# Synthesis of Acyclic Nucleoside Phosphonates as Antiviral Compounds



Frank Wormstädt,[+] Ute Brinckmann,[§] Michael Gütschow,[+] and Kurt Eger*[+]

[+]Universität Leipzig, Institut für Pharmazie, Pharmazeutische Chemie, Brüderstraße 34, D-04103 Leipzig, Germany
[§]Universität Leipzig, Institut für Virologie, Liebigstraße 24, D-04103 Leipzig, Germany

Received August 10, 1999

Reaction of 6-chloropyrimidines with diethyl [(2-aminoethoxy)methyl]phosphonate allows for a ready access to acyclic nucleoside phosphonates. A series of 5-substituted pyrimidines bearing a phosphonate side chain at position 6 were synthesized and tested against herpes simplex viruses (HSV-1 and HSV-2) and human immunodeficiency virus (HIV-1). Some compounds showed weak antiviral activity against HSV-1.

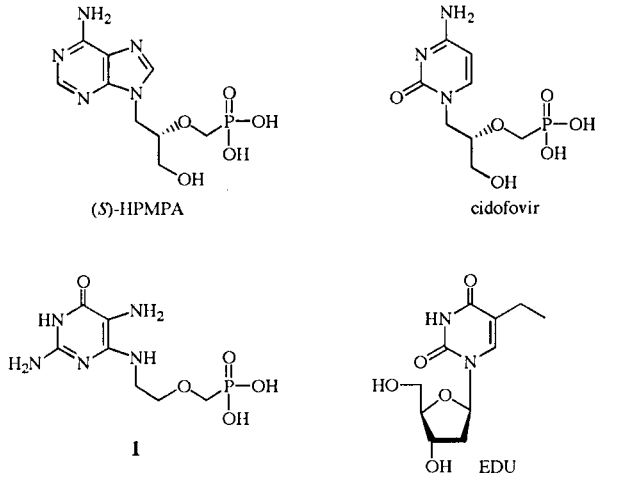
*J. Heterocyclic Chem.*, **37**, 1187 (2000).

## Introduction.

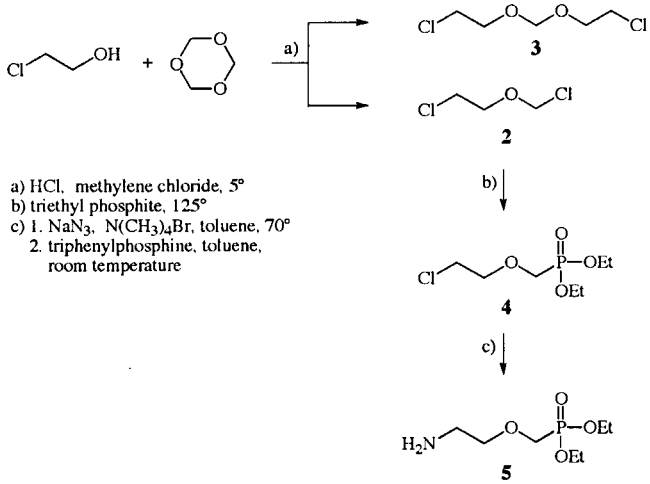
The discovery of (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine, (*S*)-HPMPA (Scheme 1), by De Clercq and Holy as a potent, broad spectrum antiviral agent has defined a new class of nucleotide analogues structurally characterized by a phosphonate side chain [1,2]. Analogues of (*S*)-HPMPA with various purine and pyrimidine bases were found to efficaciously inhibit a wide spectrum of DNA- and retro viruses [2,3]. The acyclic nucleoside phosphonates can be considered as analogues of nucleoside monophosphates whereby the first phosphate group has been built in as a phosphonate. Therefore, such compounds can bypass the initial phosphorylation, the crucial first step in the intracellular metabolism of nucleoside analogues [4]. Remarkably, (*S*)-HPMPA and related phosphonate derivatives exhibit inhibitory effects towards DNA viruses that lack viral thymidine kinase (TK) activity, including TK-deficient strains of herpes simplex virus (HSV), as well as towards viruses that do not encode TK, such as cytomegalovirus (CMV) [5]. The cytosine derivative cidofovir [6] is the first state-authorized acyclic nucleoside phosphonate and is used in the treatment of AIDS patients against CMV infections.

Previously, Eger and co-workers [7] reported on the synthesis of the phosphonate **1**, prepared as ammonium salt, that showed antiviral activity against HSV-1, assayed in mice embryo cell cultures. In this compound, the phosphonate side chain is attached at the exocyclic 6-amino group of 2,5,6-triaminopyrimidin-4(3*H*)-one. The nitrogen atoms of the 5- and 6-amino function in **1** imitate the bicyclic guanine skeleton. Herein, we report on our attemps to improve the preparation of the free phosphonic acid **1** in order to make the compound available for detailed physicochemical and biological investigations. The preparation of analogues of **1** with various substituents at positions 2 and 5 is also described in this report.

Scheme 1




Scheme 2



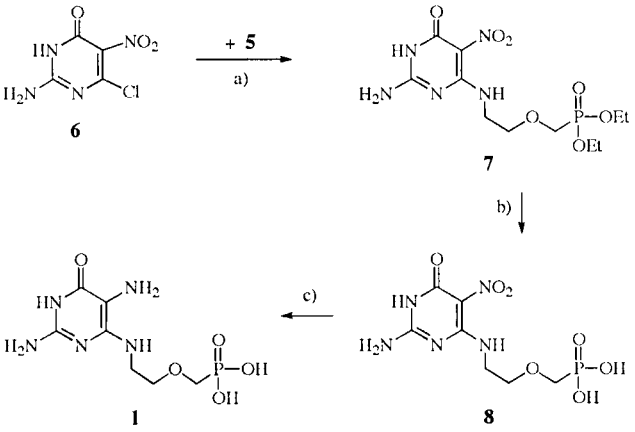

## Synthesis of 2,5-Diamino-6-{*N*-[2-(phosphonomethoxy)-ethyl]amino}pyrimidin-4(3*H*)-one (**1**) and 2-Amino-pyrimidine Derivatives.

The key compound, diethyl [(2-chloroethoxy)methyl]-phosphonate **4**, was prepared according to the described route [3]. Reaction of 2-chloroethanol with 1,3,5-trioxane and dry hydrogen chloride afforded the dichloro ether **2** (Scheme 2). However, the formation of a byproduct, 1-chloro-2-[(2-chloroethoxy)methoxy]ethane **3**, was observed. This compound, prepared from 2-chloroethanol, formaldehyd, and hydrochloric acid, has already been

described by Orlowski and co-workers [8]. Under changed reaction conditions, the yield of the desired **2** could be improved to 82%. Compound **2** was then converted with triethyl phosphite by an Arbuzov reaction to the phosphonate **4**, which was subsequently reacted with sodium azide, and reduced with triphenylphosphine to the amino phosphonate **5** [7]. The amino phosphonate **5** was utilized in several routes to prepare acyclic nucleoside phosphonates.

The synthesis of the acyclic nucleoside phosphonate **1** is outlined in Scheme 3. The transformation of the chloropyrimidine **6** into **7** [7] turned out to be difficult due to the poor solubility of **6**. However, on reacting **6** with **5** in a solvent mixture of acetone and methanol, **7** was obtained in an improved yield without chromatographic purification. The cleavage of the diester **7** with bromotrimethylsilane and subsequent hydrolysis according to McKenna and co-workers [9] produced the free phosphonic acid **8**. Final conversion of **8** to the desired **1** was accomplished with hydrogen/palladium-charcoal. In contrast to a previous report [7], the resulting derivatives **1** and **8** were not isolated in form of their ammonium salts. The free phosphonic acids **1** and **8** were well soluble in water.

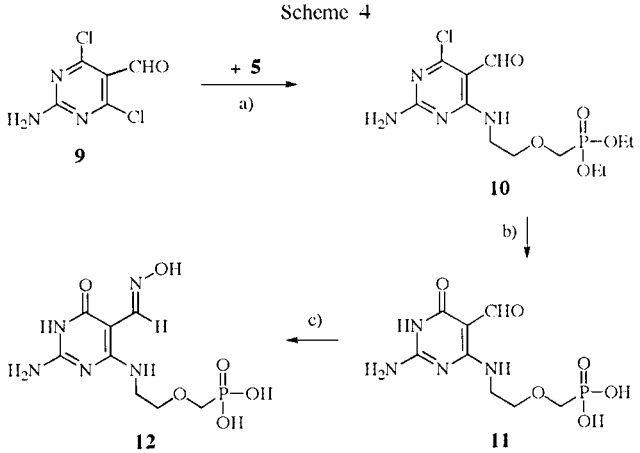
Scheme 3



a) acetone/methanol, room temperature
b) BTMS, acetonitrile, argon atmosphere, room temperature
c) $H_2$/10% Pd/C, methanol, room temperature

The synthesis of structurally related phosphonates **11** and **12** is shown in Scheme 4. These structures were choosen with respect to the strong antiviral activities of 5-formyl-2'-deoxyuridine against HSV-1, HSV-2, and CMV [10-12], as well as the activity against HSV-1 of the oxime derived from 5-formyl-2'-deoxyuridine [11]. To obtain the starting compound **9** [13], 2-amino-4,6-dihydroxypyrimidine was treated with Vilsmeyer reagent (dimethylformamide/ phosphoryl chloride) [14]. Again, the substitution reaction of **9** with the amino phosphonate **5** to form **10** was successful in an acetone/methanol mixture. Cleavage of the diester with bromotrimethylsilane in acetonitrile provided the acyclic nucleoside phosphonate **11**. Under the choosen conditions,

Scheme 4



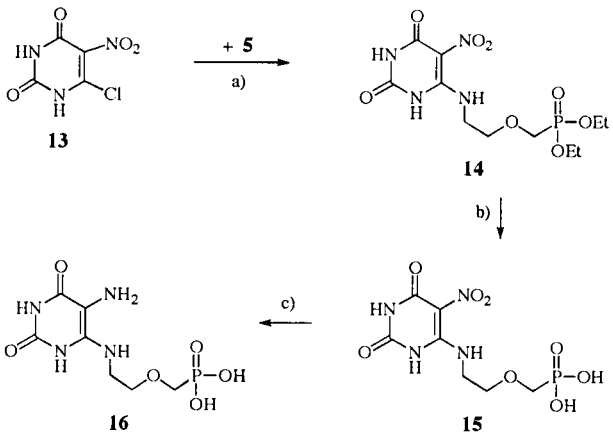
a) acetone/methanol, room temperature
b) BTMS, acetonitrile, argon atmosphere, room temperature
c) hydroxylamine hydrochloride, ethanol, room temperature

the desired lactam formation occured simultaneously. The formyl function of **11** allows for several structural variations at position 5 *via* reactions with nucleophiles. An example is the transformation to the oxime **12**, obtained by reacting **11** with hydroxylamine hydrochloride in ethanolic solution at room temperature. It was found to be advantageous to transform the 5-formyl group after the diester cleavage step. The (*E*)-configuration of the oxime **12** was concluded from the $^{13}C$ nmr shift difference between the formyl carbon of **11** and the iminyl carbon of **12**, being 42 ppm. Similar differences between aromatic aldehydes and corresponding (*E*)-oximes have been reported, whereas the iminyl carbon resonance of (*Z*)-oximes was observed at higher fields [15, 16].

Synthesis of 2-Oxopyrimidine Derivatives.

In the design of antiviral nucleoside analogues, the replacement of the 5-methyl group in thymidine by other substituents was particularly successful, leading to the development of potent therapeutic drugs, such as (*E*)-5-bromovinyl-2'-deoxyuridine (brivudine), 5-trifluoromethyl-2'-deoxyuridine (trifluridine), 5-iodo-2'-deoxyuridine (idoxuridine), and EDU (edoxudine, Scheme 1). Therefore, our attempts were directed towards the introduction of the [(phosphonomethoxy)ethyl]amino chain at position 6 of 5-substituted uracils [17]. The route to a corresponding 5-amino derivative is outlined in Scheme 5. The synthesis of the starting compound **13** was accomplished by treatment of 2,4,6-trichloropyrimidine with a mixture of aqueous sodium hydroxide and hydrogen peroxide [18] to selectively substitute the chloro atoms at positions 2 and 4. The resulting 6-chlorouracil was converted into **13** with a mixture of fuming nitric acid and concentrated sulfuric acid [19]. The nitropyrimidine **13** was then subjected to the conditions of the coupling reaction with **5** to obtain the diethyl phosphono nucleoside **14** [20]. After deprotection of **14** with bromotrimethylsilane,

Scheme 5

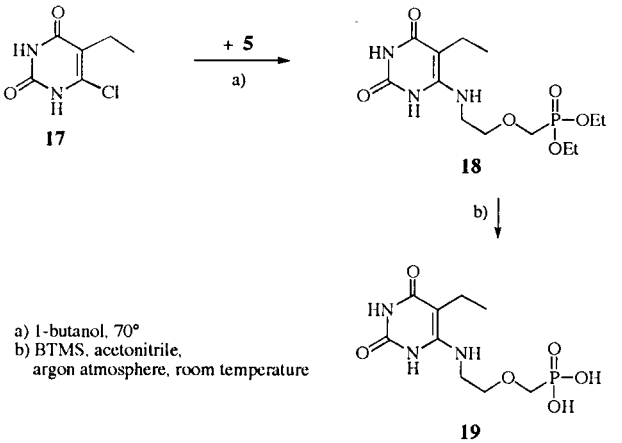



a) acetone/methanol, room temperature
b) BTMS, acetonitrile, argon atmosphere, room temperature
c) $H_2$/10% Pd/C, methanol, room temperature

the acyclic 2-oxopyrimidine phosphonate **15** was obtained. Reduction of the nitro group furnished the desired 5-aminopyrimidine derivate **16**.

Edoxudine (EDU) (Scheme 1) belongs to the antiviral agents of the first generation. Its therapeutical potential has been described as early as 1967 [21]. It is a selective inhibitor of the replication of HSV-1 and HSV-2. Remarkably, EDU is more efficient against HSV-2 than against HSV-1. Mostly, it is applied topically in cases of deep herpetic keratites. The route to an analogue of EDU, in which the (phosphonomethoxy)ethyl chain is linked *via* a 6-amino group to the uracil skeleton, is shown in Scheme 6. 1,3-Dialkyl- and 1,3,5-trialkyl-6-chlorouracils exhibit high reactivity towards nucleophilic reagents due to vinylogous acid chloride structure. On the other hand, 1-methyl-6-chloro- or 6-chlorouracil were found to be less reactive [22]. In the present case, on reacting 6-chloro-5-ethyluracil **17** with **5** in 1-butanol at 70° for three hours, suitable conditions for the preparation of **18** were found.

Scheme 6




a) 1-butanol, 70°
b) BTMS, acetonitrile, argon atmosphere, room temperature

The diester **18** was used without further purification in the final step of the sequence to obtain the acyclic nucleoside phosphonate **19**.

For a summary, the substitution of 6-chloro pyrimidines with diethyl [(2-aminoethoxy)methyl]phosphonate allows for ready access to a class of acyclic nucleoside phosphonates. Such compounds, considered as exocyclic nucleoside derivatives [7], are stable. The design of pyrimidines with a alkoxymethylamino(-NH-$CH_2$-O-R) function at position 6, according to drugs such as aciclovir, appears to be less promising due to the unstable hemiaminal moiety [7].

## Biological Methods and Results.

The phosphonic acids of the present series were examined for their inhibitory effects against HSV-1 and HSV-2. The compounds were added to Vero cell cultures at concentrations of 0.5, 5, 25, 50, 100, and 200 μg/mL and incubated for 24 hours. Cytotoxicity, indicated by growth inhibition or disruption of noninfected cells was controlled macro- and microscopically. Briefly, the cell cultures were grown in 24 well plates with minimal essential medium (MEM) containing 5% fetal calf serum (FCS) and 2.5% agarose and treated with 0-200 μg/mL of the compounds (10 mg/mL stock solutions in DMSO). After two days, the cytotoxic effect was evaluated by staining the surviving cells. The two nitro derivatives **8** and **15** were considerably cytototoxic at a concentration of 100 μg/mL and not further investigated for that reason. Compounds **1**, **11**, and **12** were not cytotoxic at a concentration of 100 μg/mL. At 200 μg/mL compounds **11** and **12** were found to be cytotoxic and compound **1** showed weak cytotoxicity. As a parameter for antiviral effects, the plaque reduction assay was carried out. Aciclovir was used as reference. The 2-aminopyrimidine derivatives **1** and **11** did exhibit antiviral activity against HSV-1. At 25 μg/mL, both compounds showed 50% reduction in plaque formation. For a comparison, aciclovir was fully active at 0.5 μg/mL. The oxime **12** showed antiviral activity only at 50 μg/mL, and the pyrimidinediones **16** and **19** were inactive. The phosphonates **1**, **11**, **12**, **16**, and **19** were also evaluated against HSV-2 and were found to be inactive except for the oxime **12**. At a relatively high concentration (100 μg/mL), **12** showed 50% reduction in plaque formation. Compounds **1** and **11** were tested against HIV-1, but found to be inactive.

## EXPERIMENTAL

Melting points were determined on a Boetius apparatus, and are uncorrected. The $^1H$ nmr spectra (300 MHz) and $^{13}C$ nmr spectra (75 MHz) were recorded on a Varian Gemini 300 spectrometer using tetramethylsilane as an internal standard. Mass spectra (EI, 70 eV) were obtained on a Varian Mat CH-6 spectrometer, and FAB spectra on a VG ZAB-HSQ spectrometer (matrix: 4-nitrobenzyl alcohol). Elemental analyses were performed at the Institute of Organic Chemistry, University of Leipzig. Thin layer chromatography was performed using silica gel 60 $F_{254}$ (Merck).

Solvents were dried by conventional methods. The FCS was purchased from Gibco/BRL. Diethyl [(2-aminoethoxy)methyl]phosphonate (**5**) was prepared as reported [7]. 6-Chloro-5-ethylpyrimidine-2,4(1*H*,3*H*)-dione (**17**) as well as the HSV strains were a gift from Robugen Company Esslingen, Germany. The following HSV strains were used, HSV-1 clone 101 and HSV-2 HG 56. The screening of compounds **1** and **11** towards HIV was carried out at the National Cancer Institute (NCI), Maryland, USA.

Diethyl [(2-Chloroethoxy)methyl]phosphonate (**4**).

2-Chloroethanol (376 g, 4.66 moles) and trioxane (140 g, 4.66 moles) were dissolved in methylen chloride (100 mL). Gaseous hydrogen chloride was introduced at 5° for 10 hours. The organic layer was separated, evaporated under reduced pressure, and dried (potassium chloride). Fractional distillation yielded 2-chloroethoxymethyl chloride **2** (493 g, 82%; bp 32-38°, 1 mbar; lit [3] bp 50-55°, 20 mbar) and 1-chloro-2-[(2-chloroethoxy)methoxy]-ethane **3** (32 g, bp 55-65°, 1 mbar, lit [8] bp 93°, 11 mbar). Compound **2** was then reacted with triethyl phosphite following the described procedure [3] to obtain **4** in 82% yield as a colorless liquid; $^1$H nmr (DMSO-$d_6$): δ 1.24 (t, J = 7.0 Hz, 6H, $CH_3$), 3.70-3.80 (m, 4H, $CH_2CH_2$), 3.87 (d, J = 8.3 Hz, 2H, $CH_2P$), 4.00-4.11 (m, 4H, $CH_2CH_3$); $^{13}$C nmr δ 16.3 ($CH_3$), 43.1 ($CH_2Cl$), 61.8 ($CH_2CH_3$), 62.8, 64.9 ($CH_2P$), 72.2, 72.3 ($CH_2CH_2O$).

2-Amino-6-{*N*-[2-(diethylphosphonomethoxy)ethyl]amino}-5-nitropyrimidin-4(3*H*)-one (**7**).

2-Amino-6-chloro-5-nitropyrimidin-4(3*H*)-one **6** [22] (2.5 g, 13 mmoles) was suspended in a mixture of acetone (60 mL) and methanol (40 mL). Compound **5** (3.15 g, 15 mmoles) was added, the mixture was stirred at room temperature for 24 hours, poured slowly onto ice-water (100 mL), and was allowed to stand overnight. The precipitate was collected by filtration and recrystallized from methanol to obtain **7** as a slightly green solid (3.1 g, 65%), mp 70°, lit [7] mp 70°.

*Anal.* Calcd. for $C_{11}H_{20}N_5O_7P$ (365.28): C, 36.17; H, 5.52; N, 19.17. Found: C, 35.86; H, 5.35; N, 19.34.

2-Amino-5-nitro-6-{*N*-[2-(phosphonomethoxy)ethyl]amino}-pyrimidin-4(3*H*)-one (**8**).

Compound **7** (250 mg, 0.68 mmol) and bromotrimethylsilane (1.0 g, 6.53 mmoles) were dissolved in dry acetonitrile (20 mL) and stirred at room temperature for 24 hours in an argon atmosphere. The solvent was removed under reduced pressure. Water (20 mL) was added and the mixture was stirred at room temperature for 4 hours. After evaporation to dryness, the residue was coevaporated three times with methanol (100 mL) to obtain **8** (73 mg, 35%) as a colorless solid, mp > 280°; $^1$H nmr (DMSO-$d_6$) δ 3.76 (d, J = 8.8 Hz, 2H, $CH_2P$), 3.80-3.95 (m, 4H, $CH_2CH_2$), 9.50 (s, 1H, NH), 10.60 (s, 1H, NH); $^{13}$C nmr δ 40.4 ($CH_2NH$), 65.3, 67.5 ($CH_2P$), 70.3, 70.5 ($CH_2CH_2O$), 110.5 (C-5), 154.1 (C-6), 156.2 (C-2), 159.2 (C-4); ms: (70eV) m/z 310 (11%, $M^+$ + 1).

2,5-Diamino-6-{*N*-[2-(phosphonomethoxy)ethyl]amino}pyrimidin-4(3*H*)-one (**1**).

Compound **8** (250 mg, 0.81 mmol) was dissolved in a mixture of water (5 mL) and methanol (25 mL). After addition of 50 mg of palladium/charcoal (10%), the mixture was stirred under a hydrogen atmosphere at room temperatur for 4 hours. The catalyst was removed by filtration and the dark solution was evaporated *in vacuo*. The residue was washed with acetone to yield **1** (170 mg, 75%) as a colorless solid, mp >280°; $^1$H nmr ($D_2O$) δ 3.76 (d, J = 8.5 Hz, 2H, $CH_2P$), 3.80-3.95 (m, 4H, $CH_2CH_2$); 7.68 (s, 1H, NH); $^{13}$C nmr δ 40.8 ($CH_2NH$), 65.2, 67.3 ($CH_2P$), 71.0, 71.1 ($CH_2CH_2O$), 101.0 (C-5), 154.2 (C-6), 159.6 (C-2), 159.8 (C-4); ms: (70eV) m/z 277 (100%, $M^+$ - 2).

2-Amino-4-chloro-6-{*N*-[2-(diethylphosphonomethoxy)ethyl}-amino]-5-pyrimidinecarboxaldehyde (**10**).

2-Amino-4,6-dichloro-5-pyrimidinecarboxaldehyde **9** [13] (2.5 g, 13 mmoles) was reacted with **5** (3.15 g, 15 mmoles) according to the procedure to prepare **7**. The crude product was recrystallized from methanol to obtain **10** (2.7 g, 57%) as a red solid, mp 83°, $^1$H nmr (DMSO-$d_6$) δ 1.21 (t, J = 7.0 Hz, 6H, $CH_3$), 3.60-3.70 (m, 4H, $CH_2CH_2$), 3.85 (d, J = 8.3 Hz, 2H, $OCH_2P$), 3.98-4.10 (m, 4H, $CH_2CH_3$), 7.70 (s, br, 2H, $NH_2$), 9.20 (s, br, 1H, NH), 9.90 (s, 1H, CH); $^{13}$C nmr δ 16.3 ($CH_3$), 40.2 ($CH_2NH$), 61.8 ($CH_2CH_3$), 62.9, 65.1 ($OCH_2P$), 70.6, 70.7 ($CH_2CH_2O$), 101.0 (C-5), 162.0 (C-6), 162.3 (C-2), 165.3 (C-4), 187.3 (CHO); ms: (70eV) m/z = 366 (8%, $M^+$).

*Anal.* Calcd. for $C_{12}H_{20}ClN_4O_5P$ (366.74): C, 39.34; H, 5.51; N, 15.30. Found: C, 40.04; H, 5.78; N, 15.17.

2-Amino-4-oxo-6-{*N*-[2-(phosphonomethoxy)ethyl]amino}-3,4-dihydro-5-pyrimidinecarboxaldehyde (**11**).

According to the procedure to prepare **8**, compound **10** (150 mg, 0.4 mmol) was reacted with bromotrimethylsilane (1.0 g, 6.53 mmoles) to obtain **11** (94 mg, 79%) as a red solid, mp 183°; $^1$H nmr (DMSO-$d_6$) δ 3.58 (d, J = 8.6 Hz, 2H, $CH_2P$), 3.60-3.68 (m, 4H, $CH_2CH_2$), 7.70 (s, br, 2H, $NH_2$), 9.19 (s, 1H, NH), 9.70 (s, 1H, CH); $^{13}$C nmr δ 65.4, 67.5 ($CH_2P$), 70.3, 70.4 ($CH_2CH_2O$), 102.5 (C-5), 159.2 (C-6), 161.4 (C-2), 161.8 (C-4), 189.6 (CHO); ms: (70eV) m/z = 290 (31%, $M^+$ - 2).

(*E*)-2-Amino-5-(hydroxyimino)methyl-6-{*N*-[2-(phosphonomethoxy)ethyl]amino}pyrimidin-4(3*H*)-one (**12**).

Compound **11** (150 mg, 0.51 mmol) was suspended in dry ethanol (20 mL). Hydroxylamine hydrochloride (210 mg, 3 mmoles) was added and the mixture was stirred for 24 hours at room temperature. The solution was evaporated to dryness and the dark red residue washed with water and dried to obtain **12** (82 mg, 52%), mp 187°; $^1$H nmr (DMSO-$d_6$) δ 3.50-3.70 (m, 6H, $CH_2P$, $CH_2CH_2$), 6.88 (s, 2H, $NH_2$), 8.25 (s, 1H, C*H*=NOH), 8.84 (s, 1H, NH); $^{13}$C nmr δ 40.7 ($CH_2NH$), 65.5, 67.7 ($CH_2P$), 70.6, 70.7 ($CH_2CH_2O$), 97. 6 (C-5), 147.4 (CH), 152.8 (C-6), 159.5 (C-2), 160.5 (C-4); ms: (70eV) m/z = 305 (20%, $M^+$ - 2).

5-Nitro-6-{*N*-[2-(diethylphosphonomethoxy)ethyl]amino}-pyrimidine-2,4(1*H*,3*H*)-dione (**14**).

6-Chloro-5-nitropyrimidine-2,4(1*H*,3*H*)-dione **13** [19] (2.5 g, 13 mmoles) was reacted with **5** (3.15 g, 15 mmoles) according to procedure described for the preparation of **7**. The crude product was recrystallized from methanol to obtain **14** (2.1g, 44%) as a slightly green solid, mp 81°; $^1$H nmr (DMSO-$d_6$) δ 1.24 (t, J = 7.1 Hz, 6H, $CH_3$), 2.97-3.03 (m, 2H, $CH_2NH$), 3.73 (t, J = 5.2 Hz, 2H, $CH_2CH_2O$), 3.88 (d, J = 7.7 Hz, 2H, $OCH_2P$), 4.00-4.12 (m, 4H, $CH_2CH_3$), 7.81 (s, 1H, NH), 9.81 (s, 1H, NH); $^{13}$C nmr δ 16.3 ($CH_3$), 38.4 ($CH_2NH$), 61.8 ($CH_2CH_3$), 62.9, 65.1 ($OCH_2P$), 68.8, 68.9 ($CH_2CH_2O$), 112.3 (C-5), 149.9 (C-6), 159.4 (C-4, C-2); ms: (70eV) m/z = 280 (37%, $M^+$ - 86).

*Anal.* Calcd. for $C_{11}H_{19}N_4O_8P$ (366.27): C, 36.06; H, 5.23; N, 15.30. Found: C, 36.40; H, 5.34; N, 16.12.

5-Nitro-6-{*N*-[2-(phosphonomethoxy)ethyl]amino}pyrimidine-2,4(1*H*,3*H*)-dione (**15**).

In an argon atmosphere, compound **14** (150 mg, 0.4 mmol) was suspended in dry acetonitrile (20 mL). Bromotrimethylsilane (700 mg, 4.57 mmoles) was added and the mixture was stirred at room temperatur for 12 hours and evaporated to dryness. The residue was dissolved in water (20 mL) and stirred for 4 hours at room temperature. After evaporation to dryness, the residue was coevaporated three times with methanol (100 mL) to obtain **15** (52 mg, 41%) as a colorless solid, mp > 280°; $^1$H nmr ($D_2O$) δ 3.24-3.30 (m, 2H, $CH_2NH$), 3.82 (d, J = 9.1 Hz, 2H, $OCH_2P$), 3.07 (t, J = 5.0 Hz, 2H, $CH_2CH_2O$); $^{13}$C nmr δ 39.3 ($CH_2NH$), 65.3, 67.4 ($CH_2P$), 68.6 ($CH_2CH_2O$), 113.7 (C-5), 151.1 (C-6), 161.8 (C-2, C-4); ms: (70eV) m/z = 310 (11%, $M^+$).

*Anal.* Calcd. for $C_7H_{11}N_4O_8P$ (310.16): C, 27.11; H, 3.57; N, 18.06. Found: C, 26.90; H, 3.72; N, 17.85.

5-Amino-6-{*N*-[2-(phosphonomethoxy)ethyl]amino}pyrimidine-2,4(1*H*,3*H*)-dione (**16**).

Following the procedure outlined for the preparation of **1**, compound **15** (250 mg, 0.81 mmol) was converted to obtain **16** (110 mg, 49%) as a colorless solid, mp >280°; $^1$H nmr (DMSO-$d_6$) δ 2.96-3.06 (m, 2H, $CH_2NH$), 3.64 (d, J = 8.0 Hz, 2H, $CH_2P$), 3.71 (t, J = 5.1 Hz, 2H, $CH_2CH_2O$), 7.77 (s, 1H, NH); $^{13}$C nmr δ 39.3 ($CH_2NH$), 65.4, 67.5 ($CH_2P$), 68.7 ($CH_2CH_2O$), 98.1 (C-5), 145.1 (C-6), 161.8 (C-2, C-4); ms: (70eV) m/z = 278 (16%, $M^+$ - 2).

5-Ethyl-6-{*N*-[2-(diethylphosphonomethoxy)ethyl]amino}-pyrimidine-2,4(1*H*,3*H*)-dione (**18**).

6-Chloro-5-ethylpyrimidine-2,4(1*H*,3*H*)-dione **17** (2.0 g, 11.46 mmoles) was suspended in 1-butanol (50 mL). Compound **5** (3.15 g, 15 mmol) was added and the mixture was stirred for 2 hours at 70° and evaporated to dryness. The residue was coevaporated three times with methanol (200 mL) to obtain crude **18** (2.1 g, 53%) as an oil; $^1$H nmr (DMSO-$d_6$) δ 0.92 (t, J = 7.3 Hz, 3H, 5-$CH_2CH_3$), 1.24 (t, J = 7.1 Hz, 6H, $OCH_2CH_3$), 2.27 (q, J = 7.3 Hz, 2H, 5-$CH_2CH_3$), 2.95-3.05 (m, 2H, $CH_2NH$), 3.74 (t, J = 5.4 Hz, 2H, $CH_2CH_2O$), 3.88 (d, J = 7.7 Hz, 2H, $OCH_2P$), 4.00-4.10 (m, 4H, $OCH_2CH_3$); $^{13}$C nmr δ 13.4 (5-$CH_2CH_3$), 16.2 ($OCH_2CH_3$), 19.1 (5-$CH_2CH_3$), 38.5 ($CH_2NH$), 61.8 ($OCH_2CH_3$), 62.9, 65.1 ($OCH_2P$), 69.4, 69.5 ($CH_2CH_2O$), 106.8 (C-5), 153.4 (C-6), 154.8 (C-2), 164.5 (C-4); ms: (70eV) m/z = 349 (28%, $M^+$).

5-Ethyl-6-{*N*-[2-(phosphonomethoxy)ethyl]amino}pyrimidine-2,4(1*H*,3*H*)-dione (**19**).

Compound **18** (150 mg, 0.43 mmol) was dissolved in dry acetonitrile (20 mL) in an argon atmosphere. Bromotrimethylsilane (1.0 g, 6.53 mmoles) was added, the mixture was stirred at room temperature for 48 hours and evaporated to dryness. Water (20 mL) was added and the mixture was stirred at room temperature for 4-hours. The solvent was removed *in vacuo* and the residue was coevaporated three times with methanol (100 mL) to obtain **19** (56 mg, 45%) as a yellow solid, mp >280°; $^1$H nmr (DMSO-$d_6$) δ 0.93 (t, J = 7.4 Hz, 3H, $CH_3$), 2.28 (q, J = 7.4 Hz, 2H, $CH_2CH_3$), 2.93-3.04 (m, 2H, $CH_2NH$), 3.63 (d, 2H, J = 8.1 Hz, $OCH_2P$), 3.71 (t, J = 5.3 Hz, 2H, $CH_2CH_2O$); $^{13}$C nmr δ 12.5 ($CH_3$), 18.5 ($CH_2CH_3$), 38.4 ($CH_2NH$), 65.1, 67.2 ($CH_2P$), 68.3, 68.4 ($CH_2CH_2O$), 111.4 (C-5), 149.6 (C-6), 162.7 (C-2), 172.2 (C-4); ms: (FAB) m/z = 294 ($MH^+$).

*Anal.* Calcd. for $C_9H_{16}N_3O_6P$ (293.22): C, 36.87; H, 5.50; N, 14.33. Found: C, 36.51; H, 5.73; N, 13.94.

Acknowledgements.

The authors would like to thank the 'Fonds der Chemischen Industrie' for financial support.

REFERENCES AND NOTES

[1] E. De Clercq, A. Holy, I. Rosenberg, T. Sakuma, J. Balzarini and P. C. Maudgal, *Nature*, **323**, 464 (1986).

[2] A. Holy, J. Günter, H. Dvorakova, M. Masojidkova, G. Andrei, R. Snoeck, J. Balzarini and E. De Clercq, *J. Med. Chem.* **42**, 2064 (1999).

[3] A. Holy, I. Rosenberg and H. Dvorakova, *Collect. Czech. Chem. Commun.* **54**, 2190 (1989).

[4] E. De Clercq, *J. Med. Chem.* **38**, 2491 (1995).

[5] J. J. Bronson, C. U. Kim, I. Ghazzouli, M. J. M. Hitchcock, E. R. Kern and J. C. Martin, in Nucleotide Analogues as Antiviral Agents, ACS Symposium, Series 401, J. C. Martin, ed., Washington 1989, 72 pp.

[6] E. Aspe, R. H. Guy, W. A. Lee, J. A. Kennedy, G. C. Visor and R. D. Ennis, *J. Pharm. Sci.* **84**, 750 (1995).

[7] K. Eger, E. M. Klünder and M. Schmidt, *J. Med. Chem.* **37**, 3057 (1994).

[8] P. Orlowski, H. Czerwinska and L. Klahr, *Przemysl. Chem.* **13**, 520 (1957); ref. *Chem. Abstr.* **52**, 8040e (1958).

[9] C. E. McKenna, M. T. Higa, N. H. Cheung and M.-C. McKenna, *Tetrahedron Lett.* 1977, 155.

[10] J. Reefschläger, D. Bärwolff, P. Engelmann, P. Langen and H. A. Rosenthal, *Antiviral Res.* **2**, 41 (1982).

[11] J. S. Park, C. T.-C. Chang, C. L. Schmidt, Y. Golander, and E. De Clercq, *J. Med. Chem.* **23**, 661 (1980).

[12] J. Neyts, A. Verbiest, A. Meerbach and E. De Clercq, *Nucleosides Nucleotides* **14**, 1153 (1995).

[13] L. Bell, H. M. McGuire and G. A. Freeman, *J. Heterocyclic Chem.* **20**, 41 (1983).

[14] The Vilsmeyer reaction with DMF/$POCl_3$ constitutes one possibility for the formation of 5-formylpyrimidines. For reports on chloronating formylation reactions on pyrimidines, activated by tautomerism, see: W. Klötzer and M. Herberz, *Monatsh. Chem.* **96**, 1567 (1965).

[15] M. S. Gordon, S. A. Sojka and J. G. Krause, *J. Org. Chem.* **49**, 97 (1984).

[16] The (*E*)-configuration of **12** was further indicated by the formation of green copper(II) complex, when **12** was treated with aqueous $CuCl_2$ solution.

[17] In a previous report, the synthesis of a sterically rigid derivative of 5-bromovinyl-2'-deoxyuridine was reported: K. Eger, M. Jalalian and M. Schmidt, *J. Heterocyclic Chem.* **32**, 211 (1995).

[18] L. F. Ovechkina, V. I. Gunar and S. I. Zavyalov, *Izv. Akad. Nauk. SSSR, Ser. Khim.* 1969, 2035; ref. *Chem. Abstr.* **72**, 21655u (1970).

[19] O. E. Schultz and P. Warnecke, *Arzneim-Forsch./Drug. Res.* **17**, 1060 (1967).

[20] The ready reactivity of 6-chloro-5-nitropyrimidines towards amines and hydrazines has been described. For examples, see ref 22.

[21] K. K. Gauri and G. Malorny, *Naunyn Schiedebergs Arch. Exp. Pathol. Pathopharmacol.* **257**, 21 (1967).

[22] W. Pfleiderer and H. Walter, *Liebigs Ann. Chem.* **677**, 113 (1964).