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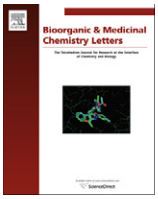

Straightforward synthesis of triazoloacyclonucleotide phosphonates as potential HCV inhibitors

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ABSTRACT

Preparation of several triazoloacyclic nucleoside phosphonates is described. The key step of the synthesis involves a copper(I)-catalysed azide–alkyne 1,3-dipolar cycloaddition between azidoalkylphosphonates and propargylated nucleobases. The antiviral properties of these new analogues have been evaluated and revealed interesting potencies.

Acyclic nucleoside phosphonates are nucleotide analogues in which the furanose ring and the readily hydrolysable ester linkage present in natural nucleotides have been, respectively, replaced by an acyclic chain and a phosphonate moiety. These analogues were developed for their ability to be resistant towards nucleolytic enzymes degradation. Initiated 25 years ago, this concept has proved to be a keystone in antiviral therapy leading ultimately to the discovery of several structurally related potent antiviral drugs namely adefovir, cidofovir and tenofovir (Fig. 1).[1,2] In this context, our group has a long experience in the design of acyclonucleosides[3–5] which eventually led in 2001 to the preparation of a series of modified 1,2,3-triazole acyclo derivatives.[6–8] The idea behind this work was to freeze the side-chain flexibility with a triazole moiety. Thus, the 1,2,3-triazolo acyclonucleosides isomers were generated through a conventional Huisgen [3 + 2] cycloaddition between propargylated nucleobases and various alkylazides. No antiviral activity was observed with these compounds probably because they did not bear the CH_2–P linkage needed to achieve high metabolic activation and biochemical stability. In the same year, independent work from Sharpless and Meldal demonstrated that copper(I) is an efficient and regiospecific catalyst for the preparation of 1,4-disubstituted 1,2,3-triazole derivatives.[9,10] Since then, triazoles have gained considerable attention in medicinal chemistry,[11,12] bioconjugation,[13] drug-delivery, or material science.[13–15] In the field of nucleic acid chemistry, the Cu-catalysed azide–alkyne 1,3-dipolar cycloaddition has been used by various research groups including ours and a large number of modified nucleosides, nucleotides and oligonucleotides have been synthesized for a broad range of applications.[16–23] However, little attention has been paid to the introduction of the triazole scaffold onto acyclic nucleotide phosphonates and to the best of our knowledge only recently was reported the synthesis of acyclonucleotides bearing a triazole unit and a diisopropyl-protected difluoromethylphosphonate group.[24] Unfortunately, the potential antiviral properties of these fluorophosphonylated compounds were not evaluated. Considering the importance of azaheterocyclic phosphonates in medicinal chemistry[25] and in continuation of our early work,[6–8] we describe here a straightforward synthesis of new acyclic nucleoside triazolylphosphonates as well as their antiviral biological evaluation against HCV and HIV infection.

The synthesis of the target molecules can be achieved by the use of either azidophosphonate or alkynylphosphonate precursors. To evaluate the influence of different linker lengths on the activities of the 1,2,3-triazoloacyclic nucleotides we choose to perform the cycloaddition between azidoalkylphosphonates and a variety of propargylated nucleobases (Table 1). Azidoalkylphosphonates were obtained by traditional substitution of an appropriate leaving group with an azidating agent following previously published procedures.[26,27] On the other hand, the synthesis of propargylic nucleobases was based on alkylation of the corresponding nucleobase by propargyl bromide under basic conditions.[28] The Cu-catalysed

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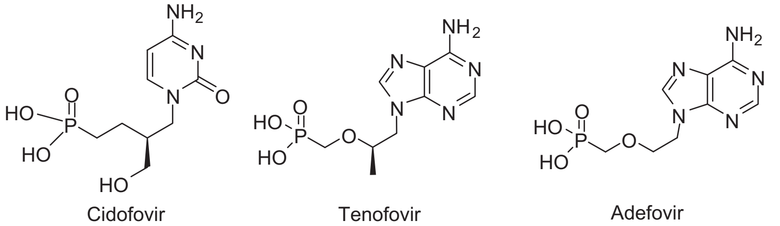
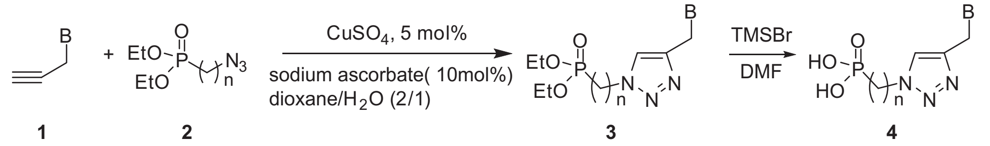


Figure 1. Marketed acyclic nucleoside phosphonates.

Table 1
Triazoloacyclic nucleoside phosphonates synthesized through the CuAAC reaction



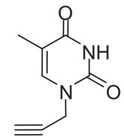
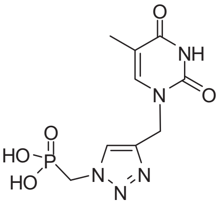
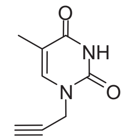
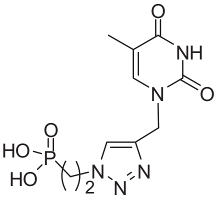
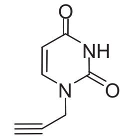
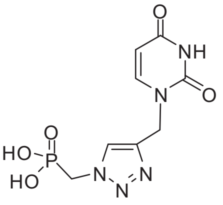
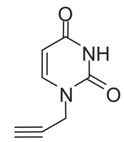
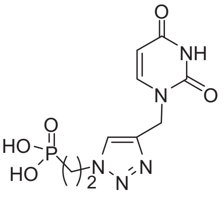
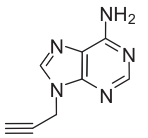
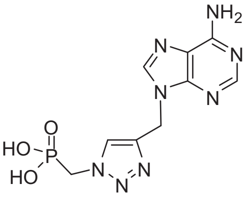
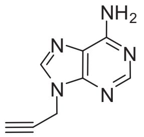
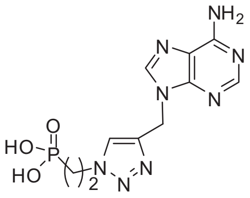
Entry	Alkyne	Azide	**3**, Yield (%)	**4**, Yield (%)
1		$n = 1$	**3a**, (86)	 **4a**, (40)
2		$n = 2$	**3b**, (65)	 **4b**, (65)
3		$n = 1$	**3c**, (82)	 **4c**, (45)
4		$n = 2$	**3d**, (88)	 **4d,** (40)
5		$n = 1$	**3e**, (72)	 **4e**, (40)

Table 1 (*continued*)

Entry	Alkyne	Azide	**3**, Yield (%)	**4**, Yield (%)
6		*n* = 2	**3f**, (70)	 **4f**, (37)

azide–alkyne 1,3-dipolar cycloadditions were conducted at room temperature in dioxane/H_2O (2/1) in the presence of Cu(II) ions (5 mol %) and sodium ascorbate (10 mol %) over 4 h (TLC monitoring). As illustrated in Table 1 this procedure furnished the expected triazoles **3a–f** in good to excellent yield. Unprotected nucleic bases are well tolerated and the regioselectivity of the reaction was demonstrated by ^{1}H and ^{31}P NMR. Finally, all diethyl esters were treated with bromotrimethylsilane in dry DMF to give the corresponding free phosphonic acids **4a–f**.

The target compounds **4a–f** were first evaluated for their antiviral effect on the replication of HCV in Huh 5.2 cells, as described previously.[29] Antiviral (IC_{50}), cytotoxicity (CC_{50}) and selectivity index (SI) properties of these compounds are listed in Table 2. As shown by the IC_{50} values listed, some of these nucleoside mimics showed selective antiviral activity against HCV replication in the Huh 5-2 HCV replicon system. Compound **4d** displayed anti-HCV activity with an IC_{50} value of 16 μM without any cytotoxicity at a concentration up to 100 μM. Interestingly, the length of the tether does not seem to have a dramatical effect on the activities and cytotoxicities of pyrimidines whereas purines seem more affected. Pyrimidine **4d** and purine **4e** are the most potent compounds suggesting a positive effect of the triazole moiety on the activity and selectivity.

In contrast, these compounds showed no anti-HIV activity when tested for their in vitro inhibition of HIV replication. Specific interactions with the biological targets due to the hydrophobic nature of the triazole ring or the reduced flexibility of the side chain might explain the differences of activities observed. Indeed, recent studies demonstrated that benzimidazole, thiophene, and benzothiadiazine heterocycles contact NS5B mainly through hydrophobic interactions.[30] Our results show that the triazoloacyclic nucleoside phosphonates described here are another class of derivatives with interesting potencies.

Table 2
Anti-HCV activity of compounds **4a**–**4f**

Compound	CC_{50} (μM)[a]	IC_{50} (μM)[b]	Max (%)[c]	SI[d]
4a	>300	26	ND	>13
4b	>300	20	79.28	>15
4c	131	29	ND	4.42
4d	>300	16	75.47	>20
4e	>300	16	80.65	>20
4f	35	>35	ND	<1

[a] CC_{50} concentrations of compound required for 50% extinction of Huh 5-2 cells.
[b] IC_{50} concentrations of compound achieving 50% inhibition of the replicon system.
[c] Max maximum percentage of inhibition observed at concentrations of compound that do not cause an antimetabolic effect. ND = no concentration of compound inhibited of the replicon without causing an antimetabolic effect.
[d] SI selectivity index = CC_{50}/IC_{50}.

In summary, we described a straightforward synthesis of a novel class of triazoloacyclic nucleoside phosphonates. A preliminary structure–activity relationship evaluation of these compounds suggests that this scaffold might have potential for further optimisation as selective inhibitors of HCV replication. The synthesis of additional and potentially more active compounds of this class of molecules is underway in our laboratory.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.046.

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