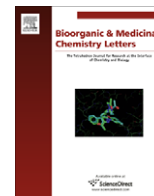




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Synthesis of 5-isoxazol-5-yl-2'-deoxyuridines exhibiting antiviral activity against HSV and several RNA viruses

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ABSTRACT

This paper describes a simple method for synthesizing a small library of 5-isoxazol-5-yl-2'-deoxyuridines from 5-iodo-2'-deoxyuridine. Nitrile oxides were generated in situ from oximes using a commercial bleaching agent; their cycloaddition with 5-ethynyl-2'-deoxyuridine yielded isoxazoles possessing activity against herpes simplex viruses 1 and 2, Encephalomyocarditis virus, Coxsackie B3, and vesicular stomatitis virus; these isoxazoles were, however, inactive against corona virus, influenza virus, and HIV.

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Herpes simplex viruses (HSVs) are among the most widespread viruses on Earth. HSV-1 and HSV-2 are two common strains of the herpes virus family, *Herpesviridae*. About 50–70% of young adults have HSV-1 antibodies in their blood. The persistent nature of herpes infection is the reason for the high probability of infection.¹ HSVs cause oral and facial lesions in humans, beginning on the skin or mucosal epithelium. Because the resistance of HSV toward antiviral drugs is increasing, the development of new drugs possessing potent antiviral activity is very important. Acyclic nucleosides, including Acyclovir (ACV), Penciclovir, and Ganciclovir, are the largest class of compounds used as anti-herpes drugs.² Another nucleoside drug class is the C5-modified pyrimidine nucleosides, including C5-iodo-2'-deoxyuridine (IdU) and Brivudine (BVDU),³ which inhibits the action of DNA polymerases after its incorporation into the viral DNA, thereby preventing viral replication. Herdewijn's group synthesized, from 5-iodo-2'-deoxyuridine, a large number of C5-heteroaromatic-substituted 2'-deoxyuridines possessing anti-HSV activity. In 1993, they synthesized 5-isoxazol-5-yl-2'-deoxyuridine **1** from 5-(3-oxo-propyn-1-yl)-2'-deoxyuridine (Fig. 1) and hydroxylamine-*O*-sulfonic acid.⁴ The [3+2] cycloaddition is a very powerful reaction for synthesizing a diverse range of heteroaromatic five-membered rings.⁵

Previously, we reported the use of [3+2] cycloadditions to efficiently synthesize sugar-modified isoxazole nucleosides and backbone-modified DNA.⁶ In this paper, we describe the synthesis of novel C5-modified nucleosides **2** through [3+2] cycloadditions.

We performed [3+2] cycloadditions using a commercial bleaching agent (containing ca. 4% NaOCl) as a practical reagent for generating the nitrile oxide. As the key intermediate and dipolarophile, we synthesized the acetyl-protected 5-ethynyl-2'-deoxyuridine **5** through Sonogashira coupling (87% yield) of 5-iodo-2'-deoxyuridine and TMS-acetylene (See Scheme 1).^{7,8} The oximes **6** were obtained from the reactions of several aldehydes with hydroxylamine (see Scheme 2). For the [3+2] cycloadditions, we employed previously established reaction conditions.⁶ Dropwise addition of the commercial bleaching agent provided higher yields—ranging from 59% to 77%—than did the use of reagent-grade 30% NaOCl (see Fig. 2 and Scheme 3).

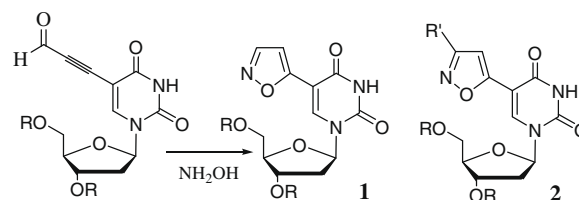
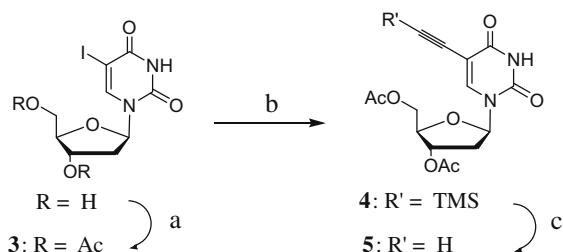
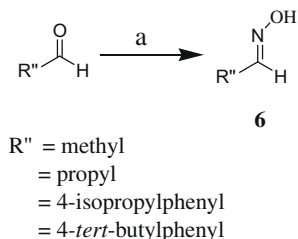


Figure 1. C-5 isoxazole nucleosides.

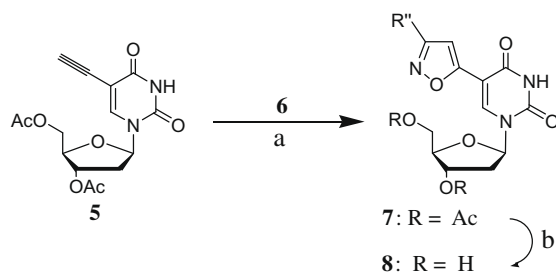
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Scheme 1. Synthesis of **3–5**. Reagents and conditions: (a) Ac₂O, pyridine, rt, 8 h; yield 92%. (b) TMS-acetylene, Pd(PPh₃)₄, Et₃N, DMF, 40 °C, 8 h; yield 87%. (c) KF, 10% MeOH in CH₂Cl₂; yield 80%.



Scheme 2. Synthesis of oximes **6**. Reagents and conditions: (a) HONH₂·HCl, 1 N NaOH, THF (1:1), rt, 6 h; yield 90–99%.



Scheme 3. Synthesis of **7** and **8**. Reagents and conditions: (a) 4% NaOCl, THF, slow dropwise addition, rt, 10 h; yield 59–77%. (b) LiOH, MeOH/water (3:1), rt 10 h; yield 60–89%.

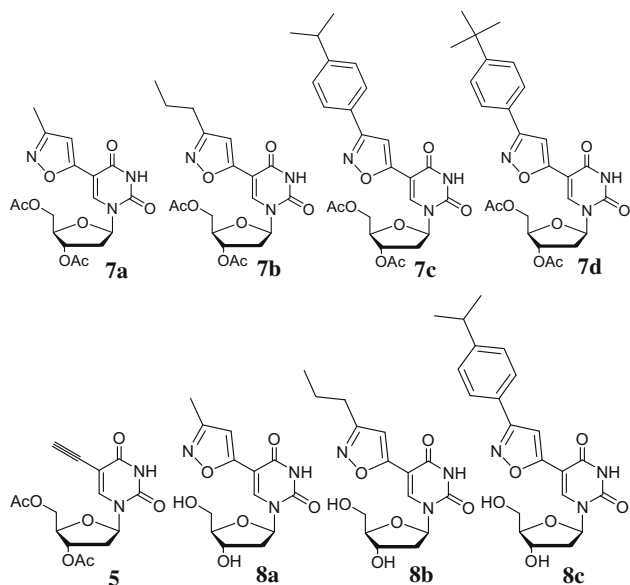


Figure 2. Compounds tested for their antiviral properties against 12 different viruses.

Table 1

In vitro EC₅₀ data for HSV-1 and HSV-2 assays

	Toxicity		Antiviral activity (EC ₅₀)		Selectivity index	
	CC ₅₀	CC ₅₀	HSV-1	HSV-2	HSV-1	HSV-2
7c	0.15	0.08	0.07	0.07	0.07	2.03
7d	0.06	>0.06	0.03	0.03	<1.0	1.81
8c	0.26	0.09	0.09	0.09	2.88	2.83
ACV>	>10.00	0.18	0.36	0.36	55.76	>27.78
Ara-C>	>10.00	0.20	0.52	0.52	49.13	>19.38

EC₅₀ (μg/mL): Concentration of test compound required to inhibit virus-induced cytopathicity by 50%; CC₅₀ (μg/mL): concentration of test compound that causes 50% cytotoxicity to uninfected cells; selectivity index: CC₅₀/EC₅₀.

Table 2

In vitro EC data for EMCV, Cox. B3, and VSV assays

	Toxicity		Antiviral activity (EC ₅₀)			Selectivity index		
	CC ₅₀	CC ₅₀	EMCV	Cox. B3	VSV	BMCV	Cox. B3	VSV
7c	0.05	0.01	0.01	0.03	0.03	4.91	5.38	1.65
7d	0.04	0.01	0.01	0.03	0.03	5.94	5.30	1.50
8c	0.07	0.02	0.03	>0.07	>0.07	3.30	2.76	<1
Rib.	0.30	0.02	0.04	0.08	0.08	>13.91	>7.69	>35.22

EC₅₀ (μg/mL): Concentration of test compound required to inhibit virus-induced cytopathicity by 50%; CC₅₀ (μg/mL): concentration of test compound that causes 50% cytotoxicity to uninfected cells; selectivity index: CC₅₀/EC₅₀.

We investigated the antiviral activities for the various compounds against 12 different viruses, namely HSV-1, HSV-2, HIV-1, HIV-2, EMCV, Cox. B3, VSV, three different influenza viruses (Taiwan, Seoul, and Panama), and two corona viruses. The isoxazole nucleosides all exhibited anti-HSV activity. The nucleoside analogues presenting bulky R'' groups possessed anti-herpes activity (Table 1). On the other hand, compound **7d**⁹ exhibits only HSV-2 activity. The antiviral activity for HSV-1 of **7d** couldn't be determined due to the high toxicity (CC₅₀ = 0.06 μg/mL). The anti-herpes activities of the isoxazole nucleoside analogues are better than those of the reference drugs ACV and Cytarabine (Ara-C); for example, **8c**⁹ and the acetylated prodrug **7c**⁹ are ca. twice as active against HSV-1, and ca. 4-fold more active than ACV and 6- to 7-fold more active than Ara-C against HSV-2. Normally the acetyl groups increase the lipophilicity and support the cell penetration due to the cleavage of the acetyl groups by carboxylesterase in the cell. The **7d**, one of isoxazole nucleoside analogues exhibited the best anti-HSV-2 activity: 12- and 17-fold more active than ACV and Ara-C, respectively.

The isoxazole nucleosides displayed anti-RNA virus activities (Table 2) against Coxsackie B3 (Cox. B3), vesicular stomatitis virus (VSV), and Encephalomyocarditis virus (EMCV). In the RNA virus assay, compound **8c** exhibited almost the same anti-EMCV and anti-Cox. B3 potencies as that of the reference, ribavirin (Rib); compounds **7c** and **7d**, however, possessed 2- to 4-fold better antiviral activities against the three RNA viruses. Although some of the isoxazole nucleosides are more active than the reference drug, the bioassay results were disappointing in terms of their cytotoxicity. The selectivity indexes for the isoxazole nucleosides were lower than those of the reference drugs. None of the compounds exhibited antiviral activity against HIV, influenza, or corona viruses. In future studies, we will use the [3+2] cycloaddition strategy to synthesize different C5-triazole-substituted nucleosides from azides, rather than oximes.

Acknowledgments

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- Selected spectroscopic data*. **5**: ^1H NMR (300 MHz, acetone- d_6) δ 10.34 (br, 1H), 8.01 (s, 1H), 6.24 (t, J = 7.20 Hz, 1H), 5.31–5.27 (m, 1H), 4.35–4.29 (m, 3H), 2.51–2.47 (m, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 2.03–2.01 (m, 1H).
Compound **7a**: ^1H NMR (300 MHz, CDCl_3) δ 8.37 (br, 1H), 6.74 (s, 1H), 6.35 (t, J = 6.77 Hz, 1H), 5.35 (br, 1H), 4.46–4.39 (m, 2H), 4.38–4.35 (m, 1H), 2.58–2.54 (m, 2H), 2.28 (s, 3H), 2.19 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 172.0, 171.8, 163.6, 162.0, 161.3, 151.0, 139.5, 126.3, 105.1, 104.4, 87.5, 84.4, 76.1, 65.4, 39.2, 21.4, 21.3, 11.8; MS (EI+) m/z 393.12, calcd 393.096.
Compound **7b**: ^1H NMR (300 MHz, CDCl_3) δ 8.30 (br, 1H), 6.79 (s, 1H), 6.39 (t, J = 5.71 Hz, 1H), 5.26 (d, J = 6.32 Hz, 1H), 4.46–4.41 (m, 1H), 4.33–4.28 (m, 2H), 2.66–2.61 (m, 2H), 2.53–2.51 (m, 1H), 2.32–2.29 (m, 1H), 2.27 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 170.8, 170.5, 165.2, 161.1, 159.1, 149.1, 137.4, 126.3, 105.1, 101.3, 85.9, 83.3, 74.6, 64.1, 38.8, 28.3, 21.9, 21.2, 21.1, 14.0; MS (EI+) m/z 421.15, calcd 421.149.
Compound **7c**: ^1H NMR (300 MHz, CDCl_3) δ 8.85 (br, 1H), 8.39 (s, 1H), 7.68 (d, J = 8.21 Hz, 2H), 7.23 (d, J = 8.08 Hz, 2H), 7.25 (s, 1H), 7.24 (s, 1H), 6.41 (t, J = 5.62 Hz, 1H), 5.27 (s, 1H), 4.42–4.37 (m, 1H), 4.36–4.32 (m, 2H), 2.90–2.85 (m, 1H), 2.54–2.48 (m, 1H), 2.26–2.23 (m, 1H), 2.21 (s, 3H), 2.05 (s, 3H), 1.20 (s, 3H), 1.18 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 170.7, 170.4, 163.4, 161.7, 159.1, 151.5, 149.1, 137.4, 127.2, 126.6, 104.9, 101.5, 86.0, 83.2, 74.5, 64.0, 38.7, 34.3, 21.1, 21.0; MS (EI+) m/z 497.18, calcd 497.180.
Compound **7d**: ^1H NMR (300 MHz, CDCl_3) δ 8.95 (br, 1H), 8.39 (s, 1H), 7.77 (d, J = 8.19 Hz, 2H), 7.46 (d, J = 8.09 Hz, 2H), 7.26 (s, 1H), 7.24 (s, 1H), 6.41 (t, J = 5.76 Hz, 1H), 5.27 (s, 1H), 4.47–4.44 (m, 1H), 4.36–4.32 (m, 2H), 2.57–2.53 (m, 1H), 2.29–2.33 (m, 1H), 2.26 (s, 3H), 2.12 (s, 3H), 1.33 (s, 9H); ^{13}C NMR (75 MHz, CDCl_3) δ 170.7, 170.4, 163.4, 161.7, 159.1, 151.5, 149.1, 137.4, 127.2, 126.6, 104.9, 101.5, 86.0, 83.2, 74.5, 64.0, 38.7, 34.3, 21.1, 21.0; MS (EI+) m/z 511.20, calcd 511.195.
Compound **8c**: ^1H NMR (300 MHz, acetone- d_6) δ 8.90 (br, 1H), 8.39 (s, 1H), 7.82 (d, J = 8.27 Hz, 2H), 7.37 (d, J = 8.20 Hz, 2H), 7.21 (s, 1H), 6.37 (t, J = 6.59 Hz, 1H), 5.27 (s, 1H), 4.57–4.54 (m, 2H), 4.51–4.49 (m, 1H), 4.05–4.03 (m, 1H), 3.90–3.87 (m, 2H), 2.98–2.91 (m, 1H), 2.41–2.37 (m, 2H), 1.26 (s, 3H), 1.24 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 170.7, 170.4, 163.4, 161.7, 159.1, 151.5, 149.1, 137.4, 127.2, 126.6, 104.9, 101.5, 86.0, 83.2, 74.5, 64.0, 38.7, 34.3, 21.1, 21.0; MS (EI+) m/z 413.16, calcd 413.159.