m, 16 H, 3xPh and H-6), 8.82 (br s, 1 H, NH). Anal. ($C_{31}H_{34}N_2O_3SSeSi$) C, H, N.

(±)-1-[4-(*tert*-Butyldiphenylsilyloxymethyl)-2*H*,5*H*-dihydrothiophen-2-yl]-1*H*-pyrimidine-2,4-dione (21a). To a solution of **20a** (200 mg, 0.32 mmol) in anhydrous CH_2Cl_2 (10 mL) was slowly added *m*-CPBA (72 mg, 0.4 mmol) in CH_2Cl_2 (3 mL) at −25 °C. The reaction mixture was stirred at the same temperature for 1 h, evaporated, and diluted with ethyl acetate (40 mL). The organic layer was washed with brine (10 mL) and dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 2.5:1) to give **21a** (129 mg, 71%): UV (MeOH) λ_{max} 267 nm; ^{1}H NMR ($CDCl_3$) δ 0.99 (s, 9 H, *tert*-butyl), 3.65 (d, 1 H, *J* = 15.2 Hz, 5-H_a), 3.93 (ddd, 1 H, *J* = 1.6, 5.2 and 15.2 Hz, 5-H_b), 4.11 (s, 2 H, SiO−CH_2), 5.65 (d, 1 H, *J* = 8.0 Hz, H-5), 5.68 (d, 1 H, *J* = 1.6 Hz, 3-H), 7.02 (d, 1 H, *J* = 1.6 Hz, 2-H), 7.37−7.69 (m, 11 H, 2xPh and H-6).

(±)-1-(4-Hydroxymethyl-2*H*,5*H*-dihydrothiophen-2-yl)-1*H*-pyrimidine-2,4-dione (22a). To a solution of **21a** (100 mg, 0.22 mmol) in THF (5 mL) was added tetra-*n*-butylammonium fluoride (1 M solution in THF, 0.3 mL, 0.3 mmol) and the mixture was stirred at 0 °C for 1 h and evaporated to a dryness. The residues were purified by silica gel column chromatography (chloroform:methanol = 10:1) to give **22a** (37 mg, 76%) as a white solid: MS *m*/*z* 249 (M + Na^+); mp 163 °C (methanol/ether); UV (H_2O) λ_{max} 266 nm (ϵ 7830); ^{1}H NMR (DMSO-d_6) δ 3.66 (d, 1 H, *J* = 15.2 Hz, 5-H_a), 3.94 (ddd, 1 H, *J* = 2.0, 5.2 and 15.2 Hz, 5-H_b), 4.12 (br s, 1 H, HOCH_2), 5.19 (t, 1 H, *J* = 5.2 Hz, OH, D_2O exchangeable), 5.66 (d, 1 H, *J* = 8.0 Hz, H-5), 5.69 (d, 1 H, *J* = 1.7 Hz, 3-H), 6.74 (m, 1 H, 2-H), 7.44 (d, 1 H, *J* = 8.0 Hz, H-6); ^{13}C NMR (DMSO-d_6) δ 37.88, 60.22, 68.45, 95.64, 122.29, 142.43, 151.53, 155.85, 166.09. Anal. ($C_9H_{10}N_2O_3S$) C, H, N.

(±)-1-[4-(*tert*-Butyldiphenylsilyloxymethyl)-3-phenylselenyltetrahydrothiophen-2-yl]-5-methyl-1*H*-pyrimidine-2,4-dione (20b). The acetate **19** (0.96 g, 1.68 mmol) was converted to **20b** (0.77 g, 72% with small amounts of *cis* isomer) as an anomeric mixture (4:1 ratio determined by ^{1}H NMR) according to the procedure used for the preparation of **20a**: ^{1}H NMR ($CDCl_3$) δ 1.06 (s, 1.8 H, *tert*-butyl), 1.12 (s, 7.2 H, *tert*-butyl), 1.77 (s, 0.6 H, 5-CH_3), 1.81 (s, 2.4 H, 5-CH_3), 2.14−2.22 (m, 0.8 H, H-4), 2.72−2.78 (m, 0.2 H, H-4), 2.92 (dd, 0.8 H, *J* = 8.0 and 10.9 Hz, 5-H_a), 3.07 (dd, 0.2 H, *J* = 4.0 and 10.9 Hz, 5-H_a), 3.27 (dd, 0.2 H, *J* = 6.4 and 10.9 Hz, 5-H_b), 3.37 (t, 0.8 H, *J* = 10.9 Hz, 5-H_b), 3.53 (dd, 0.8 H, *J* = 10.1 and 12.0 Hz, 3-H), 3.73 (dd, 0.2 H, *J* = 6.1 and 7.7 Hz, 3-H), 3.85−3.89 (m, 1 H, SiO−CH_a), 4.11−4.18 (m, 1 H, SiO−CH_b), 6.33 (d, 0.2 H, *J* = 7.7 Hz, 2-H), 6.42 (d, 0.8 H, *J* = 10.1 Hz, 2-H), 7.05−7.72 (m, 16 H, 3xPh and H-6), 7.95 (br s, 0.2 H, NH, D_2O exchangeable), 7.95 (br s, 0.8 H, NH, D_2O exchangeable). Anal. ($C_{32}H_{36}N_2O_3SSeSi$) C, H, N.

(±)-1-[4-(*tert*-Butyldiphenylsilyloxymethyl)-2*H*,5*H*-dihydrothiophen-2-yl]-5-methyl-1*H*-pyrimidine-2,4-dione (21b). Compound **20b** (485 mg, 0.76 mmol) was converted to **21b** (272 mg, 75%) according to the procedure used for the preparation of **21a**: ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 9 H, *tert*-butyl), 1.78 (s, 3 H, CH_3), 3.65 (d, 1 H, *J* = 15.2 Hz, 5-H_a), 3.98 (ddd, 1 H, *J* = 2.0, 5.6 and 15.2 Hz, 5-H_b), 4.08 (d, 1 H, *J* = 15.2 Hz, SiO−CH_a), 4.12 (d, 1 H, *J* = 15.2 Hz, SiO−CH_b), 5.65 (d, 1 H, *J* = 1.2 Hz, 3-H), 7.04 (d, 1 H, *J* = 1.2 Hz, 2-H), 7.37−7.69 (m, 11 H, 2xPh and H-6), 8.62 (br s, 1 H, NH).

(±)-1-(4-Hydroxymethyl-2*H*,5*H*-dihydrothiophen-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione (22b). Compound **21b** was converted to **22b** (35.6 mg, 71%) as a white solid according to the procedure used for the preparation of **22a**: MS *m*/*z* 263 (M + Na^+); mp 185 °C (methanol/ether); UV (H_2O) λ_{max} 270 nm (ϵ 11550); ^{1}H NMR (DMSO-d_6) δ 1.77 (s, 3 H, CH_3), 3.65 (d, 1 H, *J* = 15.6 Hz, 5-H_a), 3.99 (ddd, 1 H, *J* = 2.4, 5.6 and 15.6 Hz, 5-H_b), 4.12 (br s, 2 H, HOCH_2), 5.17 (t, 1 H, *J* = 5.7 Hz, OH, D_2O exchangeable), 5.65 (br d, 1 H, *J* = 1.6 Hz, 3-H), 6.75 (m, 1 H, 2-H), 7.24 (s, 1 H, H-6), 11.4 (br s, 1 H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 12.89, 38.21, 60.22, 67.79, 110.95, 121.64, 137.09, 151.13, 152.06, 164.36. Anal. ($C_{10}H_{12}N_2O_3S$) C, H, N.

Anti-HCMV Assay. Viruses and cells: Human cytomegalovirus (HCMV) strains AD-169 (ATCC VR-538) and Davis (ATCC VR-807) and HEL 299 (human embryonic lung fibroblast) cells (ATCC CCL137) were purchased from American Type Culture Collection (ATCC). The cells were grown in minimal essential medium (MEM; Gibco) containing nones-

sential amino acids, 40 μg/mL gentamycin (Gibco) and 10% heat-inactivated FBS (MEM/10% FBS; Gibco).

Compounds: All compounds used were dissolved with 100% dimethyl sulfoxide as 20 mg/mL of stock solution except phosphonoformic acid (PFA, foscarnet; Sigma) and ganciclovir (GCV; Synthex) with distilled H_2O.

Evaluation of anti-HCMV activity: Standard CPE inhibition assay was used.[16] HEL cells in stationary phase were infected with the virus at a multiplicity of infection of 2−4 $CCID_{50}$ (50% cell culture inhibitory dose) per well of 96-well plates. After 2 h adsorption at 37 °C, the liquid was aspirated off to remove the unadsorbed viruses and 100 μL of MEM/2% FBS containing a compound was applied to each well in duplicate for each concentration and further incubated for 6 days. Antiviral activity was measured microscopically or fluorometrically. For microscopic observation the cells were fixed with 70% ethanol, stained with 2.5% Giemsa solution for 2 h, rinsed with distilled water and air-dried. Antiviral activity was expressed as the EC_{50}, or the concentration required to inhibit virus-induced CPE by 50%. EC_{50} values were estimated from semilogarithmic graphic plots of the percentage of CPE as a function of the concentration of the test compound. For fluorometric assay,[17] the cells were washed twice with 100 μL of phosphate-buffered saline (PBS). To each well 100 μL of 5 μg/mL fluorescein diacetate (FDA; Sigma) was added and the plates were incubated for 30 min at 37 °C. The FDA solution was removed by aspiration and each well was washed with 100 μL of PBS. The fluorescence intensity (as absolute fluorescence units, AFU) in each well was measured with a fluorescent microplate reader (fluoroskan ascent, Labsystem) equipped with a 485-nm excitation filter and a 538-nm emission filter. The percentage reduction was caculated as follows: $100 - [\mathrm{AFU(dt)} - \mathrm{AFU(cc)}/\mathrm{AFU(vc)} - \mathrm{AFU(cc)} \times 100] = \mathrm{Y}$ (cc, cell control; vc, virus control; dt, drug-treated).

Cytotoxicity assay: The effect of the test compounds on host cell growth and on viability was assayed microscopically and fluorometrically by using propidium iodide (PI; Sigma). To measure cytostatic effects, HEL cells were seeded at 3000 cells/well in 96-well plates in 100 μL of MEM/10% FBS. The cells were allowed to attach to the plates by incubation at 37 °C for 1 day, different dilutions of the test compounds were added and the cells were further incubated for 3 days at 37 °C. The cells were permeabilized by freezing at −20 °C for 2 h. They were thawed at 37 °C for 30 min, 100 μL of 40 ug/mL PI diluted with medium was added to each well following incubation at room temperature for 60 min in the dark to allow dye penetration. AUF was read with a fluorescence microplate reader using a 544-nm excitation filter and a 620-nm emission filter. The concentration of compound responsible for 50% inhibition of cell growth was calculated and expressed as CS_{50} (50% cytostatic effect).

Cytocidal assay was performed as a control experiment for antiviral assay. It was carried out simultaneously with the antiviral assay described previously using mock instead of virus for infection, and cell viability was measured using PI instead of FDA. The concentration of compound responsible for 50% reduction of cell viability was calculated and expressed as CC_{50} (50% cytocidal effect).

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