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LETTER

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Synthesis of 4′-Ethynyl-2′-deoxy-4′-thioribonucleosides and Discovery of a Highly Potent and Less Toxic NRTI

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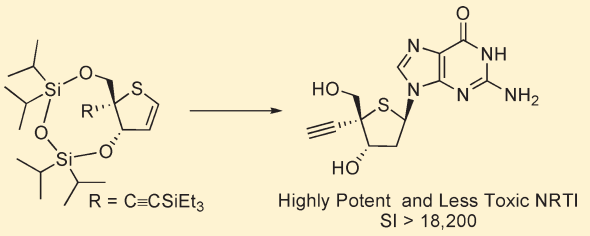
Supporting Information

ABSTRACT: The synthesis of 4′-ethynyl-2′-deoxy-4′-thioribonucleosides was carried out utilizing an electrophilic glycosidation in which 4-ethynyl-4-thiofuranoid glycal **16** served as a glycosyl donor. Electrophilic glycosidation between **16** and the silylated nucleobases (N^4-acetylcytosine, N^6-benzoyladenine, and N^2-acetyl-O^6-diphenylcarbamoylguanine) was carried out in the presence of *N*-iodosuccinimide (NIS), leading to the exclusive formation of the desired β-anomers **29**, **33**, and **36**. Anti-HIV studies demonstrated that these 4′-thio nucleosides were less cytotoxic to T-lymphocyte (i.e., MT-4 cells) than the corresponding 4′-ethynyl derivatives of 2′-deoxycytidine (**44**), 2′-deoxyadenosine (**45**), and 2′-deoxyguanosine (**46**). Comparison of the selectivity indices (SI) was made between 4′-thionucleosides (**32**, **41**, and **43**) and the corresponding 4′-oxygen analogues **44**-**46** by using the reported CC_{50} and EC_{50} values. In the case of cytosine and adenine nucleosides, comparable SI values were obtained as follows: **32** (545) and **44** (458); **41** (>230) and **45** (1630). In contrast, 4′-ethynyl-2′-deoxy-4′-thioguanosine **43** was found to possess a SI value of >18200, which is 20 times better than that of **46** (933).



KEYWORDS: 4′-Thionucleosides, glycal, electrophilic glycosidation, anti-HIV-1 activity, nucleoside reverse transcriptase inhibitors

Nucleoside analogues are recognized as an important class of biologically active compounds, especially as antiviral and antitumor agents.[1−3] Among their sugar-modified analogues, 4′-thionucleosides, in which the oxygen atom in the furanose ring is replaced with a sulfur atom, have attracted much attention since the discovery of the antiviral and antitumor activities of 4′-thiothymidine (**1**) and 2′-deoxy-4′-thiocytidine (**2**) (Figure 1).[4] Also, it has been reported that 4′-substituted thymidines such as the 4′-azido (**3**), 4′-methoxy (**4**), 4′-cyano (**5**), and 4′-ethynyl (**6**) derivatives exhibit potent anti-HIV activity.[5]

Having been stimulated by the above findings, we synthesized the 4′-substituted analogues **7**−**12** of 4′-thiothymidine (Figure 2) and found promising anti-HIV activity in the 4′-azido (**8**), the 4′-cyano (**11**), and the 4′-ethynyl (**12**) derivatives.[6] This finding led us to investigate the present study where synthesis of the 4′-ethynyl analogues having other nucleobases (cytosine, adenine, and guanine) was carried out.

In our previous study,[6] the synthesis of **7**−**12** was accomplished through nucleophilic substitution of the 4′-acetoxy derivative **13** (Figure 3). The 4′-acetoxy leaving group of **13** was introduced by diacetoxylation of the 4′,5′-anhydro derivative **14** with $Pb(OAc)_4$. Compound **14** was prepared by a series of reactions initiated with NIS-mediated electrophilic glycosidation between silylated thymine and TIPDS (1,1,3,3- tetraisopropyldisiloxane-1,3-diyl)-protected 4-thiofuranoid glycal **15**.[7] In the present study, to enable a diverse set of nucleobases to be introduced, the 4-thiofuranoid glycal **16** already substituted at the 4-position with the triethylsilylethynyl group was employed as a glycosyl donor.

Our plan to introduce an ethynyl group in a tetrahydrothiophene ring is visualized in Scheme 1. Aldol reaction between **A** and formaldehyde gives **B**, which is then converted to the *O*-silyl-protected **C**. The formyl group of **C** is reacted with dimethyl

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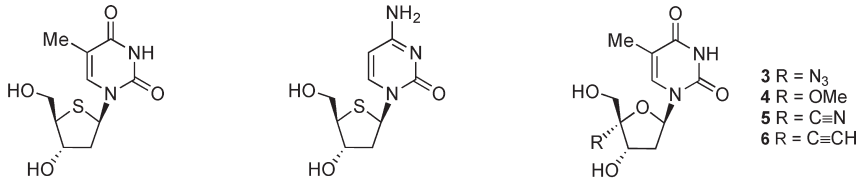

Figure 1. Structures of compounds **1**−**6**.

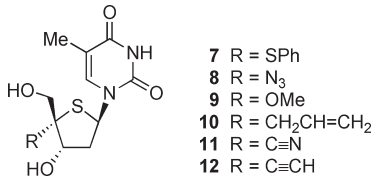

Figure 2. 4′-Substituted 4′-thiothymidines **7**−**12**.

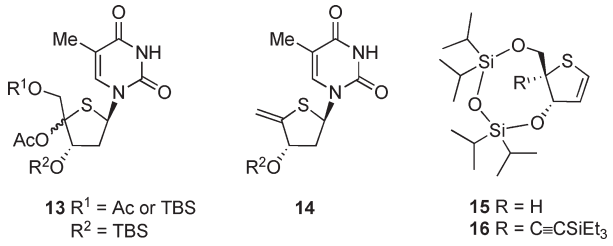

Figure 3. Structures of compounds **13**−**16**.

1-diazo(2-oxopropyl)phosphonate[8] to provide the ethynyl-substituted tetrahydrothiophene derivative **D**.

Compound **17** (Figure 4), which corresponds to the aldehyde **A** of Scheme 1, was prepared from 2,3-*O*-isopropylidene-L-lyxonolactone (**18**).[9] Namely, by following the reported procedures,[10] **18** was converted to the dimesylate **19**. Reaction of **19** with Na_2S in DMF at 80 °C led to the formation of the 1,4-anhydro-4-thio-D-ribitol derivative **20** in 66% overall yield from **18**. Compound **20** was desilylated with Bu_4NF to give **21** in 81% yield.[11] Finally, oxidation of **21** with IBX (2-iodoxybenzoic acid) in CH_3CN provided the aldehyde **17** in 83% yield.[12]

Subsequent aldol reaction between **17** and 37% aqueous formaldehyde was carried out in 60% aqueous dioxane (room temperature, overnight), and the resulting mixture was silylated with TBSCl. In the presence of K_2CO_3, the aldols **22** and **23** (Figure 5) were obtained in 21 and 13% yields, respectively, together with the silyl enol ether **24** (16%). The yield of the desired stereoisomer **22** was improved to 50% by using $NaHCO_3$, although the formation of **23** (18%) and **24** (14%) could not be eliminated.

The formyl group of **22** was converted to an ethynyl group through its reaction with dimethyl 1-diazo(2-oxopropyl)phosphonate in MeOH in the presence of K_2CO_3. Upon reacting the crude product with Bu_4NF, the 4-ethynyl derivative **25** was isolated in 73% yield from **22**.

Compound **25** was transformed to 4-thiofuranoid glycal **26** by reaction with *tert*-BuLi (4 equiv) at −70 °C in THF (Figure 6).[13] This reaction furnished the glycal **26** in 61% yield along with the ring-opened sulfide **27** (9%) and the starting material **25** (11%). The actual glycosyl donor **16** was prepared from **26** by first protecting the hydroxyl groups with the TIPDS group (yield of **28**, 72%) and then the ethynyl group with a triethylsilyl group (yield of **16**, 90%).

With the glycosyl donor **16** in hand, electrophilic glycosidation with a suitable nucleobase was examined. When **16** was reacted with N^4,O^2-bis-trimethylsily-N^4-acetylcytosine (1.5 equiv) in the presence of NIS (1.5 equiv) in CH_3CN/CH_2Cl_2 at room temperature overnight, the desired β-anomer **29** of the glycosidated product was formed as a single stereoisomer in 61% yield (Figure 7).[14] The depicted structure was confirmed by nuclear Overhauser effect (NOE) experiment: H-6/H-2′ (1%), H-6/H-3′ (5%), and H-6/CH_2-5′(0.2%). The observed exclusive formation of **29** suggested that the presence of the ethynyl group at the 4-position of 3,5-*O*-TIPDS-4-thiofuranoid glycal **15** does not influence the β-face selectivity of the electrophilic glycosidation.[7] The introduced iodine atom of **29** was removed by reaction with Bu_3SnH/Et_3B at −70 °C under an O_2 atmosphere to give **30** in 94% yield. To circumvent the difficult chromatographic separation of the free nucleoside and the side product derived from the silyl-protecting group, **30** was converted to its corresponding acetate **31** (99% isolated yield) by desilylation with Bu_4NF and subsequent acetylation in one pot. Finally, **31** was converted to the target 4′-ethynyl-2′-deoxy-4′-thiocytidine **32** (91% isolated yield) by treatment with K_2CO_3 in MeOH.

We next turned our attention to the synthesis of the adenine and guanine nucleosides. Under similar reaction conditions for the electrophilic glycosidation of N^4-acetylcytosine, bis-trimethylsilyl-N^6-benzoyladenine was reacted with **16**. In this reaction, the target nucleoside **33** could be obtained in 48% isolated yield as a single stereoisomer together with its regioisomers **34** (12%) and **35** (13%) (Figure 8).[15] The depicted structures of **33**−**35** were determined on the basis of comprehensive NMR studies including NOE, heteronuclear multiple quantum coherence, and heteronuclear multiple bond correlation experiments.[16] A similar regiochemical outcome was also observed in the glycosidation of N^2-acetyl-O^6-diphenylcarbamoylguanine,[17] where three isomeric nucleosides **36**−**38** were isolated in 25, 12, and 29% yields, respectively (Figure 9).

The N^9-glycosidated products **33** and **36** were successfully converted to 4′-ethynyl-4′-thio-2′-deoxyadenosine **41** and the respective guanosine nucleoside **43** by three steps as follows: (1) Bu_3SnH/Et_3B/PhMe, −70 °C (yield of **39**, 88%; yield of **42**, 72%), (2) Bu_4NF/Ac_2O/THF (yield of **40**, quant.), and (3) K_2CO_3/MeOH (yield of **41**, 82%; yield of **43**, 63% from **42** for two steps) (Figure 10).

The anti-HIV-1 activities of **32**, **41**, and **43** were evaluated, and the results are summarized in Table 1.[18,19] To compare the antiviral activity and cytotoxicity with the corresponding 4′-oxygen counterparts, reported biological data[20,21] of 4′-ethynyl derivatives of 2′-deoxycytidine **44**, 2′-deoxyadenosine **45**, and 2′-deoxyguanosine

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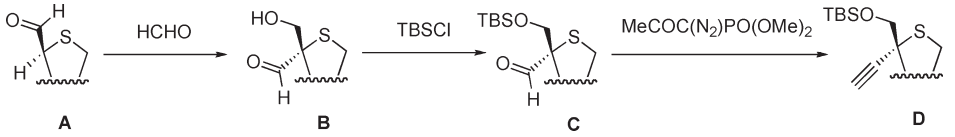
Scheme 1. Introduction of an Ethynyl Group on a Tetrahydrothiophene Ring



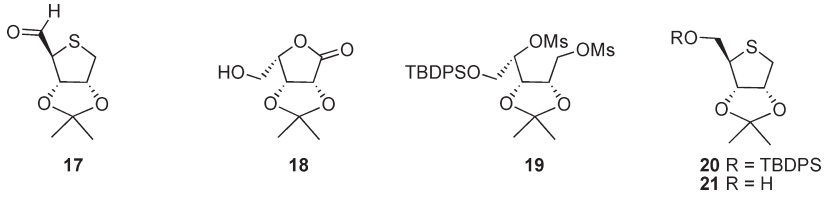


Figure 4. Structures of compounds **17**−**21**.

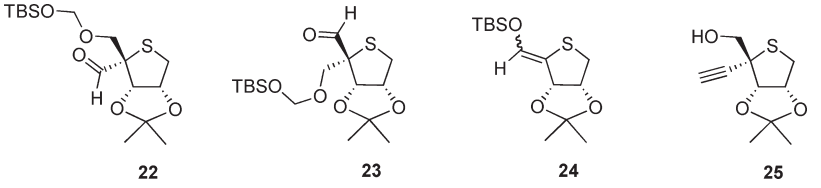


Figure 5. Structures of compounds **22**−**25**.

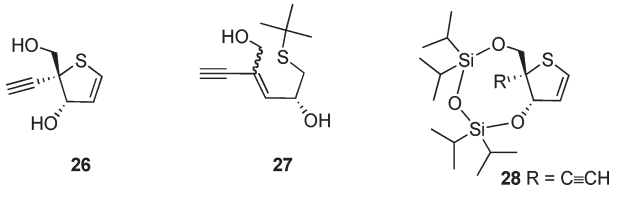


Figure 6. Structures of compounds **26**−**28**.

46 are also included in Table 1. As can be seen in entry 1, 4′-ethynyl-2′-deoxy-4′-thiocytidine **32** exhibited a 10 times lower inhibitory activity than that of the corresponding deoxycytidine derivative **44**. However, because **32** was less toxic to MT-4 cells, the SI value (545) of **32** was found to be comparable to that of **44** (458). In the case of adenine nucleosides as shown in entry 2, a similar trend was seen in terms of EC_{50} and CC_{50} values. In contrast, 4′-ethynyl-2′-deoxy-4′-thioguanosine **43** was found to be a highly promising anti-HIV agent (entry 3). Indeed, **43** exhibited comparable antiviral activity (EC_{50}: 0.0055 μM for **43** vs 0.0015 μM for **46**) and did not show any cytotoxicity to MT-4 cells up to 100 μM in contrast to the highly toxic 2′-deoxyguanosine derivative **46** (CC_{50}: 1.4 μM). The promising guanine nucleoside **43** possesses a SI value of >18200, which is 20 times better than that of 4′-ethynyl-2′-deoxyguanosine **46** (SI 933).

With the above promising anti-HIV-1 activity in hand, next, the 4′-substituted 2′-deoxy-4′-thioribonucleosides **32**, **41**, and **43** were also evaluated for their inhibitory activity against a series of other viruses including HSV-1 strain KOS, HSV-2 strain G, TK^- HSV-1 strain KOS resistant to ACV, and vaccinia virus Lederle strain, and the results were summarized in Table 2.[22] Antiviral data of ganciclovir are also included as a reference compound. As can be seen, these nucleoside derivatives also exhibited antiviral activity against herpes simplex virus and vaccinia virus without measurable cytotoxicity to the host cells up to 100 μM (entries 1−3). These potencies are at least 100 times less than that of ganciclovir, but their selectivity indices against HSV-1 and HSV-2 were >50−100 for **32** and **43**. However, it is noteworthy that all compounds synthesized in this study suppressed the replication of the thymidine kinase-deficient (TK^-) HSV-1 KOS strain at an almost equal potency as wild-type HSV-1. In contrast, the potency of ganciclovir against HSV-1 TK^- strain (1 μM) is 100-fold weaker as compared to wild-type HIV-1. These data are somewhat surprising but interesting and may suggest that the antiherpesvurus activity of **32**, **41**, and **43** is independent of the activation (phosphorylation) by the virus-encoded thymidine kinase. This may, in turn, point to another mechanism of antihepetic action of these compounds.

The compounds were not significantly inhibitory against other viruses, including parainfluenza virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus in Vero cell cultures, VSV and RSV in HeLa cell cultures, feline corona virus (FIPV) and feline herpesvirus in CrFK cell cultures, and influenza virus A (H1N1, H3N2) and B in MDCK cell cultures.

In conclusion, we have developed a novel synthetic approach to 4′-ethynyl-2′-deoxy-4′-thioribonucleosides on the basis of electrophilic glycosidation utilizing 4-ethynyl-4-thiofuranoid glycal **16** as a glycosyl donor. The synthesis of **16** was initiated with the β-face-selective aldol reaction of 1,4-anhydro-2,3-*O*-isopropylidene-4-thio-D-ribitol 5-aldehyde **17** with formaldehyde. The aldol **22** was reacted with dimethyl 1-diazo(2-oxopropyl)phosphonate to provide the ethynyl-substituted tetrahydrothiophene derivative **25**. 4-Ethynyl-4-thiofuranoid glycal **26** was obtained by the reaction of **25** with *tert*-BuLi. The actual glycosyl donor **16**

was prepared by silyl-protection of the hydroxyl and ethynyl groups of **26**.

The glycosidation between **16** and the silylated nucleobase (N^4-acetylcytosine, N^6-benzoyladenine, and N^2-acetyl-O^6-diphenylcarbamoylguanine) proceeded with facial selectivity and β-anomers

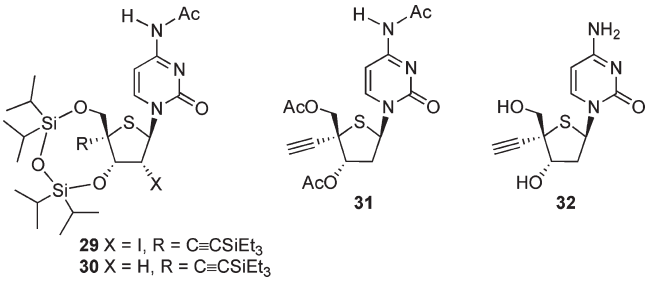


Figure 7. Structures of compounds **29**−**32**.

29, **33**, and **36** of the glycosidated products could be obtained exclusively. These glycosides were efficiently transformed into the 4′-ethynyl derivatives of 2′-deoxy-4′-thiocytidine (**32**), -adenosine (**41**), and -guanosine (**43**). It is noteworthy that these novel nucleoside analogues synthesized in this study were found to be less cytotoxic to MT-4 cells as compared to the corresponding 2′-deoxycytidine (**44**), 2′-deoxyadenosine (**45**), and 2′-deoxyguanosine (**46**) derivatives. By comparison with the reported SI value of 4′-ethynyl-2′-deoxyguanosine **46**, it was found that the SI for the 2′-deoxy-4′-thioguanosine derivative **43** has a 20-fold better value (>18200) than that of 2′-deoxyguanosine counterpart (933). These facts suggest that replacement of the furanose oxygen with sulfur atom is a promising approach for the development of less cytotoxic antiviral nucleosides. We are currently investigating the mechanism of the promising biological profile of these compounds.

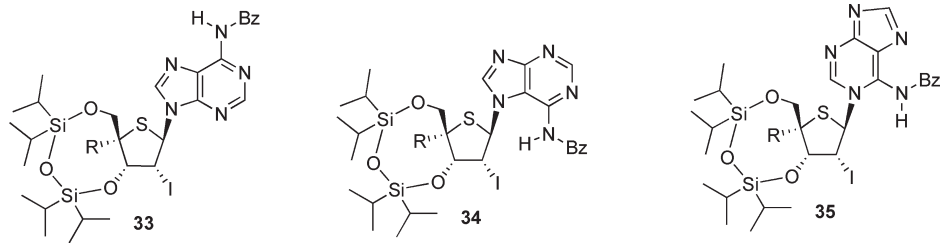


Figure 8. Structures of compounds **33**−**35** (R = C≡CSiEt$_3$).

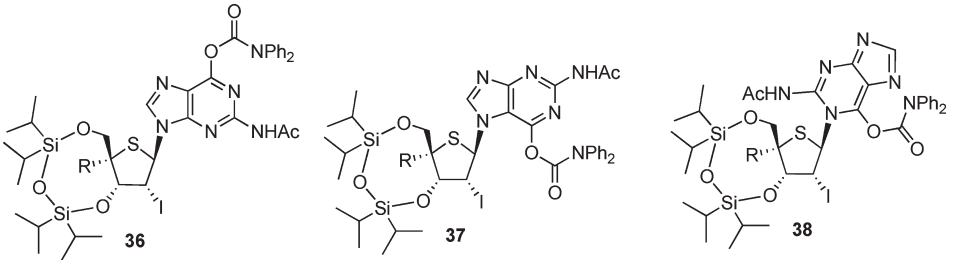


Figure 9. Structures of compounds **36**−**38** (R = C≡CSiEt$_3$).

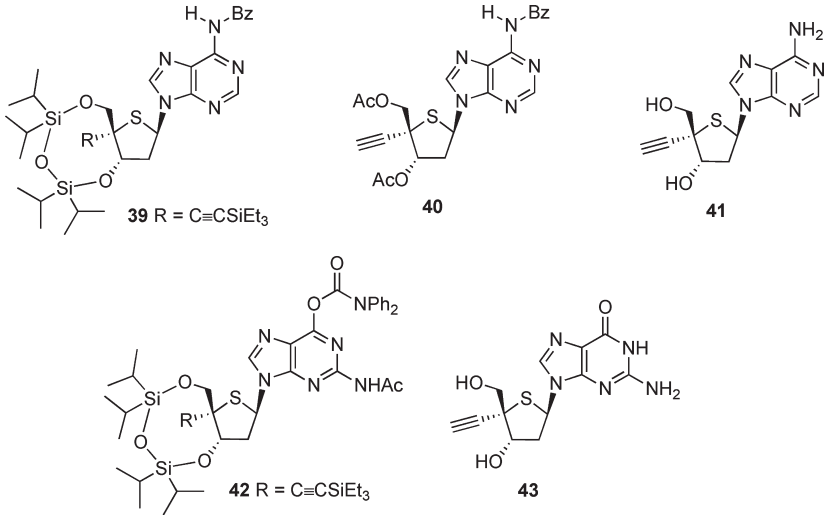


Figure 10. Structures of compounds **39**−**43**.

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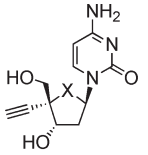
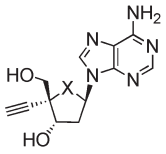
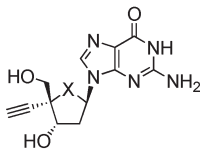
Table 1. Inhibitory Effect of 4′-Ethynyl-2′-deoxy-4′-thioribonucleosides (32, 41, and 43) and Its Oxygen Analogues (44−46) on HIV-1 in MT-4 Cells



32: X = S
44: X = O



41: X = S
45: X = O



43: X = S
46: X = O

entry	compd	EC_{50} (μM)[a]	CC_{50}(μM)[b]	SI[c]	compd	EC_{50} (μM)[d]	CC_{50}(μM)[d]	SI [c]
1	**32**	0.011 ± 0.001	6.0 ± 1.2	545	**44**	0.0048 ± 0.001	2.2 ± 1.0	458
2	**41**	0.087 ± 0.017	>20	>230	**45**	0.0098 ± 0.0043	16 ± 7.9	1630
3	**43**	0.0055 ± 0.0016	>100	>18200	**46**	0.0015 ± 0.0003	1.4 ± 0.16	933

[a] Inhibitory concentration required to achieve 50% protection of MT-4 cells against the cytopathic effect of HIV-1. [b] Cytotoxic concentration required to reduce the viability of mock-infected MT-4 cells by 50%. [c] SI = CC_{50}/EC_{50} [d] Data taken from refs 20 and 21.

Table 2. Inhibitory Effect of 4′-Ethynyl-2′-deoxy-4′-thioribonucleosides (32, 41, and 43) against Herpes Simplex Virus and Vaccinia Virus in HEL Cell Cultures[a]

		EC_{50} (μM)[b]				
entry	compd	herpes simplex virus-1 (KOS)	herpes simplex virus-2 (G)	vaccinia virus	herpes simplex virus-1 (KOS, TK^- ACV)	minimum cytotoxic concentration[c] (μM)
1	**32**	2 ± 0	1.5 ± 0.5	6.5 ± 2.5	2.5 ± 0.5	>100
2	**41**	7 ± 5	4 ± 0	52 ± 6.0	8 ± 4.0	>100
3	**43**	3 ± 1	1.5 ± 0.5	27 ± 7.0	4.5 ± 2.5	>100
4	ganciclovir	0.01	0.01	100	1	>100

[a] Data derived from two independent experiments. [b] Required to reduce virus-induced cytopathogenicity by 50%. [c] Required to cause a microscopically detectable alternation of normal cell morphology.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and full characterization for compounds **16**−**17** and **20**−**43**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) A similar regiochemical outcome was observed in PhSeCl-initiated electrophilic glycosidation between 3,5-*O*-(di-*tert*-butylsilylene)-4-thiofuranoid glycal and N^6-benzoyladenine; see ref 7.

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