4″-Benzoylureido-TSAO Derivatives as Potent and Selective Non-Nucleoside HCMV Inhibitors. Structure−Activity Relationship and Mechanism of Antiviral Action

Sonia de Castro,[†] M. Teresa Peromingo,[†] Lieve Naesens,[‡] Graciela Andrei,[‡] Robert Snoeck,[‡] Jan Balzarini,[‡] Sonsoles Velázquez,*[,†] and María-José Camarasa*[,†]

Instituto de Química Médica (C.S.I.C.), Juan de la Cierva 3, E-28006 Madrid, Spain, Rega Institute for Medical Research, K. U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

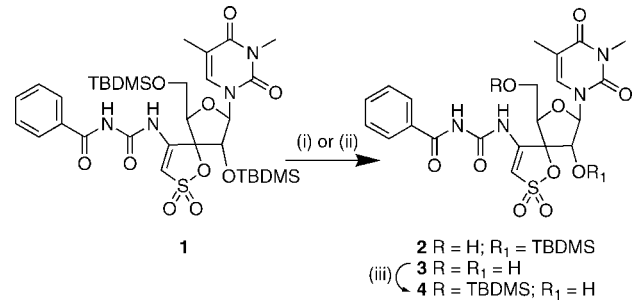
Received January 18, 2008

Analogues of the 4″-benzoyl-ureido-TSAO derivative (**1**) modified at different positions have been prepared and evaluated against wild-type strains of HCMV and murine cytomegalovirus (MCMV) in cell culture. In addition, the activity of the most active derivatives against several drug-resistant HCMV mutants has been determined. A stringent structure−antiviral activity relationship was observed for the 4″-benzoylureido-TSAO derivatives for which the concomitant presence of a highly lipophilic substituent at both 2′- and 5′-positions was required to fully preserve the antihuman cytomegalovirus efficacy. Time-of-addition studies and HCMV immediately early and early gene expression studies revealed a target at the time of viral DNA synthesis, although direct inhibition of HCMV-encoded DNA polymerase could not be observed in cell-free assays. Lack of cross-resistance against a broad variety of mutant HCMV strains points to an antiviral target that is different from those drugs that are currently approved for clinical use.

Introduction

Although it rarely causes symptomatic disease in immunocompetent individuals, human cytomegalovirus (HCMV[a]) is an opportunistic pathogen responsible for a variety of severe, often life-threatening diseases (i.e., pneumonia, retinitis) in immunocompromised or immunosuppressed hosts (i.e., transplant recipients, AIDS patients).[1] HCMV is also a major cause of congenital malformation in newborn children.[2] HCMV is a ubiquitous member of the herpes virus family. Except for the antisense RNA formivirsen, antiviral agents approved for the systemic treatment of HCMV infections are viral DNA polymerase inhibitors,[3] i.e., a pyrophosphate analogue [foscarnet (PFA)], the nucleoside analogues ganciclovir (GCV) and acyclovir (ACV) and their valine ester pro-drugs valganciclovir and valacyclovir,[4−6] and the acyclic nucleotide phosphonate cidofovir (CDV or HPMPC).[6] However, these drugs suffer from a number of drawbacks and limitations such as dose-limiting bone marrow (ganciclovir) and kidney (foscarnet and cidofovir) toxicities,[4,6] unfavorable pharmacokinetic properties, as well as the emergence of single and multiple drug resistance.[7−11] Although there are other anti-HCMV drugs in clinical development (i.e., marabivir),[6] novel and potent anti-HCMV agents, with a good selectivity, safety profile, and oral bioavailability are needed. Either inhibitors of the viral DNA polymerase with a different structure than that of the approved anti-HCMV drugs or compounds with a different mechanism of action would be good candidates to make progress in reducing the consequences of HCMV infection in the susceptible populations.We recently reported[12] that some 4″-substituted-TSAO derivatives (TSAO compounds are a particular group of potent and highly specific and selective inhibitors of HIV-1 replication)[13] lost the anti-HIV-1 activity. Surprisingly, several of these compounds gained inhibitory activity against replication of HCMV in cell culture showing pronounced antiviral activity at concentrations in the lower μM range (i.e., 0.29−2.0 μM), that is at a concentration well below their toxicity threshold. In particular, compound **1** (Scheme 1) showed the highest anti-HCMV activity lacking appreciable cytotoxicity or antiproliferative activity against HEL cell cultures.[12] The activity of the 4″-benzoyl-ureido derivative (**1**) against HCMV replication belonged to the same order of magnitude as ganciclovir.[12] The highly modified structure of these TSAO molecules (i.e., containing blocked 5′- and 2′-positions in the ribose moiety) points to a different mechanism of inhibition of HCMV than that of currently used anti-HCMV nucleoside analogues. However, it is currently unclear whether these compounds are targeted against HCMV DNA polymerase (acting as non-nucleoside inhibitors) or inhibit another viral (or cellular) function necessary for virus replication. Further studies should be pursued with these molecules to address the structural

Scheme 1[a]



[a] Reagents and conditions: (i) HCl 1N, methanol, 0 °C; (ii) TBAF, THF, r.t.; (iii) TBDMSCl, Py, r.t.

* To whom correspondence should be addressed. For M.-J.C. and S.V.: phone,(+34)-912587458;fax,(+34)-915644853;E-mail,mj.camarasa@iqm.csic.es (M.-J.C.) and iqmsv29@iqm.csic.es (S.V.).

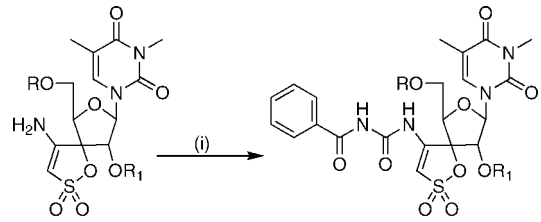
† Instituto de Química Médica (C.S.I.C.).

‡ Rega Institute for Medical Research, K. U. Leuven.

[a] Abbreviations: HCMV, human cytomegalovirus; AIDS, acquired immunodeficiency syndrome; PFA, foscarnet; GCV, ganciclovir; ACV, acyclovir; CDV or HPMPC, cidofovir; TSAO, (*tert*-butyldimethylsilyl-β-D-ribofuranosyl)-3′-spiro-4″-amino-1″,2″-oxathiole-2″,2″-dioxide; HIV, human immunodeficiency virus; HEL, human embrionic lung; MCMV, murine cytomegalovirus; TBDMS, *tert*-butyldimethylsilyl; TDS, thexyldimethylsilyl; TIPS, triisopropylsilyl; DMAP, 4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; SAR, structure−activity relationship; IE, immediately early; E, early; VZV, varizella zoster virus.

10.1021/jm800050t CCC: $40.75
Published on Web 08/16/2008

Scheme 2[a]

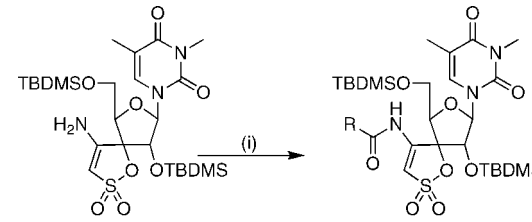


5 R = TDS; R_1 = TBDMS
6 R = TBDMS; R_1 = TDS
7 R = TIPS; R_1 = TBDMS
8 R = R_1 = H
9 R = R_1 = TDS
10 R = Bz, R_1 = Ac
11 R = Bn; R_1 = TBDMS

12 R = TDS; R_1 = TBDMS
13 R = TBDMS; R_1 = TDS
14 R = TIPS; R_1 = TBDMS
15 R = R_1 = TDS
16 R = Bz, R_1 = Ac
17 R = Bz, R_1 = H
18 R = Bz, R_1 = TBDMS
19 R = Bn; R_1 = TBDMS

[a] Reagents and conditions: (i) PhCONCO, acetonitrile, 100 °C; (ii) NH_3, methanol, 0 °C; (iii) TBDMSCl, DMAP, r.t.

Scheme 3[a]



20
(TSAO-m^{3}T)

21 R = NHPh
22 R = N(Ph)CONHPh
23 R = $NHCH_2Ph$
24 R = NH_2
25 R = NHEt
26 R = $NHCO_2Et$
27 R = $NHCH_2CO_2Et$

[a] Reagents and conditions: (i) RNCO, NaH, acetonitrile, r.t.

requirements for anti-HCMV activity and to reveal their mechanism of inhibition of viral replication.

In this report, we describe the synthesis and biological studies of analogues of the 4″-benzoyl-ureido-TSAO derivative (**1**) modified at different positions. Initial structure−activity relationships have been characterized for the *O*5′, *O*2′ (TBDMS, TDS, TIPS, Bz, Bn, Ac, H), and 4″-ureido substituents (Bz, Ph, Bn, H, Et, CO_2Et, CH_2CO_2, Et) as well as the significance of the nucleobase (N^3-methylthymine). The compounds have been evaluated against wild-type strains of HCMV and murine cytomegalovirus (MCMV) in cell culture. In addition, the activity of the most active derivatives against several drug-resistant HCMV mutants has been determined. To elucidate the mode of action of these derivatives, time-of-addition experiments, HCMV immediately early and early gene expression studies, and HCMV DNA polymerase assays have also been performed.

Results and Discussion

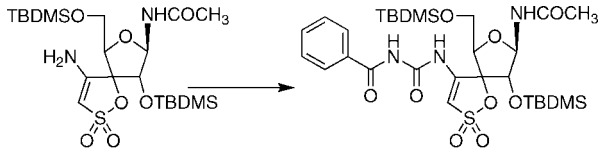
Chemistry. First, analogues of **1** modified at the 5′ and/or 2′-*O* positions were pursued (Schemes 1 and 2). Thus, treatment of **1**[12] with a 0.1N solution of HCl in methanol at 0 °C gave, selectively, the 5′-*O*-deprotected ureido derivative **2** in 59% yield (Scheme 1). Reaction of **1**[12] with tetrabutylammonium fluoride in THF afforded the fully deprotected derivative **3** (72% yield) that was silylated (TBDMSCl/pyridine) to give the 5′-*O*-TBDMS compound **4**. Next, compounds substituted with other silyl groups at 5′ or 2′ positions (Scheme 2), such as 5′-*O*- or 2′-thexyldimethylsilyl (TDS) (**12** or **13**) and 5′-*O*-triisopropylsilyl (TIPS) (**14**), were prepared in good yields (64−98%) by reaction of the corresponding 5′-*O*- or 2′-*O*-TBDMS-protected TSAO derivatives **5**,[14] or **6**,[15] or **7**[14] with benzoyl isocyanate, in dry acetonitrile in a pressure tube at 100 °C. The 2′,5′-bis-*O*-thexyldimethylsilyl derivative (**15**) was prepared from the 2′,5′-*O*-deprotected TSAO derivative **8**[16] as follows. **8**[16] was reacted with TDSCl/DMAP at 100 °C in dry acetonitrile to give compound **9** (50% yield), which was treated with benzoyl isocyanate, under similar conditions described above, to yield the target compound **15** in 75% yield. 4″-Benzoylureido-TSAO-m^{3}T derivatives bearing other groups at 2′- and/or 5′-positions were also prepared. Thus, reaction of **10** (prepared in 71% yield from 2′-*O*-acetyl-5′-*O*-benzoyl-TSAO-T[16] and methyl iodide) with benzoyl isocyanate gave the 2′-acetyl-5′-benzoyl-4″-benzoylureido derivative **16** (94% yield). Treatment of **16** with NH_3 in methanol at 0 °C gave the 5′-benzoyl-2′-*O*-deprotected derivative **17** in 90% yield. Reaction of **17** with TBDMSCl/DMAP gave the fully protected compound **18** (78% yield). The synthesis of the 4″-benzoylureido-TSAO-m^{3}T derivative **19** bearing a 5′-*O*-benzyl group (Scheme 2) was next performed. The required 5′-*O*-benzyl TSAO intermediate **11**[17] was obtained in good yield (87%) by treatment of the 5′-deprotected TSAO-m^{3}T[15] with 1.1 equiv of benzyl bromide at −40 °C in dry DMF in the presence of 1.1 equiv of K_2CO_3 as base for 15 min. It should be noted that under these conditions, alkylation of the 3″-*C* position of the sultone moiety was completely avoided, the reaction time was shortened (15 min vs 12 h), and the yield of **11** was significantly improved (87% vs 47%) compared with the previously described classical Williamson procedure in the presence of NaH as base.[17] Unfortunately, treatment of the 5′-deprotected TSAO-m^{3}T[15] with less reactive alkyl halides, such as isobutyl bromide or neopentyl bromide (in order to prepare other 5′-carbo analogues of 4″-benzoylureido-TSAO derivatives), in the presence of K_2CO_3 or NaH as base, were unsuccesful and unreacted starting material was recovered. Finally, acylation of the 5′-benzyl TSAO derivative **11** with benzoyl isocyanate, under similar conditions described above, afforded the corresponding 4″-benzoylureido-TSAO-m^{3}T derivative **19** in 59% yield.

4″-Substituted TSAO-m^{3}T derivatives, bearing different ureido groups at the 4″-position (**21**−**23**), were also prepared (Scheme 3.) When phenylisocyanate was reacted with **20**, under similar acylation conditions described above (acetonitrile, pressure tube, 100 °C), only the starting material was recovered. However, when this reaction was carried out with an excess of conveniently functionalized isocyanates, in the presence of NaH in dry THF, a mixture of **21** and **22** were isolated in 58 and 21% yield, respectively (Scheme 3). Formation of **22** could be explained by the attack of a second molecule of phenylisocyanate to the initially formed 4″-phenylureido derivative **21**. Similarly, reaction of **20** with benzylisocyanate gave compound **23** in good yield (75%). Compounds **24**−**27** were prepared as previously described[12] and are included here for comparative purposes in the SAR studies.

To determine whether the base part or the sugar part were necessary for anti-HCMV activity, the abasic analogue **29** (Scheme 4) and the 4-benzoylureido-sultone **33** (Scheme 5) were prepared. Reaction of the *N*-acetyl derivative **28**[18] (Scheme 4) with benzoyl isocyanate in dry acetonitrile in an Ace pressure tube at 100 °C gave the desired compound **29** in 90% yield.

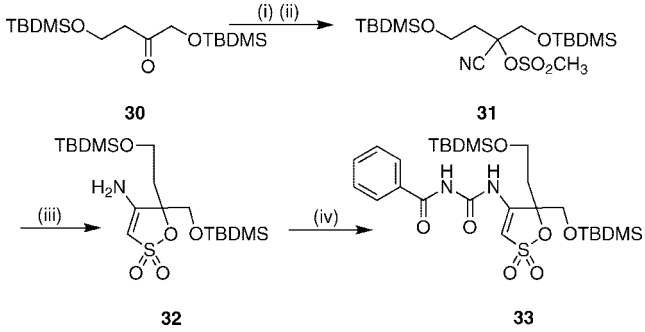
Finally, the 4-benzoylureido-sultone **33** was prepared in 67% yield as shown in Scheme 5. Thus, treatment of the ketone derivative **30**[19] with sodium cyanide in a two-phase ethyl ether/water system in the presence of sodium bicarbonate gave a racemic mixture of cyanohydrines that were used in the next

Scheme 4[a]



[a] Reagents and conditions: (i) PhCONCO, acetonitrile, 100 °C.

Scheme 5[a]



[a] Reagents and conditions: (i) NaCN, diethyl ether/water, r.t.; (ii) MsCl, TEA, dichloromethane, −20 to 0 °C; (iii) Cs_2CO_3, acetonitrile, r.t.; (iv) PhCONCO, acetonitrile, 100 °C.

Table 1. Anti-HCMV Activity of Test Compounds in HEL Cell Cultures

	EC_{50}[a] (μM)			
compd	Davis	AD-169	MCC[b] (μM)	CC_{50}[c] (μM)
1	2.4	11	≥80	>200
2	>4	>4	20	16
3	>100	>100	>100	>50
4	>100	>100	>100	22
12	1.4	1.1	≥100	>50
13	3.9	12.1	≥100	≥100
14	4.2	6.3	≥100	>100
15	1.5	1.2	≥100	>50
16	>100	>100	>100	>100
17	>100	>100	>100	>100
18	2.2	2.2	≥5 (100)	100
19	>50	>50	>50	>50
21	0.38	≥0.14	1.4	1.3
22	>10	15	46	13
23	1.1	1.3	4.0	4.1
24	2.0	>3.2	16	11
25	2.0	1.8	≥3.2	10
26	2.8	2.3	10	14
27	>3.2	>3.2	16	13
29	>20	>20	100	>100
33	>100	>100	>100	>100

[a] Effective concentration required to reduce virus-induced cytopathicity by 50%. [b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. [c] Cytotoxic concentration required to reduce cell growth by 50%.

step without further purification. Reaction of this cyanohydrine mixture with mesyl chloride in dry dichloromethane in the presence of triethylamine afforded the methyl sulfonate derivative **31**. Reaction of **31** with Cs_2CO_3 gave the β-amino-γ-sultone derivative **32** in 75% yield. Finally, treatment of **32** with benzoylisocyanate, as described for the synthesis of compounds **12**−**19** (acetonitrile, 100 °C), gave the 4-benzoylureido-sultone derivative **33** in 67% yield.

Biological Evaluation. The compounds have been evaluated against two different (AD-169 and Davis) strains of HCMV in HEL cell cultures (Table 1,) and compared with the parent compound **1**, which contain a TBDMS entity at both 2′- and 5′-positions of the 3′-spiro ribose ring. Removal of the base part (i.e., **29**, **33**) of **1** resulted in an inactive compound (data not shown). Also, removal of the TBDMS moiety at 2′ (**4**) or 5′ (**2**) or both 2′- and 5′-position (**3**) annihilated the antiviral activity of the parent compound. When one of the silylated substituents at 2′ or 5′ were replaced by the silicium containing TDS (**12**, **13**, **15**) or TIPS (**14**) substituents, the anti-HCMV activity of the test compounds was preserved. As also observed for the prototype compound **1**, the silylated derivatives **12**−**15** were devoid of significant cytotoxic or cytostatic activity in the HEL cell cultures. Whereas replacing of the silyl group at the 5′-position of the molecule by a benzoyl moiety (**18**) preserved the antiviral activity (but resulted in a higher cytotoxic activity), the antiviral activity of the compound was annihilated when the 2′-TBDMS was replaced by an acetyl (**16**) or a benzyl (**19**) or did not contain a substituent at all (free 2′-OH) (**17**). Thus, it seems that the presence of silyl groups at the 2′- and 5′-position are concomitantly required or can only be replaced by a benzoyl at the 5′-position to preserve antiviral activity of the compounds in the HCMV-infected cell cultures. In this respect, the structure−activity relationship (SAR) is remarkably similar to that for the anti-HIV-1 activity of the TSAO derivatives.[13]

If the benzoylureido group at the 3′-spiro moiety of the prototype **1** was replaced by other groups, lacking the C═O entity linked to the urea such as a phenyl (**21**), benzyl (**23**), ethyl (**25**), ethylcarboxylate (**26**), ethylcarboxymethyl (**27**), or phenylcarboxamide phenyl (**22**), or when there is no substituent at all at the ureido group (**24**), the compounds lost antiviral activity or showed antiviral activity at concentrations that are close to the toxicity threshold. Because the mechanism of antiviral (HIV-1) action of TSAO derivatives is inhibition of the virus-encoded reverse transcriptase, the DNA polymerase of HCMV[20,21] was investigated as a potential target for the test compounds. However, when examined for its inhibitory activity against the viral enzyme in the presence of activated DNA using either dGTP or dATP as the radiolabeled substrate, no appreciable inhibitory activity was observed at compound (**12** and **15**) concentrations as high as 100 μM. Under similar experimental conditions, foscarnet (PFA) proved inhibitory at a concentration of 5−7.5 μM. Therefore, it could be concluded that most likely the compounds are not directly targeted against the HCMV UL54-encoded DNA polymerase. However, it should be kept in mind that DNA synthesis in virus-infected cells requires interaction of the HCMV UL54-encoded catalytic subunit with accessory proteins. These proteins were not present in the reaction mixture of the HCMV DNA polymerase assay, and thus, it cannot be excluded that the compounds indirectly inhibit HCMV DNA polymerase activity by interfering with one of these accessory proteins. Indeed, time-of-addition studies at which the test compounds had been added to the virus-infected cell cultures at different time-points post virus infection revealed that antiviral activity was lost when the compounds were added at the time point when viral DNA synthesis occurred (Figure 1.) Thus, a delay of administration of compounds **1**, **12**, and **15** for up to 56 h postinfection did not affect the antiviral efficacy of the drugs. At longer delayed time periods, the three drugs, similarly to (*S*)-HPMPC (cidofovir) and GCV (ganciclovir) that are known to target the viral DNA polymerase, started to lose their potential to markedly reduce virus-induced cytopathicity.

Expression of immediately early (IE) and early (E) genes were determined in compound **12**-treated cells and compared to the reference compounds dextran sulfate (an inhibitor of viral entry)

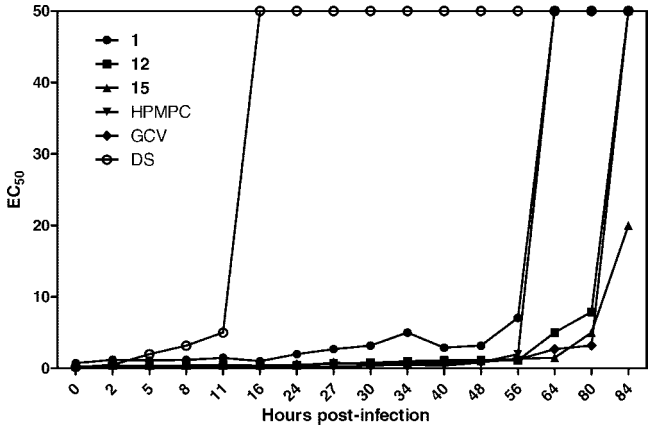


Figure 1. Time-of-addition experiments performed using cytopathic effect reduction assays in HEL cell cultures and comparing compounds **1**, **12**, and **15** to the reference drugs ganciclovir (GCV) and cidofvofir (HPMPC) and to the adsorption inhibitor dextran sulfate (DS), data are in μM.

and ganciclovir (GCV) and cidofovir (HPMPC), two inhibitors of the viral DNA polymerase. Similar to HPMPC and GCV, compound **12** was not able to inhibit IE antigen expression at 48 h postinfection as measured by flow cytometry (Figure 2.) IE antigen expression during the first cycle of viral replication, which is known to take approximately 72 h, was strongly inhibited by dextran sulfate that has been shown to inhibit viral adsorption. IE antigen expression reflects input virus and is maintained during the entire viral replicative cycle. No expression of E antigen at 48 h postinfection was detected, indicating that viral DNA synthesis have not occurred yet. After one or two complete cycles of viral replication, IE and E antigen expression was significantly inhibited by compound **12**, GCV, and HPMPC as well as by dextran sulfate. These results are in agreement with the time-of-addition experiments and suggest that compound **12** does not inhibit an early step in the HCMV replicative cycle but a step around the onset of DNA synthesis.

The 4″-benzoylureido-TSAO derivatives **12** and **15** showed also potent activity against murine cytomegalovirus (MCMV), with EC_{50} values of 0.66 and 0.54 μM, respectively. In contrast with the potent inhibitory activity of the lead compounds against human and murine cytomegalovirus, a markedly lower potency was observed against the α-herpes virus varicella zoster virus (VZV) (EC_{50} values in the order of 11−16 μM for both wild-type and thymidine-kinase deficient VZV strains). The compounds **12** and **15** were not antivirally active against herpes simplex virus types 1 and 2, vaccinia virus, or a variety of RNA viruses.

Compounds **12** and **15** were evaluated against a panel of HCMV drug-resistant mutants either isolated in vitro (Table 2) or recovered from immunocompromised patients (Table 3.) These virus strains bear mutations either in the DNA polymerase and/or in the UL97 gene, which encodes for a HCMV-specified protein kinase responsible for the phosphorylation of ganciclovir and acyclovir in HCMV-infected cells. Interestingly, all mutants remained fully sensitive to the inhibitory activity of compounds **12** and **15**, indicating a mode of action for the 4″-benzoylureido-TSAO derivatives different from that of the clinically used reference compounds.

Thus, although we were not yet able to pinpoint the exact mechanism of antiviral action of the compounds, they may most likely act at a target that is operative during the infection cycle around the onset of viral DNA synthesis. In fact, because the E antigen is a virus-encoded DNA binding protein, our data also revealed that direct interaction of the TSAO compounds with the E antigen is not required for the eventual antiviral activity. Thus, besides the viral DNA polymerase, this protein can also be excluded as a potential target for the test compounds. These findings point to a novel mechanism of action of the compounds. Further studies are ongoing to reveal their antiviral molecular target.

Experimental Section

Chemical Procedures. Microanalyses were obtained a Heraeus CHN-O-RAPID instrument. Electrospray mass spectra were measured on a quadropole mass spectrometer equipped with an electrospray source (Hewlett-Packard, LC/MC HP 1100). ^{1}H NMR spectra were recorded with, a Varian XL-300 and a Bruker 300 Avance spectrometer operating at 300 MHz with Me_4Si as internal standard. ^{13}C NMR spectra were recorded with a VARIAN XL-300 and a Bruker 300 Avance spectrometer operating at 75 MHz with Me_4Si as internal standard. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck). Separations on silica gel were performed by preparative centrifugal thin-layer chromatography (CCTLC) on a Chromatotron (Kiesegel 60 PF_{254} gypsiferous (Merck), layer thickness (1 mm), flow rate (5 mL/min). Flash column chromatography was performed with silica gel 60 (230−400 mesh)(Merck). Dichloromethane and acetonitrile were dried by refluxing over calcium hydride. Tetrahydrofurane was dried by refluxing over sodium-benzophenone. Ace pressure tubes (15 mL) were purchased from Sigma-Aldrich (catalogue no. Z181064-1EA).

[1-[(2′-*O*-*tert*-Butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (2). To a solution of **1**[12] (50 mg, 0.06 mmol) in methanol (2 mL) at 0 °C, 1N HCl in methanol (1.0 mL) was slowly added. The mixture was stirred at 0 °C for 4 h. The reaction was treated with a 1N solution of NaOH in methanol until pH ∼ 6 at 0 °C, salts were filtered, and the solvent was evaporated to dryness. The residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:3) to give **2** (22 mg, 59%) as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: 0.21(s, 6H, $2CH_3$−Si), 0.83 (s, 9H, ^{t}Bu), 1.19 (s, 3H, CH_3-5), 3.28 (s, 3H, CH_3-3), 4.00 (m, 2H, H-5′), 4.48 (m, 1H, H-4′), 5.05 (d, 1H, J = 7.9 Hz, H-2′), 5.77 (bs, 1H, OH), 6.25 (d, 1H, J = 7.9 Hz, H-1′), 7.58 (s, 1H, H-3″), 7.60 (m, 2H, Ph), 7.70 (m, 1H, Ph), 8.09 (d, 2H, J = 7.2, Ph), 8.19 (s, 1H, H-6), 10.37 (bs, 1H, NH), 11.66 (bs, 1H, NH). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ: −0.16 (CH_3−Si), −0.04 (CH_3−Si), 13.3 (CH_3-5), 18.3 (C-^{t}Bu-Si), 25.6 (^{t}Bu-Si), 28.0 (CH_3-3), 61.1 (C-5′), 75.5 (C-2′), 85.8 (C-4′), 88.0 (C-3′), 95.5, 108.4 (C-1, C-3″), 111.3 (C-5), 129.1, 129.7, 132.8, 134.4 (Ph), 135.1 (C-6), 141.8 (C-4″), 151.5, 152.3 (C-2, CO), 163.5 (C-4), 168.6 (CO). MS (ESI$^+$) *m*/*z* 637 (M + H)$^+$, 659 (M + Na)$^+$. Anal. ($C_{27}H_{36}N_4O_{10}SSi$) C, H, N, S.

[1-(β-D-Ribofuranosyl)-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (3). To a solution of **1**[12] (50 mg, 0.06 mmol) in THF (2 mL), a 1 M solution of TBAF in THF was slowly added (0.12 mL, 0.12 mmol). The mixture was stirred at room temperature for 5 min. The solvent was evaporated to dryness and the residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 99.5:0.5) to give **3** (31 mg, 72%) as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: 1.91 (s, 3H, CH_3-5), 3.26 (s, 3H, CH_3-3), 3.83 (m, 2H, H-5′), 4.38 (m, 1H, H-4′), 4.99 (m, 1H, H-2′), 5.58 (bs, 1H, OH), 6.28 (d, 1H, J = 8.4 Hz, H-1′), 6.63 (bs, 1H, OH), 7.55 (s, 1H, H-3″), 7.66 (m, 3H, Ph), 8.03 (d, 2H, J = 7.5, Ph), 8.21 (s, 1H, H-6), 10.28 (bs, 1H, NH), 11.42 (bs, 1H, NH). MS (ESI$^+$) *m*/*z* 523.0 (M + H)$^+$. Anal. ($C_{21}H_{22}N_4O_{10}S$) C, H, N, S.

[1-[(5′-*O*-*tert*-Butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (4). To a solution of **3** (50 mg, 0.10 mmol) in dry pyridine (3 mL), *tert*-butyldimethylsilyl chloride (59 mg, 0.38 mmol) was added. The mixture was stirred at room temperature for 24 h. The solvent was evaporated and ethyl acetate (5 mL) was added. The organic layer was successively washed with 0.1 N HCl

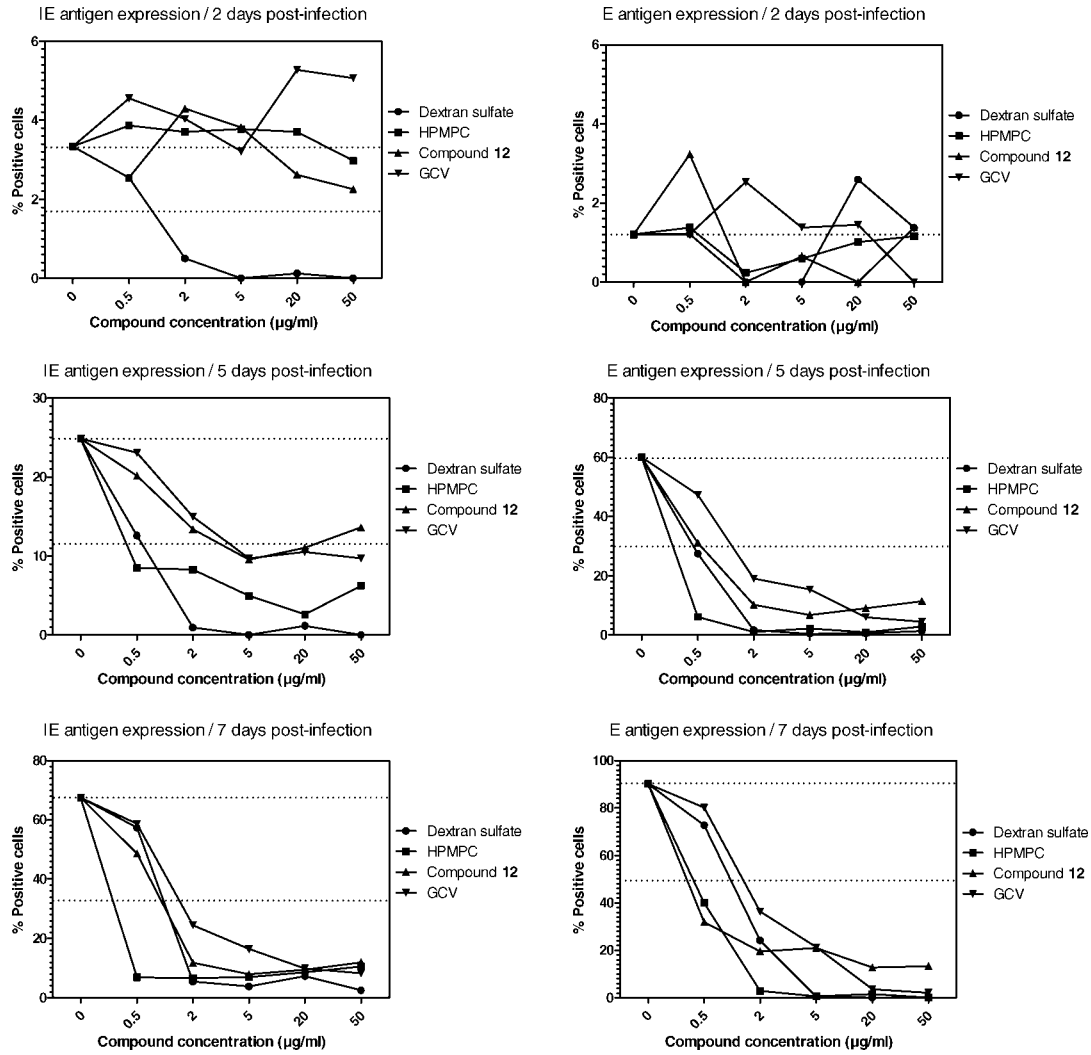


Figure 2. Inhibition of immediately early (IE) and early (E) antigen expression on days 2, 5, and 7 postinfection as measured by flow cytometry. The percentage of cells expressing IE and E antigens in treated and untreated infected cells is expressed after withdrawal of the unspecific fluorescence of uninfected cells.

Table 2. Inhibitory Activity of Compounds **12** and **15** against Drug-Resistant HCMV Mutants Selected in HEL Cell Cultures

	EC_{50} (μg/mL)[a]								
HCMV strain	(S)-HPMPC	(S)-HPMPA	GCV	ACV	PFA	PMEA	PMEDAP	compd **12**	compd **15**
wild-type (AD-169)	0.12	0.28	0.77	22	18	48	15	1.2	2.4
GCV^r (DNA pol mutant)	0.59	2.0	7.6	4.8	17	5.8	1.6	0.90	3.3
(*S*)-HPMPC^r (DNA pol mutant)	2.2	3.3	4.9	12	14	18	5.2	1.0	2.6
(*S*)-HPMPA^r (DNA pol mutant)	3.1	6.2	9.1	7.9	35	9.1	2.8	3.1	5.6
PFA^r (DNA pol mutant)	0.10	0.11	1.5	173	135	128	44	0.78	2.7
ACV^r (DNA pol mutant)	0.069	0.081	0.54	130	37	137	38	1.1	2.7

[a] Effective concentration-50, required to reduce virus-induced cytopathicity by 50%.

Table 3. Activity of Compounds **12** and **15** against Drug-Resistant HCMV Clinical Isolates

	EC_{50} (μg/mL)[a]								
HCMV strain	(S)-HPMPC	(S)-HPMPA	GCV	ACV	PFA	PMEA	PMEDAP	compd **12**	compd **15**
wild-type (Davis strain)	0.20	0.52	0.83	11	14	30	10	1.1	4.3
wild-type (AD-169 strain)	0.12	0.28	0.77	22	18	48	15	1.2	2.4
CMV-6 (UL97 mutant)	0.49	0.91	9.4	91	34	90	26	0.86	5.5
CMV-521(UL97 + DNA pol mutant)	0.79	2.3	6.0	7.7	29	20	6.7	0.67	2.9
CMV-530 (UL97 + DNA pol mutant)	0.62	1.2	6.2	22	31	42	12	1.3	5.7

[a] Effective concentration-50, required to reduce virus-induced cytopathicity by 50%.

(2 × 5 mL) and brine (2 × 5 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. Purification of the residue by CCTLC on the Chromatotron (dichloromethane/methanol 30:1) afforded 29 mg (47%) of **4** as a white amorphous solid. ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ: 0.19 (s, 6H, 2 CH_3), 0.82 (s, 9 H, *t*-Bu), 1.90 (s, 3H, CH_3-5), 3.27 (s, 3H, CH_3-3), 4.18 (dd, 1H, J = 5.5, 10.4 Hz, H-5′a), 4.29 (dd, 1H, J = 6.2, 10.4 Hz, H-5′b), 4.40 (dd, 1H, J = 5.5, 6.2 Hz, H-4′), 4.92 (d, 1H, J = 6.9 Hz, H-2′), 6.11 (d, 1H, J = 6.9 Hz, H-1′), 7.29 (s, 1H, H-3″), 7.56 (m, 2H, Ph), 7.69 (m, 1H, Ph), 7.75 (s, 1H, H-6), 8.11 (d, 2H, J = 7.5 Hz, Ph), 10.64 (bs, 1H, NH),

11.45 (bs, 1H, NH). MS (ESI^+) *m/z* 537.6 $(M + H)^+$, 659.5 $(M + Na)^+$. Anal. ($C_{27}H_{36}N_4O_{10}SSi$) C, H, N, S.

[1-[5′-*O*-(*tert*-Butyldimethylsilyl)-2′-*O*-(thexyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-amino-1″,2″-oxathiole-2″,2″-dioxide] (6). A solution of 2′-*O*-deprotected TSAO-m^3T[15] (100 mg, 0.2 mmol), thexyldimethylsilyl chloride (83 μL, 0.4 mmol), and DMAP (51 mg, 0.4 mmol) in dry acetonitrile (4 mL) was stirred at room temperature for 24 h, and the solvent was evaporated at dryness. The residue was redissolved in ethyl acetate and was successively washed with 1N HCl (2 × 10 mL), water (2 × 10 mL), and brine (2 × 10 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated. The residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1) to give 40 mg (30%) of **6** as a white foam. ^1H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.02 (s, 3H, CH_3−Si), 0.14 (s, 3H, CH_3−Si), 0.20 (s, 3H, CH_3−Si), 0.22 (s, 3H, CH_3−Si), 0.74 (s, 3H, CH_3), 0.78 (s, 3H, CH_3), 0.81 (d, 3H, *J* = 6.9 Hz, $\underline{CH_3}$-CH-Si), 0.82 (d, 3H, *J* = 6.9 Hz, $\underline{CH_3}$-CH-Si), 0.97 (s, 9H, ^{t}Bu-Si), 1.58 (m, 1H, CH_3-$\underline{CH}$-Si), 1.94 (s, 3H, CH_3-5), 3.27 (s, 3H, CH_3-3), 4.08 (m, 2H, H-5′a and H-5′b), 4.33 (t, 1H, *J* = 3.6 Hz, H-4′), 4.69 (d, 1H, *J* = 7.8 Hz, H-2′), 5.77 (s, 1H, H-3″), 6.08 (d, 1H, *J* = 7.8 Hz, H-1′), 6.49 (bs, 2H, NH_2), 7.51 (s, 1H, H-6). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ: −4.4 (CH_3−Si), −3.8 (CH_3−Si), −2.1 (CH_3−Si), −0.8 (CH_3−Si), 14.1 (CH_3-5), 15.5 (C−Si), 19.7 (CH_3), 20.0 (CH_3), 20.9 (CH_3), 21.5 (CH_3), 26.6 (C−Si), 27.4 (^{t}Bu-Si), 29.1 (CHTDS), 35.6 (CH_3-3), 64.1 (C-5′), 76.2 (C-2′), 86.1 (C-4′), 89.3 (C-3′), 93.2 (C-1′), 93.3 (C-3″), 112.3 (C-5), 135.5 (C-6), 153.1 (C-2), 153.3 (C-4″), 164.4 (C-4). MS (ESI^+) *m/z* 633.1 $(M + H)^+$, 655.1 $(M + Na)^+$. Anal. ($C_{27}H_{49}N_3O_8SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(thexyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (9). A solution of 2′,5′-*O*-deprotected TSAO-m^3T (**8**)[16] (150 mg, 0.4 mmol), thexyldimethylsilyl chloride (393 μL, 2 mmol), and DMAP (195 mg, 1.6 mmol) in dry acetonitrile (5 mL) was stirred at 100 °C for 24 h. Then, the solvent was evaporated at dryness. The residue was redissolved in ethyl acetate and washed with 1N HCl (2 × 10 mL), water (2 × 10 mL), and brine (2 × 10 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated. The residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1) to yield 131 mg (50%) of **9** as a white foam. ^1H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.02 (s, 3H, CH_3−Si), 0.14 (s, 3H, CH_3−Si), 0.21 (s, 3H, CH_3−Si), 0.22 (s, 3H, CH_3−Si), 0.75 (s, 3H, CH_3), 0.79 (s, 3H, CH_3), 0.81 (d, 3H, *J* = 6.9 Hz, $\underline{CH_3}$-CH-Si), 0.83 (d, 3H, *J* = 6.9 Hz, $\underline{CH_3}$-CH-Si), 0.91 (m, 12H, TDS), 1.57 (sept, 1H, *J* = 6.9 Hz, CH_3-$\underline{CH}$-Si), 1.68 (sept, 1H, *J* = 6.9 Hz, CH_3-$\underline{CH}$-Si), 1.94 (s, 3H, CH_3-5), 3.27 (s, 3H, CH_3-3), 4.02 (dd, 1H, *J* = 5.1 and 12.3 Hz, H-5′a), 4.11 (dd, 1H, *J* = 3.6 and 12.3 Hz, H-5′b), 4.32 (m, 1H, H-4′), 4.77 (d, 1H, *J* = 7.8 Hz, H-2′), 5.74 (s, 1H, H-3″), 6.00 (d, 1H, *J* = 7.8 Hz, H-1′), 6.49 (bs, 2H, NH_2), 7.53 (s, 1H, H-6). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ: −2.2 (CH_3−Si), −1.9 (CH_3−Si), −0.9 (CH_3−Si), 14.1 (CH_3-5), 19.6 (CH_3−Si), 19.7 (CH_3−Si), 19.9 (CH_3−Si), 20.0 (CH_3−Si), 20.9 (CH_3−Si), 21.5 (CH_3−Si), 21.6 (CH_3−Si), 21.8 (CH_3−Si), 26.5 (C−Si), 26.9 (C−Si), 29.1 (CH), 35.6 (CH-TDS), 35.7 (CH_3-3), 63.8 (C-5′), 75.8 (C-2′), 86.2 (C-4′), 89.9 (C-3′), 92.6, 92.8 (C-1′, C-3″), 112.2 (C-5), 136.0 (C-6), 153.1 (C-2), 153.6 (C-4″), 164.3 (C-4). MS (ESI^+) *m/z* 660.3 $(M + H)^+$. Anal. ($C_{29}H_{53}N_3O_8SSi_2$) C, H, N, S.

[1-(2′-*O*-Acetyl-5′-*O*-benzoyl-β-D-ribofuranosyl)-3-*N*-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (10). To a solution of 2′-*O*-acetyl-5′-*O*-benzoyl TSAO-T[16] (250 mg, 0.48 mmol) in acetone (10 mL), K_2CO_3 (33 mg, 0.24 mmol) and methyl iodide (120 μL, 1.9 mmol) were added. The reaction mixture was refluxed at 50 °C overnight. After removal of the solvent, the residue was purified by column chromatography with dicholoromethane/methanol (30:1) to give **10** (228 mg, 71%) as a white foam. ^1H NMR [300 MHz, $(CD_3)_2CO$] δ: 1.84 (s, 3H, CH_3-5), 2.03 (s, 3H, CH_3CO), 3.24 (s, 3H, CH_3-3), 4.70 (m, 3H, H-5′ and H-4′), 5.83 (s, 1H, H-3″), 5.93 (d, 1H, *J* = 7.2 Hz, H-2′), 6.15 (d, 1H, *J* = 7.2 Hz, H-1′), 6.72 (bs, 2H, NH_2), 7.51 (m, 2H, H-6 and Ph), 7.62−7.68 (m, 2H, Ph), 8.04 (d, 2H, *J* = 7.5 Hz, Ph). MS (ESI^+) *m/z* 522.1 $(M + H)^+$, 544.0 $(M + Na)^+$. Anal. ($C_{22}H_{23}N_3O_{10}S$) C, H, N, S.

[1-(5′-*O*-Benzyl-2′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)-3-*N*-methylthymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (11).[17] To a solution of 5′-*O*-deprotected TSAO-m^3T[15] (100 mg, 0.2 mmol) in dry DMF (6 mL), K_2CO_3 (30 mg, 0.22 mmol) was added and the resulting mixture was stirred for 15 min at −40 °C. Then, benzyl bromide (27 μL, 0.22 mmol) was added,l and the reaction was stirred at −40 °C for 15 min. The solvent was evaporated to dryness, and ethyl acetate (5 mL) was added. The organic layer was washed with brine (2 × 5 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. Purification of the residue by CCTLC on the Chromatotron (dichloromethane/methanol 20:1) afforded 100 mg (87%) of **11**[17] as a white foam. ^1H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.10 (s, 3H, CH_3−Si), 0.04 (s, 3H, CH_3−Si), 0.83 (s, 9 H, *t*-Bu), 1.60 (s, 3H, CH_3-5), 3.25 (s, 3H, CH_3-3), 3.90 (dd, 1H, *J* = 2.9, 11.8 Hz, H-5′a), 4.00 (dd, 1H, *J* = 1.9 Hz, H-5′b), 4.46 (m, 1H, H-4′), 4.72 (d, 1H, H-2′), 4.86 (s, 2H, CH_2Ph), 5.80 (s, 1H, H-3″), 6.25 (d, 1H, *J* = 8.2 Hz, H-1′), 6.48 (bs, 2H, NH_2), 7.40 (m, 5H, Ph), 7.72 (s, 1H, H-6).

General Procedure for the Synthesis of Substituted 4″-Ureido TSAO-m^3T Derivatives (12−19). A solution of the corresponding TSAO compound (0.16 mmol) in dry acetonitrile (8 mL) was reacted with benzoyl isocyanate (80 μL, 0.64 mmol) in an Ace pressure tube at 100 °C overnight. After evaporation, the residue was purified by CCTLC on the Chromatotron. The chromatography eluent, yield, and analytical and spectroscopic data of the isolated products are indicated below for each reaction.

[1-[2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(thexyldimethylsilyl)]-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (12). According to the general procedure, **5**[14] (100 mg, 0.16 mmol) was reacted with benzoyl isocyanate. Chromatography of the residue with hexane/ethyl acetate (3:1) afforded 121 mg (98%) of **12** as a white foam. ^1H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.15 (s, 3H, CH_3−Si), −0.02 (s, 3H, CH_3−Si), 0.12 (s, 3H, CH_3−Si), 0.13 (s, 3H, CH_3−Si), 0.80 (s, 15H, CH_3-TDS and ^{t}Bu), 0.81 (d, 6H, *J* = 7.5 Hz, $\underline{CH_3}$-CH-Si), 1.56 (sept, 1H, *J* = 7.2 Hz, CH-TDS), 1.92 (d, 3H, *J* = 1.2 Hz, CH_3-5), 3.28 (s, 3H, CH_3-3), 4.03 (dd, 1H, *J* = 5.1 and 12.0 Hz, H-5′a), 4.20 (dd, 1H, *J* = 7.8 and 12.0 Hz, H-5′b), 4.39 (dd, 1H, *J* = 5.1 and 7.8 Hz, H-4′), 5.04 (d, 1H, *J* = 7.8 Hz, H-2′), 5.98 (d, 1H, *J* = 7.8 Hz, H-1′), 7.41 (s, 1H, H-3″), 7.64 (m, 3H, H-6 and Ph), 7.77 (m, 1H, Ph), 8.15 (d, 2H, *J* = 7.5 Hz, Ph), 10.62 (bs, 1H, NH), 11.53 (bs, 1H, NH). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ: −5.5 (CH_3−Si), −5.2 (CH_3−Si), −3.6 (CH_3−Si), 12.8 (CH_3-5), 18.0, 18.5 (CH_3-TDS), 20.1, 20.2 (CH_3-TDS), 25.4 (^{t}Bu-Si), 25.5 (C-^{t}Bu), 27.7 (CH-TDS), 34.4 (CH_3-3), 61.5 (C-5′), 73.8 (C-2′), 84.9 (C-4′), 89.5, 91.6 (C-1′, C-3′), 106.9, 111.1 (C-5, C-3″), 128.9, 129.6, 131.9, 134.6 (Ph), 135.4 (C-6), 142.0 (C-4″), 150.9, 151.6 (CO, C-2), 163.1 (C-4), 170.4 (CO). MS (ESI^+) *m/z* 779.2 $(M + H)^+$. Anal. ($C_{35}H_{54}N_4O_{10}SSi_2$) C, H, N, S.

[1-[5′-*O*-(*tert*-Butyldimethylsilyl]-2′-*O*-(thexyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (13). The general procedure was followed with **6**[15] (100 mg, 0.16 mmol). Chromatography of the residue with hexane/ethyl acetate (3:1) yielded **13** (80 mg, 64%) as a white foam. ^1H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.07 (s, 3H, CH_3−Si), 0.04 (s, 3H, CH_3−Si), 0.12 (s, 3H, CH_3−Si), 0.13 (s, 3H, CH_3−Si), 0.77 (d, 3H, *J* = 7.6 Hz, $\underline{CH_3}$-CH-Si), 0.82 (d, 3H, *J* = 7.0 Hz, $\underline{CH_3}$-CH-Si), 0.85 (s, 15H, $2CH_3$-TDS and ^{t}Bu), 1.56 (sept, 1H, *J* = 7.0 Hz, CH-TDS), 1.96 (d, 3H, *J* = 1.1 Hz, CH_3-5), 3.29 (s, 3H, CH_3-3), 4.05 (dd, 1H, *J* = 4.5 and 12.3 Hz, H-5′a), 4.20 (dd, 1H, *J* = 6.4 and 12.3 Hz, H-5′b), 4.41 (dd, 1H, *J* = 4.5 and 6.4 Hz, H-4′), 5.00 (d, 1H, *J* = 7.8 Hz, H-2′), 6.08 (d, 1H, *J* = 7.8 Hz, H-1′), 7.44 (s, 1H, H-3″), 7.64 (m, 3H, H-6 and Ph), 7.76 (m, 1H, Ph), 8.16 (d, 2H, *J* = 7.5 Hz, Ph), 10.65 (bs, 1H, NH), 11.47 (bs, 1H, NH). MS (ESI^+) *m/z* 779.3 $(M + H)^+$. Anal. ($C_{35}H_{54}N_4O_{10}SSi_2$) C, H, N, S.

[1-[2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(triisopropylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (14). Following the general procedure, **7**[14] (100 mg, 0.15 mmol) was reacted with benzoyl isocyanate (75 μL, 0.60 mmol). Chromatography of the residue with hexane/ethyl acetate 3:1 yielded 82 mg (69%) of **14** as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.15 (s, 3H, CH_3−Si), −0.01 (s, 3H, CH_3−Si), 0.80 (s, 9H, ^{t}Bu), 1.00 (m, 21H, 3^{i}Pr), 1.91 (s, 3H, CH_3-5), 4.12 (dd, 1H, J = 5.8 and 11.9 Hz, H-5′a), 4.28 (dd, 1H, J = 7.3 and 11.9 Hz, H-5′b), 4.46 (dd, 1H, J = 5.8 and 7.3 Hz, H-4′), 5.07 (d, 1H, J = 7.7 Hz, H-2′), 5.96 (d, 1H, J = 7.7 Hz, H-1′), 7.41 (s, 1H, H-3″), 7.65 (m, 3H, H-6 and Ph), 7.74 (m, 1H, Ph), 8.15 (d, 2H, J = 7.5 Hz, Ph), 10.62 (bs, 1H, NH), 11.61 (bs, 1H, NH). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ: −5.2 (CH_3−Si), −4.8 (CH_3−Si), 12.5 (CH_3-5), 13.1 (3CH-^{i}Pr), 18.2 (6CH_3-^{i}Pr), 18.3 (C-^{t}Bu), 25.7 (^{t}Bu), 27.9 (CH_3-3), 62.3 (C-5′), 74.2 (C-2′), 85.4 (C-4′), 90.2, 91.4 (C-1′, C-3′), 107.0, 111.3 (C-5, C-3″), 129.2, 129.9, 132.3, 135.0 (Ph), 136.0 (C-6), 142.4 (C-4″), 151.2, 151.9 (CO, C-2), 163.4 (C-4), 170.9 (CO). MS (ESI$^+$) *m/z* 793.2 $(M + H)^+$, 815.1 $(M + Na)^+$. Anal. ($C_{36}H_{56}N_4O_{10}SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(thexyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (15). According to the general procedure, **9** (100 mg, 0.16 mmol) was reacted with benzoyl isocyanate to give after chromatography (hexane/ethyl acetate 3:1) **15** (92 mg, 75%) as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.09 (s, 3H, CH_3−Si), 0.03 (s, 3H, CH_3−Si), 0.13 (s, 3H, CH_3−Si), 0.14 (s, 3H, CH_3−Si), 0.80 (m, 24H, 8CH_3), 1.56 (m, 2H, 2CH-Si), 1.94 (s, 3H, CH_3-5), 3.29 (s, 3H, CH_3-3), 4.02 (dd, 1H, J = 5.1 and 12.1 Hz, H-5′a), 4.21 (dd, 1H, J = 7.5 and 12.1 Hz, H-5′b), 4.38 (dd, 1H, J = 5.1 and 7.5 Hz, H-4′), 5.07 (d, 1H, J = 7.7 Hz, H-2′), 5.97 (d, 1H, J = 7.7 Hz, H-1′), 7.41 (s, 1H, H-3″), 7.64 (m, 3H, H-6 and Ph), 7.76 (m, 1H, Ph), 8.16 (d, 2H, J = 7.5 Hz, Ph), 10.69 (bs, 1H, NH), 11.55 (bs, 1H, NH). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ: −2.2 (2CH_3−Si), −2.0 (CH_3−Si), −1.4 (CH_3−Si), 14.1 (CH_3-5), 19.6 (CH_3-TDS), 19.7 (CH_3-TDS), 19.8 (CH_3-TDS), 19.9 (CH_3-TDS), 21.1 (CH_3-TDS), 21.4 (CH_3-TDS), 21.5 (CH_3-TDS), 21.6 (CH_3-TDS), 26.5 (C-TDS), 26.9 (C-TDS), 29.0 (2CH-TDS), 35.6 (CH_3-3), 62.8 (C-5′), 74.9 (C-2′), 86.2 (C-4′), 90.9, 92.9 (C-1′, C-3′), 108.3, 112.4 (C-5, C-3″), 130.2, 130.9, 133.3, 135.9 (Ph), 136.9 (C-6), 143.4 (C-4″), 152.2, 152.9 (CO, C-2), 164.4 (C-4), 171.7 (CO). MS (ESI$^+$) *m/z* 807.3 $(M + H)^+$. Anal. ($C_{37}H_{58}N_4O_{10}SSi_2$) C, H, N, S.

[1-(2′-*O*-Acetyl-5′-*O*-benzoyl-β-D-ribofuranosyl)-3-*N*-methylthymine]-3′-spiro-5‴-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (16). According to the general procedure, compound **10** (100 mg, 0.19 mmol) was reacted with benzoyl isocyanate (95 μL, 0.76 mmol). Chromatography of the residue with hexane/ethyl acetate (3:1) gave **16** (119 mg, 94%) as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: 1.82 (s, 3H, CH_3-5), 2.06 (s, 3H, CH_3−CO), 3.22 (s, 3H, CH_3-3), 4.80 (m, 2H, H-5′), 4.90 (m, 1H, H-4′), 5.83 (d, 1H, J = 7.9 Hz, H-2′), 6.43 (d, 1H, J = 7.7 Hz, H-1′), 7.44−7.94 (m, 8H, H-3″ and Ph), 8.05 (m, 4H, H-6 and Ph), 11.34 (bs, 1H, NH), 11.72 (bs, 1H, NH). MS (ESI−) *m/z* 667.0 (M - H)−. Anal. ($C_{30}H_{28}N_4O_{12}S$) C, H, N, S.

[1-(5′-*O*-Benzoyl-β-D-ribofuranosyl)-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (17). A solution of **16** (50 mg, 0.08 mmol) in methanol saturated with ammonia (2 mL) was stirred at 0 °C for 1 h. The reaction mixture was evaporated to dryness and the residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 20:1) to give **17** (36 mg, 90%) as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: 1.83 (s, 3H, CH_3-5), 3.26 (s, 3H, CH_3-3), 4.62−4.83 (m, 3H, H-5′ and H-4′), 5.03 (d, 1H, J = 7.9 Hz, H-2′), 6.18 (d, 1H, J = 7.9 Hz, H-1′), 7.43−7.74 (m, 8H, H-3″, H-6 and Ph), 8.00 (m, 4H, Ph), 8.30 (bs, 1H, NH), 9.55 (bs, 1H, NH). ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ: 13.8 (CH_3-5), 28.8 (CH_3-3), 63.1 (C-5′), 74.9 (C-2′), 82.8 (C-4′), 91.1, 91.3 (C-3′, C-1′), 106.9, 111.9 (C-3″, C-5), 129.5, 129.9, 130.1, 130.2, 130.4, 130.5, 130.9, 131.2, 132.8, 133.0, 134.9, 135.5 (Ph), 135.8 (C-6, Ph), 143.8 (C-4″), 152.1, 152.9 (C-2, CO), 164.2 (CO), 166.8 (C-4), 171.4 (CO). MS (ESI$^+$) *m/z* 627.1 $(M + H)^+$. Anal. ($C_{28}H_{26}N_4O_{11}S$) C, H, N, S.

[1-[5′-*O*-Benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (18). A solution of **17** (100 mg, 0.16 mmol), *tert*-butyldimethylsilyl chloride (48 mg, 0.32 mmol), and DMAP (39 mg, 0.32 mmol) in dry acetonitrile (5 mL) was stirred at room temperature overnight. The solvent was evaporated to dryness, and ethyl acetate (5 mL) was added. The organic layer was successively washed with 0.1 N HCl (2 × 5 mL) and brine (2 × 5 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. Purification of the residue by CCTLC on the Chromatotron (hexane/ethyl acetate, 3:1) gave 92 mg (78%) of **18** as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.10 (s, 3H, CH_3−Si), 0.04 (s, 3H, CH_3−Si), 0.83 (s, 9 H, *t*-Bu), 1.86 (s, 3H, CH_3-5), 3.28 (s, 3H, CH_3-3), 4.82 (m, 3H, H-5′ and H-4′), 5.04 (d, 1H, J = 7.7 Hz, H-2′), 6.13 (d, 1H, J = 7.7 Hz, H-1′), 7.46 (m, 3H, H-3″ and Ph), 7.56 (m, 3H, H-6 and Ph), 7.74 (m, 2H, Ph), 8.03 (m, 4H, Ph), 10.62 (bs, 1H, NH), 11.70 (bs, 1H, NH). ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ: −4.2 (CH_3−Si), −3.7 (CH_3−Si), 14.1 (CH_3-5), 19.4 (C-^{t}Bu), 26.7 (^{t}Bu), 29.1 (CH_3-3), 63.3 (C-5′), 75.8 (C-2′), 83.1 (C-4′), 90.7, 91.4 (C-3′, C-1′), 107.8, 112.6 (C-3″, C-5), 130.1, 130.2, 130.4, 130.7, 131.3, 131.5, 133.2, 134.4, 135.3, 135.9 (Ph), 136.3 (C-6), 152.3, 152.9 (C-2, CO), 164.4 (CO), 167.0 (C-4), 171.8 (CO). MS (ESI$^+$) *m/z* 741.2 $(M + H)^+$. Anal. ($C_{34}H_{40}N_4O_{11}SSi$) C, H, N, S.

[1-[5′-*O*-Benzyl-2′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzoylureido)-1″,2″-oxathiole-2″,2″-dioxide] (19). Following the general procedure, **11**[17] (100 mg, 0.15 mmol) was reacted with benzoyl isocyanate (75 μL, 0.60 mmol). Purification of the residue by CCTLC on the Chromatotron (hexane/ethyl acetate 3:1) yielded 82 mg (69%) of **19** as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.10 (s, 3H, CH_3−Si), 0.04 (s, 3H, CH_3−Si), 0.80 (s, 9 H, *t*-Bu), 1.93 (s, 3H, CH_3-5), 3.29 (s, 3H, CH_3-3), 3.90 (m, 2H, 2H-5′), 4.55 (m, 1H, H-4′), 4.96 (m, 3H, H-2′, CH_2Ph), 6.33 (d, 1H, J = 7.9 Hz, H-1′), 7.27−8.17 (m, 12H, H-3″, H-6 and 2Ph), 9.85 (bs, 1H, NH), 11.55 (bs, 1H, NH). MS (ESI$^+$) *m/z* 727.1 $(M + H)^+$. Anal. ($C_{34}H_{42}N_4O_{10}SSi$) C, H, N, S.

[1-[2′,5′-Bis-*O*-*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-phenylureido)-1″,2″-oxatiole-2″,2″-dioxide] and [1-[2′,5′-Bis-*O*-*tert*-buthyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5‴-[4″-(3-phenylcarboxamide-3-phenylureido)-1″,2″-oxatiole-2″,2″-dioxide] (21 and 22). To a solution of TSAO-m^{3}T (**20**)[16] (70 mg, 0.12 mmol) in dry THF (10 mL), previously degassed under an argon atmosphere, sodium hydride 60% dispersion in mineral oil (10 mg, 0.23 mmol) was added. The mixture was stirred at room temperature for 1 h. Then, phenylisocyanate (0.03 mL, 0.23 mmol) was added and the resulting mixture was stirred at room temperature for 2.5 h. Volatiles were removed, and the residue was dissolved in ethyl acetate (10 mL) and washed, successively, with water (1 × 10 mL) and brine (2 × 10 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 4:1). The fastest moving band afforded **21** (49 mg, 58%) as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: 0.07 (s, 3H, CH_3−Si), 0.09 (s, 3H, CH_3−Si), 0.10 (s, 3H, CH_3−Si), 0.13 (s, 3H, CH_3−Si), 0.80 (s, 9H, ^{t}Bu-Si), 0.85 (s, 9H, ^{t}Bu-Si), 1.94 (s, 3H, CH_3-5), 3.40 (s, 3H, CH_3-3), 3.95 (m, 1H, H-5′), 4.13 (m, 2H, H-4′ and H-5′), 5.18 (d, 1H, J = 6.2 H, H-2′), 5.50 (d, 1H, J = 6.4, H-1′), 7.10 (m, 1H, Ph), 7.21 (s, 1H, H-3‴), 7.33 (m, 2H, Ph), 7.55 (m, 2H, Ph), 7.74 (d, 1H, J = 1.2, H-6), 8.30 (bs, 1H, NH), 9.22 (bs, 1H, NH). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ: −4.2 (CH_3−Si), −4.1 (CH_3−Si), −3.9 (CH_3−Si), −3.7 (CH_3−Si), 12.9 (CH_3-5), 18.5 (C-^{t}Bu), 18.8 (C-^{t}Bu), 25.8 (^{t}Bu), 26.1 (^{t}Bu), 28.1 (CH_3-3), 61.4 (C-5′), 73.5 (C-2′), 84.2 (C-4′), 88.7 (C-3′), 97.7 (C-1′), 100.8 (C-3‴), 111.8 (C-5), 119.7, 124.3, 129.8, 139.2, 139.6 (C-6), 145.3 (C-4″), 152.5, 151.4 (C-2, CO), 163.2 (C-4). MS (ESI$^+$) *m/z* 723.2 $(M + H)^+$, 745.3 $(M + Na)^+$. Anal. ($C_{32}H_{50}N_4O_9SSi_2$) C, H, N, S.

The slowest moving band afforded 26 mg (21%) of **22** as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.08 (s, 3H, CH_3−Si), 0.07 (s, 3H, CH_3−Si), 0.14 (s, 3H, CH_3−Si), 0.15 (s, 3H, CH_3−Si), 0.83 (s, 9H, ^{t}Bu-Si), 0.92 (s, 9H, ^{t}Bu-Si), 1.75 (s, 3H, CH_3-5), 3.28 (s, 3H, CH_3-3), 4.15 (m, 2H, H-5′), 4.42 (m, 1H, H-4′), 4.85 (d, 1H, J = 8.2, H-2′), 6.10 (d, 1H, J = 8.2, H-1′), 7.16−7.66 (m, 12H, H-6, H-3″ and Ph), 8.15 (bs, 1H, NH), 12.30 (bs, 1H, NH). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ: −3.9 (CH_3−Si), −3.7 (CH_3−Si), −3.5 (CH_3−Si), −3.3 (CH_3−Si), 13.7 (CH_3-5), 18.7 (C-^{t}Bu), 18.9 (C-^{t}Bu), 26.4 (^{t}Bu), 27.0 (^{t}Bu), 28.7 (CH_3-3), 62.8 (C-5′), 75.6 (C-2′), 86.0 (C-4′), 89.1 (C-1′), 92.7 (C-3′), 106.8 (C-3″), 112.3 (C-5), 124.8, 127.1, 130.2, 130.4, 130.5, 131.4, 131.4, 131.9 (Ph), 135.2 (C-6), 137.0 (Ph), 138.3 (Ph), 143.4 (C-4″), 152.6, 153.1 (C-2, CO), 157.2 (CO), 164.0 (C-4). MS (ESI$^+$) m/z 843.5 (M + H)$^+$, 864.0 (M + Na)$^+$. Anal. ($C_{39}H_{55}N_5O_{10}SSi_2$) C, H, N, S.

[1-[2′,5′-Bis-*O*-(*tert*-buthyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-methylthymine]-3′-spiro-5″-[4″-(3-benzylureido)-1″,2″-oxatiole-2″,2″-dioxide] (23). To a solution of **20**[16] (70 mg, 0.12 mmol) in dry THF (10 mL), previously degassed under an argon atmosphere, sodium hydride 60% dispersion in mineral oil (10 mg, 0.23 mmol) was added. The mixture was stirred at room temperature for 1 h. Then, benzylisocyanate (0.03 mL, 0.23 mmol) was added and the resulting mixture was stirred at room temperature for 3 h. Volatiles were removed, and the residue was dissolved in ethyl acetate (10 mL) and successively washed with water (1 × 10 mL) and brine (2 × 10 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 9:1) to give 64 mg (75%) of **23** as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: −0.07 (s, 3H, CH_3−Si), 0.07 (s, 3H, CH_3−Si), 0.08 (s, 3H, CH_3−Si), 0.09 (s, 3H, CH_3−Si), 0.83 (s, 9H, ^{t}Bu), 0.88 (s, 9H, ^{t}Bu), 1.90 (s, 3H, CH_3-5), 3.24 (s, 3H, CH_3-3), 3.87−4.24 (m, 3H, H-5′ and H-4′), 4.45 (dd, J = 5.8 and 6.2, CH_2Ph), 5.06 (d, 1H, J = 6.8, H-2′), 5.58 (d, 1H, J = 6.8, H-1′), 6.77 (t, 1H, J = 5.8, NHCH$_2$Ph), 7.16 (s, 1H, H-3″), 7.30 (m, 5H, Ph), 7.70 (s, 1H, H-6), 9.08 (bs, 1H, NH). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ: −4.79 (CH_3−Si), −4.76 (CH_3−Si), −4.70 (CH_3−Si), −4.15 (CH_3−Si), 13.4, (CH_3-5), 18.9 (C-^{t}Bu), 18.6 (C-^{t}Bu), 26.7 (^{t}Bu-Si), 26.2 (^{t}Bu-Si), 28.5 (CH_3-3), 44.8 (CH_2Ph), 62.2 (C-5′), 74.1 (C-2′), 85.1 (C-4′), 90.0 (C-3′), 95.8 (C-1′), 101.0 (C-3″), 112.1 (C-5), 128.4, 128.6, 129.7, 138.9 (Ph), 140.5 (C-6), 145.7 (C-4″), 152.8, 154.5 (C-2, CO), 163.7 (C-4). MS (ESI$^+$) m/z 737.1 (M + H)$^+$, 759.2 (M + Na)$^+$. Anal. ($C_{33}H_{52}N_4O_9SSi_2$) C, H, N, S.

[*N*-Acetyl-[2,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]amine]-3-spiro-5′-[4′-3-(benzoylureido)-1′,2′-oxathiole-2′,2′-dioxide] (29). Following the general procedure described for compounds **12**−**19**, compound **28**[18] (50 mg, 0.1 mmol) was reacted with benzoyl isocyanate (50 μL, 0.4 mmol) in dry acetonitrile (5 mL) in an Ace pressure tube at 100 °C overnight. Chromatography of the residue on the Chromatotron (hexane/ethyl acetate 5:1) gave **29** (60 mg, 90%) as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: 0.03 (s, 3H, CH_3−Si), 0.06 (s, 3H, CH_3−Si), 0.08 (s, 3H, CH_3−Si), 0.09 (s, 3H, CH_3−Si), 0.82 (s, 9H, ^{t}Bu-Si), 0.87 (s, 9H, ^{t}Bu-Si), 1.98 (s, 3H, CH_3−CO), 3.94 (dd, 1H, J = 5.2 and 11.9 Hz, H-5a), 4.01 (dd, 1H, J = 6.7 and 11.9 Hz, H-5b), 4.17 (dd, 1H, J = 5.2 and 6.7 Hz, H-4), 4.51 (d, 1H, J = 6.5 Hz, H-2), 5.61 (dd, 1H, J = 6.5 and 9.9 Hz, H-1), 7.32 (s, 1H, H-3′), 7.62 (m, 3H, H-6 and Ph), 7.75 (m, 1H, Ph), 8.11 (d, 1H, J = 7.2 Hz, Ph), 9.84 (bs, 1H, NH), 10.54 (bs, 1H, NH), 11.29 (bs, 1H, NH). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ: −5.8 (CH_3−Si), −5.7 (CH_3−Si), −5.2 (CH_3−Si), −5.0 (CH_3−Si), 18.2 (C-^{t}Bu), 18.4 (C-^{t}Bu), 25.6 (^{t}Bu), 25.8 (^{t}Bu), 61.4 (C-5), 76.4 (C-2), 82.6 (C-3′), 84.6 (C-4), 90.9 (C-1), 105.9 (C-3), 128.6, 128.8, 129.5, 134.4 (Ph), 153.3 (C-4′), 160.9 (CO), 170.4 (CO). MS (ESI$^+$) m/z 670 (M + H)$^+$, 692 (M + Na)$^+$. Anal. ($C_{29}H_{47}N_3O_9SSi_2$) C, H, N, S.

2-(Methanesulfonyloxy)-4-(*tert*-butyldimethylsilyloxy)-2-(*tert*-butyldimethylsilyloxymethyl)-2-butyronitrile (31). To a solution of 1,4-bis[(*tert*-butyldimethylsilyl)oxy]-2-butanone (**30**)[19] (700 mg, 2.2 mmol) in a (2:1) mixture of diethyl ether/water (12 mL), $NaHCO_3$ (550 mg, 6.6 mmol) and NaCN (160 mg, 3.3 mmol) were added. The mixture was vigorously stirred at room temperature for 24 h. The organic layer was separated, and the aqueous phase was further extracted with diethyl ether (2 × 20 mL). The combined ethereal extracts were dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in dry dichloromethane (3 mL) and treated with Et_3N (1.6 mL, 11.6 mmol) and then methanesulfonyl chloride (0.38 mL, 5 mmol) was slowly added. The mixture was stirred at −20 °C for 1 h and at 0 °C for an additional hour. Volatiles were removed, and the residue was dissolved in ethyl acetate (10 mL) and washed successively with water (1 × 10 mL) and brine (2 × 10 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by flash column chromatography (hexane:ethyl acetate, 60:1). The faster moving fractions afforded **31** (298 mg, 31%) as an amorphous solid. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: 0.14 (s, 6H, $2CH_3$−Si), 0.19 (s, 6H, $2CH_3$−Si), 0.95 (s, 9H, ^{t}Bu-Si), 0.97 (s, 9H, ^{t}Bu-Si), 2.43 (m, 2H, H-3), 3.34 (s, 3H, CH_3−S), 3.97 (m, 2H, H-4), 4.19 (s, 2H, H-1). MS (ESI$^+$) m/z 438.9 (M + 1)$^+$. Anal. ($C_{18}H_{39}NO_5SSi_2$) C, H, N, S.

The slowest moving fractions afforded 246 mg (35%) of unreacted starting material (**30**).

4-Amino-5-(*tert*-butyldimethylsilyloxyethyl)-5-(*tert*-butyldimethylsilyloxymethyl)-5*H*-1,2-oxathiole-2,2-dioxide (32). To a suspension of **31** (270 mg, 0.6 mmol) in dry acetonitrile (6 mL), cesium carbonate (300 mg, 0.9 mmol) was added and the mixture was stirred at room temperature for 3 h. The solvent was removed and the residue, thus obtained, was dissolved in ethyl acetate (20 mL) and washed successively with water (10 mL) and brine (2 × 10 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 6:1) to give **32** (200 mg, 75%) as a white foam. ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ: 0.09 (s, 3H, CH_3−Si), 0.10 (s, 3H, CH_3−Si), 0.13 (s, 3H, CH_3−Si), 0.14 (s, 3H, CH_3−Si), 0.92 (s, 9H, ^{t}Bu-Si), 0.94 (s, 9H, ^{t}Bu-Si), 2.24 (m, 2H, C$\underline{H}_2$-CH_2−O), 3.79−3.94 (m, 2H, CH_2−C$\underline{H}_2$-O), 3.99 (s, 2H, CH_2−O), 5.46 (s, 1H, H-3), 6.10 (bs, 2H, NH_2). ^{13}C NMR [75 MHz, $(CD_3)_2CO$] δ:−7.0 ($2CH_3$−Si), −6.9 ($2CH_3$−Si), 24.5 (^{t}Bu-Si), 24.6 (^{t}Bu-Si), 35.5 $\underline{C}H_2$−CH_2−O), 57.1 CH_2-$\underline{C}H_2$−O), 65.7 ($\underline{C}H_2$−O), 87.8 (C-3), 89.4 (C-5), 155.3 (C-4). MS (ESI$^+$) m/z 438.0 (M + 1)$^+$, 460.0 (M + Na)$^+$, 897.2 (2 M + Na)$^+$. Anal. ($C_{18}H_{39}NO_5SSi$) C, H, N, S.

4-Benzoylureido-5-(*tert*-butyldimethylsilyloxyethyl)-5-(*tert*-butyldimethylsilyloxymethyl)-5*H*-1,2-oxathiole-2,2-dioxide (33). According to the general procedure described for the synthesis of compounds **12**−**19**, compound **32** (100 mg, 0.22 mmol) was reacted with benzoyl isocyanate (112 μL, 0.88 mmol) in dry acetonitrile (8 mL) in an Ace pressure tube at 100 °C overnight. Chromatography of the residue on the Chromatotron (hexane/ethyl acetate 3:1) yielded 86 mg (67%) of **33** as a white foam. ^{1}H NMR [300 MHz, $CDCl_3$] δ: −0.01 (s, 3H, CH_3−Si), 0.01 (s, 3H, CH_3−Si), 0.10 (s, 3H, CH_3−Si), 0.13 (s, 3H, CH_3−Si), 0.81 (s, 9H, ^{t}Bu-Si), 0.89 (s, 9H, ^{t}Bu-Si), 2.17 (td, 1H, J = 4.4 and 15.2 Hz, $\underline{CH}_{2a}$-CH_2−O), 2.40 (ddd, 1H, J = 5.2, 8.4 and 15.2 Hz, C$\underline{H}_{2b}$-CH_2−O), 3.84 (m, 2H, CH_2−C$\underline{H}_2$-O), 3.91 and 3.95 (AB system, J = 10.4 Hz, CH_2−O), 7.00 (s, 1H, H-3), 7.57 (t, 2H, J = 8.0 Hz, Ph), 7.71 (t, 1H, J = 7.6 Hz, Ph), 7.96 (d, 2H, J = 7.6 Hz, Ph), 10.09 (bs, 1H, NH), 11.30 (bs, 1H, NH). ^{13}C NMR [75 MHz, $CDCl_3$] δ: −5.7 (CH_3−Si),−5.6 (CH_3−Si), −5.5 (CH_3−Si), −5.4 (CH_3−Si), 18.2 (C-^{t}Bu), 18.5 (C-^{t}Bu), 25.7 (^{t}Bu-Si), 25.8 (^{t}Bu-Si), 36.4 ($\underline{C}H_2$−CH_2−O), 57.2 (CH_2-$\underline{C}H_2$−O), 68.1 ($\underline{C}H_2$O), 89.8 (C-3), 104.2 (C-5), 127.9, 129.1, 131.1, 134.4 (Ph), 146.4 (C-4), 151.9 (CO), 168.4 (CO). MS (ESI$^+$) m/z 586.3 (M + 1)$^+$, 605.0 (M + Na)$^+$. Anal. ($C_{26}H_{44}N_2O_7SSi_2$) C, H, N, S.

Biological Methods. Anticytomegalovirus Assay. For the HCMV assays, confluent HEL fibroblasts were grown in 96-well microtiter plates and infected with the human cytomegalovirus strains Davis and AD-169 at 100 PFU per well. After a 2 h incubation period, residual virus was removed and the infected cells were further incubated with medium containing different concentrations of the test compounds (in duplicate). After incubation for 7 days at 37 °C, virus-induced cytopathogenicity was monitored microscopically after ethanol fixation

and staining with Giemsa. Antiviral activity was expressed as the EC_{50} or compound concentration required reducing virus-induced cytopathogenicity by 50%.

Compounds **12** and **15** were also evaluated against a panel of drug-resistant HCMV mutants isolated in vitro after serial passage of the reference strain AD-169 under increasing concentrations of anti-HCMV agents and also against several HCMV drug-resistant isolates recovered from immunocompromised patients. Different reference drugs (i.e., acyclovir (ACV), ganciclovir (GCV), foscarnet (PFA), (*S*)-3-hydroxy-2-phosphonomethoxypropyl (HPMP) derivatives of adenine (HPMPA) and cytosine ((*S*)-HPMPC, cidofovir)), and 2-phosphonomethoxyethyl (PME) derivatives of adenine (PMEA) and 2,6-diaminopurine (PMEDAP) were included in the antiviral assays.

Murine CMV (MCMV) assays were performed with the Smith strain on confluent C127I cells following the same methodology as described for HCMV.

Activity against Other Viruses. Varicella zoster virus (VZV) drug susceptibility tests were performed on confluent HEL cells in 96-well microtiter plates by the plaque reduction assay. Monolayers were infected with 20 PFU of cell-associated virus per well. For each assay, virus controls (infected-untreated cells) were included. After a 2 h incubation period, the virus inoculum was removed and the media replaced by the different dilutions (in duplicate) of the tested molecules. Serial dilutions of test compounds were incubated with the infected monolayers for 5 days. After a 5-day incubation period, the cells were fixed and stained with Giemsa, and the level of virus-induced cytopathic effect was determined by counting the number of plaques for each dilution. Activity was expressed as EC_{50} (effective compound concentration required to reduce virus plaque formation by 50%) compared to the untreated control.

Cytotoxicity Assays. Cytotoxicity measurements were based on the inhibition of HEL cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The 50% cytostatic concentration was calculated as the CC_{50}, the compound concentration required to reduce cell growth by 50% relative to the number of cells in the untreated controls. CC_{50} values were estimated from graphic plots of the number of cells (percentage of control) as a function of the concentration of the test compounds. Cytotoxicity was expressed as minimum cytotoxic concentration (MCC) or the compound concentration that causes a microscopically detectable alteration of cell morphology.

Time-of-Drug Addition Studies. Confluent HEL cell cultures were infected with approximately 100 PFU of HCMV (Davis strain) per well. A variety of concentrations of compounds were added to the infected cell cultures at the time of infection (0 h) or at several time points post infection (one set of microtiter plates per time point). Seven days after the initial infection of the cells, the different microtiter plates were fixed with ethanol and stained with Giemsa. The antiviral effects of the compounds were microscopically scored, and the EC_{50} values were calculated.

Enzyme Assay for HCMV DNA Polymerase. The procedure to determine the direct inhibitory activity of the compounds on the HCMV UL54-encoded DNA polymerase has been published elsewhere.[20] An UL54-containing expression plasmid (a kind gift from Dr. T. Cihlar, Gilead Sciences, Foster City, CA) was used to prepare the catalytic subunit of the DNA polymerase by in vitro transcription/translation. The enzyme mixture contained activated calf thymus DNA as the primer/template, 1 μM of [^{3}H]-dGTP and 100 μM of dATP, dTTP, and dCTP, or 1 μM of [^{3}H]dATP and 100 μM of dGTP, dTTP, and dCTP and serial dilutions of the test compounds.[21] After 60 min at 37 °C, nucleic acids were precipitated with trichloroacetic acid and collected on filters in which incorporated radioactivity was quantified by scintillation counting.

HCMV Antigen Expression Assay. HEL cells were grown in 24-well microtiter plates and infected with HCMV (Davis strain). At the time of infection, different dilutions of the compounds were added. Following different times of incubation at 37 °C, HCMV-infected and uninfected HEL cells were fixed in acetone at 4 °C. After resuspension of cells in PBS containing 5% gelatin, the cells were incubated at 37 °C in the presence of the desired monoclonal antibody (mAb E13 (Biosoft), directed against IE (immediately early) proteins 1 and 2, and mAb CCH2 (Dako), which reacts with the delayed early (E) DNA-binding protein p52). After a washing step, the cells were incubated for 30 min with a fluorescein-labeled rabbit antimouse IgG and finally fixed in a solution of 0.5% formaldehyde in PBS. The cells were then analyzed with a fluorescence-activated cell sorter, as described earlier.[22,23]

Acknowledgment. The authors would like to thank Susana Ruiz, Lies Van den Heurck, Steven Carmans, Anita Camps, Lizette van Berckelaer, and Elke Simons for excellent technical assistance. The Spanish MEC (project SAF2006-12713-C02), the European Commission (project HPAW-CT-2002-90001 (René Descartes Prize-2001)), and the Geconcerteerde Onderzoeksacties (GOA 05/15) of the K.U. Leuven are acknowledged for financial support.

Supporting Information Available: Elemental analysis data of of compounds **2**−**4**, **9**−**19**, **21**−**23**, **29** and **31**−**33**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Pass, R. F. Cytomegalovirus. In *Fields Virology*, 4th ed.; Lippincott, Williams & Wilkins: Philadelphia, 2001; Vol *2*, pp 2675−2705.
(2) Trincado, D. E.; Scott, G. M.; White, P. A.; Hunt, C.; Rasmussen, L.; Rawlinson, W. D. Human cytomegalovirus strains associated with congenital and perinatal infections. *J. Med. Virol.* **2000**, *61*, 481–487.
(3) Yeh, R.; Cohen, D. M. Pharmacology of viral replication. In *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*; Lippincott, Williams & Wilkins: Baltimore, 2004, pp 545−564.
(4) Griffiths, P. D. The treatment of cytomegalovirus infection. *J. Antimicrob. Chemother.* **2002**, *49*, 243–253.
(5) Cvetkovic, R. S.; Wellington, K. Valganciclovir: a review of its use in the management of CMV infection and disease in immunocompromised patients. *Drugs* **2005**, *65*, 859–878.
(6) Biron, K. K. Antiviral drugs for cytomegalovirus diseases. *Antiviral Res.* **2006**, *71*, 154–163.
(7) Villareal, E. C. Current and potential therapies for the treatment of herpes virus infections. *Prog. Drug Res.* **2003**, *60*, 263–307.
(8) Huang, E.-S. Human cytomegalovirus IV. Specific inhibition of virus induced DNA polymerase activity and viral DNA replication by phosphonoacetic acid. *J. Virol.* **1975**, *16*, 1560–1565.
(9) Mar, E. C.; Cheng, Y. C.; Huang, E.-S. Effect of 9-(1,3-dihydroxy-2-propoxymethyl)guanine on human cytomegalovirus replication in vitro. *Antimicrob. Agents Chemother.* **1983**, *24*, 518–521.
(10) Mar, E. C.; Chiou, J. F.; Cheng, Y. C.; Huang, E.-S. Inhibition of cellular DNA polymerase alpha and human cytomegalovirus-induced DNA polymerase by the triphosphates of 9-(2-hydroxyethoxymethyl)guanine and 9-(1,3-dihydroxy-2-propoxymethyl)guanine. *J. Virol.* **1985**, *53*, 776–780.
(11) Baldanti, F.; Gerna, G. Human cytomegalovirus resistance to antiviral drugs: diagnosis, monitoring and clinical impact. *J. Antimicrob. Chemother.* **2003**, *52*, 324–330.
(12) De Castro, S.; Lobatón, E.; Pérez-Pérez, M. J.; San-Félix, A.; Cordeiro, A.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J.; Camarasa, M. J.; Velázquez, S. Novel [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuronosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) derivatives with anti-HIV-1 and anti-human cytomegalovirus activity″. *J. Med. Chem.* **2005**, *48*, 1158–1168.
(13) Camarasa, M. J.; San-Félix, A.; Velázquez, A.; Pérez-Pérez, M. J.; Gago, F.; Balzarini, J. TSAO compounds: the comprehensive story of a unique family of HIV-1 specific inhibitors of reverse transcriptase. *Curr. Top. Med. Chem.* **2004**, *4*, 945–963.
(14) De Castro, S.; Lozano, A.; Jimeno, M. L.; Pérez-Pérez, M. J.; San-Félix, A.; Camarasa, M. J.; Velázquez, S. Unprecedented lability of the 5′-*O*-*tert*-butyldimethylsilyl group from 3′-spiro-5″-(4″-acylamino-1″,2″-oxathiole-2″,2″-dioxide) nucleoside derivatives via neighboring

group participation of the 4″-acylamino residue. *J. Org. Chem.* **2006**, *71*, 1407–1415.
(15) Ingate, S.; Pérez-Pérez, M. J.; De Clercq, E.; Balzarni, J.; Camarasa, M. J. Synthesis and anti-HIV-1 activity of novel TSAO-T derivatives modified at the 2′- and 5′-position of the sugar moiety. *Antiviral Res.* **1995**, *27*, 281–299.
(16) Pérez-Pérez, M. J.; San-Félix, A.; Balzarini, J.; De Clercq, E.; Camarasa, M. J. TSAO analogues. Stereospecific and anti-HIV-1 activity of 1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranoxyl]-3-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) pyrimidine and pyrimidine-modified nucleosides. *J. Med. Chem.* **1992**, *35*, 2988–2995.
(17) Chamorro, C.; Pérez-Pérez, M. J.; Rodríguez-Barrios, F.; Gago, F.; De Clercq, E.; Balzarini, J.; San-Félix, A.; Camarasa, M. J. Exploring the role of the 5′-position of TSAO-T. Synthesis and *anti*-HIV evaluation of novel TSAO-T derivatives. *Antiviral Res.* **2001**, *50*, 207–222.
(18) Velázquez, S.; Chamorro, C.; Pérez-Pérez, M. J.; Alvarez, R.; Jimeno, M. L.; Martín-Domenech, A.; Pérez, C.; Gago, F.; De Clercq, E.; Balzarini, J.; San-Félix, A.; Camarasa, M. J. Abasic analogues of TSAO-T as the first sugar derivatives that specifically inhibit HIV-1 reverse transcriptase. *J. Med. Chem.* **1998**, *41*, 4636–4647.
(19) Shimizu, N.; Mori, N.; Kuwahara, Y. Simple synthesis of mite pheromone beta-acardial and its analogs in the secretion of *Caloglyphus polyphyllae* (Acari. Acaridae). *Biosci. Biotechnol. Biochem.* **2003**, *67*, 1732–1736.
(20) Cihlar, T.; Fuller, M. D.; Cherrington, J. M. Expression of the catalytic subunit (UL54) and the accessory protein (UL44) of human cytomegalovirus DNA polymerase in a coupled in vitro transcription/translation system. *Protein Expression Purif.* **1997**, *11*, 209–218.
(21) De Bolle, L.; Manichanh, C.; Agut, H.; De Clercq, E.; Naesens, L. Human herpes virus 6 DNA polymerase: enzymatic parameters, sensitivity to ganciclovir and determination of the role of the A961V mutation in HHV-6 ganciclovir resistance. *Antiviral Res.* **2004**, *64*, 17–25.
(22) Andrei, G.; Snoeck, R.; Schols, D.; Goubau, P.; Desmyter, J.; De Clercq, E. Comparative activity of selected antiviral compounds against clinical isolates of human cytomegalovirus. *Eur. J. Clin. Microbiol. Infect. Dis.* **1991**, *10*, 1026–1033.
(23) Snoeck, R.; Andrei, G.; Bodaghi, B.; Lagneaux, L.; Daelemans, D.; De Clercq, E.; Neyts, J.; Schols, D.; Naesens, L.; Michelson, S.; Bron, D.; Otto, M. J.; Bousseau, A.; Nemecek, C.; Roy, C. 2-Chloro-3-pyridin-3-yl-5,6,7,8-tetrahydroindolizine-1-carboxamide (CMV423), a new lead compound for the treatment of human cytomegalovirus infections. *Antiviral Res.* **2002**, *55*, 413–424.

JM800050T