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Novel isoxazolidine analogues of homonucleosides and homonucleotides

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ABSTRACT

Isoxazolidine analogues of homonucleos(t)ides were synthesized from nucleobase-derived nitrones **20a-20e** (uracil, 5-fluorouracil, 5-bromouracil, thymine, adenine) employing 1,3-dipolar cycloadditions with allyl alcohol as well as with alkenylphosphonates (allyl-, allyloxymethyl- and vinyloxymethyl- and vinylphosphonate). Besides reactions with vinylphosphonate the additions proceeded regioselectively to produce mixtures of major cis and minor trans 3,5-disubstituted isoxazolidines (d.e. 28–82%). From vinylphosphonate up to 10% of 3,4-disubstituted isoxazolidines was additionally produced. Vicinal couplings, shielding effects and 2D NOE correlations were employed in configurational assignments as well as in conformational analysis to find out preferred conformations for several isoxazolidines and to observe anomeric effects (pseudoaxial orientation of phosphonylmethoxy groups) for those obtained from vinyloxymethylphosphonate. None of the tested compounds were endowed in vitro with antiviral activity against a variety of DNA and RNA viruses at subtoxic concentrations (up to 250 μM) nor exhibited antiproliferative activity towards L1210, CEM, and HeLa cells ($IC_{50} = \geq 100$ μM).

1. Introduction

A significant number of antiviral and anticancer drugs can be classified as close structural analogues of nucleosides or nucleotides. A search for new compounds has resulted in obtaining many active molecules which showed different levels of similarities to natural nucleosides.[1–10] Modifications of a nucleoside scaffold are practically unlimited since not only the sugar and nucleobase units could be altered but also additional linkers within the structure of the nucleoside can be incorporated. A list of commonly used ribofuranoside replacers includes 2′,3′-dideoxyfuranose, cyclopentane, cyclopentene, 1,3-dioxolane, 1,3-oxathiolane, isoxazolidine rings and also acyclic entities.

The idea of incorporating the isoxazolidine ring into a nucleoside framework as a sugar replacer, first proposed by Tronchet,[11] has been explored to provide several biologically active compounds (Fig. 1). A fluorouracil-containing isoxazolidine **1** was found to induce apoptosis on lymphoid and monocytoid cells and at the same time showed low cytotoxicity.[12] Antiviral nucleotides were also discovered among phosphonylated isoxazolidines **2**[13] and **3**[14] as well as among their analogues having the 1,2,3-triazole linker **4**.[15] While nucleotides **2** have been found to be potent inhibitors of the reverse transcriptase of different retroviruses,[13] its truncated analogues **3** appeared even more potent exhibiting the inhibitory activity at concentrations in the nanomolar range.[14] High cytotoxicity toward several cancer cell lines was observed for isoxazolidine nucleosides of the general formula **5**.[16] On the other hand, it is worth mentioning that the biological activity of compounds containing the isoxazolidine ring is not restricted to anticancer and antiviral properties, since it was found that they also posses antimicrobial,[17,18] antifungal,[18–21] anti-inflammatory,[22,23] antioxidant[24,25] and insecticide activity,[26] among others.

Structural modifications of nucleosides may also influence stereoelectronic effects and contribute to the anomeric effect and thus control a conformational behavior of the sugar ring and affect the biological properties of nucleosides. This is exemplified by a replacement of the ring oxygen atom by a carbon atom leading to the formation of carbanucleosides.[6,27,28] This modification results in a greater metabolic stability of nucleoside analogues lacking the natural N-glycoside bond. A similar increase in stability can be

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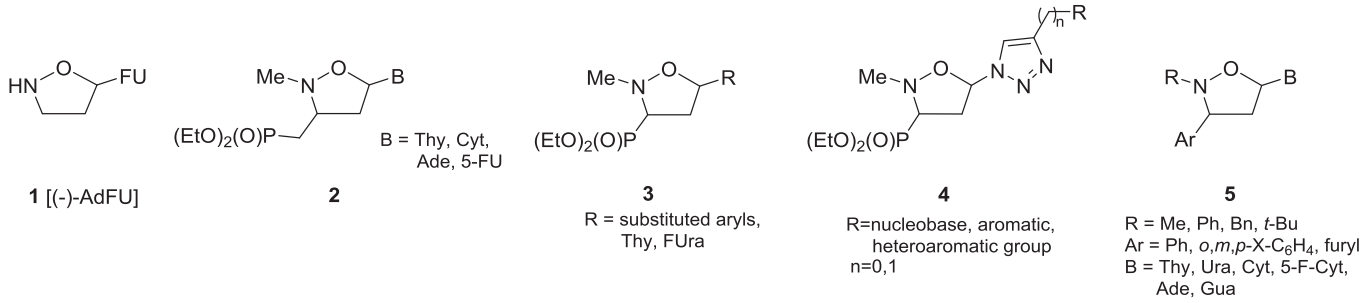


Fig. 1. Examples of biologically active isoxazolidine nucleoside/nucleotide analogues.

achieved in 1′-homonucleosides which are formed by insertion of the methylene group between the nucleobase and the sugar or sugar mimetics as illustrated by 1′-homoadenosine **6**[29,30] Moreover, the biological activity of 1′-homonucleosides is also influenced by greater conformational flexibility and slightly improved lipophilicity. Among 1′-homonucleosides[31,32] containing five-membered rings as ribofuranoside mimics several compounds showing pronounced biological activities were identified **7**–**13** (Fig. 2).[33–38] In most cases these compounds retain the hydroxymethyl group to allow for their sequential phosphorylation to active triphosphate metabolites.

Furthermore, 1′-homonucleotides containing a non-hydrolyzable P–C bond have been also synthesized to obtain analogues which could be phosphorylated to the active form, thereby omitting the first and less effective monophosphorylation step as exemplified by compounds **14**–**19** (Fig. 3).[39–45]

Recently, we have reported the synthesis of isoxazolidine-containing analogues of homonucleosides *cis*-**21**/*trans*-**22** having a nucleobase (B) at C3 of the isoxazolidine ring.[46] The synthetic approach relied on the application of the 1,3-dipolar cycloaddition of allyl alcohol to the nucleobase-derived nitrones **20**. In this paper, a full account of an already communicated preliminary study[46] is given and the reactivity of nitrones **20** with selected alkenylphosphonates **23**–**26** leading to a new series of nucleotide analogues *cis*-**27**/*trans*-**28** to *cis*-**33**/*trans*-**34** is described together with the results of their antiviral and cytostatic activities (Scheme 1).

2. Results and discussion

The synthesis of nucleobase-derived nitrones **20** has been recently described.[46] The 1,3-dipolar cycloadditions of the nitrones **20** to allyl alcohol were carried out at 60 °C or under MW irradiation (Scheme 2, Table 1). The reactions were regiospecific and produced *cis*/*trans* mixtures of diastereoisomeric cycloadducts **21** and **22** in moderate to good diastereoselectivities (d.e. 82–28%). The *cis*/*trans* ratios of the isoxazolidines were calculated from the ^{1}H NMR spectra of the reaction mixtures by comparison of integrations of diagnostic resonances of the H_2C-4 protons in the isoxazolidine ring as well as the signals of the respective protons of nucleobase moieties. The relative configurations in

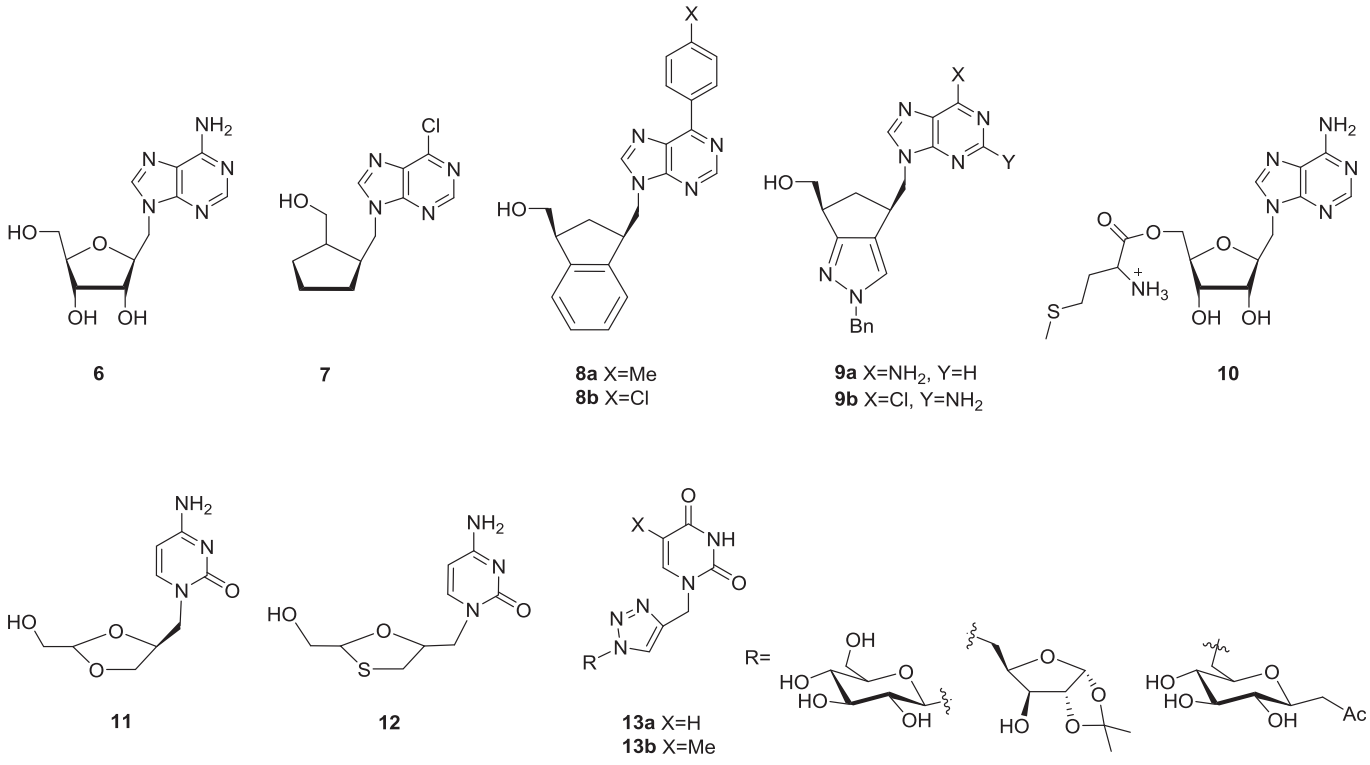


Fig. 2. Examples of structurally diversified 1′-homonucleosides.

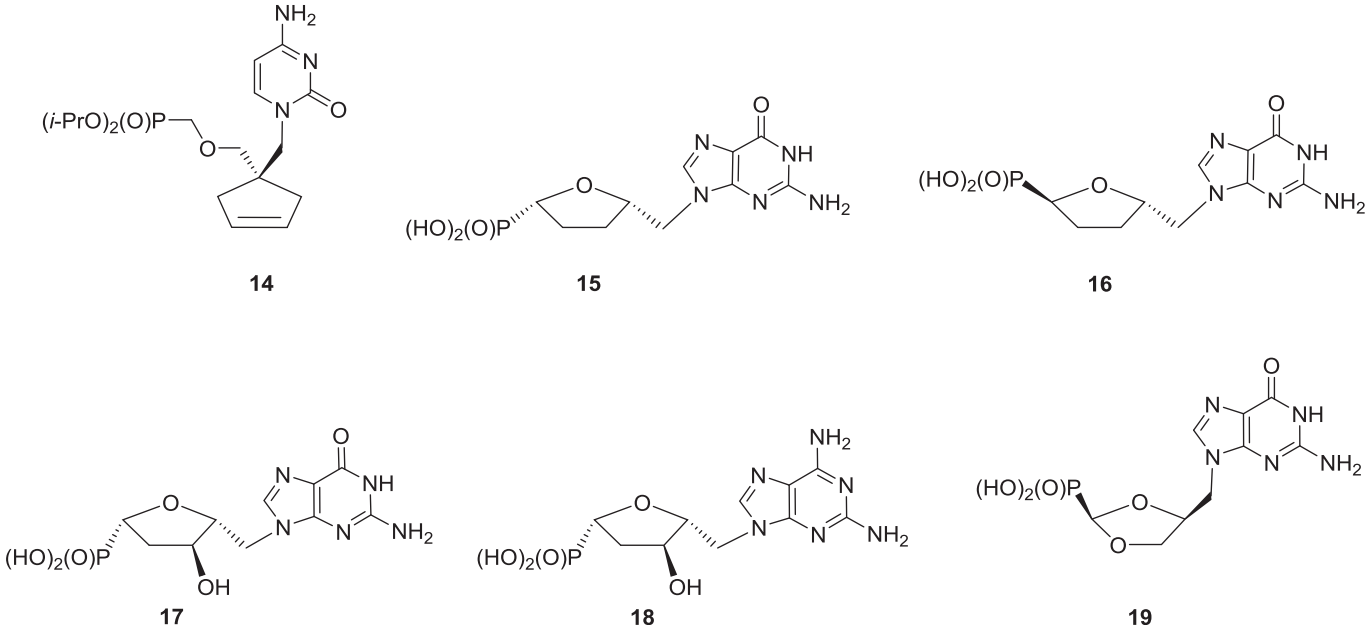
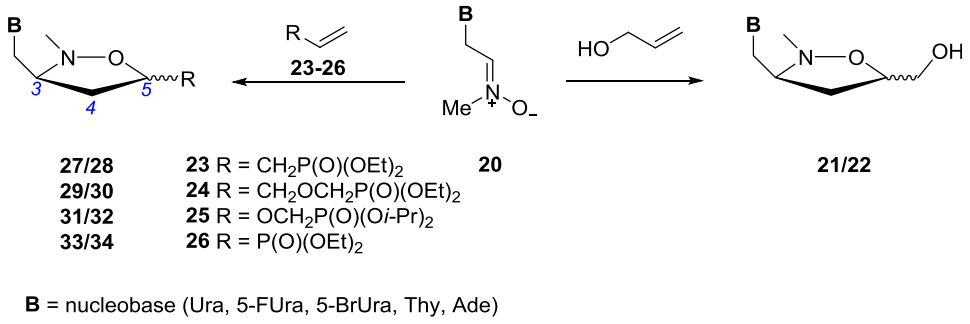
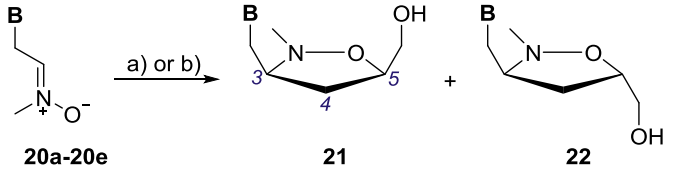


Fig. 3. Examples of 1′-homonucleotide analogues.



Scheme 1. Synthetic approach to homonucleosides **21/22** and their homonucleotide analogues *cis*-**27**/*trans*-**28** to *cis*-**33**/*trans*-**34**.



Scheme 2. Reagents and conditions: a) allyl alcohol, 60 °C, see Table 1; b) allyl alcohol MW, 60–85 °C, see Table 1.

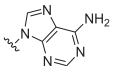
homonucleosides *cis*-**21a** and *trans*-**22a** have already been established based on 2D NOE experiments.[46] These assignments have been extended on *cis*-**21b** and *trans*-**22b**, *cis*-**21c** and *trans*-**22b**, *cis*-**21d** and *trans*-**22d** as well as *cis*-**21e** and *trans*-**22e** pairs of diastereoisomers due to almost identical spectral patterns for HC3, H_2C4 and H5 protons but also for diastereotopic protons in H_2C–B and H_2C–OH moieties in the respective ^{1}H NMR spectra.

In continuation of our studies on the reactivity of the nitrones **20**, allylphosphonate **23**, allyloxymethylphosphonate **24**, vinyloxymethylphosphonate **25** and vinylphosphonate **26** were selected as dipolarophiles to synthesize 1′-homonucleotide analogues having non-hydrolyzable P–C bonds separated by none, one, two or three bonds from C5 in the isoxazolidine ring in compounds **27**/**28**, **29**/**30**, **31**/**32** and **33**/**34**, respectively. The installation of C–O–C–P(O)(OR)$_2$ fragments in the designed compounds is additionally substantiated by their presence in nucleoside phosphonate drugs like adefovir, tenofovir and cidofovir and several other drug candidates.[47,48]

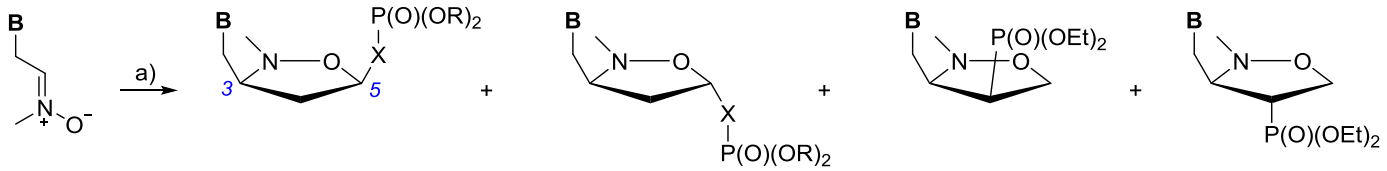
Heating the nitrone **20a** with an excess (3 equiv.) of allylphosphonate **23** at 60–80 °C for 24 h did not result in the formation of even traces of the expected products. However, cycloadditions of nitrones **20** with alkenes **23**–**26** were successfully carried out under microwave irradiation (Scheme 3).

The progress of the reactions was monitored by the ^{1}H NMR spectroscopy until the disappearance of the starting nitrone. The ratios of diastereoisomeric cycloadducts were calculated from the respective ^{31}P NMR spectra of the crude reaction mixtures. The 1,3-dipolar cycloadditions of the nitrones **20** with alkenylphosphonates **23**, **24** and **25** (Scheme 3, Table 2) were regiospecific and gave *cis*/*trans* mixtures of diastereoisomeric cycloadducts *cis*-**27**/*trans*-**28**, *cis*-**29**/*trans*-**30** and *cis*-**31**/*trans*-**32** with diastereoselectivities (d.e. 78–40%, Table 2) comparable to that found for analogous reactions with allyl alcohol (d.e. 82–28%, Table 1). In most cases chromatographic removal of the unreacted alkenylphosphonates was difficult and less effective than distilling-off an excess of allyl alcohol, and thus led to lower overall yields. In general, longer reaction times were required to achieve a full conversion of the nitrones **20a** with less reactive dipolarophiles such as **23**–**25** when compared to

Table 1
Cycloaddition of the nitrones **20a-20e** and allyl alcohol.

Nitrone	Nucleobase B	Reaction time (h)[a,b]	cis/trans ratio **21**:**22**	Yield (%)
20a		24[a] 2.5[b]	64:36[a] 69:31[b]	**21a** – 47[c]; **22a** – 28[c] **21a** – 68[c]; **22a** – 25[c]
20b		15[b]	69:31	**21b**–44[c]; **22b**–21[c]
20c		46[a] 5[b]	91:9 91:9	**21c** – 21[c]; **21c** + **22c** – 21[d] **21c** – 26[c]; **21c** + **22c** – 22[d]
20d		7.5[b]	74:26	**21d**–37[c]; **21d** + **22d**–12[d]; **22d**–22[c]
20e		5[b]	83:17	**21e** – 21[c]; **21e** + **22e** – 26[d]

[a] The cycloaddition reaction was conducted at 60 °C.
[b] The cycloaddition reaction was conducted under MW irradiation.
[c] Yield of the pure diastereoisomer.
[d] Yield of a pure mixture of diastereoisomers.



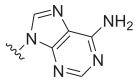
Scheme 3. Reagents and conditions: a) allylphosphonate **23**, allyloxymethylphosphonate **24**, vinyloxymethylphosphonate **25** or vinylphosphonate **26**, CH_3CN or dioxane, MW, 65–80 °C, 3–30 h; see Tables 2 and 3.

an analogous reaction with allyl alcohol. Moreover, during the reaction of the adenine-derived nitrone **20e** with allylphosphonate **23** decomposition of the starting nitrone was observed and the unreacted dipolarophile **23** was recovered almost quantitatively. When the same nitrone **20e** was treated with vinyloxymethylphosphonate formation of a complex reaction mixture was noticed from which expected pure isoxazolidine cycloadducts could not be isolated.

On the other hand, traces of 5-fluorouracil were found in crude reaction mixtures when the nitrone **20b** was treated with alkenylphosphonates **23**–**26** under MW irradiation. To verify the stability of this nitrone under conditions of the cycloaddition reaction a solution of **20b** in acetonitrile was heated under MW irradiation and the progress of the reaction was monitored by the ^{1}H NMR spectroscopy. Indeed, the formation of 5-fluorouracil was observed after 7 h (1%) and increased to 6% after an additional 14 h. The amount of 5-fluorouracil reached 15% after 18 h but the solution was contaminated with other unidentified decomposition products (up to 46%). Similarly, slow decomposition of the adenine-derived nitrone **20e** during MW irradiation of the solution in acetonitrile was observed. ^{1}H NMR spectra taken after 12 h revealed decomposition of the nitrone **20e** (c.a. 15%), since additional signals appeared in a region characteristic of adenine protons.

The relative configurations in *cis*-**27** and *trans*-**28** as well as in *cis*-**29** and *trans*-**30** can again be deduced taking into account almost identical ^{1}H NMR spectral patterns when compared to those of *cis*-**21** and *trans*-**22**. This could be predicted because the spatial and stereoelectronic influence of the substituents at C3 (CH_2–Base) and at C5 (CH_2–OH in **21**/**22**, CH_2–P in **27**/**28** and CH_2–OCH_2P in **29**/**30**) have an indistinguishable impact on the preferred conformations of the isoxazolidine rings in the *cis* and *trans* isomers. Although we were unable to unequivocally establish these conformations in addition to 2D NOE spectral data[22] further support for our configurational assignments comes from the comparison of the chemical shifts of H-C5 protons in the *cis* and *trans* diastereoisomers (Fig. 4). Thus, in the ^{1}H NMR spectra of all *trans*-configured isoxazolidines (**22**, **28**, **30**) resonances of H-C5 are significantly shifted upfield in comparison to the *cis* isomers (**21**, **27**, **29**), e.g. 4.12 ppm in **22a** vs. 4.40 ppm in **21a**, because the H-C5 protons in the *trans* isomers are positioned in the shielding cone of the heteroaromatic ring. The same phenomenon can be observed for the Hβ-C4 protons in both the *cis* and *trans* isoxazolidines but the shielding effects are much better pronounced for the *cis* isomers, e.g. 1.79 ppm for Hβ-C4 vs. 2.59 ppm for Hα-C4 in **21a** and

Table 2
Cycloaddition of the nitrones **20a-20e** and alkenylphosphonates **23**–**25**.

Nitrone	Nucleobase B	Alkene	Reaction time (h)[a]	Cis/trans ratio	Yield [%]
20a		**23**	30	80:20	**27a** – 22[b]; **27a** + **28a** – 24[c]
		24	30	72:28	**29a** – 16[b]; **29a** + **30a** – 32[c]; **30a** – 9[b]
		25	30	89:11	**31a** – 30[b]; **31a** + **32a** – 13[c]
20b		**23**	10	74:26	**27b** – 3[b]; **27b** + **28b**–10[c]
		24	16	79:21	**29b**–13[b]; **29b** + **30b**–23[c]
		25	14	86:14	**31b**–20[b]; **31b** + **32b**–26[c]
20c		**23**	8	86:14	**27c** – 4[b]; **27c** + **28c** – 15[c]
		24	21	72:28	**29c** – 12[b]; **29c** + **30c** – 3.3[c]
		25	8	70:30	**31c** – 19[b]; **31c** + **32c** – 10[c]
20d		**23**	8	71:29	**27d**–21[b]; **27d** + **28d**–15[c]
		24	10	80:20	**29d** – 5[b]; **29d** + **30d**–20[c]
		25	8	84:16	**31d**–27[b]; **31d** + **32d** – 7[c]; **32d** – 2[b]
20e		**23**	40	–	decomposition[d]
		24	26	80:20	–[e]
		25	21	69:31	–[e]

[a] Cycloaddition under MW irradiation.
[b] Yield of the pure diastereoisomer.
[c] Yield of the pure mixture of diastereoisomers.
[d] Decomposition of the starting nitrone **20e** was observed. The unreacted allylphosphonate **23** was recovered almost quantitatively.
[e] Ratio of diastereoisomeric cycloadducts **29e** and **30e** as well as **31e** and **32e** were calculated, however pure isomers could not be isolated from the mixture containing several unidentified products.

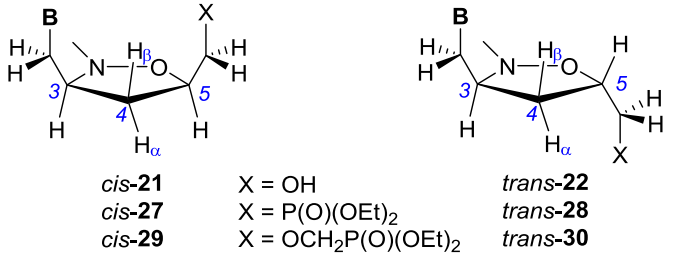


Fig. 4. Relative configurations of *cis*-**21**/**27**/**29** and *trans*-**22**/**28**/**30**.

2.10 ppm for Hβ-C4 vs. 2.31 ppm for Hα-C4 in **22a**.

Although ^{1}H and ^{13}C NMR spectra of isoxazolidines *cis*-**31** and *trans*-**32** prepared from vinyloxymethylphosphonate resembled each other regardless of a nucleobase present they significantly differed from those of the already discussed *cis*-**21**/**27**/**29** and *trans*-**22**/**28**/**30** series and for this reason their relative configurations had to be established independently. Based on the values of vicinal H-H couplings observed in the spectrum of *cis*-**31d** [*J*(HC5-HαC4) = 5.2 Hz, *J*(HC5-HβC4) = 0 Hz, *J*(HαC4-HC3) = 8.9 Hz and *J*(HβC4-HC3) = 2.1 Hz][49] the *E*[5] conformation of the isoxazolidine ring could be unequivocally assigned (Fig. 5, **37**). In a similar fashion vicinal couplings extracted from the spectrum of *trans*-**32d** [*J*(HC5-HαC4) = 1.7 Hz, *J*(HC5-HβC4) = 5.5 Hz, *J*(HαC4-HC3) = 7.6 Hz and *J*(HβC4-HC3) = 5.5 Hz] were applied to prove the E_5 conformation (Fig 5, **38**). In both conformations phosphonylmethoxy substituents at C5 are pseudoaxially oriented since the anomeric effect operates. These conformational assignments are further supported by shielding of HβC4 when compared with HαC4 and lack of shielding of HC5 protons in both isomers.

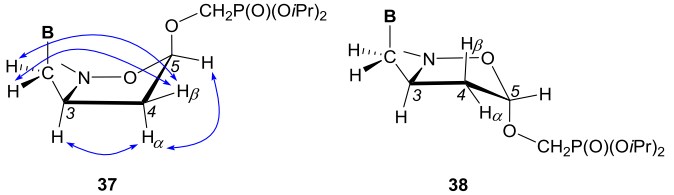
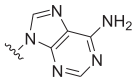


Fig. 5. Preferred conformations **37** and **38** of *cis*-**31** and *trans*-**32**, respectively; observed NOEs marked in blue.

On the other hand, when diethyl vinylphosphonate **26** was applied, in addition to major 3,5-disubstituted isoxazolidines *cis*-**33a-e** and *trans*-**34a-e** (Scheme 3, Table 3), the formation of minute amounts (less than 10%) of regioisomeric 3,4-disubstituted products **35a-e** and **36a-e** was also noticed. Their presence in the crude products as well as in the fractions obtained after column chromatography was detected by the ^{31}P NMR spectroscopy (Table 4) and additionally proved by careful analyses of the ^{1}H NMR spectra where diagnostic signals of nucleobase protons could be assigned to four different cycloadducts, namely *cis*-**33a-e**, *trans*-**34a-e**, and **35a-e**/**36a-e**.

As observed previously, ^{1}H NMR spectra of the major (*cis*-**33**) and minor (*trans*-**34**) derivatives were also similar within the series (**a**-**e**). In 2D NOE spectrum of *cis*-**33c** interactions between H_2C-B and HβC4, HαC4 and HC3 as well as HαC4 and HC5 protons were noticed thus proving their locations on the same sides of the isoxazolidine ring. These observations were further supported by significant shielding of HβC4 (2.23 ppm) when compared with HαC4 (2.84 ppm). Moreover, based on the analysis of vicinal H-H,[49] H-P[50,51] and P-C[52,53] couplings [*J*(HC5-HβC4) = 6.4 Hz, *J*(HC5-HαC4) = 10.5 Hz and *J*(P-HβC4) = 19.4 Hz, *J*(P-HαC4) = 15.8 Hz as well as *J*(HβC4-HC3) = 1.3 Hz, *J*(HαC4-HC3) = 7.7 Hz and *J*(P-C5-C4-C3) = 3.3 Hz] extracted from ^{1}H and ^{13}C NMR spectra of *cis*-**33c** one can conclude that the isoxazolidine ring adopts the E_2 conformation **39** (Fig. 6).

Table 3
Isoxazolidines **33**, **34**, **35** and **36** produced *via* Scheme 3.

Nitrone	Nucleobase B	Reaction time (h)[a]	Ratio of isomers **33**:**34**:**35**:**36**	Yield [%]
20a		3	62:31:5:2	**33a** – 14[b]; **33a** + **34a** + **35a** + **36a** – 38[c];
20b		6	62:31:6:1	**33b**–33[b]; **33b** + **34b** + **35b** + **36b**–41[c];
20c		3	60:32:6:2	**33c** – 24[b]; **33c** + **34c** + **35c** + **36c** – 16[c]; **34c** – 10[b];
20d		6	50:39:7:4	**33d** – 2[b]; **33d** + **34d** + **35d** + **36d** – 8[c]; **34d** – 1[b];
20e		8	64:30:5:1	**33e** + **34e** – 10[c]; **33e** + **34e** + **35e** + **36e** – 45[c];

[a] Cycloaddition under MW irradiation.
[b] Yield of the pure diastereoisomer.
[c] Yield of the pure mixture of two, three or four diastereoisomers.

Table 4
^{31}P NMR chemical shift values for isoxazolidines **33**, **34**, **35** and **36**.

Nitrone	δ^{31}P NMR of isoxazolidine phosphonates (ppm)			
	cis-**33**	*trans*-**34**	*cis*-**35**	*trans*-**36**
20a	23.10	22.46	27.43	26.56
20b	23.09	22.47	27.29	26.50
20c	22.68	22.05	26.45	26.00
20d	22.72	22.13	27.17	26.27
20e	22.08	21.26	26.77	25.65

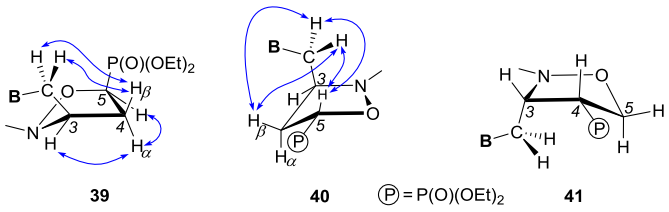


Fig. 6. Preferred conformations **39**, **40** and **41** of *cis*-**33**, *trans*-**34** and *cis*-**35**, respectively; observed NOEs marked in blue.

To establish the *trans* configuration in **34c** it is again worth noting the meaningful upfield shift (0.3 ppm) of HC5 proton in this isomer (4.18 ppm) in comparison to *cis*-**33c** (4.48 ppm) and NOE correlation peaks between H_2C-B and HC5 (not detected in *cis*-**33c**) as well as between H_2C-B and HβC4 (medium intensity) and H_2C-B and HαC4 (weak). Furthermore, the preferred conformation **40** (Fig. 6) of the isoxazolidine ring in *trans*-**34c** can be proposed after analysis of vicinal couplings [*J*(HC5-HβC4) = 8.9 Hz, *J*(HC5-HαC4) = 9.6 Hz and *J*(P-HβC4) = 7.2 Hz, *J*(P-HαC4) = 20.1 Hz as well as *J*(HβC4-HC3) = 1.4 Hz, *J*(HαC4-HC3) = 7.8 Hz and *J*(P-C5-C4-C3) = 6.3 Hz].

To unequivocally establish relative configurations in regioisomers **35** and **36** the attempts at separating them from major 3,5-disubstituted isomers *cis*-**33** and *trans*-**34** and eventually isolating at least one pure 3,4-disubstituted isomer (**35** or **36**) were undertaken. For this purpose, a 22:2:49:27 mixture of compounds **33c**, **34c**, **35c** and **36c** significantly enriched in the isomer **35c** after column chromatography was subjected to separation on an HPLC column to give minute amounts of pure **35c** sufficient enough to perform full characterization by NMR spectroscopy including the 2D NOE experiment. The *cis* configuration of this regioisomer was proved based on the vicinal H-H, H-P and P-C couplings [*J*(HC5β-HC4) = 9.2 Hz, *J*(HC5α-HC4) = 9.2 Hz and *J*(P-HβC5) = 9.2 Hz, *J*(P-HαC5) = 0 Hz as well as *J*(HC4-HC3) = 5.5 Hz, *J*(P-C4C3-H) = 16.1 Hz and *J*(P-C4C3-CH_2B) = 8.1 Hz] which allow to establish the preferred conformation **41** (Fig. 6) for this compound. To further support this assignment one should notice that since H-C4 and the CH_2B moiety are in a near antiperiplanar orientation neither shielding of H-C4 (2.57 ppm) by the heteroaromatic ring and a respective NOE cross peak nor HC5 – HC3 NOE correlation were detected.

2.1. Antiviral and cytostatic evaluation

2.1.1. Antiviral activity

Pure nitrones, as well as pure isoxazolidines or respective mixtures of isoxazolidines were evaluated for their inhibitory activity against a wide variety of DNA and RNA viruses, using the following cell-based assays: (a) human embryonic lung (HEL) cell cultures: herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, thymidine kinase deficient (acyclovir-resistant) herpes simplex virus-1 (TK^- KOS ACV^r), cytomegalovirus (AD-169 and Davis strains), varicella-zoster virus (TK^+ VZV and TK^- VZV strains); (b) HeLa cell cultures: vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus; (c) Vero cell cultures: para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus; (d) CrFK cell cultures: feline corona virus (FIPV) and feline herpes virus (FHV), (e) MDCK cell cultures: influenza A virus (H1N1 and H3N2 subtypes) and influenza B virus and (f) CEM or MT-4 cell cultures: human immunodeficiency virus-1 (III_B or NL4.3) and −2 (ROD). Ganciclovir, cidofovir, acyclovir, brivudin, (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA], oseltamivir carboxylate, amantadine, rimantadine, ribavirin, dextran sulfate (molecular weight 5,000, DS-5000),

Hippeastrum hybrid agglutinin (HHA) and Urtica dioica agglutinin (UDA) 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 cytopathicity by 50% (other viruses). None of the tested compounds showed appreciable antiviral activity toward any of the tested DNA and RNA viruses at the concentration up to 250 μM.

2.1.2. Cytostatic activity

The 50% cytostatic concentration (IC_{50}) causing a 50% inhibition of cell proliferation was determined against murine leukemia L1210, human CD_4^+ T-lymphocyte CEM, human cervix carcinoma HeLa and human dermal microvascular endothelial cells (HMEC-1). Among all compounds evaluated, marginal, if any cytostatic activity was observed. Not only compounds containing adenine, uracil, 5-bromouracil and thymine substituents as nucleobases were found inactive, but also the analogues bearing a 5-fluorouracil (5-FU) moiety showed no significant antiproliferative activity. These findings indicate that the 5-FU-containing compounds are not efficiently taken-up by the intact tumor cells and/or do not enzymatically release free 5-FU and/or do not inhibit thymidylate synthase, one of the most important target enzymes for 5-fluorodeoxyuridine-5′-monophosphate.

3. Conclusion

New nucleobase-derived nitrones **20a-e** were efficiently applied in the synthesis of isoxazolidine analogues of homonucleosides and homonucleotides which relied on the 1,3-dipolar cycloadditions of **20a-e** first to allyl alcohol and next to allyl-, allyloxymethyl-, vinyloxymethyl- and vinylphosphonates. In general cycloadditions were regioselective and led to the formation of *cis* and *trans* mixtures of 3,5-disubstituted isoxazolidines with moderate to good diastereoselectivities. However, in cycloadditions to vinylphosphonate in addition to major 3,5-disubstituted isoxazolidines also 3,4-disubstituted isomers were formed (up to 10%).

Relative (*cis* and *trans*) configurations of 3,5-disubstituted isoxazolidines were established based on the detailed analysis of ^1H and ^{13}C NMR spectral data (vicinal couplings, shielding effects and 2D NOE correlations). Several isoxazolidines exist in preferred conformations including those obtained from vinyloxymethylphosphonate in which the phosphonylmethoxy groups are oriented pseudoaxially due to the anomeric effect.

All synthesized compounds were evaluated against a broad-spectrum of DNA and RNA viruses but they were found to be inactive at concentrations up to 250 μM. Also, the compounds did not show significant cytostatic activity against murine leukemia L1210, human CD_4^+ T-lymphocyte CEM, human cervix carcinoma HeLa and human dermal microvascular endothelial cells.

Although the tested compounds contained biologically relevant fragments (nucleobases, the isoxazolidine ring and a phosphonate) they surprisingly did not show appreciable antiviral and anticancer activities. Since the isoxazolidine subunit can be also found in several structures endowed with antibacterial and antifungal activities we would progress along this line soon to hopefully discover new therapeutic applications for this class of compounds.

4. Experimental section

^1H NMR spectra were taken in $CDCl_3$, CD_3OD and D_2O on the following spectrometers: Varian Gemini 2000BB (200 MHz), Varian Mercury-300 and Bruker Avance III (600 MHz) with TMS as internal standard. ^{13}C NMR spectra were recorded for $CDCl_3$, CD_3OD and D_2O solution on the Bruker Avance III at 150 MHz and Varian Mercury-300 machine at 75 MHz. ^{31}P NMR spectra were performed in $CDCl_3$, CD_3OD and D_2O solution on the Varian Gemini 2000BB at 80.0 MHz, Varian Mercury-300 at 121 MHz or on Bruker Avance III at 242 MHz.

IR spectra were measured on an Infinity MI-60 FT-IR spectrometer. Melting points were determined on Boetius apparatus and are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory of this Faculty on Perkin-Elmer PE 2400 CHNS analyzer.

The following adsorbents were used: column chromatography, Merck silica gel 60 (70–230 mesh); analytical TLC, Merck TLC plastic sheets silica gel 60 F_{254}.

Preparative HPLC experiment was performed on a Waters apparatus equipped with Waters 2545 binary gradient module and Waters 2998 photodiode array detector (190–600 nm).

4.1. General procedure for the isoxazolidines cis-*21* and trans-*22*

A mixture of nitrone **20** (1.0 mmol) and allyl alcohol (1.0 mL) was stirred at 60 °C or irradiated in a Plazmatronika RM800 microwave reactor at 60–85 °C for the time shown in Table 1. All volatiles were removed in vacuo and the crude product was purified on silica gel column using chloroform–MeOH (10:1, 5:1, v/v) as the eluent to afford pure isoxazolidines **21** and **22**. For details see Table 1.

*4.1.1. cis-1 - 16 -((5-(Hydroxymethyl)-2-methylisoxazolidin-3-yl) methyl)pyrimidine-2,4(1H,3H)-dione (**21a**)*

Yield: 68% (0.255 g from 1.56 mmol of the nitrone **20a**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3432, 3186, 3048, 2961, 2882, 1695, 1662, 1462, 1045; ^1H NMR (300 MHz, CD_3OD) δ: 7.90 (brs, 1H, NH), 7.57 (d, 1H, J = 7.9 Hz), 5.60 (d, 1H, J = 7.9 Hz), 4.40 (dddd, 1H, J = 8.7 Hz, J = 6.6 Hz, J = 4.5 Hz, J = 3.0 Hz, H-C5), 3.83 (dd, 1H, J = 13.5 Hz, J = 4.8 Hz, *H*CHN), 3.70 (dd, 1H, J = 13.3 Hz, J = 3.0 Hz, *H*CHOH), 3.61 (dd, 1H, J = 13.5 Hz, J = 9.0 Hz, HC*H*N), 3.60 (dd, 1H, J = 13.3 Hz, J = 4.5 Hz, HC*H*OH), 3.39 (dddd, 1H, J = 9.0 Hz, J = 8.7 Hz, J = 4.8 Hz, J = 3.3 Hz, H-C3), 2.60 (s, 3H, CH_3N), 2.59 (ddd, 1H, J = 12.9 Hz, J = 8.7 Hz, J = 8.7 Hz, H_a-C4), 1.79 (ddd, 1H, J = 12.9 Hz, J = 6.6 Hz, J = 3.3 Hz, H_b-C4); ^{13}C NMR (75 MHz, CD_3OD) δ: 166.8 (C=O), 152.9 (C=O), 148.8 (C=C), 101.3 (C=C), 78.3 (C5), 66.8 (C3), 63.1 (CH_2OH), 52.2 (CH_2N), 44.6 (CH_3N), 33.2 (C4). Anal. Calcd. for $C_{10}H_{15}N_3O_4$: C, 49.79; H, 6.27; N, 17.42. Found: C, 49.62; H, 6.11; N, 17.61.

*4.1.2. trans-1 - 16 -((5-(Hydroxymethyl)-2-methylisoxazolidin-3-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**22a**)*

Yield: 25% (0.095 g from 1.56 mmol of the nitrone **20a**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3430, 3223, 3052, 2960, 2881, 1680, 1461, 1386, 1252, 1041; ^1H NMR (300 MHz, CD_3OD) δ: 7.91 (brs, 1H, NH), 7.57 (d, 1H, J = 7.8 Hz), 5.63 (d, 1H, J = 7.8 Hz), 4.12 (dddd, 1H, J = 8.1 Hz, J = 7.8 Hz, J = 5.4 Hz, J = 3.9 Hz, H-C5), 3.88 (dd, 1H, J = 13.8 Hz, J = 5.4 Hz, *H*CHN), 3.71 (dd, 1H, J = 13.8 Hz, J = 7.5 Hz, HC*H*N), 3.65 (dd, 1H, J = 12.0 Hz, J = 3.9 Hz, *H*CHOH), 3.56 (dd, 1H, J = 12.0 Hz, J = 5.4 Hz, HC*H*OH), 3.25 (dddd, 1H, J = 8.1 Hz, J = 7.5 Hz, J = 5.4 Hz, J = 4.2 Hz, HC3), 2.66 (s, 3H, CH_3N), 2.31 (ddd, 1H, J = 12.6 Hz, J = 8.1 Hz, J = 8.1 Hz, H_a-C4), 2.10 (ddd, 1H, J = 12.6 Hz, J = 7.8 Hz, J = 4.2 Hz, H_b-C4); ^{13}C NMR (75 MHz, CD_3OD) δ: 166.8 (C=O), 153.0 (C=O), 148.2 (C=C), 101.8 (C=C), 80.4 (C5), 67.2 (C3), 64.8 (CH_2-OH), 51.0 (CH_2N), 45.3 (CH_3N), 34.5 (C4). Anal. Calcd. for $C_{10}H_{15}N_3O_4$: C, 49.79; H, 6.27; N, 17.42. Found: C, 50.03; H, 6.34; N, 17.53.

*4.1.3. cis-5-Fluoro-1-((5-(hydroxymethyl)-2-methylisoxazolidin-3-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**21b**)*

Yield: 44% (0.156 g from 0.74 mmol of the nitrone **20b**); white amorphous solid (crystallized from methanol) mp 162–164 °C; IR

(KBr, cm^{-1}) ν_{max}: 3477, 3171, 3052, 2918, 2828, 1701, 1661, 1245, 1048; ^1H NMR (300 MHz, CD_3OD) δ: 7.71 (d, 1H, J = 6.3 Hz), 4.41 (dddd, 1H, J = 8.7 Hz, J = 6.6 Hz, J = 4.6 Hz, J = 3.1 Hz, H-C5), 3.81 (dd, 1H, J = 13.9 Hz, J = 4.8 Hz, *H*CHN), 3.71 (dd, 1H, J = 12.1 Hz, J = 3.1 Hz, *H*CHOH), 3.60 (dd, 1H, J = 12.1 Hz, J = 4.6 Hz, HC*H*OH), 3.56 (dd, 1H, J = 13.9 Hz, J = 9.1 Hz, HC*H*N), 3.40 (dddd, 1H, J = 9.1 Hz, J = 8.7 Hz, J = 4.8 Hz, J = 3.3 Hz, H-C3), 2.62 (s, 3H, CH_3N), 2.60 (ddd, 1H, J = 12.9 Hz, J = 8.7 Hz, J = 8.7 Hz, H_a-C4), 1.80 (ddd, 1H, J = 12.9 Hz, J = 6.6 Hz, J = 3.3 Hz, H_b-C4); ^{13}C NMR (75 MHz, CD_3OD) δ: 159.8 (d, ^2J = 25.8 Hz, C=O), 151.5 (C=O), 140.9 (d, ^1J = 229.0 Hz, (C=C), 132.8 (d, ^2J = 33.2 Hz, (C=C), 78.3 (C5), 66.8 (C3), 63.0 (CH_2OH), 52.2 (CH_2N), 44.5 (CH_3N), 33.1 (C4). Anal. Calcd. for $C_{10}H_{14}FN_3O_4$: C, 46.33; H, 5.44; N, 16.21. Found: C, 46.05; H, 5.47; N, 16.27.

4.1.4. *trans-5-Fluoro-1-((5-(hydroxymethyl)-2-methylisoxazolidin-3-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**22b**)*

Yield: 21% (0.094 g from 0.74 mmol of the nitrone **20b**); white amorphous solid (crystallized from methanol) mp 158–160 °C; IR (KBr, cm^{-1}) ν_{max}: 3418, 3063, 2991, 2920, 2825, 1696, 1662, 1470, 1227, 1026; ^1H NMR (300 MHz, CD_3OD) δ: 7.81 (d, 1H, J = 6.3 Hz), 4.13 (dddd, 1H, J = 8.1 Hz, J = 7.5 Hz, J = 5.4 Hz, J = 3.9 Hz, H-C5), 3.84 (dd, 1H, J = 13.8 Hz, J = 5.1 Hz, *H*CHN), 3.67 (dd, 1H, J = 13.8 Hz, J = 7.2 Hz, HC*H*N), 3.65 (dd, 1H, J = 12.0 Hz, J = 3.9 Hz, *H*CHOH), 3.56 (dd, 1H, J = 12.0 Hz, J = 5.4 Hz, HC*H*OH), 3.27 (dddd, 1H, J = 8.1 Hz, J = 7.2 Hz, J = 5.1 Hz, J = 4.2 Hz, H-C3), 2.67 (s, 3H, CH_3N), 2.31 (ddd, 1H, J = 12.9 Hz, J = 8.1 Hz, J = 8.1 Hz, H_a-C4), 2.11 (ddd, 1H, J = 12.9 Hz, J = 7.5 Hz, J = 4.2 Hz, H_b-C4); ^{13}C NMR (75 MHz, CD_3OD) δ: 159.8 (d, ^2J = 25.1 Hz, C=O), 151.8 (C=O), 141.2 (d, ^1J = 230.6 Hz, (C=C)), 132.1 (d, ^2J = 32.9 Hz, (C=C), 80.5 (C5), 67.2 (C3), 64.9 (CH_2OH), 51.0 (CH_2N), 46.2 (CH_3N), 34.4 (C4). Anal. Calcd. for $C_{10}H_{14}FN_3O_4$: C, 46.33; H, 5.44; N, 16.21. Found: C, 46.57; H, 5.57; N, 16.26.

4.1.5. *cis-5-Bromo-1-((-5-(hydroxymethyl)-2-methylisoxazolidin-3-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**21c**)*

Yield: 26% (0.202 g from 2.47 mmol of the nitrone **20c**); white amorphous solid (crystallized from ethyl acetate/hexane) mp 180–182 °C; IR (KBr, cm^{-1}) ν_{max}: 3397, 3143, 3060, 3037, 2992, 2825, 1703, 1680, 1621, 1428, 1346, 1115; ^1H NMR (300 MHz, CD_3OD) δ: 7.98 (s, 1H), 4.41 (dddd, 1H, J = 8.4 Hz, J = 6.6 Hz, J = 4.8 Hz, J = 2.7 Hz, H-C5), 3.84 (dd, 1H, J = 13.2 Hz, J = 4.5 Hz, *H*CHN), 3.72 (dd, 1H, J = 12.3 Hz, J = 2.7 Hz, *H*CHOH), 3.61 (dd, 1H, J = 12.3 Hz, J = 4.8 Hz, HC*H*OH), 3.59 (dd, 1H, J = 13.2 Hz, J = 9.3 Hz, HC*H*N), 3.40 (dddd, 1H, J = 9.3 Hz, J = 8.4 Hz, J = 4.5 Hz, J = 3.3 Hz, H-C3), 2.61 (s, 3H, CH_3N), 2.60 (ddd, 1H, J = 13.2 Hz, J = 8.4 Hz, J = 8.4 Hz, H_a-C4), 1.79 (ddd, 1H, J = 13.2 Hz, J = 6.6 Hz, J = 3.3 Hz, H_b-C4); ^{13}C NMR (75 MHz, CD_3OD) δ: 162.1 (C=O), 152.2 (C=O), 148.3 (C=C), 95.5 (C=C), 78.3 (C5), 66.7 (C3), 63.0 (CH_2OH), 52.4 (CH_2N), 44.5 (CH_3N), 32.9 (C4). Anal. Calcd. for $C_{10}H_{14}BrN_3O_4$: C, 37.52; H, 4.41; N, 13.13. Found: C, 37.70; H, 4.33; N, 13.10.

4.1.6. *trans-5-Bromo-1-((-5-(hydroxymethyl)-2-methylisoxazolidin-3-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**22c**)*

White amorphous solid; IR (KBr, cm^{-1}) ν_{max}: 3450, 3150, 3094, 2972, 2927, 2838, 1686, 1612, 1466, 1432, 1329, 1107; (signals of *trans*-**22c** were extracted from the spectra of an 80:20 mixture of *trans*-**22c** and *cis*-**21c**); ^1H NMR (300 MHz, CD_3OD) δ: 8.00 (s, 1H), 4.13 (dddd, 1H, J = 8.1 Hz, J = 7.5 Hz, J = 5.4 Hz, J = 4.2 Hz, H-C5), 3.89 (dd, 1H, J = 13.8 Hz, J = 4.8 Hz, *H*CHN), 3.70 (dd, 1H, J = 13.8 Hz, J = 8.4 Hz, HC*H*N), 3.66 (dd, 1H, J = 12.0 Hz, J = 4.2 Hz, *H*CHOH), 3.56 (dd, 1H, J = 12.0 Hz, J = 5.4 Hz, HC*H*OH), 3.28 (dddd, 1H, J = 8.4 Hz, J = 7.8 Hz, J = 4.8 Hz, J = 3.9 Hz, *H*-C3), 2.67 (s, 3H, CH_3N), 2.32 (ddd, 1H, J = 12.6 Hz, J = 8.1 Hz, J = 7.8 Hz, H_a-C4), 2.11 (ddd, 1H, J = 12.6 Hz, J = 7.8 Hz, J = 3.9 Hz, H_b-C4); ^{13}C NMR (75 MHz, CD_3OD) δ: 162.1 (C=O), 152.3 (C=O), 147.6 (C=C), 96.0 (C=C), 80.6 (C5), 67.1 (C3), 65.0 (CH_2OH), 51.1 (CH_2N), 46.2 (CH_3N), 34.3 (C4). Anal. Calcd. for $C_{10}H_{14}BrN_3O_4$: C, 37.52; H, 4.41; N, 13.13. Found: C, 37.54; H, 4.26; N, 12.97 (obtained on an 80:20 mixture of *trans*-**22c** and *cis*-**21c**).

4.1.7. *cis-1-((5-(Hydroxymethyl)-2-methylisoxazolidin-3-yl)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**21d**)*

Yield: 37% (0.156 g from 1.67 mmol of the nitrone **20d**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3417, 3196, 3062, 2929, 1687,1458, 1388, 1052; ^1H NMR (600 MHz, CD_3OD) δ: 7.44 (q, J = 1.2 Hz, 1H), 4.42 (dddd, 1H, J = 8.5 Hz, J = 6.7 Hz, J = 4.7 Hz, J = 3.2 Hz, H-C5), 3.82 (dd, 1H, J = 13.9 Hz, J = 4.9 Hz, *H*CHN), 3.72 (dd, 1H, J = 12.2 Hz, J = 3.2 Hz, *H*CHOH), 3.64 (dd, 1H, J = 12.2 Hz, J = 4.7 Hz, HC*H*OH), 3.63 (dd, 1H, J = 13.9 Hz, J = 9.2 Hz, HC*H*N), 3.42 (dddd, 1H, J = 9.2 Hz, J = 8.2 Hz, J = 4.9 Hz, J = 3.5 Hz, H-C3), 2.63 (s, 3H, CH_3N), 2.61 (ddd, 1H, J = 12.8 Hz, J = 8.5 Hz, J = 8.2 Hz, H_a-C4), 1.88 (d, J = 1.2 Hz, 3H, C*H*$_3$),1.82 (ddd, 1H, J = 12.8 Hz, J = 6.7 Hz, J = 3.5 Hz, H_b-C4); ^{13}C NMR (150 MHz, CD_3OD) δ: 165.5 (C=O), 151.7 (C=O), 143.4 (C=C), 108.8 (C=C), 76.9 (C5), 65.5 (C3), 61.9 (CH_2OH), 50.7 (CH_2N), 43.2 (CH_3N), 32.0 (C4), 10.9 (CH_3). Anal. Calcd. for $C_{11}H_{17}N_3O_4$: C, 51.76; H, 6.71; N, 16.46. Found: C, 51.99; H, 6.77; N, 16.36.

4.1.8. *trans-1-((5-(Hydroxymethyl)-2-methylisoxazolidin-3-yl)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**22d**)*

Yield: 22% (0.094 g from 1.67 mmol of the nitrone **20d**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3335, 2923, 2854, 1667, 1441, 1377, 1261, 1041; ^1H NMR (600 MHz, CD_3OD) δ: 7.44 (q, J = 1.0 Hz, 1H), 4.17–4.13 (m, 1H, H-C5), 3.87 (dd, 1H, J = 14.1 Hz, J = 5.6 Hz, *H*CHN), 3.72 (dd, 1H, J = 14.1 Hz, J = 6.5 Hz, HC*H*N), 3.67 (dd, 1H, J = 11.9 Hz, J = 3.9 Hz, *H*CHOH), 3.58 (dd, 1H, J = 11.9 Hz, J = 5.5 Hz, HC*H*OH), 3.30–3.24 (m, 1H, H-C3), 2.69 (s, 3H, CH_3N), 2.32 (ddd, 1H, J = 12.7 Hz, J = 7.9 Hz, J = 7.9 Hz, H_a-C4), 2.13 (ddd, 1H, J = 12.7 Hz, J = 7.7 Hz, J = 4.6 Hz, H_b-C4), 1.89 (d, J = 1.0 Hz, 3H, CH_3). ^{13}C NMR (150 MHz, CD_3OD) δ: 165.2 (C=O), 151.7 (C=O), 142.6 (C=C), 109.3 (C=C), 78.8 (C5), 65.9 (C3), 63.3 (CH_2OH), 49.5 (CH_2N), 42.2 (CH_3N), 33.2 (C4), 10.9 (CH_3). Anal. Calcd. for $C_{11}H_{17}N_3O_4$: C, 51.76; H, 6.71; N, 16.46. Found: C, 52.00; H, 6.79; N, 16.35.

4.1.9. *cis-3-((6-Amino-9H-purin-9-yl)methyl)-2-methylisoxazolidin-5-yl)methanol (**21e**)*

Yield: 21% (0.066 g from 1.20 mmol of the nitrone **20e**); white amorphous solid (crystallized from methanol) mp 225–227 °C; IR (KBr, cm^{-1}) ν_{max}: 3400, 3311, 3133, 2937, 2855, 1651, 1603, 1418, 1299, 1055; ^1H NMR (300 MHz, D_2O) δ: 7.96 (s, 1H), 7.93 (s, 1H), 4.44–4.36 (m, 1H, H-C5), 4.15 (dd, 1H, J = 14.4 Hz, J = 8.7 Hz, *H*CHN), 4.02 (dd, 1H, J = 14.4 Hz, J = 5.4 Hz, HC*H*N), 3.66 (dd, 1H, J = 12.6 Hz, J = 2.7 Hz, *H*CHOH), 3.50 (dd, 1H, J = 12.6 Hz, J = 6.0 Hz, HC*H*OH), 3.59–3.49 (m, 1H, H-C3), 2.56 (ddd, 1H, J = 13.2 Hz, J = 8.4 Hz, J = 8.4 Hz, H_a-C4), 2.48 (s, 3H, CH_3N), 1.71 (ddd, 1H J = 13.2 Hz, J = 7.2 Hz, J = 4.5 Hz, H_b-C4); ^{13}C NMR (75 MHz, D_2O) δ: 155.3, 152.3, 148.6, 142.4, 118.1, 77.3 (C5), 66.2 (C3), 61.9 (CH_2OH), 46.3 (CH_2N), 43.9 (CH_3N), 32.7 (C4). Anal. Calcd. for $C_{11}H_{16}N_6O_2$: C, 49.99; H, 6.10; N, 31.80. Found: C, 50.23; H, 6.06; N, 32.01.

4.1.10. *trans-3-((6-Amino-9H-purin-9-yl)methyl)-2-methylisoxazolidin-5-yl)methanol (**22e**)*

Yellowish amorphous solid; IR (KBr, cm^{-1}) ν_{max}: 3361, 3301, 3126, 2923, 2853, 1650, 1601, 1419, 1370, 1211, 1058; (signals of *trans*-**22e** were extracted from the spectra of a 72:28 mixture of *trans*-**22e** and *cis*-**21e**); ^1H NMR (300 MHz, D_2O) δ: 8.04 (s, 1H), 8.01 (s, 1H), 4.25–4.04 (m, 3H, CH_2N, H-C5), 3.67 (dd, 1H, J = 12.3 Hz, J = 3.3 Hz, *H*CHOH), 3.52 (dd, 1H, J = 12.3 Hz, J = 6.0 Hz, HC*H*OH), 3.51–3.40 (m, 1H, H-C3), 2.62 (s, 3H, CH_3N), 2.24 (ddd, 1H,

J = 12.6 Hz, J = 7.8 Hz, J = 7.8 Hz, H_a-C4), 2.10 (ddd, 1H, J = 12.6 Hz, J = 7.2 Hz, J = 5.7 Hz, H_b-C4); ^{13}C NMR (150 MHz, CD_3OD) δ: 155.9, 152.4, 149.5, 142.2, 118.4, 77.0 (C5), 66.2 (C3), 63.1 (CH_2OH), 46.0 (CH_2N), 43.0 (CH_3N), 32.1 (C4). Anal. Calcd. for $C_{11}H_{16}N_6O_2$: C, 49.99; H, 6.10; N, 31.80. Found: C, 50.22; H, 6.01; N, 31.53 (obtained on a 72:28 mixture of *trans*-**22e** and *cis*-**21e**).

4.2. General procedure for the cycloaddition of nitrone **20** with alkenylphosphonate **23**, **24**, **25** and **26**

A solution of a nitrone **20** (1.0 mmol) and an alkenylphosphonate **23**, **24**, **25** or **26** (3.0 mmol) in CH_3CN or dioxane was irradiated in a Plazmatronika RM800 microwave reactor at 65–80 °C for the time shown in Tables 2 and 3 All volatiles were removed in vacuo and the crude product was purified on silica gel column using chloroform–MeOH (10:1, 5:1, v/v) as the eluent to afford pure isoxazolidines **21**/**22**, **27**/**28**, **29**/**30**, **31**/**32** or **33**/**34**. For details see Tables 2 and 3

4.2.1. Diethyl cis-((3-((2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)methyl)phosphonate (**27a**)

Yield: 22% (0.130 g from 1.63 mmol of the nitrone **20a**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3454, 3164, 3051, 2984, 2872, 2823, 1689, 1632, 1455, 1250, 1025. ^{1}H NMR (300 MHz, CD_3OD) δ: 7.58 (d, 1H, J = 7.9 Hz), 5.62 (d, 1H, J = 7.9 Hz), 4.55 (ddddd, 1H, J = 7.9 Hz, J = 7.4 Hz, J = 7.2 Hz, J = 6.9 Hz, J = 6.0 Hz, *H*-C5), 4.22–4.12 (m, 4H, 2 × POC*H*$_2$CH$_3$), 3.88 (dd, 1H, J = 13.9 Hz, J = 4.6 Hz, *H*CHN), 3.60 (dd, 1H, J = 13.9 Hz, J = 8.5 Hz, HC*H*N), 3.36 (dddd, 1H, J = 8.5 Hz, J = 7.9 Hz, J = 4.6 Hz, J = 4.2 Hz, H-C3), 2.75 (ddd, 1H, J = 13.5 Hz, J = 7.9 Hz, J = 7.9 Hz, H_a-C4), 2.62 (s, 3H, CH_3), 2.22 (ddd, 1H, J = 22.2 Hz, J = 15.0 Hz, J = 6.0 Hz, *H*CHP), 2.17 (ddd, 1H, J = 22.2 Hz, J = 15.0 Hz, J = 7.2 Hz, HC*H*P), 1.78 (ddd, 1H, J = 13.5 Hz, J = 6.9 Hz, J = 4.2 Hz, H_b-C4), 1.33 (t, 6H, J = 7.0 Hz, 2 × POCH$_2$C*H*$_3$). ^{13}C NMR (75 MHz, CD_3OD) δ: 166.6 (C=O), 152.8 (C=O), 148.5 (C=C), 101.5 (C=C), 72.3 (C5), 67.5 (C3), 63.7 (d, J = 6.3 Hz, POCH$_2$), 63.5 (d, J = 6.6 Hz, POCH$_2$), 52.1 (CH_2N), 44.8 (NCH_3), 38.9 (d, J = 7.7 Hz, C4), 31.5 (d, J = 139.7 Hz, CH_2P), 16.9 (d, J = 6.0 Hz, 2 × POCH$_2$C*H*$_3$). ^{31}P NMR (121.5 MHz, CD_3OD) δ: 29.11. Anal. Calcd. for $C_{14}H_{24}N_3O_6P$: C, 46.54; H, 6.70; N, 11.63. Found: C, 46.40; H, 6.90; N, 11.61.

4.2.2. Diethyl trans-((3-((2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)methyl)phosphonate (**28a**)

Colorless oil; IR (film, cm^{-1}) ν_{max}: 3459, 3173, 3051, 2985, 2911, 2823, 1679, 1631, 1453, 1228, 1023. (signals of *trans*-**28a** were extracted from the spectra of a 6:4 mixture of *trans*-**28a** and *cis*-**27a**); ^{1}H NMR (600 MHz, CD_3OD) δ: 7.59 (d, 1H, J = 7.9 Hz), 5.66 (d, 1H, J = 7.9 Hz), 4.32–4.26 (brsx, 1H, $J \approx$ 7.0 Hz, H-C5), 4.18–4.09 (m, 4H, 2 × CH_2OP), 3.89 (dd, 1H, J = 14.1 Hz, J = 5.2 Hz, *H*CHN), 3.78 (dd, 1H, J = 14.1 Hz, J = 7.0 Hz, HC*H*N), 3.28 (brqu, 1H, $J \approx$ 7.0 Hz, H-C3), 2.70 (s, 3H, CH_3N), 2.31 (dd, 2H, J = 7.4 Hz, J = 6.7 Hz, H_2C4), 2.28 (ddd, 1H, J = 18.5 Hz, J = 15.2 Hz, J = 6.3 Hz, *H*CHP), 2.18 (ddd, 1H, J = 18.9 Hz, J = 15.2 Hz, J = 7.2 Hz, HC*H*P), 1.34 (t, 6H, J = 7.1 Hz, 2 × C*H*$_3$CH$_2$OP). ^{13}C NMR (150 MHz, CD_3OD) δ: 165.3 (C=O), 151.6 (C=O), 146.8 (C=C), 100.4 (C=C), 72.5 (C5), 65.9 (C3), 62.2 (d, J = 6.5 Hz, POCH$_2$), 62.0 (d, J = 6.5 Hz, POCH$_2$), 49.5 (CH_2N), 48.5 (NCH_3), 38.1 (d, J = 8.1 Hz, C4), 31.4 (d, J = 139.7 Hz, CH_2P), 15.3 (d, J = 6.9 Hz, 2 × POCH$_2$C*H*$_3$). ^{31}P NMR (242 MHz, CD_3OD) δ: 27.59. Anal. Calcd. for $C_{14}H_{24}N_3O_6P$: C, 46.54; H, 6.70; N, 11.63. Found: C, 46.32; H, 6.75; N, 11.53 (obtained on a 6:4 mixture of *trans*-**28a** and *cis*-**27a**).

4.2.3. Diethyl cis-((3-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)methyl)phosphonate (**27b**)

Yield: 3% (0.011 g from 0.88 mmol of the nitrone **20b**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3410, 3185, 3064, 2985, 2962, 2922, 2851, 2820, 1698, 1664, 1466, 1444, 1376, 1098, 966; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.84 (brs, 1H, NH), 7.57 (d, 1H, J = 5.8 Hz), 4.59 (ddddd, 1H, J = 9.0 Hz, J = 8.6 Hz, J = 7.9 Hz, J = 7.9 Hz, J = 4.6 Hz, H-C5), 4.21–4.11 (m, 4H, 2 × CH_2OP), 4.02 (dd, 1H, J = 13.5 Hz, J = 3.0 Hz, *H*CHN), 3.36 (dddd, 1H, J = 9.7 Hz, J = 7.9 Hz, J = 3.8 Hz, J = 3.0 Hz, H-C3), 3.30 (dd, 1H, J = 13.5 Hz, J = 9.7 Hz, HC*H*N), 2.75 (ddd, 1H, J = 13.3 Hz, J = 7.9 Hz, J = 7.9 Hz, H_a-C4), 2.64 (s, 3H, CH_3N), 2.25 (ddd, 1H, J = 19.6 Hz, J = 14.9 Hz, J = 4.6 Hz, *H*CHP), 1.99 (ddd, 1H, J = 18.2 Hz, J = 14.9 Hz, J = 8.6 Hz, HC*H*P), 1.76 (ddd, 1H, J = 13.3 Hz, J = 7.9 Hz, J = 3.8 Hz, H_b-C4), 1.37 (t, 6H, J = 7.0 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 157.0 (d, ^2J = 26.4 Hz, C=O), 149.3 (C=O), 139.8 (d, ^1J = 234.3 Hz, C=C), 130.8 (d, ^2J = 32.9 Hz, C=C), 71.0 (C5), 66.30 (C3), 62.0 (d, J = 6.8 Hz, CH_2OP), 62.0 (d, J = 6.8 Hz, CH_2OP), 51.6 (CH_2N), 44.5 (CH_3N), 37.6 (d, J = 4.5 Hz, C4), 30.8 (d, J = 139.9 Hz, CP), 16.4 (d, J = 5.8 Hz, 2 × C*H*$_3$CH$_2$OP); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 26.24. Anal. Calcd. for $C_{14}H_{23}FN_3O_6P$: C, 44.33; H, 6.11; N, 11.08. Found: C, 44.43; H, 6.18; N, 11.05.

4.2.4. Diethyl trans-((3-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)methyl)phosphonate (**28b**)

Colorless oil; IR (film, cm^{-1}) ν_{max}: 3399, 3194, 3071, 2988, 2925, 2851, 2821, 1696, 1663, 1470, 1443, 1242, 1050, 968; (signals of *trans*-**28b** were extracted from the spectra of a 2:8 mixture of *trans*-**28b** and *cis*-**27b**); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.20 (brs, 1H, NH), 7.49 (d, 1H, J = 5.7 Hz), 4.35–4.31 (m, 1H, H-C5), 4.20–4.11 (m, 4H, 2 × CH_2OP), 3.95 (dd, 1H, J = 13.9 Hz, J = 3.7 Hz, *H*CHN), 3.48 (dd, 1H, J = 13.9 Hz, J = 8.6 Hz, HC*H*N), 3.38–3.34 (m, 1H, H-C3), 2.87–2.75 (m, 1H, H_a-C4), 2.69 (s, 3H, CH_3N), 2.38–2.29 (m, 2H, *H*CHP, H_b-C4), 2.08–2.03 (m, 1H, *H*CHP), 1.37 (t, 6H, J = 7.0 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 157.2 (d, J = 26.3 Hz, C=O), 149.6 (C=O), 139.8 (d, J = 226.4 Hz, C=C), 130.4 (d, J = 32.9 Hz, C=C), 71.6 (C5), 66.0 (C3), 62.0 (d, J = 6.4 Hz, CH_2OP), 61.9 (d, J = 6.1 Hz, CH_2OP), 51.2 (CH_2N), 46.3 (CH_3N), 38.0 (d, J = 3.4 Hz, C4), 33.2 (d, J = 137.7 Hz, CP), 16.4 (d, J = 5.8 Hz, 2 × C*H*$_3$CH$_2$OP); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 25.87. Anal. Calcd. for $C_{14}H_{23}FN_3O_6P$: C, 44.33; H, 6.11; N, 11.08. Found: C, 44.47; H, 5.98; N, 11.17 (obtained on a 2:8 mixture of *trans*-**28b** and *cis*-**27b**).

4.2.5. Diethyl cis-((3-((5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)methyl)phosphonate (**27c**)

Yield: 4% (0.018 g from 1.03 mmol of the nitrone **20c**); yellow oil; IR (film, cm^{-1}) ν_{max}: 3475, 3158, 3090, 2988, 2913, 2833, 1682, 1617, 1443, 1431, 1251, 1226, 1022, 963; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.18 (brs, 1H, NH), 7.77 (s, 1H), 4.59 (ddddd, 1H, J = 8.7 Hz, J = 7.8 Hz, J = 7.8 Hz, J = 7.5 Hz, J = 4.7 Hz, H-C5), 4.19–4.11 (m, 4H, 2 × CH_2OP), 4.09–4.03 (m, 1H, *H*CHN), 3.38–3.33 (m, 2H, HC*H*N, H-C3), 2.88–2.73 (m, 1H, H_a-C4), 2.63 (s, 3H, CH_3N), 2.26 (ddd, 1H, J = 17.6 Hz, J = 14.9 Hz, J = 4.7 Hz, *H*CHP), 1.99 (ddd, 1H, J = 18.3 Hz, J = 14.9 Hz, J = 8.7. Hz, HC*H*P), 1.75 (ddd, 1H, J = 13.4 Hz, J = 7.5 Hz, J = 3.4 Hz, H_b-C4), 1.37 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP), 1.35 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.5 (C=O), 150.2 (C=O), 145.8 (C=C), 95.5 (C=C), 71.0 (C5), 66.1 (C3), 62.0 (d, J = 7.3 Hz, CH_2OP), 62.0 (d, J = 7.2 Hz, CH_2OP), 51.8 (CH_2N), 44.5 (CH_3N), 37.6 (d, J = 4.4 Hz, C4), 30.8 (d, J = 140.8 Hz, CP), 16.4 (d, J = 5.9 Hz, 2 × C*H*$_3$CH$_2$OP); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 26.19. Anal. Calcd. for $C_{14}H_{23}BrN_3O_6P$: C, 38.20; H, 5.27; N, 9.55. Found: C, 38.05; H, 5.04; N, 9.69.

4.2.6. Diethyl trans-((3-((5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)methyl)phosphonate (**28c**)

Yellow oil; IR (film, cm^{-1}) ν_{max}: 3443, 3175, 2985, 2925, 2853,

2822, 1688, 1620, 1442, 1247, 1025, 965; (signals of *trans* were extracted from the spectra of a 20:80 mixture of *trans* and *cis*); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.02 (brs, 1H, NH), 7.73 (s, 1H), 4.38–4.33 (m, 1H, H-C5), 4.21–4.12 (m, 4H, 2 × CH_2OP), 4.09–4.03 (m, 1H, *H*CHN), 3.54 (dd, 1H, J = 13.5 Hz, J = 9.1 Hz, HC*H*N), 3.37–3.34 (m, 1H, H-C3), 2.71 (s, 3H, CH_3N), 2.42–2.30 (m, 3H, H_2C4, *H*CHP), 2.08–2.03 (m, 1H, HC*H*P), 1.38–1.35 (m, 6H, 2 × CH_3CH_2OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.4 (C═O), 150.3 (C═O), 145.4 (C═C), 95.8 (C═C), 73.4 (C5), 65.8 (C3), 61.9 (d, J = 6.4 Hz, 2 × CH_2OP), 50.7 (CH_2N), 46.3 (CH_3N), 38.0 (d, J = 5.9 Hz, C4), 33.2 (d, J = 138.7 Hz, CP), 16.4 (d, J = 5.8 Hz, 2 × CH_3CH_2OP); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 25.71. Anal. Calcd. for $C_{14}H_{23}BrN_3O_6P$: C, 38.20; H, 5.27; N, 9.55. Found: C, 38.05; H, 5.02; N, 9.64 (obtained on a 2:8 mixture of *trans*-**28c** and *cis*-**27c**).

4.2.7. Diethyl cis-((2-methyl-3-((5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)isoxazolidin-5-yl)methyl) phosphonate (*27d*)

Yield: 21% (0.102 g from 1.27 mmol of the nitrone **20d**); yellow oil; IR (film, cm^{-1}) ν_{max}: 3406, 3163, 3042, 2984, 2928, 2816, 1689, 1466, 1369, 1250, 1024; ^{1}H NMR (300 MHz, CD_3OD) δ: 7.42 (q, J = 1.2 Hz, 1H), 4.55 (ddddd, 1H, J = 7.9 Hz, J = 7.4 Hz, J = 7.1 Hz, J = 7.0 Hz, J = 6.3 Hz, H-C5), 4.18–4.07 (m, 4H, 2 × CH_2OP), 3.85 (dd, 1H, J = 13.9 Hz, J = 5.0 Hz, *H*CHN), 3.59 (dd, 1H, J = 13.9 Hz, J = 8.2 Hz, HC*H*N), 3.37 (dddd, 1H, J = 8.2 Hz, J = 7.9 Hz, J = 5.0 Hz, J = 4.6 Hz, H-C3), 2.74 (ddd, 1H, J = 13.0 Hz, J = 7.9 Hz, J = 7.9 Hz, H_a-C4), 2.62 (s, 3H, CH_3N), 2.25 (ddd, 1H, J = 21.4 Hz, J = 15.3 Hz, J = 6.3 Hz, *H*CHP), 2.14 (ddd, 1H, J = 22.4 Hz, J = 15.3 Hz, J = 7.1 Hz, HC*H*P), 1.86 (d, 3H, J = 1.2 Hz, CH_3), 1.78 (ddd, 1H, J = 13.0 Hz, J = 7.0 Hz, J = 4.6 Hz, H_b-C4), 1.34 (t, 6H, J = 6.9 Hz, 2 × CH_3CH_2OP); ^{13}C NMR (75 MHz, CD_3OD) δ: 166.8 (C═O), 152.9 (C═O), 144.3 (C═C), 110.4 (C═C), 72.3 (d, J = 9.9 Hz, C5), 67.6 (C3), 63.7 (d, J = 6.4 Hz, CH_2OP), 63.5 (d, J = 6.4 Hz, CH_2OP), 52.0 (CH_2N), 44.9 (CH_3N), 39.0 (d, J = 7.4 Hz, CH_2), 31.6 (d, J = 138.9 Hz, CP), 16.9 (d, J = 6.0 Hz, CH_3CH_2OP), 16.9 (d, J = 6.0 Hz, CH_3CH_2OP), 12.4 (CH_3); ^{31}P NMR (121 MHz, CD_3OD) δ: 29.13. Anal. Calcd. for $C_{15}H_{26}N_3O_6P$: C, 48.00; H, 6.98; N, 11.19. Found: C, 48.25; H, 6.93; N, 11.23.

4.2.8. Diethyl cis-(((3-((2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) methyl)-2-methylisoxazolidin-5-yl)methoxy)methyl)phosphonate (*29a*)

Yield: 16% (0.055 g from 0.85 mmol of the nitrone **20a**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3472, 3169, 3050, 2983, 2929, 1679, 1631, 1453, 1372, 1249, 1027; ^{1}H NMR (300 MHz, CD_3OD) δ: 7.54 (d, 1H, J = 7.8 Hz), 5.61 (d, 1H, J = 7.8 Hz), 4.50 (dddd, 1H, J = 8.6 Hz, J = 6.5 Hz, J = 4.8 Hz, J = 3.5 Hz, H-C5), 4.22–4.12 (m, 4H, CH_2OP), 3.94 (d, 2H, J = 8.4 Hz, CH_2P), 3.81 (dd, 1H, J = 13.9 Hz, J = 4.9 Hz, *H*CHN), 3.72 (dAB, 1H, J_{AB} = 11.0 Hz, J = 3.5 Hz, *H*CHO), 3.70 (dAB, 1H, J_{AB} = 11.0 Hz, J = 4.8 Hz, HC*H*O), 3.64 (dd, 1H, J = 13.9 Hz, J = 8.8 Hz, HC*H*N), 3.39 (dddd, 1H, J = 8.8 Hz, J = 8.7 Hz, J = 4.9 Hz, J = 3.2 Hz, H-C3), 2.63 (ddd, 1H, J = 13.0 Hz, J = 8.6 Hz, J = 8.6 Hz, H_a-C4), 2.60 (s, 3H, CH_3N), 1.82 (ddd, 1H, J = 13.0 Hz, J = 6.5 Hz, J = 3.2 Hz, H_b-C4), 1.34 (t, 6H, J = 7.0 Hz, 2 × CH_3CH_2OP); ^{13}C NMR (75 MHz, D_2O) δ: 166.8 (C═O), 152.3 (C═O), 147.9 (C═C), 101.4 (C═C), 75.6 (C5), 73.2 (CH_2O), 65.3 (C3), 64.3 (d, J = 6.6 Hz, 2 × CH_2OP), 63.9 (d, J = 162.7 Hz, CH_2P), 51.1 (CH_2N), 43.8 (CH_3N), 32.3 (C4), 15.9 (d, J = 6.3 Hz, 2 × CH_3CH_2OP); ^{31}P NMR (121 MHz, D_2O) δ: 24.95. Anal. Calcd. for $C_{15}H_{26}N_3O_7P$: C, 46.04; H, 6.70; N, 10.74. Found: C, 45.89; H, 6.83; N, 10.61.

4.2.9. Diethyl trans-(((3-((2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)methoxy)methyl) phosphonate (*30a*)

Yield: 9% (0.030 g from 0.85 mmol of the nitrone **20a**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3472, 3173, 3051, 2984, 2929, 1681, 1631, 1454, 1370, 1245, 1023; ^{1}H NMR (600 MHz, CD_3OD) δ: 7.57 (d, 1H, J = 7.8 Hz), 5.65 (d, 1H, J = 7.8 Hz), 4.27–4.22 (m, 1H, H-C5), 4.21–4.14 (m, 4H, CH_2OP), 3.94 (d, 2H, J = 8.3 Hz, CH_2P), 3.89 (dd, 1H, J = 14.0 Hz, J = 5.4 Hz, *H*CHN), 3.74 (dd, 1H, J = 14.0 Hz, J = 6.8 Hz, HC*H*N), 3.73 (dd, 1H, J = 10.7 Hz, J = 3.5 Hz, *H*CHO), 3.66 (dd, 1H, J = 10.7 Hz, J = 5.5 Hz, HC*H*O), 3.32–3.26 (m, 1H, H-C3), 2.68 (s, 3H, CH_3N), 2.36 (ddd, 1H, J = 12.8 Hz, J = 8.0 Hz, J = 7.9 Hz, H_b-C4), 2.16 (ddd, 1H, J = 12.8 Hz, J = 7.7 Hz, J = 4.6 Hz, H_b-C4), 1.36 (t, 6H, J = 7.0 Hz, CH_3CH_2OP); ^{13}C NMR (150 MHz, CD_3OD) δ: 165.6 (C═O), 151.8 (C═O), 146.7 (C═C), 100.4 (C═C), 77.1 (C5), 74.4 (CH_2O), 65.6 (C3), 64.6 (d, J = 166.2 Hz, CH_2P), 62.7 (d, J = 6.5 Hz, 2 × CH_2OP), 49.5 (CH_2N), 44.6 (CH_3N), 33.3 (C4), 15.4 (d, J = 5.6 Hz, 2 × CH_3CH_2OP); ^{31}P NMR (242 MHz, CD_3OD) δ: 21.77. Anal. Calcd. for $C_{15}H_{26}N_3O_7P$: C, 46.04; H, 6.70; N, 10.74. Found: C, 45.81; H, 6.79; N, 10.56.

4.2.10. Diethyl cis-(((3-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)methoxy)methyl) phosphonate (*29b*)

Yield: 13% (0.026 g from 0.49 mmol of the nitrone **20b**); yellow oil; IR (film, cm^{-1}) ν_{max}: 3466, 3172, 3063, 2985, 2918, 2849, 2822, 1697, 1464, 1444, 1241, 1049, 972; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.10 (brs, 1H, NH), 7.62 (d, 1H, J = 5.9 Hz), 4.52–4.49 (m, 1H, H-C5), 4.24–4.16 (m, 4H, CH_2OP), 3.94–3.77 (m, 5H, *H*CHN, CH_2P, CH_2O), 3.55 (dd, 1H, J = 13.8 Hz, J = 9.8 Hz, HC*H*N), 3.44–3.41 (m, 1H, H-C3), 2.62 (s, 3H, CH_3N), 2.64–2.58 (m, 1H, H_a-C4), 1.88 (ddd, 1H, J = 13.0 Hz, J = 6.5 Hz, J = 3.1 Hz, H_b-C4), 1.37 (t, 6H, J = 7.0 Hz, 2 × CH_3CH_2OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 157.3 (d, J = 26.2 Hz, C═O), 149.6 (C═O), 139.7 (d, J = 233.8 Hz, C═C), 131.5 (d, J = 32.7 Hz, C═C), 75.4 (C5), 72.5 (d, J = 10.9 Hz, CH_2O), 65.7 (d, J = 166.1 Hz, CH_2P), 65.2 (C3), 62.5 (d, J = 6.7 Hz, CH_2OP), 62.4 (d, J = 6.7 Hz, CH_2OP), 51.3 (CH_2N), 44.0 (CH_3N), 31.9 (C4), 16.5 (d, J = 5.6 Hz, 2 × CH_3CH_2OP); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 20.95. Anal. Calcd. for $C_{15}H_{25}FN_3O_7P$: C, 44.01; H, 6.16; N, 10.27. Found: C, 44.25; H, 5.96; N, 10.22.

4.2.11. Diethyl cis-(((3-((5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)methoxy)methyl) phosphonate (*29c*)

Yield: 12% (0.047 g from 0.85 mmol of the nitrone **20c**); light yellow oil; IR (film, cm^{-1}) ν_{max}: 3425, 3189, 3059, 2985, 2960, 2925, 2854, 2820, 1695, 1622, 1445, 1338, 1247, 1023, 970; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.38 (brs, 1H, NH), 7.80 (s, 1H), 4.52–4.49 (m, 1H, H-C5), 4.22–4.17 (m, 4H, CH_2OP), 3.93 (dd, 1H, J = 13.9 Hz, J = 4.1 Hz, *H*CHN), 3.91–3.87 (m, 2H, CH_2P), 3.79 (dAB, 1H, J_{AB} = 10.9 Hz, J = 2.8 Hz, *H*CHO), 3.76 (dAB, 1H, J_{AB} = 10.9 Hz, J = 3.8 Hz, HC*H*O), 3.58 (dd, 1H, J = 13.9 Hz, J = 10.1 Hz, *H*CHN), 3.43–3.39 (m, 1H, *H*-C3), 2.64–2.59 (m, 1H, H_a-C4), 2.61 (s, 3H, CH_3N), 1.84 (ddd, 1H, J = 13.0 Hz, J = 6.4 Hz, J = 2.3 Hz, H_b-C4), 1.35 (t, 6H, J = 7.1 Hz, 2 × CH_3CH_2OP); (signals of *cis*-**29c** were extracted from the ^{13}C NMR spectrum of a 65:35 mixture of *cis*-**29c** and *trans*-**30c**); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.7 (C═O), 150.4 (C═O), 146.5 (C═C), 95.2 (C═C), 75.3 (C5), 72.5 (d, J = 10.6 Hz, CH_2O), 65.7 (d, J = 161.5 Hz, CP), 65.1 (C3), 62.5 (d, J = 6.6 Hz, CH_2OP), 62.5 (d, J = 6.6 Hz, CH_2OP), 51.4 (CH_2N), 44.1 (CH_3N), 31.9 (C4), 16.5 (d, J = 5.3 Hz, 2 × CH_3CH_2OP); ^{31}P NMR (80 MHz, $CDCl_3$) δ: 21.91. Anal. Calcd. for $C_{15}H_{25}BrN_3O_7P$: C, 38.31; H, 5.36; N, 8.94. Found: C, 38.36; H, 5.57; N, 8.86.

4.2.12. Diethyl trans-(((3-((5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl) methoxy)methyl)phosphonate (*30c*)

Yellowish oil; IR (film, cm^{-1}) ν_{max}: 3174, 3059, 2983, 2932, 2819, 1699, 1679, 1621, 1439, 1227, 1057; 754; (signals of *trans*-**30c** were extracted from the spectra of a 65:35 mixture of *cis*-**29c** and *trans*-

30c); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.50 (brs, 1H, NH), 7.70 (s, 1H), 4.26–4.21 (m, 1H, H-C5), 4.22–4.17 (m, 4H, CH_2OP), 3.98 (dd, 1H, J = 13.9 Hz, J = 3.9 Hz, *H*CHN), 3.93–3.83 (m, 2H, CH_2P), 3.78 (dAB, 1H, J_{AB} = 10.6 Hz, J = 4.0 Hz, *H*CHO), 3.70 (dAB, 1H, J_{AB} = 10.6 Hz, J = 5.4 Hz, HC*H*O), 3.50 (dd, 1H, J = 13.9 Hz, J = 8.7 Hz, HC*H*N), 3.33–3.29 (m, 1H, H-C3), 2.68 (s, 3H, CH_3N), 2.41 (ddd, 1H, J = 12.9 Hz, J = 8.4 Hz, J = 8.2 Hz, H_a-C4), 2.10 (ddd, 1H, J = 12.9 Hz, J = 7.6 Hz, J = 2.9 Hz, H_b-C4), 1.35 (t, 6H, J = 7.1 Hz, 2 × *C*H_3CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.5 (C=O), 150.4 (C=O), 145.6 (C=C), 95.7 (C=C), 77.6 (C5), 74.8 (d, J = 9.0 Hz, CH_2O), 65.6 (d, J = 165.1 Hz, CP), 65.5 (C3), 62.5 (d, J = 6.6 Hz, CH_2OP), 62.5 (d, J = 6.6 Hz, CH_2OP), 50.2 (CH_2N), 46.1 (CH_3N), 33.3 (C4), 16.5 (d, J = 5.3 Hz, 2 × *C*H_3CH$_2$OP); ^{31}P NMR (80 MHz, $CDCl_3$) δ: 21.80. Anal. Calcd. for $C_{15}H_{25}BrN_3O_7P$: C, 38.31; H, 5.36; N, 8.94. Found: C, 38.36; H, 5.56; N, 8.88 (obtained on a 65:35 mixture of *cis*-**29c** and *trans*-**30c**).

4.2.13. Diethyl cis-(((2-methyl-3-((5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)isoxazolidin-5-yl)methoxy)methyl)phosphonate (*29d*)

Yield: 5% (0.022 g from 1.03 mmol of the nitrone **20d**); yellowish oil; IR (film, cm^{-1}) ν_{max}: 3476, 3175, 3053, 2984, 2928, 1681, 1466, 1369, 1249, 1026; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.68 (brs, 1H, NH), 7.24 (q, 1H, J = 1.0 Hz), 4.51–4.48 (m, 1H, H-C5), 4.23–4.16 (m, 4H, CH_2OP), 3.93–3.85 (m, 2H, CH_2P), 3.88 (dd, 1H, J = 13.6 Hz, J = 4.0 Hz, *H*CHN), 3.79 (dAB, 1H, J_{AB} = 10.9 Hz, J = 2.9 Hz, *H*CHO), 3.74 (dAB, 1H, J_{AB} = 10.9 Hz, J = 4.4 Hz, HC*H*O), 3.49 (dd, 1H, J = 13.6 Hz, J = 9.7 Hz, HC*H*N), 3.44–3.40 (m, 1H, H-C3), 2.61 (s, 3H, CH_3N), 2.63–2.58 (m, 1H, H_a-C4), 1.93 (d, 3H, J = 1.0 Hz, CH_3), 1.84 (ddd, 1H, J = 13.0 Hz, J = 6.5 Hz, J = 2.8 Hz, H_b-C4), 1.35 (t, 6H, J = 7.1 Hz, 2 × *C*H_3CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 164.3 (C=O), 151.1 (C=O), 142.9 (C=C), 109.4 (C=C), 75.3 (C5), 72.9 (d, J = 10.5 Hz, CH_2O), 65.7 (d, J = 166.4 Hz, CP), 65.4 (C3), 62.6 (d, J = 5.9 Hz, CH_2OP), 62.4 (d, J = 6.1 Hz, CH_2OP), 51.4 (CH_2N), 44.2 (CH_3N), 32.5 (C4), 16.5 (d, J = 5.6 Hz, 2 × *C*H_3CH$_2$OP), 12.1 (CH_3); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 20.95. Anal. Calcd. for $C_{16}H_{28}N_3O_7P$: C, 47.41; H, 6.96; N, 10.37. Found: C, 47.56; H, 7.04; N, 10.25.

4.2.14. Diethyl trans-(((2-methyl-3-((5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)isoxazolidin-5-yl)methoxy)methyl)phosphonate (*30d*)

Yellowish oil; IR (film, cm^{-1}) ν_{max}: 3488, 3183, 3061, 2986, 2929, 1679, 1455, 1370, 1244, 1211, 1047, 1023; (signals of *trans*-**30d** were extracted from the spectra of a 32:68 mixture of *trans*-**30d** and *cis*-**29d**); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.16 (brs, 1H, NH), 7.14 (s, 1H), 4.26–4.23 (m, 1H, H-C5), 4.21–4.16 (m, 4H, CH_2OP), 3.94–3.84 (m, 3H, CH_2P, *H*CHN), 3.74 (dAB, 1H, J_{AB} = 10.6 Hz, J = 4.4 Hz, *H*CHO), 3.69 (dAB, 1H, J_{AB} = 10.6 Hz, J = 5.5 Hz, HC*H*O), 3.53 (dd, 1H, J = 12.6 Hz, J = 8.3 Hz, HC*H*N), 3.33–3.28 (m, 1H, H-C3), 2.68 (s, 3H, CH_3N), 2.37 (ddd, 1H, J = 11.6 Hz, J = 8.2 Hz, J = 8.2 Hz, H_a-C4), 2.10 (ddd, 1H, J = 11.6 Hz, J = 7.6 Hz, J = 3.7 Hz, H_b-C4), 1.92 (s, 3H, CH_3), 1.36 (t, 6H, J = 7.1 Hz, 2 × *C*H_3CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 164.3 (C=O), 151.2 (C=O), 142.0 (C=C), 110.0 (C=C), 75.3 (C5), 74.7 (d, J = 9.6 Hz, CH_2O), 65.7 (C3), 65.6 (d, J = 163.5 Hz, CP), 62.4 (d, J = 6.1 Hz, CH_2OP), 62.4 (d, J = 6.5 Hz, CH_2OP), 50.2 (CH_2N), 46.0 (CH_3N), 33.8 (C4), 16.5 (d, J = 5.5 Hz, 2 × *C*H_3CH$_2$OP), 12.2 (CH_3); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 20.89. Anal. Calcd. for $C_{16}H_{28}N_3O_7P$: C, 47.41; H, 6.96; N, 10.37. Found: C, 47.22; H, 7.20; N, 10.35 (obtained on a 32:68 mixture of *trans*-**30d** and *cis*-**29d**).

4.2.15. Diisopropyl cis-(((3-((2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)oxy)methyl)phosphonate (*31a*)

Yield: 30% (0.013 g from 0.906 mmol of the nitrone **20a**); white amorphous solid; IR (film, cm^{-1}) ν_{max}: 3432, 3113, 2984, 2930, 2854, 1714, 1680, 1454, 1236, 1027; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.95 (brs, 1H, NH), 7.55 (d, 1H, J = 7.9 Hz, C=C), 5.64 (d, 1H, J = 7.9 Hz, C=C), 5.29 (d, 1H, J = 5.1 Hz, H-C5), 4.79–4.67 (m, 2H, POC*H*$(CH_3)_2$), 3.98 (dd, 1H, J = 13.4 Hz, J = 10.4 Hz, *H*CHP), 3.95 (dAB, 1H, J_{AB} = 13.7 Hz, J = 9.4 Hz, *H*CHN), 3.93 (dAB, 1H, J_{AB} = 13.7 Hz, J = 5.3 Hz, HC*H*N), 3.62 (dd, 1H, J = 13.4 Hz, J = 8.7 Hz, HC*H*P), 3.44–3.36 (m, 1H, H-C3), 2.58 (ddd, 1H, J = 13.7 Hz, J = 8.7 Hz, J = 5.1 Hz, H_a-C4), 2.60 (s, 3H, CH_3N), 2.06 (dd, 1H, J = 13.7 Hz, J = 1.3 Hz, H_b-C4), 1.32–1.23 (m, 12H, POCH$(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 164.4 (C=O), 151.3 (C=O), 147.1 (C=C), 102.7 (d, J = 12.3 Hz, C5), 101.3 (C=C), 71.4 (d, J = 5.5 Hz, POC*H*$(CH_3)_2$), 71.3 (d, J = 5.9 Hz, POC*H*$(CH_3)_2$), 63.2 (C3), 61.1 (d, J = 171.1 Hz, CP), 51.7 (CH_2N), 47.1 (CH_3N), 37.6 (C4), 24.4 (d, J = 4.6 Hz, POCH$(CH_3)_2$), 24.3 (d, J = 3.6 Hz, POCH$(CH_3)_2$), 24.3 (d, J = 4.7 Hz, POCH$(CH_3)_2$), 24.2 (d, J = 4.5 Hz, POCH$(CH_3)_2$); ^{31}P NMR (121 MHz, $CDCl_3$) δ: 20.84. Anal. Calcd. for $C_{16}H_{28}N_3O_7P$: C, 47.41; H, 6.96; N, 10.37. Found: C, 47.63; H, 6.86; N, 10.48.

4.2.16. Diisopropyl trans-(((3-((2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)oxy)methyl)phosphonate (*32a*)

White amorphous solid; IR (film, cm^{-1}) ν_{max}: 3386, 3192, 3056, 2926, 2855, 1685, 1458, 1385, 1248, 1099; (signals of *trans*-**32a** were extracted from the spectra of a 46:54 mixture of *trans*-**32a** and *cis*-**31a**); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.53 (brs, 1H, NH), 7.34 (d, 1H, J = 7.9 Hz), 5.67 (d, 1H, J = 7.9 Hz), 5.33 (t, 1H, J = 5.3 Hz, H-C5), 4.80–4.75 (m, 2H, POC*H*$(CH_3)_2$), 3.99 (dd, 1H, J = 13.5 Hz, J = 3.7 Hz, *H*CHN), 3.97 (dd, 1H, J = 13.5 Hz, J = 9.4 Hz, *H*CHP), 3.72 (dd, 1H, J = 13.5 Hz, J = 9.0 Hz, HC*H*P), 3.53–3.46 (m, 1H, H-C3), 3.33 (dd, 1H, J = 13.5 Hz, J = 9.3 Hz, HC*H*N), 2.86 (s, 3H. CH_3N), 2.49 (ddd, 1H, J = 13.7 Hz, J = 7.6 Hz, J = 1.5 Hz, H_a-C4), 2.19 (ddd, 1H, J = 13.7 Hz, J = 5.3 Hz, J = 5.3 Hz, H_b-C4), 1.37–1.34 (m, 12H, POCH$(CH_3)_2$); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 163.3 (C=O), 150.7 (C=O), 146.1 (C=C), 105.5 (d, J = 12.3 Hz, C5), 101.1 (C=C), 71.3 (d, J = 6.6 Hz, POC*H*$(CH_3)_2$), 71.2 (d, J = 6.6 Hz, POC*H*$(CH_3)_2$), 64.5 (C3), 61.6 (d, J = 171.6 Hz, CH_2P), 51.6 (CH_2N), 48.1 (CH_3N), 39.6 (C4), 24.1 (d, J = 4.1 Hz, POCH$(CH_3)_2$), 24.0 (d, J = 4.6 Hz, POCH$(CH_3)_2$), 24.0 (d, J = 4.2 Hz, POCH$(CH_3)_2$), 23.9 (d, J = 4.9 Hz, POCH$(CH_3)_2$); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 18.92. Anal. Calcd. for $C_{16}H_{28}N_3O_7P$: C, 47.41; H, 6.96; N, 10.37. Found: C, 47.54; H, 6.75; N, 10.21 (obtained on a 46:54 mixture of *trans*-**32a** and *cis*-**31a**).

4.2.17. Diisopropyl cis-(((3-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)oxy)methyl)phosphonate (*31b*)

Yield: 20% (0.050 g from 0.60 mmol of the nitrone **20b**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3423, 3195, 3064, 2983, 2928, 2852, 2823, 1702, 1665, 1467, 1376, 1242, 1131, 990; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.69 (brs, 1H, NH), 7.85 (d, 1H, J = 5.8 Hz), 5.34 (d, 1H, J = 5.1 Hz, H-C5), 4.83–4.74 (m, 2H, C*H*$(CH_3)_2$), 4.01 (dd, 1H, J = 13.4 Hz, J = 10.6 Hz, *H*CHP), 3.98 (dd, 1H, J = 13.8 Hz, J = 9.0 Hz, *H*CHN), 3.91 (dd, 1H, J = 13.8 Hz, J = 5.3 Hz, HC*H*N), 3.67 (dd, 1H, J = 13.4 Hz, J = 8.6 Hz, HC*H*P), 3.46 (dddd, 1H, J = 8.9 Hz, J = 8.6 Hz, J = 5.3 Hz, J = 1.4 Hz, H-C3), 2.66 (s, 3H, CH_3N), 2.61 (ddd, 1H, J = 13.8 Hz, J = 8.6 Hz, J = 5.3 Hz, H_a-C4), 2.11 (dd, 1H, J = 13.8 Hz, J = 1.4 Hz, H_b-C4), 1.38–1.36 (m, 12H, 2 × CH$(CH_3)_2$); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 157.4 (d, J = 26.2 Hz, C=O), 149.8 (C=O), 139.7 (d, J = 233.9 Hz, C=C), 131.6 (d, J = 32.9 Hz, C=C), 102.6 (d, J = 12.1 Hz, C5), 71.3 (d, J = 6.4 Hz, 2 × *C*H$(CH_3)_2$), 63.0 (C3), 60.9 (d, J = 170.6 Hz, CP), 51.6 (CH_2N), 47.0 (CH_3N), 37.2 (C4), 24.1 (d, J = 4.1 Hz, 2 × CH(*C*H$_3$)$_2$); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 19.72. Anal. Calcd. for $C_{16}H_{27}FN_3O_7P$: C, 45.39; H, 6.43; N, 9.93. Found: C, 45.47; H, 6.57; N, 10.11.

*4.2.18. Diisopropyl trans-(((3-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)oxy)methyl)phosphonate (**32b**)*

Colorless oil; IR (film, cm^{-1}) ν_{max}: 3412, 3180, 3055, 2983, 2932, 2879, 2821, 1698, 1665, 1465, 1335, 1241, 1102, 990; (signals of *trans*-**32b** were extracted from the spectra of a 36:64 mixture of *trans*-**32b** and *cis*-**31b**); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.28 (brs, 1H, NH), 7.49 (d, 1H, J = 5.8 Hz), 5.34 (d, 1H, J = 5.2 Hz, H-C5), 4.82–4.74 (m, 2H, C*H*$(CH_3)_2$), 3.98 (dd, 1H, J = 13.8 Hz, J = 3.7 Hz, *H*CHN), 3.98 (dd, 1H, J = 13.8 Hz, J = 9.6 Hz, *H*CHP), 3.73 (dd, 1H, J = 13.8 Hz, J = 8.9 Hz, HC*H*P), 3.56–3.50 (m, 1H, H-C3), 3.28 (dd, 1H, J = 13.8 Hz, J = 9.2 Hz, HC*H*N), 2.87 (s, 3H, CH_3N), 2.71 (ddd, 1H, J = 13.9 Hz, J = 7.7 Hz, J = 1.9 Hz, H_a-C4), 2.19 (ddd, 1H, J = 13.9 Hz, J = 5.6 Hz, J = 4.9 Hz, H_b-C4), 1.38–1.34 (m, 12H, 2 × CH(C*H*$_3)_2$); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 157.2 (d, J = 26.9 Hz, C═O), 149.6 (C═O), 139.8 (d, J = 235.0 Hz, C═C), 130.5 (d, J = 32.8 Hz, C═C), 105.7 (d, J = 12.1 Hz, C5), 71.2 (d, J = 6.7 Hz, 2 × C*H*$(CH_3)_2$), 64.6 (C3), 62.0 (d, J = 169.8 Hz, CP), 51.0 (CH_2N), 48.0 (CH_3N), 39.4 (C4), 24.1 (d, J = 4.2 Hz, 2 × CH(C*H*$_3)_2$); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 18.88. Anal. Calcd. for $C_{16}H_{27}FN_3O_7P$: C, 45.39; H, 6.43; N, 9.93. Found: C, 45.23; H, 6.33; N, 9.78 (obtained on a 36:64 mixture of *trans*-**32b** and *cis*-**31b**).

*4.2.19. Diisopropyl cis-(((3-((5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)oxy)methyl)phosphonate (**31c**)*

Yield: 19% (0.081 g from 0.89 mmol of the nitrone **20c**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3174, 3059, 2983, 2932, 2819, 1699, 1679, 1621, 1439, 1227, 1057, 999; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.64 (brs, 1H, NH), 7.96 (s, 1H), 5.34 (d, 1H, J = 5.2 Hz, H-C5), 4.80–4.74 (m, 2H, C*H*$(CH_3)_2$), 4.00 (dd, 1H, J = 13.6 Hz, J = 10.3 Hz, *H*CHP), 3.99 (dAB, 1H, J_{AB} = 13.6 Hz, J = 9.9 Hz, *H*CHN), 3.97 (dAB, 1H, J_{AB} = 13.6 Hz, J = 5.0 Hz, HC*H*N), 3.68 (dd, 1H, J = 13.6 Hz, J = 8.4 Hz, HC*H*P), 3.41–3.38 (m, 1H, H-C3), 2.64 (s, 3H, CH_3N), 2.60 (ddd, 1H, J = 13.9 Hz, J = 8.9 Hz, J = 5.2 Hz, H_a-C4), 2.09 (dd, 1H, J = 13.9 Hz, J = 1.5 Hz, H_b-C4), 1.37–1.32 (m, 12H, 2 × CH(C*H*$_3)_2$); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.7 (C═O), 150.6 (C═O), 146.2 (C═C), 102.6 (d, J = 10.7 Hz, C5), 95.5 (C═C), 71.3 (d, J = 6.3 Hz, *C*H$(CH_3)_2$), 71.2 (d, J = 6.8 Hz, *C*H$(CH_3)_2$), 63.1 (C3), 61.0 (d, J = 170.2 Hz, CP), 51.8 (CH_2N), 46.9 (CH_3N), 37.4 (C4), 24.1 (d, J = 4.3 Hz, CH(C*H*$_3)_2$), 24.0 (d, J = 4.2 Hz, CH(C*H*$_3)_2$); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 19.73. Anal. Calcd. for $C_{16}H_{27}BrN_3O_7P$: C, 39.68; H, 5.62; N, 8.68. Found: C, 39.71; H, 5.68; N, 8.86.

*4.2.20. Diisopropyl cis-(((2-methyl-3-((5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)isoxazolidin-5-yl)oxy)methyl)phosphonate (**31d**)*

Yield: 27% (0.110 g from 0.98 mmol of the nitrone **20d**); yellow oil; IR (film, cm^{-1}) ν_{max}: 3479, 3176, 3055, 2980, 2932, 2820, 1713, 1466, 1373, 1248, 1101, 1015; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.07 (brs, 1H, NH), 7.29 (q, 1H, J = 1.0 Hz), 5.33 (d, 1H, J = 5.2 Hz, H-C5), 4.80–4.74 (m, 2H, C*H*$(CH_3)_2$), 4.02 (dd, 1H, J = 13.6 Hz, J = 9.9 Hz, *H*CHP), 3.91 (dAB, 1H, J = 13.8 Hz, J = 8.3 Hz, *H*CHN), 3.89 (dAB, 1H, J = 13.8 Hz, J = 5.3 Hz, HC*H*N), 3.68 (dd, 1H, J = 13.6 Hz, J = 8.9 Hz, HC*H*P), 3.44 (dddd, J = 8.9 Hz, J = 8.3 Hz, J = 5.3 Hz, J = 2.1 Hz, 1H, H-C3), 2.64 (s, 3H, CH_3N), 2.61 (ddd, 1H, J = 13.9 Hz, J = 8.9 Hz, J = 5.2 Hz, H_a-C4), 2.10 (dd, 1H, J = 13.9 Hz, J = 2.1 Hz, H_b-C4), 1.94 (d, 3H, J = 1.0 Hz, CH_3), 1.37–1.33 (m, 12H, 2 × CH(C*H*$_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 164.2 (C═O), 151.0 (C═O), 142.8 (C═C), 109.7 (C═C), 102.7 (d, J = 12.0 Hz, C5), 71.1 (d, J = 5.5 Hz, 2 × *C*H$(CH_3)_2$), 63.4 (C3), 61.2 (d, J = 170.7 Hz, CP), 51.6 (CH_2N), 46.8 (CH_3N), 37.6 (C4), 24.1 (d, J = 4.1 Hz, 2 × CH(C*H*$_3)_2$), 12.2 (*C*H$_3$); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 19.57. Anal. Calcd. for $C_{17}H_{30}N_3O_7P$: C, 48.68; H, 7.21; N, 10.02. Found: C, 48.43; H, 7.07; N, 9.98.

*4.2.21. Diisopropyl trans-(((2-methyl-3-((5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)isoxazolidin-5-yl)oxy)methyl)phosphonate (**32d**)*

Yield: 2% (0.008 g from 0.98 mmol of the nitrone **20d**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3429, 3180, 3055, 2980, 2926, 2853, 1683, 1465, 1247, 1101, 993; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.51 (brs, 1H, NH), 7.17 (q, 1H, J = 1.0 Hz), 5.32 (dd, 1H, J = 5.5 Hz, J = 1.7 Hz, H-C5), 4.80–4.74 (m, 2H, C*H*$(CH_3)_2$), 3.98 (dd, 1H, J = 13.6 Hz, J = 9.4 Hz, *H*CHP), 3.97 (dd, 1H, J = 13.8 Hz, J = 3.7 Hz, *H*CHN), 3.72 (dd, 1H, J = 13.6 Hz, J = 8.9 Hz, HC*H*P), 3.59–3.53 (m, 1H, H-C3), 3.34 (dd, 1H, J = 13.8 Hz, J = 9.1 Hz, HC*H*N), 2.86 (s, 3H, CH_3N), 2.69 (ddd, 1H, J = 13.7 Hz, J = 7.6 Hz, J = 1.7 Hz, H_a-C4), 2.20 (ddd, 1H, J = 13.7 Hz, J = 5.5 Hz, J = 5.5 Hz, H_b-C4), 1.94 (d, 3H, J = 1.0 Hz, CH_3), 1.38–1.34 (m, 12H, 2 × CH(C*H*$_3)_2$); (signals of *trans*-**32d** were extracted from the[13] C NMR spectrum of a 63:37 mixture of *trans*-**32d** and *cis*-**31d**) ^{13}C NMR (150 MHz, $CDCl_3$) δ: 164.0 (C═O), 150.8 (C═O), 142.0 (C═C), 109.9 (C═C), 105.5 (d, J = 12.2 Hz, C5), 71.2 (d, J = 6.6 Hz, *C*H$(CH_3)_2$), 71.1 (d, J = 6.5 Hz, *C*H$(CH_3)_2$), 64.7 (C3), 61.9 (d, J = 170.7 Hz, CP), 51.0 (CH_2N), 48.1 (CH_3N), 39.7 (C4), 24.0 (d, J = 4.0 Hz, 2 × CH(C*H*$_3)_2$), 12.2 (CH$_3$); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 18.51. Anal. Calcd. for $C_{17}H_{30}N_3O_7P$: C, 48.68; H, 7.21; N, 10.02. Found: C, 48.88; H, 7.15; N, 9.99.

*4.2.22. Diethyl cis-(3-((2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)phosphonate (**33a**)*

Yield: 14% (0.073 g from 1.69 mmol of the nitrone **20a**); white amorphous solid; mp 114–115 °C; IR (KBr, cm^{-1}) ν_{max}: 3445, 3153, 3069, 3045, 2993, 2926, 1704, 1669, 1468, 1418, 1240, 1015, 976; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.50 (brs, 1H, NH), 7.44 (d, 1H, J = 7.9 Hz), 5.63 (dd, 1H, J = 7.9 Hz, J = 2.3 Hz), 4.43 (dd, 1H, J = 10.4 Hz, J = 6.4 Hz, H-C5), 4.30–4.10 (m, 4H, CH_2OP), 3.97–3.80 (M part of ABM system, 1H, *H*CHN) and 3.69–3.51 (AB part of ABM system, 2H, HC*H*N, H-C3), 2.89–2.64 (m, 1H, H_a-C4), 2.57 (s, 3H, CH_3-N), 2.19 (dddd, 1H, J = 19.8 Hz, J = 13.4 Hz, J = 6.4 Hz, J = 1.2 Hz, H_b-C4), 1.37 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP), 1.36 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 163.9 (C═O), 151.1 (C═O), 147.0 (C═C), 101.1 (C═C), 70.2 (d, J = 171.8 Hz, C5), 65.2 (d, J = 3.9 Hz, C3), 63.4 (d, J = 6.5 Hz, CH_2OP), 62.5 (d, J = 7.1 Hz, CH_2OP), 50.9 (CH_2N), 43.5 (CH_3N), 32.3 (C4), 16.5 (d, J = 5.4 Hz, *C*H$_3$CH$_2$OP), 16.5 (d, J = 5.4 Hz, *C*H$_3$CH$_2$OP); ^{31}P NMR (80 MHz, $CDCl_3$) δ: 23.10. Anal. Calcd. for $C_{13}H_{22}N_3O_6P$: C, 44.96; H, 6.39; N, 12.10. Found: C, 44.87; H, 6.36; N, 11.94.

*4.2.23. Diethyl trans-(3-((2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)phosphonate (**34a**)*

Yellowish oil; IR (film, cm^{-1}) ν_{max}: 3422, 3055, 2984, 2920, 1682, 1458, 1392, 1234, 1023; (signals of *trans*-**34a** were extracted from the spectra of a 4:86:10 mixture of *cis*-**33a**, *trans*-**34a**, **35a**/**36a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.94 (brs, 1H, NH), 7.28 (d, 1H, J = 7.9 Hz), 5.66 (dd, 1H, J = 7.9 Hz, J = 1.9 Hz), 4.29–4.09 (m, 5H, 2 × CH$_3$C*H*$_2$OP, HC5), 4.00 (dd, 1H, J = 13.5 Hz, J = 3.5 Hz, *H*CHN), 3.54–3.42 (m, 1H, H-C3), 3.29 (dd, 1H, J = 13.5 Hz, J = 9.3 Hz, HC*H*N), 2.83 (dddd, 1H, J = 20.9 Hz, J = 13.0 Hz, J = 10.1 Hz, J = 7.3 Hz, H_a-C4), 2.71 (s, 3H, CH_3N), 2.30 (dddd, 1H, J = 13.0 Hz, J = 8.2 Hz, J = 8.2 Hz, J = 1.9 Hz, H_b-C4), 1.36 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP), 1.34 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 163.6 (C═O), 151.0 (C═O), 146.1 (C═C), 101.5 (C═C), 73.1 (d, J = 169.3 Hz, C5), 65.9 (d, J = 6.3 Hz, C3), 63.3 (d, J = 6.7 Hz, CH_2OP), 62.6 (d, J = 6.8 Hz, CH_2OP), 49.7 (CH_2N), 46.1 (CH_3N), 32.7 (C4), 16.5 (d, J = 5.3 Hz, *C*H$_3$CH$_2$OP); ^{31}P NMR (80 MHz, $CDCl_3$) δ: 22.50. Anal. Calcd. for $C_{13}H_{22}N_3O_6P$: C, 44.96; H, 6.39; N, 12.10. Found: C, 45.10; H, 6.60; N, 11.93 (obtained on a 4:86:10 mixture of *cis*-**33a**, *trans*-**34a**, **35a**/**36a**).

*4.2.24. Diethyl cis-(3-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)phosphonate (**33b**)*

Yield: 33% (0.106 g from 0.89 mmol of the nitrone **20b**); white amorphous solid, mp 126–127 °C; IR (KBr, cm^{-1}) ν_{max}: 3403, 3157, 3057, 2994, 2906, 2821,1700, 1667, 1420, 1232, 1048, 981; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.78 (brs, 1H, NH), 7.64 (d, 1H, J = 5.9 Hz), 4.45 (dd, 1H, J = 10.4 Hz, J = 6.4 Hz, *H*-C5), 4.27–4.17 (m, 4H, CH_2OP), 3.94–3.88 (M part of ABM system, 1H, *H*CHN) and 3.65–3.57 (AB part of ABM system, 2H, HC*H*N and H-C3), 2.79 (dddd, 1H, J = 15.4 Hz, J = 13.4 Hz, J = 10.5 Hz, J = 7.6 Hz, H_a-C4), 2.59 (s, 3H, CH_3N), 2.21 (dddd, 1H, J = 19.5 Hz, J = 13.4 Hz, J = 6.4 Hz, J = 1.1 Hz, H_b-C4), 1.39 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP) 1.38 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 157.5 (d, J = 26.2 Hz, C=O), 149.9 (C=O), 139.7 (d, J = 234.1 Hz, C=C), 131.4 (d, J = 32.8 Hz, C=C), 70.2 (d, J = 172.4 Hz, C5), 65.2 (d, J = 3.7 Hz, C3), 63.5 (d, J = 6.5 Hz, CH_2OP), 62.6 (d, J = 6.7 Hz, CH_2OP), 50.9 (CH_2N), 43.4 (CH_3N), 32.2 (C4), 16.5 (d, J = 5.6 Hz, *C*H$_3$CH$_2$OP), 16.5 (d, J = 5.6 Hz, *C*H$_3$CH$_2$OP); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 22.23. Anal. Calcd. for $C_{13}H_{21}FN_3O_6P$: C, 42.74; H, 5.79; N, 11.50. Found: C, 42.93; H, 5.90; N, 11.34.

*4.2.25. Diethyl trans-(3-((5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl) phosphonate (**34b**)*

Colorless oil; IR (KBr, cm^{-1}) ν_{max}: 3398, 3158, 2995, 2963, 2906, 2821, 1700, 1667, 1444, 1233, 1048, 980; (signals of *trans*-**34b** were extracted from the spectra of a 15:70:15 mixture of *cis*-**33b**, *trans*-**34b**, **35b**/**36b**); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.75 (brs, 1H, NH), 7.45 (d, 1H, J = 5.6 Hz), 4.28–4.15 (m, 5H, H-C5, CH_2OP), 4.01 (dd, 1H, J = 13.8 Hz, J = 3.5 Hz, *H*CHN), 3.52–3.45 (m, 1H, H-C3), 3.28 (dd, 1H, J = 13.8 Hz, J = 9.4 Hz, HC*H*N), 2.89–2.81 (m, 1H, H_a-C4), 2.74 (s, 3H, CH_3N), 2.33 (ddd, 1H, J = 12.8 Hz, J = 8.2 Hz, J = 8.2 Hz, H_b-C4), 1.40–1.34 (m, 6H, 2 × C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 157.3 (d, J = 25.9 Hz, C=O), 149.8 (C=O), 139.9 (d, J = 235.1 Hz, C=C), 130.5 (d, J = 32.9 Hz, C=C), 73.2 (d, J = 167.8 Hz, C5), 65.8 (d, J = 6.2 Hz, C3), 63.4 (d, J = 6.6 Hz, CH_2OP), 62.6 (d, J = 7.1 Hz, CH_2O), 49.7 (CH_2N), 46.1 (CH_3N), 32.5 (C4), 16.5 (d, J = 5.5 Hz, *C*H$_3$CH$_2$OP), 16.5 (d, J = 5.3 Hz, *C*H$_3$CH$_2$OP); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 21.58. Anal. Calcd. for $C_{13}H_{21}FN_3O_6P$: C, 42.74; H, 5.79; N, 11.50. Found: C,42.59; H, 5.68; N, 11.21 (obtained on a 15:70:15 mixture of *cis*-**33b**, *trans*-**34b**, **35b**/**36b**).

*4.2.26. Diethyl cis-(3-((5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)phosphonate (**33c**)*

Yield: 24% (0.094 g from 0.92 mmol of the nitrone **20c**); colorless crystalline solid (crystallized from ethyl acetate/hexane) mp 209–213 °C with decomposition; IR (KBr, cm^{-1}) ν_{max}: 3369, 3155, 3046, 2993, 2904, 2859, 2818, 1698, 1625, 1447, 1416, 1334, 1229, 1042, 983; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.06 (brs, 1H, NH), 7.88 (s, 1H), 4.48 (dd, 1H, J = 10.5 Hz, J = 6.4 Hz, H-C5), 4.33–4.18 (m, 4H, 2 × CH_2OP), 3.96 (dd, 1H, J = 13.9 Hz, J = 5.0 Hz, *H*CHN), 3.70 (dd, 1H, J = 13.9 Hz, J = 9.5 Hz, HC*H*N), 3.63–3.59 (m, 1H, H-C3), 2.84 (dddd, 1H, J = 15.8 Hz, J = 13.4 Hz, J = 10.5 Hz, J = 7.7 Hz, H_a-C4), 2.61 (s, 3H, CH_3N), 2.23 (dddd, 1H, J = 19.4 Hz, J = 13.4 Hz, J = 6.4 Hz, J = 1.3 Hz, H_b-C4), 1.42 (d, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP), 1.41 (d, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.4 (C=O), 150.2, (C=O), 146.0 (C=C), 95.1 (C=C), 69.9 (d, J = 176.1 Hz, C5), 64.8 (C3), 63.2 (d, J = 6.3 Hz, CH_2OP), 62.4 (d, J = 6.9 Hz, CH_2OP), 50.8 (CH_2N), 43.2 (CH_3N), 32.0 (C4), 16.4 (d, J = 5.4 Hz, *C*H$_3$CH$_2$OP), 16.3 (d, J = 5.4 Hz, *C*H$_3$CH$_2$OP); ^{31}P NMR (121 MHz, $CDCl_3$) δ: 22.68. Anal. Calcd. for $C_{13}H_{21}BrN_3O_6P$: C, 36.64; H, 4.97; N, 9.86. Found: C, 36.68; H, 4.69; N, 9.80.

*4.2.27. Diethyl trans-(3-((5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl) phosphonate (**34c**)*

Yield: 10% (0.039 g from 0.92 mmol of the nitrone **20c**); yellowish amorphous solid; IR (KBr, cm^{-1}) ν_{max}: 3418, 3158, 2992, 2906, 2819, 1698, 1622, 1446, 1230, 1046, 981; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.65 (brs, 1H, NH), 7.66 (s, 1H), 4.28–4.18 (m, 4H, 2 × CH_2OP), 4.18 (dd, 1H, J = 9.6 Hz, J = 8.9 Hz, H-C5), 4.03 (dd, 1H, J = 13.8 Hz, J = 3.6 Hz, *H*CHN), 3.51–3.45 (m, 1H, H-C3), 3.29 (dd, 1H, J = 13.6 Hz, J = 9.6 Hz, HC*H*N), 2.81 (dddd, 1H, J = 20.1 Hz, J = 12.8 Hz, J = 9.6 Hz, J = 7.8 Hz, H_a-C4), 2.72 (s, 3H, CH_3N), 2.30 (dddd, 1H, J = 12.8 Hz, J = 8.9 Hz, J = 7.2 Hz, J = 1.4 Hz, H_b-C4), 1.37 (d, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP), 1.35 (d, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 159.4 (C=O), 150.4 (C=O), 145.5 (C=C), 95.8 (C=C), 73.2 (d, J = 167.9 Hz, C5), 65.7 (C3), 63.4 (d, J = 6.7 Hz, CH_2OP), 62.6 (d, J = 7.0 Hz, CH_2OP), 49.9 (CH_2N), 46.2 (CH_3N), 32.5 (C4), 16.5 (d, J = 5.6 Hz, *C*H$_3$CH$_2$OP), 16.5 (d, J = 5.6 Hz, *C*H$_3$CH$_2$OP); ^{31}P NMR (121 MHz, $CDCl_3$) δ: 22.05. Anal. Calcd. for $C_{13}H_{21}BrN_3O_6P$: C, 36.64; H, 4.97; N, 9.86. Found: C, 36.68; H, 4.64; N, 9.56.

*4.2.28. Diethyl cis-(2-methyl-3-((5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)isoxazolidin-5-yl)phosphonate (**33d**)*

Yield: 2% (0.009 g from 1.11 mmol of the nitrone **20d**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3481, 3171, 3054, 2985, 2931,2818, 1688, 1468, 1239, 1048; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.81 (brs, 1H, NH), 7.27 (q, 1H, J = 1.2 Hz), 4.44 (dd, 1H, J = 10.3 Hz, J = 6.6 Hz, H-C5), 4.29–4.15 (m, 4H, 2 × CH_2OP), 3.92–3.83 (M part of ABM system, 1H, *H*CHN) and 3.63–3.54 (AB part of ABM system, 2H, HC*H*N and H-C3), 2.87–2.71 (m, 1H, H_a-C4), 2.57 (s, 3H, CH_3N), 2.19 (ddd, 1H, J = 19.6 Hz, J = 12.8 Hz, J = 6.6 Hz, H_b-C4), 1.91 (d, 3H, J = 1.2 Hz, CH_3), 1.38 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP) 1.37 (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 164.3 (C=O), 151.0 (C=O), 143.0 (C=C), 109.4 (C=C), 70.2 (d, J = 171.9 Hz, C5), 65.4 (d, J = 3.7 Hz, C3), 63.3 (d, J = 6.4 Hz, CH_2OP), 62.5 (d, J = 6.7 Hz, CH_2OP), 51.0 (CH_2N), 43.6 (CH_3N), 32.4 (C4), 16.6 (d, J = 5.5 Hz, *C*H$_3$CH$_2$OP), 16.5 (d, J = 5.4 Hz, *C*H$_3$CH$_2$OP), 12.1 (CH_3); ^{31}P NMR (121 MHz, $CDCl_3$) δ: 22.73. Anal. Calcd. for $C_{14}H_{24}N_3O_6P$: C, 46.54; H, 6.70; N, 11.63. Found: C, 46.32; H, 6.73; N, 11.58.

*4.2.29. Diethyl trans-(2-methyl-3-((5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)isoxazolidin-5-yl)phosphonate (**34d**)*

Yield: 1% (0.005 g from 1.11 mmol of the nitrone **20d**); colorless oil; IR (film, cm^{-1}) ν_{max}: 3463, 3178, 3058, 2985, 2930, 2830, 1687, 1468, 1234, 1024; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.39 (brs, 1H, NH), 7.10 (q, 1H, J = 1.0 Hz), 4.27–4.12 (m, 5H, 2 × CH_2OP, H-C5), 3.95 (dd, 1H, J = 13.7 Hz, J = 3.9 Hz, *H*CHN), 3.53–3.41 (m, 1H, H-C3), 3.30 (dd, 1H, J = 13.7 Hz, J = 9.1 Hz, HC*H*N), 2.79 (dddd, 1H, J = 20.7 Hz, J = 12.9 Hz, J = 9.6 Hz, J = 7.3 Hz, H_a-C4), 2.72 (s, 3H, CH_3N), 2.31 (dddd, 1H, J = 12.9 Hz, J = 8.2 Hz, J = 8.2 Hz, J = 2.1 Hz, H_b-C4), 1.95 (d, 3H, J = 1.0 Hz, CH_3), 1.37 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP), 1.35 (t, 3H, J = 7.0 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 163.9 (C=O), 150.8 (C=O), 141.9 (C=C), 110.1 (C=C), 73.2 (d, J = 169.1 Hz, C5), 66.0 (d, J = 5.8 Hz, C3), 63.3 (d, J = 6.8 Hz, CH_2OP), 62.6 (d, J = 6.8 Hz, CH_2OP), 49.7 (CH_2N), 46.0 (CH_3N), 32.9 (C4), 16.5 (d, J = 5.4 Hz, *C*H$_3$CH$_2$OP), 16.5 (d, J = 5.4 Hz, *C*H$_3$CH$_2$OP), 12.1 (CH_3); ^{31}P NMR (121 MHz, $CDCl_3$) δ: 22.11. Anal. Calcd. for $C_{14}H_{24}N_3O_6P$: C, 46.54; H, 6.70; N, 11.63. Found: C, 46.45; H, 6.98; N, 11.82.

*4.2.30. Diethyl cis-(3-((6-amino-9H-purin-9-yl)methyl)-2-methylisoxazolidin-5-yl)phosphonate (**33e**)*

Yellow oil; IR (film, cm^{-1}) ν_{max}: 3323, 3177, 2984, 1650, 1599, 1476, 1416, 1327, 1300, 1242, 1047, 970; (signals of *cis*-**33e** were extracted from the spectra of a 65:35 mixture of *cis*-**33e** and *trans*-

34e); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.36 (s, 1H), 8.08 (s, 1H), 5.78 (brs, 2H, NH_2), 4.49 (dd, 1H, J = 10.1 Hz, J = 6.7 Hz, H-C5), 4.31–4.15 (m, 6H, CH_2N, 2 × CH_2OP), 3.74–3.68 (m, 1H, H-C3), 2.84–2.72 (m, 1H, H_a-C4), 2.56 (s, 3H, CH_3N), 2.29 (dddd, 1H, J = 19.9 Hz, J = 13.2 Hz, J = 6.7 Hz, J = 1.9 Hz, H_b-C4), 1.41 (t, 3H, J = 6.7 Hz, CH_3CH_2OP), 1.39 (t, 3H, J = 6.7 Hz, CH_3CH_2OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 155.6 (CNH_2), 152.8, 149.9, 142.2, 119.5, 70.4 (d, J = 170.4 Hz, C5), 66.1 (C3), 63.4 (d, J = 6.5 Hz, CH_2OP), 62.6 (d, J = 6.0 Hz, CH_2OP), 46.2 (CH_2N), 43.6 (CH_3N), 32.5 (C4), 16.5 (d, J = 4.9 Hz, CH_3CH_2OP), 16.4 (d, J = 5.3 Hz, CH_3CH_2OP); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 22.08. Anal. Calcd. for $C_{14}H_{23}N_6O_4P$: C, 45.40; H, 6.26; N, 22.69. Found: C, 45.63; H, 6.08; N, 22.50 (obtained on a 65:35 mixture of *cis*-**33e** and *trans*-**34e**).

4.2.31. Diethyl trans-(3-((6-amino-9H-purin-9-yl)methyl)-2-methylisoxazolidin-5-yl)phosphonate (**34e**)

Yellow oil; IR (film, cm^{-1}) ν_{max}: 3323, 3177, 2984, 1650, 1599, 1476, 1416, 1327, 1300, 1242, 1047, 970; (signals of *trans*-**34e** were extracted from the spectra of a 65:35 mixture of *cis*-**33e** and *trans*-**34e**); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.37 (s, 1H), 7.94 (s, 1H), 5.78 (brs, 2H, NH_2), 4.31–4.10 (m, 7H, CH_2N, 2 × CH_2OP, H-C5), 3.56–3.50 (m, 1H, H-C3), 2.84–2.72 (m, 1H, H_a-C4), 2.70 (s, 3H, CH_3-N), 2.35 (dddd, 1H, J = 11.9 Hz, J = 9.1 Hz, J = 9.1 Hz, J = 2.9 Hz, H_b-C4), 1.37 (t, 3H, J = 6.7 Hz, CH_3CH_2OP), 1.35 (t, 3H, J = 6.7 Hz, CH_3CH_2OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 155.7 (CNH_2), 153.0, 150.0, 141.4, 119.3, 72.7 (d, J = 161.9 Hz, C5), 66.5 (d, J = 5.7 Hz, C3), 63.2 (d, J = 6.6 Hz, CH_2OP), 62.6 (d, J = 6.8 Hz, CH_2OP), 45.8 (CH_2N), 43.9 (CH_3N), 33.3 (C4), 16.5 (d, J = 4.9 Hz, CH_3CH_2OP), 16.4 (d, J = 5.3 Hz, CH_3CH_2OP); ^{31}P NMR (242 MHz, $CDCl_3$) δ: 21.25. Anal. Calcd. for $C_{14}H_{23}N_6O_4P$: C, 45.40; H, 6.26; N, 22.69. Found: C, 45.31; H, 6.05; N, 22.58 (obtained on a 65:35 mixture of *cis*-**33e** and *trans*-**34e**).

4.2.32. Diethyl cis-(4-((5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)methyl)-2-methylisoxazolidin-5-yl)phosphonate (**35c**)

A 22:2:49:27 mixture of isoxazolidines **33c**, **34c**, **35c** and **36c** (0.030 g) was subjected to the separation on a X Bridge Prep, C18, 5 μm, OBD, 19 × 100 mm column using water/methanol (70:30, v/v) to provide *cis*-**35c** (0.002 g) as a colorless oil (retention time = 6.50 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.71 (s, 1H), 4.28–4.10 (m, 4H, 2 × CH_2OP), 4.16 (ddd, 2H, J = 9.2 Hz, J = 9.2 Hz, J = 9.2 Hz, H_2C5), 4.08 (dd, 1H, J = 13.3 Hz, J = 2.5 Hz, *H*CHN), 3.49 (dddd, J = 16.1 Hz, J = 9.0 Hz, J = 5.5 Hz, J = 2.5 Hz, H-C3), 3.44 (dd, J = 13.4 Hz, J = 9.0 Hz, HC*H*N), 2.67 (s, 3H, CH_3N), 2.57 (dddd, J = 15.5 Hz, J = 9.2 Hz, J = 9.2 Hz, J = 5.5 Hz, H-C4), 1.36 (d, J = 7.0 Hz, 3H, CH_3CH_2OP), 1.35 (d, J = 7.0 Hz, 3H, CH_3CH_2OP); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 2 signals of *C*=O not detected due to very low concentration, 146.0 (C=C), 96.2 (C=C), 67.1 (C5), 65.1 (C3), 62.8 (d, J = 6.7 Hz, CH_2OP), 62.7 (d, J = 6.7 Hz, CH_2OP), 51.9 (d, J = 8.1 Hz, CH_2N), 44.3 (d, J = 149.5 Hz, C4), 44.0 (CH_3N), 16.5 (d, J = 5.6 Hz, CH_3CH_2OP), 16.4 (d, J = 5.6 Hz, CH_3CH_2OP); ^{31}P NMR (121 MHz, $CDCl_3$) δ: 26.45.

4.3. Antiviral activity assays

The compounds were evaluated against different herpes viruses, including 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, varicella-zoster virus (VZV) strains Oka and YS, TK- VZV strains 07-1 and YS-R, human cytomegalovirus (HCMV) strains AD-169 and Davis as well as feline herpes virus (FHV), the poxvirus vaccinia virus (Lederle strain), para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, respiratory syncytial virus (RSV), feline coronavirus (FIPV) and influenza A virus subtypes H1N1 (A/PR/8), H3N2 (A/HK/7/87) and influenza B virus (B/HK/5/72) and human immunodeficiency virus (HIV-1/III_B and HIV-2/ROD). The antiviral assays, other than HIV, were based on inhibition of virus-induced cytopathicity or plaque formation (for VZV) in human embryonic lung (HEL) fibroblasts, African green monkey kidney cells (Vero), human epithelial cervix carcinoma cells (HeLa), Crandell-Rees feline kidney cells (CRFK), or Madin Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) (for VZV) and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation (VZV) were 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 cytopathicity or viral plaque formation by 50%.

4.4. Anti-HIV activity assays

Inhibition of HIV-1 (NL4.3)- and HIV-2 (ROD)-induced cytopathicity in $CD4^+$ T-lymphocyte MT-4 cell cultures was determined in microtiter 96-well (200-μl) plates containing ~ 10^6 MT-4 cells/ml and a variety of test compound concentrations. Thirty min after exposure of the MT-4 cells to the test compounds, the cell cultures were infected with HIV-1 (NL4.3) at 3 pg p24/well (or 60 pg/ml). The virus dose affords full cytopathicity after 4–5 days of incubation in the absence of the test compounds (control). Therefore, after 4–5 days incubation at 37 °C in a CO_2-controlled atmosphere, cytopathicity was microscopically recorded. Concomitantly, 100 μl of the supernatants of each of the cell cultures was removed from the wells and 50 μl of a MTS solution was added to the remaining cell suspension. After 2–3 h incubation at 37 °C, 50 μl Triton X-100 (0.5%) was added and absorbancy measured using a Soft Max Pro programme.

5. Cytostatic activity assays

Cytostatic measurements were based on the inhibition of murine leukemia L1210, human CD_4^+ T-lymphocyte CEM, human cervix carcinoma HeLa and human dermal microvascular endothelial cell proliferation. Cells were seeded at ~5 × 10^3 cells/well into 96-well (200 μl) microtiter plates. Then, medium containing different concentrations of the test compounds was added. After 2–4 days of further incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, or the compound concentration required to inhibit cell proliferation by 50% relative to the number of cells in the untreated controls. Alternatively, cytotoxicity of the test compounds in confluent (HEL, Vero, HeLa and CRFK) cell cultures (used for the antiviral assays) was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that caused a microscopically detectable alteration of cell morphology.

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