Communications to the Editor

Synthesis, Anti-Human Immunodeficiency Virus and Anti-Hepatitis B Virus Activities of Novel Oxaselenolane Nucleosides

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Since dioxolane[1−4] and oxathiolane[5−10] nucleosides have exhibited promising antiviral and anticancer activities, it was of interest to synthesize an isosteric class of compounds, oxaselenolane nucleosides in search of biologically interesting nucleosides. Despite their structural similarity to the known 3′-heteroatom-substituted nucleosides, the synthesis of oxaselenolane nucleosides has been elusive. A plausible method for the construction of oxaselenolane ring with proper substituents has never been reported to the best of our knowledge. 1,6-Anhydro and 1,6-epithio sugars have been successfully used for the asymmetric syntheses of dioxolane[1−4] and oxathiolane[7] nucleosides, respectively. Recently, a 1,6-episeleno sugar has also been reported.[11] However, similar procedures used for asymmetric syntheses of dioxolane and oxathiolane nucleosides which involved oxidation reactions could not be applied in the synthesis of oxaselenolane nucleosides because one of the characteristic reactions of selenides is oxidative elimination.[12] We describe herein our preliminary results on the synthesis and antiviral activities of racemic oxaselenolane pyrimidine nucleosides against HIV and HBV *in vitro*.

Retrosynthetic analysis of the title compounds suggested that one of the key steps for the synthesis of oxaselenolane nucleosides is to construct the oxaselenolane ring with proper substituents. Thus, selenol acetic acid was selected as the precursor of the key intermediate, oxaselenolanone **5** (Scheme 1). Selenocyanate **2** was prepared by the method of Kirby[13] in excellent yield. In order to construct lactone **5**, we initially attempted to reduce the selenocyanate **2** with $NaBH_4$[14] and hydrolyze the resulting ester with aqueous NaOH to selenol acetic acid, which could be used for the construction of the oxaselenolane ring system **5**. However, it was found that selenol acetic acid decomposed during the acidification with HCl at pH 2. It was reported[15] that selenols could be readily oxidized by oxygen in air to stable dimers which can be reduced back to the selenols by H_3PO_2. On the basis of this property of the selenols and the instability of selenoacetic acid, we designed and explored an approach in which reduction of the bis(selenoacetic acid) to selenol, as well as the method that cyclization take place in an one-pot reaction without isolation. Thus, dimer **3** was prepared in 81% yield by refluxing **1** with KSeCN in ethanol for 1 h followed by reduction with $NaBH_4$ at 0 °C for 20−30 min. In comparison to recently reported procedure for the preparation of diselenides,[16] our method has advantages of milder reaction conditions, higher yield, and an easier workup. Lactone **5** was then prepared in 33% yield by the hydrolysis of **3** with refluxing aqueous acetic acid (50%) for 24 h followed by the reduction with H_3PO_2 to selenol acetic acid, which was condensed *in situ* with 2-(benzoyloxy)acetaldehyde under nitrogen. Diisobutylaluminum hydride (DIBAL-H) has been the regent of choice for reduction of lactone to lactol. However, no selective reduction of lactone in the presence of ester was reported. For reduction of the lactone **5**, fortunately, it was found that DIBAL-H can selectively reduce the lactone in THF, while no selectivity was observed in toluene. Thus, we were able to prepare the cyclic acetate **7** by DIBAL-H reduction of **5** in THF followed by *in situ* acetylation with acetic anhydride. Condensation of the acetate **7**, without purification, with silylated bases in the presence of $SnCl_4$ or TMSOTf gave inseparable mixtures of α- and β-isomers **8a** and **8b**. Removal of the benzoyl protecting group of **8a** and **8b** by methylamine or ammonia in methanol gave the final nucleosides as an α/β mixture. The α-cytosine nucleoside **10a** was obtained by repeated recrystallization of the α/β mixture from MeOH/Et_2O followed by methanol, while β-cytosine nucleoside **9a** was obtained by HPLC separation of the mother liquor (C_{18} column, 20% MeOH in H_2O). For the separation of the β- and α-5-fluorocytosine nucleosides (**9b** and **10b**), analytical HPLC (C_{18} column, 5−10% MeOH or CH_3CN in H_2O) gave differences of retention times of 3−5 min. However, preparative HPLC showed only a broad peak under the same conditions. Fortunately, the individual compounds were obtained by vacuum silica gel chromatographic separation of the α/β mixture (EtOAc−hexanes−$CHCl_3$−MeOH, 20−20−20−1, v/v). The structures of the synthesized oxaselenolane nucleosides were confirmed by elemental analyses and ^1H and ^{13}C NMR. Stereochemical assignments were determined based on 2D-NOESY experiments, in which a correlation between 2′-H and 5′-H of the β-isomer **9b** was observed while an absence of this correlation in α-isomer **10b** was noted. Additionally, a correlation between 6-H and 2′-H of the α-isomer **10b** was observed while no such correlation exhibited for the β-isomer **9b**. The assignment of stereochemistry was also supported by the upfield chemical shifts of 2′-H (β-form) in **9a** and **9b** compared to that (α-form) of **10a** and **10b** due to deshielding effects by heterocyclic bases. Single-crystal

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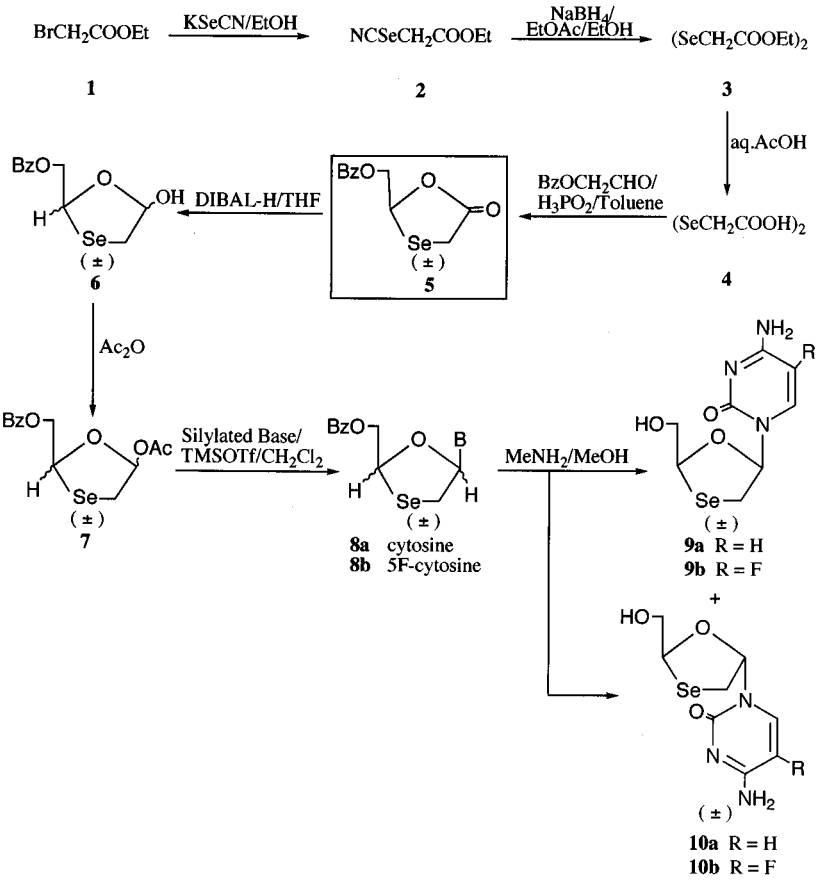
Scheme 1. Synthesis of Oxaselenolane Nucleosides



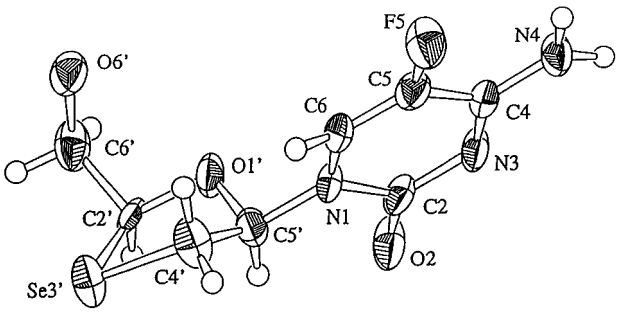


Figure 1. ORTEP drawing of oxaselenolane 5-fluorocytosine nucleoside **9b**.

X-ray crystallography ($P = 121.5°$, corresponding to the conformation $_1$E, Figure 1) of 5-fluorocytosine analog (β-**9b**) further supported the above stereochemical assignments.

Antiviral evaluations of the cytosine and 5-fluorocytosine analogs (**9a** and **9b**) showed potent anti-HIV activity (EC_{50} 0.88 and 0.51 μM, respectively) and anti-HBV activity (EC_{50} 1.2 μM for both compounds) with no toxicities up to 100 μM in various cell lines (PBM, CEM, and Vero). It is interesting to note that the α-isomer (**10a**) also exhibited moderately potent antiviral activity against HIV with no toxicities up to 100 μM in PBM, CEM, and Vero cells, which is akin to the previous studies.[17] As with racemic BCH-189 and FTC, the 5-fluoro derivative **9b** was more potent than the cytosine analog **9a** against HIV-1 infected primary lymphocytes (Table 1).[8−10] In order to compare the relative activities of the synthesized oxaselenolane nucleosides against HIV and HBV, the activity data of 3TC were also included in Table 1.

Table 1. Anti-HIV and Anti-HBV Activities of Racemic Oxaselenolane Nucleosides *in Vitro*

compd	anti-HIV activity in PBM cells (EC_{50}, μM)[a]	anti-HBV activity in 2.2.15 cells (EC_{50}, μM)	toxicity (IC_{50}, μM)		
			PBM	CEM	Vero
9a (β)	0.88	1.2	>100	>100	>100
9b (β)	0.51	1.2	>100	>100	>100
10a (α)	2.4	ND[b]	>100	>100	>100
10b (α)	>10	ND	>100	>100	>100
3TC	0.07[10]	0.008[9]	>100	>100	>100

[a] PBM = peripheral blood mononuclear. [b] ND = not determined.

In summary, the first synthetic method for novel class of oxaselenolane nucleosides has been developed, which led to cytosine and 5-fluorocytosine nucleosides with potent anti-HIV and anti-HBV activities. The synthesis and biological evaluation of related purine and pyrimidine analogs, as well as the preparation of optically pure oxaselenolane nucleosides are in progress in our laboratories.

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Supporting Information Available: Experimental details (4 pages). Ordering information is given on any current masthead page.

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