Synthesis of New Acyclic Pyrimidine Nucleoside Analogs as Potential Antiviral Drugs†

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Received September 15, 1993®

The synthesis of 6-[*N*-(4-hydroxybutyl)amino]pyrimidinone derivatives **18–23** and the acyclic phosphonate nucleoside analogs **29–30** is reported. Their cytotoxic and antiviral effects were investigated. 2,5-Diamino-6-[*N*-[2-(phosphonomethoxy)ethyl]amino]pyrimidin-4(3*H*)-one (**30**) showed strong antiviral effects, and **21** showed significant cytotoxic activity.

Introduction

Aciclovir (**1**; Chart 1) is the most effective drug in the treatment of diseases caused by herpes simplex virus (HSV) type 1 and 2. It is also effective in the therapy of infections with varizella zoster (VZV) and cytomegalie virus.[1]

As an acyclic guanosine analog, aciclovir represents a prodrug. The first activation step, a monophosphorylation, is performed by virus-encoded thymidine kinase (TK) only, while cellular kinases are not envolved. The active triphosphate form of **1** is then produced with the aid of mammalian cellular kinases. Aciclovir triphosphate leads to chain termination reactions in DNA replication; furthermore, it inhibits viral DNA polymerase. Because of the poor bioavailability of **1** and due to the occurrence of aciclovir resistant strains of HSV in hospitals, the search for new antiviral compounds is going on worldwide.

In 1988, the working groups of Kamikawa[2] and Moss[3] independently reported on the synthesis of clitocine (**2**; Chart 1), a natural exocyclic amino nucleoside, which was isolated from the mushroom Clytocybe inversa.[4] **2** has interesting biological properties: it shows strong insecticidal activity against the pink bollworm Pectinophora gossypiella. **2** also inhibits L1210 cell growth in vitro (ID_{50}: 301 nM) and was found to be a substrate and inhibitor of adenosine kinase ($K_i = 3\ \mu M$). Despite being a nucleoside analog, the compound showed no activity against parainfluenza, vaccinia, or HSV-2.[3]

Palmer et al. published the synthesis of a carbocyclic analog of **2**, without giving biological data.[5] V. E. Marquez and co-workers used **2** as a template for the potentially antiviral compounds **3-8** (Chart 1). Compound **6** showed some activity against HSV-1, HSV-2, and human cytomegalovirus (HCMV).[6]

To our knowledge, up to now no other attempt was made to use exocyclic uracil nucleosides as a template for antiviral compounds, though a large number of N-1-substituted pyrimidine nucleoside analogs can be found in the literature.[7–10] Our aim was to fill this gap by the synthesis of some acyclic substituted derivatives of **2**.

Computer-Assisted Modeling

With the aid of computer models, it can be deduced that the distance between N-9 and O-5′ in **1** is 8.0 Å but only 6.7 Å between N-1 and O-5′ in N-1-substituted pyrimidine nucleosides. The model compound **2**-amino-6-[*N*-[(2-hydroxyethoxy)methyl]amino]pyrimidin-4-one (**12**; Chart 2) posesses the same sugar moiety as **1**. Furthermore, a comparison of the geometry-optimized structures of **1** and **12** with respect to their electrostatic potentials, calculated with semiempirical methods (MOPAC), showed that both, **1** and **12**, should have the same orientation and interaction at the binding site of TK.

Unfortunately we were not able to synthesize **12** and attributed this failure to the instability of the hemiaminal side chain.

Chart 1

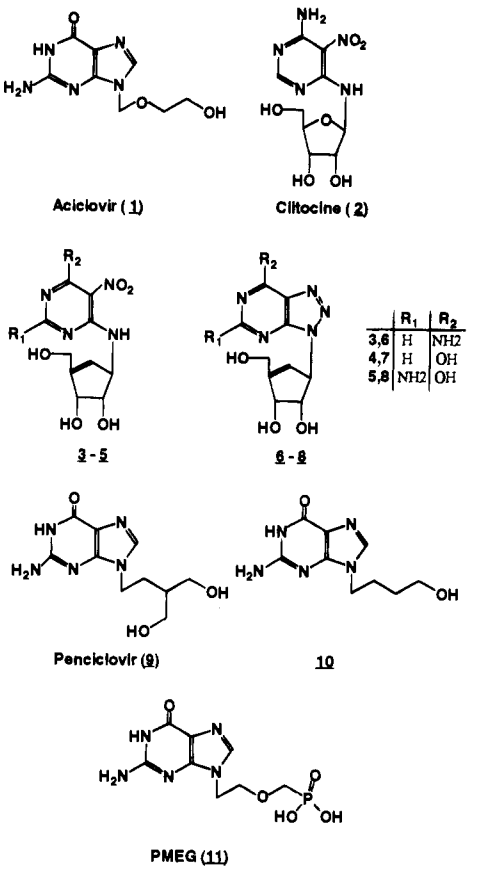
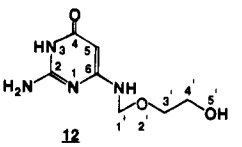


Chart 2

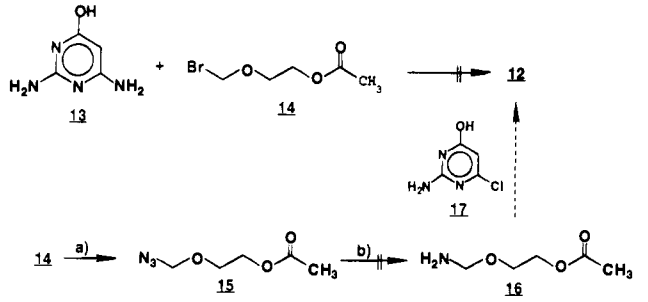


† Dedicated to Prof. Dr. H. J. Roth, University of Tübingen, on the occasion of his 65th birthday.

® Abstract published in *Advance ACS Abstracts,* August 1, 1994.

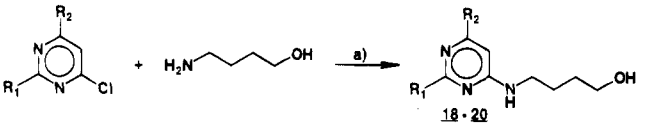
0022-2623/94/1837-3057$04.50/0

Scheme 1[a]



[a] (a) NaN_3, toluene, 70 °C; (b) $(CH_3)_3P$.

Scheme 2



(a) Triethylamine, butanol, reflux

	R_1	R_2
18	OH	OH
19	NH_2	OH
20	NH_2	Cl

Chemistry

We approached the synthesis of **12** by two different ways: the first was to react 2,6-diaminouracil (**13**) with (2-acetoxyethoxy)methyl bromide (**14**).[11] This procedure did not yield any product, possibly due to the extremely poor solubility of **13** in both lipophilic and hydrophilic solvents (Scheme 1).

A second way to obtain **12** could have been introducing 2-(aminomethoxy)ethyl acetate (**16**) into 2-amino-6-chlorouracil (**17**). In order to prepare **16**, we exchanged the bromine atom of **14** by azide in a nucleophilic substitution, using tetramethylammonium bromide as a phase transfer catalyst.[12] On reduction of (2-acetoxyethoxy)methyl azide (**15**) with trimethylphosphine, only ethylene glycol acetate was formed. As we also failed to obtain **16** via Delepine- or Gabriel-type reactions, we left the idea of preparing **12** and switched over to more stable carbo analogous side chains.

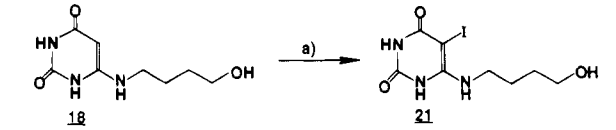
4-Aminobutanol as a Model for a Simple Side Chain. Penciclovir (**9**) and 9-(4-hydroxybutyl)guanine (**10**; Chart 1) are both antiviral agents[13,14] and can be defined as carbo analogs to **1**.

An aminobutanol side chain as in **9** can be introduced in 6-chloropyrimidine derivatives by refluxing the reactants (Scheme 2) in butanol in the presence of triethylamine.[15] The structures of the resulting products given in the schemes were assigned by means of NMR spectroscopy. Thus, **18** and **19** (Scheme 2) were found to have the 2-hydroxy and 2-amino configuration, respectively, whereas **21**–**23** (Schemes 3 and 4) can be assigned the tautomeric 2-oxo and 2-imino form, respectively.

21 was prepared by iodination of **18** with iodine and silver sulfate in methanol at ambient temperature (Scheme 3), without previous protection of the hydroxy groups.[16] **21** is very sensitive to heat and light; even on storage in the refrigerator, it was not stable for more than a few weeks.

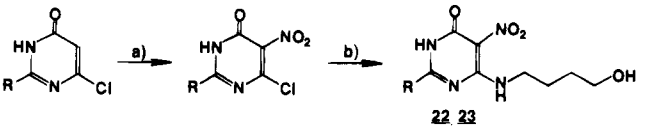
We also succeeded in introducing a nitro group in **18** and **19** by nitration of the appropriate pyrimidines with nitrating acid[17] followed by the condensation reaction with 4-aminobutanol, thus obtaining **22** and **23** in high yields (Scheme 4).

Scheme 3[a]



[a] (a) I_2, Ag_2SO_4, MeOH.

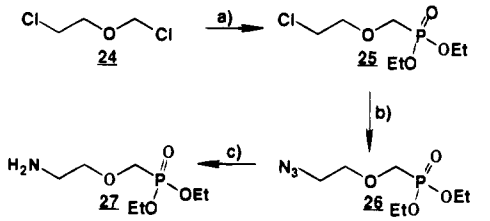
Scheme 4



(a) H_2SO_4/HNO_3
(b) aminobutanol, methanol, reflux

	R_1
22	OH
23	NH_2

Schem 5[a]



[a] (a) Triethyl phosphite, reflux; (b) NaN_3, $N(CH_3)_4Br$, toluene, 90 °C; (c) triphenylphosphine, toluene.

(Phosphonomethoxy)ethyl as Side Chain. In 1989, E. De Clercq, A. Holy, and I. Rosenberg[18] described 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG, **11**; Chart 1) as a potent antiviral phosphonate isoster of aciclovir. Like the aminobutanol side chain, this phosphonate has no aminalic substructure, and thus no problems with the instability of the sugar moiety should occur. Furthermore, as the phosphonate substructure imitates natural monophosphates, the activation step by viral TK is omitted, as **11** is converted into its active triphosphate form directly via cellular kinases. The active form of **11** inhibits viral DNA polymerase and can thus be used in virus strains without viral TK.

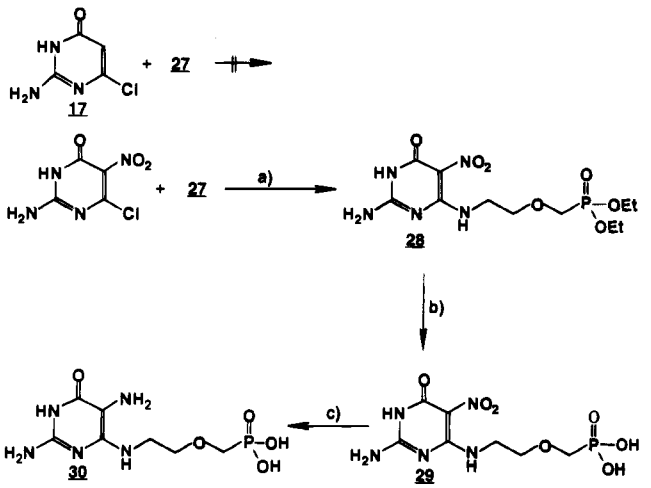
Introducing the phosphonate side chain into 2-amino-6-chlorouracil (**17**) yields a derivative of **11** with an opened imidazole ring. We refluxed (chloromethoxy)-ethyl chloride (**24**) with triethyl phosphite in a Michael Arbusow reaction.[19] The resulting diethyl [(2-chloroethoxy)methyl]phosphonate (**25**) was converted into the azide **26** as described above and then reduced to the amino compound **27** (Scheme 5).

Refluxing **27** with **17** failed to give a product because **27** is not stable at elevated temperatures and **17** is not reactive enough at room temperature. In order to activate the uracil derivative, a nitro group was introduced in C-5.[17] Now the condensation to **28** proceeded smoothly (Scheme 6). Saponification of the phosphonate ester groups with trimethylsilyl bromide in dry acetonitrile yielded the ammonium salt of **29**, which was subsequently reduced to the desired **30**. Like in the case of the aminobutanol side chain, the tautomeric structures of **28**–**30** were derived from the NMR spectra.

Biological Results and Discussion

The aminobutanol-substituted pyrimidine nucleoside analogs **18**–**23** and the pyrimidine phosphonates **29**–

Scheme 6[a]



[a] (a) Acetone, room temperature; (b) trimethylsilyl bromide, acetonitrile, argon atmosphere, room temperature; (c) palladium/charcoal, hydrogen, methanol.

Table 1. In Vitro Antiviral Activity against HSV-1

compound	0.05 μg/mL (%)	0.5 μg/mL (%)	5 μg/mL (%)	50 μg/mL (%)	500 μg/mL (%)
EDU	0	25	100	100	100
PMEG	100	100	(−)[a]	(−)	(−)
1	100	100	100	100	100
18–23, 29	0	0	0	0	0
30	50	100	100	(−)	(−)

[a] (−): cytotoxicity predominates antiviral activity.

Table 2. In Vitro Cytotoxicity

compound	5 μg/mL (%)	50 μg/mL (%)	500 μg/mL (%)
PMEG	25	50	75
EDU, **1**	0	0	0
18	0	0	25
19, 22, 29	0	0	0
20	0	0	50
21	0	100	100
23	0	0	100
30	0	50	75

30 were tested for their cytotoxicity and antiviral effects against HSV-1, using aciclovir, ethyldeoxyuridine (EDU), and PMEG as references (Tables 1 and 2).

18–23 did not show any antiviral or cytotoxic activity. These results are in agreement with the biological results of **10**, which in comparison to **1** also showed only moderate antiviral effects.[1] Only the 5-iodo derivative **21** was considerably cytotoxic at a concentration of 50 μg/mL. Such effects of 5-halogenated pyrimidines are known from other 5-halogenouracils. 5-Fluorouracil, for example, is registered as an antitumor drug.

While the acyclic 5-nitro phosphonate **29** had neither antiviral nor cytotoxic effects, the 5-amino compound **30** showed a biological activity comparable to that of PMEG: at a concentration of 50 μg/mL, both compounds showed 100% growth inhibition of HSV, and at 0.05 μg/mL, PMEG still kept its full activity while **30** was half as effective. The differences in the effectiveness of **29** and **30**, despite the closely related structure, may be explained by the 5-amino function, which might imitate the imidazole nitrogen in position 7 of guanine derivatives like PMEG.

Experimental Section

Computer Modeling. Molecular modeling was done on a MicroVAX 3500 instrument with a PS390 graphical output system. For geometry optimizing, Sybyl Modelling Software 5.4, Tripos Assoc. Inc., St. Louis, Mo., was used and calculations of electrostatic fields were done with MOPAC 5.0, QCPE No. 455.

Biological Methods.[20] The compounds were added to mice embryo cell cultures in concentrations of 0.05, 0.5, 5, 50, and 500 μg/mL and incubated for 3 days. Subsequently the cell plaques were controlled macro- and microscopically. Growth inhibition or disruption of noninfected cells indicated cytotoxicity. As a parameter for antiviral effects, the reduction or inhibition of cell destruction of HSV-1-infected plaque assays was used. Aciclovir, 5-ethyldeoxyuridine (EDU), and 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG) were used as references.

Synthesis. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. The IR spectra (potassium bromide) were recorded on a Perkin-Elmer 1750 FTIR spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured on a Bruker AC 200 spectrometer using tetramethylsilane as an internal standard. For the resonance signals, the following abbreviations are used, s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. In all cases, the solvent was DMSO-d_6. The structural assignment derived from the spectra was confirmed by comparison to literature data. Mass spectra were obtained on a Finnigen MAT 711A spectrometer (modified by AMD Intectra GmbH) using a direct inlet system. They were recorded by the Abteilung Massenspektroskopie, Organisch-Chemisches Institut der Universität Tübingen. Elemental analysis was performed by the Abteilung Elementaranalyse, Anorganisch-Chemisches Institut der Universität Tübingen. Thin layer chromatograms were run on Merck silica gel 60 F_{254} alumina plates, with the following eluants: (A) EtOH/CH_2Cl_2, 1 + 4; (B) EtOH/CH_2Cl_2, 4 + 1; (C) ethyl acetate/formic acid, 9 + 1; (D) ethyl acetate; (E). Chemicals were purchased from E. Merck and Aldrich Chemie, FRG.

(2-Acetoxyethoxy)methyl Azide (15). (2-Acetoxyethoxy)-methyl bromide[11] (5.0 g, 25 mmol) and 1.0 g (3 mmol) of tetrabutylammonium bromide as phase transfer catalyst were dissolved in 25 mL of toluene. Sodium azide (2.5 g, 38 mmol) was added, and the mixture was stirred at 70 °C for 6 h, the mixture turning slightly yellow. After cooling, the organic layer was extracted three times with 25 mL of water. The organic layer was dried (Na_2SO_4), and the solvent was evaporated in vacuo. The crude product was distilled at 45 °C/0.04 mbar. **15** was obtained as a colorless oil. Yield: 6.0 g (75%). n^{20}D: 1.4409. R_f 0.86 (E). IR (cm^{-1}): 2959 (CH_2), 2124 (azide), 1742 (C=O), 1230 (ester), 1055 (ether). ^{1}H-NMR: δ 2.02 (s, 3H, CO-**CH$_3$**), 3.76 (m, 2H, **CH$_2$**-O-CH$_2$), 4.14 (m, 2H, CO-O-**CH$_2$**), 4.77 (s, 2H, O-**CH$_2$**-N$_3$). $C_5H_9N_3O_3$: 159.1. Anal. C, H, N.

2-Hydroxy-6-[*N*-(4-hydroxybutyl)amino]pyrimidine-4-(3*H*)-one (18). 6-Chloropyrimidine-2,4-(1*H*,3*H*)-dione (1.0 g, 7 mmol) and 1.0 g (10 mmol) of freshly distilled triethylamine were suspended in 20 mL of butan-1-ol; 1.0 g (12 mmol) of 4-aminobutanol was added and the mixture refluxed for 4 h until a yellow solution resulted. The solvent was removed in vacuo, and acetone was added to the oily residue. The off-white precipitates were collected by filtration and recrystallized from methanol. Yield: 1.13 g (81%). Mp: 250 °C. R_f: 0.5 (B). IR (cm^{-1}): 3250, 3000 (OH, NH), 2920 (CH_2), 1700, 1617 (lactam). ^{1}H-NMR: δ 1.47 (m, 4H, N-CH$_2$-**CH$_2$**-**CH$_2$**-CH$_2$-O), 2.98 (m, 2H, **CH$_2$OH**), 3.40 (m, 2H, NH-**CH$_2$**), 4.37 (s, 1H, CO-**CH**=C), 6.38 (t, 1H, NH-butyl, J = 5.3 Hz), 10.0 (s, br, 1H, **OH**). ^{13}C-NMR: δ 25.6 (NH-CH$_2$-**CH$_2$**), 30.4 (**CH$_2$**-CH$_2$-OH), 42.0 (NH-**CH$_2$**), 61.0 (**CH$_2$**-OH), 73.0 (CO-**CH**=C), 152.1 (N=**COH**-NH), 155.5 (CH=**C**-N), 164.3 (NH-**CO**-CH=). $C_8H_{13}N_3O_3$: 199.2. Anal. C, H, N.

2-Amino-6-[*N*-(4-hydroxybutyl)amino]pyrimidin-4(3*H*)-one (19). 2-Amino-6-chloro-4-hydroxypyrimidine (1.5 g, 10 mmol), 30 mL of butan-1-ol, 1.5 g (15 mmol) of freshly distilled triethylamine, and 1.33 g (15 mmol) of 4-aminobutanol were refluxed for 4 h. For further procedure, see **18**. **19** was

obtained as off-white crystals. Yield: 1.68 g (84%). MP: 206 °C. R_f 0.48 (B), 0.25 (C). IR (cm^{-1}): 3440 (NH_2), 3200 (OH), 2960 (CH_2), 1640 (C=N). ^{1}H-NMR: δ 1.40 (m, 4H, O-CH_2-**CH_2-CH_2**-CH_2-N), 3.00 (m, 2H, **CH_2**-NH), 3.35 (m, 2H, **CH_2**-OH), 4.40 (s, 1H, CO-**CH**=C), 6.06 (s, 2H, **NH_2**), 6.27 (t, 1H, **NH**-butyl, J = 5.5 Hz), 9.7 (s, 1H, NH). ^{13}C-NMR: δ 25.6 (NH-CH_2-**CH_2**), 30.0 (**CH_2**-CH_2OH), 40.7 (NH-**CH_2**), 60.5 (**CH_2**-OH), 78.8 (CO-**CH**=C), 154.9 (CH=**C**-N), 163.0 (N=**C**-**NH_2**-NH), 164.2 (NH-**CO**-CH=). $C_8H_{14}N_4O_2$: 198.2. Anal. C, H, N.

2-Amino-6-[*N*-(4-hydroxybutyl)amino]-4-chloropyrimidine (20).[15] 2-Amino-4,6-dichloropyrimidine (0.9 g, 6 mmol), 15 mL of butan-1-ol, 0.8 g (8 mmol) of freshly distilled triethylamine, and 0.5 g (6 mmol) of 4-aminobutanol were refluxed for 4 h. After evaporation of the solvent, 5 mL of water was added to the oily residue. On cooling, the product precipitated as yellow crystals, which were collected by filtration and recrystallized from water. Yield: 1.02 g (78%, lit.[15] yield 76%). Mp: 140 °C (lit.[15] mp 138 °C). R_f: 0.48 (C), 0.77 (B). IR (cm^{-1}): 3360 (NH), 3180 (OH), 2900 (CH), 1590 (C=N). ^{1}H-NMR: δ 1.45 (m, 4H, O-CH_2-**CH_2**-**CH_2**-CH_2-N), 3.18 (m, 2H, **CH_2**-NH), 3.39 (m, 2H, **CH_2**-OH), 4.36 (t, 1H, OH, J = 4.9 Hz), 5.71 (s, 1H, CCl-**CH**=C), 6.30 (s, 2H, N=C-**NH_2**), 7.0 (t, 1H, **NH**-butyl, J = 5.5 Hz). ^{13}C-NMR: δ 25.5 (NH-CH_2-**CH_2**), 29.8 (**CH_2**-CH_2OH), 40.3 (NH-**CH_2**), 60.5 (**CH_2**-OH), 92.6 (CCl-**CH**=C), 162.9 (CH=**C**-N), 164.0 (N=**C**-**NH_2**-NH), 183.0 (N=**CCl**-CH=). $C_8H_{13}ClN_4O$: 216.7. Anal. C, H, N, Cl.

6-[*N*-(4-Hydroxybutyl)amino]-5-iodopyrimidine-2,4-(1*H*,3*H*)-dione (21). Light and temperature >20 °C must be avoided in this reaction! Iodine (0.62 g, 2.5 mmol) and 0.77 g (2.5 mmol) of silver sulfate were dissolved in 40 mL of methanol. A solution of **18** in 15 mL of water was added slowly, and the mixture was stirred at 20 °C for 15 min. The color of the mixture changed from dark brown to yellow, and silver iodide precipitated. AgI was separated by filtration, and the yellow solution was evaporated in vacuo without heating. The yellow crystalline residue was stirred with CH_2Cl_2 in order to remove residual iodine, and recollected by suction, and the off-white crystals were washed with a few milliliters of cold ethanol. The product is sensitive to light and temperature. Yield: 0.35 g (40%). Mp: 165 °C dec. R_f: 0.72 (B). IR (cm^{-1}): 3390 (OH, NH), 2980 (CH), 1720, 1603 (lactam). ^{1}H-NMR: δ 1.46 (m, 4H, N-CH_2-**CH_2**-**CH_2**-CH_2-O), 3.39 (m, 4H, **CH_2**-NH, **CH_2**-OH), 4.48 (s, 1H, **OH**), 6.25 (t, 1H, **NH**-butyl, J = 6.1 Hz), 10.59 (s, 2H, **NH**-CO-**NH**). $C_8H_{12}IN_3O_3$: 325.1. Anal. C, H, N, I.

6-[*N*-(4-Hydroxybutyl)amino]-5-nitropyrimidine-2,4-(1*H*,3*H*)-dione (22). 6-Chloro-5-nitropyrimidine-2,4(1*H*,3*H*)-dione[17a] (0.7 g, 3.6 mmol) was dissolved in 20 mL of methanol, and 1.8 g (20 mmol) of 4-aminobutanol was added. The solution became warm and colorless. It was refluxed for 15 min until white crystals precipitated. The mixture was neutralized with acetic acid; the product was collected by filtration and recrystallized from water. Yield: 0.2 g (25%). Mp 260 °C dec. R_f: 0.5 (C). IR (cm^{-1}): 3420 (NH), 3180 (OH), 2990 (CH), 1640, 1560 (lactam), 1420, 1340 (NO_2). ^{1}H-NMR: δ 1.50 (m, 4H, N-CH_2-**CH_2**-**CH_2**-CH_2-O), 3.41 (m, 5H, **CH_2**-NH, **CH_2**-**OH**), 4.70 (s, br, **NH**-butyl), 9.47, 9.76 (2 × s, 2 × 1H, **NH**-CO-**NH**). ^{13}C-NMR: δ 25.5 (NH-CH_2-**CH_2**), 29.9 (**CH_2**-CH_2OH), 40.4 (NH-**CH_2**), 60.5 (**CH_2**-OH), 110.5 (CO-**CNO_2**=C), 154.9 (CNO_2=**C**-N), 159.0 (NH-**CO**-NH), 160.0 (HN-**CO**-CNO_2=). $C_8H_{12}N_4O_5{\cdot}2H_2O$: 244.2. Anal. C, H, N.

6-[*N*-(4-Hydroxybutyl)amino]-2(1*H*)-imino-5-nitropyrimidin-4(3*H*)-one (23). 4-Aminobutanol (1.8 g, 20 mmol) was added to a suspension of 1.0 g (5 mmol) of 2-amino-6-chloro-5-nitropyrimidin-4(3*H*)-one[17b] in 20 mL of ethanol. For further procedure, see **22**. Yield: 0.83 g (68%) as off-white crystals. Mp: 270 °C. R_f: 0.47 (C). IR (cm^{-1}): 3100 (NH), 1690 (CO), 1650 (lactam), 1590 (C=C), 1510, 1320 (NO_2). ^{1}H-NMR: δ 1.53 (m, 4H, N-CH_2-**CH_2**-**CH_2**-CH_2-O), 3.46 (m, 4H, **CH_2**-NH, **CH_2**-OH), 4.45 (t, 1H, **OH**, J = 4.9 Hz), 6.61 (s, br, 1H, **NH**-butyl), 7.76, 9.60 (s, br, 2 × 1H, **NH**-C-**NH**), 10.57 (s, 1H, C=**NH**). ^{13}C-NMR: δ 25.6 (NH-CH_2-**CH_2**), 29.8 (**CH_2**-CH_2OH), 40.7 (NH-**CH_2**), 60.4 (**CH_2**-OH), 110.4 (CO-**CNO_2**=C), 154.0 (CNO_2=**C**-N), 156.3 (NH-**C**=**NH**), 159.1 (HN-**CO**-CNO_2=). $C_8H_{13}N_5O_4$: 243.2. Anal. C, H, N.

Diethyl [(2-Azidoethoxy)methyl]phosphonate (26). A suspension of 5.0 g (20 mmol) of diethyl [(2-chloroethoxy)-methyl]phosphonate,[19] 1.0 g (3 mmol) of tetrabutylammonium bromide as a phase transfer catalyst, and 2.5 g of sodium azide in 50 mL of toluene was heated to 90 °C for 4 h. After cooling, the toluene layer was extracted three times with 25 mL of water. The aqueous layer was reextracted three times with 20 mL of toluene. The organic layers were dried over anhydrous sodium sulfate, and the solvent was removed in vacuo. Yield: 3.5 g (68%) of a slightly yellow oil. IR (cm^{-1}): 2950 (CH), 2100 (azide), 1250 (P=O), 1060 (R-O-R). ^{1}H-NMR: δ 1.25 (t, 6 H, 2 × CH_2-**CH_3**, J = 7.0 Hz), 3.40 (m, 2H, CH_2-N_3), 3.74 (m, 2H, **CH_2**-O), 3.92 (dd, 2H, O-**CH_2**-P, $J_{H\text{-}H}$ = 8.0 Hz, $J_{H\text{-}P}$ = 14.4 Hz), 4.11 (q, 4H, **CH_2**-CH_3, J = 7.0 Hz). $C_7H_{16}N_3O_4P$: 237.2.

Diethyl [(2-Aminoethoxy)methyl]phosphonate (27). 26 (5.63 g, 24 mmol), dissolved in 5 mL of toluene, was added to a solution of 9.5 g (36 mmol) of triphenylphosphine in 35 mL of toluene within 30 min. The mixture was then stirred at room temperature for 1 h. The reaction was terminated by the addition of 50 mL of water and vigorous stirring for 15 min. The aqueous layer was separated and washed with 2 × 20 mL of ether in order to remove residues of triphenylphosphine oxide. The water was evaporated in vacuo. Traces of water were removed by repeated addition and evaporation of methanol. Yield: 3.9 g (75%) as a slightly yellow oil. IR (cm^{-1}): 3410 (NH), 2950 (CH), 1240 (P=O), 1050 (R-O-R). ^{1}H-NMR: δ 1.23 (t, 6 H, 2 × CH_2-**CH_3**, J = 7.1 Hz), 1.93 (s, 2H, **NH_2**), 2.65 (t, 2H, **CH_2**-NH_2, J = 5.8 Hz), 3.46 (t, 2H, **CH_2**-O, J = 5.8 Hz), 3.88 (dd, 2H, O-**CH_2**-P, $J_{H\text{-}H}$ = 8.0 Hz, $J_{H\text{-}P}$ = 16.8 Hz), 4.09 (q, 4H, **CH_2**-CH_3, J = 7.1 Hz). $C_7H_{18}NO_4P{\cdot}H_2O$: 211.2. Anal. C, H, N.

6-[*N*-[2-[(Diethylphosphono)methoxy]ethyl]amino]-2-(1*H*)-imino-5-nitropyrimidin-4(3*H*)-one (28). 2-Amino-6-chloro-5-nitropyrimidin-4(3*H*)-one[17b] (2.3 g, 12 mmol) was suspended in 100 mL of acetone. Adding 4.2 g (20 mmol) of **27** led to a yellow solution, which was stirred at room temperature for 12 h. The color turned slightly brownish. The solvent was evaporated without heating and the residue chromatographed on a silica gel column (150 g), eluant (C). The fractions containing the product were evaporated, and the crude oil product was dissolved in a few milliliters of methanol. Precipitation was induced by addition of diethyl ether and petroleum ether. Yield: 2.2 g (50.2%) of white crystals. Mp: 70 °C. R_f: 0.33 (C). IR (cm^{-1}): 3250 (NH), 2920 (CH), 1685, 1580 (lactam), 1520, 1340 (NO_2), 1230 (P=O), 1040 (R-O-R). ^{1}H-NMR: δ 1.21 (t, 6 H, 2 × CH_2-**CH_3**, J = 7.1 Hz), 3.53 (s, br, 4H, N-**CH_2**-**CH_2**-O), 3.86 (d, 2H, O-**CH_2**-P, $J_{H\text{-}P}$, = 8.4 Hz), 4.03 (q, 4H, **CH_2**-CH_3, J = 7.1 Hz), 7.69 (s, br, 1H, NH-ethoxy), 8.06, 8.38 (2 × s, br, **NH**-C-**NH**), 9.56 (s, br, 1H, C=**NH**). ^{13}C-NMR: δ 16.3 (CH_2-**CH_3**), 61.75 (CH_3-**CH_2**-O-P, $J_{C\text{-}O\text{-}P}$ = 6.2 Hz), 62.6 (O-CH_2-**CH_2**-N), 65.2 (O-**CH_2**-CH_2-N), 70.75 (O-**CH_2**-P, $J_{C\text{-}P}$ = 11.6 Hz), 110.6 (CO-**CNO_2**=C), 155.2 (CNO_2=**C**-N), 157.8 (NH-**C**=**NH**), 159.3 (**CO**-CNO_2=C). $C_{11}H_{20}N_5O_7P$: 365.3. MS (FD): m/z 366.1 (M^+ + 1). Anal. C, H, N.

2(1*H*)-Imino-6-[*N*-[2-(phosphonomethoxy)ethyl]amino]-5-nitropyrimidin-4(3*H*)-one (29). 28 (0.4 g, 1 mmol) and 1.0 g (7 mmol) of trimethylsilyl bromide were dissolved in 20 mL of dry acetonitrile in an argon atmosphere. The orange solution was stirred at ambient temperature for 2 days. The mixture was neutralized with 2 N aqueous ammonia, and the solvent was removed in vacuo. The off-white ammonium salt of **29** was washed with methanol. Yield: 0.25 g (69%). Mp: 180 °C dec. IR (cm^{-1}): 3100 (OH), 1670 (lactam), 1510, 1320 (NO_2), 1230 (P=O), 1060 (R-O-R). ^{13}C-NMR: δ 43.2 (O-CH_2-**CH_2**-NH), 70.4 (O-**CH_2**-CH_2-NH), 73.3 (O-**CH_2**-P, $J_{C\text{-}P}$ = 30.4 Hz), 115.0 (CO-**CNO_2**=C), 163.1 (CNO_2=**C**-N), 165.4 (NH-**C**=**NH**), 172.5 (**CO**-CNO_2=C). $C_7H_{12}N_5O_7P{\cdot}2NH_3$: 309.2. MS (FAB): m/z 310.1. Anal. C, H, N.

2,5-Diamino-6-[*N*-[2-(phosphonomethoxy)ethyl]amino]-pyrimidin-4(3*H*)-one (30). 29 (0.25 g, 68 mmol) was dissolved in a mixture of 5 mL of water and 25 mL of methanol; 50 mg of palladium/charcoal (10%) was added and the mixture stirred under a hydrogen atmosphere at 1023 mbar and room temperature for 1 h. The catalyst was collected by filtration, and the solvent was evaporated in vacuo. **30** was isolated as

a green crystalline ammonium salt, washed with methanol, and dried. Yield: 0.1 g (69%). Mp >280 °C dec. IR (cm^{-1}): 3390 (NH), 3170 (OH), 2970 (CH), 1660 (lactam), 1100 (P=O), 980 (R-O-R). ^{13}C-NMR: δ 65.2 (O-CH_2-**CH_2**-N), 67.7 (O-**CH_2**-CH_2-N), 71.2 (O-**CH_2**-P, J_{C-P} = 10.6 Hz), 84.0 (CO-C**NH_2**=C), 153.8 (CNH_2=**C**-N), 156.5 (**NH**=**C**-**NH_2**), 158.2 (**CO**-CNH_2=C). $C_7H_{14}N_5O_5P \cdot 1NH_3$: 279.2. MS (FAB): *m/z* 279.6.

Acknowledgment. The authors wish to thank Dr. U. Schloz, Robugen company Esslingen, for biological tests. This project was supported by the Bundesministerium für Forschung und Technologie der Bundesrepublik Deutschland.

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