Review

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PHOSPHONATED ISOXAZOLIDINYL NUCLEOSIDES, A NEW CLASS OF MODIFIED NUCLEOSIDES

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SUMMARY

This review offers an overview of the synthesis of phosphonated isoxazolidinyl nucleosides, a new class of interesting and potentially antiviral/antitumor agents. The synthetic methodology relies on a 1,3-dipolar cycloaddition reaction as a key step. The cycloaddition process involves substituted nitrones, phosphonated nitrones, or nitrones containing functional groups easily convertible into a phosphonated group. Biological assays are presented, which show that phosphonated isoxazolidinyl nucleosides represent a new promising template of nucleoside analogues.

Introduction

Modified nucleosides have received a great deal of attention in the search for new antiviral/anticancer agents.[1-12] Many compounds have been described in literature; however, often, the clinical exploitation of most of the biologically active compounds is prevented by significant and undesirable side effects, and by different emerging problems such as tolerance, toxicity and cross-resistance. For this reason, a great research effort is still being dedicated to the design and development of new nucleoside analogues endowed with improved efficiency and reduced side effects.

With regard to this, structural modifications have been performed with respect to natural nucleosides at the level of both the heterocyclic base and the sugar residue, such as a) the replacement of the C-N glycosidic bond with a hydrolytically stable C-C bond;[13-18] b) the substitution of the ribose ring with an acyclic system;[19-21] c) the displacement of the oxygen atom of the ribose unit with a methylene group;[22,23] d) the insertion of unnatural nucleobases;[24,25] e) the replacement of the furanose ring with different heterocyclic systems.[26,27]

In particular, the substitution of the sugar moiety with an isoxazolidine ring has allowed for the development of a new class of N,O-nuclosides, which have been shown to be endowed with important biological features.[28-43]

Most of the nucleoside analogues possessing antiviral activities rely upon specific phosphorylation by a virally encoded kinase.[44] Of the three successive phosphorylation steps, the first is rate-limiting, while the conversion to di- and tri-phosphates, which interact with RT or interfere with cell growth, is catalyzed by less specific kinases. Consequently, a good delivery system for a nucleoside across cellular membranes is represented by the design of phosphate analogues, where the phosphate moiety is changed to isosteric and isoelectronic phosphonates, which mimic the nucleoside monophosphates, and are able

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to bypass the initial selective enzymatic monophosphorylation step.

The purpose of this review is to give an overview of the synthesis of phosphonated isoxazolidinyl nucleosides, with particular focus on the biologically interesting compounds, covering the recent literature.

Synthetic approach to Phosphonated Isoxazolidinyl Nucleosides

Phosphonated isoxazolidinyl nucleosides can be classified in different groups depending on the relative position of the nucleobase and the phosphonated moiety (Figure 1).

Compounds of structure I.

Truncated phosphonated *N,O*-nucleosides (**I**), containing a diethylphosphonate group directly linked at C-3 of the isoxazolidine ring, have been synthesized by Romeo and Chiacchio's group.[45] These compounds were obtained, starting from the phosphonated nitrone **1**, according to two different routes. Route a) involves the reaction of **1** with vinyl nuleobases, under microwave irradiation, and leads to the α -nucleosides **2** as main adducts (58-60%); route b), on the contrary, produces the β -anomers **3** as main products in 42-45% overall yield, by a two-step procedure involving the 1,3-dipolar cycloaddition of nitrone **1** with vinyl acetate and the subsequent Vorbrüggen nucleosidation (Scheme 1).

A modification of this series of compounds is represented by the insertion of an alkoxy carbonyl group at the anomeric

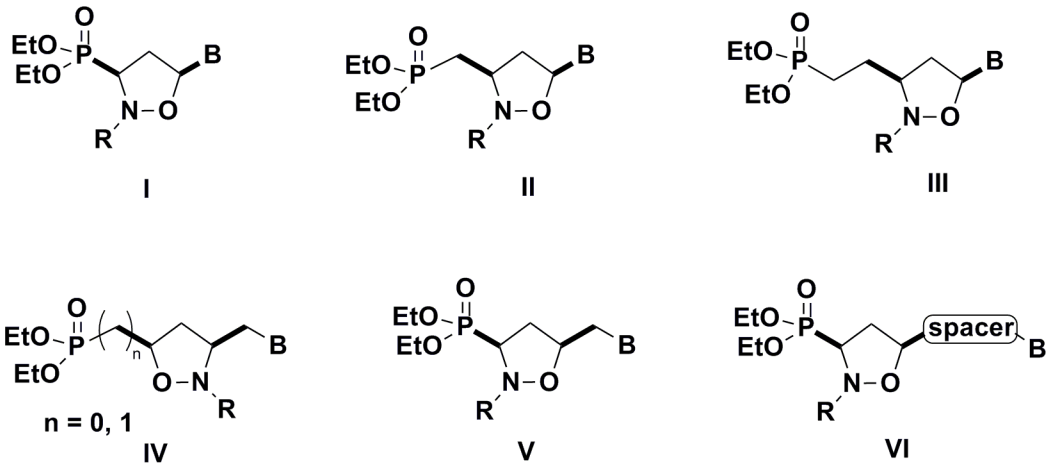
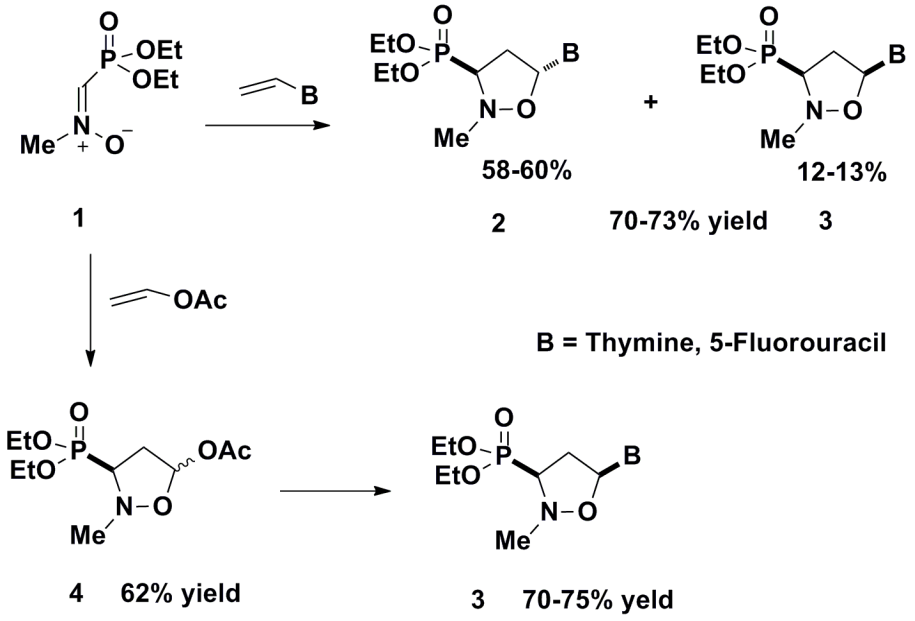


Figure 1: Phosphonated isoxazolidinyl nucleosides

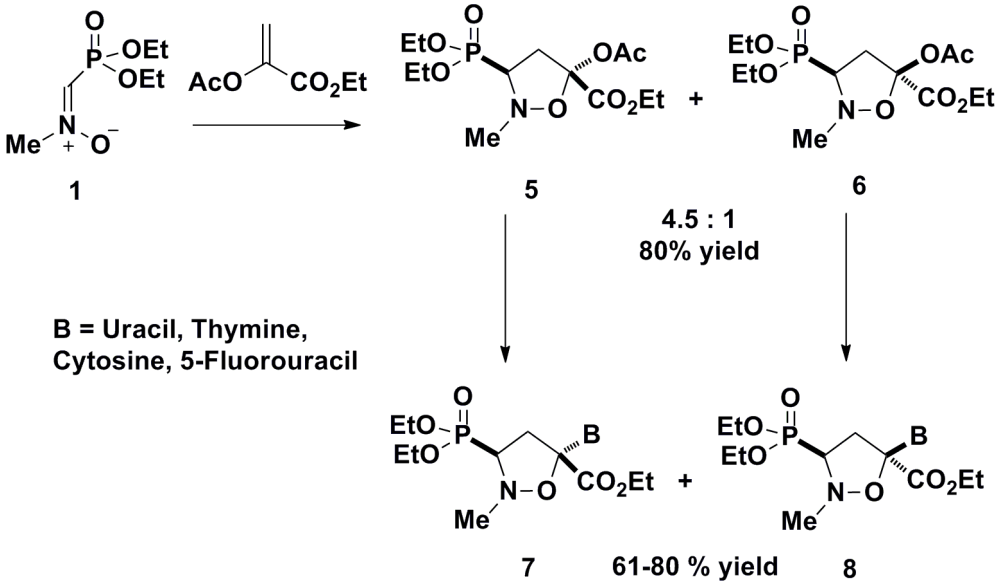


Scheme 1

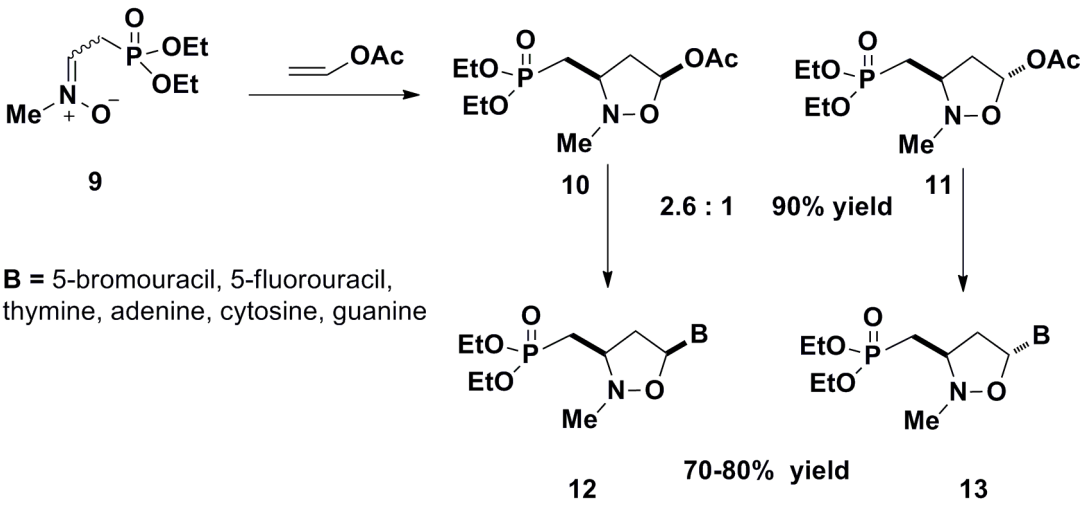
center of the isoxazolidine ring to produce the so called phosphonated C-1-branched *N,O*-nucleosides.[46] These nucleosides are synthesized by reaction of the phosphonated nitrone **1** with ethyl 2-acetyloxyacrylate, followed by the usual Vorbrüggen procedure.[47] The cycloaddition approach produces a mixture of *trans/cis* isoxazolidines **5** and **6** in an isomeric ratio of 4.5:1 and a global yield of 80%. The subsequent condensation with silylated thymine, uracil or acetylcytosine affords α and β–anomers **7** and **8** in a 2:3 relative ratio, while, with silylated 5-fluorouracil, the β–anomer **8** is almost the only compound (Scheme 2).

Compounds of structure II

These compounds are considered mimetic of monophosphate nucleosides and contain a diethylphosphonate group linked at C-3' of the isoxazolidine ring.[48] Their synthesis involves the 1,3-dipolar cycloaddition of the phosphonated nitrone **9** with vinyl acetate, with formation of a mixture of epimeric isoxazolidines **10** and **11**. Their independent coupling with silylated nucleobases affords phosphonated N,O-nucleosides **12** and **13**. The anomeric distribution of **12** and **13** is dependent on the nucleobase used. With 5-fluorouracil, the β –anomer is almost the only product, while, with thymine and *N*-acetylcytosine, a sig-



Scheme 2



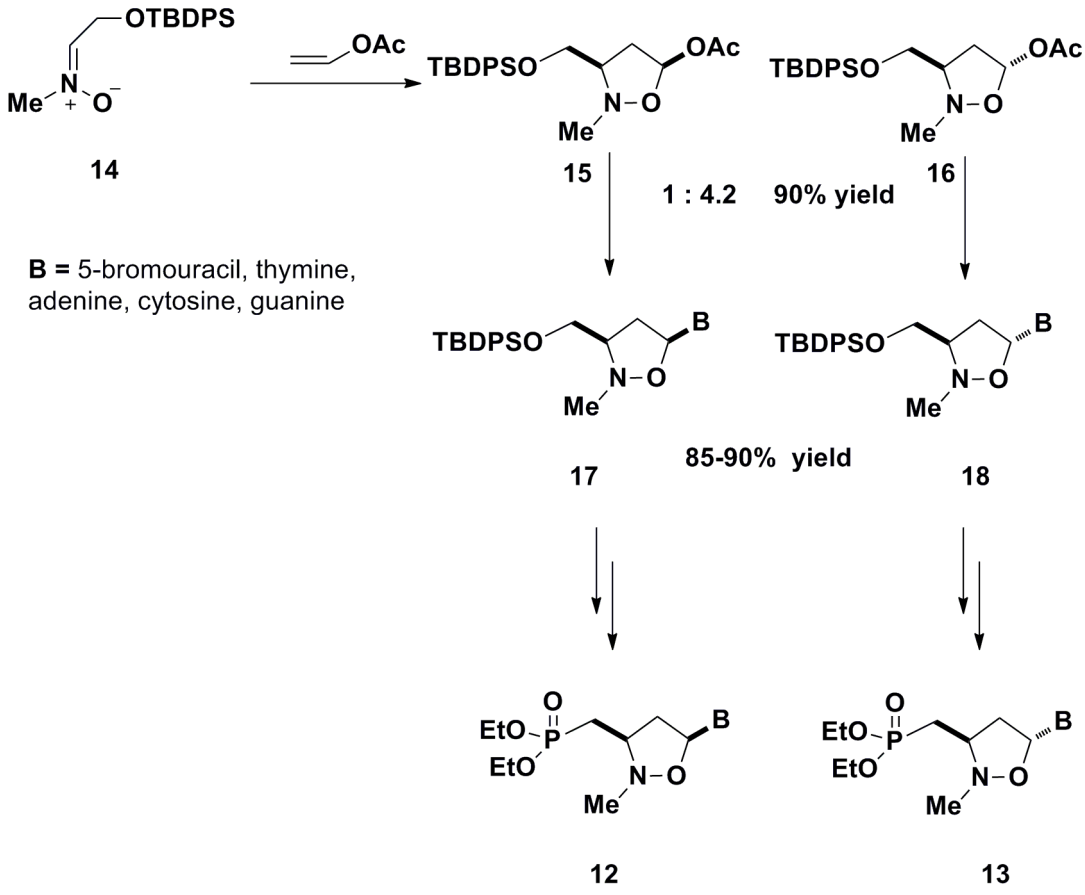
Scheme 3

nificant amount of the α–anomer has been obtained (Scheme 3).[49]

A successful implementation of the synthetic scheme, leading to phosphonated *N,O*-nucleosides **12** and **13,** has been achieved starting from the *C*-[(*tert*-butyldiphenylsilyl)oxy]-*N*-methylnitrone **14**, and proceeds in five steps. In the first step, the nitrone **14** is reacted with vinyl acetate affording a mixture of epimeric isoxazolidines **15** and **16** (Scheme 4) in a relative ratio of 1:4.2 (global yield 90%). In the second step, the crude mixture of isoxazolidines is nucleosidated with purine or pyrimidine nucleobases, to give nucleosides **17** and **18**, in acetonitrile at 50-60 ° C, in presence of trimethylsilytriflate as catalyst. In these conditions, the stereochemical outcome of the nucleosidation process depends on the nucleobase. Thus, with purine nucleobases, a mixture of α and β–anomers in a 2:3 relative ratio has been obtained, while, a better stereoselection in favour of β–anomers occurs when pyrimidine nucleobases are used (α:β = 3:7). Finally, the targets *N,O*-nucleosides **12** and **13** have been obtained from nucleosides **17** and **18** in three consecutive reactions: a) desilylation; b) tosylation; c) Arbuzov reaction.[50]

Compounds of structure III

The synthetic approach leading to compounds of structure III, which contain the phosphonate group linked at C-3" of the isoxazolidine ring, exploits the 1,3-dipolar cycloaddition reaction of nitrone **19**. This compound (*E/Z* mixture, 85% yield) is prepared from the commercially available 3-chloro propanaldehyde diethyl acetal by reaction with triethyl phosphite, acidic hydrolysis to the corresponding phosphonated aldehyde, and reaction with *N*-methyl hydroxylamine. The nitrone **19**, thus obtained, is converted into the target compounds **22** and **23**, by treatment with vinyl acetate and subsequent coupling with silylated nucleobases.[47] The β– anomers **23** have been obtained in higher yields with respect to α–anomers **22**, in a ratio varying from 9:1 (N-acetylcytosine) to 7:3 (thymine



Scheme 4

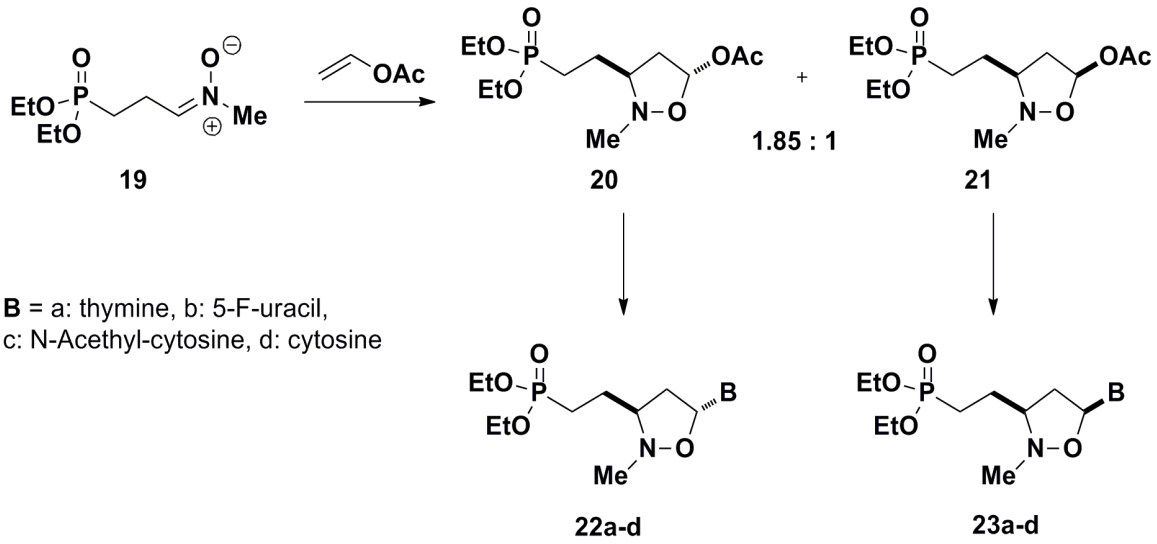
and 5-fluorouracil). The N-acetylcitosine derivatives **23c** have been further deacetylated to **23d** by treatment with 5% aqueous sodium carbonate.[51]

Compounds of structure IV, V and VI

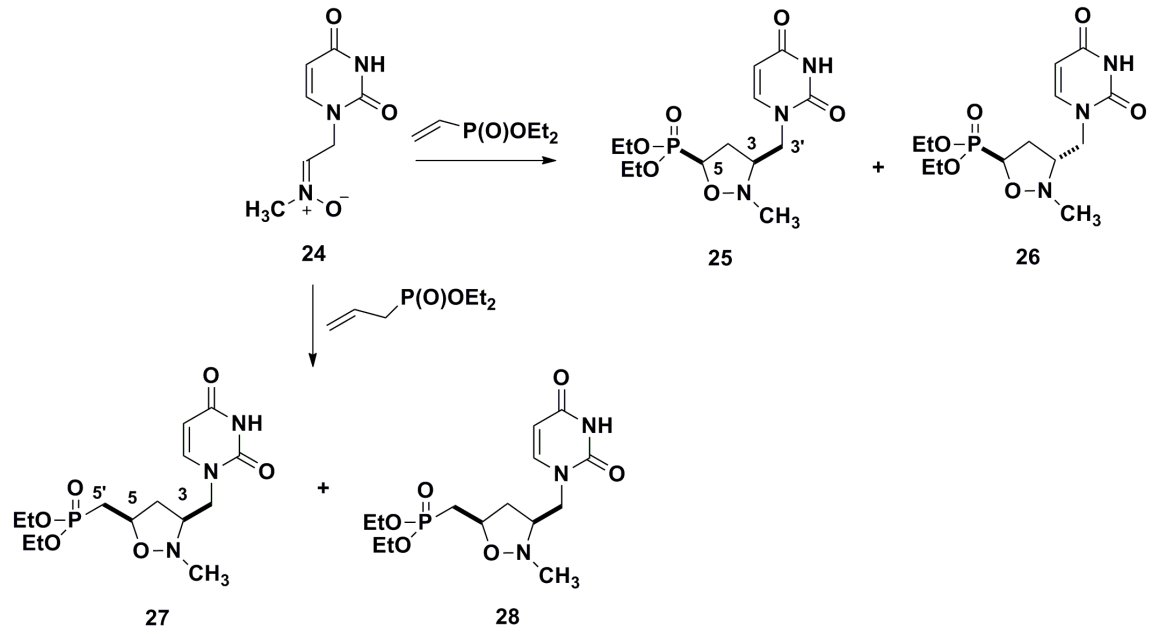
The development of synthetic strategies aimed at the preparation of new phosphonated *N,O*-nucleoside analogues has also been investigated by Piotrowska and coworkers. In particular, phosphonated *N,O*-homonucleosides, phosphonated *N,O*-nucleotide analogues with a 1,2,3-triazole linker, and phosphonated *N,O*-nucleotide analogues with a carbamoyl linker were synthesized.[52,53]

Phosphonated *N,O*-homonucleosides (Structures IV) were prepared according to two synthetic approaches. The first leads to the formation of *N,O*-homonucleosides having the nucleobase at C-3', with the phosphonic group linked at the C-5 position, while the second one affords *N,O*-homonucleosides containing the phosphonic group at C-5' of the isoxazolidinic ring.[54]

The 1,3-dipolar cycloaddition of nitrone **24** with diethyl vinyl-, and dietyl allylphosphonate was performed under microwave irradiation to produce a *cis/trans* mixture



Scheme 5



Scheme 6

of diastereoisomeric isoxazolidines. When diethyl vinylphosphonate was used, a 64:36 mixture of isoxazolidines **25** and **26** in 30% yield was obtained. The cycloaddition with diethyl allylphosphonate affords an 80:20 mixture of isoxazolidines **27** and **28** in 24% yield. (Scheme 6).

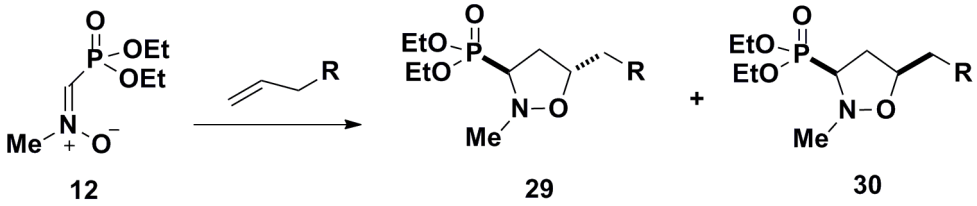
Reversed phosphonated *N,O*-homonucleosides, in which the phosphonate group is linked at C-3, while the heterocyclic bases are linked at C-5 of isoxazolidine ring, have also been reported (Structures V).[55] The 1,3-dipolar cycloaddition of the phosphonated nitrone **1** with *N*-allylnucleobase was carried out in thermal conditions in a single solvent or in a solvents mixture, depending on the solubility of the respective *N*-nucleobase, to afford a mixture of diastereoisomeric phosphonated *N,O*-homonucleosides **29** and **30** in good to excellent yield, with low to moderate *trans/cis* diastereoselectivity (Scheme 7).

In this regard, a new class of phosphonated *N,O*-nucleotide analogues, characterized by the presence of a 1,2,3-triazole linker, was synthesized (Structures VI). The new compounds present the 1,2,3-triazole linker between the isoxazolidine spacer and the purine/pyrimidine nucleobase.[56]

The synthetic approach was based on the preparation of 5-azidoisoxazolidines **31** and **32**, in 1:1 anomeric mixture, from 5-acetoxyisoxazolidines **4** by reaction with $TMSN_3$, and further transformation of the azides into the respective nucleoside mimetics by a click chemistry process.[57,58] (Scheme 8).

Piotrowska and co-workers have also investigated the synthesis and the biological activity of a new series of phosphonated 5-(arylcarbamoyl)-isoxazolidines, with a carbamoyl linker between the isoxazolidine spacer and the purine/pyrimidine nucleobase or other aromatic compounds as nucleobase replacers.[52,53]

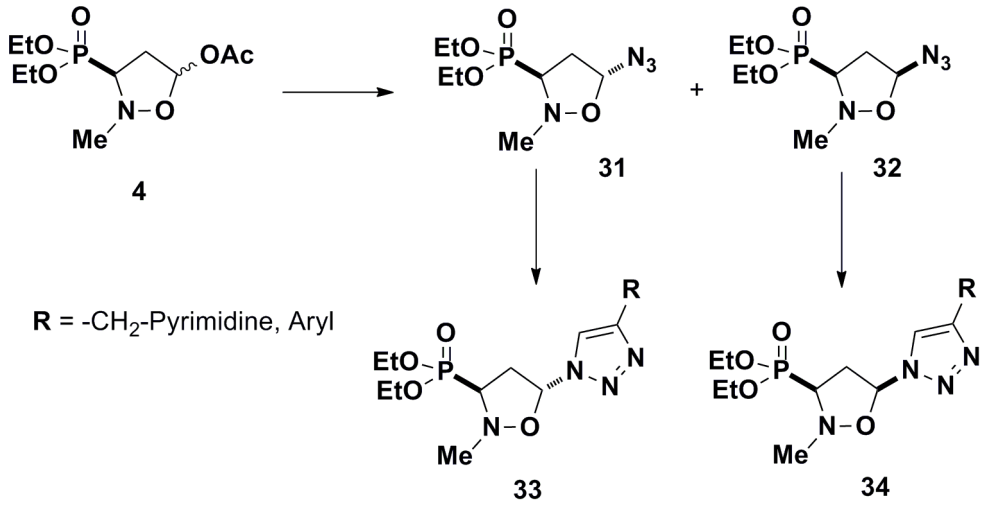
The synthetic method exploits the 1,3-dipolar cycloaddition of the nitrone **1** with



12 **29** **30**

R = pyrimidine, purine, heterocyclic ring

Scheme 7



R = -CH_2-Pyrimidine, Aryl

Scheme 8

substituted acrylamides **35**, which have been synthesized from commercially available substituted anilines and acryloyl chloride in the presence of triethylamine.[57,58] The cycloaddition reaction is carried out in toluene at 70°C and affords mixtures of diastereoisomeric *trans/cis* phosphonated 5-(arylcarbamoyl)-isoxazolidine **36** and **37** with a 50-80% diastereoselectivity.

Biological Properties of Phosphonated Isoxazolidinyl Nucleosides

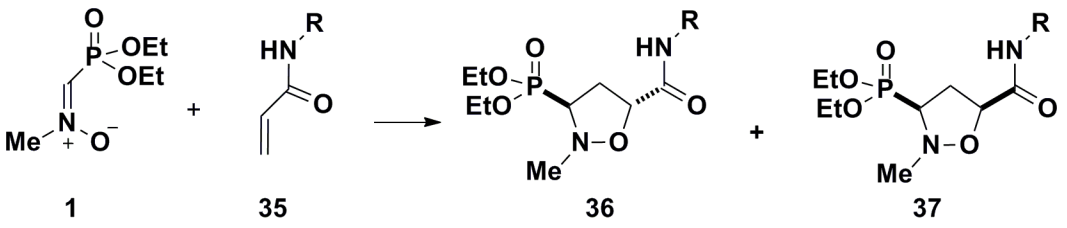
Biological assays performed on the series of compounds **I**, indicate that β-anomers **3** completely inhibit the RT of Avian Moloney Virus (AMV) and Human Immunodeficiency Virus (HIV), at concentrations 1±0.1 nM, at a level comparable with that of tenofovir (1nM) and 10-fold lower than AZT (10 nM). Moreover, MTS assays have indicated a very low toxicity (CC_{50} > 500 μM) in comparison with AZT (CC_{50} 12.14 μM).[45]

Biological assays performed on the β-anomers **8**, indicated that these compounds are able to inhibit the RT of AMV, HTLV-1 and HIV. In particular, the 5-fluorouracil derivative is shown to be the most promising derivative, acting on AMV and on HIV at concentration 1 and 10nM, respectively. The level of the inhibitory activity towards HTLV-1 and HIV was 10-fold higher than that of tenofovir and similar to that of AZT. Moreover, this compound does not show any cytoxicity according to MTS assays.

Also, biological properties of the series of compounds **II** have been evaluated. In particular, the cytotoxicity and the RT-inhibitory activity of β-anomers **12** were investigated. These compounds show low levels of cytotoxicity, assessed by conventional methods to detect viability. Noteworthy, the synthesized *N,O*-phosphonated nucleosides were as powerful as AZT in inhibiting the RT activity of the human T-cell leukemia/lymphotropic virus type 1 and in protecting human peripheral blood mononuclear cells against human T-cell leukemia/lymphotropic virus type 1 transmission *in vitro*.[49,50]

All the compounds of structure **III** have been evaluated for their ability to inhibit RT of avian myeloblastosis retrovirus, but no significant antiviral activity was observed.[51] The lack of antiviral activity in this class of compounds can be inferred from the considerations reported by Sigel.[60,61] Chelation with metal ions seems to play a determinant role: viral polymerases recognize and use triphosphate nucleosides, complexed with metal ions (Mg^{++} and Mn^{++}). The type of complexation determines the reaction pattern of nucleotides. Thus, for phosphonated N,O-nucleosides, the proximity of the N- atom to P- atom appears to be determinant for the biological activity. In full length nucleotides **23**, the N atom cannot make a certain contribution because a 7-membered ring should be formed by chelation, while short length and truncated PCOAN could form 6-six or 5-membered chelates, thus facilitating the bond break between P-α and P-β and allowing the transfer of the nucleotide group with release of pyrophosphate.

Phosphonated *N,O*-homonucleosides, (structure **V**) resulting from the cycloaddition with *N*-allylnucleobases, were screened for activity against a variety of DNA and RNA viruses; the compounds have been found to be non-toxic up to a concentration of 250 μM. Proliferation inhibitory effect on murine leukemia (L1210), human T-lymphocyte (CEM) and human cervix carcinoma cells (HeLa) was detected with an IC_{50} in the 33-192 μM



1 **35** **36** **37**

R = pyrimidine, purine, heterocyclic ring

Scheme 9

range.
For the series of compounds of structure **VI**, no inhibitory activity against any virus was shown at 250 μM. Derivatives in which the nucleobase was replaced by an aromatic system are endowed with a 50% cytostatic concentration (CC_{50}), against murine Leukemia L1210, human lymphocyte CEM, human cervix carcinoma Hela and human lung fibroblast HEL cells, in the range of 40-250 μM.
Cytostatic activity, of derivatives of phosphonated 5-(arylcarbamoyl)-isoxazolidines **36** and **37**, with a carbamoyl linker, was measured on three tumor cell lines (L1210, CEM and HeLa). An IC_{50} above 100 μM was shown.

Conclusions

In this review, the reported synthetic approaches to regard to phosphonated *N,O*-nucleoside analogues have been summarized. The key step of the synthetic process is the 1,3-dipolar cycloaddition reaction of suitably substituted nitrones with functionalized dipolarophiles. Better results were obtained through the use of microwave irradiation compared to classical thermal conditions.
The potential antiviral and anticancer activities of phosphonated *N,O*-nucleosides have been examined. Biological tests show that some of these derivatives are able to inhibit the infection of HIV or HTLV-1 viruses, while the derivatives in which the natural nucleobase has been replaced with heteroaromatic systems are able to inhibit cell proliferation of different tumor cell lines.
The obtained results show that phosphonated *N,O*-nucleosides represent a promising new template of nucleoside analogues which deserve further investigation as lead compounds for the preparation of potent inhibitors of biological targets.

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