obtained via the procedure described for compound 4 from 60 mg (0.13 mmol) of the preceding compound. A white solid was obtained after recrystallization (MeOH/H_2O) (30 mg, 76%): mp 95 °C; R_f (F) 0.65; ^1H NMR δ 2.45 ($COCH_2$), 2.68 ($CH_2\beta$), 2.88 (CH_2 malonate), 3.02 (NCH_3), 4.17 ($CH\alpha$), 7.13 (Ph), 7.97 (NH), 9.72 ppm (OH); HPLC $AcONH_4$/MeCN (85/15) 8 min 12 s. Anal. ($C_{14}H_{18}N_2O_5$) C, H, N.

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Registry No. (*R*)-1, 115364-50-6; (*S*)-1, 115364-82-4; (*R*)-2, 115364-52-8; (*S*)-2, 58207-46-8; (*R*)-3, 115364-53-9; (*S*)-3, 115364-85-7; 4, 115364-54-0; 5, 115364-55-1; (*R,R*)-6, 115364-56-2; (*R,S*)-6, 115364-89-1; (*S,R*)-6, 115364-90-4; (*S,S*)-6, 115364-91-5; (*R,R*)-7, 115364-57-3; (*R,S*)-7, 115364-86-8; (*S,R*)-7, 115364-87-9; (*S,S*)-7, 115364-88-0; (*R,R*)-8, 115364-58-4; (*R,S*)-8, 115364-83-5; (*S,R*)-9, 115364-59-5; (*S,S*)-9, 115364-84-6; 10, 115364-60-8; 11, 115364-62-0; 12, 115364-63-1; 13, 115364-64-2; (*R*)-14, 101555-61-7; (*S*)-14, 51871-62-6; (*R*)-15, 115364-65-3; (*S*)-15, 115364-92-6; (*R*)-16, 115364-67-5; (*S*)-16, 115364-94-8; (*R*)-17, 115364-68-6; (*S*)-17, 115364-95-9; 18, 115364-69-7; 19, 115364-70-0; (*R,R*)-20, 115364-71-1; (*R,S*)-20, 115364-97-1; (*R,R*)-21, 115364-72-2; (*R,S*)-21, 115383-46-5; (*R,R*)-22, 115364-73-3; (*R,S*)-22, 115364-99-3; (*R,R*)-23, 115364-74-4; (*R,S*)-23, 115364-98-2; (*R,R*)-24, 115364-75-5; (*R,S*)-24, 115364-96-0; (*R,R*)-25, 115364-76-6; (*R,S*)-25, 115365-00-9; 26, 115364-77-7; 27, 115364-79-9; 28, 115364-80-2; 29, 115364-81-3; DAP, 9032-67-1; BOC-D-Phe-OH, 18942-49-9; BOC-Phe-OH, 13734-34-4; H_2NOCH_2Ph·HCl, 2687-43-6; $MeNHOCH_2Ph$, 22513-22-0; $HOOCCH_2COOCH_2Ph$, 40204-26-0; (±)-HOOCCH(CH_3)COOEt, 81110-31-8; (±)-HOOCCH(CH_2Ph)COOEt, 67682-05-7; (*R*)-(BOC)NHCH(CH_2Ph)$COCHN_2$, 115313-19-4; (*S*)-(BOC)NHCH(CH_2Ph)$COCHN_2$, 60398-41-6; EC 3.4.24.11, 82707-54-8; EC 3.4.11.2, 9054-63-1.

Phosphonoformate and Phosphonoacetate Derivatives of 5-Substituted 2′-Deoxyuridines: Synthesis and Antiviral Activity

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The synthesis of potential "combined prodrugs" wherein phosphonoformate or phosphonoacetate was attached to the 5′-position of 2′-deoxyuridine, 2′-deoxythymidine, 5-iodo-2′-deoxyuridine (IDU), 5-(2-chloroethyl)-2′-deoxyuridine (CEDU), or 5-(2-bromovinyl)-2′-deoxyuridine (BVDU) or to the 3′-position of CEDU is described. The antiviral activities of these derivatives and of reference compounds were compared in Vero, HEp-2, and primary rabbit kidney cells against herpes simplex virus types 1 and 2 (HSV-1 and -2). The CEDU and BVDU analogues were also evaluated against systemic and intracutaneous HSV-1 infection in mice. The nature of the 5-substituent proved critical for antiviral activity, since only the 5-iodo-, 5-(2-bromovinyl)-, and 5-(2-chloroethyl)-substituted derivatives were inhibitory to the herpesviruses. Furthermore, the type specificity is determined by the nature of the 5-substituent: the IDU analogues were similarly inhibitory to HSV-1 and -2 whereas the CEDU and BVDU analogues inhibited HSV-2 replication only at considerably higher concentrations than HSV-1. In vivo, several derivatives were shown to possess significant antiviral activity; however, none surpassed its respective parent compound, CEDU or BVDU, in potency. It seems improbable, therefore, that a synergistic effect between PFA or PAA and the nucleoside analogue occurred. The extent of in vitro and in vivo activity of the CEDU and BVDU 5′-phosphonoformates and 5′-phosphonoacetates is most plausibly explained by the ease by which the "combined prodrugs" are hydrolyzed and the parent compound, CEDU an BVDU, respectively, is released.

The class of 5-substituted pyrimidine nucleoside analogues comprises many compounds that possess significant and therapeutically useful antiherpesvirus activity.[1] Two of the most potent and selective antiviral representatives of this class are (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU)[2] and the recently described 5-(2-chloroethyl)-2′-deoxyuridine (CEDU).[3] Both compounds effectively inhibit herpes simplex virus type 1 (HSV-1) in vitro and in vivo.[2-10] Their selectivity is attested by their high antiviral indexes, which are 2000 and 5000 for CEDU and BVDU, respectively, as determined by the ratio of the minimum toxic dose for the normal host cell to the minimum inhibitory dose for HSV-1. In vivo CEDU is effective against systemic HSV-1 infection at a dose that is about 10-fold lower than those required for BVDU and the reference compound acyclovir (ACV), whereas in vitro BVDU is active at about 1/10 the concentration of CEDU.[3,9,10]

Phosphonoformic acid (PFA) and phosphonoacetic acid (PAA) have been reported to be inhibitory to herpesvirus replication in tissue culture and to be effective in the treatment of several herpesvirus infections of animals.[11]

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(1) De Clercq, E. In *Approaches to Antiviral Agents*; Harnden, M. R., Ed.; Macmillan: London, 1985, p 57.
(2) De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P. J.; Jones, A. S.; Walker, R. T. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2947.
(3) Griengl, H.; Bodenteich, M.; Hayden, W.; Wanek, E.; Streicher, W.; Stütz, P.; Bachmayer, H.; Ghazzouli, I.; Rosenwirth, B. *J. Med. Chem.* **1985**, *28*, 1679.
(4) Maudgal, P. C.; De Clercq, E.; Descamps, J.; Missotten, L.; De Somer, P.; Busson, R.; Vanderhaeghe, H.; Verhelst, G.; Walker, R. T.; Jones, A. S. *Antimicrob. Agents Chemother.* **1980**, *17*, 8.
(5) De Clercq, E.; Zhang, Z.-X.; Descamps, J.; Huygen, K. *J. Infect. Dis.* **1981**, *143*, 846.
(6) Park, N.-H.; Pavan-Langston, D.; Boisjoly, H. M.; De Clercq, E. *J. Infect. Dis.* **1982**, *145*, 909.
(7) De Clercq, E.; Zhang, Z.-X.; Sim, I. S. *Antimicrob. Agents Chemother.* **1982**, *22*, 421.
(8) Soike, K. F.; Gibson, S.; Gerone, P. J. *Antiviral Res.* **1981**, *1*, 325.
(9) De Clercq, E.; Rosenwirth, B. *Antimicrob. Agents Chemother.* **1985**, *28*, 246.
(10) Rosenwirth, B.; Griengl, H.; Wanek, E.; De Clercq, E. *Antiviral Res.* **1985**, Suppl. 1, 21.
(11) Öberg, B. *Pharmacol. Ther.* **1983**, *19*, 387.

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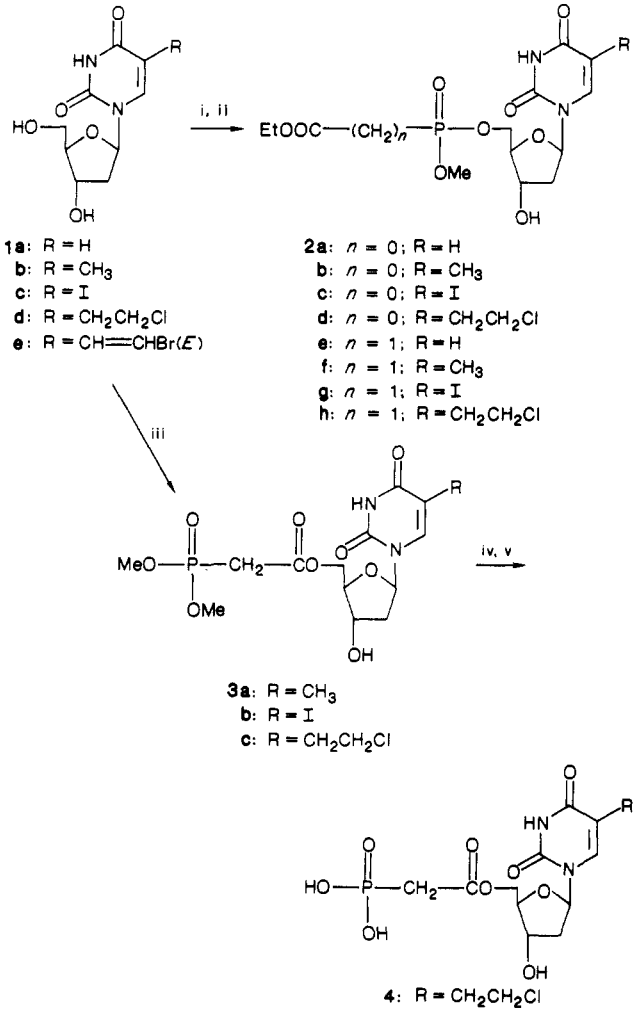
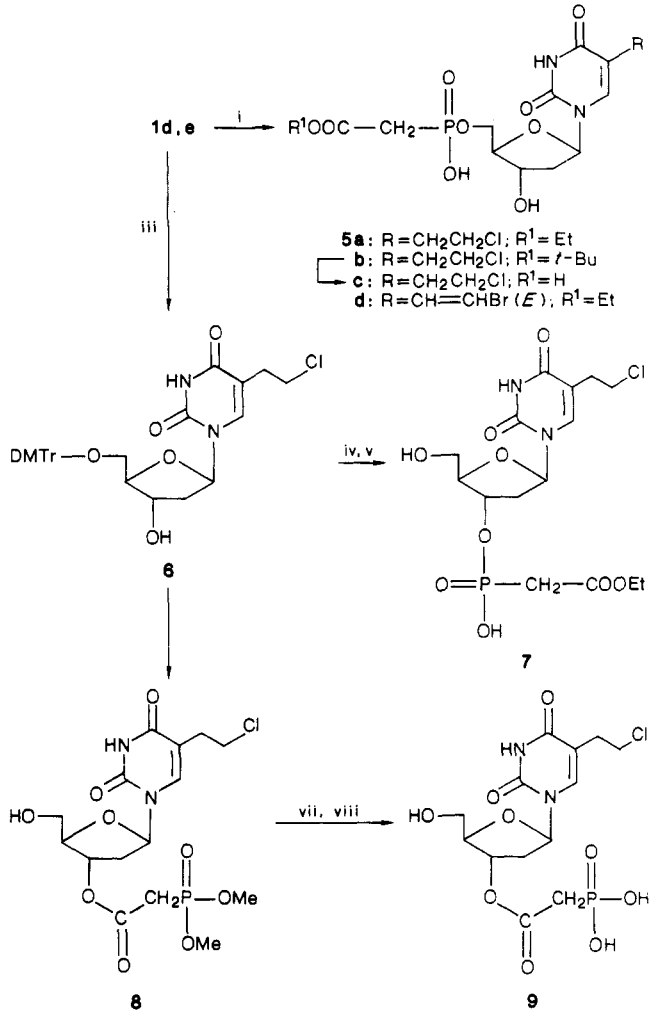
Scheme I[a]



[a] (i) $EtOOC(CH_2)_nP(O)(OMe)Cl$; (ii) $KHCO_3$; (iii) $(MeO)_2P(O)CH_2COOH$, DCC; (iv) Me_3SiCl/KI; (v) MeOH.

Scheme II[a]



[a] (i) $R^1OOCCH_2P(O)(OH)_2$/Pyr, DCC; (ii) TFA; (iii) DMTrCl/Pyr; (iv) $EtOOCCH_2P(O)(OH)_2$/Pyr, DCC; (v) HCl; (vi) $(MeO)_2P(O)CH_2COOH$/Pyr, DCC; (vii) Me_3SiCl/KI; (viii) MeOH.

An attractive approach to antiviral chemotherapy is the combination of two inhibitory substances with the aim of potentiating antiviral activity while minimizing toxic effects and preventing the emergence of resistant mutants. The administration of pairs of drugs, which interfere with viral replication in different ways, may result in enhancement of synergism. The combination of acyclovir (ACV) and PFA has been shown to inhibit HSV-1 in a synergistic manner in vitro[12] and in vivo.[13] Similarly, BVDU and PFA have been reported to be highly synergistic against herpes simplex virus type 2 (HSV-2).[14] Nucleoside analogues such as ACV and BVDU in their triphosphate form interact with the viral DNA polymerase. Resistance to these drugs may be mediated by a mutation in the polymerase gene but also by a mutation in the thymidine kinase gene.[15,16] PFA presumably binds to the pyrophosphate exchange site of the viral DNA polymerase.[17] The positions of the loci for resistance to ACV, BVDU, and PFA have been mapped in the polymerase gene in close proximity,[18] although the loci are not identical:[19] Certain mutants being resistant to ACV as a result of an altered DNA polymerase have been found to be sensitive to PFA.[20,21] Thus, the modes of action of ACV and BVDU, on the one hand, and PFA, on the other, may be different enough to explain the observed synergistic effects.[12,13]

The chemical "combination" of PFA or PAA with antivirally active nucleoside analogues could result in compounds, which act as "combined prodrugs". Within the cells or the organism, metabolic conversion might generate both active parts, namely the nucleoside analogue and the pyrophosphate analogue, which could then exert their antiviral effects in a synergistic manner. In this perspective, we envisaged the synthesis of "combined prodrugs" whereby phosphonoformate or phosphonoacetate were attached to the 5′-position of 2′-deoxyuridine, 2′-deoxythymidine, 5-iodo-2′-deoxyuridine (IDU), CEDU, or BVDU or to the 3′-position of CEDU and investigated

(12) Janz, C.; Wigand, R. *Arch. Virol.* **1982**, *73*, 135.
(13) Burkhardt, U.; Wigand, R. *J. Med. Virol.* **1983**, *12*, 137.
(14) Smith, K. O.; Galloway, K. S.; Ogilvie, K. K.; Cheriyan, U. O. *Antimicrob. Agents Chemother.* **1982**, *22*, 1026.
(15) Schnipper, L. E.; Crumpacker, C. S. *Proc. Nat. Acad. Sci. U.S.A.* **1980**, *77*, 2270.
(16) Coen, D.; Schaffer, P. A. *Proc. Nat. Acad. Sci. U.S.A.* **1980**, *77*, 2273.
(17) Mao, J. C. H.; Robishaw, E. E.; Overby L. R. *J. Virol.* **1975**, *15*, 1281.
(18) Crumpacker, C. S.; Chartrand, P.; Subak-Sharpe, J. H.; Wilkie, N. M. *Virology* **1980**, *105*, 171.
(19) Crumpacker, C. S.; Schnipper, L. E.; Kowalsky, P. N.; Sherman, D. M. *J. Infect. Dis.* **1982**, *146*, 167.
(20) Furman, P. A.; Coen, D. M.; St. Clair, M. H.; Schaffer, P. A. *J. Virol.* **1981**, *40*, 936.
(21) Larder, B. A.; Darby, G. *Antimicrob. Agents Chemother.* **1986**, *29*, 894.

Table I. Antiviral Activity of the Test Compounds CEDU, IDU, PAA, PFA, and ACV in Vero and HEp Cells

compd	minimum cytotoxic concentration,[a] μM	minimum inhibitory concentration,[b] μM							
		HSV-1 (Brand)		HSV-2 (K979)		TK⁻ HSV-1		TK⁻ HSV-2	
		Vero	HEp-2	Vero	HEp-2	Vero	HEp-2	Vero	HEp-2
2a	≥872	>264	>264	>264	264	nt	nt	nt	nt
2b	≥842	>255	>255	>255	>255	nt	nt	nt	nt
2c	198	0.6	2.0	5.9	5.9	19.8	2.0	5.9	5.9
2d	749	0.7	0.7	227	22.7	68.1	>227	>227	>227
2e	≥842	>255	>255	>255	>255	nt	nt	nt	nt
2f	≥812	>246	>246	>246	>246	nt	nt	nt	nt
2g	193	1.9	5.8	57.9	57.9	57.9	1.9	>193	193
2h	726	6.6	66.0	>220	>220	66.0	>220	>220	>220
3a	≥842	>255	>255	>255	>255	nt	nt	nt	nt
3b	655	0.6	0.2	5.9	5.9	198	2.0	>198	59.5
3c	749	6.8	6.8	>227	>227	227	>227	>227	>227
4	800	24.2	24.2	>242	>242	72.7	242	72.7	242
5a	749	>227	>227	>227	>227	nt	nt	nt	nt
5b	≥704	>213	>213	>213	>213	nt	nt	nt	nt
5c	766	2.3	2.3	232	69.6	23.2	23.2	>232	232
7	749	>227	>227	>227	>227	nt	nt	nt	nt
8	227	0.7	68.1	22.7	6.8	227	>227	227	>227
9	800	24.2	72.7	242	72.7	242	>242	>242	>242
10	658	2.0	2.0	>199	59.8	59.8	>199	199	>199
11a	≥800	24.2	24.2	>242	242	242	>242	>242	>242
11b	≥773	23.4	23.4	>234	234	70.3	>234	234	>234
11c	675	20.5	20.5	>205	>205	20.5	>205	205	>205
12a	≥800	>242	>242	>242	>242	nt	nt	nt	nt
12b	≥773	234	>234	>234	>234	nt	nt	nt	nt
1c(IDU)	281	0.3	0.8	2.8	0.8	8.4	0.8	>281	8.4
1d(CEDU)	1127	0.03	0.1	102	10.2	34.2	>342	>342	>342
PAA	2.356	71.4	214	71.4	71.4	71.4	214	71.4	214
PFA	2.619	238	794	238	238	79.4	794	79.4	238
ACV	440	0.01	4.4	1.3	13.2	44	440	132	440

[a,b] The lowest concentration of compound causing at least 25% inhibition of virus-induced cytopathic effect (MIC) and the lowest concentration causing microscopically visible toxic effects on uninfected cells (MTC); nt = not tested.

their antiviral activity in vitro and in vivo .

Chemistry

Phosphonoformates **2a–d** and phosphonoacetates **2e–h** were prepared by reaction of nucleosides or nucleoside analogues **1** with the corresponding phosphonic acid ester chloride (Scheme I). On the other hand, linkage by a carboxylic ester bond was accomplished by condensation of **1b–d** with dimethoxyphosphinyl acetic acid with DDC to give phosphonates **3**. The free phosphonic acid **4** was obtained from **3c** by treatment with chlorotrimethylsilane/potassium iodide and subsequent methanolysis.[22-24]

Application of the DCC method was also successful for the preparation of monophosphonates **5a,b,d** and **11a,c–e** (Schemes II and III). With **1d** and (ethoxycarbonyl)-phosphonic acid as starting materials, the same reaction yielded both 3'- and 5'-phosphorylated compounds **11b** and **12b**, which were separated by column chromatography. Similar reactions of phosphonic acids with nucleosides were reported in the literature with tris(triisopropyl)benzenesulfonyl chloride,[25] 1-(4-tolylsulfonyl)-1*H*-1,2,4-triazole,[26] or mesitylene-1,3-disulfonyl chloride[27] as condensation agent.

When anilinium (ethoxycarbonyl)phosphonate was used as starting material in DMF/pyridine as solvent, phosphonamide **10** was obtained instead of the corresponding hydrogen phosphonate **11b**.

Table II. Antiviral Activity of the Test Compounds BVDU, CEDU, PAA, and PFA in PRK Cells

compd	minimum cytotoxic concentration,[a] μM	minimum inhibitory concentration,[b] μM		
		HSV-1	HSV-2	TK⁻HSV-1
5d	≥828	41.4	>828	>828
11d	≥87.9	1.5	>87.9	>87.9
11e	≥81.1	4.1	>81.1	>81.1
1e (BVDU)	>1302	0.07	32.6	>1302
1d (CEDU)	>1366	0.3	34.2	>1366
PAA	>2856	286	143	286
PFA	>3174	238	159	317

[a,b] The lowest concentration of compound causing 50% inhibition of virus-induced cytopathic effect (MIC) and the lowest concentration causing microscopically visible toxic effects on uninfected cells (MTC). Average values for three HSV-1 (KOS, F. McIntyre), three HSV-2 (G, 196, Lyons), and two TK⁻HSV-1 (B2006, VMW 1837) strains.

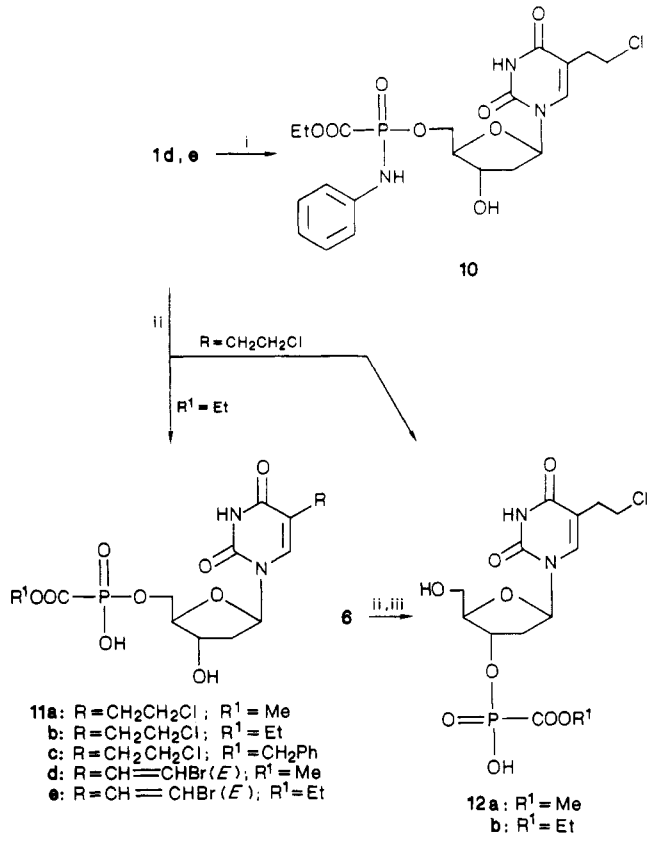
With the aim to synthesize a nucleoside derivative with a free carboxylic acid function, the *tert*-butyl ester **5b** was cleaved under acidic conditions, yielding the phosphonoacetic acid **5c**.[28]

Some representative 3'-phosphonates **7**, **8**, and **12** were prepared in a protection–condensation–deprotection sequence via standard procedures.[29,30] 3'-Phosphonate **9** was obtained from **8** in the same manner as outlined above for **4**.

The structures of all commpounds were confirmed by ^{1}H NMR spectroscopy. 3'- and 5'-derivatives were dis-

(22) Sekine, M.; Hata, T. *J. Chem. Soc., Chem. Commun.* **1978**, 285.
(23) Machida, Y.; Nomoto, S.; Saito, I. *Synth. Commun.* **1979**, *9*, 97.
(24) Morita, T.; Okamoto, Y.; Sakurai, H. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 267.
(25) Heimer, E. P.; Grove, C.; Nussbaum, A. L.; Montclair, U. US Pat. 4056637, 25 Oct 1977, *Chem. Abstr.* **1978**, *88*, 51130s.
(26) Sekine, M.; Mori, H.; Hata, T. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 239.
(27) Kume, A.; Fujii, M.; Sekine, M.; Hata, T. *J. Org. Chem.* **1984**, *49*, 2139.
(28) Michalski, J.; Musierowicz, S. *Angew. Chem.* **1967**, *79*, 1070; *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 1079.
(29) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821.
(30) Sekine, M.; Matsuzaki, J.; Hata, T. *Tetrahedron Lett.* **1981**, 3209.

Scheme III[a]



[a] (i) $EtOOCP(O)(OH)_2 \cdot PhNH_2$/DCC, DMF/Pyr; (ii) $ROOCP(O)(OH)_2$/Pyr, DCC; (iii) HCl.

tinguished easily by the spectral data. The 3′-CH multiplet of 5′-phosphonates is embodied in a 3.3–4.7 ppm multiplet whereas 3′-phosphonates showed a clearly separated downfield-shifted signal at 4.8–4.9 ppm. In compounds **8** and **9** the 3′-CH resonance appeared at 5.15 ppm. Regarding the OH groups, the assignment of which was confirmed by D_2O exchange, a broad doublet at 5.3–5.5 ppm (OH-3′) was obtained for 5′-phosphonates **3c**, **5a,b,d**, while 3′-derivatives **7**, **8**, **12** gave a broad triplet at 5.0–5.2 ppm (OH-5′).

Biological Results and Discussion

The antiviral activities of test and reference compounds and their toxic effects on host cells were determined in Vero and HEp-2 cells (Table I) and in primary rabbit kidney (PRK) cells (Table II). It is evident that the nature of the 5-substituent is critical for antiviral activity, since only the 5-iodo (**2c**, **2g**, **3b**), 5-(*E*)-(2-bromovinyl) (**5d**, **11d**, **11e**), and the 5-(2-chloroethyl) (**2d**, **2h**, **3c**) derivatives proved inhibitory to the herpesviruses. Furthermore, the type-specificity is determined by the nature of the 5-substituent: the IDU analogues were similarly inhibitory to HSV-1 and -2 whereas the CEDU and BVDU analogues, like CEDU (**1d**) and BVDU (**1e**) themselves,[1-3] inhibited HSV-2 replication only at considerably higher concentrations than HSV-1. None of the phosphonate derivatives surpassed their parent compound IDU, CEDU, or BVDU, respectively, in antiviral activity in vitro.

The compounds were also evaluated for inhibition of thymidine kinase (TK) deficient herpesvirus mutants, which are resistant to nucleoside analogues depending on virus-specific phosphorylation like BVDU and CEDU but are sensitive to PAA and PFA. Only in two cases, namely **4** and **5c**, lower MIC values were obtained for the derivatives than for the parent compound, indicating that in these cases the phosphonate moiety may have contributed to antiviral activity. Both derivatives contain PAA in a completely unblocked form in the 5′-position; in **4** PAA is attached via its carboxylic group, in **5c** it is attached via its phosphate group. These two derivatives as well as **9** are the only compounds in which the phosphonate moiety is unblocked.

The derivatives substituted with 2-chloroethyl in the C-5 position of the pyrimidine ring can be divided in the following four classes: (i) 5′-phosphono, (ii) 5′-carboxylic, (iii) 3′-phosphono, and (iv) 3′-carboxylic derivatives. The classes may be subdivided in PAA (n = 1) and PFA (n = 0) derivatives. These compounds, as well as the phosphono derivatives of BVDU, were evaluated in vivo against systemic (ip) HSV-1 infection of NMRI mice (Table III) and some also against intracutaneous infection of hairless mice (Table IV).

Of the PFA derivatives (n = 0) of class i, **2d** emerged as the most potent antiviral compound in vitro and in vivo. The two derivatives carrying additional blocking groups at the phosphate group (**2d**, **10**) were more active in vitro than those blocked only at the carboxylic group (**11b**). In vivo, the order of antiviral activities within this group of derivatives was for po administration: **2d** > **10** > **11c** > **11b** > **11a**, where **11a** was inactive and **11b** only marginally active; and for ip administration: **2d** > **11b** > **11c** > **10** ≥ **11a**, where **2d** was almost equally potent as its parent compound CEDU; **2d** was also as potent as CEDU when applied topically in the cutaneous infection model. Compounds **11b** and **11c** proved inactive in this model. Interestingly, for **10** the antiviral activity largely depended on the route of administration of the compound. There was no clear correlation between in vitro and in vivo activity, as is evident from a comparison of the activities of compounds **10** and **11c** when given ip versus in vitro.

Of the PAA-related compounds (n = 1) of class i, the completely unblocked derivative **5c** was the most potent in vitro and in vivo. Upon po administration, all compounds of this class were only marginally active (**5c**, **5a**) or inactive (**5b**, **2h**); given ip **5b** and **2h** were inactive, **5a** was marginally active, and **5c** was about 10 times less active than CEDU. When applied topically **5c** showed antiviral activity and **2h** was inactive. As was observed for the PFA-related group of compounds, an additional blocking group at the free oxygen bond of the phosphate group proved advantageous for in vitro activity (**2h** versus **5a**), although for **2h** the activity was highly cell line dependent and, again, not predictive for in vivo potency.

Among the 5′-carboxylic CEDU derivatives ii, only PAA-related compounds (n = 1) were synthesized and evaluated. Again, the compound carrying methyl groups at the free oxygen bonds of the phosphate group (**3c**) was slightly more active in vitro against HSV-1 (Brand) than its deblocked analogue (**4**), although in vivo they were about equipotent (ip) against this virus strain; **4** was even more potent than **3c** when administered orally. Compound **4** was also active when applied topically in the cutaneous model. Thus, again the in vitro and in vivo activities did not covary for this class of compounds.

Of the 3′-phosphono derivatives iii (n = 0 and n = 1), none showed in vitro antiviral activity. In vivo, the PFA-related compound **12b** proved inactive when given po and moderately active when given ip. When **12b** was applied topically, no beneficial effect could be detected, which was also true for the PAA-related analogue **7**. Compound **12b** resembles **11b**, its 5′ analogue, in activity. Thus, for this pair of compounds the position of the PFA moiety seems to be irrelevant for in vivo antiviral activity.

Table III. Activity of the Test Compounds CEDU, BVDU, and ACV against Systemic (Intraperitoneal) HSV-1 Infection in Mice

test compd	treatment regimen: route of administration	treatment regimen: daily dose, mg/kg	cumulative mortality in percent of infected mice (*p* value)	mean survival time in days (*p* value)
2d	po	10	13 (<0.001)	18.9 (<0.001)
		5	38 (<0.001)	16.0 (0.001)
		1	63 (ns)	13.9 (0.015)
2h	po	10	67 (ns)	11.5 (ns)
		5	75 (ns)	12.8 (ns)
		1	100 (ns)	8.9 (ns)
3c	po	10	75 (ns)	12.2 (ns)
		5	83 (ns)	11.2 (ns)
		1	58 (ns)	13.5 (ns)
4	po	10	42 (0.017)	16.3 (0.003)
		5	50 (0.032)	15.3 (0.014)
		1	67 (ns)	11.8 (ns)
5a	po	10	42 (0.017)	15.9 (0.003)
		5	58 (ns)	13.6 (ns)
		1	83 (ns)	11.9 (ns)
5b	po	10	58 (ns)	13.3 (ns)
		5	83 (ns)	11.3 (ns)
		1	83 (ns)	11.5 (ns)
5c	po	10	50 (0.032)	15.3 (0.013)
		5	92 (ns)	11.2 (ns)
		1	100 (ns)	8.6 (ns)
5d	po	100	80[a] (ns)	
10	po	10	25 (<0.001)	17.8 (<0.001)
		5	50 (0.032)	15.4 (0.005)
		1	100 (ns)	11.4 (ns)
11a	po	10	75 (ns)	13.7 (ns)
		5	67 (ns)	12.7 (ns)
		1	75 (ns)	12.6 (ns)
11b	po	10	50 (0.032)	15.3 (0.010)
		5	75 (ns)	11.9 (ns)
		1	92 (ns)	10.1 (ns)
11c	po	10	33 (0.001)	16.8 (0.006)
		5	50 (0.032)	14.3 (ns)
		1	67 (ns)	12.7 (ns)
11d	po	100	50[a] (ns)	
11e	po	100	70[a] (ns)	
12b	po	10	75 (ns)	11.8 (ns)
		5	75 (ns)	11.9 (ns)
		1	83 (ns)	11.3 (ns)
BVDU (**1e**)	po	100	30[a] (<0.025)	
CEDU (**1d**)	po	5	10 (<0.001)	19.0 (<0.001)
ACV	po	100	10 (<0.001)	18.9 (<0.001)
placebo[b]	po		84	10.8
2d	ip	5	0 (<0.001)	20.0 (<0.001)
		1	42 (0.002)	15.6 (0.007)
		0.5	33 (<0.001)	16.7 (<0.001)
2h	ip	5	83 (ns)	11.8 (ns)
		1	92 (ns)	11.9 (ns)
		0.5	58 (ns)	13.9 (ns)
3c	ip	5	8 (<0.001)	19.2 (<0.001)
		1	75 (ns)	13.0 (ns)
		0.5	67 (ns)	13.3 (ns)
4	ip	5	17 (<0.001)	18.3 (<0.001)
		1	33 (0.003)	16.0 (0.039)
		0.5	75 (ns)	12.2 (ns)
5a	ip	5	42 (0.014)	15.5 (ns)
		1	83 (ns)	11.3 (ns)
		0.5	75 (ns)	12.2 (ns)
5b	ip	5	50 (ns)	15.4 (ns)
		1	67 (ns)	12.8 (ns)
		0.5	67 (ns)	12.9 (ns)
5c	ip	5	25 (<0.001)	17.3 (0.004)
		1	58 (ns)	14.1 (ns)
		0.5	50 (ns)	14.2 (ns)
10	ip	5	25 (<0.001)	18.0 (<0.001)
		1	67 (ns)	13.9 (ns)
		0.5	75 (ns)	13.1 (ns)
11a	ip	5	33 (0.003)	16.3 (0.013)
		1	58 (ns)	14.1 (ns)
		0.5	75 (ns)	11.8 (ns)
11b	ip	5	0 (<0.001)	20.0 (<0.001)
		1	33 (0.003)	16.5 (0.011)
		0.5	58 (ns)	13.8 (ns)
11c	ip	5	8 (<0.001)	19.3 (<0.001)
		1	33 (0.003)	16.3 (0.015)
		0.5	67 (ns)	13.8 (ns)
12b	ip	5	8 (<0.001)	18.9 (<0.001)
		1	42 (0.014)	15.6 (ns)
		0.5	83 (ns)	11.5 (ns)
CEDU (**1d**)	ip	0.5	35 (<0.001)	16.7 (<0.001)
ACV	ip	25	15 (<0.001)	18.6 (<0.001)
placebo	ip		82	11.4

[a] As compared to 90% cumulative mortality for the placebo group; mice inoculated with KOS strain of HSV-1. [b] The vehicle was used as placebo.

Table IV. Activity of Selected Test Compounds and CEDU against Intracutaneous HSV-1 Infection in Mice

test compd	daily dose[a]	cumulative lesions in percent of infected mice (*p* value)	mean duration of lesions in days (*p* value)
2d	0.3	17 (0.001)	1.4 (0.001)
	0.1	33 (0.018)	3.3 (0.003)
2h	0.3	58 (ns)	6.0 (ns)
	0.1	83 (ns)	10.6 (ns)
4	0.1	40 (ns)	2.4 (0.001)
5c	0.1	60 (ns)	5.3 (0.005)
7	0.1	80 (ns)	9.4 (ns)
11b	0.1	80 (ns)	6.7 (ns)
11c	0.1	100 (ns)	8.1 (ns)
12b	0.1	90 (ns)	9.7 (ns)
CEDU (**1d**)	0.3	17 (0.001)	0.9 (<0.001)
	0.1	40 (ns)	2.4 (0.001)
placebo[b]		81	9.8

[a] Expressed in percentage (wt/vol) of active compound in its vehicle (AZDMSO) (see the Experimental Section for details). [b] AZDMSO was used as placebo.

Within the class of the 3′-carboxylic derivatives iv, the PAA-related compound carrying methyl groups at the free oxygen bonds of the phosphate group, **8**, was more potent in vitro than its deblocked analogue **9**; the activity was highly cell line dependent as was also true for **2h**. Neither **8** nor **9** was evaluated in vivo.

The too limited number of (*E*)-5-(2-bromovinyl)-substituted derivatives that were synthesized (**5d**, **11d**, **11e**) did not allow a similar structure–function analysis as with the 5-(2-chloroethyl)-substituted derivatives. For those (*E*)-5-(2-bromovinyl) derivatives that were synthesized, the in vivo activity paralleled the in vitro activity; thus, in order of decreasing antiviral activity, **1e** > **11d** > **11e** > **5d**.

In conclusion, we postulate that the in vitro and in vivo activity of the CEDU and BVDU 5′-phosphonoformates and 5′-phosphonoacetates is due to the release of their parent compounds, CEDU and BVDU, respectively. The extent of in vitro and in vivo activity can best be explained by the ease by which the "combined prodrugs" are hydrolyzed. None of the derivatives surpassed CEDU or BVDU in in vitro or in in vivo antiviral activity. It seems improbable, therefore, that a synergistic effect between PFA or PAA and the nucleoside analogue has occurred. Apparently, the antiviral potency of CEDU (and BVDU) is so much higher than those of PFA or PAA that the additional presence of the latter compounds at the doses released from the "combined prodrugs" does not contribute

to the antiviral effects observed. Vice versa, the antiviral effects observed with the 5′-phosphonoformate derivatives of adenosine, guanosine, 2′-deoxyadenosine, and 2′-deoxyguanosine, as recently reported by Vaghefi et al.,[31] may entirely be accounted for by PFA released from the derivatives upon hydrolysis, as, in this case, the nucleosides would not be contributory to the antiviral activity.

Experimental Section[41]

Chemistry. Melting points were determined on a Büchi melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Hitachi Perkin-Elmer R-24B (60 MHz) and a Bruker WH-90 (90 MHz) spectrometer with DMSO-d_6 as solvent and TMS as standard. The spectral data were in full agreement with the assigned structures. The analytical results obtained for all new compounds were within ±0.4% of the theroretical values (C, H, N). All solvents were dried and distilled prior to their use. Column chromatography was performed on silica gel 60 (230–400 mesh, Merck, Darmstadt) with the following solvents: A, $CHCl_3$/MeOH (9:1, v/v); B, $CHCl_3$/MeOH (2:1, v/v).

The phosphonic acid chlorides [(chloromethoxyphosphinyl)-acetic acid ethyl ester, chloromethoxyphosphinecarboxylic acid ethyl ester oxide, respectively] were prepared via known procedures.[32] (Dimethoxyphosphinyl)acetic acid was synthesized according to Malevannaya et al.[33] The pyridinium salts of the phosphonic acids were obtained by reaction of the corresponding *O,O*-bis(trimethylsilyl)phosphonic acid esters[22-24] with methanol/pyridine.

General Procedure for the Preparation of 2a–d. A solution of 5.0 mmol of the corresponding nucleoside 1 in dry DMF (20 mL) was cooled to −50 °C, and 0.62 mL (5.0 mmol) of chloromethoxyphosphinecarboxylic acid ethyl ester oxide was added. After being stirred at −50 °C for 20 min, the reaction was quenched by the addition of $KHCO_3$ (0.50 g, 5.0 mmol). The solvent was removed in vacuo (0.1 mmHg) at room temperature, and the residue was stirred with 50 mL of $CHCl_3$ and filtered. The esters were obtained as glass after column chromatography with a $CHCl_3$/MeOH gradient (start, 100/0; end, 95/5) as eluent.

[[1-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy]methoxyphosphinecarboxylic acid ethyl ester oxide (2a): yield 1.28 g (68%); glass. Anal. ($C_{13}H_{19}N_2O_9P$) C, H, N.

[[1-(2,4-Dioxo-5-methyl-1,2,3,4-tetrahydropyrimidin-1-yl)-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy]methoxyphosphinecarboxylic acid ethyl ester oxide (2b): yield 1.25 g (65%); glass. Anal. ($C_{14}H_{21}N_2O_9P$) C, H, N.

[[1-(2,4-Dioxo-5-iodo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy]methoxyphosphinecarboxylic acid ethyl ester oxide (2c): yield 1.69 g (62%); glass. Anal. ($C_{13}H_{18}IN_2O_9P$) C, H, N.

[[1-[5-(2-Chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy]methoxyphosphinecarboxylic acid ethyl ester oxide (2d): yield 1.45 g (66%); glass. Anal. ($C_{15}H_{22}ClN_2O_9P$) C, H, N.

Reaction of the Nucleosides and Nucleoside Analogues 1 with (Chloromethoxyphosphinyl)acetic Acid Ethyl Ester. A 5.0-mmol portion of the corresponding 1 (dissolved in 20 mL of dry DMF) was treated with 0.7 mL (5.0 mmol) of (chloromethoxyphosphinyl)acetic acid ethyl ester for 2 h at room temperature. The mixture was quenched with $KHCO_3$ (0.50 g, 5.0 mmol) and worked up as described above.

[[[1-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy]methoxyphosphinyl]acetic acid ethyl ester (2e): yield 1.00 g (51%); glass. Anal. ($C_{14}H_{21}N_2O_9P$) C, H, N.

[[[1-(2,4-Dioxo-5-methyl-1,2,3,4-tetrahydropyrimidin-1-yl)-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy]methoxyphosphinyl]acetic acid ethyl ester (2f): yield 1.12 g (55%); glass. Anal. ($C_{15}H_{23}N_2O_9P$) C, H, N.

[[[1-(2,4-Dioxo-5-iodo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy]methoxyphosphinyl]acetic acid ethyl ester (2g): yield 1.20 g (46%); glass. Anal. ($C_{14}H_{20}IN_2O_9P$) C, H, N.

[[[1-[5-(2-Chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy]methoxyphosphinyl]acetic acid ethyl ester (2h): yield 1.14 g (50%); glass. Anal. ($C_{16}H_{24}ClN_2O_9P$) C, H, N.

Esterification of Nucleosides and Nucleoside Analogues 1 with (Dimethoxyphosphinyl)acetic Acid. A 5.0-mmol portion of the corresponding 1, (dimethoxyphosphinyl)acetic acid (1.08 g, 6.5 mmol) and dicyclohexylcarbodiimide (2.06 g, 10.0 mmol) were stirred in 30 mL of dry pyridine for 1 h at 45 °C. The precipitated urea was removed by filtration, and the solution was concentrated in vacuo. Purification was performed by column chromatography with eluant A. The resulting hygroscopic esters were obtained as glass.

(Dimethoxyphosphinyl)acetic acid 1-(2,4-dioxo-5-methyl-1,2,3,4-tetrahydropyrimidin-1-yl)-2-deoxy-β-D-*erythro*-pentofuranos-5-yl ester (3a): yield 1.37 g (61%); glass.

(31) Vaghefi, M. M.; McKernan, P. A.; Robins, R. K. *J. Med. Chem.* **1986**, *29*, 1389.
(32) Petrov, K. A.; Maklyaev, F. L.; Korshunov, M. A. *Zh. Obshch. Khim.* **1959**, *29*, 301; *J. Gen. Chem. USSR (Engl. Transl.)* **1959**, 304.
(33) Malevannaya, R. A.; Tsvetkov, E. N.; Kabachnik, M. I. *Zh. Obshch. Khim.* **1972**, *42*, 765; *J. Gen. Chem. USSR (Engl. Transl.)* **1972**, 757.
(34) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. *J. Infect. Dis.* **1980**, *141*, 563.
(35) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature (London)* **1986**, *323*, 464.
(36) Jones, A. S.; Verhelst, G.; Walker, R. T. *Tetrahedron Lett.* **1979**, *45*, 4415.
(37) Schaeffer, H. J.; Beauchamp, L.; de Miranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. *Nature (London)* **1978**, *272*, 583.
(38) Sacks, L. *Angewandte Statistik*, 6th ed.; Springer-Verlag KG: Berlin, 1984; pp 269–281.
(39) De Clercq, E. *Antimicrob. Agents Chemother.* **1984**, *26*, 155.
(40) Swain, A. P.; Braun, D. F.; Naegele, S. K. *J. Org. Chem.* **1953**, *18*, 1087.

(41) Following the recommendations of a referee and consistent with common practice,[31] an abbreviated chemical nomenclature may be used, although this naming is not in accordance with IUPAC rules and Chemical Abstracts practice and cannot be used consequently for all compounds of this paper: **2a**, 2′-deoxyuridine 5′-[(ethoxycarbonyl)-*O*-methyl]phosphonate; **2b**, thymidine 5′-[(ethoxycarbonyl)-*O*-methyl]phosphonate; **2c**, 5-iodo-2′-deoxyuridine 5′-[(ethoxycarbonyl)-*O*-methyl]-phosphonate; **2d**, 5-(2-chloroethyl)-2′deoxyuridine 5′-[(ethoxycarbonyl)-*O*-methyl]phosphonate; **2e**, 2′-deoxyuridine 5′-[[(ethoxycarbonyl)methyl]-*O*-methyl]phosphonate; **2f**, thymidine 5′-[[(ethoxycarbonyl)methyl)-*O*-methyl]phosphonate; **2g**, 5-iodo-2′-deoxyuridine 5′-[[(ethoxycarbonyl)methyl]-*O*-methyl]phosphonate; **2h**, 5-(2-chloroethyl)-2′-deoxyuridine 5′-[[(ethoxycarbonyl)methyl]-*O*-methyl]phosphonate; **3a**, (dimethoxyphosphinyl)acetic acid (thymidin-5′-yl) ester; **3b**, (dimethoxyphosphinyl)acetic acid 5-iodo-2′-deoxyuridin-5′-yl) ester; **3c**, (dimethoxyphosphinyl)acetic acid 5-(2-chloroethyl)-2′-deoxyuridin-5′-yl ester; **4**, [[[5-(2-chloroethyl)-2′-deoxyuridin-5′-yl]carbonyl]methyl]phosphonic acid; **5a**, [(ethoxycarbonyl)methyl]phosphonic acid 5-(2-chloroethyl)-2′-deoxyuridin-5′-yl ester; **5b**, [(*tert*-butoxycarbonyl)methyl]-phosphonic acid 5-(2-chloroethyl)-2′-deoxyuridin-5′-yl ester; **5c**, (carboxymethyl)phosphonic acid 5-(2-chloroethyl)-2′-deoxyuridin-5′-yl ester; **5d**, [(ethoxycarbonyl)methyl]phosphonic acid 5-(*E*)-(2-bromovinyl)-2′-deoxyuridin-5′-yl ester; **6**, 5-(2-chloroethyl)-5′-(4,4′-dimethoxytrityl)-2′-deoxyuridine; **7**, [(ethoxycarbonyl)methyl]phosphonic acid 5-(2-chloroethyl)-2′-deoxyuridin-3′-yl ester; **8**, (dimethoxyphosphinyl)acetic acid 5-(2-chloroethyl)-2′-deoxyuridin-3′-yl ester; **9**, [[[5-(2-chloroethyl)-2′-deoxyuridin-3′-yl]carbonyl]methyl]phosphonic acid; **10**, [5-(2-chloroethyl)-2′-deoxyuridin-5′-yl](phenylamino)-phosphinecarboxylic acid ethyl ester oxide; **11a**, (methoxycarbonyl)phosphonic acid 5-(2-chloroethyl)-2′-deoxyuridin-5′-yl ester; **11b**, (ethoxycarbonyl)phosphonic acid 5-(2-chloroethyl)-2′-deoxyuridin-5′-yl ester; **11c**, [(benzyloxy)carbonyl]-phosphonic acid 5-(2-chloroethyl)-2′-deoxyuridin-5′-yl ester; **11d**, (methoxycarbonyl)phosphonic acid 5-(*E*)-(2-bromovinyl)-2′-deoxyuridin-5′-yl ester; **11e**, (ethoxycarbonyl)-phosphonic acid 5-(*E*)-(2-bromovinyl)-2′-deoxyuridin-5′-yl ester; **12a**, (methoxycarbonyl)phosphonic acid 5-(2-chloroethyl)-2′-deoxyuridin-3′-yl ester; **12b**, (ethoxycarbonyl)-phosphonic acid 5-(2-chloroethyl)-2′-deoxyuridin-3′-yl ester.

Table V. ^{1}H NMR Data

compd	^{1}H NMR δ values	compd	^{1}H NMR δ values
2a	1.30 (3 H, t, $COCH_2CH_3$), 2.21 (2 H, m, H-2'), 3.42 (2 H, m), 3.86 (3 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 4.0–4.6 (7 H, m), 5.67 (1 H, d, H-5), 6.23 (1 H, t, H-1'), 7.58 (1 H, dd, H-6), 10.1 (1 H, NH)	**5c**	2.18 (2 H, m, H-2'), 2.74 (2 H, d, $^2J_{HP}$ = 20 Hz, $POCH_2CO$), 2.8 (2 H, t, CH_2CH_2Cl), 3.3–4.5 (6 H, m), 6.21 (1 H, t, H-1'), 7.63 (1 H, s, H-6), 10.1–10.9 (2 H, br, D_2O exchangable), 11.2 (1 H, NH)
2b	1.37 (3 H, t, $COCH_2CH_3$), 1.90 (3 H, br s, 5-CH_3), 2.24 (2 H, m, H-2'), 3.96 (3 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 4.1–4.6 (7 H, m), 6.39 (1 H, t, H-1'), 7.20 (1 H, br s, H-6), 10.15 (1 H, NH)	**5d**	1.10 (3 H, t, $COCH_2CH_3$), 2.08 (2 H, m, H-2'), 2.74 (2 H, d, $^2J_{HP}$ = 21 Hz, $POCH_2CO$), 3.7–4.3 (6 H, m), 5.5 (1 H, d, 3'-OH), 6.10 (1 H, t, H-1'), 6.79 (1 H, d, J = 14 Hz, vinylic H), 7.25 (1 H, d, J = 14 Hz, vinylic H), 7.84 (1 H, s, H-6), 11.0 (1 H, NH)
2c	1.37 (3 H, t, $COCH_2CH_3$), 2.3 (2 H, m, H-2'), 4.0 (3 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 4.1–4.7 (7 H, m), 6.27 (1 H, t, H-1'), 7.97 (1 H, s, H-6), 10.0 (1 H, NH)	**6**	2.05–2.6 (4 H, m), 3.1–3.4 (5 H, m), 3.73 (6 H, s, OCH_3), 4.3 (1 H, m, H-3'), 5.3 (1 H, d, 3'-OH), 6.17 (1 H, t, H-1'), 7.65–8.5 (14 H, m, aromatic H and H-6), 11.6 (1 H, NH)
2d	1.21 (3 H, t, $COCH_2CH_3$), 2.20 (2 H, m, H-2'), 2.65 (2 H, m, CH_2CH_2Cl), 3.55 (2 H, t, CH_2Cl), 3.80 (3 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 3.95 –4.5 (7 H, m), 6.24 (1 H, t, H-1'), 7.45 (1 H, s, H-6), 10.2 (1 H, NH)	**7**	1.18 (3 H, t, $COCH_2CH_3$), 2.26 (2 H, m, H-2'), 2.63 (2 H, t, CH_2CH_2Cl), 2.65 (2 H, d, $^2J_{HP}$ = 20 Hz, $POCH_2CO$), 3.4–4.2 (7 H, m), 4.89 (1 H, m, H-3'), 5.0 (1 H, t, 5'-OH), 6.17 (1 H, t, H-1'), 7.87 (1 H, s, H-6), 11.5 (1 H, NH)
2e	1.10 (3 H, t, $COCH_2CH_3$), 2.09 (2 H, m, H-2'), 2.90 (2 H, d, $^2J_{HP}$ = 21 Hz, $POCH_2CO$), 3.65 (3 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 3.8–4.5 (7 H, m), 5.60 (1 H, d, H-5), 6.14 (1 H, t, H-1'), 7.53 (1 H, dd, H-6), 10.3 (1 H, NH)	**8**	2.22 (2 H, m, H-2'), 2.59 (2 H, t, CH_2CH_2Cl), 3.16 (2 H, d, $^2J_{HP}$ = 22 Hz, $POCH_2CO$), 3.5–4.05 (5 H, m), 3.61 (6 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 5.15 (1 H, m, H-3'), 5.25 (1 H, t, 5'-OH), 6.13 (1 H, t, H-1'), 7.74 (1 H, s, H-6), 11.5 (1 H, NH)
2f	1.30 (3 H, t, $COCH_2CH_3$), 1.90 (3 H, br s, 5-CH_3), 2.31 (2 H, m, H-2'), 3.08 (2 H, d, $^2J_{HP}$ = 20 Hz, $POCH_2CO$), 3.82 (3 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 4.0–4.6 (7 H, m), 6.35 (1 H, t, H-1'), 7.42 (1 H, br s, H-6), 10.1 (1 H, NH)	**9**	2.23 (2 H, m, H-2'), 2.63 (2 H, t, CH_2CH_2Cl), 2.78 (2 H, d, $^2J_{HP}$ = 21 Hz, $POCH_2CO$), 3.4–4.05 (5 H, m), 5.17 (1 H, m, H-3'), 6.08 (1 H, t, H-1'), 7.69 (1 H, s, H-6), 7.8 (3 H, br s, D_2O exchangable), 11.5 (1 H, NH)
2g	1.10 (3 H, t, $COCH_2CH_3$), 2.20 (2 H, m, H-2'), 3.03 (2 H, d, $^2J_{HP}$ = 21 Hz, $POCH_2CO$), 3.71 (3 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 3.9–4.5 (7 H, m), 6.13 (1 H, t, H-1'), 7.90 (1 H, s, H-6), 10.5 (1 H, NH)	**10**	1.05 (3 H, t, $COCH_2CH_3$), 2.04 (2 H, m, H-2'), 2.59 (2 H, t, CH_2CH_2Cl), 3.3–4.3 (8 H, m), 5.32 (1 H, 3'-OH), 6.04 (1 H, t, H-1'), 7.49 (1 H, s, H-6), 8.4 (1 H, d, $^2J_{HP}$ = 8 Hz, aromatic NH), 11.2 (1 H, NH)
2h	1.10 (3 H, t, $COCH_2CH_3$), 2.12 (2 H, m, H-2'), 2.60 (2 H, t, CH_2CH_2Cl), 2.90 (2 H, d, $^2J_{HP}$ = 21 Hz, $POCH_2CO$), 3.50 (2 H, t, CH_2Cl), 3.60 (3 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 3.85–4.45 (7 H, m), 6.12 (1 H, t, H-1'), 7.35 (1 H, d, H-6), 9.6 (1 H, NH)	**11a**	2.13 (2 H, m, H-2'), 2.67 (3 H, m, CH_2CH_2Cl and H-5'), 3.1–4.1 (4 H, m), 3.53 (3 H, s, OCH_3), 4.26 (1 H, m, H-3'), 5.39 (1 H, d, 3'-OH), 6.13 (1 H, t, H-1'), 7.74 (1 H, s, H-6), 11.4 (1 H, NH)
3a	1.90 (3 H, br s, 5-CH_3), 2.23 (2 H, m, H-2'), 3.10 (2 H, d, $^2J_{HP}$ = 22 Hz, $POCH_2CO$), 3.76 (6 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 4.0–4.6 (5 H, m), 6.28 (1 H, t, H-1'), 7.38 (1 H, s, H-6), 10.4 (1 H, NH)	**11b**	1.15 (3 H, t, $COCH_2CH_3$), 2.10 (2 H, m, H-2'), 2.69 (3 H, m, CH_2CH_2Cl and H-5'a), 3.4–4.1 (6 H, m), 4.23 (1 H, m, H-3'), 5.36 (1 H, d, 3'-OH), 6.10 (1 H, t, H-1'), 7.73 (1 H, s, H-6), 11.4 (1 H, NH)
3b	2.41 (2 H, m, H-2'), 3.20 (2 H, d, $^2J_{HP}$ = 21 Hz, $POCH_2CO$), 3.82 (6 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 4.0–4.75 (5 H, m), 6.22 (1 H, t, H-1'), 8.0 (1 H, s, H-6), 10.0 (1 H, NH)	**11c**	2.12 (2 H, m, H-2'), 2.68 (3 H, m, CH_2CH_2Cl and H-5'a), 3.2–4.2 (4 H, m), 4.29 (1 H, m, H-3'), 5.05 (2 H, s, CH_2Ph), 5.10 (1 H, d, 3'-OH), 6.10 (1 H, t, H-1'), 7.28 (5 H, br s, aromatic H), 7.79 (1 H, s, H-6), 11.4 (1 H, NH)
3c	2.20 (2 H, m, H-2'), 2.70 (2 H, t, CH_2CH_2Cl), 3.23 (2 H, d, $^2J_{HP}$ = 21 Hz, $POCH_2CO$), 3.5–4.4 (6 H, m), 3.67 (6 H, d, $^3J_{HP}$ = 11 Hz, $POCH_3$), 5.40 (1 H, d, 3'-OH), 6.18 (1 H, t, H-1'), 7.89 (1 H, s, H-6), 11.4 (1 H, NH)	**11d**	2.13 (2 H, m, H-2'), 3.32 (3 H, s, OCH_3), 3.9–4.4 (4 H, m), 5.40 (1 H, d, 3'-OH), 6.22 (1 H, t, H-1'), 7.05 (1 H, d, J = 14 Hz, vinylic H), 7.43 (1 H, d, J = 14 Hz, vinylic H), 8.20 (1 H, s, H-6), 11.6 (1 H, NH)
4	2.17 (2 H, m, H-2'), 2.67 (2 H, t, CH_2CH_2Cl), 2.83 (2 H, d, $^2J_{HP}$ = 21 Hz, $POCH_2CO$), 3.7–4.4 (5 H, m), 6.13 (1 H, t, H-1'), 7.65 (1 H, s, H-6), 7.7 (3 H, br s, D_2O exchangable), 11.5 (1 H, NH)	**11e**	1.24 (3 H, t, $COCH_2CH_3$), 2.16 (2 H, m, H-2'), 3.8–4.4 (6 H, m), 5.45 (1 H, br, 3'-OH), 6.24 (1 H, t, H-1'), 7.05 (1 H, d, J = 14 Hz, vinylic H), 7.41 (1 H, d, J = 14 Hz, vinylic H), 8.24 (1 H, s, H-6), 11.6 (1 H, NH)
5a	1.20 (3 H, t, $COCH_2CH_3$), 2.07 (2 H, m, H-2'), 2.68 (2 H, t, CH_2CH_2Cl), 2.70 (2 H, d, $^2J_{HP}$ = 20 Hz, $POCH_2CO$), 3.4–4.3 (8 H, m), 5.50 (1 H, d, 3'-OH), 6.05 (1 H, t, H-1'), 7.67 (1 H, s, H-6), 11.3 (1 H, NH)	**12a**	2.25 (2 H, m, H-2'), 2.65 (2 H, t, CH_2CH_2Cl), 3.3–4.2 (5 H, m), 3.50 (3 H, s, OCH_3), 4.81 (1 H, m, H-3'), 5.2 (1 H, t, 5'-OH), 6.09 (1 H, t, H-1'), 7.75 (1 H, s, H-6), 11.4 (1 H, NH)
5b	1.38 (9 H, s, *t*-Bu), 2.10 (2 H, m, H-2'), 2.66 (2 H, t, CH_2CH_2Cl), 2.70 (2 H, d, $^2J_{HP}$ = 21 Hz, $POCH_2CO$), 3.4–4.3 (6 H, m), 5.40 (1 H, d, 3'-OH), 6.13 (1 H, t, H-1'), 7.56 (1 H, s, H-6), 11.4 (1 H, NH)	**12b**	1.20 (3 H, t, $COCH_2CH_3$), 2.28 (2 H, m, H-2'), 2.66 (2 H, t, CH_2CH_2Cl), 3.45–4.25 (7 H, m), 4.77 (1 H, m, H-3'), 5.23 (1 H, d, 5'-OH), 6.05 (1 H, t, H-1'), 7.72 (1 H, s, H-6), 11.3 (1 H, NH)

Anal. ($C_{14}H_{21}N_2O_9P$) C, H , N.

(Dimethoxyphosphinyl)acetic acid 1-(2,4-dioxo-5-iodo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-deoxy-β-D-*erythro*-pentofuranos-5-yl ester (3b): yield 1.58 g (54%); glass. Anal. ($C_{13}H_{18}IN_2O_9P$) C, H, N.

(Dimethoxyphosphinyl)acetic acid 1-[5-(2-chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl ester (3c): yield 1.32 g (60%); glass. Anal. ($C_{15}H_{22}ClN_2O_9P$) C, H, N.

[[[[1-[5-(2-Chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy]carbonyl]methyl]phosphonic Acid (4). To a mixture of **3c** (0.44 g, 1.0 mmol) and finely powdered KI (0.37 g, 2.2 mmol) in 5 mL of dry acetonitrile was added dropwise Me_3SiCl (0.56 ml, 4.4 mmol). After the mixture was stirred for 45 min at room temperature, the inorganic salts were removed by filtration and the solution was evaporated. The remaining oil was dissolved in 7 mL of MeOH and concentrated under reduced pressure after 30 min. Treating of the residue with 5 mL of dry acetonitrile yielded 0.33 g (80%) of **4** as colorless, hygroscopic oil. Anal. ($C_{13}H_{18}ClN_2O_9P$) C, H, N.

General Procedure for the Preparation of 5a,b,d and 11a,c–e. A stirred solution of **1d**, **1e**, respectively (5.0 mmol), dicyclohexylcarbodiimide (2.06 g, 10.0 mmol), and 6.0 mmol of the corresponding pyridinium phosphonate in 30 mL of dry pyridine was kept at 50 °C for 3 h. The precipitated urea was filtered off, and the resulting solution was evaporated twice with 100 mL of water. Filtration and shaking with Dowex WGR ion exchange resin (formiate form) led to an aqueous solution, which was evaporated to dryness (50 °C, 0.1 mmHg). Column chromatography of the residue with solvent B as eluant gave the phosphonates as crystalline solids after treatment with dry ether.

[(Ethoxycarbonyl)methyl]phosphonic acid 1-[5-(2-chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl ester (5a): yield 0.93 g (42%); mp 175–180 °C dec. Anal. ($C_{15}H_{22}ClN_2O_9P$) C, H, N.

[(*tert*-Butoxycarbonyl)methyl]phosphonic acid 1-[5-(2-chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl ester (5b): yield 0.63 g (27%); foam. Anal. ($C_{17}H_{26}ClN_2O_9P$) C, H, N.

[(Ethoxycarbonyl)methyl]phosphonic acid 1-[5-(*E*)-(2-bromovinyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl ester (5d): yield 1.02 g (42%); foam. Anal. ($C_{15}H_{20}BrN_2O_9P$) C, H, N.

(Methoxycarbonyl)phosphonic acid 1-[5-(2-chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl ester (11a): yield 0.58 g (28%); mp 217–218 °C dec. Anal. ($C_{13}H_{18}ClN_2O_9P$) C, H, N.

[(Benzyloxy)carbonyl]phosphonic acid 1-[5-(2-chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl ester (11c): yield 0.64 g (26%); mp 197–200 °C dec. Anal. ($C_{19}H_{22}ClN_2O_9P$) C, H, N.

(Methoxycarbonyl)phosphonic acid 1-[5-(*E*)-(2-bromovinyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl ester (11d): yield 0.61 g (27%); mp 225–230 °C dec. Anal. ($C_{13}H_{16}BrN_2O_9P$) C, H, N.

(Ethoxycarbonyl)phosphonic acid 1-[5-(*E*)-(2-bromovinyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl ester (11e): yield 0.71 g (30%); mp 220–225 °C dec. Anal. $C_{14}H_{18}BrN_2O_9P$) C, H, N.

Trifluoroacetic Acid Cleavage of 5b. To 5 mL of ice-cold TFA was added **5b** (0.47 g, 1.0 mmol). After 5 min the TFA was distilled off in vacuo, and the residue was coevaporated twice with 10 mL of dry CH_2Cl_2. The resulting foam was dried under reduced pressure: yield 0.37 g of **[[[1-[5-(2-chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy]hydroxyphosphinyl]acetic acid (5c)** as extremely hygroscopic foam. Anal. ($C_{13}H_{18}ClN_2O_9P·H_2O$) C, H, N.

1-[5-(2-Chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-5-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranose (6). Compound **1d** (1.45 g, 5.0 mmol) was dissolved in 20 mL of dry pyridine. After addition of 4,4′-dimethoxytrityl chloride (2.03 g, 6.0 mmol) the mixture was stirred at room temperature for 1 h. Evaporation and column chromatography with eluant A afforded 2.20 g (80%) of crude **6**, which was used without further purification.

General Procedure for the Preparation of Phosphonates 7 and 12a. To a mixture of **6** (1.78 g, 3.0 mmol) and dicyclohexylcarbodiimide (1.24 g, 6.0 mmol) in 15 mL of dry pyridine was added a solution of the corresponding pyridinium phosphonate (3.5 mmol) in 5 mL of the same solvent. After 5 h at 50 °C, the precipitated urea was filtered off and the solvent was removed by azeotropic distillation with water in vacuo. To the resulting aqueous solution was added 3 mL of 1 N HCl. Neutralization ($NaHCO_3$) and centrifugation gave an opalescent solution, which was evaporated to dryness. The phosphonates could be isolated as a crystalline solid after column chromatography with solvent B and treatment of the remaining oils with dry ether.

[(Ethoxycarbonyl)methyl]phosphonic acid 1-[5-(2-chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-3-yl ester (7): yield 0.75 g (57%); mp 216–217 °C dec. Anal. ($C_{15}H_{22}ClN_2O_9P$) C, H, N.

(Methoxycarbonyl)phosphonic acid 1-[5-(2-chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-3-yl ester (12a): yield 0.59 g (48%); mp 228–230 °C dec. Anal. ($C_{13}H_{18}ClN_2O_9P$) C, H, N.

(Ethoxycarbonyl)phosphonic Acid 1-[5-(2-Chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl Ester (11b) and (Ethoxycarbonyl)phosphonic Acid 1-[5-(2-Chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-3-yl] Ester (12b). Via the procedure described for **11a** the two isomeric compounds **11b** and **12b** could be separated by column chromatography with eluant B. **11b**: yield 0.45 g (21%); mp 186–190 °C dec.; R_f 0.19 (solvent B). Anal. ($C_{14}H_{20}ClN_2O_9P$) C, H, N. **12b**: yield 0.26 g (12%); mp 243–244 °C dec.; R_f 0.28 (solvent B). Anal. ($C_{14}H_{20}ClN_2O_9P$) C, H, N.

(Dimethoxyphosphinyl)acetic Acid 1-[5-(2-Chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-3-yl Ester (8). To a solution of **6** (1.78 g, 3.0 mmol) and DCC (1.24 g, 6.0 mmol) in 15 mL of dry pyridine was added (dimethoxyphosphinyl)acetic acid (0.58 g, 3.5 mmol) dissolved in 5 mL of pyridine. The mixture was stirred for 4 h at 45 °C, filtered, and coevaporated twice with 50 mL of water. The remaining residue was dissolved in 20 mL of acetone, and 10 mL of 0.1 N HCl was added. After 1 h the mixture was neutralized by addition of $NaHCO_3$, filtered, and evaporated to dryness. The resulting crude product was purified by column chromatography with solvent A: yield 0.61 g (46%); glass. Anal. ($C_{15}H_{22}ClN_2O_9P$) C, H, N.

[[[[1-[5-(2-Chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-3-yl]oxy]carbonyl]methyl]phosphonic Acid (9). Via the procedure described for compound **4**, 0.44 g (1.0 mmol) of **8** gave 0.32 g (68%) of **9** as crystalline solid (mp 125–130 °C), which decomposed when stored for some days at room temperature. Anal. ($C_{13}H_{18}ClN_2O_9P$) C, H, N.

[[1-[5-(2-Chloroethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]-2-deoxy-β-D-*erythro*-pentofuranos-5-yl]oxy](phenylamino)phosphinecarboxylic Acid Ethyl Ester Oxide (10). Compound **1d** (1.45 g, 5.0 mmol), DCC (2.06 g, 10.0, mmol), and anilinium [(ethoxycarbonyl)methyl]phosphonate (1.47 g, 6.0 mmol) were stirred in a mixture of DMF/pyridine (30 mL, 2:1 v/v) for 24 h at room temperature. After workup as described for **5a**, the resulting oil was chromatographed on silica gel with solvent A. The pure compound crystallized on treating with dry ether: yield 0.75 g (30%); mp 162–164 °C. Anal. ($C_{20}H_{25}ClN_3O_8P$), C, H, N.

Antiviral Evaluation. Materials. Cells. Vero and HEp-2 cells were obtained from the American Type Culture Collection, Rockville, MD. PRK refer to primary rabbit kidney cell cultures.

Viruses. Herpes simplex virus type 1 (HSV-1) strain Brand was originally isolated by H. zur Hausen in Erlangen, FRG; TK^- HSV-1 is B 2006, a standard TK-deficient strain; HSV-2 strain K 979 was isolated by B. Vestergaard in Copenhagen, Denmark; TK^- HSV-2 is the BrdC-resistant mutant C5A of strain 72 isolated in Giessen, FRG, and was shown to be TK-deficient by M. Scriba, Vienna, Austria. All virus strains were kindly provided by M. Scriba. The origin of the HSV-1 strains KOS, F, McIntyre, the HSV-2 strains G, 196, Lyons, and the TK^- HSV-1 strain B 2006 has been described previously,[34] as has been the origin of TK^- HSV-1 VMW 1837 [referred to as TK^- HSV-1 (ACV^r, $BVDU^r$) in ref 35].

Compounds. The reference compound (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) was synthesized according to Jones et al.[36] and 9-[(2-hydroxyethoxy)methyl]guanosine (Acyclovir, ACV) according to Schaeffer et al.[37] CEDU (**1d**) was prepared as described previously.[3] 5-Iodo-2′-deoxyuridine (IDU) was purchased from Mack, Illertissen, FRG, phosphonoacetic acid (PAA) from ICN Pharmaceuticals Inc., Plainview, NY, and phosphonoformic acid (PFA) from Sigma Chemical Co., St. Louis, MO. For in vitro assays the test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted further in cell culture medium. Up to 1% DMSO was tolerated by the cells without visible toxic effects.

Methods. In Vitro Tests. Cytopathic effect (CPE) inhibition assays in Vero and HEp-2 cells were performed as follows: Serial 3-fold dilutions of the test compound in Eagle's minimum essential medium (E-MEM) were prepared in flat-bottomed microtiter plates. Equal parts of virus in E-MEM and cell suspension in E-MEM plus 15% fetal calf serum were added. Virus-infected cultures without compound were included as controls as were uninfected cells treated with compound. The cell input was adjusted so as to give a confluent monolayer after 1–2 days of incubation, and the virus input was so as to cause 90–100% CPE in the infected control cell culture after 3 days. At this time, the plates were fixed and stained, and the extent of virus-induced CPE in infected controls and in drug-treated wells was estimated. The CPE-inhibition assays in PRK cell cultures were carried out as described previously.[34]

In Vivo Tests. For systemic treatment of systemic HSV-1 or HSV-2 infection, NMRI mice inoculated intraperitoneally (ip) with HSV-1 (Brand), and, in some instances, with HSV-1 (KOS), as indicated in the footnote to Table III, at 1.3×10^5 PFU/0.1 mL per mouse or with HSV-2 (K 979) at 4.7×10^3 PFU/0.1 mL per mouse and treated either perorally (po) (via gavage) or ip twice a day (at 9 a.m. and 4 p.m.) with the indicated doses of the test

compound for 5 days, starting on the day of virus infection. The test compounds were formulated in 0.2% (w/w) sodium (carboxymethyl)cellulose, 0.2% Tween 80 in H_2O to give a solution or a homogeneous suspension. Twenty mice were used per group. Statistical significance of the differences in the final mortality rates (after 20 days of observation) was assessed by the χ^2 test with Yates correction for small numbers.[38]

The procedure for topical treatment of cutaneous HSV-1 or HSV-2 infection in hairless mice has been recently described.[39] The mice were inoculated intracutaneously in the lumbosacral area with either HSV-1 (Brand) at 1×10^6 PFU/0.025 mL per mouse or HSV-2 (K 979) at 1.8×10^5 PFU/0.025 mL per mouse. The test compounds were formulated in AZDMSO (5% azone [1-dodecylazacycloheptan-2-one], synthesized at the Sandoz Forschungsinstitut by the method of Swain et al.,[40] in dimethyl sulfoxide). They were applied in a volume of 50 μL topically four times a day (at 9 a.m., 11 a.m., 2 p.m., and 4 p.m.) for 5 days, starting immediately after virus infection. Ten mice were used per group.

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Registry No. **1a**, 951-78-0; **1b**, 50-89-5; **1c**, 54-42-2; **1d**, 90301-59-0; **1e**, 69304-47-8; **2a**, 115365-13-4; **2b**, 115365-14-5; **2c**, 115365-15-6; **2d**, 115365-16-7; **2e**, 115365-18-9; **2f**, 115365-19-0; **2g**, 115365-20-3; **2h**, 115365-21-4; **3a**, 115365-22-5; **3b**, 115365-23-6; **3c**, 115383-47-6; **4**, 115365-24-7; **5a**, 115365-25-8; **5b**, 115365-26-9; **5c**, 115365-27-0; **5d**, 115365-28-1; **6**, 115365-33-8; **7**, 115365-34-9; **8**, 115365-38-3; **9**, 115365-39-4; **10**, 115365-40-7; **11a**, 115365-29-2; **11b**, 115365-36-1; **11c**, 115365-30-5; **11d**, 115365-31-6; **11e**, 115365-32-7; **12a**, 115365-35-0; **12b**, 115365-37-2; $EtOCOP(O)(OMe)Cl$, 115365-12-3; $EtOCOCH_2P(O)(OMe)Cl$, 115365-17-8; $HOOCCH_2P(O)(OMe)_2$, 34159-46-1; $EtOCOCH_2P(O)(OH)_2$, 35752-46-6; t-$BuOCOCH_2P(O)(OH)_2$, 77530-32-6; $EtOCOP(O)(OH)_2$, 55920-71-3; $EtOCOP(O)(OH)_2 \cdot PhNH_2$, 67472-32-6; $MeOCOP(O)(OH)_2$, 55920-68-8; $PhCH_2OCOP(O)(OH)_2$, 55920-74-6.

Synthesis and Biological Activity of Some Transition-State Inhibitors of Human Renin

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A series of renin inhibitors containing the dipeptide transition state mimics (2*S*,4*S*,5*S*)-5-amino-4-hydroxy-2-isopropyl-7-methyloctanoic acid (Leu$\overset{OH}{—}$Val) and (2*S*,4*S*,5*S*)-5-amino-4-hydroxy-2-isopropyl-6-cyclohexylhexanoic acid (Cha$\overset{OH}{—}$Val) was prepared. A structure–activity study with Boc-Phe-His-Leu$\overset{OH}{—}$Val-Ile-His-NH_2 (**8a**) as starting material led to *N*-[(2*S*)-2-[(*tert*-butylsulfonyl)methyl]-3-phenylpropionyl]-His-Cha$\overset{OH}{—}$Val-NHC_4H_9-*n* (**8i**) which has the length of a tetrapeptide and contains only one natural amino acid. Compound **8i** had an IC_{50} of 2×10^{-9} M against human renin and showed high enzyme specificity; IC_{50} values against the related aspartic proteinases pepsin and cathepsin D were (8×10^{-6} and 3×10^{-6} M, respectively). In salt-depleted marmosets, **8i** inhibited plasma renin activity PRA and lowered blood pressure for up to 2 h after oral administration of a dose of 10 mg/kg.

Renin, the rate-determining enzyme in the cascade leading to the vasopressor substance angiotensin II, plays a key role in the regulation of blood pressure.[1] Interruption of the renin-angiotensin system by inhibition of angiotensin converting enzyme (ACE) has led to the development of effective antihypertensive agents.[2] In principle, renin inhibitors should also provide a means of controlling blood pressure. Animal studies comparing an ACE inhibitor with a renin inhibitor have shown the two agents to be equieffective.[3] In addition, renin inhibitors may have advantages over ACE inhibitors, since, unlike ACE, which hydrolyzes a variety of bioactive peptides, renin is specific, having angiotensinogen as its only known substrate.[4] Human renin hydrolyzes the Leu[10]-Val[11] amide bond of angiotensinogen. A number of nonhydrolyzable equivalents of this dipeptide based on the transition state inhibitor concept have been prepared and incorporated into small peptides. Szelke and co-workers were the first to apply this concept to the synthesis of renin inhibitors.[5] These and subsequent efforts by others have produced a number of potent inhibitors of renin, but none of these have shown good oral activity.[6] Herein we report some

(1) (a) Peach, M. J. *Physiol. Rev.* **1977**, *57*, 313. (b) Ondetti, M. A.; Cushman, D. W. *Annu. Rev. Biochem.* **1982**, *51*, 283.

(2) (a) Frohlich, E. D.; Copper, R. A.; Lewis, E. J. *Arch. Intern. Med.* **1984**, *144*, 1441. (b) Vlasses, P. H.; Rotmensch, H. H.; Swanson, B. N.; Lee, R. B.; Koplin, J. R.; Ferguson, R. F. *J. Clin. Pharmacol.* **1983**, *23*, 227. (c) Watthey, J. W. H.; Stanton, J. L.; Desai, M.; Babiarz, J. E.; Finn, B. M. *J. Med. Chem.* **1985**, *28*, 1511. (d) Stanton, J. L.; Watthey, J. W. H.; Desai, M. N.f Finn, B. M.; Babiarz, J. E.; Tomaselli, H. C. *J. Med. Chem.* **1985**, *28*, 1603. (e) Teetz, V.; Geiger, R.; Henning, R.; Urbach, H. *Arzneim. Forsch.* **1984**, *34*(II), 1399. (f) Schaller, M. D.; Nussberger, J.; Waeber, B.; Bussien, J. P.; Turini, G. A.; Brunner, H.; Brunner, H. R. *Eur. J. Clin. Pharmacol.* **1985**, *28*, 267.

(3) (a) Hofbauer, K. G.; Fuhrer, W.; Heusser, C.; Wood, J. M. *J. Cardiovasc. Pharmacol.*, **1985**, *7*(Supple. 4), 562. (b) Wood, J. M.; Forgiarini, P.; Hofbauer, K. G. *J. Hypertens.* **1983**, *1*-(Suppl. 2), 189. (c) Sweet, C. S.; Ludden, C. T.; Frederick, C. M.; Bush, L. R.; Ribeiro, L. G. T. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 1067.

(4) Foltmann, B.; Pedersen, V. B. *Acid Proteases, Structure, Function, and Biology*; Tang, J., Ed.; Plenum: New York, 1977; pp 3–22.

(5) Szelke, M.; Leckie, B. J.; Hallett, A.; Jones, D. M.; Sueiras, J.; Atrash, B.; Lever, A. F. *Nature (London)* **1982**, *299*, 555.

0022-2623/88/1831-1839$01.50/0