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Original article

Design, synthesis, antiviral and cytostatic evaluation of novel isoxazolidine nucleotide analogues with a 1,2,3-triazole linker

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ABSTRACT

Azidation ($TMSN_3$, $SnCl_4$) of a 9:1 mixture of *trans*- and *cis*-5-acetoxy-2-methylisoxazolidin-3-yl-3-phosphonates at the anomeric carbon atom led to the formation of the equimolar mixture of *cis*- and *trans*-5-azido-2-methylisoxazolidin-3-yl-3-phosphonates, which were efficiently separated. The 1,3-dipolar cycloaddition of pure *trans*- and *cis*-5-azidoisoxazolidin-3-yl-3-phosphonates with selected alkynes gave the respective nucleoside mimetics containing a 1,2,3-triazole linker. The (1,2,3-triazolyl) isoxazolidine phosphonates obtained herein were evaluated in vitro for activity against a variety of DNA and RNA viruses. None of the compounds were endowed with antiviral activity at subtoxic concentrations. Compounds **15f**–**j** and **16f**–**j** were cytostatic in the higher micromolar range.

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1. Introduction

An intensive search for drugs effective in chemotherapy of viral infections and/or various cancers has been under way for decades. The idea of structural modifications of nucleosides and nucleotides gained interest fairly early and appeared fruitful supplying a wide variety of marketed compounds such as ribavirin, zidovudine, adefovir and many others (Fig. 1). In general, the modifications included replacements of the phosphate P(O)–O–C bond for the non-hydrolysable phosphonate P(O)–C linkage, a furanose ring for other heterocyclic or even acyclic fragments or natural nucleobases for unnatural, substituted heteroaromatic or even homoaromatic systems. Several functionalities have been tried as linkers and aliphatic chains as well as 3- to 6-membered aliphatic and aromatic homo- and heterocyclic systems exemplify a long list of examples [1–4].

Among commonly applied linkers a 1,2,3-triazole ring is of special interest since it is extremely easily to prepare by a 1,3-dipolar cycloaddition and biological activity of many 1,2,3-triazole derivatives has been recognised (Fig. 2). The idea of retaining the natural nucleobases with simultaneous introduction of the functionalised 1,2,3-triazole unit into a furanose ring led to compounds **1** and **2** which showed an antiviral activity [5,6]. On the other hand 2′,3′-diethanethio-2′,3′,5′-trideoxy-5′-triazolonucleosides **3** displayed antitumour activity [7]. Recently, Kim et al. designed novel 1,2,3-triazole-appended C5-modified nucleosides **4** and their anticancer activity was demonstrated [8,9]. Various acyclonucleosides [10,11] and acyclonucleoside phosphonates [12] containing a 1,2,3-triazole unit were obtained from *N*-propargyl nucleobases and their antiviral activity was evaluated. Among them, pyrimidine- and purine-containing derivatives **5** and **6** appeared to be the most potent so far and exhibited anti-HCV activity (IC_{50} values of 16 μM) without any cytotoxicity at a concentration up to 100 μM. On the basis of a similar idea, novel 1,2,3-triazole nucleosides **7** linked to DNA nucleobases as a recognition element have been synthesised and their antiviral activity was evaluated [13].

Recently, we succeeded in the synthesis of *N*-substituted *C*-phosphorylated nitrone **8** [14] and its application in the 1,3-dipolar cycloaddition with vinyl acetate was described [15]. Furthermore, the usefulness of C5-acetoxyisoxazolidines **9** as precursors in the synthesis of isoxazolidine nucleoside analogues **10** has recently been demonstrated (Scheme 1) [15,16].

In continuation of these efforts, a novel class of 1,2,3-triazole-containing isoxazolidine nucleosides **11** (Scheme 2) was designed. Although glycosyl as well as furanosyl azides have commonly

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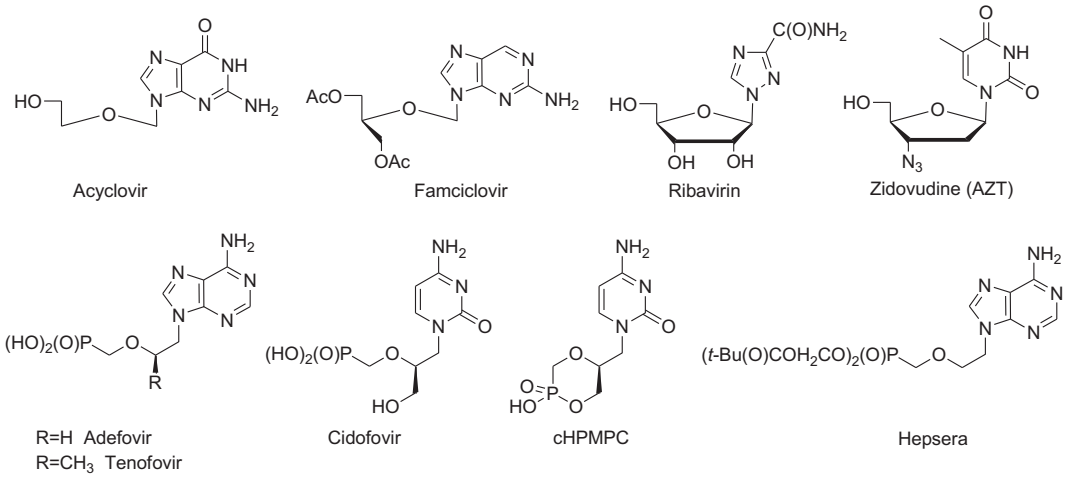


Fig. 1. Examples of marketed nucleoside and nucleotide analogues.

been used in a direct synthesis of substituted 1,2,3-triazoles [13,17–20], to the best of our knowledge isoxazolidine analogues of 1-azidofuranose remain unknown. Herein, we wish to report the efficient synthesis of 5-azidoisoxazolidines **12** from 5-acetoxyisoxazolidines **9** and further transformation of the azides **12** into the respective nucleoside mimetics **11**. The aim of this study was the preparation of a small library of nucleotide analogues **11** which was based on the idea of replacing of a furanose ring by an isoxazolidine system and introduction of a 1,2,3-triazole linker between isoxazolidine and the respective purine or pyrimidine nucleobase or other aromatic function as a nucleobase replacer (Scheme 2).

2. Results and discussion

2.1. Chemistry

A 9:1 mixture of *trans*- and *cis*-5-acetoxyisoxazolidines **9a** and **9b** was obtained from *N*-methyl-*C*-phosphorylated nitrone **8** and vinyl acetate as previously described [21]. This mixture was immediately treated with $TMSN_3$ in the presence of $SnCl_4$ as a catalyst to give 5-azidoisoxazolidines **12** and **13** in a ~1:1 ratio (Scheme 3) [22]. They were cleanly separated on a silica gel column into the less polar diastereoisomer **13** (50%) and more polar **12** (46%).

Since almost an equimolar mixture of both anomeric azides **12** and **13** was detected in a crude product, the replacement of the acetoxy group at C5 with an azide function occurred via the formation of the respective oxonium intermediate. Under these circumstances the nucleophile can attack both faces of the isoxazolidinium ion to form the azides **12** and **13** as a ca. 1:1 anomeric mixture.

The relative configurations in the diastereoisomeric isoxazolidines **12** and **13** were established based on ^1H and ^{13}C NMR spectroscopic data [23,24]. Analysis of vicinal coupling constants extracted from the spectra of the isomer **12** [$J(H_3-H_{4\alpha}) = 6.8$ Hz, $J(H_3-H_{4\beta}) = 10.8$ Hz, $J(H_{4\alpha}-P) = 4.0$ Hz, $J(H_{4\beta}-P) = 17.0$ Hz, $J(H_{4\alpha}-H_5) = 0.8$ Hz, $J(H_{4\beta}-H_5) = 5.3$ Hz and $J(CCCP) = 10.3$ Hz] shows that the isoxazolidine ring exists in the single ^4E conformation. In this conformation the $P(O)(OEt)_2$ and

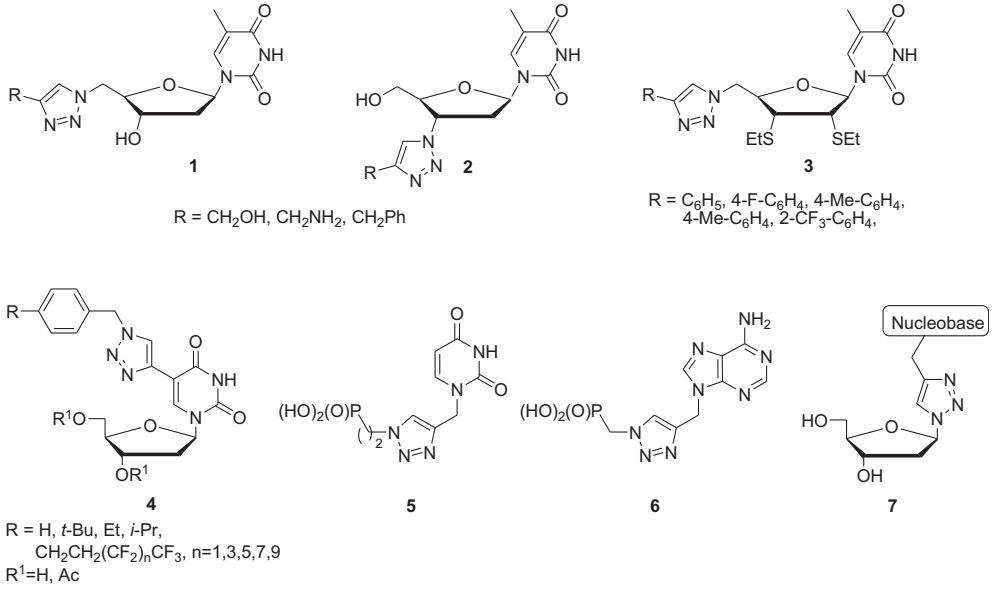
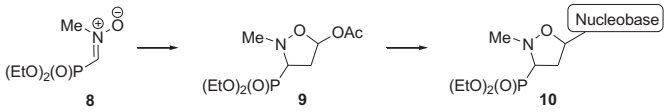
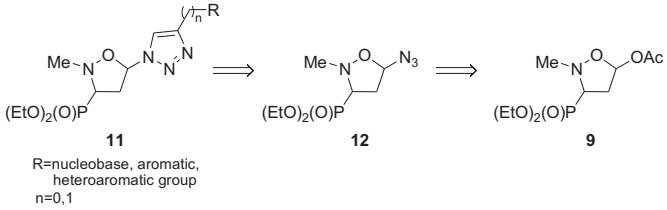


Fig. 2. Biologically active nucleoside mimetics having functionalised 1,2,3-triazole unit.



Scheme 1. Transformation of the *C*-phosphorylated nitrone **8** into isoxazolidine nucleoside analogues **10**.



Scheme 2. Retrosynthesis of nucleoside mimetics **11**.

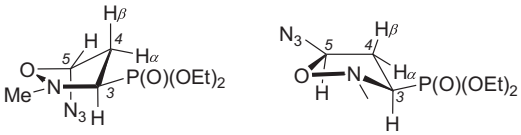
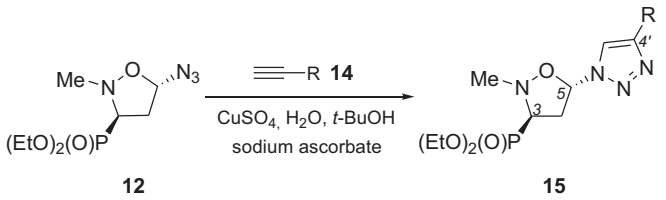


Fig. 3. The preferred conformations of 5-azidoisoxazolidines **12** (left) and **13** (right).



Scheme 4. 1,3-Dipolar cycloadditions of the 5-azidoisoxazolidines **12** with propargylated nucleobases and selected ethynyl aryls.

azido groups occupy the pseudoequatorial and pseudoaxial positions, respectively (Fig. 3) and thus reveal the *trans* relationship of H_3 and H_5. On the other hand, from the NMR spectra of the isoxazolidine **13** the following couplings were calculated: $J(H_3–H_{4\alpha}) = 9.3$ Hz, $J(H_3–H_{4\beta}) = 9.1$ Hz, $J(H_{4\alpha}–P) = 4.8$ Hz, $J(H_{4\beta}–P) = 17.0$ Hz, $J(H_{4\alpha}–H_5) = 7.5$ Hz, $J(H_{4\beta}–H_5) = 2.4$ Hz and $J(CCCP) = 10.3$ Hz. On the basis of these values, a preferred E_3 conformation having the diethoxyphosphoryl and azido groups in equatorial and pseudoequatorial positions was established (Fig. 3). At the same time, the *cis* relationship of the substituents at C3 and C5 in **13** was unambiguously proved.

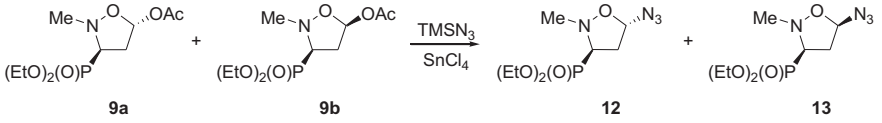
Next the 1,3-dipolar cycloadditions of the 5-azidoisoxazolidines **12** and **13** with propargylated nucleobases and selected ethynyl aryls were examined (Scheme 4, Table 1). Because we were interested in the formation of single regioisomers of C4′-substituted 1,2,3-triazoles, all reactions were carried out with equimolar amounts of the respective dipolarophiles **14** using Cu(I) as a catalyst, according to the procedure described by Sharpless [25,26]. Indeed, 5-azidoisoxazolidin-3-yl-3-phosphonate *trans*-**12** was cleanly transformed into C4′-substituted 1,2,3-triazoles *trans*-**15** in good to excellent yields, whereas from *cis*-**13** and the respective alkynes **14**, isoxazolidines *cis*-**16** were produced exclusively. In all cycloadditions no epimerization at C5 in the isoxazolidine ring was observed.

Additional evidence supporting our configurational assignments in the azidoisoxazolidines **12** and **13** comes from the comparison of the ^1H and ^{13}C NMR spectroscopic data of the respective (1,2,3-triazol-1-yl)isoxazolidines **15** and **16**. Based on the vicinal couplings [$J(H_3–H_{4\alpha}) = 6.8–6.9$ Hz, $J(H_3–H_{4\beta}) = 10.5–11.1$ Hz, $J(H_{4\alpha}–P) = 4.5–6.8$ Hz, $J(H_{4\beta}–P) = 16.8–71.7$ Hz, $J(H_{4\alpha}–H_5) = 1.2–1.8$ Hz, $J(H_{4\beta}–H_5) = 6.6–6.9$ Hz and $J(CCCP) = 9.4–10.5$ Hz] observed in the ^1H and ^{13}C NMR spectra of the isoxazolidines **15** it was concluded that they exist in the ^4E conformation, the same as observed for the azidoisoxazolidine **12** (Fig. 4). On the other hand, the preferred E_3 conformation for all (1,2,3-triazol-1-yl)isoxazolidines **16** was established based on the vicinal coupling constants derived from their NMR spectra [$J(H_3–H_{4\alpha}) = 9.0–9.9$ Hz, $J(H_3–H_{4\beta}) = 8.1–9.0$ Hz, $J(H_{4\alpha}–P) = 5.4–6.0$ Hz, $J(H_{4\beta}–P) = 17.2–18.0$ Hz, $J(H_{4\alpha}–H_5) = 7.8–7.9$ Hz, $J(H_{4\beta}–H_5) = 2.0–2.4$ Hz and $J(CCCP) = 8.6–9.7$ Hz] (Fig. 4).

2.2. Antiviral and cytostatic evaluation

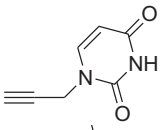
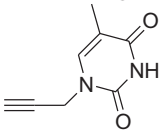
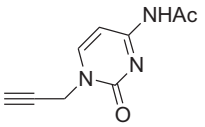
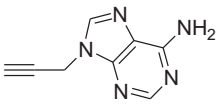
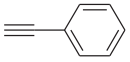
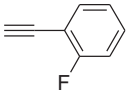
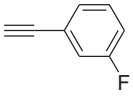
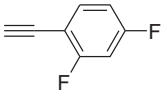
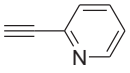
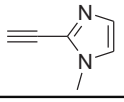
All (1,2,3-triazol-1-yl)isoxazolidines *trans*-**15** and *cis*-**16** were evaluated for inhibitory activity against a wide variety of DNA and RNA viruses, using the following cell-based assays: (a) human embryonic lung (HEL) cells: herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), herpes simplex virus-1 (TK^- KOS ACV^r), vaccinia virus and vesicular stomatitis virus, cytomegalovirus (AD-169 strain and Davis strain) and varicella–zoster virus (TK^+ VZV strain Oka, and TK^- VZV strain 07/1); (b) Vero cell cultures: parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus; (c) HeLa cell cultures: vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus; (d) MDCK cell cultures: influenza A virus (H1N1 and H3N2) and influenza B virus; (e) CrFK cell cultures: feline herpes virus (FHV) and feline corona virus (FIPV) and (f) CEM cell cultures: human immunodeficiency virus type 1 (HIV-1) and HIV-2. Ganciclovir, cidofovir, acyclovir, brivudin, (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA], oseltamivir and ribavirin 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) by 50% or to reduce virus-induced cytopathogenicity by 50% (other viruses). Unfortunately, no inhibitory activity against any virus was detected for the evaluated compounds at 250 μM.

The cytotoxicity of the tested compounds towards the uninfected host cells was defined as the minimum compound concentration (MCC) that caused a microscopically detectable alteration of normal cell morphology. The 50% cytostatic concentration (CC_{50}), causing a 50% decrease in cell proliferation was determined against murine leukaemia L1210, human lymphocyte CEM, human cervix carcinoma HeLa and human lung fibroblast HEL cells. None of the tested compounds affected cell morphology of HEL, HeLa, Vero, MDCK and CrFK cells at concentrations up to 100 μM. However, several compounds (*trans*-**15f–j** and *cis*-**16f–j**) were able to inhibit



Scheme 3. Synthesis of 5-azidoisoxazolidines **12** and **13**.

Table 1
Isoxazolidines **15** and **16** produced via Scheme 4.

Entry	Alkyne	Azide	Product; yield [%]
a		12 13	**15a**; 85 **16a**; 91
b		12 13	**15b**; 77 **16b**; 98
c		12 13	**15c**; 77 **16c**; 93
d		12 13	**15d**; 82 **16d**; 83
e		12 13	**15e**; 85 **16e**; 98
f		12 13	**15f**; 87 **16f**; 89
g		12 13	**15g**; 82 **16g**; 98
h		12 13	**15h**; 81 **16h**; 98
i		12 13	**15i**; 85 **16i**; 94
j		12 13	**15j**; 76 **16j**; 87
k		12 13	**15k**; 61 **16k**; 65

cell proliferation by 50% (CC_{50}) at concentrations ranging from 40 to 78 μM for HEL cells, and from 120 to 250 μM for L1210, CEM and HeLa cells (Table 2).

Active as cell proliferation inhibitors isomeric isoxazolidines **15**/**16f**–**j** exhibit several common structural features such as a six-membered aromatic ring (benzene or pyridine) and substitution

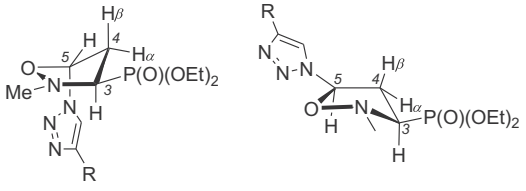


Fig. 4. The preferred conformations of (1,2,3-triazol-1-yl)isoxazolidines **15** (left) and **16** (right).

Table 2
Cytostatic activity of compounds **15a**–**k** and **16a**–**k**.

Compound	CC_{50}[a] (μM)			
	L1210	CEM	HeLa	HEL
15a	>250	>250	≥250	>100
15b	≥250	>250	>250	>100
15c	≥250	>250	>250	>100
15d	140	>250	>250	>100
15e	191	>250	>250	>100
15f	>250	>250	>250	40
15g	120	>250	202	73
15h	≥250	>250	≥250	40
15i	131	≥250	162	59
15j	≥250	>250	>250	62
15k	≥250	>250	>250	>100
16a	235	>250	>250	>100
16b	280	>250	>250	>100
16c	>250	>250	>250	>100
16d	147	>250	≥250	>100
16e	166	>250	>250	>100
16f	172	>250	>250	54
16g	122	>250	247	41
16h	104	>250	212	43
16i	124	≥250	135	42
16j	206	>250	>250	78
16k	≥250	>250	>250	>100

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

with fluorine. The aromatic fragments of these compounds fulfil the requirements to be considered as "nonpolar nucleoside isosteres" [27].

3. Conclusions

Azidation of a 9:1 mixture of *trans*- and *cis*-5-acetoxy-2-methylisoxazolidin-3-yl-3-phosphonates with trimethylsilyl azide in the presence of $SnCl_4$ gave an easily separable 1:1 mixture of diethyl 5-azido-2-methylisoxazolidin-3-yl-3-phosphonates *trans*-**12** and *cis*-**13**. A series of 1,2,3-triazole-containing isoxazolidin-3-yl-3-phosphonates was obtained in good to excellent yields from azides *trans*-**12** and *cis*-**13** via 1,3-dipolar cycloaddition with the respective propargylated nucleobases and ethynyl aryls.

The relative configuration in the 5-azidoisoxazolidines *trans*-**12** and *cis*-**13** as well as the 1,2,3-triazolyl cycloadducts *trans*-**15** and *cis*-**16** was established based on the conformational analysis using vicinal coupling constants extracted from ^1H and ^{13}C NMR spectra.

All synthesised *trans*- and *cis*-(1,2,3-triazolyl)isoxazolidinephosphonates *trans*-**15** and *cis*-**16** were evaluated against a broad-spectrum of viruses but found not active at 250 μM. The unsubstituted and fluoro-substituted phenyl derivatives proved slightly cytostatic (middle to higher micromolar range: 40–250 μM).

4. Experimental section

4.1. Chemistry

The ^1H NMR spectra were taken in $CDCl_3$ or C_6D_6 on the Varian Mercury-300 spectrometer with TMS as an internal standard. The ^{13}C NMR spectra were recorded for $CDCl_3$ solutions on a Varian Mercury-300 machine at 75.5 MHz. The ^{31}P NMR spectra were taken in $CDCl_3$ or C_6D_6 on Varian Mercury-300 at 121.5 MHz.

IR spectra 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 Perkin–Elmer PE 2400 CHNS analyser.

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}.

Starting Materials. All solvents were dried according to the literature methods. The nitrone **8** and isoxazolidines **9a** and **9b** were previously reported [21].

*4.1.1. Synthesis of 5-azidoisoxazolidines **12** and **13***

To a solution of a 9:1 mixture of 5-acetoxyisoxazolidines **10** and **11** (3.384 g, 12.03 mmol) in dry methylene chloride (40 mL) trimethylsilyl azide (3.97 mL, 30.08 mmol) was added at 0 °C under argon followed by $SnCl_4$ (0.704 mL, 6.02 mmol). The reaction mixture was stirred at this temperature for 12 h and then saturated aqueous $NaHCO_3$ (70 mL) was added at 0 °C. After stirring for 30 min at this temperature organic phase was separated, and an aqueous layer was extracted with methylene chloride (4 × 20 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2 × 20 mL), dried over $MgSO_4$, filtered and solvent was removed under reduced pressure. Purification on a silica gel column with toluene:isopropanol (200:1 and then 100:1, v/v) gave azidoisoxazolidine **13** (1.587 g, 50%) and azidoisoxazolidine **12** (1.468 g, 46%), both as colourless oils.

*4.1.1.1. Diethyl 5-azido-2-methylisoxazolidin-3-yl-3-phosphonate **12***. IR (film, cm^{-1}) ν_{max}: 3473, 2962, 2114, 1443, 1260, 1240, 1100, 1054, 1026, 964, 800; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (t, 6H, J = 6.9 Hz, 2× $POCH_2CH_3$), 2.47 (dddd, 1H, J = 12.6, 6.8, 4.0, 0.8 Hz, *Hb*C4), 2.63 (dddd, 1H, J = 17.0, 12.6, 10.8, 5.3 Hz, *Ha*C4), 3.04 (d, 3H, J = 1.0 Hz, CH_3–N), 3.27 (ddd, 1H, J = 10.8 Hz, 6.8, 2.4 Hz, *H*C3), 4.15–4.30 (m, 4H, 2× $POCH_2CH_3$), 5.58 (dd, 1H, J = 5.3, 0.8 Hz, *H*C5); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.6 (d, J = 6.2 Hz), 40.2 (d, $^2J_{PCC}$ = 1.9 Hz, C4), 49.5 (d, J = 6.9 Hz, CH_3–N), 62.2 (d, $^1J_{PC}$ = 175.2 Hz, C3), 62.8 (d, J = 7.0 Hz, C–O–P), 63.4 (d, J = 6.9 Hz, C–O–P), 89.8 (d, $^3J_{PCCC}$ = 10.3 Hz, C5); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 22.39. Anal. Calcd for $C_8H_{17}N_4O_4P$: C, 36.37; H, 6.49; N, 21.20. Found: C, 36.25; H, 6.59; N, 20.98.

*4.1.1.2. Diethyl 5-azido-2-methylisoxazolidin-3-yl-3-phosphonate **13***. IR (film, cm^{-1}) ν_{max}: 3580, 2981, 1442, 1257, 1053, 1027, 968; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.36 (t, 3H, J = 7.1 Hz, $POCH_2CH_3$), 1.38 (t, 3H, J = 7.1 Hz, $POCH_2CH_3$), 2.40–2.60 (m, 1H, *Hb*C4), 2.80–2.90 (m, 1H, *Ha*C4), 2.85–2.98 (m, 1H, *H*C3), 2.96 (s, 3H, CH_3–N), 4.15–4.30 (m, 4H, 2× $POCH_2CH_3$), 5.41 (very br d, 1H, *H*C5); ^{1}H NMR (300 MHz, C_6D_6) δ: 0.99 (t, 3H, J = 7.1 Hz, $POCH_2CH_3$), 1.11 (t, 3H, J = 7.1 Hz, $POCH_2CH_3$), 2.16 (dddd, 1H, J = 12.8, 9.3, 7.5, 4.8 Hz, *Hb*C4), 2.42 (dddd, 1H, J = 17.0, 12.8, 9.1, 2.4 Hz, *Ha*C4), 2.55 (ddd, 1H, J = 9.3, 9.1, 2.0 Hz, *H*C3), 2.94 (d, 3H, J = 1.2 Hz, CH_3–N), 3.80–3.98 (m, 2H, $POCH_2CH_3$), 4.00–4.23 (m, 2H, $POCH_2CH_3$), 4.64 (dd, 1H, J = 7.5, 2.4 Hz, *H*C5); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.6 (d, J = 6.4 Hz), 16.7 (d, J = 6.0 Hz), 40.5 (d, $^2J_{PCC}$ = 2.8 Hz, C4), 45.8 (d, J = 2.3 Hz, CH_3–N), 62.6 (d, J = 6.5 Hz, C–O–P), 63.7 (d, J = 6.5 Hz, C–O–P), 63.9 (d, $^1J_{PC}$ = 164.8 Hz, C3), 88.0 (d, $^3J_{PCCC}$ = 10.3 Hz, C5); ^{31}P NMR (121.5 MHz, C_6D_6) δ: 21.03; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.38. Anal. Calcd for $C_8H_{17}N_4O_4P$: C, 36.37; H, 6.49; N, 21.20. Found: C, 36.45; H, 6.69; N, 21.43.

*4.1.2. General procedure for the synthesis of (1,2,3-triazolyl) isoxazolidines **15** and **16***

To a solution of the azidoisoxazolidine **12** or **13** (1.00 mmol) in *tert*-butanol (0.5 mL), $CuSO_4 \cdot 4H_2O$ (0.10 mmol) in water (1 mL) was added followed by sodium ascorbate (0.20 mmol) and the respective alkyne **14** (1.00 mmol). The reaction mixture was stirred at room temperature for 48 h, concentrated and co-evaporated with ethanol (3 × 10 mL). The residue was dissolved in chloroform (10 mL), dried over $MgSO_4$, filtered through a pad of Celite and the solution was evaporated under reduced pressure. The crude product was purified on a silica gel column with chloroform:methanol (from 100:1 to 20:1, v/v) as eluent.

*4.1.2.1. Diethyl 5-(4-((3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl) methyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate **15a***. From azidoisoxazolidine **12** (0.166 g, 0.628 mmol) and N^1-propargyluracil (0.094 g, 0.628 mmol), phosphonate **15a** (0.222 g, 85%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). IR (film, cm^{-1}) ν_{max}: 3485, 3152, 2993, 1680, 1468, 1441, 1250, 1220, 1050, 1026, 971, 753; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (t, J = 7.1 Hz, 3H), 1.36 (t, J = 7.1 Hz, 3H), 2.88 (s, 3H), 3.00–3.25 (m, 1H), 3.40–3.55 (m, 1H), 3.58–3.65 (m, 1H), 4.15–4.30 (m, 4H), 4.95 (AB, 1H, J_{AB} = 15.5 Hz), 5.03 (AB, 1H, J_{AB} = 15.5 Hz), 5.70 (d, 1H, J = 7.9 Hz), 6.15 (d, 1H, J = 6.1 Hz), 7.51 (d, 1H, J = 7.9 Hz), 7.94 (s, 1H), 9.20 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.5 (d, J = 5.7 Hz), 16.6 (d, J = 5.7 Hz), 38.8, 43.1, 47.8 (d, J = 5.4 Hz), 62.5 (d, $^1J_{PC}$ = 171.5 Hz, C3), 63.0 (d, J = 6.9 Hz, C–O–P), 63.4 (d, J = 6.6 Hz, C–O–P), 86.3 (d, $^3J_{PCCC}$ = 9.7 Hz, C5), 102.6, 123.9, 142.1, 144.4, 151.0, 164.1; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.33. Anal. Calcd for $C_{15}H_{23}N_6O_6P$: C, 43.48; H, 5.59; N, 20.28. Found: C, 43.56; H, 5.76; N, 20.38.

*4.1.2.2. Diethyl 5-(4-((3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl) methyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate **16a***. From azidoisoxazolidine **13** (0.164 g, 0.621 mmol) and N^1-propargyluracil (0.093 g, 0.621 mmol), phosphonate **16a** (0.233 g, 91%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (20:1, v/v). IR (film, cm^{-1}) ν_{max}: 3464, 3155, 2996, 1706, 1690, 1457, 1390, 1235, 1110, 1044, 1020, 959, 755; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (t, 3H, J = 7.1 Hz), 1.34 (t, 3H, J = 7.1 Hz), 2.96 (d, 3H, J = 0,8 Hz), 3.00–3.20 (m, 2H), 3.20–3.40 (m, 1H), 4.00–4.25 (m, 4H), 5.00 (s, 2H), 5.70 (d, J = 7.9 Hz, 1H), 6.37 (d, J = 6.0 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 8.20 (s, 1H), 8.87 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.4 (d, J = 5.7 Hz), 40.2, 42.6, 45.6, 62.9 (d, J = 6.9 Hz, C–O–P), 63.4 (d, $^1J_{PC}$ = 166.6 Hz, C3), 63.5 (d, J = 6.6 Hz, C–O–P), 85.4 (d, $^3J_{PCCC}$ = 9.2 Hz, C5), 102.4, 122.3, 141.9, 144.4, 150.8, 164.2; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.36. Anal. Calcd for $C_{15}H_{23}N_6O_6P$: C, 43.48; H, 5.59; N, 20.28. Found: C, 43.46; H, 5.53; N, 20.44.

*4.1.2.3. Diethyl 5-(4-((3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate **15b***. From azidoisoxazolidine **12** (0.165 g, 0.628 mmol) and N^1-propargylthymine (0.103 g, 0.628 mmol), phosphonate **15b** (0.206 g, 77%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). IR (film, cm^{-1}) ν_{max}: 3454, 3143, 2986, 1689, 1458, 1390, 1320, 1232, 1067, 1026, 973, 809, 783; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (t, 3H, J = 7.2 Hz), 1.36 (t, 3H, J = 7.2 Hz), 1.90 (s, 3H), 2.89 (s, 3H), 3.14 (dddd, 1H, J = 17.7, 12.9, 10.2, 6.6 Hz), 3.39–3.48 (m, 1H), 3.60 (ddd, 1H, J = 10.2, 7.2, 3.3 Hz), 4.10–4.35 (m, 4H), 4.91 (AB, 1H, J_{AB} = 15.0 Hz), 5.02 (AB, 1H, J_{AB} = 15.0 Hz), 6.15 (d, 1H, J = 6.7 Hz), 7.33 (s, 1H), 7.91 (s, 1H), 8.77 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 12.4, 16.4 (d, J = 5.7 Hz), 16.5 (d, J = 5.7 Hz), 38.8, 47.7 (d, J = 5.7 Hz), 50.2, 62.5 (d, $^1J_{PC}$ = 171.5 Hz, C3), 63.0 (d, J = 6.9 Hz, C–O–P), 63.4 (d, J = 6.6 Hz, C–O–P), 86.3 (d, $^3J_{PCCC}$ = 10.0 Hz, C5), 111.1, 123.9, 140.2, 142.4, 151.2, 164.5; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.23. Anal. Calcd for $C_{16}H_{25}N_6O_6P$: C, 44.86; H, 5.88; N, 19.62. Found: C, 45.07; H, 5.70; N, 19.83.

4.1.2.4. Diethyl 5-(4-((3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2H)-yl)methyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-

3-phosphonate ***16b***. From azidoisoxazolidine **13** (0.150 g, 0.568 mmol) and N^1-propargylthymine (0.093 g, 0.568 mmol), phosphonate **16b** (0.243 g, 98%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). IR (film, cm^{-1}) ν_{max}: 3484, 3156, 3058, 2984, 1690, 1679, 1468, 1367, 1236, 1110, 1050, 1024, 972, 786, 734; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.30 (t, 3H, $J = 7.1$ Hz), 1.34 (t, 3H, $J = 7.1$ Hz), 1.90 (s, 3H), 2.96 (s, 3H), 2.95–3.20 (m, 2H), 3.20–3.40 (m, 1H), 4.05–4.25 (m, 4H), 4.98 (s, 2H), 6.36 (d, 1H, $J = 6.3$ Hz), 7.36 (s, 1H), 8.19 (s, 1H), 8.42 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 12.4, 16.5 (d, $J = 5.7$ Hz), 40.2, 42.4, 45.6, 62.9 (d, $J = 6.9$ Hz, C–O–P), 63.4 (d, $^1J_{PC} = 166.3$ Hz, C3), 63.5 (d, $J = 6.6$ Hz, C–O–P), 85.4 (d, $^3J_{PCCC} = 9.2$ Hz, C5), 110.9, 122.2, 140.2, 142.2, 150.9, 164.6; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.32. Anal. Calcd for $C_{16}H_{25}N_6O_6P$: C, 44.86; H, 5.88; N, 19.62. Found: C, 44.77; H, 5.90; N, 19.60.

4.1.2.5. Diethyl 5-(4-((acetylamino)-2-oxopyrimidin-1(2H)-yl) methyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***15c***. From azidoisoxazolidine **12** (0.169 g, 0.640 mmol) and *N*-(1,2-dihydro-2-oxo-1-(prop-2-ynyl)pyrimidin-4-yl)acetamide (0.122 g, 0.640 mmol), phosphonate **15c** (0.224 g, 77%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). IR (film, cm^{-1}) ν_{max}: 3446, 2928, 1654, 1559, 1490, 1374, 1307, 1221, 1024, 962, 792; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.35 (t, $J = 7.2$ Hz, 3H), 1.36 (t, $J = 7.2$ Hz, 3H), 2.25 (s, 3H), 2.87 (s, 3H), 3.14 (dddd, 1H, $J = 17.4$, 13.3, 10.9, 6.7 Hz), 3.40 (dddd, 1H, $J = 13.3$, 6.9, 5.0, 1.8 Hz), 3.63 (ddd, 1H, $J = 10.5$, 6.9, 3.0 Hz), 4.15–4.27 (m, 4H), 5.10 (AB, 1H, $J_{AB} = 14.5$ Hz), 5.20 (AB, 1H, $J_{AB} = 14.5$ Hz), 6.20 (dd, 1H, $J = 6.7$, 1.8 Hz), 7.42 (d, 1H, $J = 7.1$ Hz), 7.96 (d, 1H, $J = 7.1$ Hz), 8.09 (s, 1H), 9.40 (br s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.6 (d, $J = 3.7$ Hz), 24.9, 38.9, 45.1, 47.8 (d, $J = 6.0$ Hz, *C*H_3–N), 62.7 (d, $^1J_{PC} = 171.5$ Hz, C3), 63.1 (d, $J = 6.9$ Hz, C–O–P), 63.4 (d, $J = 6.6$ Hz, C–O–P), 86.4 (d, $^3J_{PCCC} = 9.4$ Hz, C5), 97.3, 124.3, 142.1, 148.9, 155.8, 163.0, 171.1; ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ: 20.82. Anal. Calcd for $C_{17}H_{26}N_7O_6P$: C, 44.84; H, 5.75; N, 21.53. Found: C, 44.93; H, 5.76; N, 21.67.

4.1.2.6. Diethyl 5-(4-((acetylamino)-2-oxopyrimidin-1(2H)-yl) methyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***16c***. From azidoisoxazolidine **13** (0.203 g, 0.768 mmol) and *N*-(1,2-dihydro-2-oxo-1-(prop-2-ynyl)pyrimidin-4-yl)acetamide (0.147 g, 0.768 mmol), phosphonate **16c** (0.325 g, 93%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). IR (film, cm^{-1}) ν_{max}: 3446, 2982, 2924, 1718, 1684, 1662, 1600, 1497, 1374, 1308, 1240, 1050, 1025, 952, 797; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.31 (t, 3H, $J = 7.1$ Hz,), 1.34 (t, 3H, $J = 7.1$ Hz,), 2.24 (s, 3H), 2.96 (d, 3H, $J = 1.0$ Hz,), 3.00–3.10 (m, 2H), 3.10–3.20 (m, 1H), 4.00–4.20 (m, 4H), 5.17 (s, 2H), 6.35 (dd, 1H, $J = 7.3$, 2.2 Hz), 7.38 (d, 1H, $J = 7.3$ Hz), 7.96 (d, 1H, $J = 7.3$ Hz), 8.25 (s, 1H), 9.00 (br s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.4 (d, $J = 5.7$ Hz), 24.8, 40.0, 44.8, 45.5 (s, *C*H_3–N), 62.9 (d, $J = 6.9$ Hz, C–O–P), 63.4 (d, $^1J_{PC} = 166.3$ Hz, C3), 63.5 (d, $J = 6.6$ Hz, C–O–P), 85.4 (d, $^3J_{PCCC} = 9.2$ Hz, C5), 97.0, 122.6, 141.8, 148.9, 155.6, 162.9, 171.2; ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ: 20.53. Anal. Calcd for $C_{17}H_{26}N_7O_6P$: C, 44.84; H, 5.75; N, 21.53. Found: C, 44.79; H, 5.64; N, 21.60.

4.1.2.7. Diethyl 5-(4-((6-amino-9H-purin-9-yl)methyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***15d***. From azidoisoxazolidine **12** (0.150 g, 0.568 mmol) and N^9-(propargyl) adenine (0.098 g, 0.568 mmol), phosphonate **15d** (0.204 g, 82%) was obtained as a white amorphous solid after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). M.p.: 100–102 °C. IR (KBr, cm^{-1}) ν_{max}: 3445, 3301, 3137, 2984, 1665, 1600, 1474, 1418, 1322, 1249, 1050, 1023, 969, 776; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.33 (t, 3H, $J = 7.0$ Hz), 1.34 (t, 3H, $J = 7.0$ Hz), 2.82 (s, 3H), 3.10 (dddd, 1H, $J = 17.4$, 12.6, 10.2, 6.6 Hz), 3.60–4.30 (m, 2H), 4.10–4.30 (m, 4H), 5.50 (s, 2H), 6.01 (s, 2H), 6.08 (d, 1H, $J = 6.6$ Hz, *H*-C5), 7.88, (s, 1H), 8.00 (s, 1H), 8.35 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.5 (d, $J = 5.4$ Hz), 16.6 (d, $J = 5.7$ Hz), 38.5, 38.7, 47.9 (d, $J = 5.7$ Hz), 62.5 (d, $^1J_{PC} = 172.0$ Hz, C3), 63.0 (d, $J = 6.9$ Hz, C–O–P), 63.4 (d, $J = 6.6$ Hz, C–O–P), 86.4 (d, $^3J_{PCCC} = 9.7$ Hz, C5), 119.0, 123.1, 140.1, 142.7, 149.2, 152.7, 155.7; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.38. Anal. Calcd for $C_{16}H_{24}N_9O_4P$: C, 43.94; H, 5.53; N, 28.82. Found: C, 44.03; H, 5.30; N, 29.01.

4.1.2.8. Diethyl 5-(4-((6-amino-9H-purin-9-yl)methyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***16d***. From azidoisoxazolidine **13** (0.150 g, 0.568 mmol) and N^9-(propargyl) adenine (0.098 g, 0.568 mmol), phosphonate **16d** (0.207 g, 83%) was obtained as a white amorphous solid after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). M.p.: 139–140 °C. IR (KBr, cm^{-1}) ν_{max}: 3292, 2983, 1665, 1600, 1475, 1304, 1243, 1115, 1050, 1019, 960, 777; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.21 (t, 3H, $J = 7.1$ Hz), 1.29 (t, 3H, $J = 7.1$ Hz), 2.93 (s, 3H), 2.98–3.17 (m, 2H), 3.20–3.40 (m, 1H), 4.00–4.20 (m, 4H), 5.49 (s, 2H), 5.99 (br s, 2H), 6.35 (d, 1H, $J = 6.1$ Hz, *H*-C5), 8.01 (s, 1H), 8.18 (s, 1H), 8.35 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.4 (d, $J = 5.4$ Hz), 16.2 (d, $J = 5.7$ Hz), 38.4, 40.2, 45.6 (s, *C*H_3–N), 62.8 (d, $J = 6.9$ Hz, C–O–P), 63.3 (d, $J = 6.9$ Hz, C–O–P), 63.3 (d, $^1J_{PC} = 166.6$ Hz, C3), 85.4 (d, $^3J_{PCCC} = 9.2$ Hz, C5), 119.1, 121.6, 140.2, 142.4, 149.4, 152.8, 155.8; ^{31}P NMR (121.5 MHz, $CDCl_3$): 21.44. Anal. Calcd for $C_{16}H_{24}N_9O_4P$: C, 43.94; H, 5.53; N, 28.82. Found: C, 43.76; H, 5.60; N, 28.71.

4.1.2.9. Diethyl 5-(4-(methoxycarbonyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***15e***. From azidoisoxazolidine **12** (0.150 g, 0.568 mmol) and methyl propiolate (0.051 mL, 0.568 mmol), phosphonate **15e** (0.168 g, 85%) was obtained as colourless needles after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). M.p.: 78–80 °C. IR (KBr, cm^{-1}) ν_{max}: 3457, 2989, 2923, 1744, 1445, 1257, 1202, 1046, 1020, 804; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.37 (t, 3H, $J = 7.0$ Hz, $POCH_2CH_3$), 1.38 (t, 3H, $J = 7.0$ Hz, $POCH_2CH_3$), 2.90 (s, 3H, *C*H_3–N), 3.10 (dddd, 1H, $J = 17.4$, 13.1, 10.8, 6.9 Hz, *Hb*C4), 3.45–3.60 (m, 2H), 3.98 (s, 3H, $COOCH_3$), 4.17–4.30 (m, 4H, 2× $POCH_2CH_3$), 6.20 (dd, 1H, $J = 6.5$, 1.0 Hz, *H*C5), 8.31 (s, 1H, *H*C5′); ^1H NMR (300 MHz, C_6D_6,) δ: 1.00 (t, 3H, $J = 6.9$ Hz, $POCH_2CH_3$), 1.54 (t, 3H, $J = 6.9$ Hz, $POCH_2CH_3$), 2.69 (d, 3H, $J = 0.4$ Hz, *C*H_3–N), 2.84 (dddd, 1H, $J = 17.4$, 13.1, 10.8, 6.9 Hz, *Hb*C4), 3.13 (dddd, 1H, $J = 13.1$, 6.8, 4.7, 1.6 Hz, *Ha*C4), 3.47 (ddd, 1H, $J = 10.8$, 6.8, 2.8 Hz, *H*C3), 3.16 (s, 3H, $COOCH_3$), 3.86–3.99 (m, 2H, $POCH_2CH_3$), 4.01–4.07 (m, 2H, $POCH_2CH_3$), 5.24 (dd, 1H, $J = 6.9$, 1.6 Hz, *H*C5), 7.55 (s, 1H, *H*C5′); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.70 (d, $J = 5.8$ Hz), 16.8 (d, $J = 5.7$ Hz), 39.3 (s, C4), 48.2 (s, *C*H_3–N), 52.6 (s, $COOCH_3$), 62.7 (d, $^1J_{PC} = 171.8$ Hz, C3), 63.2 (d, $J = 6.9$ Hz, C–O–P), 63.7 (d, $J = 6.6$ Hz, C–O–P), 87.0 (d, $^3J_{PCCC} = 10.0$ Hz, C5), 127.4 (s, C5′), 140.5 (s, C4′), 160.9 (s, C=O); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.03. ^{31}P NMR (C_6D_6, 121.5 MHz): 21.33. Anal. Calcd for $C_{12}H_{21}N_4O_6P$: C, 41.38; H, 6.08; N, 16.09. Found: C, 41.42; H, 5.99; N, 16.20.

4.1.2.10. Diethyl 5-(4-(methoxycarbonyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***16e***. From azidoisoxazolidine **13** (0.150 g, 0.568 mmol) and methyl propiolate (0.051 mL, 0.568 mmol), phosphonate **16e** (0.168 g, 85%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). IR (film, cm^{-1}) ν_{max}: 3480, 3156, 2983, 1741, 1545, 1440, 1368, 1241, 1200, 1163, 1098, 1050, 1026, 972, 809, 779; ^1H NMR (300 MHz, C_6D_6) δ: 0.96 (t, 3H, $J = 7.2$ Hz, $POCH_2CH_3$), 1.01 (t, 3H, $J = 7.2$ Hz, $POCH_2CH_3$), 2.41 (dddd, 1H, $J = 13.5$, 9.9, 7.9, 5.4 Hz, *Hb*C4), 2.53 (ddd, 1H, $J = 9.9$, 8.1, 1.8 Hz,

*H*C3), 2.74 (dddd, 1H, $J = 17.2$, 13.5, 8.1, 2.0 Hz, *Ha*C4), 2.76 (d, 3H, $J = 1.0$ Hz, CH_3–N), 3.48 (s, 3H, COOCH_3), 3.93–3.75 (m, 4H, 2× POCH_2CH$_3$), 5.87 (dd, 1H, $J = 7.9$, 2.0 Hz, *H*C5), 8.74 (s, 1H, *H*C5′); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.70 (d, $J = 5.5$ Hz), 16.6 (d, $J = 5.7$ Hz), 40.8 (d, $^2J_{PCC} = 2.0$ Hz, C4), 45.7 (d, $J = 1.7$ Hz, CH_3–N), 52.3 (s, COOCH_3), 63.0 (d, $J = 6.8$ Hz, C–O–P), 63.4 (d, $^1J_{PC} = 166.9$ Hz, 140.0 (s, C4′), C3), 63.5 (d, $J = 6.9$ Hz, C–O–P), 85.7 (d, $^3J_{PCCC} = 8.6$ Hz, C5), 126.6 (s, C5′), 161.1 (s, C=O); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.12; ^{31}P NMR (121.5 MHz, C_6D_6) δ:21.30. Anal. Calcd for $C_{12}H_{21}N_4O_6P$: C, 41.38; H, 6.08; N, 16.09. Found: C, 41.23; H, 6.18; N, 16.28.

4.1.2.11. Diethyl 2-methyl-5-(4-phenyl-1H-1,2,3-triazol-1-yl)isoxazolidin-3-yl-3-phosphonate ***15f***. From azidoisoxazolidine **12** (0.130 g, 0.492 mmol) and phenylacetylene (0.054 mL, 0.492 mmol), phosphonate **15f** (0.157 g, 87%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). IR (film, cm^{-1}) ν_{max}: 3469, 3129, 2983, 2910, 1484, 1441, 1250, 1235, 1160, 1060, 1027, 972, 768, 698; ^{1}H NMR (300 MHz, C_6D_6) δ: 1.02 (t, 3H, $J = 7.2$ Hz, POCH$_2$CH_3), 1.08 (t, 3H, $J = 7.2$ Hz, POCH$_2$CH_3), 2.77 (s, 3H, CH_3–N), 2.93 (dddd, 1H, $J = 17.1$, 12.9, $J = 10.8$, 6.6 Hz, *Hb*C4), 3.38 (dddd, 1H, $J = 12.9$, 6.9, 5.4, 1.5 Hz, *Ha*C4), 3.66 (ddd, 1H, $J = 10.8$, 6.9, 2.4 Hz, *H*C3), 3.90–3.98 (m, 2H, POCH_2CH$_3$), 4.01–4.12 (m, 2H, POCH_2CH$_3$), 5.40 (dd, 1H, $J = 6.6$, 1.5 Hz, *H*C5), 7.08 (s, 1H), 7.09–7.42 (m, 3H), 7.90–7.93 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.8 (d, $J = 5.7$ Hz), 16.9 (d, $J = 5.7$ Hz), 38.9 (s, C4), 48.4 (d, $J = 6.3$ Hz, CH_3–N), 63.0 (d, $^1J_{PC} = 172.6$ Hz, C3), 63.3 (d, $J = 6.9$ Hz, C–O–P), 63.7 (d, $J = 6.9$ Hz, C–O–P), 86.7 (d, $^3J_{PCCC} = 9.7$ Hz, C5), 119.7, 125.9, 129.0, 129.1, 130.2, 148.5; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.63; ^{31}P NMR (121.5 MHz, C_6D_6) δ: 21.68. Anal. Calcd for $C_{16}H_{23}N_4O_4P$: C, 52.46; H, 6.33; N, 15.29. Found: C, 52.45; H, 6.32; N, 15.25.

4.1.2.12. Diethyl 2-methyl-5-(4-phenyl-1H-1,2,3-triazol-1-yl)isoxazolidin-3-yl-3-phosphonate ***16f***. From azidoisoxazolidine **13** (0.100 g, 0.378 mmol) and phenylacetylene (0.042 mL, 0.378 mmol), phosphonate **16f** (0.124 g, 89%) was obtained as a white amorphous solid after purification on silica gel with chloroform–methanol (from 100:1 to 50:1, v/v). M.p.: 80–81 °C. IR (KBr, cm^{-1}) ν_{max}: 3522, 2455, 2988, 2891, 1609, 1430, 1250, 1053, 1027, 949; ^{1}H NMR (300 MHz, C_6D_6) δ: 0.93 (t, 3H, $J = 7.2$ Hz, POCH$_2$CH_3), 0.95 (t, $J = 7.2$ Hz, 3H, POCH$_2$CH_3), 2.41 (dddd, 1H, $J = 13.8$, 9.9, 7.8, 6.0 Hz, *Ha*C4), 2.82 (d, 3H, $J = 1.0$ Hz, CH_3–N), 2.56 (ddd, 1H, $J = 9.9$, 8.4, 2.4 Hz, *H*C3), 2.92 (dddd, 1H, $J = 18.0$, 13.8, 8.4, 2.4 Hz, *Hb*C4), 3.75–3.92 (m, 4H, POCH_2CH$_3$), 5.95 (dd, 1H, $J = 7.8$, 2.4 Hz, *H*C5), 7.00–7.20 (m, 3H), 8.02–8.06 (m, 2H), 8.46 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.8 (d, $J = 5.7$ Hz), 40.6 (s, C4), 45.9 (d, $J = 1.7$ Hz, CH_3–N), 63.1 (d, $J = 6.9$ Hz, C–O–P), 63.5 (d, $J = 6.9$ Hz, C–O–P), 63.7 (d, $^1J_{PC} = 166.9$ Hz, C3), 85.6 (d, $^3J_{PCCC} = 9.2$ Hz, C5), 118.6, 125.9, 128.2, 128.9, 130.7, 148.1; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.04; ^{31}P NMR (121.5 MHz, C_6D_6) δ: 21.99. Anal. Calcd for $C_{16}H_{23}N_4O_4P$: C, 52.46; H, 6.33; N, 15.29. Found: C, 52.29; H, 6.52; N, 15.20.

4.1.2.13. Diethyl 5-(4-(2-fluorophenyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***15g***. From azidoisoxazolidine **12** (0.102 g, 0.386 mmol) and 1-ethynyl-2-fluorobenzene (0.043 mL, 0.386 mmol), phosphonate **15g** (0.122 g, 82%) was obtained as a white amorphous solid after purification on silica gel with chloroform–methanol (from 100:1 to 50:1, v/v). M.p.: 102–105 °C. IR (KBr, cm^{-1}) ν_{max}: 3426, 2983, 2922, 1488, 1437, 1243, 1047, 1027, 948, 762; ^{1}H NMR (300 MHz, C_6D_6) δ: 1.01 (t, 3H, $J = 7.1$ Hz, POCH$_2$CH_3), 1.07 (t, 3H, $J = 7.1$ Hz, POCH$_2$CH_3), 2.78 (s, 3H, CH_3–N), 2.90 (dddd, 1H, $J = 16.9$, 13.2, 11.1, 6.9, *Hb*C4), 3.30 (dd, 1H, $J = 13.2$, 6.9, 4.5, 1.5 Hz, *Ha*C4), 3.67 (ddd, 1H, $J = 10.8$, 6.9, 2.1 Hz, *H*C3), 3.87–4.10 (m, 4H, 2× POCH_2CH$_3$), 5.34 (dd, 1H, $J = 6.9$, 1.5 Hz, *H*C5), 6.83–6.93 (m, 3H), 7.69 (d, $J = 3.8$ Hz, 1H), 8.62–8.68 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.7 (d, $J = 5.7$ Hz), 16.8 (d, $J = 5.4$ Hz), 38.9 (d, $J = 1.7$ Hz, C4), 48.3 (d, $J = 6.3$ Hz, CH_3–N), 62.9 (d, $^1J_{PC} = 172.6$ Hz, C3), 63.1 (d, $J = 6.7$ Hz, C–O–P), 63.6 (d, $J = 6.7$ Hz, C–O–P), 86.6 (d, $^3J_{PCCC} = 9.7$ Hz, C5), 115.7 (d, $J = 21.5$ Hz), 118.1 (d, $J = 12.6$ Hz), 122.6 (d, $J = 12.9$ Hz), 124.6 (d, $J = 3.4$ Hz), 127.7 (d, $J = 3.4$ Hz), 129.6 (d, $J = 8.6$ Hz), 141.6 (s, C4′), 159.2 (d, $J = 247.9$ Hz, CF); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.47; ^{31}P NMR (121.5 MHz, C_6D_6) δ: 21.79. Anal. Calcd for $C_{16}H_{22}FN_4O_4P$: C, 50.00; H, 5.77; N, 4.94. Found: C, 49.90; H, 5.84; N, 5.01.

4.1.2.14. Diethyl 5-(4-(2-fluorophenyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***16g***. From azidoisoxazolidine **13** (0.153 g, 0.579 mmol) and 1-ethynyl-2-fluorobenzene (0.066 mL, 0.579 mmol), phosphonate **16g** (0.122 g, 98%) was obtained as a white amorphous solid after purification on silica gel with chloroform–methanol (from 100:1 to 50:1, v/v). M.p.: 97–98 °C. IR (KBr, cm^{-1}) ν_{max}: 3482, 3092, 2983, 1620, 1590, 1479, 1454, 1360, 1240, 1129, 1068, 1034, 971, 864, 790; ^{1}H NMR (300 MHz, C_6D_6) δ: 0.98 (t, 3H, $J = 7.2$ Hz, POCH$_2$CH_3), 1.05 (t, 3H, $J = 7.2$ Hz, POCH$_2$CH_3), 2.43 (dddd, 1H, $J = 13.5$, 9.0, 7.8, 5.4 Hz, *Hb*C4), 2.58 (dd, 1H, $J = 9.0$, 9.0, 2.4 Hz, *H*C3), 2.83 (d, 3H, $J = 1.0$ Hz, CH_3–N), 2.93 (dddd, 1H, $J = 17.2$, 13.5, 9.0, 2.1 Hz, *Ha*C4), 3.80–3.90 (m, 2H, POCH_2CH$_3$), 3.93–4.00 (m, 2H, POCH_2CH$_3$), 5.95 (dd, 1H, $J = 7.8$, 2.1 Hz, *H*C5), 6.80–6.92 (m, 3H), 8.61 (d, $J = 3.9$ Hz, 1H), 8.70–8.65 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.6 (d, $J = 6.3$ Hz), 16.7 (d, $J = 5.7$ Hz), 40.7 (d, $J = 2.6$ Hz, C4), 45.8 (s, CH_3–N), 62.9 (d, $J = 6.7$ Hz, C–O–P), 63.7 (d, $J = 6.8$ Hz, C–O–P), 63.8 (d, $^1J_{PC} = 166.9$ Hz, C3), 85.6 (d, $^3J_{PCCC} = 9.7$ Hz, C5), 115.7 (d, $J = 21.7$ Hz), 118.7 (d, $J = 13.0$ Hz), 121.4 (d, $J = 13.2$ Hz), 124.6 (d, $J = 3.6$ Hz), 127.9 (d, $J = 3.9$ Hz), 129.4 (d, $J = 8.5$ Hz), 141.6 (s, C4′), 159.2 (d, $J = 247.6$ Hz, CF); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.41; ^{31}P NMR (121.5 MHz, C_6D_6) δ: 21.37. Anal. Calcd for $C_{16}H_{22}FN_4O_4P$: C, 50.00; H, 5.77; N, 4.94. Found: C, 50.13; H, 6.01; N, 5.12.

4.1.2.15. Diethyl 5-(4-(3-fluorophenyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***15h***. From azidoisoxazolidine **12** (0.103 g, 0.390 mmol) and 1-ethynyl-3-fluorobenzene (0.045 mL, 0.390 mmol), phosphonate **15h** (0.122 g, 81%) was obtained as colourless plates after purification on silica gel with chloroform–methanol (from 100:1 to 50:1, v/v). M.p.: 93–95 °C. IR (KBr, cm^{-1}) ν_{max}: 3123, 2988, 1618, 1590, 1486, 1448, 1343, 1242, 1052, 1023, 976, 945, 862; ^{1}H NMR (300 MHz, C_6D_6) δ: 1.02 (t, 3H, $J = 6.9$ Hz, POCH$_2$CH_3); 1.09 (t, 3H, $J = 6.9$ Hz, POCH$_2$CH_3), 2.78 (s, 3H, CH_3–N), 2.94 (dddd, 1H, $J = 17.7$, 13.2, 10.8, 6.9 Hz, *Hb*C4), 3.36 (dddd, 1H, $J = 13.2$, 6.9, 5.7, 1.5 Hz, *Ha*C4), 3.65 (ddd, 1H, $J = 10.8$, 6.9, 2.7 Hz, *H*C3), 3.89–4.00 (m, 2H, POCH_2CH$_3$), 4.01–4.11 (m, 2H, POCH_2CH$_3$), 5.39 (dd, 1H, $J = 6.9$, 1.5 Hz, *H*C5), 6.72–6.80 (m, 1H), 6.95 (s, 1H), 6.93–7.00 (m, 1H), 7.58 (ddd, 1H, $J = 9.9$, 2.6, 1.6 Hz), 7.67 (dt, 1H, $J = 7.9$, 1.2 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.7 (d, $J = 6.4$ Hz), 16.8 (d, $J = 6.4$ Hz), 38.8 (s, C4), 48.3 (d, $J = 5.4$ Hz, CH_3–N), 62.9 (d, $^1J_{PC} = 172.4$ Hz, C3), 63.1 (d, $J = 6.9$ Hz, C–O–P), 63.6 (d, $J = 6.9$ Hz, C–O–P), 86.7 (d, $^3J_{PCCC} = 10.4$ Hz, C5), 112.8 (d, $J = 22.9$ Hz), 115.3 (d, $J = 21.7$ Hz), 119.9, 121.4 (d, $J = 3.6$ Hz), 130.5 (d, $J = 8.5$ Hz), 132.3 (d, $J = 8.7$ Hz), 147.3, 163.1 (d, $J = 245.4$ Hz, CF); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.56; ^{31}P NMR (121.5 MHz, C_6D_6) δ: 21.75. Anal. Calcd for $C_{16}H_{22}FN_4O_4P$: C, 50.00; H, 5.77; N, 4.94. Found: C, 50.03; H, 5.60; N, 4.91.

4.1.2.16. Diethyl 5-(4-(3-fluorophenyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***16h***. From azidoisoxazolidine **13** (0.148 g, 0.560 mmol) and 1-ethynyl-3-fluorobenzene (0.065 mL, 0.560 mmol), phosphonate **16h** (0.213 g, 98%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (from 100:1 to 50:1, v/v). IR (film, cm^{-1})

ν_{max}: 3470, 2980, 1619, 1589, 1478, 1454, 1440, 1359, 1240, 1044, 1020, 972, 954, 863; ^{1}H NMR (300 MHz, C_6D_6) δ: 0.92 (t, 3H, $J = 6.9$ Hz, $POCH_2CH_3$), 0.94 (t, 3H, $J = 6.9$ Hz, $POCH_2CH_3$), 2.39 (dddd, 1H, $J = 13.5, 9.6, 7.8, 6.0$ Hz, *Hb*C4), 2.55 (ddd, 1H, $J = 9.6, 8.4, 1.8$ Hz, *H*C3), 2.81 (s, 3H, CH_3–N), 2.88 (dddd, 1H, $J = 17.7, 13.5, 8.4, 2.1$ Hz, *Ha*C4), 3.77–3.87 (m, 4H, 2× $POCH_2CH_3$), 5.91 (dd, 1H, $J = 7.8, 2.1$ Hz, *H*C5), 6.68–6.75 (m, 1H), 6.88–6.95 (m, 1H), 7.73–7.77 (m, 2H), 8.41 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.6 (d, $J = 6.3$ Hz), 16.7 (d, $J = 6.3$ Hz), 40.5 (d, $J = 2.4$ Hz, C4), 45.8 (d, $J = 2.5$ Hz, CH_3–N), 63.1 (d, $J = 6.9$ Hz, C–O–P), 63.4 (d, $J = 6.9$ Hz, C–O–P), 63.5 (d, $^1J_{PC} = 166.7$ Hz, C3), 85.6 (d, $^3J_{PCCC} = 9.2$ Hz, C5), 112.7 (d, $J = 22.7$ Hz), 115.0 (d, $J = 21.1$ Hz), 119.0, 121.4 (d, $J = 3.4$ Hz), 130.4 (d, $J = 8.6$ Hz), 132.8 (d, $J = 8.0$ Hz), 146.9 (d, $J = 3.4$ Hz), 163.1 (d, $J = 245.2$ Hz, CF); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.93; ^{31}P NMR (121.5 MHz, C_6D_6) δ: 22.00. Anal. Calcd for $C_{16}H_{22}FN_4O_4P$: C, 50.00; H, 5.77; N, 4.94. Found: C, 49.87; H, 5.60; N, 4.93.

4.1.2.17. Diethyl 5-(4-(2,4-difluorophenyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***15i***. From azidoisoxazolidine **12** (0.105 g, 0.397 mmol) and 1-ethynyl-2,4-difluorobenzene (0.055 g, 0.397 mmol), phosphonate **15i** (0.136 g, 85%) was obtained as colourless needles after purification on silica gel with chloroform–methanol (20:1, v/v). M.p.: 112–113 °C. IR (KBr, cm^{-1}) ν_{max}: 3435, 3140, 2984, 2924, 1621, 1561, 1495, 1444, 1390, 1336, 1243, 1142, 1065, 1029, 951; ^{1}H NMR (300 MHz, C_6D_6) δ: 1.01 (t, 3H, $J = 6.9$ Hz, $POCH_2CH_3$), 1.07 (t, 3H, $J = 6.9$ Hz, $POCH_2CH_3$), 2.80 (s, 3H, CH_3–N), 2.92 (dddd, 1H, $J = 17.7, 12.9, 10.8, 6.9$ Hz, *Hb*C4), 3.30 (dddd, 1H, $J = 12.9, 6.9, 4.5, 1.5$ Hz, *Ha*C4), 3.66 (ddd, 1H, $J = 10.8, 6.9, 2.7$ Hz, *H*C3), 3.90–4.00 (m, 2H, $POCH_2CH_3$), 4.03–4.10 (m, 2H, $POCH_2CH_3$), 5.35 (dd, 1H, $J = 6.9, 1.5$ Hz, *H*C5), 6.49–6.89 (m, 2H), 7.55 (dt, 1H, $J = 3.8$ Hz), 8.36–8.44 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.7 (d, $J = 5.7$ Hz), 16.8 (d, $J = 5.7$ Hz), 38.9 (s, C4), 48.3 (d, $J = 5.7$ Hz, CH_3–N), 63.0 (d, $^1J_{PC} = 172.4$ Hz, C3), 63.1 (d, $J = 6.9$ Hz, C–O–P), 63.6 (d, $J = 6.6$ Hz, C–O–P), 86.7 (d, $^3J_{PCCC} = 9.7$ Hz, C5), 104.3 (t, $J = 25.4$ Hz), 112.2 (dd, $J = 21.7, 3.9$ Hz), 114.7 (dd, $J = 13.6, 3.8$ Hz), 122.1 (d, $J = 13.3$ Hz), 128.8 (dd, $J = 9.7, 5.1$ Hz), 141.2 (d, $J = 4.0$ Hz), 159.3 (dd, $J = 254.4, 12.0$ Hz, CF), 162.9 (dd, $J = 254.4, 12.5$ Hz, CF); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.45; ^{31}P NMR (121.5 MHz, C_6D_6) δ: 21.68. Anal. Calcd for $C_{16}H_{21}F_2N_4O_4P$: C, 47.76; H, 5.26; N, 13.93. Found: C, 48.00; H, 5.37; N, 13.85.

4.1.2.18. Diethyl 5-(4-(2,4-difluorophenyl)-1H-1,2,3-triazol-1-yl)-2-methylisoxazolidin-3-yl-3-phosphonate ***16i***. From azidoisoxazolidine **13** (0.152 g, 0.575 mmol) and 1-ethynyl-2,4-difluorobenzene (0.079 g, 0.575 mmol), phosphonate **16i** (0.218 g, 94%) was obtained as colourless plates after purification on silica gel with chloroform–methanol (20:1, v/v). M.p.: 72–74 °C. IR (KBr, cm^{-1}) ν_{max}: 3445, 3121, 3100, 2983, 2905, 1625, 1600, 1559, 1492, 1447, 1355, 1257, 1140, 1046, 1025, 967, 946; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32 (t, 3H, $J = 6.9$ Hz), 1.36 (t, 3H, $J = 6.9$ Hz), 3.00 (s, 3H), 3.00–3.21 (m, 2H), 3.25–3.40 (m, 1H), 4.12–4.30 (m, 4H), 6.46 (dd, 1H, $J = 7.8, 2.4$ Hz), 6.85–6.95 (m, 1H), 6.96–7.03 (m, 1H), 8.28 (dt, 1H, $J = 8.7, 6.3$ Hz), 8.44 (d, 1H, $J = 3.6$ Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.4 (d, $J = 5.5$ Hz), 40.3, 45.6, 62.7 (d, $J = 6.6$ Hz, C–O–P), 63.4 (d, $J = 6.6$ Hz, C–O–P), 63.5 (d, $^1J_{PC} = 166.0$ Hz, C3), 85.4 (d, $^3J_{PCCC} = 9.1$ Hz, C5), 104.0 (t, $J = 25.8$ Hz), 111.7 (dd, $J = 21.2, 3.4$ Hz), 115.0 (dd, $J = 13.5, 4.0$ Hz), 120.8 (d, $J = 12.0$ Hz), 128.6 (dd, $J = 11.5, 5.2$ Hz), 140.6, 158.9 (dd, $J = 238.5, 13.1$ Hz, CF), 162.2 (dd, $J = 242.2, 12.7$ Hz, CF); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 20.75. Anal. Calcd for $C_{16}H_{21}F_2N_4O_4P$: C, 47.76; H, 5.26; N, 13.93. Found: C, 47.67; H, 5.06; N, 13.99.

4.1.2.19. Diethyl 2-methyl-5-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl) isoxazolidin-3-yl-3-phosphonate ***15j***. From azidoisoxazolidine **12** (0.152 g, 0.575 mmol) and 2-ethynylpyridine (0.058 mL, 0.575 mmol), phosphonate **15j** (0.160 g, 76%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (100:1, v/v). IR (film, cm^{-1}) ν_{max}: 3466, 3138, 2983, 2930, 1603, 1572, 1474, 1424, 1343, 1254, 12,110, 1160, 1050, 1025, 972, 788; ^{1}H NMR (300 MHz, C_6D_6) δ: 0.99 (t, 3H, $J = 6.9$ Hz, $POCH_2CH_3$), 1.06 (t, 3H, $J = 6.9$ Hz, $POCH_2CH_3$), 2.76 (s, 3H, CH_3–N), 2.85 (dddd, 1H, $J = 17.7, 12.9, 11.1, 6.9$ Hz, *Hb*C4), 3.22 (dddd, 1H, $J = 12.9, 6.9, 4.5, 1.2$ Hz, *Ha*C4), 3.58 (ddd, 1H, $J = 10.5, 6.6, 1.8$ Hz, *H*C3), 3.86–3.99 (m, 2H, $POCH_2CH_3$), 4.00–4.08 (m, 2H, $POCH_2CH_3$), 5.39 (d, 1H, $J = 6.9, 1.2$ Hz, *H*C5), 6.59–6.64 (m, 1H), 7.09–7.16 (m, 1H), 8.18 (s, 1H), 8.36 (d, 1H, $J = 7.9$ Hz), 8.44–8.47 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.5 (d, $J = 6.2$ Hz), 16.6 (d, $J = 5.8$ Hz), 38.9 (d, $J = 2.4$ Hz, C4), 48.0 (d, $J = 6.0$ Hz, CH_3–N), 62.6 (d, $^1J_{PC} = 172.4$ Hz, C3), 62.9 (d, $J = 7.2$ Hz, C–O–P), 63.4 (d, $J = 6.9$ Hz, C–O–P), 86.5 (d, $^3J_{PCCC} = 10.5$ Hz, C5), 120.2, 121.7, 123.0, 136.9, 148.7, 149.2, 149.5; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.44; ^{31}P NMR (121.5 MHz, C_6D_6) δ: 21.62. Anal. Calcd for $C_{15}H_{22}N_5O_4P$: C, 49.04; H, 6.04; N, 19.07. Found: C, 48.97; H, 5.95; N, 19.19.

4.1.2.20. Diethyl 2-methyl-5-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl) isoxazolidin-3-yl-3-phosphonate ***16j***. From azidoisoxazolidine **13** (0.156 g, 0.590 mmol) and 1-ethynylpyridine (0.060 g, 0.590 mmol), phosphonate **16j** (0.188 g, 87%) was obtained as white amorphous solid after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). M.p.: 108–109 °C. IR (KBr, cm^{-1}) ν_{max}: 3447, 3113, 2983, 2906, 1595, 1446, 1358, 1252, 1167, 1041, 969, 804; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.31 (t, 3H, $J = 7.2$ Hz), 1.33 (t, 3H, $J = 7.2$ Hz), 2.99 (s, 3H), 3.00–3.22 (m, 2H), 3.24–3.40 (m, 1H), 4.10–4.25 (m, 4H), 6.43 (dd, $J = 6.9, 1.8$ Hz, 1H), 7.20–7.25 (m, 1H), 7.75–7.82 (m, 1H), 8.17 (d, $J = 8.1$ Hz, 1H), 8.57–8.60 (m, 1H), 8.70 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.4 (d, $J = 6.3$ Hz), 16.5 (d, $J = 6.0$ Hz), 40.6 (d, $J = 2.3$ Hz, C4), 45.6 (s, CH_3–N), 62.7 (d, $J = 6.9$ Hz, C–O–P), 63.6 (d, $J = 6.3$ Hz, C–O–P), 63.6 (d, $^1J_{PC} = 166.0$ Hz, C3), 85.4 (d, $^3J_{PCCC} = 9.2$ Hz, C5), 120.1, 120.9, 122.8, 136.7, 148.4, 149.3, 150.1; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.19. Anal. Calcd for $C_{15}H_{22}N_5O_4P$: C, 49.04; H, 6.04; N, 19.07. Found: C, 49.05; H, 5.90; N, 19.01.

4.1.2.21. Diethyl 2-methyl-5-(4-(1-methyl-1H-imidazol-5-yl)-1H-1,2,3-triazol-1-yl)isoxazolidin-3-yl-3-phosphonate ***15k***. From azidoisoxazolidine **12** (0.151 g, 0.571 mmol) and 5-ethynyl-1-methyl-1*H*-imidazole (0.058 mL, 0.571 mmol), phosphonate **15k** (0.128 g, 61%) was obtained as a colourless oil after purification on silica gel with chloroform–methanol (from 50:1 to 20:1, v/v). IR (film, cm^{-1}) ν_{max}: 3113, 2980, 1692, 1613, 1555, 1444, 1239, 1160, 1037, 1020, 967; ^{1}H NMR (300 MHz, C_6D_6) δ: 1.02 (t, 3H, $J = 7.1$ Hz, $POCH_2CH_3$), 1.08 (t, 3H, $J = 7.1$ Hz, $POCH_2CH_3$), 2.80 (s, 3H, CH_3–N), 2.94 (dddd, 1H, $J = 17.4, 13.2, 10.8, 6.9$ Hz, *Hb*C4), 3.28 (dddd, 1H, $J = 13.2, 6.9, 5.1, 1.8$ Hz, *Ha*C4), 3.29 (s, 3H, CH_3–N), 3.66 (ddd, 1H, $J = 10.8, 6.9, 2.7$ Hz, *H*C3), 3.91–3.99 (m, 2H, $POCH_2CH_3$), 3.99–4.10 (m, 2H, $POCH_2CH_3$), 5.47 (dd, 1H, $J = 6.9, 1.8$ Hz, *H*C5), 6.97 (s, 1H), 7.08 (s, 1H), 7.44 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.6 (d, $J = 6.4$ Hz), 16.7 (d, $J = 6.3$ Hz), 33.8, 38.8 (s, C4), 48.1 (d, $J = 5.4$ Hz, CH_3–N), 62.8 (d, $^1J_{PC} = 172.1$ Hz, C3), 63.0 (d, $J = 6.8$ Hz, C–O–P), 63.5 (d, $J = 6.6$ Hz, C–O–P), 86.5 (d, $^3J_{PCCC} = 10.0$ Hz, C5), 120.9, 122.9, 128.5, 138.9, 139.5; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 21.41; ^{31}P NMR (121.5 MHz, C_6D_6) δ: 21.68. Anal. Calcd for $C_{14}H_{23}N_6O_4P$: C, 45.40; H, 6.26; N, 22.69. Found: C, 45.45; H, 6.09; N, 22.50.

4.1.2.22. Diethyl 2-methyl-5-(4-(1-methyl-1H-imidazol-5-yl)-1H-1,2,3-triazol-1-yl)isoxazolidin-3-yl-3-phosphonate ***16k***. From azidoisoxazolidine **13** (0.135 g, 0.511 mmol) and 5-ethynyl-1-methyl-1*H*-imidazole (0.052 mL, 0.511 mmol), phosphonate **16k** (0.122 g, 65%) was obtained as a colourless oil after purification on silica gel

with chloroform–methanol (from 50:1 to 20:1, v/v). IR (film, cm^{-1}) ν_{max}: 3110, 2982, 1612, 1502, 1442, 1240, 1161, 1114, 1045, 1020, 975; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.32 (t, 3H, $J = 7.1$ Hz), 1.36 (t, 3H, $J = 7.1$ Hz), 2.99 (d, 3H, $J = 1.0$ Hz), 3.00–3.20 (m, 2H), 3.20–3.40 (m, 1H), 3.93 (s, 3H), 4.10–4.25 (m, 2H), 6.45 (dd, $J = 7.8$, 2.1 Hz, 1H), 7.54 (s, 1H), 8.30 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 16.4 (d, $J = 6.2$ Hz), 33.5, 40.4 (d, $J = 2.2$ Hz, C4), 45.6 (d, $J = 2.0$ Hz, CH_3–N), 62.8 (d, $J = 7.0$ Hz, C–O–P), 63.2 (d, $J = 6.9$ Hz, C–O–P), 63.5 (d, $^1J_{PC} = 166.8$ Hz, C3), 85.5 (d, $^3J_{PCCC} = 9.5$ Hz, C5), 119.8, 123.5, 128.7, 138.8, 139.4; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ: 20.97. Anal. Calcd for $C_{14}H_{23}N_6O_4P$: C, 45.40; H, 6.26; N, 22.69. Found: C, 45.30; H, 6.21; N, 22.80.

4.2. Antiviral activity assays

The antiviral assays [except anti-human immunodeficiency virus (HIV) 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 (para-influenza-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 (feline herpes virus; 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 methodology of the anti-HIV assays was as follows: human CEM ($\sim 3 \times 10^5$ cells/cm^3) cells were infected with 100 $CCID_{50}$ of HIV-1(III_B) or HIV-2(ROD)/mL and seeded in 200 μL 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.

4.3. Cytostatic activity assays

Murine leukaemia L1210, human T-lymphocyte CEM, human cervix carcinoma (HeLa) and human lung fibroblast (HEL) cells were suspended at 300,000–500,000 cells/mL of culture medium, and 100 μL of a cell suspension was added to 100 μL of an appropriate dilution of the test compounds in wells of 96-well microtiter plates. After incubation at 37 °C for two (L1210) or three (CEM, HeLa, HEL) days, the cell number was determined using a Coulter counter. The IC_{50} was defined as the compound concentration required to inhibit cell proliferation by 50%.

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