Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 1771–1779
Bioorganic & Medicinal Chemistry

Synthesis of the 5-phosphono-pent-2-en-1-yl nucleosides: A new class of antiviral acyclic nucleoside phosphonates☆

Hyunah Choo,[a,b,c] James R. Beadle,[a,b] Youhoon Chong,[d] Julissa Trahan[a,b] and Karl Y. Hostetler[a,b,*]

[a]*Department of Medicine, Division of Infectious Disease, University of California, San Diego, La Jolla, CA 92093-0676, USA*
[b]*Veterans Medical Research Foundation, San Diego, CA 92161, USA*
[c]*Life Sciences Division, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea*
[d]*Division of Biosciences and Biotechnology, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea*

Received 18 September 2006; accepted 27 November 2006
Available online 30 November 2006

Abstract—A new class of acyclic nucleoside phosphonates, the 5-phosphono-pent-2-en-1-yl nucleosides and their hexadecyloxypropyl esters, were synthesized from butyn-1-ol. Only the hexadecyloxypropyl esters showed antiviral activity against herpes simplex virus type 1, in vitro. Hexadecyloxypropyl 1-(5-phosphono-pent-2-en-1-yl)-thymine was the most active and selective compound among the synthesized nucleotides with an EC_{50} value of 0.90 μM.

1. Introduction

The herpes simplex viruses (HSV) belong to the family of the *Herpesviridae*.[1] There are eight known human herpesviruses, including the two herpes simplex viruses HSV-1 and HSV-2. HSV virions are 180–200 nm in diameter and contain an icosahedral capsid surrounding the linear double-stranded DNA genome. Cellular infection is initiated when the virus binds to heparin sulfate chains on cell surface proteoglycans.[2] HSV-1 is responsible for the lesions at orofacial sites that are commonly known as cold sores. Viral transmission is caused by kissing, or by sharing the same utensils and towels. HSV-2 is responsible for mucocutaneous genital infections and usually is spread by intimate sexual contact.

Nucleoside analogs became the mainstay for chemotherapy of viral diseases in 1982 with the introduction of acyclovir, the first selective antiviral agent.[3] Valaciclovir, an oral prodrug of acyclovir, and famciclovir, an oral form of penciclovir, are also available now as effective medications that can reduce the duration and severity of HSV-1 infections.[4] The general mode of action of nucleoside analogs is through inhibition of viral DNA polymerases by acting as competitive inhibitors and/or DNA chain terminators. Nucleosides require intracellular conversion to their triphosphate form to be active as antivirals, with the rate-determining step often being the first phosphorylation which affords the corresponding 5′-monophosphate. The effectiveness of nucleoside analogs is often limited by either poor cellular penetration, inefficient phosphorylation or weak interaction with the viral DNA polymerase.

Resistance to selective HSV-1 agents such as acyclovir and penciclovir[5] is mostly mediated by deficiency of HSV thymidine kinase, the enzyme normally responsible for the initial phosphorylation in HSV-1-infected cells.[6] The emergence of acyclovir-resistant variants, especially in immunocompromised patients, has forced the development of new drugs with activity against acyclovir-resistant viruses.[7]

In contrast to nucleoside analogs, acyclic nucleoside phosphonates, such as cidofovir and adefovir, possess a phosphonomethyl ether moiety instead of the nucleotide phosphate ester and have advantages over the nucleoside analogs because they bypass the limiting first

Keywords: Antivirals; Nucleosides; Nucleoside phosphonates; Prodrugs.
☆ Portions of this paper were presented in abstract form at the International Conference on Antiviral Research, April 11–14, 2005, Barcelona, Spain.
* Corresponding author. Tel.: +1 858 552 8585x2616; fax: +1 858 534 6133; e-mail: khostetl@ucsd.edu

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.11.038

phosphorylation step. As a result, acyclic nucleoside phosphonates inhibit viruses that do not encode a thymidine kinase, as well as thymidine kinase deficient strains of HSV-1. However, their double negative charge at physiological pH reduces penetration into the cell, reducing their antiviral activity. To overcome this problem, nucleotides are converted to phosphonoester forms which are designed to penetrate into the cell by virtue of their reduced polarity. The esters are hydrolyzed during oral absorption by intestinal enzymes and the nucleotides circulate as the antiviral nucleoside phosphonate. After cellular uptake and anabolic phosphorylation to corresponding diphosphate nucleotides, they can be incorporated into the growing viral DNA strand causing chain termination or otherwise inhibiting antiviral activity. One example is tenofovir disoproxil fumarate, which is approved by the FDA for the treatment of HIV (Fig. 1).[8] Tenofovir disoproxil is a phosphonodiester form of tenofovir. After hydrolysis and two anabolic phosphorylations, tenofovir diphosphate shows antiviral activity especially against wild type and drug-resistant HIV.

Our laboratory recently reported a new type of phosphonoester, alkoxyethyl and alkoxypropyl phosphonoester prodrugs which greatly increase the penetration of nucleotide analogs into cells.[9] This strategy increases antiviral activity dramatically and makes acyclic nucleoside phosphonates orally active.[10] Cidofovir (CDV), which is used intravenously for the treatment of cytomegalovirus (CMV) retinitis, was converted to hexadecyloxypropyl-CDV (HDP-CDV) or octadecyloxyethyl-CDV (ODE-CDV) (Fig. 1), resulting in a >100-fold increase of antiviral activity against CMV.[11] HDP-CDV and ODE-CDV were orally active against lethal poxvirus infections and in murine CMV infection.[12] Acyclic nucleoside phosphonates are very promising antiviral agents, especially when their high polarity is minimized by esterification with alkoxyalkyl groups.

Herein we describe for the first time the synthesis and anti-HSV activity of a new type of acyclic nucleoside phosphonates, the 5-phosphono-pent-2-en-1-yl nucleosides (PPen-Ns) and their hexadecyloxypropyl derivatives (HDP-PPen-Ns).

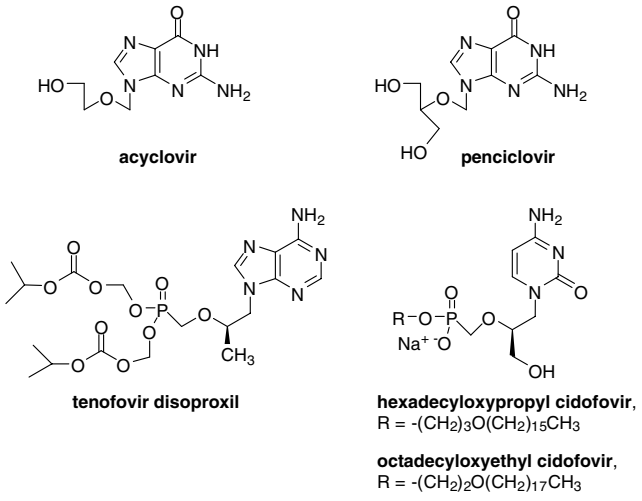


Figure 1. Structures of antiviral nucleosides and acyclic nucleoside phosphonates.

2. Results and discussion

2.1. Synthetic chemistry

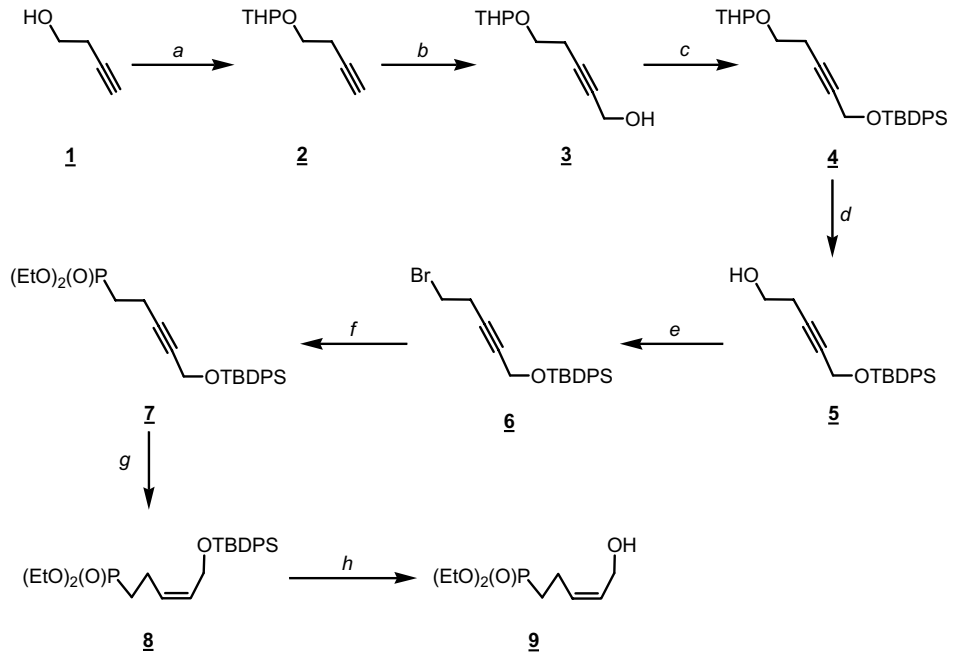
Our synthetic strategy, shown in Scheme 1, was to introduce the *cis*-double bond through partial hydrogenation of a triple bond to give an intermediate (**9**) which could be coupled to various nucleobases. Thus, 3-butyn-1-ol (**1**) was treated with 3,4-dihydropran and PPTS to give THP-protected butynol (**2**) in 86% yield. Reaction of compound **2** with paraformaldehyde gave hydroxymethylated compound **3** in 62% yield. Compound **3** was protected (**4**, in 96% yield) and then treated with PPTS/methanol to give alcohol **5** in 78% yield. Compound **5** was converted to bromide **6** (99% yield), which underwent Arbuzov reaction in $P(OEt)_3$ to afford diethyl phosphonate **7** in 98% yield. Partial hydrogenation of **7** over Lindlar's catalyst[13] gave **8** with a *cis*-double bond. Finally, key intermediate **9** was obtained in 99% yield after deprotection of **8** with TBAF.

Scheme 2 illustrates Mitsunobu reaction of compound **9** with various nucleobases to give the PPen nucleosides. Adenine and 2-amino-6-chloropurine were coupled with **9** to give compounds **10** and **11** in 42% and 50% yield, respectively. Compound **10** was then dealkylated using bromotrimethylsilane (TMSBr) to give 9-(5-phosphonopent-2-en-1-yl)-adenine (**13**, PPen-A). Each 5-phosphonopent-2-en-1-yl nucleoside was purified by DOWEX-1X2 ion-exchange column chromatography with gradient elution (0–0.25 M HCO_2H). Compound **11** was hydrolyzed with 88% HCO_2H at 100 °C for 8 h to give guanine derivative **12** which was dealkylated to give 9-(5-phosphono-pent-2-en-1-yl)-guanine (**15**, PPen-G). Hexadecyloxypropyl 9-(5-phosphono-pent-2-en-1-yl)-adenine (**16**, HDP-PPen-A) was obtained by DCC coupling of PPen-A (**13**) and 3-(hexadecyloxy)-1-propanol. Similarly, compound **11** was hydrolyzed by TMSBr, esterified with 3-hexadecyl-1-propanol, and hydrolyzed with HCO_2H to afford HDP-PPen-G **18**.

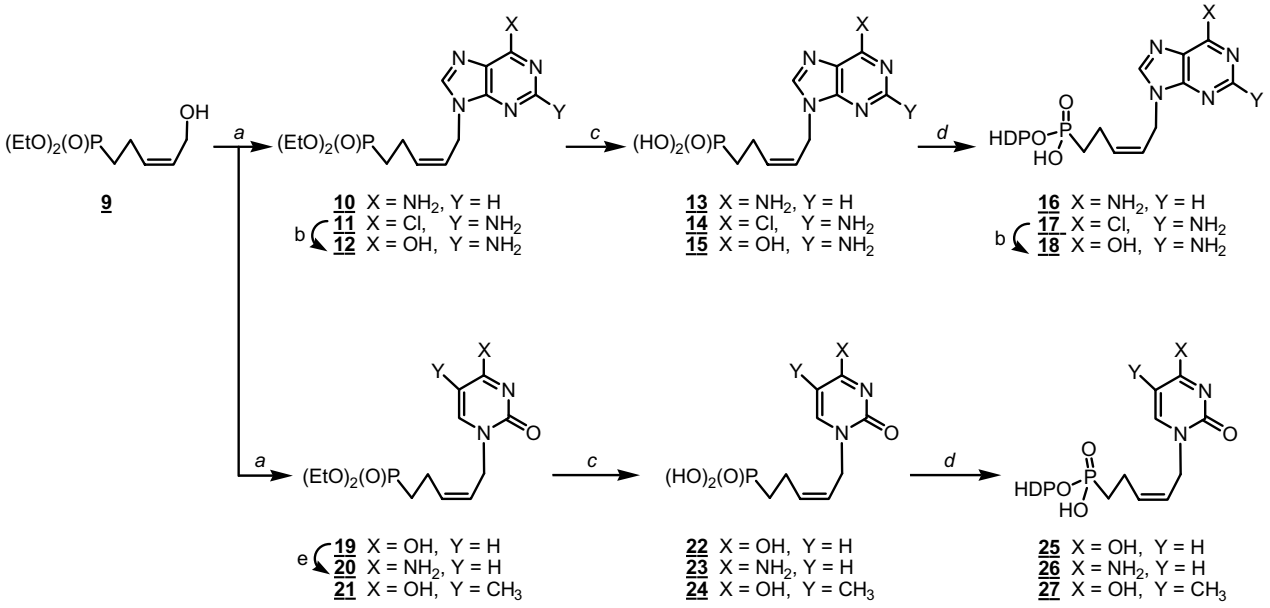
Intermediate **9** was also coupled with 3-benzoyluracil and 3-benzoylthymine under Mitsunobu conditions, followed by treatment with methanolic ammonia, to give compounds **19** and **21** in 81% and 78% yield, respectively. PPen-U (**22**) and PPen-T (**24**) were obtained by treatment with TMSBr in acetonitrile in 98% and 85% yield, respectively. Compound **19** was treated with 2,4,6-triisopropylbenzenesulfonyl chloride, TEA, and DMAP followed by NH_4OH to give the cytosine derivative **20** in 75% yield. Compound **20** was converted to PPen-C (**23**) in 71% yield. Finally, hexadecyloxypropyl derivatives **25**, **26**, and **27** were obtained by DCC coupling in 44%, 38%, and 15% yield, respectively.

2.2. Antiviral evaluation

The antiviral activity of the synthesized phosphonopentenyl nucleosides (PPen-Ns) and their hexadecyloxypro-



Scheme 1. Synthesis of the key intermediate **9**. Reagents and conditions: (a) DHP, PPTS, CH_2Cl_2, rt; (b) *n*-BuLi, $(CH_2O)_n$, 0 °C; (c) TBDPSCl, imidazole, CH_2Cl_2, rt; (d) MeOH, PPTS, rt; (e) CBr_4, PPh_3, CH_2Cl_2, −78 °C; (f) $P(OEt)_3$, reflux; (g) H_2, Lindlar's catalyst, MeOH, rt; (h) TBAF, acetonitrile, 0 °C.



Scheme 2. Synthesis of 5-phosphono-pent-2-en-1-yl nucleosides and their phosphonoesters. Reagents and conditions: (a) DIAD, PPh_3, nucleobases, DMF, 0 °C or rt; (b) HCOOH, reflux; (c) TMSBr, acetonitrile, rt; (d) HDP-OH, DCC, DMAP, DMF, 60 °C; (e) i—2,4,6-triisopropylbenzenesulfonyl chloride, TEA, DMAP, acetonitrile, rt; ii—NH_4OH.

pyl derivatives (HDP-PPen-Ns) was tested against HSV-1 in vitro using a DNA reduction assay method as reported previously[11] and the results are summarized in Table 1. The unmodified PPen nucleosides, such as compounds **13**, **15**, **22**, **23**, and **25**, show no inhibitory activity against HSV-1 and no cytotoxicity up to 100 μM. Nucleoside phosphonates sometimes fail to exhibit biological activity in vitro because they carry a double negative charge at physiological pH conditions and do not readily enter cells. Previously, it was reported that lipid-modified cidofovir, HDP-CDV, enters cells rapidly and is metabolized to the active form, cidofovir diphosphate, resulting in increased antiviral activity versus CDV.[9,11] Therefore, we prepared hexadecyloxypropyl esters of the PPen compounds. Some of the esterified PPen nucleosides showed potent inhibitory activity against HSV-1. Of these, HDP-PPen-T (**27**) is the most active compound against HSV-1 with an

Table 1. Antiviral activity and selectivity of the PPen nucleosides[a]

Compounds	Anti-HSV-1 activity[b] EC_{50}, μM	Cytotoxicity[c] CC_{50}, μM	Selective index
PPen-A **13**	>30	>100	—
PPen-G **15**	>30	>100	—
PPen-U **22**	>30	>100	—
PPen-C **23**	>30	>100	—
PPen-T **24**	>30	>100	—
HDP-PPen-A **16**	18 ± 4.5	17.3 ± 4.2	0.96
HDP-PPen-G **18**	5.8 ± 2.2	12.2± 6.2	2.10
HDP-PPen-U **25**	>30	51.0 ± 11.8	—
HDP-PPen-C **26**	4.7 ± 5.4	11.2 ± 4.2	2.38
HDP-PPen-T **27**	0.90 ± 0.5	7.4 ± 2.4	8.22

[a] The values are the means ± SD of 3 experiments.
[b] Anti-HSV-1 activity was determined by DNA reduction assay in MRC-5 cells.
[c] Antiproliferative activity and cytotoxicity was determined by neutral red uptake assay method in MRC-5 cells.

EC_{50} value of 0.90 μM. The lack of antiviral activity of HDP-PPen-U (**25**) may be explained by the fact that uridine triphosphate (RNA) is not a HSV-1 DNA polymerase substrate. The other HDP-PPen nucleosides such as compounds **16**, **18**, and **26** show anti-HSV-1 activity with EC_{50} values of 18.0, 5.8, and 4.7 μM. Selective indexes were low for HDP-PPen-A and HDP-PPen-U, intermediate with HDP-PPen-C and HDP-PPen-G, and high with HDP-PPen-T, the most active compound.

Based on the observed HSV-1 antiviral activity, it is reasonable to suggest that the active HDP-PPen nucleosides enter cells and are metabolized to the corresponding PPen nucleoside diphosphates and bind tightly to the HSV-1 DNA polymerase to inhibit HSV-1 replication. The compounds may also be incorporated resulting in termination of the growing DNA chain. Figure 2 shows a model of the replication complex of HSV-1 DNA polymerase complexed with PPen-T diphosphate constructed by following the protocol of Liu et al.[14] The model suggests a binding mode of PPen-T diphosphate to the target enzyme that is similar to that of acyclovir. In addition, a possible hydrophobic π–π interaction between the *cis*-double bond of PPen-T diphosphate and the aromatic side chain of the steric gate (Tyr722), which lies beneath the incoming nucleotide binding site, might increase the binding interaction of PPen-T diphosphate with the target enzyme.[15]

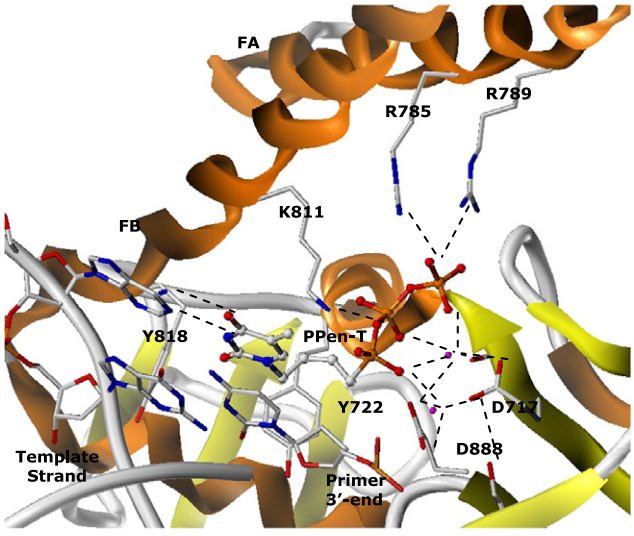


Figure 2. A model of PPen-T diphosphate · HSV-1 polymerase complex. HSV-1 polymerase is shown in ribbons and the key enzyme residues are represented as sticks. Hydrogen bonds involving PPen-T diphosphate are shown in black dashes.

3. Conclusion

In summary, hexadecyloxypropyl 5-phosphono-pent-2-en-1-yl nucleosides (HDP-PPen-Ns) were synthesized and identified as novel antivirals with activity against HSV-1, in vitro. HDP-PPen-T (**27**) was the most active compound among the synthesized nucleotides with an EC_{50} value of 0.90 μM. A model of PPen-T bound at the active site of HSV-1 polymerase suggests that its binding mode is similar to that of acyclovir and the *cis*-double bond of HDP-PPen-Ns might serve to increase the binding interaction to the target enzyme. Further modification of the synthesized nucleotides is in progress and their antiviral activity against other viruses is being explored.

4. Experimental

4.1. General chemistry methods

^{1}H and ^{31}P nuclear magnetic resonance (NMR) spectra were recorded on a Varian HG-300 spectrometer at 300 MHz for ^{1}H NMR. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Mass spectra were recorded on a Finnigan LCQDECA mass spectrometer and a ThermoFinnigan MAT900XL high-resolution mass spectrometer at the small molecule facility in Department of Chemistry at University of California, San Diego. Thin-layer chromatography (TLC) was performed on silica gel-GF Uniplates (250 μm) purchased from Analtech Inc. (Newark, DE) and visualized by UV light, phospray (Supelco, Bellefonte, PA), and charring at 400 °C. Flash column chromatography was performed using silica gel-60 (240–400 mesh).

4.2. Synthesis of 2-but-3-ynyloxy-tetrahydro-pyran (2)

A solution of 3-butyn-1-ol (**1**, 1.00 g, 14.3 mmol) and PPTS (0.72 g, 2.9 mmol) in CH_2Cl_2 (20 ml) was treated dropwise with 3,4-dihydropyran (1.7 ml, 19 mmol) and the resulting mixture was stirred overnight. The reaction mixture was diluted with CH_2Cl_2 (80 ml) and washed with 0.02 N NaOH (50 ml) and brine (100 ml). The organic layer was dried over $MgSO_4$ and concentrated, and the residue was purified by flash chromatography (3–5% EtOAc in hexanes) to give 1.90 g of compound **2** (12.3 mmol, 86% yield); ^{1}H NMR ($CDCl_3$) δ 4.63 (t, J = 3.4 Hz, 1H), 3.92–3.77 (m, 2H), 3.59–3.45 (m, 2H), 2.47 (td, J = 7.1, 2.8 Hz, 2H), 1.96 (t, J = 2.5 Hz, 1H), 1.90–1.44 (m, 6H).

4.3. Synthesis of 5-(tetrahydro-pyran-2-yloxy)-pent-2-yn-1-ol (3)

A solution of compound **2** (11.0 g, 71.3 mmol) in THF (80 ml) was treated with a 1.6 M solution of *n*-BuLi in hexane (58 ml, 92.8 mmol) dropwise at 0 °C for 20 min. After 30 min, paraformaldehyde (6.4 g) was added to the reaction mixture at 0 °C. After 5 h, the reaction mixture was quenched with aqueous NH_4Cl at 0 °C, diluted with EtOAc (200 ml), and washed with aq NH_4Cl and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified with 20% EtOAc in hexanes by flash chromatography to give 8.15 g of compound **3** (44.2 mmol, 62% yield); ^{1}H NMR ($CDCl_3$) δ 4.63 (t, J = 3.4 Hz, 1H), 4.22 (t, J = 2.1 Hz, 2H), 3.91–3.76 (m, 2H), 3.59–3.46 (m, 2H), 2.51 (tt, J = 7.1, 2.2 Hz, 2H), 1.86–1.46 (m, 6H).

4.4. Synthesis of *tert*-butyl-diphenyl-[5-(tetrahydro-pyran-2-yloxy)-pent-2-ynyloxy]-silane (4)

A solution of compound **3** and imidazole in CH_2Cl_2 was treated with TBDPSCl dropwise at 0 °C for 2 h. The reaction mixture was diluted with CH_2Cl_2 (300 ml) and washed with water (200 ml). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified with 10% EtOAc in hexanes by flash chromatography to give 18.0 g of compound **4** (42.6 mmol, 96% yield); ^{1}H NMR ($CDCl_3$) δ 7.80–7.64 (m, 4H), 7.49–7.34 (m, 6H), 4.63 (t, J = 3.6 Hz, 1H), 4.31 (t, J = 1.9 Hz, 2H), 3.93–3.83 (m, 1H), 3.77 (dt, J = 9.7, 7.1 Hz, 1H), 3.55–3.44 (m, 2H), 2.48 (tt, J = 7.1, 2.1 Hz, 2H), 1.89–1.46 (m, 6H), 1.06 (s, 9H).

4.5. Synthesis of 5-(*tert*-butyl-diphenyl-silanyloxy)-pent-3-yn-1-ol (5)

A solution of compound **4** (6.12 g, 14.5 mmol) in MeOH (100 ml) was treated with PPTS (0.36 g, 1.43 mmol) at room temperature overnight. After concentration, the residue was purified by flash chromatography with 20% EtOAc in hexanes to give 3.84 g of product **5** (11.3 mmol, 78%); ^{1}H NMR ($CDCl_3$) δ 7.80–7.66 (m, 4H), 7.49–7.35 (m, 6H), 4.33 (t, J = 1.9 Hz, 1H), 3.60 (t, J = 6.0 Hz, 2H), 2.40 (tt, J = 2.2, 6.1 Hz, 2H) 1.05 (s, 9H).

4.6. Synthesis of (5-bromo-pent-2-ynyloxy)-*tert*-butyl-diphenyl-silane (6)

A solution of compound **5** (1.09 g, 3.22 mmol) and CBr_4 (1.28 g, 3.86 mmol) in CH_2Cl_2 (70 ml) was treated with a solution of Ph_3P (1.27 g, 4.84 mmol) in CH_2Cl_2 (30 ml) dropwise at −78 °C. After 30 min, the reaction mixture was slowly warmed up to room temperature for 2 h and then stirred for overnight. The reaction mixture was filtered through a silica gel pad. The filtrate was concentrated to dryness. After concentration, the residue was purified with 0–2% EtOAc in hexanes by flash chromatography to give 1.28 g of product **6** (3.19 mmol, 99% yield); ^{1}H NMR ($CDCl_3$) δ 7.80–7.65 (m, 4H), 7.50–7.32 (m, 6H), 4.34 (t, J = 1.9 Hz, 1H), 3.35 (t, J = 5.8 Hz, 2H), 2.73 (tt, J = 2.1, 6.0 Hz, 2H) 1.08 (s, 9H).

4.7. Synthesis of [5-(*tert*-butyl-diphenyl-silanyloxy)-pent-3-ynyl]-phosphonic acid diethyl ester (7)

A mixture of compound **6** (3.24 g, 8.07 mmol) and triethyl phosphite (40 ml) was refluxed under nitrogen atmosphere overnight. After evaporation, the residue was purified with 50% EtOAc in hexanes by flash chromatography to give 3.26 g of product **7** (7.90 mmol, 98% yield); ^{1}H NMR ($CDCl_3$) δ 7.77–7.64 (m, 4H), 7.46–7.32 (m, 6H), 4.29 (t, J = 1.9 Hz, 2H), 4.16–4.02 (m, 4H), 2.50–2.34 (m, 2H), 1.96–1.82 (m, 2H), 1.31 (t, J = 7.1 Hz, 6H), 1.04 (s, 9H); ^{31}P NMR ($CDCl_3$) δ 30.43.

4.8. Synthesis of [5-(*tert*-butyl-diphenyl-silanyloxy)-pent-3-enyl]-phosphonic acid diethyl ester (8)

A mixture of compound **7** (5.00 g, 10.9 mmol) and Lindlar's catalyst (5% palladium on calcium carbonate poisoned with lead) in MeOH was treated with H_2 using a balloon. After overnight, the reaction mixture was filtered and concentrated to dryness to give 2.50 g of product **8** (5.5 mmol, 50% yield); ^{1}H NMR (MeOH-d_4) 7.73–7.56 (m, 4H), 7.44–7.32 (m, 6H), 5.68–5.57 (m, 1H), 5.45–5.34 (m, 1H), 4.25 (d, J = 6.0 Hz, 2H), 4.08–3.97 (m, 4H), 2.24–2.10 (m, 2H), 1.74–1.61 (m, 2H), 1.26 (t, J = 7.1 Hz, 6H), 1.03 (s, 9H); ^{31}P NMR (MeOH-d_4) δ 32.17.

4.9. Synthesis of (5-hydroxy-pent-3-enyl)-phosphonic acid diethyl ester (9)

A solution of compound **8** (0.67 g, 1.46 mmol) in acetonitrile (20 ml) was treated with a 1.0 M solution of TBAF in THF (1.7 ml) at 0 °C. After 1 h, the reaction mixture was concentrated and purified with 5% MeOH in CH_2Cl_2 by flash chromatography to give 0.32 g of product **9** (1.44 mmol, 99% yield); ^{1}H NMR ($CDCl_3$) 5.66–5.47 (m, 2H), 4.16–4.02 (m, 6H), 2.43–2.28 (m, 2H), 1.94–1.79 (m, 2H), 1.32 (t, J = 7.1 Hz, 6H); ^{31}P NMR ($CDCl_3$) δ 33.47.

4.10. Synthesis of 9-(5-phosphono-pent-2-en-1-yl)-adenine diethyl phosphonoester (10)

A solution of adenine (0.49 g, 3.6 mmol) in DMF was added to a flask containing compound **9** (0.32 g, 1.4 mmol). The resulting mixture was treated with Ph_3P (0.94 g, 3.6 mmol) and DIAD (0.70 ml, 3.6 mmol)

successively at 0 °C. After overnight, the mixture was concentrated and the residue was purified with 5–10% MeOH in CH_2Cl_2 by flash chromatography to give 0.20 g of product **10** (0.589 mmol, 42% yield); ^{1}H NMR ($CDCl_3$) δ 8.36 (s, 1H), 7.86 (s, 1H), 5.83 (br s, 2H), 5.82–5.61 (m, 2H), 4.86 (d, J = 6.6 Hz, 2H), 4.18–4.02 (m, 4H), 2.63–2.49 (m, 2H), 1.88 (dt, J = 17.9, 7.4 Hz, 2H), 1.32 (t, J = 6.9 Hz, 6H); ^{31}P NMR ($CDCl_3$) δ 31.71; MS (ESI) m/z 340 $(M+H)^+$.

4.11. Synthesis of 2-amino-6-chloro-9-(5-phosphono-pent-2-en-1-yl)-purine diethyl phosphonoester (11)

A solution of 2-amino-6-chloropurine (0.61 g, 3.6 mmol) in DMF was added to a flask containing compound **9** (0.32 g, 1.4 mmol). The resulting mixture was treated with Ph_3P (0.94 g, 3.6 mmol) and DIAD (0.70 ml, 3.6 mmol) successively at 0 °C. After overnight, the mixture was concentrated and the residue was purified with 5–10% MeOH in CH_2Cl_2 by flash chromatography to give 0.33 g of product **11** (0.92 mmol, 66 % yield) ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 1H), 5.75 (br s, 2H), 5.72–5.48 (m, 2H), 4.67 (d, J = 6.1 Hz, 2H), 4.17–4.01 (m, 4H), 2.74–2.56 (m, 2H), 1.94–1.78 (m, 2H), 1.30 (t, J = 7.1 Hz, 6H); ^{31}P NMR ($CDCl_3$) δ 32.37; MS (ESI) m/z 374 $(M+H)^+$, 372 $(M-H)^-$.

4.12. Synthesis of 9-(5-phosphono-pent-2-en-1-yl)-guanine diethyl phosphonoester (12)

A solution of compound **11** (0.200 g, 0.53 mmol) in 30 ml of 88% HCO_2H was stirred at 100 °C for 8 h. After concentration, the residue was purified with 10% MeOH in CH_2Cl_2 by flash chromatography to give 0.170 g of product **12** (0.478 mmol, 89% yield); ^{1}H NMR (MeOH-d_4) δ 8.97 (s, 1H), 5.90–5.77 (m, 1H), 5.75–5.63 (m, 1H), 4.89 (d, J = 7.1 Hz, 1H), 4.17–4.04 (m, 4H), 2.70–2.50 (m, 2H), 2.06–1.87 (m, 2H), 1.33 (t, J = 6.9 Hz, 6H); ^{31}P NMR ($CDCl_3$) δ 33.48; MS (ESI) m/z 356 $(M+H)^+$, 354 $(M-H)^-$.

4.13. Synthesis of 9-(5-phosphono-pent-2-en-1-yl)-adenine (13, PPen-A)

A solution of compound **10** (0.300 g, 0.884 mmol) in acetonitrile (10 ml) was treated with TMSBr (5 ml) at room temperature overnight. After concentration, the residue was dissolved in water (20 ml) and the resulting mixture was stirred for 1 h. The reaction mixture was concentrated to dryness. The residue was dissolved in water (4 ml) and adjusted to ca. pH 8. The resulting mixture was loaded to a column containing DOWEX-1X2 resin and purified with gradient eluent (0–0.25 M HCO_2H) to give 0.180 g of product **13** (0.636 mmol, 72% yield); ^{1}H NMR (MeOH-d_4) δ 8.20 (s, 1H), 8.13 (s, 1H), 5.74–5.62 (m, 1H), 5.53–5.41 (m, 1H), 4.78 (d, J = 6.9 Hz, 1H), 2.33–2.19 (m, 2H), 1.57–1.43 (m, 2H); ^{31}P NMR (DMSO-d_6) δ 26.31; MS (ESI) m/z 284 $(M+H)^+$, 282 $(M-H)^-$.

4.14. Synthesis of 2-amino-6-chloro-9-(5-phosphono-pent-2-en-1-yl)-purine (14)

See the procedure for the preparation of compound **13**. Compound **14** was obtained on 0.576 mmol-scale. Without further purification, compound **14** was used for the next reaction; ^{1}H NMR (MeOH-d_4) δ 9.09 (s, 1H), 5.93–5.81 (m, 1H), 5.78–5.63 (m, 1H), 4.91 (d, J = 7.4 Hz, 1H), 2.72–2.54 (m, 2H), 1.96–1.81 (m, 2H); ^{31}P NMR (MeOH-d_4) δ 30.23.

4.15. Synthesis of 9-(5-phosphono-pent-2-en-1-yl)-guanine (15, PPen-G)

See the procedure for the preparation of compound **13**. Compound **15** was obtained on 0.563 mmol-scale in 95% yield; ^{1}H NMR (MeOH-d_4) δ 8.71 (s, 1H), 5.84–5.73 (m, 1H), 5.54–5.48 (m, 1H), 4.74 (d, J = 6.9 Hz, 1H), 2.44–2.29 (m, 2H), 1.79–1.65 (m, 2H); ^{31}P NMR (MeOH-d_4) δ 29.97; MS (ESI) m/z 300 $(M+H)^+$, 298 $(M-H)^-$.

4.16. Synthesis of 9-(5-phosphono-pent-2-en-1-yl)-adenine mono-(3-hexadecyloxy-1-propyl) phosphonoester (16)

A solution of compound **13** (0.120 g, 0.424 mmol), 3-hexadecyloxy-propan-1-ol (HDP-OH) (0.191 g, 0.64 mmol), and DMAP (0.078 g, 0.64 mmol) in DMF (10 ml) was treated with DCC (0.262 g, 1.26 mmol) at room temperature. The reaction mixture was warmed up to 80 °C and stirred overnight. After concentration, the residue was purified with a gradient mixture of chloroform, methanol, con NH_4OH, and water (100:40:3:3 to 80:20:1:1) by flash chromatography to give 0.065 g of product **16** (0.115 mmol, 27% yield); ^{1}H NMR (MeOH-d_4) δ 8.21 (s, 1H), 8.19 (s, 1H) 5.84–5.74 (m, 1H), 5.65–5.57 (m, 1H), 4.92 (d, J = 7.0 Hz, 2H), 3.94 (q, J = 6.2 Hz, 2H), 3.52 (t, J = 6.2 Hz, 2H), 3.37 (t, J = 6.6 Hz, 2H), 2.58–2.44 (m, 2H), 1.90–1.78 (m, 2H), 1.74–1.62 (m, 2H), 1.54–1.43 (m, 2H), 1.36–1.14 (m, 12H), 0.89 (t, J = 7.0 Hz, 3H); ^{31}P NMR (MeOH-d_4) δ 25.89; MS (ESI) m/z 566 $(M+H)^+$, 564 $(M-H)^-$.

4.17. Synthesis of 2-amino-6-chloro-9-(5-phosphono-pent-2-en-1-yl)-purine mono-(3-hexadecyloxy-1-propyl) phosphonoester (17)

See the procedure for the preparation of compound **16**. Compound **17** was obtained on 0.598 mmol-scale in 56% yield; ^{1}H NMR (MeOH-d_4) δ 7.79 (s, 1H) 5.76–5.68 (m, 1H), 5.57–5.49 (m, 1H), 4.72 (d, J = 7.3 Hz, 2H), 3.95 (q, J = 6.6 Hz, 2H), 3.53 (t, J = 6.2 Hz, 2H), 3.39 (t, J = 6.6 Hz, 2H), 2.62–2.52 (m, 2H), 1.90–1.82 (m, 2H), 1.74–1.64 (m, 2H), 1.56–1.46 (m, 2H), 1.36–1.12 (m, 12H), 0.89 (t, J = 6.6 Hz, 3H); ^{31}P NMR (MeOH-d_4) δ 26.46.

4.18. Synthesis of 9-(5-phosphono-pent-2-en-1-yl)-guanine mono-(3-hexadecyloxy-1-propyl) phosphonoester (18, HDP-PPen-G)

A solution of compound **17** (0.200 g, 0.333 mmol) in 88% HCO_2H (40 ml) was stirred at 100 °C overnight. After concentration, the residue was purified with a mixture of chloroform, methanol, con NH_4OH, and water (80:20:1:1 to 70:58:8:8) to give 0.120 g of product **18** (0.206 mmol, 62% yield); ^{1}H NMR (MeOH-d_4) δ 7.75 (s, 1H) 5.76–5.66 (m, 1H), 5.56–5.48 (m, 1H), 4.71 (d, J = 7.3 Hz, 2H), 3.94 (q, J = 5.9 Hz, 2H), 3.53 (t, J = 6.6 Hz, 2H), 3.39 (t,

J = 6.6 Hz, 2H), 2.62–2.52 (m, 2H), 1.90–1.82 (m, 2H), 1.72–1.62 (m, 2H), 1.54–1.47 (m, 2H), 1.34–1.22 (m, 12H), 0.89 (t, J = 7.0 Hz, 3H); ^{31}P NMR (MeOH-d_4) δ 26.14; MS (ESI) m/z 582 $(M+H)^+$, 580 $(M-H)^-$.

4.19. Synthesis of 1-(5-phosphono-pent-2-en-1-yl)-uracil diethyl phosphonoester (19)

A solution of compound **9** (0.20 g, 0.90 mmol), 3-benzoyl-uracil (0.24 g, 1.1 mmol), and Ph_3P (0.29 g, 1.1 mmol) in DMF was treated with DIAD (0.21 ml, 1.1 mmol) dropwise at 0 °C. After 2 h, the reaction mixture was concentrated and purified with 2% MeOH in CH_2Cl_2 by flash chromatography. The benzoyl-protected intermediate was dissolved in methanolic ammonia (50 ml) and stirred overnight. The resulting mixture was concentrated and purified with 5% MeOH in CH_2Cl_2 by silica gel column chromatography to give 0.23 g of product **19** (0.73 mmol, 81% yield); ^{1}H NMR ($CDCl_3$) δ 8.66 (br s, 1H), 7.31 (d, J = 8.1 Hz, 1H), 5.76–5.66 (m, 1H), 5.69 (dd, J = 7.7, 2.0 Hz, 1H), 4.41 (d, J = 7.0 Hz, 2H), 4.16–4.02 (m, 4H), 2.53–2.42 (m, 2H), 1.89–1.80 (m, 2H), 1.32 (t, J = 7.0 Hz, 3H); ^{31}P NMR ($CDCl_3$) δ 31.80; MS (ESI) m/z 317 $(M+H)^+$, 315 $(M-H)^-$.

4.20. Synthesis of 1-(5-phosphono-pent-2-en-1-yl)-cytosine diethyl phosphonoester (20)

A solution of compound **19** (0.73 g, 2.3 mmol), TEA (0.97 ml, 7.0 mmol), and DMAP (0.28 g, 2.3 mmol) in acetonitrile was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (2.1 g, 6.9 mmol) at room temperature for 3 h. Concentrated NH_4OH (5 ml) was added to the reaction mixture. The resulting mixture was stirred for 1 h. After concentration, the residue was purified with a mixture of chloroform, methanol, con NH_4OH, and water (240:20:1:1) by flash chromatography to give 0.55 g of product **20** (1.74 mmol, 75% yield); ^{1}H NMR ($CDCl_3$) δ 7.34 (d, J = 7.1 Hz, 1H), 5.80 (d, J = 7.4 Hz, 1H), 5.72–5.61 (m, 1H), 5.56–5.46 (m, 1H), 4.44 (d, J = 6.9 Hz, 2H), 4.16–4.02 (m, 4H), 2.54–2.39 (m, 2H), 2.17 (br s, 2H), 1.91–1.78 (m, 2H), 1.32 (t, J = 7.1 Hz, 6H); ^{31}P NMR ($CDCl_3$) δ 32.06; MS (ESI) m/z 316 $(M+H)^+$, 314 $(M-H)^-$.

4.21. Synthesis of 1-(5-phosphono-pent-2-en-1-yl)-thymine diethyl phosphonoester (21)

See the procedure for the preparation of compound **19**. Compound **21** was obtained on 1.44 mmol-scale in 78% yield; ^{1}H NMR ($CDCl_3$) δ 8.46 (br s, 1H), 7.11 (s, 1H), 5.78–5.66 (m, 1H), 5.52–5.41 (m, 1H), 4.38 (d, J = 6.6 Hz, 2H), 4.20–4.02 (m, 4H), 2.55–2.41 (m, 2H), 1.91 (s, 3H), 1.91–1.78 (m, 2H), 1.33 (t, J = 7.1 Hz, 6H); ^{31}P NMR ($CDCl_3$) δ 31.84; HRMS (ESI) obsd, m/z 330.1352, calcd for $C_{14}H_{23}N_2O_5P$, m/z 330.1339 M^+.

4.22. Synthesis of 1-(5-phosphono-pent-2-en-1-yl)-uracil (22, PPen-U)

See the procedure for the preparation of compound **13**. Compound **22** was obtained on 1.26 mmol-scale in 98% yield; ^{1}H NMR (DMSO-d_6) δ 11.23 (s, 1H), 7.69 (d, J = 7.6 Hz, 1H), 5.68–5.57 (m, 1H), 5.52 (d, J = 6.7 Hz, 1H), 5.39–5.18 (m, 1H), 4.28 (d, J = 6.6 Hz, 2H), 2.36–2.22 (m, 2H), 1.64–1.51 (m, 2H); ^{31}P NMR (DMSO-d_6) δ 26.38; MS (ESI) m/z 261 $(M+H)^+$, 259 $(M-H)^-$.

4.23. Synthesis of 1-(5-phosphono-pent-2-en-1-yl)-cytosine (23, PPen-C)

See the procedure for the preparation of compound **13**. Compound **23** was obtained on 1.30 mmol-scale in 71% yield; ^{1}H NMR (DMSO-d_6) δ 7.52 (d, J = 7.1 Hz, 1H), 7.04 (br s, 1H), 6.98 (br s, 1H), 5.62 (d, J = 7.7 Hz, 1H), 5.66–5.53 (m, 1H), 5.37–5.26 (m, 1H), 4.26 (d, J = 6.9 Hz, 2H), 2.37–2.23 (m, 2H), 1.63–1.50 (m, 2H); ^{31}P NMR (DMSO-d_6) δ 26.14; MS (ESI) m/z 260 $(M+H)^+$, 258 $(M-H)^-$.

4.24. Synthesis of 1-(5-phosphono-pent-2-en-1-yl)-thymine (24, PPen-T)

See the procedure for the preparation of compound **13**. Compound **24** was obtained on 1.12 mmol-scale in 85% yield; ^{1}H NMR (MeOH-d_4) δ 7.41 (s 1H), 5.80–5.65 (m, 1H), 5.53–5.41 (m, 1H), 4.39 (d, J = 5.5 Hz, 2H), 2.56–2.40 (m, 2H), 1.85 (s, 3H), 1.85–1.74 (m, 2H); ^{31}P NMR (MeOH-d_4) δ 30.40; HRMS (ESI) obsd, m/z 274.0727, calcd for $C_{10}H_{15}N_2O_5P$, m/z 274.0713 M^+.

4.25. Synthesis of 1-(5-phosphono-pent-2-en-1-yl)-uracil mono-(3-hexadecyloxy-1-propyl) phosphonoester (25, HDP-PPen-U)

See the procedure for the preparation of compound **16**. Compound **25** was obtained on 0.50 mmol-scale in 44% yield; ^{1}H NMR (MeOH-d_4) δ 7.62 (d, J = 8.0 Hz, 1H), 5.82–5.71 (m, 1H), 5.65 (d, J = 8.0 Hz, 1H), 5.50–5.37 (m, 1H), 4.44 (d, J = 5.8 Hz, 2H), 3.93 (q, J = 6.3 Hz, 2H), 3.53 (t, J = 6.3 Hz, 2H), 3.41 (t, J = 6.6 Hz, 2H), 2.50–2.36 (m, 2H), 1.90–1.81 (m, 2H), 1.70–1.48 (m, 4H), 1.40–1.20 (m, 12H), 0.89 (t, J = 6.2 Hz, 3H); ^{31}P NMR (MeOH-d_4) δ 25.90; MS (ESI) m/z 543 $(M+H)^+$, 541 $(M-H)^-$.

4.26. Synthesis of 1-(5-phosphono-pent-2-en-1-yl)-cytosine mono-(3-hexadecyloxy-1-propyl) phosphonoester (26, HDP-PPen-C)

See the procedure for the preparation of compound **16**. Compound **17** was obtained on 0.58 mmol-scale in 38% yield; ^{1}H NMR (MeOH-d_4) δ 7.86 (d, J = 7.4 Hz, 1H), 5.96 (d, J = 7.6 Hz, 1H), 5.84–5.72 (m, 1H), 5.52–5.51 (m, 1H), 4.50 (d, J = 6.6 Hz, 2H), 3.92 (q, J = 6.3 Hz, 2H), 3.53 (t, J = 6.0 Hz, 2H), 3.41 (t, J = 6.6 Hz, 2H), 2.49–2.35 (m, 2H), 1.91–1.80 (m, 2H), 1.70–1.45 (m, 4H), 1.36–1.22 (m, 12H), 0.89 (t, J = 6.6 Hz, 3H); ^{31}P NMR (MeOH-d_4) δ 25.93; HRMS (ESI) obsd, m/z 541.3645, calcd for $C_{28}H_{52}N_3O_5P$, m/z 541.3639 M^+.

4.27. Synthesis of 1-(5-phosphono-pent-2-en-1-yl)-thymine mono-(3-hexadecyloxy-1-propyl) phosphonoester (27, HDP-PPen-T)

See the procedure for the preparation of compound **16**. Compound **17** was obtained on 0.58 mmol-scale in 15%

yield; ^{1}H NMR (MeOH-d_4) δ 7.44 (d, J = 1.1 Hz, 1H), 5.80–5.69 (m, 1H), 5.47–5.36 (m, 1H), 4.42 (d, J = 6.9 Hz, 2H), 3.92 (q, J = 6.3 Hz, 2H), 3.53 (t, J = 6.3 Hz, 2H), 3.41 (t, J = 6.6 Hz, 2H), 2.50–2.36 (m, 2H), 1.91–1.81 (m, 2H), 1.87 (d, J = 1.1 Hz, 3H), 1.70–1.48 (m, 4H), 1.35–1.24 (m, 12H), 0.89 (t, J = 6.6 Hz, 3H); ^{31}P NMR (MeOH-d_4) δ 25.62; HRMS (ESI) obsd, m/z 556.3643, calcd for $C_{29}H_{53}N_2O_6P$, m/z 556.3636 M^+.

4.28. Molecular modeling

All molecular modeling of the enzyme-ligand complexes was carried out on a Linux enterprise operation system using SYBYL 7.2 software packages (Tripos Inc. St Louis, Mo.). The crystal structure of herpes polymerase was downloaded from the protein data bank (PDB ID: 2GV9), and a model of the replication complex of HSV POL which includes template, primer, and inhibitor was constructed by following the protocol reported by Liu et al.[14] Thus, individual domains of HSV POL were superimposed onto those of the replication complex of RB69 POL (PDB ID: 1IG9).[16] The palm domain of the replicating RB69 POL complex was chosen as the reference, and the HSV POL domains were individually superimposed onto the RB69 POL structure. The key active site residues of RB69 POL (Asp411, Leu412, Leu415, Tyr416, Arg482, Lys486, Lys560, Asn564, Tyr567, Asp623, and Ser624) were in good match with the corresponding residues in HSV POL (Asp717, Phe718, Leu721, Tyr722, Arg785, Arg789, Asn815, Tyr818, Asp888, and Ser889, respectively). Template, primer, active site calcium ions, and thymidine-5′-triphosphate (TTP) of the RB69 POL were merged into the HSV POL to provide a model of catalytically active HSV POL structure. Crystallographic structure of TTP bound at the active site was modified to construct PPen-T, which was energy-minimized to give the stable conformation inside the active site of HSV POL. The resulting HSV POL:PPen-T complex was used for investigation of the possible binding mode of PPen-T to the active site of HSV POL.

4.29. DNA reduction antiviral assays for activity against HSV-1 in vitro

Antiviral activities of the compounds were determined against HSV-1 by DNA reduction with MRC-5 human lung fibroblast cells using HSV-1 DNA probes as described previously.[17] Briefly, subconfluent MRC-5 cells in 24-well culture plates were inoculated by removing the medium and adding HSV-1 at a dilution that causes 3–4+ cytopathic effect in a nondrug well in 24 h. The virus was absorbed for 1 h at 37 °C, aspirated, and replaced with various concentrations of drugs as indicated in Eagles MEM containing 2% FBS. After 20–24 h, HSV DNA was quantified in triplicate by nucleic acid hybridization using an HSV antiviral susceptibility kit from Diagnostic Hybrids (Athens, Ohio) according to the manufacturer's instructions. Results are expressed as a percentage of the untreated, HSV-infected controls.

4.30. Neutral red uptake assay for cytotoxicity

MRC-5 cells were seeded into 96-well tissue culture plates at 2.5×10^4 cells/well. After 24-h incubation, media were replaced with MEM containing 2% FBS, drug was added to the first row, and then diluted serially fivefold from 100 μM to 0.03 μM. The plates were incubated for 7 days and cells stained with neutral red and incubated for 1 h. Plates were shaken on a plate shaker for 15 min. and neutral red was solubilized with 1% glacial acetic acid/50% ethanol. The optical density was read at 540 nm. The concentration of drug that reduced cell viability by 50% (CC_{50}) was calculated using computer software. Since the cells were plated sparse and are dividing during the 7 day period, the results reflect both the antiproliferative and cytotoxicity of the compounds.

Acknowledgments

The studies were supported in part by NIH Grants AI-66499, and AI-64615 from National Institute of Allergy and Infectious Disease and EY-11832 and EY-07366 from the National Eye Institute.

References and notes

1. Roizman, B.; Pellett, P. E.. In *Fields' Virology*; Knipe, D. M., Howley, P. M., Eds.; Lippincott Williams and Wilkins: Philadelphia, PA, 2001; Vol. 2, p 2381.
2. (a) Spear, P. G. *Cell. Microbiol.* **2004**, *6*, 401; (b) Spear, P. G.; Longnecker, R. *J. Virol.* **2003**, *77*, 10179.
3. (a) Elion, G. B.; Furman, P. A.; Fyfe, J. A.; De Miranda, P.; Beauchamp, L.; Schaeffer, H. J. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5716; (b) Schaeffer, H. J.; Beauchamp, L.; De Miranda, P.; Elion, G. B.; Buaer, D. J.; Collins, P. *Nature* **1978**, *272*, 583; (c) Furman, P. A.; Miranda, P.; De Clair, M. H. S.; Elion, G. B. *Antimicrob. Agents Chemother.* **1981**, *20*, 518.
4. De Clercq, E. *J. Clin. Virol.* **2004**, *30*, 115–133.
5. (a) Harnden, M. R.; Jarvest, L. R.; Bacon, T. H.; Boyd, M. R. *J. Med. Chem.* **1987**, *30*, 1636; (b) Tippie, M. A.; Martin, J. C.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *Nucleosides Nucleotides* **1984**, *3*, 525.
6. Bae, P. K.; Kim, J. H.; Kim, H. S.; Chung, I. K.; Paik, S. G.; Lee, C.-K. *Antiviral Res.* **2006**, *70*, 93.
7. Kimberlin, D. W. *Pediatr. Clin. North Am.* **2005**, *52*, 837.
8. De Clerq, E. *J. Med. Chem.* **2005**, *48*, 1297.
9. Aldern, K. A.; Ciesla, S. L.; Winegarden, K. L.; Hostetler, K. Y. *Mol. Pharmacol.* **2003**, *63*, 678.
10. Ciesla, S. L.; Trahan, J.; Wan, W. B.; Beadle, J. R.; Aldern, K. A.; Painter, G. R.; Hostetler, K. Y. *Antiviral Res.* **2003**, *59*, 163.
11. (a) Beadle, J. R.; Rodriquez, N.; Aldern, K. A.; Hartline, C.; Harden, E.; Kern, E. R.; Hostetler, K. Y. *Antimicrob. Agents Chemother.* **2002**, *46*, 2381; (b) Wan, W. B.; Beadle, J. R.; Hartline, C. B.; Kern, E. R.; Ciesla, S. L.; Valiaeva, N.; Hostetler, K. Y. *Antimicrob. Agents Chemother.* **2005**, *49*, 656.
12. (a) Quenelle, D. C.; Collins, D. J.; Wan, W. B.; Beadle, J. R.; Hostetler, K. Y.; Kern, E. R. *Antimicrob. Agents Chemother.* **2004**, *48*, 404; (b) Kern, E. R.; Collins, D. J.; Wan, W. B.; Beadle, J. R.; Hostetler, K. Y.; Quenelle, D. C. *Antimicrob. Agents Chemother.* **2004**, *48*, 3516; (c) Buller, R. M.; Owens, G.; Schriewer, J.;

Melman, L.; Beadle, J. R.; Hostetler, K. Y. *Virology* **2004**, *318*, 474.
13. Lindlar, H. *Helv. Chim. Acta* **1952**, *35*, 446.
14. Liu, S.; Knafels, J. D.; Chang, J. S.; Waszak, G. A.; Baldwin, E. T.; Deibel, M. R., Jr.; Thomsen, D. R.; Homa, F. L.; Wells, P. A.; Tory, M. C.; Poorman, R. A.; Gao, H.; Qiu, X.; Seddon, A. P. *J. Biol. Chem.* **2006**, *281*, 18193.
15. Chong, Y.; Choo, H.; Choi, Y.; Mathew, J.; Schinazi, R. F.; Chu, C. K. *J. Med. Chem.* **2002**, *45*, 4888.
16. Franklin, M.; Wang, J.; Steitz, T. A. *Cell* **2001**, *105*, 657.
17. Hostetler, K. Y.; Kini, G. D.; Beadle, J. R.; Aldern, K. A.; Gardner, M. F.; Border, R.; Barshak, L.; Kumar, R.; Sridhar, C. N.; Wheeler, C. J.; Richman, D. D. *Antiviral Res.* **1996**, *31*, 59.