Synthesis and Antiviral Activity of Some New S-Adenosyl-L-homocysteine Derivatives

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A series of new S-adenosyl-L-homocysteine (AdoHcy) analogues with modifications to amino acid and nucleoside moieties was prepared via condensation of appropriate nucleoside precursors and suitably protected L-homocystine derivatives.The AdoHcy derivatives as well as the nucleoside precursors were evaluated for their antiviral activity. Some of the compounds, in particular S-tubercidinyl-L-homocysteine propyl ester (**36**), N-(trifluoroacetyl)-S-tubercidinyl-L-homocysteine isopropyl ester (**27**), S-3'-deoxytubercidinyl-L-homocysteine (**58**), N-(trifluoroacetyl)-S-tubercidinyl-L-homocysteine propyl ester (**26**), and N-(methoxyacetyl)-S-tubercidinyl-L-homocysteine ethyl ester (**31**) showed potent and selective activity against HSV, VV, and VSV.It is likely that they exert their antiviral effect via selective inhibition of the methyltransferases which are required for the maturation of viral mRNAs.

Introduction

S-Adenosyl-L-homocysteine (AdoHcy) markedly inhibits S-adenosylmethionine (AdoMet)-dependent transmethylations.[1] It is rapidly hydrolyzed and metabolized by cellular enzymes such as S-adenosyl-L-homocysteine hydrolase, adenosine deaminase, and purine nucleoside phosphorylase.[2] In addition, owing to a zwitterionic nature of the amino acid moiety, AdoHcy and its analogues hitherto reported are poorly transported across the cell membrane.[3]

There is evidence that the 2'- and 3'-hydroxyl functions of the pentose moiety as well as the exocyclic amino group of the heterocyclic base are crucial for binding of AdoHcy to several methyltransferases.[4,5] Replacement of these groups by other substituents may therefore alter the compounds' activity spectrum against their target enzymes and result in more selective antiviral (or cytotoxic) action.

Modifications to the heterocyclic base such as isosteric replacement of N-3, N-7, or N-9 nitrogens with "CH" render the compounds resistant to catabolism by cellular enzymes.[2] With the aim of developing a metabolically stable analogue with selective antiviral activity, we prepared a series of new AdoHcy analogues containing different heterocyclic bases such as pyrrolo[2,3-*d*]pyrimidine, imidazo[4,5-*c*]pyridine, and pyrazolo[4,3-*d*]pyrimidine as well as a modified pentose moiety in which the 3'-hydroxyl function is replaced by hydrogen.

A new approach to the synthesis of AdoHcy analogues based on the condensation of an unprotected nucleoside with a suitably protected L-homocystine[6,7] enabled preparation of novel derivatives having carboxyl and/or amino functions of the amino acid moiety protected with alkyl or acyl groups. In order to examine the potential of such compounds as prodrugs, which would be readily transported across the cell membrane and then converted intracellularly into their parent zwitterionic AdoHcy counterparts, we also prepared a series of new S-tubercidinyl-L-homocysteine (TubHcy) derivatives with carboxyl and/or amino functions protected with various alkyl or acyl groups. The parent TubHcy is the most potent and metabolically stable inhibitor of several methyltransferases described so far.[2,8]

Since the nucleoside precursors in the synthesis of the AdoHcy derivatives were potentially capable of exerting some antiviral or cytotoxic effect in their own right upon intracellular conversion to the corresponding AdoHcy derivatives or 5'-triphosphates, they were also evaluated for their antiviral activity.

Chemistry

Our structure–activity studies required the preparation of new S-tubercidinyl-L-homocysteine (TubHcy) analogues **19–42** having carboxy and/or amino functions of the amino acid moiety protected with various protecting groups R_1 and R_2, respectively (Table II). The purpose of the studies was to determine the effect of those protecting groups on compounds' antiviral activity. The synthesis of compounds **19–42** (Scheme II) required prior preparation of suitably protected L-homocystine derivatives **3–17**. The compounds **3–17** were prepared from L-homocystine (**1**) according to the route outlined in Scheme I. At first

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(6) Serafinowski, P. A convenient preparation of S-adenosylhomocysteine and its analogues. *Synthesis* **1985**, 926–928.

(7) Serafinowski, P. Synthesis of some S-3'-deoxyadenosyl-L-homocysteine analogues. *Nucleic Acids Res.* **1987**, *15*, 1121–1137.

(8) Borchardt, R. T. In *The Biochemistry of S-Adenosylmethionine*; Salvatore, F., Borek, E., Zappia, V., Williams-Ashmann, H. G., Schlenk, F., Eds., Columbia University Press: New York, 1977.

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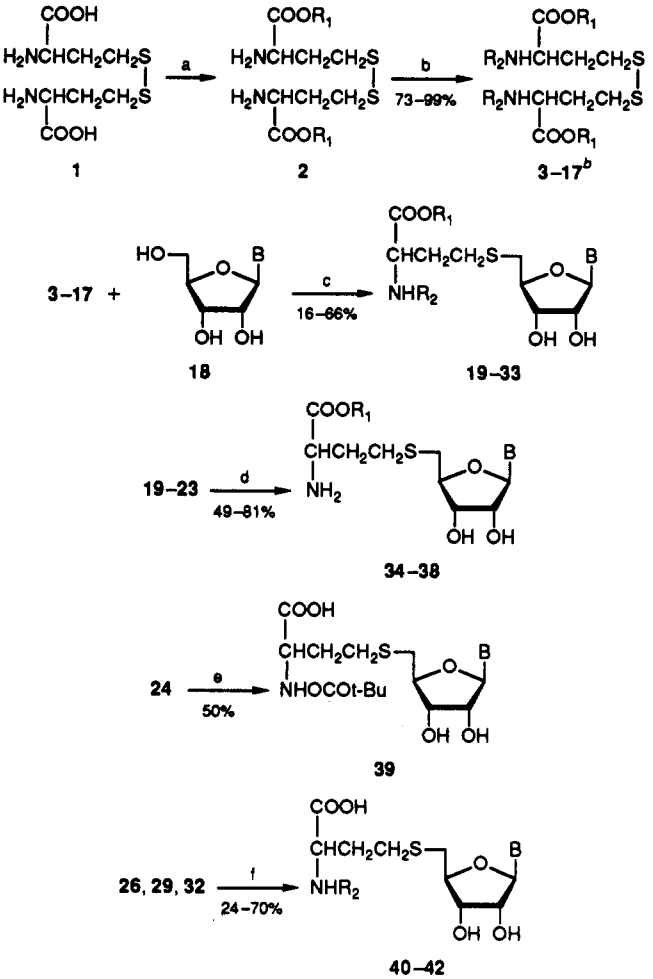
Scheme I [a]



B = 7-deazaadenin-9-yl

R_1 = CH_3, CH_2CH_3, $CH_2CH_2CH_3$, $CH(CH_3)_2$, $CH_2CH_2CH_2CH_3$, CH_2CH_2TMS[c]

R_2 = $OCOC(CH_3)_3$, CF_3CO, CH_3CO, CH_3OCH_2CO[c]

[a] (a) R_1OH/H^+ reflux, 3 h; (b)$(t\text{-}BuOCO)_2O/Na_2CO_3$, 18 h, rt or$(CF_3CO)_2O$, 1/2 h reflux or CH_3COCl/DMAP 2 h, room temperature or $(CH_3OCH_2CO)_2O$/DMAP, 2 h, room temperature; (c) $(Et)_3P$/pyridine room temperature, 48–120 h; (d) F_3CCOOH, 1 h, room temperature (e) 1 M $(t\text{-}Bu)_4NF$/THF, 15 min, room temperature (f) 0.25 M $Ba(OH)_2$ 50% aqueous MeOH, room temperature, 15 min.[b] Compound 8 was prepared by successive reactions of 1 with di-*tert*-butyl pyrocarbonate and (trimethylsilyl)ethanol/DCC (please see the Experimental Section).[c] Please see Tables I and II to find out which numbered structures correspond to which R_1 and R_2.

L-homocystine (1) was esterified with an excess of an appropriate alcohol in the presence of an acidic catalyst.[9,10] The esters 2, thus obtained, were subsequently acylated in a one-pot procedure with di-*tert*-butyl pyrocarbonate in aqueous sodium carbonate,[11] trifluoroacetic anhydride,[6] acetic anhydride in the presence of dimethylaminopyridine (DMAP), or methoxyacetyl chloride in the presence of DMAP.[12] The resulting amino acid precursors 3–17 were obtained in high yields as crystalline solids (Table I).

Subsequently the compounds 3–17 were condensed with tubercidin (18) in the presence of triethylphosphine[6,7] (Scheme I). The protected *S*-tubercidinyl-L-homocysteine derivatives 19–33 were obtained in good yields after column chromatography on silica gel (Table II). The structures of compounds 19–33 were established on the basis of their ^{1}H-NMR spectra which revealed the presence of signals deriving from both the amino acid and nucleoside moieties (see Experimental Section). The fact that the substitution had occurred at the 5′-position was confirmed by the absorption at 5.2–5.5 ppm which corresponds to the free 2′- and 3′-hydroxyl groups (see Experimental Section).

Some of the carboxyl or amino protecting groups R_1 or R_2, respectively, could be removed selectively from 19–33 so that TubHcy derivatives with a semiprotected amino acid moiety were also obtained. Thus, *tert*-butoxycarbonyl group was removed from 19–23 by the action of trifluoroacetic acid[13] to give hitherto unknown TubHcy esters 34–38 in good yields (Table II), after purification on a reverse-phase S10 C8 column or an anion-exchange Sephadex A-25 column. The compounds gave positive tests with ninhydrin which confirmed the presence of a free amino group. It was also possible to remove selectively the (trimethylsilyl)ethyl group from 24 with 1 M tetrabutylammonium fluoride in THF[13] to obtain a compound with a free carboxyl function, *N*-(*tert*-butoxycarbonyl)-*S*-tubercidinyl-L-homocysteine (39). It proved, however, difficult to prepare the (trimethylsilyl)ethyl ester of *N*,*N*′-bis(trifluoroacetyl)-L-homocystine and prepare *N*-(trifluoroacetyl)-*S*-tubercidinyl-L-homocysteine (40) in the manner similar to that described for 39. Instead, the compound 40 was obtained when *N*-(trifluoroacetyl)-*S*-tubercidinyl-L-homocysteine propyl ester (26) was allowed to react with 0.25 M barium hydroxide in 50% aqueous methanol[6] for 15 min. Although after that period considerable amounts of the starting material were still present in the reaction mixture and the product of full deprotection, *S*-tubercidinyl-L-homocysteine, was already formed, it was possible to isolate the required *N*-(trifluoroacetyl)-*S*-tubercidinyl-L-homocysteine (40) in 24% yield on a Sephadex A-25 column.

Similarly, *N*-acetyl- and *N*-(methoxyacetyl)-*S*-tubercidinyl-L-homocysteine propyl esters 29 and 32, were partially deprotected with 0.25 M barium hydroxide to give *N*-acetyl- and *N*-(methoxyacetyl)-*S*-tubercidinyl-L-homocysteine 41 and 42 in considerably higher yields, 70 and 65%, respectively.

N-1-[(Pivaloyloxy)methyl]formycin A (43) and its 3′-deoxy counterpart 44 were prepared in high yields by reaction of formycin A or its 3′-deoxy analogue with (pivaloyloxy)methyl chloride in DMF in the presence of potassium carbonate (Scheme II).[15]

Syntheses of 3′-deoxyadenosine (45), 3′-deoxytubercidin (46), 3′-deoxy-3-deazaadenosine (47), 2′-deoxy-3-deazaadenosine (48), 3′-deoxyformycin A (49), 2′-deoxyformycin A (50), 2′,3′-dideoxy-3-deazaadenosine (51), 5′-chloro-3′,5′-dideoxyadenosine (52), 5′-chloro-5′-deoxyformycin A (53), 5′-chloro-3′,5′-dideoxyformycin A (54), *S*-formycinyl-L-homocysteine (55), *S*-(3′-deoxyformycinyl)-L-homocysteine (56), *S*-(3′-deoxyadenosyl)-L-homocysteine (57), *S*-(3′-

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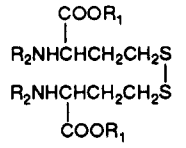
Table I. Yields and Physical Data of Intermediates 3–17



no.	R_1	R_2	% yield	mp °C (solvent)	formula[a]
3	CH_3	$C(O)OC(CH_3)_3$	82	indefinite (MeOH/H_2O)	$C_{20}H_{36}H_2O_8S_2$
4	CH_2CH_3	$C(O)OC(CH_3)_3$	73	123–124 (EtOH/H_2O)	$C_{22}H_{40}N_2O_8S_2$
5	$CH_2CH_2CH_3$	$C(O)OC(CH_3)_3$	89	106–107 (EtOH)	$C_{24}H_{44}N_2O_8S_2$
6	$CH(CH_3)_2$	$C(O)OC(CH_3)_3$	73	105–106 (EtOH)	$C_{24}H_{44}N_2O_8S_2$
7	$CH_2CH_2CH_2CH_3$	$C(O)OC(CH_3)_3$	90	93–94 (EtOH)	$C_{26}H_{48}N_2O_8S_2$
8	$CH_2CH_2Si(CH_3)_3$	$C(O)OC(CH_3)_3$	83	75–76 (EtOH)	$C_{28}H_{56}N_2O_8S_2Si_2$
9	CH_2CH_3	$C(O)CF_3$	91	87–88 (EtOH)	$C_{16}H_{22}F_6N_2O_6S_2$
10	$CH_2CH_2CH_3$	$C(O)CF_3$	80	99 (PrOH)	$C_{18}H_{26}F_6N_2O_6S_2$
11	$CH(CH_3)_2$	$C(O)CF_3$	96	94 (iPrOH)	$C_{18}H_{26}F_6N_2O_6S_2$
12	CH_2CH_3	$C(O)CH_3$	78	92–95 (EtOAc)	$C_{16}H_{28}N_2O_6S_2$
13	$CH_2CH_2CH_3$	$C(O)CH_3$	98	98–100 (PrOH)	$C_{18}H_{32}N_2O_6S_2$
14	$CH(CH_3)_2$	$C(O)CH_3$	99	97–99 (iPrOH)	$C_{18}H_{32}N_2O_6S_2$
15	CH_2CH_3	$C(O)CH_2OCH_3$	92	74–76 (EtOH)	$C_{18}H_{32}N_2O_8S_2$
16	$CH_2CH_2CH_3$	$C(O)CH_2OCH_3$	98	101–102.5 (PrOH)	$C_{20}H_{36}N_2O_8S_2$
17	$CH(CH_3)_2$	$C(O)CH_2OCH_3$	98	120–122 (iPrOH)	$C_{20}H_{36}N_2O_8S_2$

[a] Analyses for C, H, and N within ±0.4%.

deoxytubercidinyl)-L-homocysteine (**58**), *S*-(3′-deoxy-3-deazaadenosyl)-L-homocysteine (**59**), *N*-(trifluoroacetyl)-*S*-3′-deoxytubercidinyl methyl ester (**60**), and 4-amino-3-(2-deoxy-β-D-erythropentofuranosyl)-1*H*-imidazo[4,5-*c*]pyridine (**61**), were described elsewhere (Table III).[6,7,10,16]

Antiviral Activity

All the compounds were evaluated for activity against herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2), vaccinia virus (VV), and vesicular stomatitis virus (VSV) in primary rabbit kidney (PRK) or embryonic skin-muscle (E_6SM) fibroblast cultures, against VSV, polio virus type 1, and Coxsackie B4 virus in HeLa cell cultures, against Coxsackie B4 virus, parainfluenza virus type 3, reovirus type 1, Sindbis virus, and Semliki forest virus (SFV) in Vero cell cultures, and against human immunodeficiency virus type 1 (HIV-1) in MT-4 cells [MT-4 being a human T4 lymphocyte line chronically infected with HTLV-I (human T-cell leukemia virus type 1)].

Under the conditions used, none of the compounds proved inhibitory to HIV-1 replication in MT-4 cells at a concentration which was not toxic to the proliferating host (MT-4) cells (Table IV). In the other cell systems (PRK, E_6SM, HeLa, Vero), which were used as monolayers, antiviral effects were noted at compound concentrations which were ≥10-fold lower than the concentrations required to alter normal cell morphology: with **46** against reovirus in Vero cells, with **43** against parainfluenza and reovirus in Vero cells; with **55** against VSV in PRK cells; with **31**, **37**, **38**, **40**, and **41** against VV in E_6SM cells; with **33** and **58** against VV and VSV in PRK (of E_6SM) cells; with **26**, **28**, **30**, and **34** against HSV-2, VV, and VSV in PRK (or E_6SM) cells; with **25**, **27**, **29**, **32**, **35**, and **36** against HSV-1, HSV-2, VV, and VSV in E_6SM cells; and with **25–28**, **30**, **34**, **37**, **38**, and **58** against VSV in HeLa cells.

As the most potent antiviral agent, with MIC_{50} values of 1–2 μg/mL against HSV-2, VV, or VSV emerged compound **36**, that was virtually noncytotoxic in PRK/E_6SM cells (MIC: 400 μg/mL), but it inhibited MT-4 cell proliferation at an IC_{50} of 12 μg/mL. Compounds **26** and **31** were among the most selective anti-VV compounds tested but did not show anti HSV-1 and HSV-2 activity. Thus, compound **36** can be considered as the most selective HSV, VV, and VSV inhibitor of all compounds tested.

The mechanism of action of the test compounds (in particular **26**, **31**, and **36**) remains the subject of further study. If the test compounds act as prodrugs of TubHcy they do not appear to be cleaved effectively inside the virally infected cells to release the parent compound at marked levels. As they are analogues of *S*-adenosyl-L-homocysteine (AdoHcy), it is obvious that they may interfere directly in the methyltransferase reactions with *S*-adenosylmethionine (AdoMet) as the methyl donor.[17] These AdoMet-dependent methyl transfers are required for the maturation of mRNAs, including viral mRNA (i.e. 5′-cap formation). AdoHcy analogues, such as *S*-arister-

(16) Serafinowski, P. Synthesis of S-formycinyl-L-homocysteine and its 3′-deoxy derivative. *Synthesis* **1987**, 879–883.

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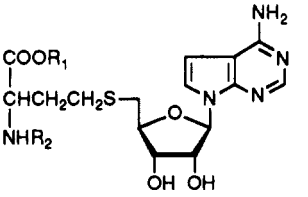
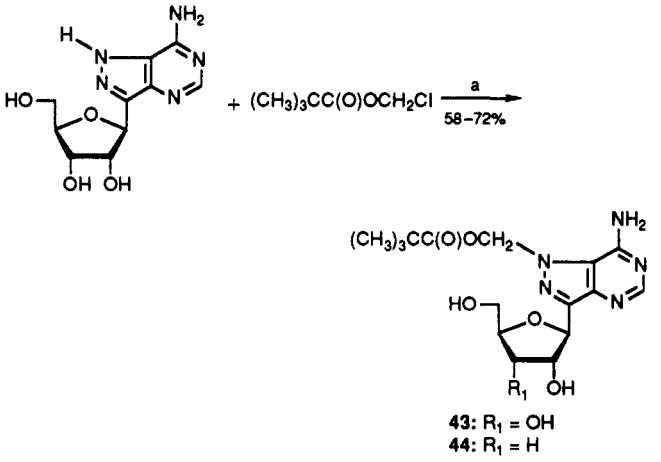
Table II. Yields and Physical Data of Intermediates and Compounds Tested (19–42)



no.[a]	R_1	R_2	reaction time (h)	% yield	formula[b]
19	CH_3	$(H_3C)_3COCO$	120	47	$C_{21}H_{31}N_5O_7S\cdot0.75H_2O$
20	CH_2CH_3	$(H_3C)_3COCO$	120	66	$C_{22}H_{33}N_5O_7S\cdot1.1H_2O$
21	$CH_2CH_2CH_3$	$(H_3C)_3COCO$	96	48	$C_{23}H_{35}N_5O_7S\cdot0.5H_2O$
22	$CH(CH_3)_2$	$(CH_3)_3COCO$	120	27	$C_{23}H_{35}N_5O_7S\cdot0.7H_2O$
23	$CH_2CH_2CH_2CH_3$	$(CH_3)_3COCO$	120	48	$C_{24}H_{37}N_5O_7S\cdot0.5H_2O$
24	$CH_2CH_2Si(CH_3)_3$	$(CH_3)_3COCO$	96	23	$C_{25}H_{41}N_5O_7SSi$[c]
25	CH_2CH_3	CF_3CO	70	53	$C_{19}H_{24}F_3N_5O_6S$
26	$CH_2CH_2CH_3$	CF_3CO	96	16	$C_{20}H_{26}F_3N_5O_6S$
27	$CH(CH_3)_2$	CF_3CO	96	40	$C_{20}H_{26}F_3N_5O_6S$
28	CH_2CH_3	CH_3CO	96	22	$C_{19}H_{27}N_5O_6S$
29	$CH_2CH_2CH_3$	CH_3CO	96	48	$C_{20}H_{29}N_5O_6S$
30	$CH(CH_3)_2$	CH_3CO	96	55	$C_{20}H_{29}N_5O_6S$
31	CH_2CH_3	CH_3OCH_2CO	60	54	$C_{20}H_{29}N_5O_7S$
32	$CH_2CH_2CH_3$	CH_3OCH_2CO	96	54	$C_{21}H_{31}N_5O_7S$
33	$CH(CH_3)_2$	CH_3OCH_2CO	96	53	$C_{21}H_{31}N_5O_7S$
34	CH_3	H	1	76	$C_{16}H_{23}N_5O_5S\cdot0.5H_2O$
35	CH_2CH_3	H	1	81	$C_{17}H_{25}N_5O_5S$
36	$CH_2CH_2CH_3$	H	1	61	$C_{18}H_{27}N_5O_5S\cdot1.6H_2O$
37	$CH(CH_3)_2$	H	1	61	$C_{18}H_{27}N_5O_5S\cdot H_2O$
38	$CH_2CH_2CH_2CH_3$	H	1	49	$C_{19}H_{29}N_5O_5S\cdot0.5H_2O$
39	H	$(CH_3)_3COCO$	3 min	50	$C_{20}H_{29}N_5O_7S$[c]
40	H	CF_3CO	15 min	24	$C_{17}H_{20}F_3N_5O_6S$[c]
41	H	CH_3CO	15 min	70	$C_{17}H_{23}N_5O_6S$[c]
42	H	CH_3OCH_2CO	15 min	65	$C_{18}H_{25}N_5O_7S$[c]

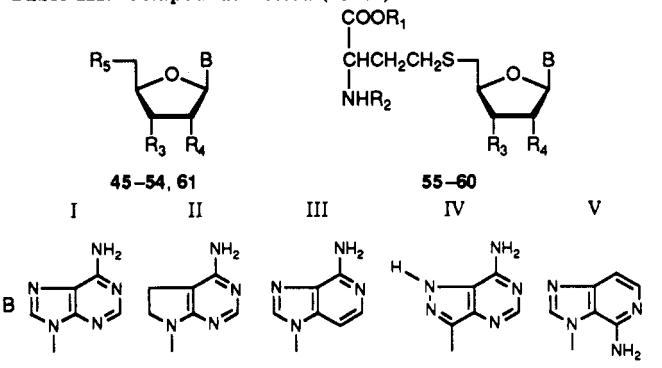
[a] The compounds were obtained as amorphous powders having indefinite melting points. [b] Analyses of C, H, and N with ±0.4% except where characterized by high-resolution mass spectra. [c] Characterized by high-resolution FAB-MS: **24** calcd for $C_{25}H_{41}N_5O_7SSi(M+H)^+$ 584.25735, found 584.2595; **39** calcd for $C_{20}H_{29}N_5O_7S(M+H)^+$ 484.18657, found 484.1888; **40** calcd for $C_{17}H_{20}F_3N_5O_6S(M+H)^+$ 480.11642, found 480.1142; **41** calcd for $C_{17}H_{23}N_5O_6S(M+H)^+$ 426.14471, found 426.1460; **42** calcd for $C_{18}H_{25}N_5O_7S(M+H)^+$ 456.15522, found 456.1574.

Scheme II [a]



[a] (a) K_2CO_3/DMF

Table III. Compounds Tested (45–61)



compd	R_1	R_2	R_3	R_4	R_5	B
45	–	–	H	OH	OH	I
46	–	–	H	OH	OH	II
47	–	–	H	OH	OH	III
48	–	–	OH	H	OH	I
49	–	–	H	OH	OH	IV
50	–	–	OH	H	OH	IV
51	–	–	H	H	OH	III
52	–	–	H	OH	Cl	I
53	–	–	OH	OH	Cl	IV
54	–	–	H	H	Cl	IV
55	H	H	OH	OH	–	IV
56	H	H	H	OH	–	IV
57	H	H	H	OH	–	I
58	H	H	H	OH	–	II
59	H	H	H	OH	–	III
60	CH_3	$C(O)CF_3$	H	OH	–	II
61	–	–	OH	H	OH	V

omycinyl-L-homocysteine,[18,19] have been previously shown to block these methyltransferase reactions. Depending on the individual needs of the various viral mRNAs for such methylations, AdoHcy analogues may be expected to differentially inhibit the replication of different viruses.

Apparently, HSV, VV, and VSV are more dependent on such methylations than other viruses. This may explain why these viruses are particularly sensitive to the inhibitory effects of the AdoHcy analogue **36**. However, it remains

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Table IV. Antiviral Activity of Test Compounds in Different Assays Systems

	MIC_{50} (μg/mL)[a]												
	PRK/E_6SM[b]				HeLa			Vero					MT-4
compd no.	HSV-1 (KOS)	HSV-2 (G)	VV	VSV	VSV	polio-1	coxsackie B4	Coxsackie B4	parainfluenza-3	reo-1	Sindbis	SFV	HIV-1 (III_B)
24	>10 (10)	>10	2	>10	>40 (100)	>40	>40	70 (100)	>40	>40	>40	>40	>8 (18)
25	7 (100)	10	7	2	4 (100)	20	20	7 (40)	>20	>20	>20	>40	>4 (6)
26	>10 (>40)	>10	0.7	>10	20 (>200)	>100	>100	150 (>200)	70	>100	70	>200	*c*
27	20 (200)	10	1	5	20 (200)	>100	>100	>50 (200)	>50	>50	>70	>100	>4 (6)
28	70 (400)	20	15	5	20 (>200)	70	70	20 (100)	>40	>30	>70	>100	>4 (9)
29	40 (400)	20	7	7	20 (100)	40	20	20 (40)	20	15	>40	>40	>4 (8)
30	100 (>400)	40	10	10	20 (200)	100	70	40 (200)	>100	>70	>100	>200	>4 (8.5)
31	>100 (>200)	>100	2	70	70 (>400)	>200	150	150 (>400)	>400	300	300	>400	*c*
32	20 (200)	10	4	2	20 (>200)	100	100	15 (70)	20	>30	>40	>70	>0.8 (1.8)
33	150 (>400)	70	7	20	100 (400)	>200	>200	>100 (400)	>200	>200	>200	>200	>100 (86)
34	100 (>400)	20	4	20	20 (400)	>200	>200	>200 (200)	>200	>200	>200	>200	>20 (30)
35	20 (400)	20	7	7	20 (100)	70	70	20 (100)	>100	>70	>100	>100	*c*
36	10 (400)	2	1	2	20 (100)	>100	>100	70 (200)	>70	>70	>70	>150	>20 (12)
37	>10 (40)	>10	0.2	7	7 (100)	>100	>40	>100 (100)	20	>40	>40	>100	>1.6 (3.7)
38	20 (40)	>10	2	20	7 (100)	>100	>40	>200 (400)	150	70	>200	>200	>1.6 (3.5)
39	>100 (100)	>100	20	>100	>400 (>400)	>400	>400	>400 (>400)	>400	>400	>400	>400	>200 (>200)
40	>200 (400)	>200	20	>200	>400 (>400)	>400	>400	>400 (>400)	>400	>400	>400	>400	>200 (>200)
41	>200 (400)	>200	7	>200	>400 (>400)	>400	>400	>400 (>400)	>400	>400	>400	>400	>200 (>200)
42	>400 (400)	>400	>400	>400	>400 (>400)	>400	>400	>400 (>400)	>400	>400	>400	>400	>200 (>200)
43	30 (40)	>40	>40	30	30 (40)	>40	>40	>10 (40)	1	3	>10	>10	>0.8 (1.5)
44	>100 (200)	>100	>100	>100	>100 (200)	>100	>100	>100 (200)	>100	>100	>100	>100	>20 (35)
45	>40 (100)	>40	>100	>40	>100 (200)	>100	>100	>100 (200)	>100	>100	>100	>100	>4 (7)
46	>10 (40)	7	7	>10	>10 (40)	7	7	7 (40)	7	4	30	>40	–
47	>100 (200)	70	>40	>100	>40 (40)	>40	>40	>100 (200)	>100	>100	>100	>100	>20 (30)
48	150 (>400)	100	150	>200	>200 (400)	>200	>200	>200 (400)	>200	>200	>200	>200	>20 (42)
49	>400 (>400)	>400	>400	>400	>400 (>400)	>400	>400	>200 (400)	300	>200	>400	>400	>100 (>100)
50	>400 (>400)	>400	>400	>400	>400 (>400)	>400	>400	300 (400)	>400	>400	>400	>400	>100 (>100)
51	>200 (200)	>100	>100	>100	>200 (400)	>200	>200	>100 (200)	>100	>100	>100	>100	>100 (>100)
52	>200 (400)	>100	>200	>400	>200 (200)	>200	>200	>40 (40)	>40	>40	>40	>40	>100 (80)
53	>200 (400)	>200	300	>400	>200 (400)	>400	>400	70 (200)	70	>100	>100	>100	>100 (>100)
54	>200 (>400)	>200	>400	>400	>400 (400)	>400	>400	>200 (400)	>200	>200	>200	>400	>100 (>100)
55	200 (400)	400	100	20	150 (400)	>400	>400	>100 (100)	30	>100	>100	>100	>4 (6)
56	>40 (40)	>40	>40	>40	>40 (100)	>40	>40	>40 (40)	>40	>40	>40	>40	>100 (>100)
57	>100 (200)	>100	>200	>100	>200 (400)	>200	>200	>200 (200)	>200	>200	>200	>100	>100 (>100)
58	>100 (200)	>100	2	20	20 (200)	>200	>200	>200 (400)	100	70	>200	>200	–
59	>400 (400)	>400	>400	>200	>400 (>400)	>400	>400	>400 (400)	>400	>400	>400	>400	>100 (>100)
60	>200 (400)	>200	70	20	300 (400)	>400	>200	>200 (400)	>200	40	>400	>400	>100 (>100)
61	150 (400)	100	>200	>200	>200 (>200)	>200	>200	>400 (>400)	>400	>400	>400	>400	50 (>100)
tubercidin	>0.2 (0.4)	0.2	>0.1	0.07	0.07 (0.4)	0.2	0.2	0.07 (0.1)	0.02	0.02	>0.1	>0.1	–
(*S*)-DHPA	>400 (>400)	>400	40	20	70 (>400)	>400	>400	70 (>400)	10	7	>400	>400	–
ribavirin	>400 (>400)	>400	30	50	20 (400)	70	40	70 (>400)	70	40	200	300	>20 (20)
C-^{3}Ado	>400 (400)	100	1	0.7	2 (>400)	>400	>400	>200 (400)	0.4	0.4	70	>400	–

[a] Minimum inhibitory concentration, required to inhibit virus-induced cytopathicity by 50%. Listed in parentheses are the minimum cytotoxic concentrations, required to cause a microscopically detectable alteration of normal cell morphology (PRK, $E_7$6SM, HeLa, or Vero), or the 50% inhibitory concentrations (IC_{50}) for cell growth (MT-4). [c] Compounds **34**, **43–47**, **49**, **50**, **52–60** were evaluated in PRK, whereas compounds **24–33**, **35–42**, **48**, **51**, **61** were evaluated in E_6SM. [c] Not determined.

puzzling why compounds **26** and **31** are only effective against VV but not against HSV-1 and HSV-2. As an interesting avenue of further research, the AdoHcy analogues (described here or elsewhere) should be explored for their full antiviral activity spectrum. In particular, their activity spectrum should be compared with that of the adenosine (i.e. neplanocin A) analogues that are targeted at the AdoHcy hydrolase reaction,[20-22] and thus indirectly interfere with the AdoMet-dependent transmethylations.[17]

Experimental Section

Chemistry. Melting points were determined on a Reichert micro-hot stage apparatus and were uncorrected. UV spectra were measured in 95% ethanol with a Pye-Unicam SP-8-150 UV-vis spectrometer. ^{1}H-NMR spectra were recorded at 250 MHz with a Bruker WH-250 spectrometer with TMS as an internal standard and DMSO-d_6 as a solvent. In cases where analytical data are given for hydrates the presence of water was confirmed by ^{1}H-NMR. The protons of 2'-OH, 3'-OH, NH_2, H_2O, and NHCO were exchangeable with D_2O. Mass spectra were obtained using a VG 7070 H with either EI or FAB ionization. HPTLC was run on Merck silica gel 60 F_{254} analytical plates in the following systems: (A) $CHCl_3$/EtOH (4:1) or (B) $CHCl_3$/EtOH (9:1). Short-column chromatography was carried out on silica gel 60 H (Merck), the 10-μm octyl S10 C_8 24/39 820028 (Phase Sep:) was used for reverse-phase columns, whereas Sephadex A25 was used for anion-exchange columns. HPLC analysis was performed on the system comprising Waters Model S10 pump, Model 680 automated gradient controller, Model 46K injector, and Model 490 programmable wavelength detector. Retention times (t_R) were determined on a Trilab 3000 multichannel chromatography data system (Trivector). The column, 5 μM APEX ODS 250 × 4.6 mm, Jones Chromatography U.K., was eluted with 0.025 M NH_4OAc buffer/CH_3CN (31:19) (C), (4:1) (D), and (3:1) (E) under isocratic conditions. Solvent removal was performed in vacuo at 30–40 °C. L-Homocystine and tubercidin were purchased from Sigma.

***N,N'*-Bis(*tert*-butoxycarbonyl)-L-homocystine Alkyl Esters (3–7). General Procedure.** L-Homocystine (1) (5.0 g, 18.6 mmol) was suspended in the appropriate dry alcohol (150 mL) and cooled to 0–5 °C. Dry hydrogen chloride was bubbled through the suspension for 0.5 h, and the resulting solution was heated under reflux for 3 h. The solvent was removed under reduced pressure and the residue dissolved in dioxane/water (2:1, 60 mL). Sodium carbonate (4 g, 37.7 mmol) in water (20 mL) was added to the stirred solution, and the mixture was cooled to 0–5 °C. Di-*tert*-butyl pyrocarbonate (9.5 g, 43.5 mmol) was then added in one portion, and the stirring was continued for 18 h at room temperature. The mixture was concentrated to about 15 mL in vacuo, the precipitate was filtered off and suspended in ethanol (50 mL), and the suspension heated briefly under reflux. Insoluble particles were filtered off, and the filtrate was stored at 0–5 °C for 18 h. The resulting colorless crystals were collected by filtration and dried in a desiccator (Table I). **3**: ^{1}H NMR δ 1.38 (s, 18 H, $C(CH_3)_3$), 2.00 (m, 4 H, CH_2-β), 2.72 (t, 4 H, J = 7.75 Hz, CH_2-γ), 3.63 (s, 6 H, OCH_3), 4.10 (m, 2 H, CH-α), 7.35 (d, 2 H, J = 7.89 Hz, N-H).

***N,N'*-Bis(*tert*-butoxycarbonyl)-L-homocystine (Trimethylsilyl)ethyl Ester (8).** To a stirred and cooled (0 °C) solution of L-homocystine (1) (5.0 g, 18.6 mmol) in aqueous 1 M NaOH (37.3 mL) and dioxane/water (2:1, 60 mL) was added di-*tert*-butyl pyrocarbonate (8.9 g, 41 mmol) in one portion. The cooling was removed, and the solution was stirred for 18 h at room temperature. The precipitate was filtered off, the filtrate was concentrated in vacuo, and ethyl acetate (75 mL) was added. The pH of the mixture was adjusted to 3 with aqueous 2 M $KHSO_4$, the aqueous layer was extracted with ethyl acetate (2 × 75 mL), and the combined organic layers were washed with brine (75 mL) and water (75 mL), dried (Na_2SO_4), and evaporated to give the product *N,N'*-bis(*tert*-butoxycarbonyl)-L-homocystine as a colorless glass: yield 5.74 g (66%); mp 149–151 °C (EtOH); ^{1}H-NMR δ 1.38 (s, 18 H, $C(CH_3)_3$), 1.97 (m, 4 H, CH_2-β), 2.72 (m, 4 H, CH_2-γ), 4.01 (m, 2 H, CH-α), 7.18 (d, 2 H, J = 8.1 Hz, NH), 11.68 (bs, 2 H, COOH).

To a solution of *N,N'*-bis(*tert*-butoxycarbonyl)-L-homocystine (5.0 g, 13.6 mmol) in acetonitrile (35 mL) and DMF (10.2 mL) were added pyridine (4.4 mL, 54 mmol) and (trimethylsilyl)-ethanol (4.7 mL, 32 mmol). Subsequently the mixture was cooled to 0 °C and after 10 min, DCC (6.2 g, 30 mmol) was added in one portion. The mixture was stirred for an additional 1 h at room temperature and stored at 4 °C for 18 h. 5 M Oxalic acid in DMF (0.82 mL) was added, and the mixture was stirred for 30 min at room temperature. The precipitate was filtered off and washed with ethyl acetate (100 mL), and the combined filtrate and washings were washed with 0.5 M HCl (100 mL), 5% aqueous $NaHCO_3$ (100 mL), water (100 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was applied to a short column of silica gel, and the product was eluted with ethyl acetate/hexane (2:8) to give **8** as a colorless glass; crystallization of the crude product from ethyl acetate gave **8** as colorless crystals (Table I): ^{1}H-NMR δ 0.04 (s, 18 H, $Si(CH_3)_3$), 0.95 (t, 4 H, J = 8.3 Hz, CH_2Si), 1.38 (s, 18 H, $C(CH_3)_3$), 1.99 (m, 4 H, CH_2-β), 2.73 (m, 4 H, CH_2-γ), 4.12 (m, 6 H, OCH_2, CH-α), 7.23 (d, 2 H, J = 7.71 Hz, NH).

***N,N'*-Bis(trifluoroacetyl)-L-homocystine Alkyl Esters (9–11). General Procedure.** L-Homocystine (1) (5 g, 18.6 mmol) was suspended in the appropriate dry alcohol (150 mL) and cooled to 0–5 °C. Dry hydrogen chloride was bubbled through the suspension for 0.5 h, and te resulting solution was heated under reflux for 3 h. The solvent was removed under reduced pressure, and the residue was dried in vacuo, dissolved in trifluoroacetic anhydride (50 mL), and heated under reflux for 30 min. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate (250 mL), washed with water (150 mL), 5% aqueous sodium bicarbonate (150 mL), and water (150 mL), dried (Na_2SO_4), and concentrated to give the oily residues which usually solidified on standing and were crystallized from the appropriate alcohols to afford **9–11** as colorless crystals (Table I). **9**: ^{1}H-NMR δ 1.93 (t, 6 H, J = 7.12, CH_3), 2.18 (m, 4 H, CH_2-β), 2.77 (m, 4 H, CH_2-γ), 4.14 (q, 4 H, J = 7.08 Hz, CH_2), 4.46 (m, 2 H, CH-α), 9.86 (bs, 2 H, NH).

***N,N'*-Diacetyl-L-homocystine Alkyl Esters (12–14) and *N,N'*-Bis(methoxyacetyl)-L-homocystine Alkyl Esters (15–17). General Procedure.** L-Homocystine (1) (5.0 g, 18.6 mmol) was suspended in the appropriate dry alcohol (150 mL) and cooled to 0–5 °C. Dry hydrogen chloride was bubbled through the suspension for 0.5 h, the resulting solution heated under reflux for 3 h, the solvent removed under reduced pressure, and the residue dried in vacuo. The residue was suspended in dry acetonitrile (60 mL), and then (dimethylamino)pyridine (9.1 g, 74 mmol) followed by a solution of acetic acid anhydride (5.6 g, 55.8 mmol) or methoxyacetyl chloride (61 g, 55.8 mmol) in dry acetonitrile (20 mL) were added to the stirred suspension. The mixture was stirred for 2 h at room temperature, the solvent was removed in vacuo, and the residue was partitioned between ethyl acetate (320 mL) and water (80 mL). The organic layer was washed with cold 1 M HCl (2 × 80 mL), water (80 mL), sodium bicarbonate (80 mL), water (80 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give the products **12–17** as pale yellow or white solids. The crude products were crystallized from appropriate solvents to give colorless crystals (Table I). **12**: ^{1}H-NMR δ 1.19 (m, 6 H, CH_3), 1.85 (s, 6 H, C(O)-CH_3), 2.01 (m, 4 H, CH_2-β), 2.71 (m, 4 H, CH_2-γ), 4.08 (m, 4 H, CH_2, 4.33 (m, 2 H, CH-α), 8.23 (d, 2 H, J = 7.59 Hz, NH).

Condensation of Protected L-Homocystine Derivatives (3–17) with Tubercidin. General Procedure. Tubercidin (18)

(20) De Clercq, E.; Cools, M.; Balzarini, J.; Marquez, V. E.; Borcherding, D. R.; Borchardt, R. T.; Drach, J. C.; Kitaoka, S.; Konno, T. Broad-spectrum antiviral activities of neplanocin A, 3-deazaneplanocin A, and their 5'-nor derivatives. *Antimicrob. Agents Chemother.* **1989**, *33*, 1291–1297.

(21) Cools, M.; De Clercq, E. Correlation between the antiviral activity of acyclic and carbocyclic adenosine analogues in murine L929 cells and their inhibitory effect on L929 cell S-adenosylhomocysteine hydrolase. *Biochem. Pharmacol.* **1989**, *38*, 1061–1067.

(22) Cools, M.; De Clercq, E. Influence of S-adenosylhomocysteine hydrolase inhibitors on S-adenosylhomocysteine and S-adenosylmethionine pool levels in L929 cells. *Biochem. Pharmacol.* **1990**, *40*, 2259–2264.

(500 mg, 1.88 mmol) and the appropriate amino acid precursor (3–17) (5.64 mmol) were suspended in dry pyridine (20 mL), and the suspension was concentrated under reduced pressure with rigorous exclusion of moisture. The procedure was repeated three times, the oily residue was dissolved in dry pyridine (7 mL), triethylphosphine (1.6 mL, 11.3 mmol) was added, and the mixture was stirred at room temperature for 48–120 h.

Methanol (20 mL) was added, the stirring was continued for another 10 min, and the solvents were removed in vacuo. The residue was coevaporated with pyridine (3 × 15 mL) and toluene (3 × 15 mL), dissolved in a small amount of chloroform, and applied to a short column of silica gel. The products were eluted with $CHCl_3$/EtOH 92:8 for **19**, 91:9 for **20**, 90:10 for **25**, **32**, **33**, 88:12 for **26**, **27**, **31**, 85:15 for **21–23** and 80:20 for **24**, **28–30**. The appropriate fractions were pooled and evaporated to give the required products as colorless oils. Each colorless oil was dissolved in a small amount of chloroform (1–2 mL) and added dropwise to a stirred petroleum ether (bp 30–40 °C, 70 mL). The resulting colorless amorphous precipitates were collected by centrifugation and dried in a desiccator to give analytically pure **19–33** (Table II). **19**: R_f 0.35 (A), 0.11 (B); t_R 205 (C); UV λ_{max} = 270 nm, log ϵ = 4.04; MS (70 eV) *m/z* (%) 488 (M^+, 32), 163 (24), 135 (100); ^{1}H-NMR δ 1.37 (s, 9 H, $C(CH_3)_3$), 1.85 (m, 2 H, CH_2-β), 2.88 (m, 2 H, CH_2-γ), 2.82 (m, 2 H, H-5′a, H-5′b), 3.60 (s, 3 H, OCH_3), 3.95 (m, 1 H, H-4′), 4.03 (m, 2 H, H-3′, CH-α), 4.44 (m, 1 H, H-2′), 5.26 (bs, 1 H, 3′-OH), 5.36 (bs, 1 H, 2′-OH), 6.06 (d, 1 H, J = 5.86 Hz, H-1′), 6.65 (d, 1 H, J = 3.61 Hz, H-7), 7.20 (bs, 2 H, NH_2), 7.29 (d, 1 H, J = 7.91 Hz, NH), 7.35 (d, 1 H, J = 3.66 Hz, H-8), 8.09 (s, 1 H, H-2).

Deprotection of N-(*tert*-Butoxycarbonyl)-*S*-tubercidinyl-L-homocysteine Alkyl Esters (19–23) with Trifluoroacetic Acid. General Procedure. The *N*-(*tert*-butoxycarbonyl)-*S*-tubercidinyl-L-homocysteine alkyl esters (**19–23**) (0.2 mmol) were dissolved in dry trifluoroacetic acid (3 mL), and the solution was kept at room temperature for 1 h. The solvent was removed in vacuo, and the residue was coevaporated with toluene (3 × 5 mL) and dissolved in methanol (20 mL). The pH of the solution was brought to 6–7 by the addition of Bio-Rad AG-1X-2 (100–200 mesh) OH^- resin. The resin was filtered off and washed with methanol (4 × 3 mL), and the combined filtrate and washings were concentrated under reduced pressure to give the crude products **34–38** as colorless glasses.

Each colorless glass was dissolved in a small amount of water (1–2 mL) and applied to a Sephadex A-25 column (270 × 17 mm). The products were eluted with a 0.001–0.3 M gradient of triethylammonium bicarbonate buffer. The appropriate fractions were combined, concentrated, and coevaporated with methanol (3 × 20 mL) to give a colorless residue which was redissolved in a small amount of water (1–2 mL) and lyophilized to give products **34–38** as a colorless powder (Table II).

Compounds **34** and **35** were also purified using reverse-phase chromatography. Thus, each of the crude products **34** and **35** was dissolved in water (1–2 mL) and applied to a reverse-phase column (22 × 24 mm). The products were eluted with H_2O/CH_3OH (75:25) for **34** and 65:35 for **35**. The appropriate fractions were combined, concentrated, and lyophilized as described above (Table II). **34**: t_R = 139 (E); UV λ_{max} = 271 nm, log ϵ = 3.99; MS (70 eV) *m/z* (%), 398 (M^+, 36), 163 (13), 135 (30); ^{1}H-NMR δ 1.73 (m, 2 H, CH_2-β), 2.59 (t, 2 H, J = 7.65 Hz, CH_2-γ), 2.83 (m, 2 H, H-5′a, H-5′b), 3.36 (m, 1 H, CH-α), 3.60 (m, 3 H, OCH_3), 3.95 (m, 1 H, H-4′), 4.04 (m, 1 H, H-3′), 4.43 (m, 1 H, H-2′), 6.05 (d, 1 H, J = 5.86 Hz, H-1′), 6.61 (d, 1 H, J = 3.64 Hz, H-7), 7.01 (bs, 2 H, NH_2), 7.31 (d, 1 H, J = 3.65 Hz, H-8), 8.06 (s, 1 H, H-2).

***N*-(*tert*-Butoxycarbonyl)-*S*-tubercidinyl-L-homocysteine (39).** The *N*-(*tert*-butoxycarbonyl)-*S*-tubercidinyl-L-homocysteine (trimethylsilyl)ethyl ester (**24**) (60 mg, 0.103 mmol) was dissolved in DMF (2 mL), and 1 M tetrabutylammonium fluoride in THF (0.5 mL) was added. After 3 min the solution was cooled to 0–5 °C and diluted with water (2 mL). The solvent was removed in vacuo, the residue was coevaporated with toluene (3 × 5 mL), dissolved in a small amount of water, and applied to a Sephadex A-25 column (270 × 17 mm). The product was eluted with a 0.001–0.3 M gradient of triethylammonium bicarbonate buffer. The appropriate fractions were concentrated and coevaporated with methanol (3 × 20 mL). The residue was redissolved in a small amount of water and lyophilized, to give the product **39** as a colorless powder (Table II): t_R = 155 (D); UV λ_{max} = 280 nm, log ϵ = 3.89; MS (70 eV) *m/z* (%) 484 (M^+, 86), 163 (100); ^{1}H-NMR δ 1.37 (s, 9 H, $C(CH_3)_3$), 1.82 (m, 2 H, CH_2-β), 2.54 (m, 2 H, CH_2-γ), 2.82 (m, 2 H, H-5′a, H-5′b), 3.94 (m, 3 H, H-3′, H-4′, CH-α), 4.43 (t, 1 H, J = 5.34 Hz, H-2′), 6.06 (d, 1 H, J = 5.73 Hz, H-1′), 6.61 (d, 1 H, J = 3.54 Hz, H-7), 6.95 (s, 2 H, NH_2), 7.30 (d, 1 H, J = 3.50 Hz, H-8), 8.06 (s, 1 H, H-2).

Deprotection of *N*-(Trifluoroacetyl)-, *N*-Acetyl-, and *N*-(Methoxyacetyl)-*S*-tubercidinyl-L-homocysteine Propyl Esters (26, 29, and 32) with 0.25 M Barium Hydroxide in 50% Aqueous Methanol. General Procedure.

A suspension of the *N*-(trifluoroacetyl)-, *N*-acetyl-, or *N*-(methoxyacetyl)-*S*-tubercidinyl-L-homocysteine propyl ester, **26**, **29**, or **32**, respectively (0.2 mmol), was dissolved in 0.25 M barium hydroxide in aqueous methanol (1:1, 10 mL) and stirred for 15 min at room temperature. The pH of the solution was adjusted to 6–7 with 1 M sulfuric acid, and the resulting suspension was centrifuged. The supernatant was concentrated in vacuo, redissolved in a small amount of water (1–2 mL), and applied to a Sephadex A-25 column (270 × 17 mm). The products were eluted with a 0.001–0.3 M gradient of triethylammonium bicarbonate buffer. The appropriate fractions were pooled, concentrated, and lyophilized to give the products **40–42** as a colorless powder (Table II). **40**: t_R = 132 (D); UV λ_{max} = 270, log ϵ = 3.92; MS (70 eV) *m/z* (%) 480 (M^+, 100). ^{1}H-NMR δ 1.94 (m, 2 H, CH_2-β), 2.65 (t, 2 H, J = 7.58 Hz, CH_2-γ), 2.83 (ddd, 2 H, J = 6.27, 6.50, 16.78, H-5′a, H-5′b), 3.54 (m, 1 H, CH-α), 3.98 (m, 1 H, H-4′), 4.06 (m, 1 H, H-3′), 4.46 (t, 1 H, J = 5.48 Hz, H-2′), 6.06 (d, 1 H, J = 5.61 Hz, H-1′), 6.62 (d, 1 H, J = 3.44 Hz, H-7), 7.01 (s, 2 H, NH_2), 7.31 (d, 1 H, J = 3.59 Hz, H-8), 8.07 (s, 1 H, H-2).

***N*-[(Pivaloyloxy)methyl]formycin A (43) and *N*-1-[(Pivaloyloxy)methyl]-3′-deoxyformycin A (44).** Formycin A or 3′-deoxyformycin A (1 mmol), anhydrous potassium carbonate (0.42 g, 3 mmol), and chloromethyl pivalate (0.32 g, 2 mmol) were dissolved in dry DMF (6 mL) and stirred for 2.5 h (formycin A) or 5 h (3′-deoxyformycin A) at room temperature. Chloroform (10 mL) was added, the precipitate was filtered off, and ethanol (5 mL) was added to the filtrate.

The solvents were removed in vacuo, and the residue was coevaporated with toluene (3 × 10 mL), dissolved in a small amount of chloroform (2 mL), and applied to a short column of silica gel. The products were eluted with $CHCl_3$/EtOH (22:3) to give **43** (0.27 g, 72%) as a colorless glass or with $CHCl_3$/EtOH (23:2) to give **44** (0.21 g, 58%) as a colorless glass. Analytically pure samples were obtained when the crude products were dissolved in chloroform (1–2 mL), and the solution was added dropwise to a stirred petroleum ether (bp 30–40 °C) (50 mL). The resulting precipitates were collected by centrifugation and dried in a desiccator.

43: mp 109–121 °C (acetone); ^{1}H-NMR δ 1.09 (s, 9 H, $C(CH_3)_3$), 3.52 (m, 1 H, H-5′a), 3.64 (m, 1 H, H-5′b), 3.96 (m, 1 H, H-4′), 4.10 (m, 1 H, H-3′), 4.64 (m, 1 H, H-2′), 4.89 (d, 1 H, J = 7.65 Hz, H-1′), 6.44 (s, 2 H, CH_2N), 7.55 (bs, 2 H, NH_2), 8.23 (s, 1 H, H-5). Anal. $C_{16}H_{23}N_5O_6$ (C, H, N). FW 381.38.

44: mp indefinite; ^{1}H-NMR δ 1.08 (s, 9 H, $C(CH_3)_3$), 1.95 (m, 1 H, H-3′a), 2.25 (m, 1 H, H-3′b), 3.45 (m, 1 H, H-5′a), 3.63 (m, 1 H, H-5′b), 4.28 (m, 1 H, H-4′), 4.62 (m, 1 H, H-2′), 4.90 (d, 1 H, J = 4.87 Hz, H-1′), 6.41 (s, 2 H, CH_2N), 8.24 (s, 1 H, H-5). Anal. $C_{16}H_{23}N_5O_5$ (C, H, N). FW 365.38.

Antiviral Activity Assays. To assess the antiviral effects of the compounds, inhibition of virus-induced cytopathicity was measured in either (PRK, E_6SM, HeLa, Vero) cell monolayers or (MT-4) cell suspensions. The sources of the viruses and methods used have been described previously: herpes simplex and vaccinia virus,[23] vesicular stomatitis and other RNA viruses,[24] and retroviruses (i.e. human immunodeficiency virus).[25,26]

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Supplementary Material Available: ^{1}H-NMR spectra of intermediates **3–17** (Table V), R_f values and retention times of intermediates and compounds tested (**19–42**, Table VI), UV, ^{1}H-NMR, and mass spectra of intermediates and compounds tested (**19–42**, Table VII) (7 pages). Ordering information is given on any current masthead page.