Full Paper

1-Amino-3-(1*H*-1,2,3-triazol-1-yl)propylphosphonates as Acyclic Analogs of Nucleotides

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A new series of 1-amino-3-(1*H*-1,2,3-triazol-1-yl)propylphosphonates (*R*)- and (*S*)-**16** were obtained from enantiomerically pure (*R*)- and (*S*)-1-*tert*-butoxycarbonyl (Boc)-amino-3-azidopropylphosphonates and *N*-propargylated nucleobases in good yields. All 1,2,3-triazolylphosphonates (*R*)- and (*S*)-**16** were evaluated for their activities against a broad range of DNA and RNA viruses. Compound (*R*)-**16g** (**B** = 3-acetylindole) was moderately active against vesicular stomatitis virus in HeLa cell cultures (EC_{50} = 45 μM). In addition, (*S*)-**16c** (**B** = adenine), (*R*)-**16f** (**B** = N^3-Bz-benzuracil), (*R*)-**16g** (**B** = 3-acetylindole), and (*R*)-**16h** (**B** = 5,6-dimethylbenzimidazole) were cytotoxic toward Crandell-Rees feline kidney (CRFK) cells (CC_{50} = 2.9, 45, 72, and 96 μM, respectively). Compounds (*R*)-**16g**, (*S*)-**16g**, and (*S*)-**16h** were slightly cytostatic to different tumor cell lines.

Keywords: Antiproliferative agents / Antiviral activity / Cycloaddition / Cytotoxic activity / Triazoles

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Introduction

Acyclic analogs of nucleosides and nucleotides are the most important and widely explored groups of potential antiviral compounds [1, 2].

Over decades, several antiviral agents have been discovered and some of them found application in clinical practice. Among them, nucleoside analogs such as acyclovir, ganciclovir, and penciclovir as well as phosphonylated nucleosides such as adefovir, tenofovir, and cidofovir belong to the clinically approved drugs with a broad spectrum and high level of antiviral potency (Fig. 1) [3–8].

In case of treatment of viral infections, the primary advantage of acyclic nucleoside and nucleotide analogs relies on their stability to enzymatic degradation because of absence of a glycosidic bond as well as on conformational flexibility of the acyclic side-chain, which facilitates binding to enzymes involved in the synthesis of nucleic acids, including inhibition of reverse transcriptase and DNA polymerases. Problems of low selectivity, drug resistance, and long-term toxicity of approved drugs still impose serious limitations on their application [9].

In search of novel analogs with improved pharmacological properties, various modified nucleosides have been obtained. It has been well recognized that the physiological properties of nucleosides/nucleotides may change dramatically if additional heteroatoms or selected functional groups are incorporated into the structure of the known compounds. Thus, acyclic nucleosides in which a nitrogen atom was included into an alkyl chain or placed as an additional amino group were synthesized and some of them showed antiviral and anticancer activity as well as anti-parasitic properties (Fig. 2) [10–17].

AHPBU **1** exhibits inhibitory activity against uridine phosphorylases isolated from Sarcoma 180 neoplastic cells while showing low cytotoxicity [10]. Its close structural analog **2** was evaluated as an inhibitor in the treatment of parasite infections [11]. Both adenine **3** and **4** [12] as well as guanine **5** and **6** [13] nucleoside analogs did not show any pronounced activity against HSV-1 and HSV-2. On the other hand, formamide derivative **7** appeared to be active toward both HSV-1 and HSV-2 in MRC-5 cells [14]. Among various nitrogen-containing nucleotides **8**–**10** obtained by Zhou, only analog **10** (**B** = Gua) showed slight inhibitory activity against HSV-1,

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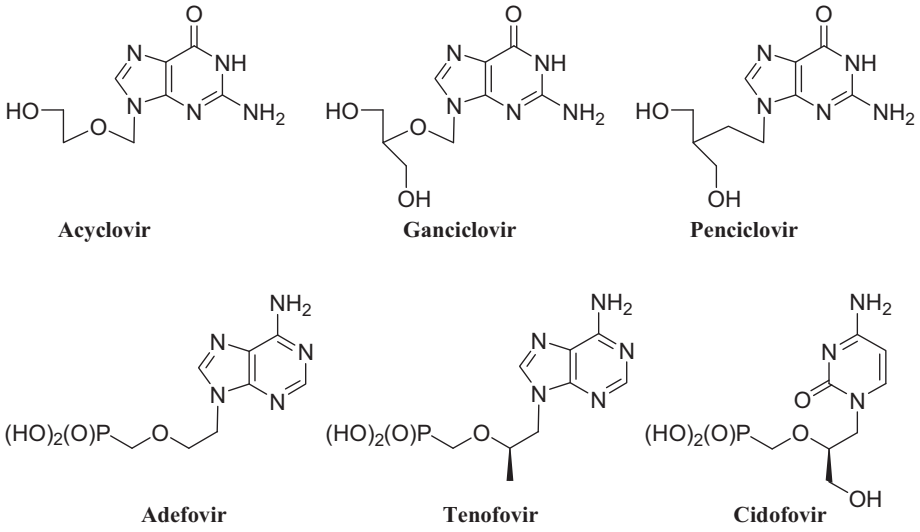


Figure 1. Examples of clinically applied acyclic nucleoside/nucleotide analogs.

HSV-2, and CMV [15]. Aza-nucleotide **11** with nitrogen incorporated into the phosphonoalkyl residue [16] and its **N**-branched derivative **12** [17] appeared to be selective inhibitors of phosphoribosyltransferase P*f*HGXPRT, a key enzyme for the growth of *Plasmodium falciparum*, and at the same time did not show detectable inhibitory activity against human HGPRT. Moreover, it was shown that the protection of the amino functionalities in **13** with bulky groups such as

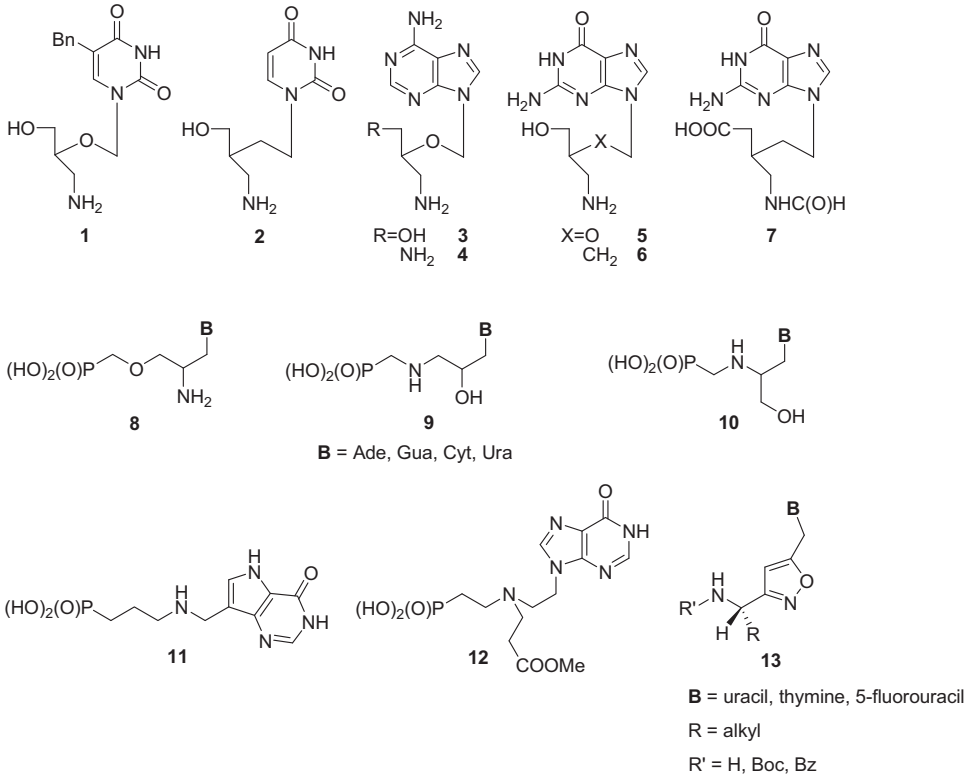


Figure 2. Examples of nitrogen-containing nucleoside/nucleotide analogs.

tert-butoxycarbonyl (Boc) or benzoyl (Bz) leads to the enhancement of antiviral activities of the protected compounds [18].

Recently, we succeeded in the synthesis of several series of acyclic nucleotide analogs equipped with a 1,2,3-triazolyl linker between nucleobases and diethoxyphosphonoalkyl units [19]. Among them, nucleotide analog **14** with a trimethylene chain appeared to be active against both herpes simplex viruses (HSV-1 and HSV-2) and feline herpes virus (FHV) [19]. Moreover, several other compounds, namely **15a–f**, have been found to inhibit proliferation of CEM as well as HeLa cells (Fig. 3) [19, 20].

In continuation of our ongoing program directed toward synthesis of biologically active acyclic 1,2,3-triazolyl nucleotides, a new series of 1-amino-(1,2,3-triazol-1-yl)propylphosphonates (*R*)- and (*S*)-**16a–h** were designed (Scheme 1) and their antiviral and cytostatic properties were evaluated. *N*-Boc-protection of the amino groups was deliberately left to be in line with previous observations made by Lee and Kim [18]. Although biological activity of phosphonate nucleoside

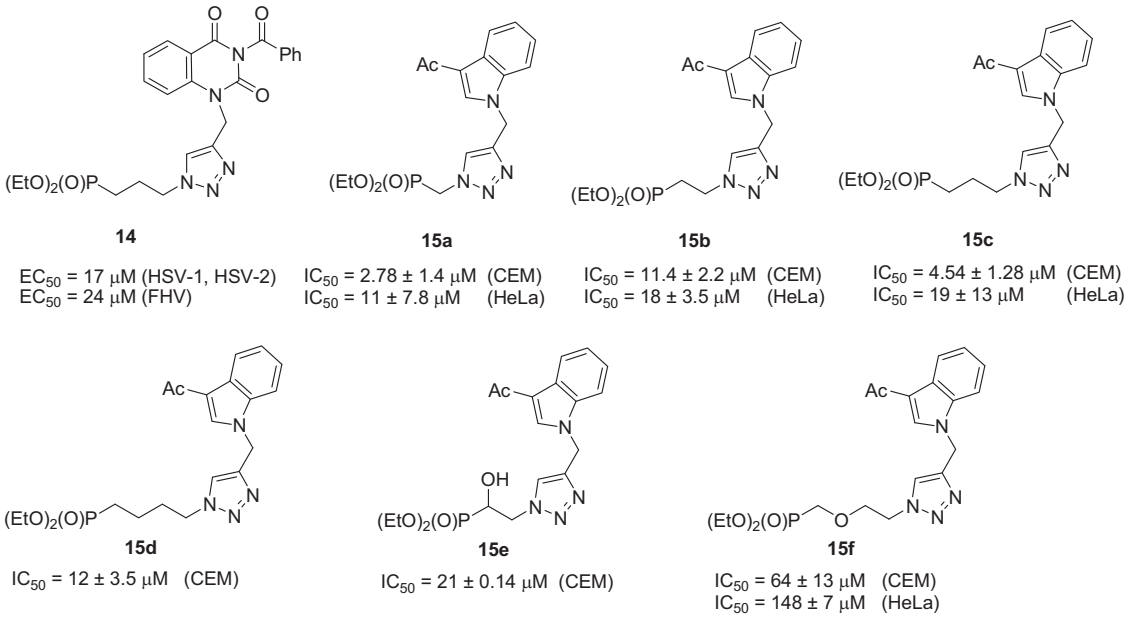
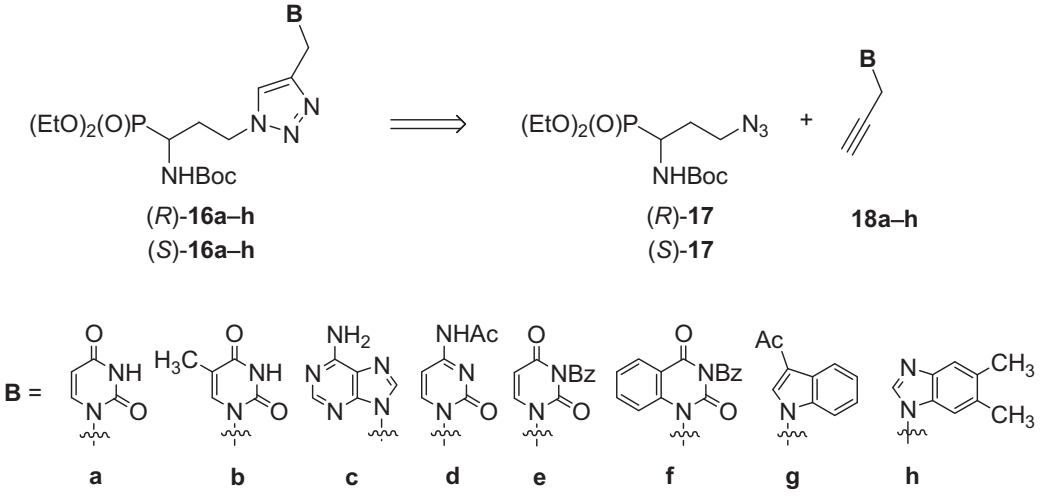


Figure 3. Biologically active acyclic 1,2,3-triazolyl analogs of nucleotides, recently obtained in our group.



Scheme 1. Retrosynthesis of the designed 1,2,3-triazolyl analogs of nucleotides (*R*)- and (*S*)-**16**.

analogs is usually discussed for the free acids, in order to improve their bioavailability, they are administered as prodrugs as diesters or diamides [4, 5, 7, 8, 21, 22]. For these reasons and encouraged by our recent observations [19, 23, 24] in current studies, diethyl esters **16** are subjected to biological evaluation to improve low membrane permeability of diacids [$P(O)(OH)_2$].

Results and discussion

Chemistry

Enantiomerically pure diethyl 1-(Boc-amino)-3-azidopropylphosphonates (*R*)- and (*S*)-**17** were obtained in good overall yields from the *N*-Boc-protected 1-amino-3-hydroxyphosphonates (*R*)-**19** [25] and (*S*)-**19** [25, 26] by tosylation followed by a nucleophilic substitution with sodium azide in dimethylformamide (DMF) (Scheme 2) [27].

All propargylated nucleobases **18a–d** (N^1-propargyluracil **18a**, N^1-propargylthymine **18b**, N^9-propargyladenine **18c**, N^4-acetyl-N^1-propargylcytosine **18d**), and propargylated nucleobase analogs **18e–18h** (N^3-benzoyl-N^1-propargyluracil **18e**, N^3-benzoyl-N^1-propargylbenzuracil **18f**, 3-acetyl-*N*-propargylindole **18g**, and 5,6-dimethyl-N^1-propargylbenzimidazole **18h**) have already been described in the literature and were prepared according to the known procedures [19, 28–31].

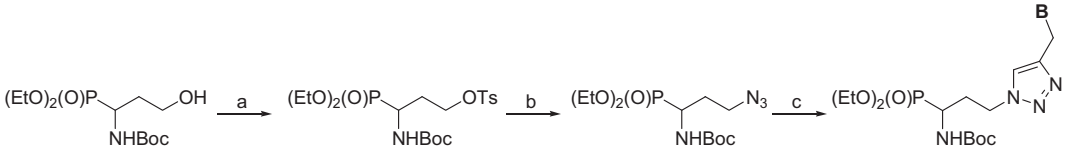
1,3-Dipolar cycloadditions of azides (*R*)- and (*S*)-**17** with *N*-propargyl nucleobases **18a–18d** and propargylated nucleobase analogs **18e–18h** were carried out in a microwave oven leading to the formation of 1,2,3-triazole derivatives (*R*)- and (*S*)-**16a–h**, respectively. The crude reaction mixtures were subjected to column chromatography, and in all cases, good to excellent overall yields of final products were obtained (Scheme 2). Structure and purity of all (1,2,3-triazolyl)-acyclonucleotides (*R*)- and (*S*)-**16a–h** were established by ^1H, ^{13}C, and ^{31}P NMR and IR techniques as well as by elemental analysis.

Antiviral activity and cytostatic/cytotoxic evaluation

All enantiomerically pure (1,2,3-triazolyl)acyclonucleotide analogs (*R*)- and (*S*)-**16a–16h** 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) cell cultures: herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, and herpes simplex virus-1 (TK^- KOS ACV^r); (b) HeLa cell cultures: vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus; (c) Vero cell cultures: parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, and Punta Toro virus; (d) Crandell-Rees feline kidney (CRFK) cell cultures: feline corona virus (FIPV), and FHV; (e) 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), and (f) CEM cell cultures: human immunodeficiency virus [HIV-1 and HIV-2]. 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-induced cytopathogenicity by 50% (other viruses).

The cytotoxicity of the tested compounds toward the 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.

All synthesized compounds were tested against a broad variety of viruses but only analog (*R*)-**16g** (**B** = 3-acetylindole) showed moderate activity against vesicular stomatitis virus in HeLa cell cultures ($EC_{50} = 45\ \mu M$). When compared with our



B = a. uracil; **b.** thymine; **c.** adenine; **d.** N^4-acetylcytosine; **e.** N^3-benzoyluracil; **f.** N^3-benzoylbenzuracil; **g.** 3-acetyloindole; **h.** 5,6-dimethylbenzimidazole

Scheme 2. Reactions and conditions: (a) TsCl, NEt_3, CH_2Cl_2; (b) NaN_3, DMF; and (c) *N*-propargylated nucleobases **18a–18d** and propargylated nucleobase analogs **18e–18h**, $CuSO_4 \cdot 5H_2O$ (0.05 equiv.), sodium ascorbate (0.1 equiv.), H_2O–EtOH (1:1), MW, 40–45°C, 15 min.

entire collection of phosphonate nucleoside analogs having 3-acetylindole as a nucleobase mimetic, compound (*R*)-**16g** is the very first antiviral hit. Since its enantiomer (*S*)-**16g** was not active one may conclude that the spatial orientations of the *N*-Boc-amino and diethoxyphosphoryl groups are discriminative to exert antiviral response (Table 1).

On the other hand, although none of the phosphonate analogs **16** was active against feline corona and feline herpes viruses, several compounds from the (*R*)-**16** series, namely (*R*)-**16f** (**B** = N^3-Bz-benzuracil), (*R*)-**16g** (**B** = 3-acetylindole), and (*R*)-**16h** (**B** = 5,6-dimethylbenzimidazole) appeared slightly cytotoxic to CRFK cells (CC_{50} = 45, 72, and 96 μM, respectively), while compound (*S*)-**16c** (**B** = adenine) exhibited significant cytotoxicity (CC_{50} = 2.9 μM) to these cell cultures (Table 2).

The cytostatic activity of the tested compounds was defined as the 50% cytostatic inhibitory concentration (IC_{50}), causing a 50% decrease in cell proliferation and was determined against murine leukemia L1210, human lymphocyte CEM and human cervix carcinoma HeLa cells.

Compound (*R*)-**16g** and (**B** = 3-acetylindole) appeared slightly cytostatic against murine leukemia L1210, human CEM and HeLa cells at an IC_{50} of 172 ± 6, 193 ± 81, and 122 ± 3 μM, respectively, whereas its enantiomer (*S*)-**16g** affected human CEM cell proliferation only at an IC_{50} of 172 ± 26 (Table 3). However, phosphonate nucleoside analogs having 3-acetylindole as a nucleobase mimetic **21a–21g** (Fig. 4) [19] appeared cytostatic against human CEM line at IC_{50} of 2.78 ± 1.4 to 100 ± 2.8 μM for the entire series depending on the length (CH_2, CH_2CH_2, $CH_2CH_2CH_2$, and $CH_2CH_2CH_2CH_2$) and substitution [$CH(OH)CH_2$, $CH(OH)CH_2CH_2$, and $CH_2CH(OH)CH_2$] of the linker between the phosphorus atom and an 1,2,3-triazole ring [19]. The same series of phosphonate analogs was found at least an order of magnitude less active against HeLa cells, and the most active were phosphonates with CH_2, CH_2CH_2 and $CH_2CH_2CH_2$ linkers (IC_{50} of 11.76 ± 7.6, 18 ± 3.5, and 19 ± 13 μM, respectively) [19]. Cytostatic activity of the phosphonates **21a–g** against murine leukemia L1210 cells was even lower [19] and in general comparable with that of (*R*)-**16g**.

Table 1. Cytotoxicity and antiviral activity of enantiomerically pure (*R*)- and (*S*)-1-Boc-amino-3-(1*H*-1,2,3-triazol-1-yl)propylphosphonates **16** in HeLa cell cultures.

			EC_{50} (μM)		
Compound	Base (B)	MCC (μM)	Vescicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
(*R*)-**16g**		>100	45	>100	>100
(*S*)-**16g**		>100	>100	>100	>100
(*S*)-DHPA		>250	126	>250	>250
Ribavirin		>250	7	112	6

Table 2. Cytotoxicity and antiviral activity of enantiomerically pure (*R*)- and (*S*)-1-Boc-amino-3-(1*H*-1,2,3-triazol-1-yl)propylphosphonates **16** in CRFK cell cultures.

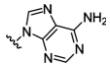
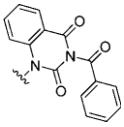
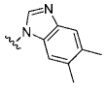
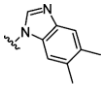
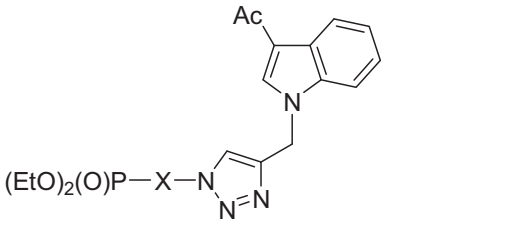
			EC_{50} (μM)	
Compound	Base (B)	CC_{50} (μM)	Feline corona virus	Feline herpes virus
(*R*)-**16c**		>100	>100	>100
(*S*)-**16c**		2.9	>0.8	>0.8
(*R*)-**16f**		45.5	>20	>20
(*S*)-**16f**		100	>100	>100
(*R*)-**16g**		71.6	>20	>20
(*S*)-**16g**		>100	>100	>100
(*R*)-**16h**		96.3	>20	>20
(*S*)-**16h**		>100	>100	>100
UDH (μg/mL)		>100	0.8	0.4
Ganciclovir		>100	>100	4.0

Table 3. Inhibitory effect of enantiomerically pure (*R*)- and (*S*)-1-Boc-amino-3-(1*H*-1,2,3-triazol-1-yl)propylphosphonates **16** against the proliferation of murine leukemia (L1210), human T-lymphocyte (CEM), and human cervix cells (HeLa).

		IC_{50} (μM)		
Compound	Base (B)	L1210	CEM	HeLa
(*R*)-**16g**		172 ± 6	193 ± 81	122 ± 3
(*S*)-**16g**		>250	172 ± 26	≥250
(*R*)-**16h**		>250	>250	>250
(*S*)-**16h**		>250	189 ± 34	>250
5-Fluorouracil		0.33 ± 0.17	18 ± 5	0.54 ± 0.12

Conclusions

Enantiomerically pure 1-Boc-amino-3-azidopropylphosphonates (*R*)- and (*S*)-**17** were obtained from the *N*-Boc-protected 1-aminohydroxyphosphonates (*R*)- and (*S*)-**19** and applied to



21a–g **a.** X = CH_2
b. X = $(CH_2)_2$
c. X = $(CH_2)_3$
d. X = $(CH_2)_4$
e. X = $CH(OH)CH_2$
f. X = $CH_2CH(OH)CH_2$
g. X = $CH(OH)CH_2CH_2$

Figure 4. Acyclic phosphonate nucleoside analogs having 3-acetylindole as a nucleobase mimetic **21a**–**21g** [19].

cycloaddition with *N*-propargylated nucleobases **18a**–**18d** and *N*-propargylated nucleobase analogs **18e**–**18h** to provide a series of 1-amino-3-(1*H*-1,2,3-triazol-1-yl)propylphosphonates (*R*)- and (*S*)-**16a**–**h** as acyclic analogs of nucleotides.

All synthesized 1,2,3-triazolylphosphonates (*R*)- and (*S*)-**16a**–**h** were evaluated for activity against a variety of DNA and RNA viruses, and compound (*R*)-**16g** (**B** = 3-acetylindole) was found moderately active against vesicular stomatitis virus in HeLa cell cultures ($EC_{50} = 45\,\mu M$). Moreover, compounds (*S*)-**16c** (**B** = adenine), (*R*)-**16f** (**B** = N^3-Bz-benzuracil), (*R*)-**16g** (**B** = 3-acetylindole), and (*R*)-**16h** (**B** = 5,6-dimethylbenzimidazole) appeared cytotoxic to CRFK cell cultures ($CC_{50} = 2.9$, 45, 72, and 96 μM, respectively). Furthermore, (*R*)-**16g** exhibited slight inhibitory activity on proliferation of L1210, CEM, and HeLa cells, whereas (*S*)-**16g** and (*S*)-**16h** were cytostatic to CEM cell cultures only.

Experimental

Chemistry

^{1}H NMR were taken in $CDCl_3$ or CD_3OD 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 $CDCl_3$, CD_3OD, or DMSO-d_6 solutions on a Varian Mercury-300 and Bruker Avance III (600 MHz) spectrometer at 75.5 and 151 MHz, respectively. ^{31}P NMR spectra were taken in $CDCl_3$ or CD_3OD on Varian Mercury-300 at 121.5 MHz. IR spectral data were measured on an Infinity MI-60 FT-IR spectrometer. Melting points were determined on a Boetius apparatus and are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory of this Faculty on a Perkin Elmer PE 2400 CHNS analyzer. 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}. TLC plates were developed in chloroform–methanol solvent systems. Visualization of spots was effected with iodine vapors. All solvents were purified by methods described in the literature. Microwave irradiation experiments were carried out in 50 mL glass vials in a microwave reactor Plazmatronika RM 800.

Starting materials

Diethyl 1-Boc-amino-3-hydroxypropylphosphonates (*R*)- and (*S*)-**19** were prepared as previously reported [25]. All propargylated nucleobases **18a**–**18d** and propargylated nucleobase analogs **18e**–**18h** were obtained according to the procedures described in the literature [19, 28–31].

*Preparation of tosylates (R)- and (S)-**20***

To a solution of *N*-Boc-aminohydroxypropylphosphonates (*R*)- or (*S*)-**19** (1.00 mmol) in methylene chloride (3 mL), triethylamine (2.00 mmol) was added followed by tosyl chloride (2.00 mmol). The reaction mixture was stirred at room temperature for 16 h; the solution was washed with water (2 × 2 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. A crude product was chromatographed on a silica gel column (chloroform–methanol, 200:1, v/v) to give pure tosylates (*R*)- or (*S*)-**20**.

*tert-Butyl (R)-1-(O,O-diethylphosphono)-3-(p-toluenesulfonyloxy)propylcarbamate (R)-**20***

Yield: 86%; colorless oil; $[\alpha]_D^{20} = -25.4$ (*c* 1.33, $CHCl_3$). IR (film): $\nu = 3288$, 3028, 2992, 2969, 1683, 1230, 1026, 779, 696 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.80$ (d, $J = 8.1$ Hz, 1H), 7.33 (d, $J = 8.1$ Hz, 1H), 4.70 (brd, $J = 9.3$ Hz, 1H, NH), 4.17–4.04 (m, 7H, 2 × POCH_2CH$_3$, PC*H*, PCCCH_2), 2.44 (s, 3H, CH_3), 2.39–2.18 (m, 1H, PCCH_aH$_b$), 1.99–1.76 (m, 1H, PCCH$_a$$H_b$), 1.45 (s, 9H, 3 × CH_3), 1.31 (t, $J = 7.0$ Hz, 6H, 2 × POCH$_2$CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 155.0$ (d, $J = 5.4$ Hz, C–O), 144.7, 132.8, 129.8, 127.9, 80.1, 66.7 (d, $J = 14.9$ Hz, PCC*C*), 62.9 (d, $J = 7.1$ Hz, POC), 62.7 (d, $J = 7.1$ Hz, POC), 43.9 (d, $J = 158.3$ Hz, PC), 29.7 (d, $J = 4.3$ Hz, PC*C*), 28.3, 21.7, 16.6 (d, $J = 6.0$ Hz, POC*C*), 16.4 (d, $J = 6.0$ Hz, POC*C*); ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 24.11$ ppm. Anal. calcd. for $C_{19}H_{32}NO_8PS$: C, 49.02; H, 6.93; N, 3.01. Found: C, 49.06; H, 6.96; N, 3.17.

*tert-Butyl (S)-1-(O,O-diethylphosphono)-3-(p-toluenesulfonyloxy)propylcarbamate (S)-**20***

Yield: 90%; colorless oil; $[\alpha]_D^{20} = +24.9$ (*c* 1.11, $CHCl_3$). Anal. calcd. for $C_{19}H_{32}NO_8PS$: C, 49.02; H, 6.93; N, 3.01. Found: C, 49.27; H, 6.85; N, 3.24.

*Preparation of azides (R)- and (S)-**17***

To a solution of tosylate (*R*)- or (*S*)-**20** (1.00 mmol) in DMF (4 mL) NaN_3 (2.00 mmol) was added and the suspension was stirred at room temperature for 24 h. A crude reaction mixture was co-evaporated *in vacuo* with toluene (3 × 5 mL) and the residue was dissolved in methylene chloride (5 mL), washed with water (2 × 2 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The products (*R*)- or (*S*)-**17** were used in the next step without further purification.

*tert-Butyl (R)-3-azido-1-(O,O-diethylphosphono)-propylcarbamate (R)-**17***

Yield: 94%; colorless oil; $[\alpha]_D^{20} = -23.4$ (*c* 0.87, $CHCl_3$). IR (film): $\nu = 3301$, 2994, 2984, 2899, 2190, 1678, 1225, 1024 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.75$ (brd, $J = 9.9$ Hz, 1H, NH), 4.20–4.10 (m, 4H, 2 × POCH_2CH$_3$), 3.48–3.37 (m, 3H, PC*H* and PCCCH_2), 2.36–2.18 (m, 1H, PCCH_aH$_b$), 1.81–1.78 (m, 1H, PCCH$_a$$H_b$), 1.45 (s, 9H, 3 × CH_3), 1.31 (t, $J = 6.9$ Hz, 6H, 2 × POCH$_2$CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$):

$\delta = 155.2$ (d, $J = 5.7$ Hz, C=O), 80.1, 62.9 (d, $J = 7.1$ Hz, POC), 62.5 (d, $J = 7.1$ Hz, POC), 48.1 (d, $J = 14.3$ Hz, PCC*C*), 44.6 (d, $J = 157.7$ Hz, PC), 29.6 (d, $J = 4.3$ Hz, PC*C*), 28.3, 16.5 (d, $J = 6.3$ Hz, POC*C*), 16.4 (d, $J = 6.3$ Hz, POC*C*); ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 25.20$ ppm. Anal. calcd. for $C_{12}H_{25}N_4O_5P$: C, 42.85; H, 7.49; N, 16.66. Found: C, 42.72; H, 7.35; N, 16.42.

*tert-Butyl (S)-3-azido-1-(O,O-diethylphosphono)-propylcarbamate (S)-**17***

Yield: 98%; colorless oil; $[\alpha]_D^{20} = +21.2$ (*c* 0.82, $CHCl_3$). Anal. calcd. for $C_{12}H_{25}N_4O_5P$: C, 42.85; H, 7.49; N, 16.66. Found: C, 42.69; H, 7.56; N, 16.58.

*General procedure for the preparation of 1,2,3-triazoles (R)- and (S)-**16a–h***

To a solution of azidoalkylphosphonates (*R*)- or (*S*)-**17** (1.00 mmol) in EtOH (1 mL) and H_2O (1 mL) $CuSO_4 \cdot 5H_2O$ (0.05 mmol), sodium ascorbate (0.10 mmol), and alkynes (1.00 mmol) were added. The reaction mixture was irradiated in a microwave reactor at 40–45°C for 15 min. The solvent was removed by vacuum evaporation and the residue was suspended in chloroform (5 mL) and filtered through a layer of celite. The solution was concentrated *in vacuo* and the crude product was purified on a silica gel column with chloroform–methanol mixtures (50:1, 20:1, or 10:1 v/v) to give the respective 1,2,3-triazoles (*R*)- or (*S*)-**16**.

*tert-Butyl (R)-1-(O,O-diethylphosphono)-3-{4-[(3,4-dihydro-2,4-dioxopyrimidin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylcarbamate (R)-**16a***

Yield: 88%; amorphous solid, m.p. 76–79°C; $[\alpha]_D^{20} = -12.1$ (*c* 1.13, $CHCl_3$). IR (KBr): $\nu = 3223$, 3143, 3038, 2933, 2812, 1694, 1235, 1028 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.12$ (brs, 1H, NH), 7.83 (s, 1H, *H*C5′), 7.52 (d, $J = 8.0$ Hz, 1H, *H*C=CH), 5.71 (d, $J = 8.0$ Hz, 1H, *H*C=CH), 5.14 (dd, $J = 9.8$ Hz, $J = 2.6$ Hz, 1H, NH), 5.00 (AB, $J_{AB} = 15.1$ Hz, 1H, C*H*$_a$H$_b$), 4.98 (AB, $J_{AB} = 15.1$ Hz, 1H, CH$_a$*H*$_b$), 4.53–4.45 (m, 2H, PCCC*H*$_a$*H*$_b$), 4.17–3.98 (m, 5H, 2 × POC*H*$_2$CH$_3$, PC*H*), 2.58–2.47 (m, 1H, PCC*H*$_a$H$_b$), 2.30–2.06 (m, 1H, PCCH$_a$*H*$_b$), 1.46 (s, 9H, 3 × CH$_3$), 1.32 (t, $J = 6.9$ Hz, 6H, 2 × POCH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 165.1$ (s, C=O); 156.1 (d, $J = 4.1$ Hz, C=O), 151.2, 145.4, 142.3, 124.3, 101.4, 79.6, 63.1 (d, $J = 7.2$ Hz, POC), 62.9 (d, $J = 7.2$ Hz, POC), 46.6 (d, $J = 15.6$ Hz, PC*C*C), 44.3 (d, $J = 160.5$ Hz, PC), 42.6, 29.5 (d, $J = 4.8$ Hz, PC*C*), 27.4, 15.4 (d, $J = 5.4$ Hz, POC*C*), 15.3 (d, $J = 5.4$ Hz, POC*C*); ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 24.49$ ppm. Anal. calcd. for $C_{19}H_{31}N_6O_7P$: C, 46.91; H, 6.42; N, 17.28. Found: C, 47.06; H, 6.64; N, 17.40.

*tert-Butyl (S)-1-(O,O-diethylphosphono)-3-{4-[(3,4-dihydro-2,4-dioxopyrimidin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylcarbamate (S)-**16a***

Yield: 80%; amorphous solid, m.p. 79–81°C; $[\alpha]_D^{20} = +11.9$ (*c* 1.72, $CHCl_3$). Anal. calcd. for $C_{19}H_{31}N_6O_7P$: C, 46.91; H, 6.42; N, 17.28. Found: C, 46.80; H, 6.52; N, 17.24.

*tert-Butyl (R)-1-(O,O-diethylphosphono)-3-{4-[(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylcarbamate (R)-**16b***

Yield: 73%; white powder, m.p. 86–89°C; $[\alpha]_D^{20} = -14.3$ (*c* 1.13, $CHCl_3$). IR (KBr): $\nu = 3287$, 2982, 2932, 2820, 1696, 1122, 1025 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 9.09$ (s, 1H, NH), 7.82 (s, 1H, *H*C5′), 7.35 (d, $J = 1.2$ Hz, 1H, *H*C=CCH$_3$), 5.11 (dd, $J = 10.3$ Hz, $J = 3.0$ Hz, 1H, NH), 5.00 (AB, $J_{AB} = 15.1$ Hz, 1H, C*H*$_a$H$_b$), 4.94 (AB, $J_{AB} = 15.1$ Hz, 1H, CH$_a$*H*$_b$), 4.53–4.42 (m, 2H, PCCC*H*$_a$*H*$_b$), 4.19–3.93 (m, 5H, 2 × POC*H*$_2$CH$_3$, PC*H*), 2.60–2.38 (m, 1H, PCC*H*$_a$H$_b$), 2.28–2.04 (m, 1H, PCCH$_a$*H*$_b$), 1.91 (d, $J = 1.2$ Hz, 3H, HC=C*H*$_3$), 1.45 (s, 9H, 3 × CH$_3$), 1.32 (t, $J = 6.9$ Hz, 6H, 2 × POCH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 165.3$ (s, C=O); 156.1 (d, $J = 4.1$ Hz, C=O), 151.3, 142.5, 141.2, 124.2, 110.2, 79.6, 63.0 (d, $J = 7.4$ Hz, POC), 62.8 (d, $J = 7.4$ Hz, POC), 46.5 (d, $J = 15.5$ Hz, PC*C*C), 44.3 (d, $J = 160.6$ Hz, PC), 42.3, 29.4 (d, $J = 4.5$ Hz, PC*C*), 27.3, 15.4 (d, $J = 5.3$ Hz, POC*C*), 15.3 (d, $J = 5.3$ Hz, POC*C*), 12.2; ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 24.47$ ppm. Anal. calcd. for $C_{20}H_{33}N_6O_7P \cdot H_2O$: C, 46.33; H, 6.80; N, 16.21. Found: C, 46.09; H, 6.92; N, 16.40.

*tert-Butyl (S)-1-(O,O-diethylphosphono)-3-{4-[(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylcarbamate (S)-**16b***

Yield: 72%; white powder, m.p. 78–80°C; $[\alpha]_D^{20} = +13.8$ (*c* 0.98, $CHCl_3$). Anal. calcd. for $C_{20}H_{33}N_6O_7P \cdot H_2O$: C, 46.33; H, 6.80; N, 16.21. Found: C, 46.13; H, 7.10; N, 16.35.

*tert-Butyl (R)-1-(O,O-diethylphosphono)-3-{4-[(6-amino-9H-purin-9-yl)methyl]-1H-1,2,3-triazol-1-yl}-propylcarbamate (R)-**16c***

Yield: 99%; colorless oil; $[\alpha]_D^{20} = -13.8$ (*c* 1.28, $CHCl_3$). IR (film): $\nu = 3329$, 3210, 2982, 1704, 1644, 1601, 1242, 1165, 1027 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.37$ (s, 1H), 8.00 (s, 1H), 7.79 (s, 1H), 5.82 (brs, 2H), 5.52 (AB, $J_{AB} = 15.5$ Hz, 1H, C*H*$_a$H$_b$), 5.49 (AB, $J_{AB} = 15.5$ Hz, 1H, CH$_a$*H*$_b$), 5.20 (dd, $J = 10.5$ Hz, $J = 3.2$ Hz, 1H, NH), 4.57–4.47 (m, 1H, PCCC*H*$_a$H$_b$), 4.38–4.17 (m, 1H, PCCCH$_a$*H*$_b$), 4.09–3.93 (m, 5H, 2 × POC*H*$_2$CH$_3$, PC*H*), 2.49–2.39 (m, 1H, PCC*H*$_a$H$_b$), 2.20–2.04 (m, 1H, PCCH$_a$*H*$_b$), 1.44 (s, 9H, 3 × CH$_3$), 1.31 (t, $J = 6.9$ Hz, 3H, POCH$_2$C*H*$_3$), 1.28 (t, $J = 6.9$ Hz, 3H, POCH$_2$C*H*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 155.6$ (d, $J = 5.5$ Hz, C=O), 153.0, 149.6, 142.2, 140.4, 123.7, 119.3, 80.5, 63.2 (d, $J = 6.8$ Hz, POC), 62.8 (d, $J = 6.8$ Hz, POC), 47.0 (d, $J = 15.1$ Hz, PC*C*C), 44.6 (d, $J = 158.8$ Hz, PC), 38.6, 30.9 (d, $J = 4.2$ Hz, PC*C*), 28.2, 16.4 (d, $J = 5.4$ Hz, POC*C*), 16.3 (d, $J = 5.4$ Hz, POC*C*); ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 23.76$ ppm. Anal. calcd. for $C_{20}H_{32}N_9O_5P$: C, 47.15; H, 6.33; N, 24.74. Found: C, 46.88; H, 6.14; N, 24.86.

*tert-Butyl (S)-1-(O,O-diethylphosphono)-3-{4-[(6-amino-9H-purin-9-yl)methyl]-1H-1,2,3-triazol-1-yl}-propylcarbamate (S)-**16c***

Yield: 81%; colorless oil; $[\alpha]_D^{20} = +13.1$ (*c* 0.94, $CHCl_3$). Anal. calcd. for $C_{20}H_{32}N_9O_5P$: C, 47.15; H, 6.33; N, 24.74. Found: C, 47.42; H, 6.47; N, 24.96.

*tert-Butyl (R)-1-(O,O-diethylphosphono)-3-{4-[(4-acetylamino-2-oxopyrimidin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylcarbamate (R)-**16d***

Yield: 72%; white powder, m.p. 197–199°C; $[\alpha]_D^{20} = -40.9$ (*c* 1.55, $CHCl_3$). IR (KBr): $\nu = 3221$, 3046, 2982, 2932, 1676, 1623, 1224, 1048 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 10.05$ (brs, 1H, NH), 7.96 (d, $J = 7.4$ Hz, 1H, *H*C=CH), 7.93 (s, 1H, *H*C5′), 7.42 (d, $J = 7.4$ Hz, 1H, *H*C=CH), 5.94 (brd, $J = 10.1$ Hz, 1H, NH), 5.10 (AB, $J_{AB} = 14.4$ Hz, 1H, C*H*$_a$H$_b$), 5.08 (AB, $J_{AB} = 14.4$ Hz, 1H, CH$_a$*H*$_b$), 4.54–4.40 (m, 2H,

PCCCH_aH_b), 4.17–3.85 (m, 5H, 2 × POCH_2CH$_3$, PC*H*), 2.46–2.40 (m, 1H, PCCH_aH_b), 2.32–2.06 (m, 1H, PCCH_aH_b), 2.20 (s, 3H, CH$_3$), 1.45 (s, 9H, 3 × CH$_3$), 1.32 (t, J = 6.9 Hz, 6H, 2 × POCH$_2$CH_3); ^{13}C NMR (151 MHz, CDCl$_3$): δ = 171.2 (s, C=O); 163.0 (s, C=O); 155.8 (d, J = 6.0 Hz, C=O), 155.7, 148.7, 145.4, 141.5, 125.1, 97.0, 79.7, 63.3 (d, J = 6.9 Hz, POC), 62.7 (d, J = 6.9 Hz, POC), 47.1 (d, J = 17.4 Hz, PC*C*), 44.9 (d, J = 159.8 Hz, PC), 44.7, 30.8 (d, J = 4.3 Hz, PC*C*), 28.3, 24.6, 16.3 (d, J = 5.5 Hz, POC*C*); ^{31}P NMR (121.5 MHz, CDCl$_3$): δ = 24.24 ppm. Anal. calcd. for $C_{21}H_{34}N_7O_7P$: C, 47.81; H, 6.50; N, 18.59. Found: C, 48.10; H, 6.62; N, 18.31.

*tert-Butyl (S)-1-(O,O-diethylphosphono)-3-{4-[(4-acetylamino-2-oxopyrimidin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylcarbamate (S)-**16d***

Yield: 74%; white powder, m.p. 221–223°C; $[\alpha]_D^{20}$ = +40.5 (*c* 2.13, CHCl$_3$). Anal. calcd. for $C_{21}H_{34}N_7O_7P$: C, 47.81; H, 6.50; N, 18.59. Found: C, 47.63; H, 6.40; N, 18.81.

*tert-Butyl (R)-1-(O,O-diethylphosphono)-3-{4-[(3,4-dihydro-3-benzoyl-2,4-dioxopyrimidin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylcarbamate (R)-**16e***

Yield: 72%; white powder, m.p. 131–133°C; $[\alpha]_D^{20}$ = −14.0 (*c* 1.17, CHCl$_3$). IR (KBr): ν = 3263, 2982, 2932, 1748, 1704, 1662, 1235, 1026, 975, 754 cm^{-1}, ^{1}H NMR (600 MHz, CDCl$_3$): δ = 7.91–7.89 (m, 2H, H$_{aromat.}$), 7.75 (s, 1H, *H*C5′), 7.65–7.63 (m, 1H, H$_{aromat.}$), 7.63 (d, J = 8.1 Hz, 1H, *H*C=CH), 7.49–7.46 (m, 2H, H$_{aromat.}$), 5.79 (d, J = 8.0 Hz, 1H, HC=C*H*), 5.16 (brd, J = 9.5 Hz, 1H, NH), 5.04 (AB, J_{AB} = 15.1 Hz, 1H, CH_aH_b), 4.97 (AB, J_{AB} = 15.1 Hz, 1H, CH$_a$H$_b$), 4.53–4.48 (m, 1H, PCCCH_aH_b), 4.43–4.37 (m, 1H, PCCCH$_a$H$_b$), 4.14–4.07 (m, 4H, 2 × POCH_2CH$_3$), 4.03–3.96 (m, 1H, PC*H*), 2.48–2.42 (m, 1H, PCCH_aH_b), 2.18–2.10 (m, 1H, PCCH$_a$H$_b$), 1.43 (s, 9H, 3 × CH$_3$), 1.29 (t, J = 6.9 Hz, 6H, 2 × POCH$_2$CH_3); ^{13}C NMR (151 MHz, CDCl$_3$): δ = 168.7, 162.3 (s, C=O); 155.5 (d, J = 5.8 Hz, C=O), 149.8, 144.2, 141.3, 135.1, 130.4, 129.2, 124.3, 102.4, 80.6, 63.0 (d, J = 6.7 Hz, POC), 62.8 (d, J = 6.7 Hz, POC), 47.0 (d, J = 14.9 Hz, PC*C*), 44.6 (d, J = 157.9 Hz, PC), 43.3, 31.0 (d, J = 3.7 Hz, PC*C*), 28.2, 16.4 (d, J = 5.5 Hz, POC*C*), 16.3 (d, J = 5.5 Hz, POC*C*); ^{31}P NMR (243 MHz, CDCl$_3$): δ = 23.32 ppm. Anal. calcd. for $C_{26}H_{35}N_6O_8P$: C, 52.88; H, 5.97; N, 14.23. Found: C, 52.61; H, 5.72; N, 13.98.

*tert-Butyl (S)-1-(O,O-diethylphosphono)-3-{4-[(3,4-dihydro-3-benzoyl-2,4-dioxopyrimidin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylcarbamate (S)-**16e***

Yield: 75%; colorless oil; $[\alpha]_D^{20}$ = +14.1 (*c* 1.72, CHCl$_3$). Anal. calcd. for $C_{26}H_{35}N_6O_8P$: C, 52.88; H, 5.97; N, 14.23. Found: C, 53.10; H, 5.79; N, 14.00.

*tert-Butyl (R)-1-(O,O-diethylphosphono)-3-{4-[(3,4-dihydro-3-benzoyl-2,4-dioxoquinazolin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylcarbamate (R)-**16f***

Yield: 85%; colorless oil; $[\alpha]_D^{20}$ = −4.6 (*c* 0.95, CHCl$_3$). IR (film): ν = 3267, 3034, 2990, 1748, 1710, 1665, 1230, 1027, 754, 688 cm^{-1}; ^{1}H NMR (600 MHz, CDCl$_3$): δ = 8.18 (dd, J = 7.8 Hz, J = 1.5 Hz, 1H), 7.97–7.95 (m, 2H, H$_{aromat.}$), 7.74–7.24 (m, 1H, H$_{aromat.}$), 7.73 (s, 1H, *H*C5′), 7.65–7.63 (m, 2H, H$_{aromat.}$), 7.28 (dt, J = 7.8 Hz, J = 0.5 Hz, 1H, H$_{aromat.}$), 5.41 (AB, J_{AB} = 15.9 Hz, 1H, CH_aH_b), 5.37 (AB, J_{AB} = 15.9 Hz, 1H, CH$_a$H$_b$), 5.06 (brd, J = 8.8 Hz, 1H, NH), 4.49–4.44 (m, 1H, PCCCH_aH_b), 4.40–4.35 (m, 1H, PCCCH$_a$H$_b$), 4.14–4.07 (m, 4H, 2 × POCH_2CH$_3$), 4.04–3.96 (m, 1H, PC*H*), 2.47–2.40 (m, 1H, PCCH_aH_b), 2.16–2.08 (m, 1H, PCCH$_a$H$_b$), 1.41 (s, 9H, 3 × CH$_3$), 1.29 (t, J = 7.1 Hz, 3H, POCH$_2$CH_3), 1.28 (t, J = 7.1 Hz, 3H, POCH$_2$CH_3); ^{13}C NMR (151 MHz, CDCl$_3$): δ = 168.6 (s, C=O), 161.1 (s, C=O); 155.4 (d, J = 5.7 Hz, C=O), 149.5, 142.3, 140.3, 136.2, 135.0, 129.2, 128.9, 124.1, 123.7, 115.6, 115.4, 80.5, 62.9 (d, J = 6.7 Hz, POC), 62.7 (d, J = 6.7 Hz, POC), 47.0 (d, J = 14.6 Hz, PC*C*), 44.7 (d, J = 158.4 Hz, PC), 38.9, 31.1 (d, J = 3.7 Hz, PC*C*), 28.2, 16.4 (d, J = 5.5 Hz, POC*C*), 16.3 (d, J = 5.5 Hz, POC*C*); ^{31}P NMR (243 MHz, CDCl$_3$): δ = 23.31 ppm. Anal. calcd. for $C_{30}H_{37}N_6O_8P \times H_2O$: C, 54.71; H, 5.97; N, 12.76. Found: C, 54.89; H, 6.12; N, 12.56.

*tert-Butyl (S)-1-(O,O-diethylphosphono)-3-{4-[(3,4-dihydro-3-benzoyl-2,4-dioxoquinazolin-1-yl)methyl]-1H-1,2,3-triazol-1-yl}propylcarbamate (S)-**16f***

Yield: 75%; white powder, m.p. 90–93°C; $[\alpha]_D^{20}$ = +4.5 (*c* 1.08, CHCl$_3$). Anal. calcd. for $C_{30}H_{37}N_6O_8P \cdot H_2O$: C, 54.71; H, 5.97; N, 12.76. Found: C, 54.82; H, 5.73; N, 12.86.

*tert-Butyl (R)-1-(O,O-diethylphosphono)-3-{4-[(3-acetylindol-1-yl)methyl]-1H-1,2,3-triazol-1-yl}-propylcarbamate (R)-**16g***

Yield: 81%; white powder, m.p. 160–163°C; $[\alpha]_D^{20}$ = −20.8 (*c* 1.14, CHCl$_3$). IR (KBr): ν = 3271, 3112, 2982, 2931, 1722, 1630, 1025, 746 cm^{-1}; ^{1}H NMR (600 MHz, CDCl$_3$): δ = 8.38–8.35 (m, 1H), 7.87 (s, 1H), 7.57 (s, 1H), 7.44–7.42 (m, 1H), 7.28–7.25 (m, 2H), 5.43 (s, 2H), 5.10 (brd, J = 10.5 Hz, 1H, NH), 4.52–4.48 (m, 1H, PCCCH_aH_b), 4.34–4.29 (m, 1H, PCCCH$_a$H$_b$), 4.11–4.06 (m, 4H, 2 × POCH_2CH$_3$), 3.95–3.86 (m, 1H, PC*H*), 2.49 (s, 3H, CH$_3$), 2.47–2.37 (m, 1H, PCCH_aH_b), 2.14–2.04 (m, 1H, PCCH$_a$H$_b$), 1.43 (s, 9H, 3 × CH$_3$), 1.28 (t, J = 7.1 Hz, 3H, POCH$_2$CH_3), 1.25 (t, J = 7.1 Hz, 3H, POCH$_2$CH_3); ^{13}C NMR (151 MHz, CDCl$_3$): δ = 193.3 (s, C=O), 155.5 (d, J = 5.0 Hz, C=O), 142.7, 136.6, 134.7, 126.5, 123.5, 123.2, 122.7, 122.6, 117.6, 109.8, 80.6, 62.9 (d, J = 7.0 Hz, POC), 62.7 (d, J = 7.0 Hz, POC), 46.8 (d, J = 14.6 Hz, PC*C*), 44.4 (d, J = 157.7 Hz, PC), 42.3, 31.0 (d, J = 4.3 Hz, PC*C*), 28.2, 27.5, 16.3 (d, J = 5.7 Hz, POC*C*), 16.2 (d, J = 5.5 Hz, POC*C*); ^{31}P NMR (243 MHz, CDCl$_3$): δ = 23.24 ppm. Anal. calcd. for $C_{25}H_{36}N_5O_6P \cdot H_2O$: C, 54.44; H, 6.94; N, 12.70. Found: C, 54.63; H, 6.98; N, 12.48.

*tert-Butyl (S)-1-(O,O-diethylphosphono)-3-{4-[(3-acetylindol-1-yl)methyl]-1H-1,2,3-triazol-1-yl}-propylcarbamate (S)-**16g***

Yield: 87%; white powder, m.p. 164–165°C; $[\alpha]_D^{20}$ = +20.3 (*c* 1.63, CHCl$_3$). Anal. calcd. for $C_{25}H_{36}N_5O_6P \cdot H_2O$: C, 54.44; H, 6.94; N, 12.70. Found: C, 54.40; H, 7.00; N, 12.90.

*tert-Butyl (R)-1-(O,O-diethylphosphono)-3-{4-[(5,6-dimethylbenzimidazol-1-yl)methyl]-1H-1,2,3-triazol-1-yl}-propylcarbamate (R)-**16h***

Yield: 94%; colorless very thick oil; $[\alpha]_D^{20}$ = −12.9 (*c* 1.31, CHCl$_3$). IR (film): ν = 3268, 3122, 2977, 2932, 1715, 1224, 1021, 973, 840 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.90 (s, 1H), 7.55 (s, 1H), 7.50 (s, 1H), 7.22 (s, 1H), 5.43 (s, 2H), 4.91 (dd, J = 10.5 Hz, J = 3.6 Hz, 1H, NH), 4.54–4.45 (m, 1H, PCCCH_aH_b), 4.35–4.25 (m, 1H, PCCCH$_a$H$_b$), 4.14–4.05 (m, 4H, 2 × POCH_2CH$_3$), 4.04–3.86 (m, 1H, PC*H*), 2.47–2.33 (m, 1H, PCCH_aH_b), 2.37 (s, 3H, CH$_3$), 2.35 (s, 3H, CH$_3$), 2.16–2.00 (m, 1H, PCCH$_a$H$_b$), 1.43 (s, 9H, 3 × CH$_3$), 1.29

(t, $J = 7.1$ Hz, 3H, $POCH_2CH_3$), 1.26 (t, $J = 7.1$ Hz, 3H, $POCH_2CH_3$); ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 155.5$ (d, $J = 6.0$ Hz, C=O), 143.1, 132.5, 131.3, 123.0, 120.5, 110.1, 80.8, 63.3 (d, $J = 7.0$ Hz, POC), 63.0 (d, $J = 7.0$ Hz, POC), 47.1 (d, $J = 15.1$ Hz, PCCC), 44.7 (d, $J = 157.7$ Hz, PC), 40.8, 31.4 (d, $J = 4.6$ Hz, PCC), 28.5, 20.8, 20.5, 16.7 (d, $J = 5.7$ Hz, POCC), 16.6 (d, $J = 5.5$ Hz, POCC); ^{31}P NMR (121.5 MHz, $CDCl_3$): $\delta = 23.67$ ppm. Anal. calcd. for $C_{24}H_{37}N_6O_5P$: C, 55.37; H, 7.16; N, 16.14. Found: C, 55.44; H, 7.18; N, 16.43.

*tert-Butyl (S)-1-(O,O-diethylphosphono)-3-{4-[(5,6-dimethylbenzimidazol-1-yl)methyl]-1H-1,2,3-triazol-1-yl}-propylcarbamate (S)-**16h***

Yield: 88%; colorless very thick oil; $[\alpha]_D^{20} = +13.0$ (*c* 1.31, $CHCl_3$). Anal. calcd. for $C_{24}H_{37}N_6O_5P$: C, 55.37; H, 7.16; N, 16.14. Found: C, 55.33; H, 7.36; N, 16.15.

Pharmacology

Antiviral activity assays

The antiviral assays were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia virus, and vesicular stomatitis virus], Vero (parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, and Punta Toro virus), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus), MDCK (influenza A (H1N1 and H3N1) and influenza B virus) or CRFK (FHV; feline corona virus (FIPV)) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100-cell culture inhibitory dose-50 ($CCID_{50}$) of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) in the presence of varying concentrations (250, 50, 10, ... μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The antiviral concentration was expressed as the EC_{50} or 50%-effective compound concentration required to inhibit virus-induced cytopathicity by 50%.

Cytotoxicity and cytostatic assay

The cytotoxicity of the test compounds was monitored as a microscopically visible alteration of cell morphology, and expressed as the MCC or compound concentration required to afford a microscopically detectable alteration of cell culture morphology.

The cytostatic activity of the test compounds was determined as the 50% cytostatic concentration (IC_{50}) or compound concentration required to inhibit cell proliferation by 50%. For this purpose, cells were seeded in 200-μL wells of 96-well microtiter plates and allowed to proliferate for 2 (L1210) to 3 (CEM) or 4 (HeLa) days in the absence or presence of different serial concentrations of the test compounds. At the end of the exponential proliferation phase, the cells were counted by an automated Coulter ZI particle counter (Analis, Ghent, Belgium).

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