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ORIGINAL PAPER

Synthesis, antiviral, cytotoxic and cytostatic evaluation of N^1-(phosphonoalkyl)uracil derivatives

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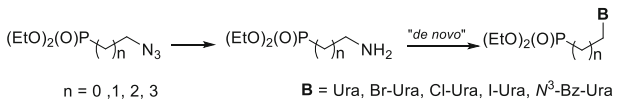
Abstract A series of N^1-(phosphonoalkyl)uracils was prepared in a two-step reaction sequence from ω-aminoalkylphosphonates and (*E*)-3-ethoxyacryloyl isocyanate followed by the uracil ring closure. Under standard conditions (NCS; NBS; I_2/CAN) all N^1-(phosphonoalkyl)uracils were transformed into the respective 5-halogeno derivatives to be later benzoylated at N3. All compounds were evaluated in vitro for activity against a broad variety of DNA and RNA viruses. One compound was slightly active against human cytomegalovirus in HEL cell cultures ($EC_{50} = 45$ μM) while another showed weak activity against varicella-zoster virus (TK^+ VZV strain OKA and TK^- VZV strain 07-1) with $EC_{50} = 43$ and 53 μM, respectively. In addition, several compounds exhibited noticeable inhibitory effects on the proliferation of human cervical carcinoma cells (HeLa) at a concentration lower than 200 μM.

Graphical abstract



Keywords Nucleotides · Halogenation · Antiviral activity · NMR spectroscopy · Cyclizations

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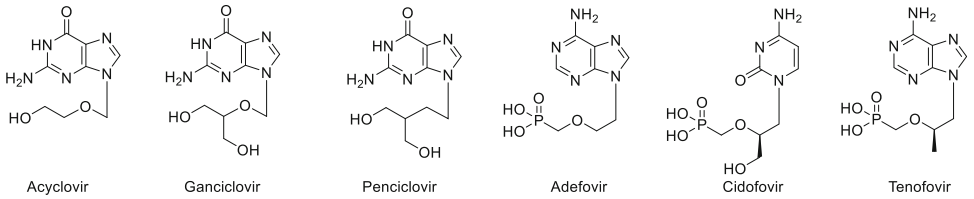
Introduction

Acyclic nucleoside and nucleotide analogues have been used in clinical practice for almost 50 years and have become cornerstones in the treatment of patients with viral infections [1–3]. Nucleoside analogues such as acyclovir, ganciclovir, and penciclovir as well as acyclic nucleoside phosphonates (ANPs), namely adefovir, cidofovir, and tenofovir, belong to the most widely used antiviral drugs (Fig. 1) [4–6]. The ribose moiety in natural nucleosides/nucleotides was replaced by linear or branched aliphatic units to obtain analogues with specific activity following activation by viral and/or cellular kinases. The antiviral activity of these compounds relies on enzyme inhibition or on incorporation as active metabolites into the viral nucleic acids resulting in inhibition of viral genome replication.

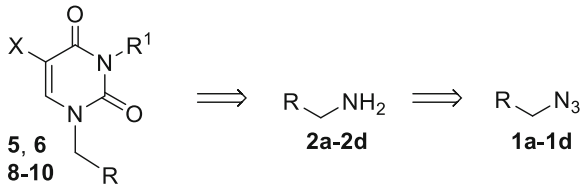
In the last decade, several new drugs, including nucleoside and nucleotide analogues, have been approved for the treatment of viral infections and in cancer chemotherapy. Many nucleoside/nucleotide analogues and their derivatives are currently under preclinical and clinical trials [7] which demonstrates how rapidly this area of research grows. However, some of these analogues have poor oral bioavailability and are associated with toxicity, limiting their application for treatment of cancer and viral infections. Moreover, a long-term treatment with antiviral drugs may result in lower sensitivity of the viruses to chemotherapeutics (drug resistance) [8, 9]. For this reason, a search for new compounds with higher antiviral and antitumor activities, good bioavailability, better solubility, and proper balance between efficacy and long-term toxicity still continues.

The known synthetic strategies to ANPs commonly apply alkylation of heterocyclic bases [10] while the construction of nucleobase skeletons from the appropriate terminal primary amines has been less frequently used [11–

Fig. 1 Acyclic nucleoside and nucleotide analogues



Scheme 1



5a-5d X = H, R^1 = H
6a-6d X = Cl, R^1 = H
8a-8d X = Br, R^1 = H
9a-9d X = I, R^1 = H
10a-10d X = H, R^1 = Bz

a: R = $(EtO)_2(O)P$
b: R = $(EtO)_2(O)PCH_2$
c: R = $(EtO)_2(O)PCH_2CH_2$
d: R = $(EtO)_2(O)PCH_2CH_2CH_2$

15]. Herein, we report on the efficient synthesis and biological evaluation of a new series of acyclic nucleotide analogues by application of the latter approach (Scheme 1).

Results and discussion

ω-Aminoalkylphosphonates can be prepared by several methods. Arbuzov reaction [16] of *N*-(ω-bromoalkyl)phthalimides with trialkyl phosphites seems to be most frequently applied. Alternatively, ω-aminoalkylphosphonates can be synthesized from nitriles [17] or from ω-azidophosphonates employing hydrogenolysis [18].

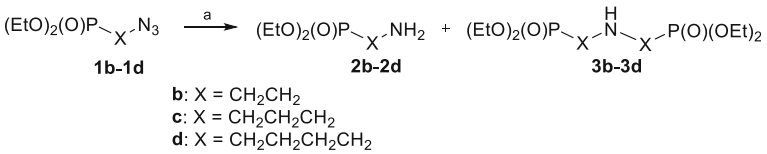
Diethyl aminoalkylphosphonates **2a–2d** (aminomethyl-, 2-aminoethyl-, 3-aminopropyl-, and 4-aminobutylphosphonates), used in this study, are known and have already been described in the literature [19–27]. Thus, diethyl aminomethylphosphonate (**2a**) was prepared in total 90 % yield from *N*-(bromomethyl)phthalimide followed by the treatment with hydrazine [19–21] whereas ω-aminoalkylphosphonates **2b–2d** were synthesized from the corresponding ω-azidoalkylphosphonates **1b–1d** [28–32] by catalytic hydrogenation [23, 26]. However, several literature reports [33, 34] noticed the formation of significant amounts of symmetrical secondary amines as by-products during hydrogenolysis of azides depending on the azide concentration and the azide to catalyst ratio. When ω-azidophosphonates **1b–1d** were subjected to hydrogenation in the presence of 10 % Pd–C, symmetrical secondary amines **3b–3d** contaminated (11–33 %) the major phosphonates **2b–2d** as judged from the ^{31}P NMR spectra (Scheme 2). The mixtures of ω-aminoalkylphosphonates **2b–2d** containing various amounts of symmetrical secondary amines **3b–3d** were separated on silica gel columns.

Alkylation of nucleobases with substituted alkylphosphonates is generally recognized as a capricious process since dealkylation of phosphonate esters accompany the formation of a carbon-nucleobase bond and this was the primary reason to replace *O*,*O*-dimethyl and *O*,*O*-diethyl esters with *O*,*O*-diisopropyl phosphonates [35, 36]. Based on this experience and that of other groups, we concluded that the alternative approach to independently construct the pyrimidine ring through functionalization of ω-aminoalkylphosphonates is more feasible than the alkylation.

The N^1-(phosphonoalkyl)uracils **5a–5d** were synthesized from ω-aminoalkylphosphonates **2b–2d** in a two-step procedure which involved reaction with (*E*)-3-ethoxyacryloyl isocyanate [37] in situ generated from (*E*)-3-ethoxyacryloyl chloride [38–40] and silver cyanate followed by the uracil ring closure in the presence of 2 M H_2SO_4 (Scheme 3) [18, 39, 40].

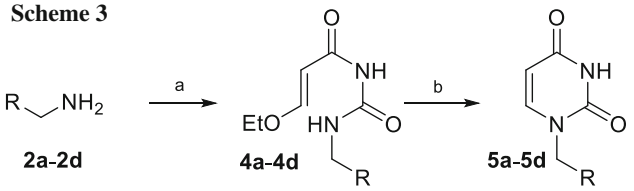
The conversion of uracil phosphonates **5a–5d** into 5-chlorouracils **6a–6d** and 5-bromouracils **8a–8d** was achieved by treatment with *N*-chlorosuccinimide (NCS) [41] and *N*-bromosuccinimide (NBS) [41] respectively,

Scheme 2



Reagents and conditions: a. H_2, 10% Pd-C, EtOH, r.t. 20 h

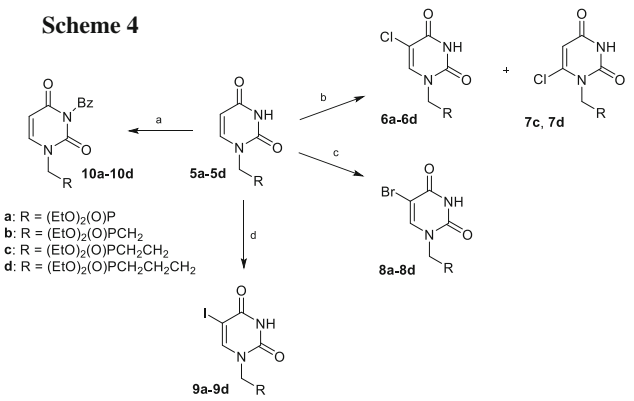
Scheme 3



a: R = $(EtO)_2(O)P$
b: R = $(EtO)_2(O)PCH_2$
c: R = $(EtO)_2(O)PCH_2CH_2$
d: R = $(EtO)_2(O)PCH_2CH_2CH_2$

Reagents and conditions: a. (*E*)-3-ethoxyacryloyl isocyanate, DMF, 0 °C → r.t, 12 h b. 2M H_2SO_4, dioxane, reflux, 20 h

Scheme 4



a: R = $(EtO)_2(O)P$
b: R = $(EtO)_2(O)PCH_2$
c: R = $(EtO)_2(O)PCH_2CH_2$
d: R = $(EtO)_2(O)PCH_2CH_2CH_2$

Reagents and conditions: a. benzoyl chloride, NEt_3, CH_2Cl_2, 0 °C to r.t., 72 h. b. NCS, pyridine, 100 °C, 0.5 h. c. NBS, pyridine, 100 °C, 0.5 h. d. I_2, CAN, CH_3CN, reflux, 2 h

whereas 5-iodouracils **9a**–**9d** were synthesized using iodine and cerium(IV) ammonium nitrate (CAN) [42] (Scheme 4). Although iodination and bromination of uracils **5a**–**5d** proceeded regioselectively at C5, chlorination of uracil phosphonates **5c**, **5d** under the applied conditions provided mixtures of C5 and C6 regioisomers **7c**, **7d** in an approximately 8:2 ratio.

Moreover, based on the literature report [43] and on our previous observations regarding the effect of the benzoyl substituent at N-3 of a nucleobase on the antiviral activity [29, 44], the N^3-benzoyluracil analogues **10a**–**10d** were synthesized from phosphonates **5a**–**5d** as shown on Scheme 4.

Structures and purity of all synthesized compounds were established by ^1H, ^{31}P and ^{13}C NMR and IR techniques as well as by elemental analysis.

Antiviral activity and cytotoxic evaluation

All the synthesized compounds **5a**–**5d**, **6a**–**6d**, and **8a**–**10d** were evaluated for their antiviral activities against a wide variety of DNA and RNA viruses, using the following cell-based assays: (a) human embryonic lung (HEL) cells: herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), herpes simplex virus-1 (TK^- ACV^r KOS), vaccinia virus and vesicular stomatitis virus, human cytomegalovirus (AD-169 strain and Davis strain), varicella-zoster virus (TK^+ VZV strain OKA and TK^- VZV strain 07-1); (b) CEM cell cultures: human immunodeficiency virus (HIV-1 and HIV-2); (c) Vero cell cultures: para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus; (d) HeLa cell cultures: vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus (RSV); (e) Crandell-Rees Feline Kidney (CRFK) cell cultures: feline corona virus (FIPV) and feline herpes virus (FHV); (f) Madin Darby Canine Kidney (MDCK) cell cultures: influenza A virus H1N1 subtype (A/PR/8), influenza A virus H3N2 subtype (A/HK/7/87) and influenza B virus (B/HK/5/72). Ganciclovir, cidofovir, acyclovir, brivudin, (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA], *Hippeastrum* hybrid agglutinin (HHA), *Urtica dioica* agglutinin (UDA), dextran sulfate (molecular weight 5000, DS-5000), ribavirin, oseltamivir carboxylate, amantadine, and rimantadine were used as the reference compounds. The antiviral activity was expressed as the EC_{50}: the compound concentration required to reduce virus-plaque formation (VZV) or virus-induced cytopathogenicity by 50 % (other viruses). It was found that the acyclic phosphonate analogues **10a** and **10d** containing the N^3-benzoyluracil group as a modified nucleobase exhibited noticeable activity. Thus, compound **10a** showed activity against cytomegalovirus (AD-169 strain) in human embryonic lung (HEL) cells ($EC_{50} = 45$ μM), whereas compound **10d** proved to be active against VZV (TK^+ strain OKA and TK^- strain 07-1) with $EC_{50} = 43$ and 53 μM, respectively. The antiviral activity of compound **10d** against VZV TK^- strain 07-1 appeared to be slightly better than the activity of acyclovir and brivudin used as reference compounds ($EC_{50} = 160$ and 103 μM, respectively). To rationalize the observed activity of the N^3-benzoyl derivatives **10a** and **10d** one can refer to their easier transport through cell membranes in comparison to the precursors **5a** and **5d** which appeared inactive. After entering into the cell debenzoylation would provide nucleoside analogues capable of Watson-Crick bas pairing. Our previous results [44] support this conclusion.

The cytotoxicity of the test compounds toward uninfected host cells was defined as the minimum cytotoxic concentration (MCC) that causes a microscopically detectable alteration of normal cell morphology. The 50 % cytotoxic concentration (CC_{50}), causing a 50 % decrease in cell viability was determined using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) assay system. None of the tested compounds affected cell morphology of HEL,

HeLa, Vero, MDCK, and CRFK cells (MCC or CC_{50}) at compound concentrations up to 100 μM.

Evaluation of cytostatic activity

The cytostatic activity of the tested compounds was defined as the 50 % inhibitory concentration (IC_{50}), or compound concentration causing a 50 % decrease in cell proliferation. IC_{50} values were determined against murine leukemia L1210 cells, human lymphocyte CEM cells and human cervical carcinoma HeLa cells. Several synthesized compounds showed marginal inhibitory effect on the proliferation of HeLa cells at concentrations lower than 200 μM (Table 1).

Conclusion

A series of N^1-(phosphonoalkyl)uracils **5a–5d** has been efficiently obtained from ω-aminoalkylphosphonates **2a–2d** in a two-step process which involved a reaction with (*E*)-3-ethoxyacryloyl isocyanate followed by the uracil ring closure. The uracil phosphonates **5a–5d** were successfully transformed into 5-chloro-, 5-bromo-, and 5-iodouracils as well as N^3-benzoyluracils. All synthesized compounds were tested in vitro for their antiviral activities against a broad variety of DNA and RNA viruses and compound **10a** was slightly active against human cytomegalovirus in HEL cell cultures (EC_{50} = 45 μM) while **10d** showed activity against both TK^+ and TK-VZV strains with EC_{50} values of 43 μM (Oka strain) and of 53 μM (07-1 strain), which was two to threefold better than the potency of acyclovir and brivudin against 07-1 (EC_{50} = 160 and 103 μM, respectively).

Among them, several compounds exhibited a very poor inhibitory effect on the proliferation of human cervical carcinoma cells (HeLa) at a concentration lower than 200 μM. In contrast, none of the compounds affected the proliferation of CEM cells and L1210 cells up to a concentration of 250 μM.

Table 1 Inhibitory effect of the tested compounds against the proliferation of murine leukemia (L1210), human T-lymphocyte (CEM), and human cervix carcinoma cells (HeLa)

Compounds	IC_{50}^{a} (μM)		
	L1210	CEM	HeLa
5a	>250	>250	213 ± 52
6a	>250	>250	≥250
8a	>250	>250	≥250
9a	>250	>250	208 ± 59
10a	≥250	≥250	130 ± 8
5b	>250	>250	158 ± 1
6b	>250	>250	195 ± 78
8b	>250	>250	195 ± 78
9b	>250	>250	233 ± 23
10b	>250	>250	117 ± 81
5c	>250	>250	99 ± 51
6c	>250	>250	100 ± 55
8c	>250	≥250	90 ± 56
9c	>250	>250	129 ± 97
10c	≥250	>250	117 ± 61
5d	>250	>250	126 ± 87
6d	>250	200 ± 66	97 ± 69
8d	>250	>250	≥250
9d	≥250	≥250	199 ± 73
10d	>250	>250	166 ± 118
5-Fluorouracil	0.33 ± 0.17	18 ± 5	0.54 ± 0.12

[a] 50 % Inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50 %

Experimental

^{1}H NMR was taken in $CDCl_3$ on the following spectrometers: Varian Mercury-300 and Bruker Avance III (600 MHz) with TMS as an internal standard; chemical shifts δ in ppm with respect to TMS; coupling constants *J* in Hz. ^{13}C NMR spectra were recorded for $CDCl_3$ solution on the Varian Mercury-300 and Bruker Avance III (600 MHz) spectrometer at 75.5 and 151 MHz, respectively. ^{31}P NMR spectra were taken in $CDCl_3$ on the Varian Mercury-300 and Bruker Avance III (600 MHz) spectrometer at 121.5 and 243 MHz, respectively. IR spectra were measured on an Infinity MI-60 FT-IR spectrometer as KBr pellets or as films, and absorptions are given in cm^{-1}. Melting points were determined on a Boetius apparatus. Elemental analyses were performed by Microanalytical Laboratory of this Faculty on Perkin Elmer PE 2400 CHNS analyzer and their results were found to be in good agreement (±0.3 %) with the calculated values. The following adsorbents were used: column chromatography, Merck silica gel 60 (70–230 mesh); analytical TLC, Merck TLC plastic sheets silica gel 60 F_{254}.

Applied reagents such as silver cyanate (Alfa Aesar, catalogue number: 45411), CAN (Tokyo Chemical Industry (TCI) catalogue number: C1806) and inorganic reagents are commercially available and were used as received. Solvents were dried according to the literature methods. (*E*)-3-Ethoxyacryloyl chloride was obtained according to the known protocol [18]. Diethyl aminomethylphosphonate (**2a**) was prepared according to literature (colorless oil, yield 90 %) [19–24].

General procedure for the preparation of aminoalkylphosphonates 2b–2d

The ω-azidoalkylphosphonates **1b–1d** (1.0 mmol) were dissolved in 10 cm^3 ethanol and 10 % Pd–C (16 mg) was

added. The suspension was stirred under hydrogen atmosphere (balloon) at room temperature for 20 h. The catalyst was filtered off through a layer of Celite and the solvent was evaporated to give a mixture of ω-aminoalkylphosphonates **2b–2d** and **3b–3d** (ca. 8:2). The crude products were chromatographed on silica gel columns with chloroform–methanol mixtures (50:1, 20:1, 10:1, v/v) to give pure ω-aminoalkylphosphonates **2b–2d** and pure compounds **3b–3d**.

Diethyl 2-aminoethylphosphonate (**2b**) [22–24]
Colorless oil; yield 80 %.

Bis[2-(O,O-diethylphosphoryl)ethyl]amine (**3b**) [45]
Pale yellow oil; yield 11 %.

Diethyl 3-aminopropylphosphonate (**2c**) [19, 24–26]
Yellowish oil; yield 80 %.

Bis[3-(O,O-diethylphosphoryl)propyl]amine
(**3c**, $C_{14}H_{33}NO_6P_2$)
Pale yellow oil; yield 13 %; IR (film): $\bar{\nu}$ = 3436, 2984, 2936, 2910, 1648, 1227, 1053, 1027, 964 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ = 4.14–4.04 (m, 8H, 4 × POCH_2-CH_3), 2.68 (t, J = 6.4 Hz, 4H, 2 × C$H_2$$CH_2$$CH_2$P), 2.03 (br s, 1H, NH), 1.82–1.73 (m, 8H, 2 × C$H_2$$CH_2$P), 1.32 (t, J = 7.1 Hz, 12H, 4 × POCH_2CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ = 61.5 (d, J = 6.4 Hz, POC), 49.7 (d, J = 16.7 Hz, *C*CCP), 23.4 (d, J = 142.1 Hz, CP), 22.8 (d, J = 4.7 Hz, *C*CP), 16.4 (d, J = 5.7 Hz, POC*C*) ppm; ^{31}P NMR (243 MHz, $CDCl_3$): δ = 32.20 ppm.

Diethyl 4-aminobutylphosphonate (**2d**) [19, 27]
Yellowish oil; yield 70 %.

Bis[4-(O,O-diethylphosphoryl)butyl]amine
(**3d**, $C_{16}H_{36}NO_6P_2$)
Pale yellow oil; yield 23 %; IR (film): $\bar{\nu}$ = 3441, 2982, 2936, 1642, 1230, 1052, 1026, 962 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ = 4.13–4.03 (m, 8H, 4 × POC$H_2$$CH_3$), 2.59 (t, J = 7.1 Hz, 4H, 2 × C$H_2$$CH_2$$CH_2$$CH_2$P), 2.00 (br s, 1H, NH), 1.76–1.70 (m, 4H, 2 × CH_2P), 1.66–1.54 (m, 8H, 2 × CH_2C$H_2$$CH_2$P), 1.31 (t, J = 7.1 Hz, 12H, 4 × POCH_2-CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ = 61.4 (d, J = 6.5 Hz, POC), 49.3 (s, *C*CCCP), 30.9 (d, J = 16.0 Hz, *C*CCP), 25.6 (d, J = 140.9 Hz, CP), 20.3 (d, J = 5.2 Hz, *C*CP), 16.4 (d, J = 6.2 Hz, POC*C*) ppm; ^{31}P NMR (243 MHz, $CDCl_3$): δ = 32.07 ppm.

General procedure for the preparation of acryloylureas 4a–4d

A solution of 1.009 g 3-ethoxyacryloyl chloride (7.50 mmol) in 20 cm^3 dry toluene was refluxed under an argon atmosphere with 2.173 g silver cyanate (14.50 mmol) previously dried in vacuo for 2 h with protection from light. After 45 min, the mixture was allowed to cool and then was transferred via cannula to a solution of the respective ω-aminoalkylphosphonate **2** (2.50 mmol) in 10 cm^3 dry DMF at 0 °C. Solid AgCl was washed with dry toluene (2 × 8 cm^3) to remove the residual 3-ethoxyacryloyl isocyanate which was added to the reaction flask. The reaction mixture was stirred at room temperature overnight. The solid residue was filtered through a layer of Celite and the solvent was evaporated. The crude product was chromatographed on a silica gel column with chloroform–methanol mixtures (100:1, 20:1 v/v) to afford phosphonates **4a–4d**.

Diethyl (E)-[3-(3-ethoxyacryloyl)ureido]methylphosphonate (**4a**, $C_{11}H_{21}N_2O_6P$)
Creamy solid; yield 79 %; m.p.: 56–58 °C; IR (KBr): $\bar{\nu}$ = 3428, 3239, 3133, 2984, 2936, 1684, 1617, 1563, 1304, 1229, 1168, 1120, 1054, 1023, 979, 807 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.00 (br s, 1H, NH), 9.01 (dt, J = 5.9, 1.9 Hz, 1H, *H*NCH_2), 7.66 (d, J = 12.3 Hz, 1H, EtOC*H*=CH), 5.39 (d, J = 12.3 Hz, 1H, EtOCH=C*H*), 4.22–4.12 (m, 4H, 2 × POC$H_2$$CH_3$), 3.97 (q, J = 6.9 Hz, 2H, OC$H_2$$CH_3$), 3.77 (dd, J = 11.9, 5.9 Hz, 2H, CH_2P), 1.37 (t, J = 6.9 Hz, 3H, OCH_2CH_3), 1.34 (t, J = 7.2 Hz, 6H, 2 × POCH_2CH_3) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 168.3 (s, C=O), 162.9 (s, EtO*C*H=CH), 155.5 (d, J = 6.6 Hz, C=O), 98.0 (s, EtOCH=*C*H), 67.7 (s, OCH_2-CH_3), 62.7 (d, J = 6.3 Hz, POC), 35.3 (d, J = 157.2 Hz, CP), 16.6 (d, J = 5.7 Hz, POC*C*), 14.7 (s, OCH_2CH_3) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 23.25 ppm.

Diethyl (E)-2-[3-(3-ethoxyacryloyl)ureido]ethylphosphonate (**4b**, $C_{12}H_{23}N_2O_6P$)
Colorless solid; yield 65 %; m.p.: 61–63 °C; IR (KBr): $\bar{\nu}$ = 3297, 3231, 3149, 3102, 2984, 2938, 1705, 1680, 1618, 1536, 1227, 1164, 1032, 965, 846 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 9.81 (br s, 1H, NH), 8.92 (t, J = 5.7 Hz, 1H, *H*NCH_2), 7.64 (d, J = 12.3 Hz, 1H, EtOC*H*=CH), 5.38 (d, J = 12.3 Hz, 1H, EtOCH=C*H*), 4.21–4.00 (m, 4H, 2 × POC$H_2$$CH_3$), 3.97 (q, J = 7.2 Hz, 2H, OC$H_2$$CH_3$), 3.58 (dt, J = 14.7, 7.2 Hz, 2H, C$H_2$$CH_2$P), 2.08 (dt, J = 18.0, 7.2 Hz, 2H, CH_2P), 1.36 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.34 (t, J = 7.2 Hz, 6H, 2 × POCH_2CH_3) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 168.1 (s, C=O), 162.7 (s, EtO*C*H=CH), 155.4 (s, C=O), 98.2 (s, EtOCH=*C*H), 67.6 (s, O$CH_2$$CH_3$), 62.0 (d, J = 6.6 Hz, POC), 34.2 (d, J = 3.1 Hz, *C*CP), 26.5 (d, J = 139.1 Hz, CP), 16.7 (d, J = 6.0 Hz, POC*C*), 14.8 (s, OCH_2CH_3) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 29.76 ppm.

Diethyl (E)-3-[3-(3-ethoxyacryloyl)ureido]propylphosphonate (**4c**, $C_{13}H_{25}N_2O_6P$)
Yellow oil; yield 73 %; IR (film): $\bar{\nu}$ = 3285, 3140, 2981, 1681, 1613, 1548, 1242, 1167, 1105, 1027, 966 cm^{-1}; ^1H

NMR (300 MHz, $CDCl_3$): $\delta = 9.67$ (br s, 1H, NH), 8.76 (t, $J = 6.0$ Hz, 1H, $HNCH_2$), 7.64 (d, $J = 12.3$ Hz, 1H, EtOC*H*=CH), 5.38 (d, $J = 12.3$ Hz, 1H, EtOCH=C*H*), 4.16–4.04 (m, 4H, 2 × $POCH_2CH_3$), 3.98 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3), 3.38 (dt, $J = 12.6$, 6.0 Hz, 2H, $CH_2CH_2CH_2P$), 1.94–1.72 (m, 4H, CH_2CH_2P), 1.36 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 1.32 (t, $J = 7.0$ Hz, 6H, 2 × $POCH_2CH_3$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 168.4$ (s, C=O), 162.5 (s, EtO*C*H=CH), 155.5 (s, C=O), 98.1 (s, EtOCH=*C*H), 67.4 (s, OCH_2CH_3), 61.7 (d, $J = 6.3$ Hz, POC), 39.9 (d, $J = 18.0$ Hz, *C*CCP), 23.2 (d, $J = 142.6$ Hz, CP), 23.0 (d, $J = 4.9$ Hz, *C*CP), 16.6 (d, $J = 6.0$ Hz, POC*C*), 14.6 (s, OCH_2CH_3) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 32.56$ ppm.

Diethyl (E)-4-[3-(3-ethoxyacryloyl)ureido]butylphosphonate (**4d**, $C_{14}H_{27}N_2O_6P$)
Yellowish oil; yield 72 %; IR (film): $\bar{\nu} = 3421$, 3283, 3144, 3103, 2982, 2941, 1704, 1679, 1617, 1549, 1234, 1168, 1105, 1057, 1027, 965, 819 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 9.52$ (br s, 1H, NH), 8.68 (t, $J = 5.8$ Hz, 1H, $HNCH_2$), 7.63 (d, $J = 12.2$ Hz, 1H, EtOC*H*=CH), 5.36 (d, $J = 12.2$ Hz, 1H, EtOCH=C*H*), 4.15–4.03 (m, 4H, 2 × $POCH_2CH_3$), 3.97 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3), 3.31 (dt, $J = 6.4$, 5.8 Hz, 2H, $CH_2CH_2CH_2CH_2P$), 1.81–1.62 (m, 6H, $CH_2CH_2CH_2P$), 1.36 and 1.32 (2t, $J = 6.9$ Hz, 6H, 2 × $POCH_2CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 168.4$ (s, C=O), 162.4 (s, EtO*C*H=CH), 155.5 (s, C=O), 98.2 (s, EtOCH=*C*H), 67.2 (s, OCH_2CH_3), 61.5 (d, $J = 6.6$ Hz, POC), 38.9 (s, *C*CCCP), 30.4 (d, $J = 15.5$ Hz, *C*CCP), 25.2 (d, $J = 141.8$ Hz, CP), 19.8 (d, $J = 5.5$ Hz, *C*CP), 16.3 (d, $J = 6.3$ Hz, POC*C*), 14.4 (s, OCH_2CH_3) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 32.19$ ppm.

General procedure for the preparation of uracil derivatives 5a–5d

To a solution of the respective acryloylurea **4a**–**4d** (1.0 mmol) in 8 cm^3 dioxane, 8 cm^3 H_2SO_4 (2 M) was added and the mixture was refluxed for 20 h. NaOH (2 M) was added to reach pH 7 and the reaction mixture was concentrated to dryness. To the solid residue, chloroform was added and the mixture was stirred for 15 min, then filtered through a layer of Celite and concentrated. The product was purified by column chromatography with chloroform–methanol mixtures (50:1, 20:1 v/v) to give pure uracil derivatives **5a**–**5d**.

Diethyl [3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]methylphosphonate (**5a**, $C_9H_{15}N_2O_5P$)
White solid; yield 58 %; m.p.: 122–124 °C; IR (KBr): $\bar{\nu} = 3428$, 3172, 3057, 2991, 2870, 2822, 1689, 1632, 1452, 1382, 1343, 1239, 1049, 977, 732 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 9.29$ (br s, 1H, NH), 7.35 (d, $J = 7.9$ Hz, 1H, HC-6), 5.76 (dd, $J = 7.9$, 1.4 Hz, 1H, HC-5), 4.19 (dq, $J = 7.9$, 6.9 Hz, 4H, 2 × $POCH_2CH_3$), 4.17 (d, $J = 11.5$ Hz, 2H, CH_2P), 1.34 (t, $J = 6.9$ Hz, 6H, 2 × $POCH_2CH_3$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 163.8$ (s, C=O), 150.6 (d, $J = 2.0$ Hz, C=O), 144.2 (s, C-6), 102.9 (s, C-5), 63.5 (d, $J = 6.6$ Hz, POC), 42.0 (d, $J = 156.3$ Hz, CP), 16.6 (d, $J = 6.0$ Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 19.35$ ppm.

Diethyl 2-[3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]ethylphosphonate (**5b**) [46, 47]
Colorless oil; yield: 83 %.

Diethyl 3-[3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]propylphosphonate (**5c**) [46, 47]
Colorless oil; yield: 84 %.

Diethyl 4-[3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]butylphosphonate (**5d**) [46, 47]
Colorless oil; yield: 85 %.

General procedure for the chlorination and bromination reactions

To the solution of the respective phosphonate **5a**–**5d** (0.30 mmol) in 5 cm^3 pyridine, *N*-halogenosuccinimide (NCS or NBS, 0.45 mmol) was added and the mixture was stirred for 30 min at 100 °C. Saturated aqueous $NaHCO_3$ solution (10 cm^3) was added and the mixture was extracted with chloroform (3 × 20 cm^3). The combined organic phases were dried over $MgSO_4$, filtered, and the solvent was removed. The crude product was purified by column chromatography or crystallization.

Diethyl [5-chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]methylphosphonate (**6a**, $C_9H_{14}ClN_2O_5P$)
Colorless solid; yield 72 % [after column chromatography (ethyl acetate) and crystallization from ethyl acetate-petroleum ether mixture]; m.p.: 136–138 °C; IR (KBr): $\bar{\nu} = 3406$, 3172, 3043, 2984, 2945, 2831, 1712, 1690, 1633, 1338, 1224, 1061, 1012, 971 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 9.92$ (br s, 1H, NH), 7.61 (s, 1H, HC-6), 4.21 (dq, $J = 7.9$, 6.9 Hz, 4H, 2 × $POCH_2CH_3$), 4.19 (d, $J = 10.7$ Hz, 2H, CH_2P), 1.35 (t, $J = 6.9$ Hz, 6H, 2 × $POCH_2CH_3$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 159.4$ (s, C=O), 149.9 (d, $J = 2.0$ Hz, C=O), 141.0 (s, C-6), 109.4 (s, C-5), 63.6 (d, $J = 6.3$ Hz, POC), 42.2 (d, $J = 156.0$ Hz, CP), 16.6 (d, $J = 6.0$ Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 18.80$ ppm.

Diethyl 2-[5-chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]ethylphosphonate (**6b**, $C_{10}H_{16}ClN_2O_5P$)

Creamy solid; yield 69 % [after column chromatography (chloroform/methanol 100:1 v/v)]; m.p.: 149–151 °C; IR (KBr): $\bar{\nu}$ = 3171, 3029, 2994, 2926, 2839, 1701, 1665, 1631, 1368, 1250, 1069, 1018, 963, 793 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ = 9.18 (br s, 1H, NH), 7.61 (s, 1H, HC-6), 4.19–4.10 (m, 4H, 2 × POC$H_2$$CH_3$), 4.05 (dt, J = 16.1, 6.8 Hz, 2H, C$H_2$$CH_2$P), 2.26 (dt, J = 18.2, 6.8 Hz, 2H, CH_2P), 1.35 (t, J = 7.1 Hz, 6H, 2 × POCH_2-CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ = 159.6 (s, C=O), 149.9 (s, C=O), 142.2 (s, C-6), 108.4 (s, C-5), 62.3 (d, J = 6.4 Hz, POC), 44.6 (d, J = 3.0 Hz, *C*CP), 25.0 (d, J = 140.9 Hz, CP), 16.4 (d, J = 5.7 Hz, POC*C*) ppm; ^{31}P NMR (243 MHz, $CDCl_3$): δ = 26.78 ppm.

Diethyl 3-[5-chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]propylphosphonate (**6c**, $C_{11}H_{18}ClN_2O_5P$)
Colorless solid; yield 53 % (after column chromatography (ethyl acetate) and crystallization from ethyl acetate-petroleum ether mixture); m.p.: 64–66 °C; IR (KBr): $\bar{\nu}$ = 3545, 3044, 2988, 1701, 1654, 1624, 1458, 1272, 1242, 1227, 1015, 962 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 9.95 (br s, 1H, NH), 7.63 (s, 1H, HC-6), 4.24–4.07 (m, 4H, 2 × POC$H_2$$CH_3$), 3.90 (t, J = 7.2 Hz, 2H, C$H_2$$CH_2$-$CH_2$P), 2.06 (dqu, J = 14.6, 7.2 Hz, 2H, C$H_2$$CH_2$P), 1.81 (dt, J = 18.2, 7.2 Hz, 2H, CH_2P), 1.36 (t, J = 6.9 Hz, 6H, 2 × $POCH_2CH_3$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.7 (s, C=O), 150.2 (s, C=O), 141.6 (s, C-6), 108.8 (s, C-5), 62.2 (d, J = 6.6 Hz, POC), 49.1 (d, J = 14.6 Hz, *C*CCP), 22.4 (d, J = 142.8 Hz, CP), 22.3 (d, J = 4.9 Hz, *C*CP), 16.7 (d, J = 5.7 Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 31.35 ppm.

Diethyl 3-[6-chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]propylphosphonate (**7c**, $C_{11}H_{18}ClN_2O_5P$)
Colorless oil; yield 6 % [after column chromatography (ethyl acetate)]; IR (film): $\bar{\nu}$ = 3229, 3077, 2925, 2852, 1745, 1696, 1471, 1240, 1098, 1024, 970 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.18 (br s, 1H, NH), 5.14 (s, 1H, HC-5), 4.19–4.02 (m, 4H, 2 × POC$H_2$$CH_3$), 3.86 (dt, J = 13.9, 7.0 Hz, 1H, C$H_a$$H_b$$CH_2CH_2CH_2P$), 3.47 (dt, J = 13.9, 6.8 Hz, 1H, $CH_aH_bCH_2CH_2CH_2P$), 2.18–1.84 (m, 4H, CH_2CH_2P), 1.34 and 1.32 (2 × t, J = 7.0 Hz, 6H, 2 × $POCH_2CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ = 161.5 (s, C=O), 150.6 (s, C=O), 86.3 (s, C-6), 79.5 (s, C-5), 62.5 (d, J = 6.4 Hz, POC), 62.2 (d, J = 6.8 Hz, POC), 47.8 (d, J = 13.5 Hz, *C*CCP), 22.1 (d, J = 142.4 Hz, CP), 21.1 (d, J = 4.3 Hz, *C*CP), 16.4 (d, J = 6.6 Hz, POC*C*), 16.3 (d, J = 5.3 Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 33.88 ppm.

Diethyl 4-[5-chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]butylphosphonate (**6d**, $C_{12}H_{20}ClN_2O_5P$)
White solid; yield 65 % (after column chromatography (hexane/ethyl acetate 2:1, 1:2 v/v) and crystallization from ethyl acetate-hexane mixture); m.p.: 139–142 °C; IR (KBr): $\bar{\nu}$ = 3150, 2982, 2947, 2818, 1688, 1627, 1451, 1429, 1355, 1245, 1210, 1187, 1045, 1020, 971 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 9.38 (br s, 1H, NH), 7.45 (s, 1H, HC-6), 4.17–4.04 (m, 4H, 2 × POC$H_2$$CH_3$), 3.76 (t, J = 7.3 Hz, 2H, C$H_2$$CH_2CH_2CH_2P$), 1.88–1.61 (m, 6H, $CH_2CH_2CH_2P$), 1.33 (t, J = 7.1 Hz, 6H, 2 × $POCH_2CH_3$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.6 (s, C=O), 150.1 (s, C=O), 141.2 (s, C-6), 108.8 (s, C-5), 61.9 (d, J = 6.6 Hz, POC), 48.8 (s, *C*CCCP), 29.7 (d, J = 14.3 Hz, *C*CCP), 25.2 (d, J = 141.7 Hz, CP), 19.7 (d, J = 4.9 Hz, *C*CP), 16.7 and 16.6 (2 × d, J = 5.9 Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 31.46 ppm.

Diethyl 4-[6-chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]butylphosphonate (**7d**, $C_{12}H_{20}ClN_2O_5P$)
White solid; yield 12 % [after column chromatography (hexane/ethyl acetate 2:1, 1:2 v/v)]; m.p.: 138–141 °C; IR (KBr): $\bar{\nu}$ = 3412, 3231, 2961, 2924, 2854, 1745, 1698, 1482, 1439, 1254, 1027 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.60 (br s, 1H, NH), 5.11 (s, 1H, HC-5), 4.16–3.98 (m, 4H, 2 × POC$H_2$$CH_3$), 3.67 (dt, J = 13.7, 7.0 Hz, 1H, C$H_a$$H_b$$CH_2CH_2CH_2P$), 3.49 (dt, J = 13.7, 7.1 Hz, 1H, $CH_aH_bCH_2CH_2CH_2P$), 1.94–1.65 (m, 6H, CH_2CH_2CH_2P), 1.32 and 1.31 (2 × t, J = 7.0 Hz, 6H, 2 × $POCH_2CH_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ = 161.6 (s, C=O), 150.6 (s, C=O), 85.6 (s, C-6), 79.5 (s, C-5), 62.2 (d, J = 6.5 Hz, POC), 62.0 (d, J = 7.1 Hz, POC), 46.3 (s, *C*CCCP), 28.2 (d, J = 11.7 Hz, *C*CCP), 24.3 (d, J = 141.3 Hz, CP), 19.1 (d, J = 4.8 Hz, *C*CP), 16.4 (d, J = 6.6 Hz, POC*C*), 16.3 (d, J = 6.6 Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 33.19 ppm.

Diethyl [5-bromo-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]methylphosphonate (**8a**, $C_9H_{14}BrN_2O_5P$)
White solid; yield 69 % (after column chromatography (hexane/ethyl acetate 1:2 and ethyl acetate v/v) and crystallization from ethyl acetate-petroleum ether mixture); m.p.: 120–122 °C; IR (KBr): $\bar{\nu}$ = 3415, 3164, 3040, 2943, 2851, 1707, 1626, 1422, 1338, 1224, 1012, 985 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.06 (br s, 1H, NH), 7.72 (s, 1H, HC-6), 4.21 (dq, J = 8.1, 7.0 Hz, 4H, 2 × POCH_2-CH_3), 4.20 (d, J = 11.1 Hz, 2H, CH_2P), 1.35 (t, J = 7.0 Hz, 6H, 2 × $POCH_2CH_3$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.5 (s, C=O), 150.1 (d, J = 2.0 Hz, C=O), 143.5 (s, C-6), 97.2 (s, C-5), 63.6 (d, J = 6.6 Hz, POC), 42.2 (d, J = 156.0 Hz, CP), 16.6 (d, J = 6.0 Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 18.82 ppm.

Diethyl 2-[5-bromo-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]ethylphosphonate (**8b**, $C_{10}H_{16}BrN_2O_5P$)
White solid; yield 75 % (after column chromatography (chloroform/methanol 200:1 v/v) and crystallization from

ethyl acetate-petroleum ether mixture); m.p.: 134–135 °C; IR (KBr): $\bar{\nu}$ = 3162, 3038, 2990, 2834, 1704, 1660, 1623, 1251, 1054, 1016, 962 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.06 (br s, 1H, NH), 7.73 (s, 1H, HC-6), 4.19–4.03 (m, 4H, 2 × POCH_2CH$_3$), 4.02 (dt, J = 16.2, 6.9 Hz, 2H, CH_2CH$_2$P), 2.23 (dt, J = 18.0, 6.9 Hz, 2H, CH$_2$P), 1.30 (t, J = 6.8 Hz, 6H, 2 × POCH$_2$CH_3) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.9 (s, C=O), 150.3 (s, C=O), 144.8 (s, C-6), 96.1 (s, C-5), 62.5 (d, J = 6.6 Hz, POC), 44.6 (d, J = 3.4 Hz, *C*CP), 25.2 (d, J = 140.3 Hz, CP), 16.6 (d, J = 6.0 Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 27.46 ppm.

Diethyl 3-[5-bromo-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]propylphosphonate (**8c**, $C_{11}H_{18}BrN_2O_5P$)
Creamy solid; yield 85 % [after column chromatography (chloroform/methanol 200:1 v/v)]; m.p.: 48–50 °C; IR (KBr): $\bar{\nu}$ = 3549, 3504, 3162, 3052, 2986, 2824, 1702, 1618, 1452, 1346, 1272, 1242, 1180, 1014, 962 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 9.84 (br s, 1H, NH), 7.72 (s, 1H, HC-6), 4.22–4.04 (m, 4H, 2 × POCH_2CH$_3$), 3.88 (t, J = 7.2 Hz, 2H, CH_2CH$_2$CH$_2$P), 2.04 (dqu, J = 15.0, 7.2 Hz, 2H, CH_2CH$_2$P), 1.78 (dt, J = 18.0, 7.2 Hz, 2H, CH$_2$P), 1.34 (t, J = 6.8 Hz, 6H, 2 × POCH$_2$CH_3) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.9 (s, C=O), 150.5 (s, C=O), 144.2 (s, C-6), 96.5 (s, C-5), 62.2 (d, J = 6.6 Hz, POC), 49.0 (d, J = 15.2 Hz, *C*CCP), 22.4 (d, J = 142.6 Hz, CP), 22.3 (d, J = 4.9 Hz, *C*CP), 16.6 (d, J = 6.0 Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 31.36 ppm.

Diethyl 4-[5-bromo-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]butylphosphonate (**8d**, $C_{12}H_{20}BrN_2O_5P$)
White solid; yield 85 % (after column chromatography (chloroform/methanol 200:1 v/v) and crystallization from ethyl acetate-petroleum ether mixture); m.p.: 112–114 °C; IR (KBr): $\bar{\nu}$ = 3141, 3074, 2980, 2928, 2817, 1705, 1686, 1621, 1424, 1353, 1247, 1211, 1040, 959 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 9.36 (br s, 1H, NH), 7.56 (s, 1H, HC-6), 4.17–4.02 (m, 4H, 2 × POCH_2CH$_3$), 3.77 (t, J = 7.2 Hz, 2H, CH_2CH$_2$CH$_2$CH$_2$P), 1.88–1.61 (m, 6H, CH_2CH_2CH_2P), 1.33 (t, J = 7.0 Hz, 6H, 2 × POCH$_2$CH_3) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 159.6 (s, C=O), 150.3 (s, C=O), 143.8 (s, C-6), 96.6 (s, C-5), 61.9 (d, J = 6.6 Hz, POC), 48.8 (s, *C*CCCP), 29.8 (d, J = 14.3 Hz, *C*CCP), 25.2 (d, J = 141.7 Hz, CP), 19.7 (d, J = 4.9 Hz, *C*CP), 16.7 and 16.6 (2 × d, J = 6.0 Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 31.46 ppm.

General procedure for the iodination reactions

A solution of the respective phosphonate **5a**–**5d** (0.30 mmol), iodine (0.18 mmol) and $(NH_4)_2Ce(NO_3)_6$ (0.15 mmol) in 10 cm^3 anhydrous acetonitrile was refluxed for 2 h. After the solvent was removed, the residue was washed with diethyl ether (3 × 15 cm^3) and purified by column chromatography with 2-propanol-hexane mixture (1:3, v/v) to give phosphonates **9a**–**9d**.

Diethyl [5-iodo-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]methylphosphonate (**9a**, $C_9H_{14}IN_2O_5P$)
Colorless solid; yield 72 % (after crystallization from ethyl alcohol-petroleum ether); m.p.: 116–118 °C; IR (KBr): $\bar{\nu}$ = 3156, 3008, 2984, 2929, 2827, 1694, 1613, 1418, 1352, 1226, 1055, 1017, 967 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 9.24 (br s, 1H, NH), 7.78 (s, 1H, HC-6), 4.20 (dq, J = 8.1, 7.0 Hz, 4H, 2 × POCH_2CH$_3$), 4.17 (d, J = 11.3 Hz, 2H, CH$_2$P), 1.35 (t, J = 7.0 Hz, 6H, 2 × POCH$_2$CH_3) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 160.4 (s, C=O), 150.3 (d, J = 2.0 Hz, C=O), 148.5 (s, C-6), 68.8 (s, C-5), 63.6 (d, J = 6.6 Hz, POC), 42.2 (d, J = 155.7 Hz, CP), 16.6 and 16.5 (2 × d, J = 6.0 Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 18.02 ppm.

Diethyl 2-[5-iodo-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]ethylphosphonate (**9b**, $C_{10}H_{16}IN_2O_5P$)
Colorless oil; yield 77 %; IR (film): $\bar{\nu}$ = 3473, 3167, 3051, 2984, 2817, 1691, 1613, 1443, 1420, 1359, 1284, 1226, 1097, 1051, 1024, 973, 794 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 10.04 (br s, 1H, NH), 7.84 (s, 1H, HC-6), 4.21–4.04 (m, 4H, 2 × POCH_2CH$_3$), 4.04 (dt, J = 15.7, 6.9 Hz, 2H, CH_2CH$_2$P), 2.25 (dt, J = 18.2, 6.9 Hz, 2H, CH$_2$P), 1.32 (t, J = 7.0 Hz, 6H, 2 × POCH$_2$CH_3) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 161.0 (s, C=O), 150.6 (s, C=O), 149.7 (s, C-6), 67.7 (s, C-5), 62.5 (d, J = 6.3 Hz, POC), 44.5 (d, J = 2.6 Hz, *C*CP), 25.2 (d, J = 140.3 Hz, CP), 16.6 (d, J = 5.7 Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 27.49 ppm.

Diethyl 3-[5-iodo-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]propylphosphonate (**9c**) *[42]*
White solid; yield 71 % (after crystallization from chloroform–diethyl ether mixture); m.p.: 82–84 °C.

Diethyl 4-[5-iodo-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]butylphosphonate (**9d**, $C_{12}H_{20}IN_2O_5P$)
Colorless oil; yield 91 %; IR (film): $\bar{\nu}$ = 3445, 3165, 3050, 2984, 2816, 1690, 1612, 1441, 1420, 1228, 1051, 1026, 966 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 9.63 (br s, 1H, NH), 7.67 (s, 1H, HC-6), 4.18–4.02 (m, 4H, 2 × POCH_2CH$_3$), 3.77 (t, J = 7.1 Hz, 2H, CH_2CH$_2$CH$_2$-CH$_2$P), 1.88–1.61 (m, 6H, CH_2CH_2CH_2P), 1.33 (t, J = 7.1 Hz, 6H, 2 × POCH$_2$CH_3) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 160.9 (s, C=O), 150.7 (s, C=O), 148.8 (s, C-6), 68.1 (s, C-5), 61.9 (d, J = 6.6 Hz, POC), 48.6 (s, *C*CCCP), 29.8 (d, J = 14.9 Hz, *C*CCP), 25.1 (d, J = 142.0 Hz, CP), 19.6 (d, J = 3.7 Hz, *C*CP), 16.8 and

16.7 (2 × d, $J = 5.9$ Hz, POC*C*) ppm; ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 31.55$ ppm.

General procedure for the benzoyl derivatives 10a–10d

To a solution of the respective phosphonate **5a**–**5d** (0.20 mmol) in 6 cm^3 dichloromethane, 0.022 g triethylamine (0.22 mmol) and 0.030 g benzoyl chloride (0.21 mmol) were added at 0 °C and the mixture was stirred at room temperature for 72 h. The solution was washed with water (3 × 15 cm^3), dried over $MgSO_4$, and purified by column chromatography with chloroform–methanol mixtures (200:1, 20:1, v/v) to give pure phosphonates **10a**–**10d**.

Diethyl [3-benzoyl-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]methylphosphonate (**10a**, $C_{16}H_{19}N_2O_6P$)
Colorless oil; yield 63 %; IR (film): $\bar{\nu} = 3482, 3090, 2988, 2939, 1750, 1706, 1668, 1598, 1437, 1381, 1341, 1241, 1047, 1023, 976$ cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.95$–7.94 (m, 2H), 7.68–7.66 (m, 1H), 7.53–7.50 (m, 2H), 7.44 (d, $J = 8.0$ Hz, 1H, HC-6), 5.87 (d, $J = 8.0$ Hz, 1H, HC-5), 4.16–4.09 (m, 4H, 2 × POCH_2CH$_3$), 4.18 (d, $J = 11.3$ Hz, 2H, CH_2P), 1.34 (t, $J = 7.1$ Hz, 6H, 2 × POCH$_2$CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 168.2$ (s, C=O), 162.0 (s, C=O), 149.5 (d, $J = 2.3$ Hz, C=O), 143.8 (s, C-6), 135.2, 131.3, 130.5, 129.2, 102.7 (s, C-5), 63.4 (d, $J = 6.5$ Hz, POC), 42.2 (d, $J = 155.7$ Hz, CP), 16.3 (d, $J = 5.7$ Hz, POC*C*) ppm; ^{31}P NMR (243 MHz, $CDCl_3$): $\delta = 17.86$ ppm.

Diethyl 2-[3-benzoyl-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]ethylphosphonate (**10b**, $C_{17}H_{21}N_2O_6P$)
Colorless oil; yield 64 %; IR (film): $\bar{\nu} = 3494, 3088, 2984, 2931, 2910, 1746, 1705, 1664, 1441, 1391, 1353, 1242, 1051, 1026, 987$ cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.96$–7.95 (m, 2H), 7.68–7.66 (m, 1H), 7.53–7.50 (m, 2H), 7.41 (d, $J = 8.0$ Hz, 1H, HC-6), 5.81 (d, $J = 8.0$ Hz, 1H, HC-5), 4.18–4.09 (m, 4H, 2 × POCH_2CH$_3$), 4.05 (dt, $J = 15.9$, 7.0 Hz, 2H, CH_2CH$_2$P), 2.26 (dt, $J = 18.2$, 7.0 Hz, 2H, CH_2P), 1.36 (t, $J = 7.0$ Hz, 6H, 2 × POCH$_2$CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 168.6$ (s, C=O), 162.4 (s, C=O), 149.6 (s, C=O), 144.9 (s, C-6), 135.1, 131.5, 130.5, 129.2, 101.9 (s, C-5), 62.2 (d, $J = 6.5$ Hz, POC), 44.8 (d, $J = 4.0$ Hz, *C*CP), 24.9 (d, $J = 140.7$ Hz, CP), 16.4 (d, $J = 6.0$ Hz, POC*C*) ppm; ^{31}P NMR (243 MHz, $CDCl_3$): $\delta = 26.76$ ppm.

Diethyl 3-[3-benzoyl-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]propylphosphonate (**10c**, $C_{18}H_{23}N_2O_6P$)
Colorless oil; yield 56 %; IR (film): $\bar{\nu} = 3448, 3083, 2984, 2932, 1747, 1704, 1663, 1439, 1242, 1028, 967$ cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.95$–7.94 (m, 2H), 7.68–7.66 (m, 1H), 7.53–7.51 (m, 2H), 7.43 (d, $J = 8.0$ Hz, 1H, HC-6), 5.83 (d, $J = 8.0$ Hz, 1H, HC-5), 4.18–4.08 (m, 4H, 2 × POCH_2CH$_3$), 3.91 (t, $J = 7.5$ Hz, 2H, CH_2CH$_2$CH$_2$P), 2.07 (dqu, $J = 14.9$, 7.5 Hz, 2H, CH_2CH$_2$P), 1.79 (dt, $J = 18.5$, 7.5 Hz, 2H, CH_2P), 1.35 (t, $J = 7.1$ Hz, 6H, 2 × POCH$_2$CH_3) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 168.7$ (s, C=O), 162.4 (s, C=O), 149.8 (s, C=O), 144.4 (s, C-6), 135.1, 131.5, 130.4, 129.2, 102.2 (s, C-5), 61.9 (d, $J = 6.6$ Hz, POC), 49.0 (d, $J = 13.7$ Hz, *C*CCP), 22.3 (d, $J = 143.0$ Hz, CP), 22.2 (d, $J = 4.6$ Hz, *C*CP), 16.5 (d, $J = 5.8$ Hz, POC*C*) ppm; ^{31}P NMR (243 MHz, $CDCl_3$): $\delta = 29.96$ ppm.

Diethyl 4-[3-benzoyl-3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl]butylphosphonate (**10d**, $C_{19}H_{25}N_2O_6P$)
Colorless oil; yield 73 %; IR (film): $\bar{\nu} = 3451, 3083, 2983, 2939, 2872, 1747, 1704, 1663, 1439, 1241, 1027, 963$ cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.94$–7.93 (m, 2H), 7.67–7.65 (m, 1H), 7.52–7.50 (m, 2H), 7.30 (d, $J = 8.0$ Hz, 1H, HC-6), 5.81 (d, $J = 8.0$ Hz, 1H, HC-5), 4.14–4.04 (m, 4H, 2 × POCH_2CH$_3$), 3.78 (t, $J = 7.3$ Hz, 2H, CH_2CH$_2$CH$_2$CH$_2$P), 1.88–1.83 (m, 2H, CH_2CH$_2$CH$_2$P), 1.81–1.76 (m, 2H, CH_2P), 1.72–1.64 (m, 2H, CH_2CH$_2$P), 1.32 (t, $J = 7.0$ Hz, 6H, 2 × POCH$_2$CH_3) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 168.8$ (s, C=O), 162.4 (s, C=O), 149.8 (s, C=O), 144.1 (s, C-6), 135.0, 131.6, 130.4, 129.2, 102.2 (s, C-5), 61.6 (d, $J = 6.6$ Hz, POC), 48.5 (s, *C*CCCP), 29.5 (d, $J = 14.6$ Hz, *C*CCP), 25.0 (d, $J = 141.9$ Hz, CP), 19.5 (d, $J = 4.7$ Hz, *C*CP), 16.4 (d, $J = 5.7$ Hz, POC*C*) ppm; ^{31}P NMR (243 MHz, $CDCl_3$): $\delta = 30.88$ ppm.

Antiviral activity assays

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strains Lyons and G, varicella-zoster virus (VZV) strain Oka, TK^- VZV strain 07-1, human cytomegalovirus (HCMV) strains AD-169 and Davis, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie B4, Parainfluenza 3, Influenza virus A (subtypes H1N1, H3N2), influenza virus B, Reovirus-1, Sindbis, Reovirus-1, Punta Toro, human immunodeficiency virus type 1 strain III_B, and human immunodeficiency virus type 2 strain ROD. The antiviral, other than anti-HIV, assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey cells (Vero), human epithelial cells (HeLa), or Madin-Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$

of virus (1 $CCID_{50}$ being the virus dose to infect 50 % of the cell cultures) or with 20 plaque forming units (PFU) (VZV) in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50 %.

The methodology of the anti-HIV assays was as follows: human CEM ($\sim 3 \times 105$ cells/cm^3) cells were infected with 100 CCID50 of HIV(IIIB) or HIV-2(ROD)/cm^3 and seeded in 200 mm^3 wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

Cytostatic activity assays

All assays were performed in 96-well microtiter plates. To each well were added (5–7.5) $\times$ 10^4 tumor cells and a given amount of the test compound. The cells were allowed to proliferate for 48 h (murine leukemia L1210 cells) or 72 h (human lymphocytic CEM and human cervix carcinoma HeLa cells) at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC_{50} (50 % inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50 %.

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