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ORIGINAL PAPER

Design, synthesis, antiviral and cytotoxic evaluation of novel acyclic phosphonate nucleotide analogues with a 5,6-dihydro-1*H*-[1,2,3]triazolo[4,5-*d*]pyridazine-4,7-dione system

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Abstract A series of diethyl 2-(4,5-dimethoxycarbonyl-1*H*-1,2,3-triazol-1-yl)alkylphosphonates was synthesised from *ω*-azidoalkylphosphonates and dimethyl acetylenedicarboxylate and was further transformed into the respective diamides, dihydrazides, and 5,6-dihydro-1*H*-[1,2,3]triazolo[4,5-*d*]pyridazine-4,7-diones as phosphonate analogues of acyclic nucleosides having nucleobases replaced with substituted 1,2,3-triazoles. All compounds containing P–C–C–triazole or P–C–C–CH_2–triazole moieties exist in single conformations in which the diethoxyphosphoryl and substituted 1,2,3-triazolyl or substituted (1,2,3-triazolyl)methyl groups are oriented *anti*. All phosphonates were evaluated in vitro for activity against a variety of DNA and RNA viruses. None of the compounds were endowed with antiviral activity. They were not cytostatic at 100 μM.

Keywords Cycloadditions · Cyclizations · Heterocycles · NMR spectroscopy · Conformation

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Introduction

In the past two decades acyclic nucleoside phosphonates (ANPs) have become one of the most important classes of antiviral drugs [1]. Three of them (adefovir, cidofovir, tenofovir; Fig. 1) have been marketed for treatment of viral infection caused by HIV, HBV, HSV and other DNA viruses [2–5]. The concept of acyclic nucleosides is based on the assumption that an acyclic moiety most often bearing an oxygen atom mimics the furanose ring at least partially. Acyclic nucleoside phosphonates require conversion in vivo to their triphosphate metabolites to become active [2, 6]. The replacement of the natural phosphate moiety by a phosphonate group makes analogues less susceptible to enzymatic hydrolysis [7, 8].

Further studies in this field brought a new generation of nucleotide/nucleoside analogues in which natural nucleobases were modified as exemplified by the 2,4-diaminopyrimidine framework present in antiviral HPMPO-DAPy and PMPO-DAPy [9–11] and the 1,2,4-triazole ring in ribavirin (Fig. 2) [12].

The antiviral activity of ribavirin stimulated interest in replacing the 1,2,4-triazole system with an isomeric 1,2,3-triazole ring because several 1,2,3-triazoles exhibit antibacterial [13–15], antifungal [15–17], anticancer [18–20], anti-inflammatory [21, 22] and antiviral [23–26] properties. It was found that carbocyclic analogues **1** and phosphonocarbocyclic analogues **2** of ribavirin displayed antiviral activity against HIV-1 (Fig. 3) [27].

Furthermore, preliminary structure–activity relationship evaluation of 1,2,3-triazole nucleoside phosphonates **3** and **4** suggested that this scaffold could be further optimised to afford selective inhibitors of HCV replication (Fig. 4) [28].

On the other hand, it was reported that nucleoside analogues **5** and **6** containing the 5,6-dihydro-1*H*-imidazo[4,5-

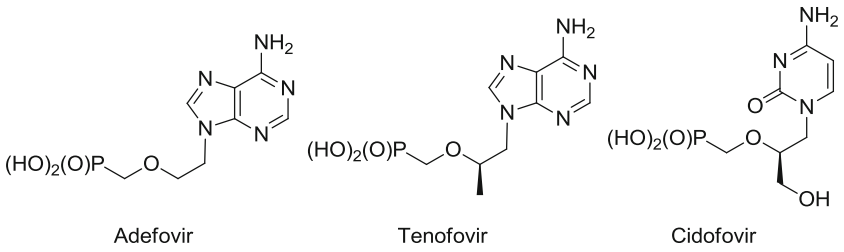


Fig. 1 ANPs marketed for treatment of viral infections

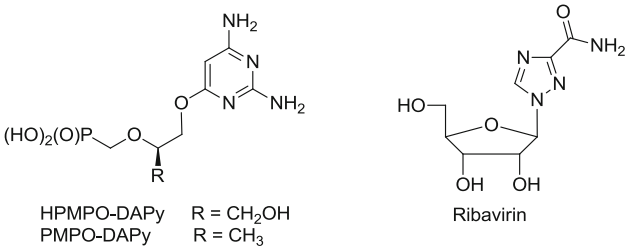


Fig. 2 Structures of HPMPO-DAPy, PMPO-DAPy and ribavirin

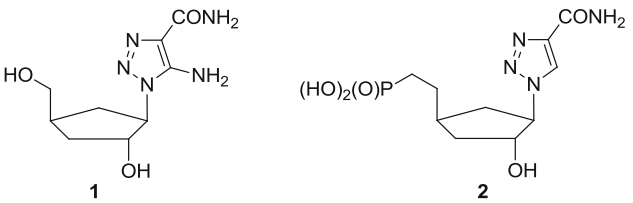


Fig. 3 Antiviral analogues of ribavirin having the 1,2,3-triazole ring

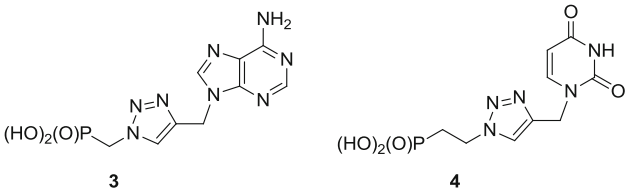


Fig. 4 1,2,3-Triazole nucleoside phosphonates as potential inhibitors of HCV replication

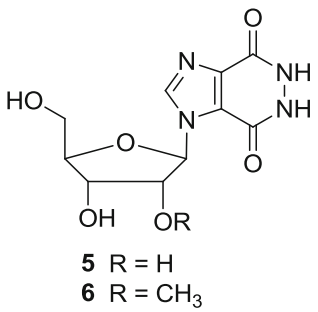


Fig. 5 Cyclic nucleoside analogues based on the 5,6-dihydro-1*H*-imidazo[4,5-*d*]pyridazine-4,7-dione framework

d]pyridazine-4,7-dione ring instead of the natural purine framework exhibited antiviral activity by inhibition of a viral helicase from West Nile Virus (WNV) and HCV (Fig. 5). These observations may be useful in designing a lead structure for the development of new classes of antiviral agents [29].

Based on the active compounds already discussed, a novel series of phosphonate analogues **11** having the 5,6-dihydro-1*H*-[1,2,3]triazolo[4,5-*d*]pyridazine-4,7-dione system was designed as potential antiviral agents (Scheme 1). Furthermore, because their immediate precursor dihydrazides **10** as well as diamides **9** share several common structural features with ribavirin, they also may show antiviral activity. The key step of our synthetic plan involves the 1,3-dipolar cycloaddition of dimethyl acetylenedicarboxylate and ω-azidoalkylphosphonates **7** which contain structurally diversified alkyl chains to provide the intermediate diesters **8**.

Results and discussion

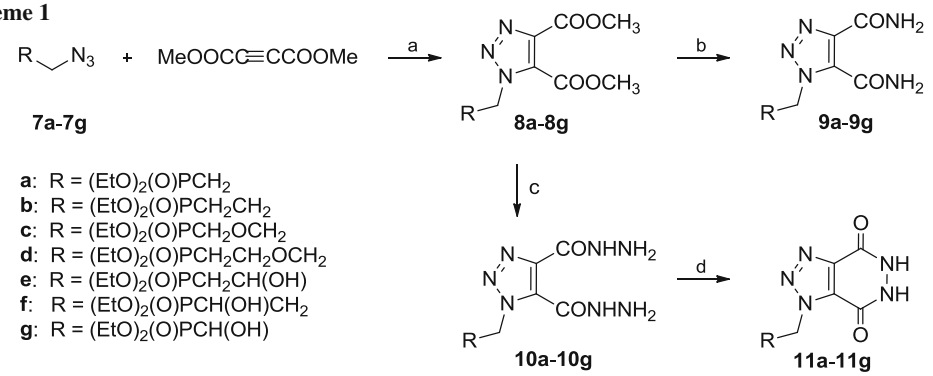
Chemistry

The 1,2,3-triazoles **8a**–**8d** and **8f** were synthesised in 64–98 % yield employing the Huisgen 1,3-dipolar cycloaddition of the corresponding azidophosphonates **7** and dimethyl acetylenedicarboxylate at 110 °C in the same way as the known compounds **8e** and **8g** [30, 31]. They were finally purified either by chromatography on a silica gel column or by crystallisation (Scheme 1).

The required azidoalkylphosphonates **7a**–**7e** and **7g** have already been described in the literature [30, 32–36]. Azidophosphonate **7f** was obtained in the Abramov reaction [37, 38] from 3-azidopropanal [39] and diethyl phosphite in 34 % yield (Scheme 2). It was found that 3-azidopropanal is unstable in the presence of triethylamine used as a catalyst in this reaction. For this reason only 0.4 equiv. of diethyl phosphite was applied to avoid tedious separation of **7f** from the reaction mixture.

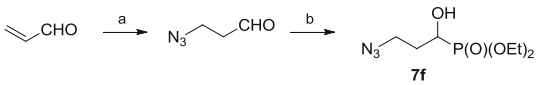
The diamides **9a**–**9g** were obtained from diesters **8a**–**8g** by ammonolysis according to a standard protocol (Scheme 1) [6, 40]. The crude products were subjected to column chromatography on silica gel and finally purified

Scheme 1



Reagents and conditions: a. toluene, 110 °C, 4 h; b. aqueous ammonia, EtOH, rt, 24 h; c. hydrazine hydrate, EtOH, reflux, 2 h; d. 10% HCl, 90 °C, 2.5 h.

Scheme 2



Reagents and conditions: a. NaN_3, acetic acid, -10 °C, 2 h; b. $(EtO)_2P(O)H$, NEt_3.

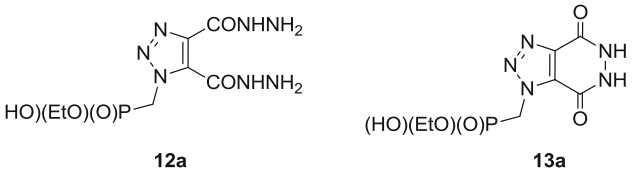


Fig. 6 Compounds **12a** and **13a**

by crystallisation to give **9a**–**9g** in 37–61 % yields. The ^{1}H NMR spectra of diamides **9a**–**9g** in chloroform-*d* confirmed the nonequivalence of protons from the carbamoyl groups because in the 6.0–10.8 ppm region four broad singlets were always observed.

The diesters **8a**–**8g** were also converted to the corresponding dihydrazides **10a**–**10g** with hydrazine hydrate (Scheme 1). Preliminary attempts at synthesising dihydrazide **10a** followed the literature procedure [41] and showed that refluxing phosphonate **8a** and hydrazine hydrate in ethanolic solution for 5 h led to the formation of several products. The ^{31}P NMR spectrum of the reaction mixture revealed the presence of the expected phosphonate **10a** (76 %), bicyclic 1,2,3-triazolopyridazinedione **11a** (11 %) and their monodealkylated counterparts **12a** and **13a** (10 and 3 %, respectively) (Fig. 6). When hydrazinolysis of the diester **8a** was conducted for 2 h only phosphonates **10a** (79 %) and **11a** (21 %) were produced. For this reason syntheses of dihydrazides **10b**–**10g** were performed under the same conditions. However, it appeared that for compounds **8c** and **8g** significant dealkylation still occurred. In both cases the respective crude reaction mixtures contained almost 50 % of by-products. Purifications on silica gel columns and crystallisations gave dihydrazides **10a**–**10g** in 28–66 % yields.

Finally, heating dihydrazides **10a**–**10g** with 10 % hydrochloric acid for 2.5 h gave [1,2,3]triazolo[4,5-*d*]pyridazine-4,7-diones **11a**–**11g** (Scheme 1) in 30–66 % yields [41].

Conformational analysis

Detailed analyses of ^{1}H and ^{13}C NMR spectral data revealed conformational preferences of phosphonates described in this paper. Compounds **8a**–**11a** contain an 1,2-substituted ethylene fragment which does not freely rotate around a C–C bond because their ^{1}H NMR spectra display AA′XX′P patterns. A similar spectrum was also noticed for 2-azidoethylphosphonate [31]. Antiperiplanar disposition of the diethoxyphosphoryl groups and substituted 1,2,3-triazoles **14a** (Fig. 7) was proved by the presence of two identical $^3J(P–H_X) = 10.5$ Hz couplings which were calculated from the $^1H\{^{31}P\}$ NMR spectrum of **8a**.

In the ^{1}H NMR spectra of compounds **8g**, **10g**, and **11g** three hydrogen atoms attached to the two-carbon linker appeared as deceptively simple but very similar ABXP spectral patterns. However, the relevant ^3J(H1–H2a) and ^3J(H1–H2b) coupling constants were precisely calculated (10.2 and 3.6 Hz, respectively) from the ^{1}H NMR spectrum of diamide **9g**. These values [42] together with small couplings for ^3J(P–H2a) and ^3J(P–H2b) [43, 44] allowed us to unequivocally establish **14g** (Fig. 7) as the preferred conformation of phosphonate **9g** although other phosphonates from this series very likely adopt the same *anti* conformation.

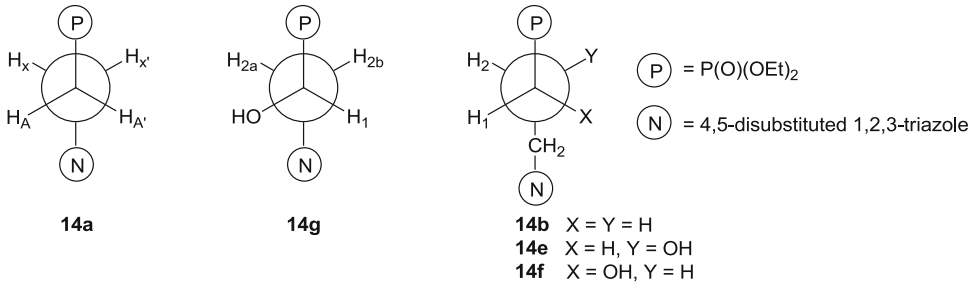


Fig. 7 Preferred conformations of the phosphonates described in this study

Large values (16.9–19.2 Hz) of ^{3}J(P–CC–C3) [44–46] observed in the ^{13}C NMR spectra of phosphonates **8–11** containing a three-carbon fragment between the phosphorus atom and the 1,2,3-triazole ring (series **b**, **e**, and **f**) evidenced the preference of antiperiplanar conformations **14b**, **14e**, and **14f** (Fig. 7) for these compounds. This conclusion was further supported by vicinal H1–H2 couplings calculated for 2- and 1-hydroxyphosphonates (series **e** and **f**) which clearly indicated *gauche* (3.0–3.6 Hz) and *anti* (9.6–10.8 Hz) arrangements of the respective H–C1C2–H protons.

Although values (9.3–12.1 Hz) of ^{3}J(P–C–O–C) were easily extracted from the ^{13}C NMR spectra of phosphonates **8–11** (series **c**) they could not be unequivocally applied in the estimation of the extent to which rotation around the PC–OC bond is hindered because the angular dependence of ^{3}J(P–C–O–C) has not been established so far. However, the rotation around the OC–CN bond is not restricted because vicinal H_2C–CH_2 coupling constants observed for phosphonates **8c–11c** fall in the 4.8–5.2 Hz range. On the other hand, on the basis of the values of ^{3}J(HC–CH) found for PH_2C–CH_2O and OH_2C–CH_2N units (7.2–7.8 and 5.1–5.7 Hz, respectively), full conformational freedom within a five-atom linker in phosphonates **8–11** (series **d**) is anticipated.

Antiviral activity evaluation

The synthesised compounds **9a–9g**, **10a–10g**, and **11a–11g** 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: herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), herpes simplex virus-1 (TK^- ACV^r KOS), vaccinia virus, vesicular stomatitis virus, varicella-zoster virus (TK^+ VZV strain OKA and TK^- VZV strain 07-1) and cytomegalovirus (CMV) (strain AD-169 and Davis); (b) CEM cell cultures: human immunodeficiency virus-1 (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, Coxackie virus B4 and respiratory syncytial virus; (e) Crandel-Rees feline kidney (CRFK) cell cultures: feline corona virus (FIPV) and feline herpes virus (FHV) and (f) Madin Darby Canine kidney (MDCK) cell culture: influenza A virus H1N1 subtype A/PR/8, influenza 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], 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 plaques formation (VZV, CMV) by 50 % or to reduce virus-induced cytopathogenicity by 50 % (other viruses). None of the compounds showed appreciable antiviral activity at subtoxic concentrations.

Evaluation of cytotoxicity

The cytotoxicity of the tested compounds towards the uninfected host cells was defined as the minimum cytotoxic concentration (MMC) that causes a microscopically detectable alteration of normal cell morphology. The 50 % cytotoxic concentration (CC_{50}), i.e. 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 the cell morphology of Vero, HEL, HeLa or CRFK cells (MCC or CC_{50}) at compound concentrations up to 100 μM. They were also not cytostatic against murine leukemia and human CEM and HeLa cells at 100 μM.

Conclusions

Phosphonylated 1,2,3-triazoles **8a–8g** were obtained in good to excellent yields by 1,3-dipolar cycloaddition between ω-azidophosphonates **7a–7g** and dimethyl acetylenedicarboxylate. New series of diamides **9a–9g**, dihydrazides **10a–10g** and 1,2,3-triazolopyridazinediones **11a–11g** were efficiently synthesised as phosphonate analogues of acyclic nucleosides in which nucleobases were replaced by substituted 1,2,3-triazoles.

Compounds **8–11** (series **a**, **b**, **e**, **f**, **g**) exist in single conformations in which diethoxyphosphoryl and substituted 1,2,3-triazolyl (series **a** and **b**) or (1,2,3-triazolyl)methyl groups (series **e**, **f**, **g**) prefer the *anti* orientation.

All synthesised compounds were evaluated for their antiviral activity against DNA and RNA viruses and were inactive. None of the compounds were cytotoxic (Vero, HEL, HeLa) or cytostatic (L1210, CEM, HeLa) at a concentration up to 100 μM.

Experimental

The ^{1}H NMR spectra were recorded in $CDCl_3$, CD_3OD or D_2O 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. The ^{13}C NMR spectra were recorded for $CDCl_3$, CD_3OD or D_2O solutions on Varian Mercury-300 and Bruker Avance III (600 MHz) machines at 75.5 and 150.5 MHz, respectively. The ^{31}P NMR spectra were recorded in $CDCl_3$, CD_3OD or D_2O on Varian Mercury-300 and Bruker Avance III (600 MHz) spectrometers at 121.5 and 243 MHz, respectively.

IR spectra were measured on an Infinity MI-60 FT-IR spectrometer. Melting points were determined on the Boetius apparatus. Elemental analyses were performed by the microanalytical laboratory of the host institution on Perkin Elmer PE 2400 CHNS analyzer and the results were found to be in good agreement (±0.3 %) with the calculated values.

The following absorbents 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 vapours. All solvents were dried according to standard literature methods.

Diethyl 3-azido-1-hydroxypropylphosphonate (**7f**, $C_7H_{16}N_3O_4P$)

A mixture of 5.77 g 3-azidopropanal [39] (0.0582 mol), 3.0 cm^3 diethyl phosphite (0.023 mol) and 0.81 cm^3 triethylamine (0.0058 mol) was stirred at 5 °C for 24 h. The crude product was subjected to chromatography on silica gel with chloroform/methanol (100:1 and 50:1, v/v) to give a yellow oil (4.671 g, 34 %). IR (film): $\bar{V}$ = 3,272, 2,985, 2,934, 2,911, 2,874, 2,102, 1,227, 1,028 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.35 (t, J = 7.1 Hz, 6H, 2 × $POCH_2CH_3$), 1.87–2.06 (m, 3H, CH_2, OH), 3.55 (t, J = 6.3 Hz, 2H, CH_2N_3), 4.01 (dt, J = 9.8, 4.6 Hz, 1H, H-1), 4.12–4.24 (m, 4H, 2 × $POCH_2CH_3$) ppm; ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ = 16.6 and 16.7 (2 d, J = 5.4 Hz, POC*C*), 30.8 (d, J = 2.6 Hz, C-2), 47.7 (d, J = 15.5 Hz, C-3), 62.9 and 63.2 (2 d, J = 7.2 Hz, POC), 64.6 (d, J = 164.6 Hz, C-1) ppm; ^{31}P NMR ($CDCl_3$, 121.5 MHz): δ = 25.28 ppm.

*Synthesis of 1,2,3-triazoles **8a–8g** (general procedure)*

A solution of azidophosphonate **7a–7g** (1.00 mmol) and dimethyl acetylenedicarboxylate **13** (1.00 mmol) in 4 cm^3 toluene was refluxed for 4 h. The reaction mixtures were concentrated to dryness to leave yellow oils which were purified on silica gel columns with chloroform/methanol (100:1, v/v) or were crystallised to give 1,2,3-triazoles **8a–8g**.

Diethyl 2-(4,5-dimethoxycarbonyl-1H-1,2,3-triazol-1-yl)ethylphosphonate (**8a**, $C_{12}H_{20}N_3O_7P$)

From 0.450 g azidophosphonate **7a** (2.17 mmol) and 0.309 g dimethyl acetylenedicarboxylate **13** (2.17 mmol) phosphonate **8a** was obtained as a yellowish oil (0.674 g, 89 %) after chromatography on a silica gel column with chloroform/methanol (100:1, v/v). IR (film): $\bar{V}$ = 3,462, 2,985, 2,958, 1,736, 1,468, 1,447, 1,225, 1,140, 1,060, 1,025, 957 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.33 (t, J = 7.1 Hz, 6H, CH_3CH_2OP), 2.40–2.52 (m, 2H, PCH_2), 3.98 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 4.08–4.18 (m, 4H, CH_3CH_2OP), 4.82–4.91 (m, 2H, CH_2N) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 16.6 (d, J = 5.7 Hz, *C*COP), 27.2 (d, J = 140.6 Hz, PC), 45.4 (s, PC*C*N), 52.9 (s, OCH_3), 53.7 (s, OCH_3), 62.4 (d, J = 6.3 Hz, C*C*OP), 129.8 and 140.2 (2 s, C=C), 158.6 and 160.4 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 121 MHz): δ = 25.9 ppm.

Diethyl 3-(4,5-dimethoxycarbonyl-1H-1,2,3-triazol-1-yl)propylphosphonate (**8b**, $C_{13}H_{22}N_3O_7P$)

From 0.785 g azidophosphonate **7b** (3.565 mmol) and 0.507 g dimethyl acetylenedicarboxylate **13** (3.565 mmol) phosphonate **8b** was obtained as a yellowish oil (1.067 g, 83 %) after chromatography on a silica gel column with chloroform/methanol (100:1, v/v). IR (film): $\bar{V}$ = 2,953, 2,836, 1,736, 1,463, 1,226, 1,030 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.34 (t, J = 7.1 Hz, 6H, CH_3CH_2OP), 1.72–1.84 (m, 2H, PCH_2CH_2), 2.16–2.31 (m, 2H, PCH_2), 4.00 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 4.05–4.18 (m, 4H, CH_3CH_2OP), 4.71 (t, J = 7.1 Hz, 2H, CH_2N) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 16.5 (d, J = 6.0 Hz, POC*C*), 22.6 (d, J = 143.1 Hz, PC), 23.5 (d, J = 4.3 Hz, PC*C*), 50.4 (d, J = 16.9 Hz, PCC*C*), 52.7 (s, OCH_3), 53.2 (s, OCH_3), 61.8 (d, J = 6.3 Hz, POC), 129.7 and 139.8 (2 s, C=C), 158.6 and 160.3 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 121 MHz): δ = 30.7 ppm.

Diethyl 2-(4,5-dimethoxycarbonyl-1H-1,2,3-triazol-1-yl)ethoxymethylphosphonate (**8c**, $C_{13}H_{22}N_3O_8P$)

From 1.027 g azidophosphonate **7c** (4.330 mmol) and 0.615 g dimethyl acetylenedicarboxylate **13** (4.330 mmol) phosphonate **8c** was obtained as a yellowish oil (1.538 g, 93 %) after chromatography on a silica gel column with chloroform/

methanol (100:1, v/v). IR (film): $\bar{V}$ = 3,459, 2,983, 2,957, 2,908, 1,732, 1,462, 1,225, 1,117, 1,060, 1,027, 960 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ = 1.31 (t, J = 7.0 Hz, 6H, CH_3CH_2OP), 3.74 (d, J = 7.9 Hz, 2H, PCH_2), 3.98 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 4.02 (t, J = 5.2 Hz, 2H, OCH_2CH_2N), 4.08–4.13 (m, 4H, CH_3CH_2OP), 4.86 (t, J = 5.2 Hz, 2H, OCH_2CH_2N) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 16.5 (d, J = 5.7 Hz, POC*C*), 49.8 (s, CN), 52.8 (s, OCH_3), 53.5 (s, OCH_3), 62.6 (d, J = 6.5 Hz, PO*C*C), 65.2 (d, J = 164.7 Hz, PC), 71.0 (d, J = 9.3 Hz, PCO*C*), 131.0 and 139.6 (2 s, C=C), 158.8 and 160.3 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 243 MHz): δ = 20.04 ppm.

Diethyl 2-[2-(4,5-dimethoxycarbonyl-1H-1,2,3-triazol-1-yl)-ethoxy]ethylphosphonate (**8d**, $C_{14}H_{24}N_3O_8P$)
From 0.647 g azidophosphonate **7d** (2.68 mmol) and 0.382 g dimethyl acetylenedicarboxylate **13** (2.68 mmol) phosphonate **8d** (1.030 g, 98 %) was obtained as a yellowish oil. The crude product was sufficiently pure and was used in the next step without further purification. IR (film): $\bar{V}$ = 3,459, 2,983, 2,957, 2,909, 1,736, 1,466, 1,229, 1,118, 1,063, 1,027, 963 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ = 1.32 (t, J = 7.1 Hz, 6H, CH_3CH_2OP), 2.00 (dt, J = 15.3, 7.7 Hz, 2H, PCH_2), 3.63 (dt, J = 10.4, 7.7 Hz, 2H, PCH_2CH_2O), 3.82 (t, J = 5.2 Hz, 2H, OCH_2CH_2N), 4.00 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.05–4.12 (m, 4H, CH_3CH_2OP), 4.82 (t, J = 5.2 Hz, 2H, OCH_2CH_2N) ppm; ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 16.3 (d, J = 5.7 Hz, POC*C*), 26.7 (d, J = 139.3 Hz, PC), 49.8 (s, OC*C*N), 52.6 (s, OCH_3), 53.3 (s, OCH_3), 61.6 (d, J = 6.4 Hz, C*C*OP), 65.3 (s, PC*C*), 68.7 (s, O*C*CN), 131.4 and 139.5 (2 s, C=C), 159.1 and 160.3 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 243 MHz): δ = 27.58 ppm.

Diethyl 1-hydroxy-3-(4,5-dimethoxycarbonyl-1H-1,2,3-triazol-1-yl)propylphosphonate (**8f**, $C_{13}H_{22}N_3O_8P$)
From 0.560 g azidophosphonate **7f** (2.36 mmol) and 0.335 g dimethyl acetylenedicarboxylate **13** (2.36 mmol) phosphonate **8f** (0.850 g, 95 %) was obtained as a white amorphous solid after chromatography on a silica gel column with chloroform/methanol (100:1, v/v). M.p.: 107–109 °C; IR (film): $\bar{V}$ = 3,420, 3,218, 3,081, 2,985, 2,878, 1,675, 1,451, 1,280, 1,223, 1,030, 970 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.33 and 1.34 (2 t, J = 7.2 Hz, 6H, CH_3CH_2OP), 2.28–2.47 (m, 2H, PCCH_2), 3.70 (dd, J = 6.0, 5.1 Hz, 1H, OH), 3.84 (dddd, J = 10.5, 6.3, 6.0, 3.3 Hz, 1H, PCH), 3.98 (s, OCH_3), 4.00 (s, OCH_3), 4.10–4.22 (m, 4H, CH_3CH_2OP), 4.75–4.90 (m, 2H, CH_2N) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 16.6 and 16.7 (2 d, J = 5.4 Hz, *C*COP), 31.9 (d, J = 3.2 Hz, PC*C*), 47.2 (d, J = 16.3 Hz, PCC*C*), 52.8 (s, OCH_3), 53.6 (s, OCH_3), 63.0 and 63.3 (2 d, J = 7.3 Hz, C*C*OP), 64.6 (d, J = 164.2 Hz, PC), 130.3 and 139.7 (2 s, C=C), 158.8 and 160.4 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 121 MHz): δ = 23.5 ppm.

*Synthesis of diamides **9a–9g** (general procedure)*

To a solution of the diester **8a–8g** (1.00 mmol) in 14 cm^3 ethanol 16 cm^3 was added aqueous ammonia. The reaction mixtures were stirred at room temperature for 24 h. Ethanol and excess ammonia were evaporated in vacuo. Diamides **9a–9g** were purified on silica gel columns with chloroform/methanol or by crystallisation.

Diethyl 2-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)ethylphosphonate (**9a**, $C_{10}H_{18}N_5O_5P$)
From 0.165 g diester **8a** (0.472 mmol) diamide **9a** was obtained as a white amorphous solid (0.065 g, 42 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, v/v) followed by crystallisation from ethanol/diethyl ether. M.p.: 172–173 °C; IR (KBr): $\bar{V}$ = 3,428, 3,228, 3,113, 2,986, 2,932, 1,687, 1,451, 1,221, 1,023 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.34 (t, J = 6.9 Hz, 6H, CH_3CH_2OP), 2.41–2.53 (m, 2H, PCH_2), 4.09–4.21 (m, 4H, CH_3CH_2OP), 5.08–5.17 (m, 2H, CH_2N), 6.01 (s, 2H, NH_2), 7.58 (s, 1H, NH), 10.77 (s, 1H, NH) ppm; ^{13}C NMR (CD_3OD, 75 MHz): δ = 16.8 (d, J = 6.0 Hz, *C*COP), 27.3 (d, J = 140.3 Hz, PC), 47.2 (s, PC*C*N), 63.8 (d, J = 6.6 Hz, C*C*OP), 132.1 and 140.4 (2 s, C=C), 160.3 and 165.2 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 121 MHz): δ = 26.5 ppm.

Diethyl 3-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)propylphosphonate (**9b**, $C_{11}H_{20}N_5O_5P$)
From 0.114 g diester **8b** (0.314 mmol) diamide **9b** was obtained as a white amorphous solid (0.070 g, 61 %) after chromatography on a silica gel column with chloroform/methanol (50:1, v/v) and crystallisation from ethanol. M.p.: 143–144 °C; IR (KBr): $\bar{V}$ = 3,455, 3,270, 2,984, 2,909, 1,694, 1,629, 1,592, 1,454, 1,205, 1,018, 961 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.31 (t, J = 6.9 Hz, 6H, CH_3CH_2OP), 1.74–1.86 (m, 2H, PCH_2CH_2), 2.18–2.32 (m, 2H, PCH_2), 4.04–4.16 (m, 4H, CH_3CH_2OP), 4.98 (t, J = 6.9 Hz, 2H, CH_2N), 6.13 (s, 1H, NH), 6.25 (s, 1H, NH), 7.62 (s, 1H, NH), 10.79 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 16.7 (d, J = 6.6 Hz, *C*COP), 23.0 (d, J = 141.9 Hz, PC), 23.8 (d, J = 4.3 Hz, PC*C*), 51.8 (d, J = 19.2 Hz, PCC*C*), 62.0 (d, J = 6.6 Hz, C*C*OP), 130.7 and 138.7 (2 s, C=C), 158.6 and 163.7 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 121 MHz): δ = 31.4 ppm.

Diethyl [2-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)ethoxy]-methylphosphonate (**9c**, $C_{11}H_{20}N_5O_6P$)
From 0.280 g diester **8c** (0.738 mmol) diamide **9c** was obtained as a white amorphous solid (0.139 g, 54 %) after chromatography on a silica gel column with chloroform/methanol (50:1, v/v). M.p.: 100–101 °C; IR (KBr): $\bar{V}$ = 3,361, 3,259, 3,110, 2,990, 2,959, 2,901, 1,689, 1,613, 1,454, 1,303, 1,219, 1,118, 1,048, 1,019, 967,

943 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz): $\delta = 1.29$ (t, $J = 7.1$ Hz, 6H, CH_3CH_2OP), 3.89 (d, $J = 8.5$ Hz, 2H, PCH_2), 4.06 (t, $J = 5.2$ Hz, 2H, OCH_2CH_2N), 4.06–4.10 (m, 4H, CH_3CH_2OP), 5.14 (t, $J = 5.2$ Hz, 2H, CH_2N) ppm; ^{13}C NMR (CD_3OD, 150 MHz): $\delta = 15.3$ (d, $J = 5.9$ Hz, *C*COP), 50.3 (s, CN), 62.8 (d, $J = 6.5$ Hz, C*C*OP), 64.0 (d, $J = 164.7$ Hz, PC), 71.1 (d, $J = 11.9$ Hz, PCO*C*), 131.0 and 139.0 (2 s, C=C), 159.1 and 163.9 (2 s, C=O) ppm; ^{31}P NMR (CD_3OD, 243 MHz): $\delta = 21.3$ ppm.

Diethyl 2-[2-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)ethoxy]ethylphosphonate (**9d**, $C_{12}H_{22}N_5O_6P$)
From 0.264 g diester **8d** (0.671 mmol) diamide **9d** was obtained as a white amorphous solid (0.090 g, 37 %) after chromatography on a silica gel column with chloroform/methanol (50:1, v/v) and crystallisation from ethyl acetate. M.p.: 102–103 °C; IR (KBr): $\bar{V} = 3{,}400$, 3,306, 3,214, 2,986, 2,906, 1,672, 1,608, 1,453, 1,245, 1,109, 1,024, 961 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 1.31$ (t, $J = 6.9$ Hz, 6H, CH_3CH_2OP), 2.05 (dt, $J = 15.3$, 7.8 Hz, 2H, PCH_2), 3.69 (dt, $J = 11.1$, 7.8 Hz, 2H, PCH_2CH_2O), 3.91 (t, $J = 5.4$ Hz, 2H, OCH_2CH_2N), 4.01–4.12 (m, 4H, CH_3CH_2OP), 5.11 (t, $J = 5.4$ Hz, 2H, OCH_2CH_2N), 6.00 (s, 1H, NH), 6.07 (s, 1H, NH), 7.60 (s, 1H, NH), 10.77 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 16.6$ (d, $J = 6.0$ Hz, CCOP), 26.9 (d, $J = 139.1$ Hz, PC), 51.0 (s, OC*C*N), 61.9 (d, $J = 6.3$ Hz, C*C*OP), 65.0 (s, PC*C*O), 69.1 (s, O*C*CN), 130.9 and 139.7 (2 s, C=C), 158.7 and 163.7 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 29.2$ ppm.

Diethyl 3-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)-2-hydroxypropylphosphonate (**9e**, $C_{11}H_{20}N_5O_6P$)
From 0.145 g diester **8e** (0.382 mmol) diamide **9e** was obtained as a white amorphous solid (0.060 g, 45 %) after chromatography on a silica gel column with chloroform/methanol (50:1, v/v) and crystallisation from methanol. M.p.: 142–143 °C; IR (KBr): $\bar{V} = 3{,}428$, 3,308, 3,034, 2,988, 1,686, 1,667, 1,599, 1,443, 1,375, 1,293, 1,113, 1,083, 1,055, 1,029, 960 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz): $\delta = 1.32$ (t, $J = 7.2$ Hz, 6H, CH_3CH_2OP), 2.06 (ddd, $J = 22.8$, 15.3, 7.8 Hz, 1H, PCH_aH_b), 2.18 (ddd, $J = 20.4$, 15.3, 5.1 Hz, 1H, PCH_aH_b), 4.07–4.19 (m, 4H, CH_3CH_2OP), 4.37–4.50 (m, 1H, PCC*H*), 4.99 (dd, $J = 13.5$, 8.1 Hz, 1H, $PCCCH_aH_b$), 5.02 (dd, $J = 13.5$, 4.2 Hz, 1H, $PCCCH_aH_b$) ppm; ^{13}C NMR (CD_3OD, 75 MHz): $\delta = 16.9$ (d, $J = 6.3$ Hz, *C*COP), 32.3 (d, $J = 141.7$ Hz, PC), 58.1 (d, $J = 18.0$ Hz, PCC*C*), 63.4 and 63.7 (2 d, $J = 6.6$ Hz, C*C*OP), 67.0 (d, $J = 4.0$ Hz, PC*C*), 132.6 and 140.4 (2 s, C=C), 160.6 and 165.3 (2 s, C=O) ppm; ^{31}P NMR (CD_3OD, 121 MHz): $\delta = 30.1$ ppm.

Diethyl 3-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)-1-hydroxypropylphosphonate (**9f**, $C_{11}H_{20}N_5O_6P$)
From 0.180 g diester **8f** (0.475 mmol) diamide **9f** was obtained as a white amorphous solid (0.093 g, 56 %) after crystallisation from methanol. M.p.: 179–180 °C; IR (KBr): $\bar{V} = 3{,}433$, 3,319, 3,212, 2,974, 2,930, 2,874, 1,668, 1,605, 1,455, 1,399, 1,221, 1,167, 1,066, 1,014, 953 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz): $\delta = 1.32$ (t, $J = 7.2$ Hz, 6H, CH_3CH_2OP), 2.13–2.28 (m, 1H, $PCCH_aH_b$), 2.31–2.44 (m, 1H, $PCCH_aH_b$), 3.89 (ddd, $J = 10.5$, 7.2, 3.0 Hz, 1H, PCH), 4.10–4.21 (m, 4H, CH_3CH_2OP), 4.95–5.14 (m, 2H, CH_2N) ppm; solubility of **9f** in D_2O or CD_3OD was not sufficient to measure the ^{13}C NMR spectrum; ^{31}P NMR (CD_3OD, 121 MHz): $\delta = 25.4$ ppm.

Diethyl 2-(4,5-dicarbamoyl-1H-1,2,3-triazol-1-yl)-1-hydroxyethylphosphonate (**9g**, $C_{10}H_{18}N_5O_6P$)
From 0.230 g diester **8g** (0.630 mmol) diamide **9g** was obtained as a white amorphous solid (0.112 g, 53 %) after chromatography on a silica gel column with chloroform/methanol (20:1, v/v) and crystallisation from methanol. M.p.: 200–202 °C; IR (KBr): $\bar{V} = 3{,}431$, 3,223, 3,081, 2,990, 2,847, 1,671, 1,599, 1,452, 1,393, 1,289, 1,225, 1,031, 979 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz): $\delta = 1.39$ and 1.40 (2 t, $J = 7.2$ Hz, 6H, CH_3CH_2OP), 4.22-4.29 (m, 4H, CH_3CH_2OP), 4.55 (ddd, $J = 10.2$, 9.9 Hz, 3.6 Hz, 1H, PCH), 5.10 (ddd, $J = 13.5$, 10.2, 8.7 Hz, 1H, $PCCH_aH_b$), 5.22 (ddd, $J = 13.5$, 3.6, 3.1 Hz, 1H, $PCCH_aH_b$) ppm; solubility of **9g** in D_2O and CD_3OD was not sufficient to measure the ^{13}C NMR spectrum; ^{31}P NMR (CD_3OD, 243 MHz): $\delta = 21.2$ ppm.

*Synthesis of dihydrazides **10a–10g** (general procedure)*

A solution of the diester **8a–8g** (0.73 mmol) and 0.530 cm^3 hydrazine hydrate (10.8 mmol) in 6 cm^3 ethanol was refluxed for 2 h. The reaction mixtures were concentrated to give yellow oils or solids which were subjected to chromatography on a silica gel column with chloroform/methanol or crystallisation to obtain dihydrazides **10a–10g**.

Diethyl 2-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]ethylphosphonate (**10a**, $C_{10}H_{20}N_7O_5P$)
From 0.115 g diester **8a** (0.330 mmol) dihydrazide **10a** was obtained (0.070 g, 61 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v) as a white amorphous solid. M.p.: 84–86 °C; IR (KBr): $\bar{V} = 3{,}335$, 3,282, 2,984, 2,932, 1,661, 1,606, 1,551, 1,491, 1,262, 1,223, 1,056, 1,020, 963 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz): $\delta = 1.32$ (t, $J = 6.6$ Hz, 6H, CH_3CH_2O), 2.48–2.59 (m, 2H, PCH_2), 4.07–4.18 (m, 4H, CH_3CH_2O), 5.03–5.14 (m, 2H, CH_2N) ppm; ^{13}C NMR

(CD_3OD, 75 MHz): $\delta = 16.9$ (d, $J = 6.0$ Hz, *C*COP), 27.4 (d, $J = 140.0$ Hz, PC), 47.1 (s, PC*C*N), 63.8 (d, $J = 6.3$ Hz, C*C*OP × 2), 130.8 and 139.3 (2 s, C=C), 157.6 and 161.8 (2 s, C=O) ppm; ^{31}P NMR (CD_3OD, 121 MHz): $\delta = 28.2$ ppm.

Diethyl 3-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]propylphosphonate (**10b**, $C_{11}H_{22}N_7O_5P$)
From 0.170 g diester **8b** (0.468 mmol) dihydrazide **10b** was obtained as a white amorphous solid (0.113 g, 66 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v). M.p.: 73–75 °C; IR (KBr): $\bar{V} = 3{,}300$, 3,192, 2,983, 2,931, 1,656, 1,551, 1,209, 1,024, 969 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.28$ (t, $J = 6.9$ Hz, 6H, C*H*$_3$CH$_2$OP), 1.71–1.82 (m, 2H, PCH$_2$C*H*$_2$), 2.13–2.27 (m, 2H, PCH$_2$), 4.00–4.13 (m, 4H, CH$_3$C*H*$_2$OP), 4.94 (t, $J = 7.2$ Hz, 2H, CH$_2$N), 5.15–6.03 (brs, 6H, NHNH$_2$) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 16.7$ (d, $J = 6.0$ Hz, *C*COP), 22.9 (d, $J = 139.9$ Hz, PC), 23.9 (d, $J = 7.4$ Hz, PC*C*), 51.8 (d, $J = 18.9$ Hz, PCC*C*), 62.0 (d, $J = 6.6$ Hz, C*C*OP), 129.5 and 137.6 (2 s, C=C), 156.3 and 161.3 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 31.5$ ppm.

Diethyl [2-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]ethoxy]methylphosphonate (**10c**, $C_{11}H_{22}N_7O_6P$)
From 0.430 g diester **8c** (1.13 mmol) dihydrazide **10c** was obtained as a colourless oil (0.139 g, 32 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v). IR (film): $\bar{V} = 3{,}326$, 3,248, 2,985, 2,918, 2,890, 1,666, 1,544, 1,440, 1,300, 1,238, 1,117, 1,024, 968 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz): $\delta = 1.28$ (t, $J = 7.0$ Hz, 6H, C*H*$_3$CH$_2$OP), 3.89 (d, $J = 8.5$ Hz, 2H, PCH$_2$), 4.05–4.10 (m, 6H, CH$_3$C*H*$_2$OP and OC*H*$_2$CH$_2$N), 5.14 (t, $J = 4.8$ Hz, 2H, OCH$_2$C*H*$_2$N) ppm; ^{13}C NMR (CD_3OD, 150 MHz): $\delta = 15.3$ (d, $J = 5.5$ Hz, *C*COP), 50.2 (s, CN), 62.8 (d, $J = 6.5$ Hz, C*C*OP), 64.0 (d, $J = 164.9$ Hz, PC), 71.1 (d, $J = 11.9$ Hz, PCO*C*), 129.9 and 137.9 (2 s, C=C), 156.5 and 160.5 (2 s, C=O) ppm; ^{31}P NMR (CD_3OD, 243 MHz): $\delta = 21.4$ ppm.

Diethyl 2-[2-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]ethoxy]ethylphosphonate (**10d**, $C_{12}H_{24}N_7O_6P$)
From 0.216 g diester **8d** (0.549 mmol) dihydrazide **10d** was obtained as a white amorphous solid (0.128 g, 59 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, v/v) and crystallisation from ethyl acetate. M.p: 95–96 °C; IR (KBr): $\bar{V} = 3{,}300$, 3,232, 2,983, 2,913, 2,827, 1,667, 1,545, 1,550, 1,252, 1,023, 966, 918 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 1.31$ (t, $J = 7.2$ Hz, 6H, C*H*$_3$CH$_2$OP), 2.03 (dt, $J = 15.0$, 7.5 Hz, 2H, PCH$_2$), 3.69 (dt, $J = 11.1$, 7.5 Hz, 2H, PCH$_2$C*H*$_2$O), 3.91 (t, $J = 5.7$ Hz, 2H, OC*H*$_2$CH$_2$N), 4.01–4.13 (m, 4H, CH$_3$C*H*$_2$OP), 4.20 (brs, 4H, NH$_2$), 5.13 (t, $J = 5.7$ Hz, 2H, OCH$_2$C*H*$_2$N), 8.81 (s, 1H, NH), 12.0 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 16.6$ (d, $J = 6.0$ Hz, *C*COP), 27.0 (d, $J = 138.5$ Hz, PC), 51.0 (s, OC*C*N), 61.9 (d, $J = 6.3$ Hz, C*C*OP), 65.0 (s, PC*C*O), 69.1 (s, O*C*CN), 129.9 and 137.5 (2 s, C=C), 156.5 and 161.3 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 28.5$ ppm.

Diethyl 3-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]-2-hydroxypropylphosphonate (**10e**, $C_{11}H_{22}N_7O_6P$)
From 0.290 g diester **8e** (0.764 mmol) dihydrazide **10e** was obtained as a white amorphous solid (0.140 g, 48 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, v/v) and crystallisation from ethanol. M.p.: 140–142 °C; IR (KBr): $\bar{V} = 3{,}336$, 3,275, 2,980, 2,930, 1,668, 1,578, 1,549, 1,443,1,260, 1,217, 1,067, 1,027, 957 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz): $\delta = 1.34$ and 1.35 (2 t, $J = 7.1$ Hz, 6H, C*H*$_3$CH$_2$OP), 2.12 (ddd, $J = 23.6$, 15.4, 8.2 Hz, 1H, PC*H*$_a$H$_b$), 2.18 (ddd, $J = 20.1$, 15.4, 4.7 Hz, 1H, PCH$_a$*H*$_b$), 4.11–4.19 (m, 4H, CH$_3$C*H*$_2$OP), 4.44–4.47 (m, 1H, PCC*H*), 4.96 (dd, $J = 13.4$, 8.4 Hz, 1H, PCCC*H*$_a$H$_b$), 5.05 (dd, $J = 13.4$, 4.1 Hz, 1H, PCCCH$_a$*H*$_b$) ppm; ^{13}C NMR (CD_3OD, 150 MHz): $\delta = 15.3$ (d, $J = 5.8$ Hz, *C*COP), 30.8 (d, $J = 141.6$ Hz, PC), 56.4 (d, $J = 17.3$ Hz, PCC*C*), 61.9 and 62.2 (2 d, $J = 6.5$ Hz, C*C*OP), 65.6 (d, $J = 3.6$ Hz, PC*C*), 130.1 and 137.9 (2 s, C=C), 156.7 and 160.6 (2 s, C=O) ppm; ^{31}P NMR (CD_3OD, 243 MHz): $\delta = 28.9$ ppm.

Diethyl 3-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]-1-hydroxypropylphosphonate (**10f**, $C_{11}H_{22}N_7O_6P$)
From 0.262 g diester **8f** (0.691 mmol) dihydrazide **10f** was obtained as a white amorphous solid (0.155 g, 60 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 30:1, v/v). M.p.: 119–120 °C; IR (KBr): $\bar{V} = 3{,}316$, 3,266, 3,160, 2,976, 2,930, 1,659, 1,572, 1,532, 1,249, 1,210, 1,072, 1,022, 955 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz): $\delta = 1.31$ (t, $J = 7.5$ Hz, 6H, C*H*$_3$CH$_2$OP), 2.13-2.29 (m, 1H, PCC*H*$_a$H$_b$), 2.31–2.44 (m, 1H, PCCH$_a$*H*$_b$), 3.91 (ddd, $J = 10.8$, 7.8, 3.3 Hz, 1H, PCH), 4.09–4.21 (m, 4H, CH$_3$C*H*$_2$OP), 4.96–5.15 (m, 2H, CH$_2$N) ppm; ^{13}C NMR (CD_3OD, 75 MHz): $\delta = 17.0$ (d, $J = 5.4$ Hz, *C*COP), 33.4 (s, PC*C*), 48.4 (d, $J = 18.9$ Hz, PCC*C*), 64.2 and 64.5 (d, $J = 7.1$ Hz, C*C*OP), 65.6 (d, $J = 166.7$ Hz, PC), 130.9 and 139.2 (2 s, C=C), 157.7 and 161.8 (2 s, C=O × 2) ppm; ^{31}P NMR (CD_3OD, 121 MHz): $\delta = 25.5$ ppm.

*Diethyl 2-[4,5-bis(hydrazinocarbonyl)-1H-1,2,3-triazol-1-yl]-1-hydroxyethylphosphonate (**10g**, $C_{10}H_{20}N_7O_6P$)*
From 0.450 g diester **8g** (1.23 mmol) dihydrazide **10g** was obtained as a white amorphous solid (0.128 g, 28 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 30:1, v/v). M.p.: 149–152 °C; IR (KBr): $\bar{V} = 3{,}334$, 3,207, 2,980, 2,930, 2,870, 1,668, 1,578, 1,449,

1,217, 1,067, 1,028, 953 cm^{-1}; ^1H NMR (D_2O, 600 MHz): $\delta = 1.31$ (t, $J = 7.0$ Hz, 6H, CH_3CH_2OP), 4.18–4.24 (m, 4H, CH_3CH_2OP), 4.53–4.58 (m, 1H, PCH), 4.94–5.01 (m, 1H, $PCCH_aH_b$), 5.11–5.16 (m, 1H, $PCCH_aH_b$) ppm; ^{13}C NMR (D_2O, 150 MHz): $\delta = 15.7$ (d, $J = 4.6$ Hz, *C*COP), 51.9 (d, $J = 11.2$ Hz, PC*C*N), 64.7 and 64.8 (2 d, $J = 7.4$ Hz, C*C*OP), 65.5 (d, $J = 165.8$ Hz, PC), 130.4 and 137.5 (2 s, C=C), 156.5 and 160.2 (2 s, C=O) ppm; ^{31}P NMR (D_2O, 243 MHz): $\delta = 21.8$ ppm.

*Synthesis of 1,2,3-triazolopyridazinediones **11a–11g** (general procedure)*

A mixture of the dihydrazide **10a–10g** (0.50 mmol) and 5 cm^3 10 % hydrochloric acid was heated at 90 °C for 2.5 h. After concentration in vacuo, crude products were purified on a silica gel column with chloroform/methanol or crystallised from the appropriate solvent.

Diethyl 2-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo[4,5-d]pyridazin-1-yl)ethylphosphonate (**11a**, $C_{10}H_{16}N_5O_5P$)
From 0.167 g dihydrazide **10a** (0.478 mmol) compound **11a** was obtained as a white amorphous solid (0.045 g, 30 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v) followed by crystallisation from ethanol. M.p.: 198–200 °C; IR (KBr): $\bar{V} = 3{,}385$, 2,922, 2,672, 1,687, 1,582, 1,460, 1,262, 1,214, 1,014, 1,019, 980 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz): $\delta = 1.28$ (t, $J = 6.6$ Hz, 6H, CH_3CH_2OP), 2.58 (dt, $J = 14.7$ Hz, $J = 7.5$ Hz, PCH_2), 4.03–4.13 (m, 4H, CH_3CH_2OP), 5.12 (dt, $J = 12.6$ Hz, $J = 7.5$ Hz, CH_2N) ppm; ^{13}C NMR (CD_3OD, 150 MHz): $\delta = 17.5$ (d, $J = 5.7$ Hz, *C*COP), 28.2 (d, $J = 141.0$ Hz, PC), 46.5 (d, $J = 3.0$ Hz, PC*C*N), 64.7 (d, $J = 6.6$ Hz, C*C*OP), 131.3 and 141.8 (2 s, C=C), 152.0 and 154.6 (2 s, C=O) ppm; ^{31}P NMR (CD_3OD, 121 MHz): $\delta = 27.7$ ppm.

Diethyl 3-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo[4,5-d]pyridazin-1-yl)propylphosphonate
(**11b**, $C_{11}H_{18}N_5O_5P$)
From 0.055 g dihydrazide **10b** (0.151 mmol) compound **11b** was obtained as a white amorphous solid (0.033 g, 66 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v). M.p.: 148–150 °C; IR (KBr): $\bar{V} = 3{,}446$, 3,074, 2,991, 2,960, 1,662, 1,555, 1,462, 1,294, 1,218, 1,032, 972, 805 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz): $\delta = 1.31$ (t, $J = 6.9$ Hz, 6H, CH_3CH_2OP), 1.84–1.95 (m, 2H, PCH_2CH_2), 2.24–2.38 (m, 2H, PCH_2), 4.02–4.15 (m, 4H, CH_3CH_2OP), 4.93 (t, $J = 6.9$ Hz, 2H, CH_2N) ppm; ^{13}C NMR (CD_3OD, 75 MHz): $\delta = 16.7$ (d, $J = 5.7$ Hz, *C*COP), 22.7 (d, $J = 141.9$ Hz, PC), 23.9 (s, PC*C*), 50.5 (d, $J = 18.5$ Hz, PCC*C*), 62.6 (d, $J = 6.5$ Hz, C*C*OP), 129.1 and 139.7 (2 s, C=C), 150.5 and 152.7 (2 s, C=O) ppm; ^{31}P NMR (CD_3OD, 121 MHz): $\delta = 32.6$ ppm.

Diethyl [2-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo-[4,5-d]pyridazin-1-yl)ethoxy]methylphosphonate
(**11c**, $C_{11}H_{18}N_5O_6P$)
From 0.090 g dihydrazide **10c** (0.237 mmol) compound **11c** was obtained as a white amorphous solid (0.043 g, 52 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, v/v). M.p.: 170–172 °C; IR (KBr): $\bar{V} = 3{,}348$, 2,985, 2,930, 2,918, 1,658, 1,552, 1,420, 1,260, 1,070, 1,028, 975 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz): $\delta = 1.25$ (t, $J = 7.0$ Hz, 6H, CH_3CH_2OP), 3.89 (d, $J = 8.5$ Hz, 2H, PCH_2), 4.01–4.04 (m, 4H, CH_3CH_2OP), 4.16 (t, $J = 5.1$ Hz, 2H, OCH_2CH_2N), 5.09 (t, $J = 5.1$ Hz, 2H, OCH_2CH_2N) ppm; ^{13}C NMR (CD_3OD, 150 MHz): $\delta = 15.2$ (d, $J = 5.7$ Hz, *C*COP), 49.2 (s, CN), 62.7 (d, $J = 6.6$ Hz, C*C*OP), 63.9 (d, $J = 164.8$ Hz, PC), 70.9 (d, $J = 12.1$ Hz, PCO*C*), 129.2 and 139.3 (2 s, C=C), 149.6 and 152.3 (2 s, C=O) ppm; ^{31}P NMR (CD_3OD, 243 MHz): $\delta = 21.2$ ppm.

Diethyl 2-[2-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo[4,5-d]pyridazin-1-yl)ethoxy]ethylphosphonate
(**11d**, $C_{12}H_{20}N_5O_6P$)
From 0.165 g dihydrazide **10d** (0.419 mmol) compound **11d** was obtained as a white amorphous solid (0.087 g, 58 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, v/v) and crystallisation from ethyl acetate/chloroform. M.p.: 134–135 °C; IR (KBr): $\bar{V} = 3{,}433$, 2,974, 2,928, 2,872, 1,715, 1,645, 1,206, 1,113, 1,065, 1,026, 982, 952 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 1.26$ (t, $J = 7.2$ Hz, 6H, CH_3CH_2OP), 2.07 (dt, $J = 15.0$, 7.2 Hz, 2H, PCH_2), 3.73 (dt, $J = 13.5$, 7.5 Hz, 2H, PCH_2CH_2O), 3.96–4.10 (m, 6H, CH_3CH_2OP and OCH_2CH_2N), 5.01 (t, $J = 5.1$ Hz, 2H, OCH_2CH_2N), 10.49 (brs, 2H, NH) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 16.6$ (d, $J = 6.3$ Hz, *C*COP), 26.8 (d, $J = 138.9$ Hz, PC), 49.9 (s, OC*C*N), 62.2 (d, $J = 6.5$ Hz, C*C*OP), 65.0 (s, PC*C*O), 68.8 (s, O*C*CN), 129.4 and 139.4 (2 s, C=C), 151.1 and 152.8 (2 s, C=O) ppm; ^{31}P NMR ($CDCl_3$, 121 MHz): $\delta = 29.3$ ppm.

Diethyl 3-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo[4,5-d]pyridazin-1-yl)-2-hydroxypropylphosphonate
(**11e**, $C_{11}H_{18}N_5O_6P$)
From 0.270 g dihydrazide **10e** (0.719 mmol) compound **11e** was obtained as a white amorphous solid (0.087 g, 39 %) after chromatography on a silica gel column with chloroform/methanol (20:1, 10:1, v/v) and crystallisation from water. M.p.: 168–170 °C; IR (KBr): $\bar{V} = 3{,}476$, 2,982, 2,932, 2,867, 1,655, 1,550, 1,427, 1,405, 1,256, 1,222, 1,108, 1,054, 957 cm^{-1}; ^1H NMR (D_2O, 600 MHz):

$\delta = 1.30$ and 1.31 (2 t, $J = 6.1$ Hz, 6H, CH_3CH_2OP), 2.25 (td, $J = 16.1$, 8.8 Hz, 1H, PCH_aH_b), 2.35 (td, $J = 15.8$, 3.2 Hz, 1H, PCH_aH_b), 4.10–4.17 (m, 4H, CH_3CH_2OP), 4.52–4.59 (m, 1H, PCCH), 4.91 (dd, $J = 12.8$, 9.2 Hz, 1H, $PCCCH_aH_b$), 4.98 (dd, $J = 12.8$, 2.6 Hz, 1H, $PCCCH_aH_b$) ppm; ^{13}C NMR (D_2O, 150 MHz): $\delta = 15.6$ (d, $J = 5.7$ Hz, *C*COP), 30.0 (d, $J = 139.9$ Hz, PC), 55.7 (d, $J = 17.8$ Hz, PCC*C*), 63.5 and 63.6 (2 d, $J = 6.4$ Hz, C*C*OP), 65.4 (d, $J = 3.8$ Hz, PC*C*), 130.1 and 140.1 (2 s, C=C), 149.8 and 153.4 (2 s, C=O) ppm; ^{31}P NMR (D_2O, 243 MHz): $\delta = 30.3$ ppm.

Diethyl 3-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo-[4,5-d]pyridazin-1-yl)-1-hydroxypropylphosphonate (**11f**, $C_{11}H_{18}N_5O_6P$)

From 0.163 g dihydrazide **10f** (0.430 mmol) compound **11f** was obtained as a white amorphous solid (0.086 g, 58 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 20:1, 10:1, v/v). M.p.: 66–68 °C; IR (KBr): $\bar{V} = 3{,}362$, 2,987, 2,920, 1,666, 1,580, 1,449, 1,212, 1,050, 1,021, 970 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz): $\delta = 1.32$ (t, $J = 7.2$ Hz, 6H, CH_3CH_2OP), 2.19–2.28 (m, 1H, $PCCH_aH_b$), 2.31–2.44 (m, 1H, $PCCH_aH_b$), 3.89 (ddd, $J = 10.5$, 7.2, 3.0 Hz, 1H, PCH), 4.10–4.21 (m, 4H, CH_3CH_2OP), 4.95–5.12 (m, 2H, CH_2N) ppm; ^{13}C NMR (CD_3OD, 75 MHz): $\delta = 17.0$ (d, $J = 5.4$ Hz, *C*COP), 33.3 (d, $J = 4.0$ Hz, PC*C*), 48.1 (d, $J = 16.2$ Hz, PCC*C*), 64.2 and 64.5 (d, $J = 6.9$ Hz, C*C*OP), 65.4 (d, $J = 167.3$ Hz, PC), 130.2 and 140.8 (2 s, C=C), 148.9 and 152.2 (2 s, C=O) ppm; ^{31}P NMR (CD_3OD, 121 MHz): $\delta = 25.3$ ppm.

Diethyl 2-(4,7-dioxo-5,6-dihydro-1H-1,2,3-triazolo-[4,5-d]pyridazin-1-yl)-1-hydroxyethylphosphonate (**11g**, $C_{10}H_{16}N_5O_6P$)

From 0.100 g dihydrazide **10g** (0.274 mmol) compound **11g** was obtained as a colourless oil (0.036 g, 40 %) after chromatography on a silica gel column with chloroform/methanol (50:1, 10:1, v/v). IR (film): $\bar{V} = 3{,}362$, 2,988, 2,971, 1,667, 1,580, 1,212, 1,021 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz): $\delta = 1.38$ and 1.39 (2 t, $J = 7.0$ Hz, 6H, CH_3CH_2OP), 4.23–4.29 (m, 4H, CH_3CH_2OP), 4.64–4.69 (m, 1H, PCH), 5.09–5.15 (m, 2H, CH_2N) ppm; ^{13}C NMR (CD_3OD, 150 MHz): $\delta = 15.3$ and 15.4 (d, $J = 5.2$ Hz, *C*COP), 51.3 (d, $J = 11.6$ Hz, PC*C*N), 63.3 and 63.5 (2 d, $J = 6.8$ Hz, COP), 66.4 (d, $J = 166.2$ Hz, PC), 129.2 and 139.4 (2 s, C=C), 150.1 and 152.4 (2 s, C=O) ppm; ^{31}P NMR (CD_3OD, 243 MHz): $\delta = 20.5$ ppm.

Assays for antiviral activity other than HIV

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) strain G, cytomegalovirus (strains AD-169 and Davis), varicella-zoster virus (VZV) (strains OKA and YS), vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), coxsackie b4 virus, parainfluenza 3, influenza virus A (subtypes H1N1, H3N2), influenza virus B, reovirus-1, Sindbis virus and Punta Toro virus. The antiviral 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 cervix carcinoma cells (HeLa) or 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 20 or 100 plaque forming units (PFU) (for VZV and CMV, respectively) 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 %.

Anti-HIV activity assays

Inhibition of HIV-1(III_B)- and HIV-2(ROD)-induced cytopathicity in CEM cell cultures was measured in microtiter 96-well plates containing 3×10^5 CEM cells/cm^3 infected with 100 CCID50 of HIV per cm^3 and containing appropriate dilutions of the test compounds. After 4–5 days of incubation at 37 °C in a CO_2-controlled humidified atmosphere, CEM giant cell (syncytium) formation was examined microscopically. The EC_{50} (50 % effective concentration) was defined as the compound concentration required to inhibit HIV-induced giant cell formation by 50 %.

Cytostatic activity assays

All assays were performed in 96-well microtiter plates. To each well were added (5–7.5) $\times 10^4$ tumour 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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