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Heterocyclic Nucleoside Analogues: Design and Synthesis of Antiviral, Modified Nucleosides Containing Isoxazole Heterocycles

Yoon-Suk Lee and Byeang Hyean Kim*

National Research Laboratory, Department of Chemistry, Center for Integrated Molecular Systems, Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang 790-784, South Korea

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Abstract—We have designed and synthesized novel antiviral nucleoside analogues, which consist of isoxazole rings as modified sugars and nucleobases (thymine, uracil, and 5-fluorouracil) with a methylene linker between them. These compounds represent a new class of modified nucleoside analogues and some of them show potent antiviral activities against Polio virus (Coxsackie B type 3 and Vesicular Stomatitis).

Modification of naturally occurring nucleosides is an important area for the development of antiviral agents such as anti-HIV drugs. Especially heterocyclic replacement of the nucleoside ribose moiety has resulted in an excellent anti-HIV drug, 3TC and is a promising field for searching antiviral lead compounds.[1] Adams and coworkers reported on the preparation of heterocyclic nucleoside analogues by using cycloaddition reactions of 1-vinylthymine with 1,3-dipoles.[2] Recently Zhao and coworkers have successfully synthesized isoxazolidinyl nucleosides **1** and dihydroisoxazole nucleosides **2** (Fig. 1), and investigated their anti-HIV-1 activities.[3] We designed and synthesized novel isoxazole nucleosides **3** (Fig. 1), and report here their efficient syntheses by using nitrile oxide cycloadditions with the propargylic dipolarophiles. We also investigated antiviral activities of these heterocyclic nucleoside analogues and reveal some bioassay results in this letter.

Most of the nucleoside analogues possessing antiviral activities rely upon specific phosphorylation of a virally encoded kinase.[4] Thus, it is well known that most antiviral nucleoside analogues should have 5′-hydroxyl groups for kinase phosphorylation. However, TSAO analogues[5] and HEPT analogues[6] which bind to the allosteric binding site are exceptional antiviral compounds having no 5′-free hydroxyl group. In these cases, 5′-hydroxyl groups are protected with bulky groups such as *t*-butyldimethylsilyl group. We first synthesized isoxazole and isoxazoline nucleosides with 5′-hydroxyl groups. Their antiviral activities are low. Then we turned our attention to the design and synthesis of isoxazole nucleosides **3** as antiviral compounds with allosteric binding activities shown in TSAO and HEPT analogues. The design of isoxazole nucleosides **3** as novel heterocyclic nucleoside analogues were based on the following considerations: (1) use of rigid isoxazole heterocycles as surrogates for conformationally restricted deoxyriboses, (2) introduction of the additional methylene unit between isoxazole and nucleobase to render conformational flexibility, (3) easy incorporation of chiral amino functionality by using α-amino acids as chiral starting materials.

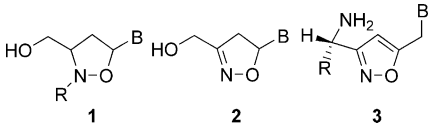

Figure 1. Modified nucleoside analogues with heterocyclic rings.

The *N*-protected amino aldoximes,[7] the precursors of nitrile oxides, were prepared from the corresponding amino acids in a four-step procedure as reported by us before.[7]

*Corresponding author. Tel.: +82-54-279-2115; fax: +82-54-279-3399; e-mail: bhkim@postech.ac.kr

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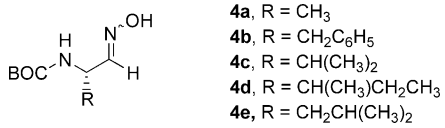
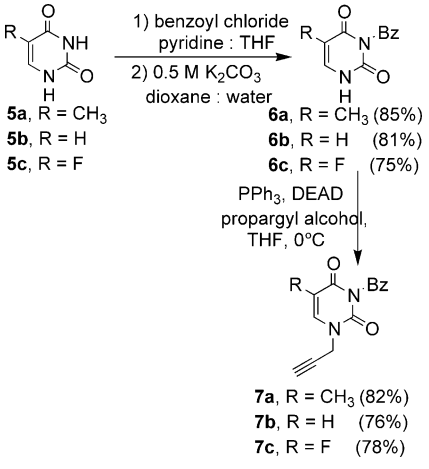


Figure 2. Amino aldoximes prepared.

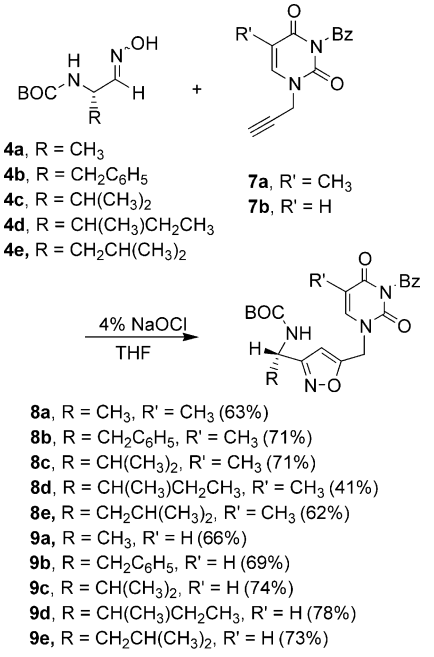
Starting from L-alanine, L-phenylalanine, L-valine, L-leucine and L-isoleucine, we obtained the corresponding *N*-Boc amino aldoximes **4a**–**4e** as mixtures of *E* and *Z* isomers (Fig. 2). The dipolarophilic N^1-propargyl N^3-benzoyl pyrimidines **7a**–**7c** were synthesized from corresponding N^3-benzoyl pyrimidines **6a**–**6c** by treating with propargyl alcohol in the presence of diethyl azodicarboxylate and triphenylphosphine.[8] The required N^3-benzoyl pyrimidines **6a**–**6c** were prepared by dibenzoylation of nucleobases **5a**–**5c** at N^1 and N^3 positions followed by selective deprotection at the N^1 position with weak base (Scheme 1).[9]

With both *N*-Boc amino aldoximes and N^1-propargyl N^3-benzoyl pyrimidines in hand, we next carried out [3 + 2] nitrile oxide cycloadditions by using a commercial bleaching agent (typically contains 4% NaOCl) as a practical reagent for nitrile oxide generation (Scheme 2). We obtained ten isoxazole nucleosides having thymine and uracil bases in the range of 41–78% yield. 5-Fluorouracil has been widely used for the development of pharmaceutically active compounds.[10] Thus we designed and synthesized isoxazole nucleoside analogues having a 5-fluorouracil base and a bulky group at the chiral carbon. Scheme 3 shows the preparation of compounds **10a** and **10b** and fully deprotected compounds **11a** and **11b**. In order to investigate the effect of protecting groups at N^3 of pyrimidine base and amino site in the antiviral activity, we also prepared several compounds with different protection patterns at two nitrogen sites (Fig. 3).

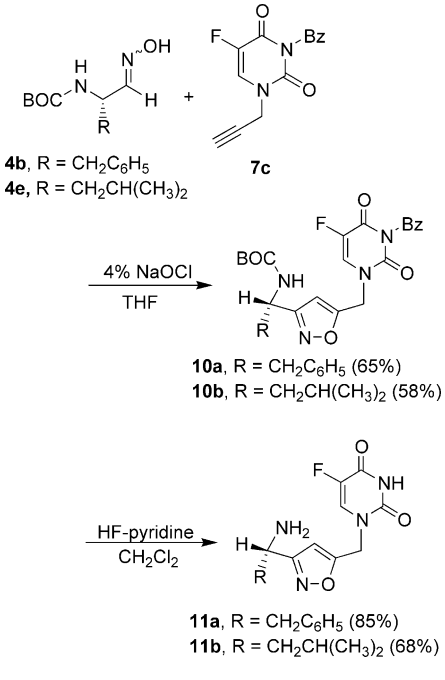
With 17 modified nucleosides (**8a**–**8e**, **9a**–**9e**, **10a**–**10b**, **11a**–**11b**, **12**–**14**) in hand, we next investigated their antiviral (anti-HIV, anti-HSV, anti-Polio) activities. From the bioassay results, we recognized that the bulky protection group (Boc or benzoyl) of the amino functionality played a vital role in the antiviral activities and complete deprotecition gave much poorer antiviral activities as shown in compounds **11a**–**11b**. Among 17 isoxazole nucleoside analogues, compounds **8b** and **9c** showed most potent anti-polio activities (Table 1). Especially compound **8b** is better than the reference drug, ribavirin in terms of EC_{50} value but have poorer selectivity index (SI) values. Although we do not know



Scheme 1.



Scheme 2.



Scheme 3.

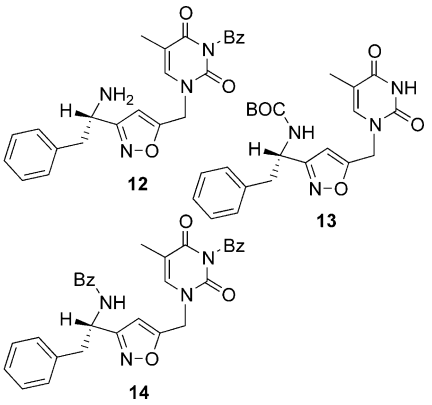


Figure 3. Prepared compounds with different protection patterns.

Table 1. Antiviral activities in terms of EC_{50} values

Compd	Anti-polio activity (EC_{50}, μg/mL)		Selectivity index (CC_{50}/EC_{50})	
	COX.B3[a]	VSV[b]	COX.B3[a]	VSV[b]
8b	10.61	7.94	2.46	3.29
9c	98.07	80.02	1.02	1.25
Ribavirin[c]	92.45	15.62	5.58	27.26

[a]Coxsackie B virus type 3.
[b]Vesicula stomatitis virus.
[c]Average values.

about the exact mechanism of action for antiviral activity of our heterocyclic nucleoside analogues, we speculate that these heterocyclic nucleoside analogues may bind at allosteric sites rather than the catalytic sites.

We are carrying out further modification of these compounds to improve antiviral activity and selectivity index and will report the results in due course.

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