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SYNTHESIS AND ANTI-HIV ACTIVITY OF DIHYDROISOXAZOLE 6-CHLOROPURINE AND ADENINE

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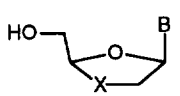
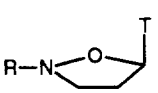
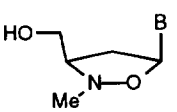
Abstract. *Dihydroisoxazole 6-chloropurine and adenine were efficiently prepared via 1,3-dipolar cycloaddition reaction of a nitrile oxide with vinyl 6-chloropurine, and found to be moderately effective against HIV-1 in acutely infected primary human lymphocytes.* Copyright © 1996 Elsevier Science Ltd

A variety of potent antiviral agents has been developed from naturally occurring nucleosides. Modification of the furanose substituents has resulted in several 2',3'-dideoxynucleoside analogs such as AZT, ddC, ddI and d4T, and the preservation of the furanose ring is the common feature of these potential antiviral agents (**1**, Figure).[1] Recently, the introduction of an oxygen or sulfur atom into the furanose skeleton resulted in dioxolanyl (**2a**)[2] and oxathiolanyl nucleosides (**2b**),[3,4] some of these diheteroatom-substituted analogs or their corresponding L-isomers were potent virus inhibitors.[2-4] However, little is known of nucleosides containing nitrogen as the second heteroatom, with the exception of the work of Tronchet's group, who first synthesized the *N*-alkyl-substituted isoxazolidine nucleosides (**3**) and their derivatives.[5] We have recently reported the synthesis and antiviral evaluation of the *hydroxymethyl* isoxazolidine analog (**4**).[6] The current report provides a facile approach to a new class of nucleosides (**5**) containing a dihydroisoxazole skeleton and nucleoside bases (B = 6-chloropurine and adenosine).







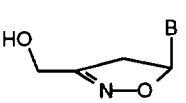
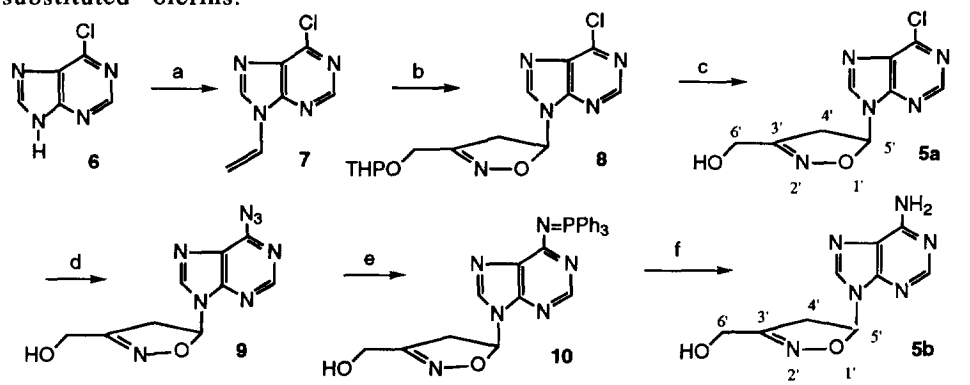


Figure **1**: X = CH_2
2a: X = O; **2b**: X = S **3**: R = Me, Ph **4** **5**

The design and synthesis of compounds (**5**) were based on the consideration of the steric and electronic factors of dihydroisoxazole as well as the feasibility of producing stable final products. Firstly, the nitrogen atom of the C=N-O moiety requires no substituents for a minimum of steric perturbation. Secondly, the direct

connection of nitrogen and oxygen atoms makes them weakly nucleophilic, resulting in possible stabilization of the glycosidic bond in **5**. Moreover, it appears that 1,3-dipolar cycloaddition reactions can be used for the preparation of dihydroisoxazole nucleosides (**5**). Although a variety of substituted olefins reacts with nitrile oxides,[7] a strategy using vinyl purine derivatives for the preparation of nucleoside analogs has not been reported. Our successful approach to **5** is shown in the Scheme below. The desired vinyl 6-chloropurine **7** was prepared by using a slightly modified procedure of Pitha and Ts'O,[8] which consists of treatment of 6-chloropurine **6** and vinyl acetate in the presence of catalytic concentrated sulfuric acid and mercuric acetate. The subsequent key 1,3-dipolar cycloaddition proceeded smoothly *via* the phenyl isocyanate-promoted reaction of $THPOCH_2CH_2NO_2$ and vinyl 6-chloropurine at room temperature to yield **8** in 68% yield. Deprotection of **8** was achieved by Dowex 50 (H^+) in methanol to obtain nucleoside **5a** in quantitative yield.[9] The regiochemistry of **5a** was determined from the ^{13}C NMR spectrum in which a resonance at 56 ppm is consistent with the non-heteroatom-substituted CH_2 at the 4'-position while the other possible regioisomer would produce an oxygen-substituted methylene group. This result is also consistent with other known 1,3-dipolar cycloaddition reactions, where the less sterically demanding oxygen is connected to the more hindered side of monosubstituted olefins.[7]



Scheme: (a) Vinyl acetate, conc. H_2SO_4 (cat.), $Hg(OAc)_2$ (cat.), reflux, 5 h; (b) $THPOCH_2CH_2NO_2$, PhNCO, Et_3N, THF, rt, overnight, 68%; (c) Dowex 50 (H^+), MeOH, rt, 1 h, 100%; (d) NaN_3, DMF, 80 °C, 15 min or NaN_3, EtOH, H_2O, reflux, 1 h; (e) Ph_3P, rt, 30 min. (f) AcOH, reflux, 30 min, 51% from **5a**.

An initial attempt to directly convert **5a** to the desired product **5b**, using methanolic ammonia under heating conditions, was unsuccessful. This was attributed to a weak glycosidic bond in **5a** that could be cleaved under basic conditions. To circumvent this problem, a different strategy was pursued in which compound **9** was prepared by treatment of **5a** with sodium azide. The reduction of azide **9** using 1,3-propanedithiol and catalytic triethylamine[10] gave adenine **5b** in low and inconsistent yield (<37%). A successful pathway was, however, found in the Staudinger reaction.[11]

Reaction of **9** with triphenylphosphine produced the stable ylide **10**, which was readily converted to the desired product **5b** by acidic hydrolysis.[12] Evaluation of **5a-b** *in vitro* exhibited moderate anti-HIV-1 activity and the adenine derivative **5b** showed no cytotoxicity up to 100 μM in activated primary human lymphoblastoid CEM cells or Vero cells (Table).[13] Although less toxic than AZT, none of the purines **5** were more potent. The synthesis of additional purine and pyrimidine derivatives is now underway which will hopefully produce more potent and selective analogs.

Table. Median Effective (EC_{50}) and Inhibitory (IC_{50}) Concentration for Dihydroisoxazole nucleosides **5** in Anti-HIV-1 Primary Human Lymphocytes (PMB cells), and Cytotoxicity in CEM and Vero Cells.

Compound	Anti-HIV-1 activity in PBM cells EC_{50} (μM)	Cytotoxicity in [IC_{50} (μM)]		
		PBM	CEM	Vero
5a	34.6	73.5	35.2	>100
5b	54.8	>100	>100	>100
AZT	0.004	>100	14.0	27.7

Nucleosides **5a-b** represent the first examples of potential antiviral agents which derive from dihydroisoxazole. The reported 1,3-dipolar cycloaddition method appears suitable for the construction of C=N-O containing nucleosides, and will be further applied towards the synthesis of other base-modified analogs. The use of the N-O moiety allows successful replacement of the methylene group with a nitrogen atom in the furanose ring of nucleoside derivatives. Consequently, it becomes possible to design and synthesize other classes of such analogs for the investigation of their antiviral activity.

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9. Compound **5a**: ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.83 (s, 1H, H-8), 8.76 (s, 1H, H-2), 7.01 (dd, *J* = 4.1, 8.5 Hz), 5.50 (t, *J* = 5.9 Hz, 1H, OH), 4.44-4.47 (m, 2H, H-6'), 3.79-3.83 (m, 2H, H-4'). ^{13}C NMR (50 MHz, DMSO-d_6/D_2O) δ 161, 152, 151, 150, 146, 131, 108, 84, 56. Anal. calcd for $C_9H_8ClN_5O_2$: C, 42.62; H, 3.17; N, 27.61. Found: C, 42.66; H, 3.23; N, 27.62.

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12. Compound **5b**: ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.19 (s, 1H, H-8), 8.18 (s, 1H, H-2), 7.34 (s, 2H, NH_2), 6.85 (dd, *J* = 8.8, 4.3 Hz, 1H, H-5'), 5.48 (t, *J* = 6.1 Hz, 1H, OH), 4.43 (d, *J* = 3.2 Hz, 1H, H-6'), 4.40 (d, *J* = 3.2 Hz, 1H, H-6'), 3.72 (d, *J* = 9.1 Hz, 1H, H-4'), 3.69 (d, *J* = 4.3 Hz, 1H, H-4'). Anal. calcd for $C_9H_{10}N_6O_2 \cdot 0.25H_2O$: C, 45.28; H, 4.43; N, 35.20. Found: C, 45.43; H, 4.37; N, 35.24.

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