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Synthesis of 5-radioiodoarabinosyl uridine analog for probing HSV-1 thymidine kinase gene: an unexpected chelating effect

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Abstract

Tumor cells transduced with herpes simplex virus thymidine kinase gene has been intensively applied to the field of positron emission tomography via imaging of its substrate. As a pilot synthesis approach, a facial preparation of 5-[^{125}I]iodoarabinosyl uridine starting from commercial available uridine is reported herein. Interestingly, the tin group in 5-trimethylstannyl arabinosyluridine was easily removed during purification. The destannylation through the formation of a six-ligand coordination involving 2′ -hydroxyl and tin was thereby proposed.

Keywords: Arabinosyl; Chelating; HSV-1 TK; PET; Tin; Uridine

1. Introduction

Herpes simplex virus (HSV-1) thymidine kinase (TK) has become of great interest in medical research as a suicide or reporter gene in therapeutic or imaging technique [1,2]. The rationale of the medical application is based on the observation that viral TK presents a broader substrate specificity profile compared to mammalian TK. Moreover, in vivo gene expression of HSV-1 TK in cell models can be successfully monitored by imaging its substrate analog, usually a nucleoside analog. After being phosphorylated by viral TK, the metabolite can be randomly incorporated into elongating DNA chains by cellular enzymes, causing a premature termination of DNA replications known as gene suicide mechanism [3,4]. The localization of this event can be traced down by using a tagged nucleoside analog. The unique decay characteristics of positron emitters make a labeled nucleoside analog an excellent candidate for providing high-resolution quantifiable images of in vivo studies. Particularly, through positron emission tomography (PET), a superior temporal and spatial resolution for imaging can be obtained. Also, the assessment of gene expression levels in animal models can be successfully quantified by using PET [5,6].

The most widely used imaging probes can be categorized into two groups: ^{124}I-labeled pyrimidine nucleosides [4,7] and ^{18}F-labeled acyclopurine nucleosides [8–10]. Although pyrimidine nucleosides such as [^{124}I]FIAU have shown to accumulate with a significant level in HSV-1 TK gene-expressed cell lines, purine nucleosides such as [^{18}F]FHBG have exerted a better effect in mutant gene such as HSV-1 sr39TK [11]. Intensive studies have suggested that different transduction protocols, for instance, liposome-wrapped or virus-mediated transduction methods, may contribute to these contradictory outcomes [11,12]. In addition to the variation of transduction methods, other factors such as the sensitivity of imaging probes might also lead to the development of a more powerful gene expression technique. To address this issue and, in return, expedite the research in the molecular cell biology, we are interested to establish an imaging probe library as fundamental tools.

Our immediate goal was to synthesize a variety of thymidine analog derivatives. The main drawback of these deoxyuridines is their instabilities upon encountering cellular catabolism enzymes that mediate a hydrolytic phosphorylation at their glycosidic bonds. Yet, introduction of electronegative groups, for instance, fluorine or hydroxyl

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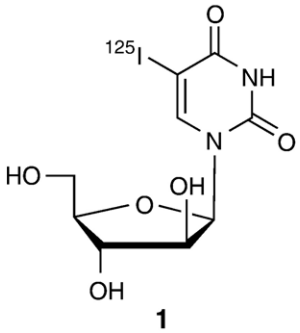


Fig. 1. Target compound to be synthesized.

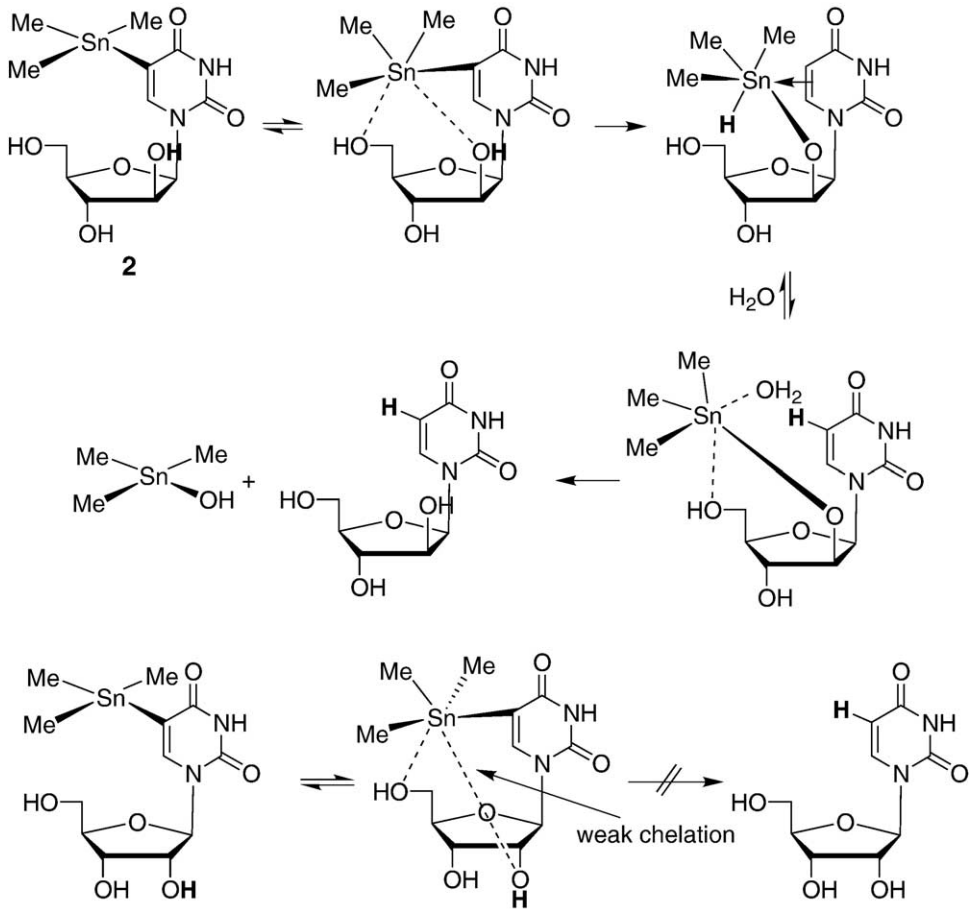
groups at the C-2′ position, can prevent deoxyuridines from forming the carbocation intermediates, thereby increasing their resistance to the catabolism processing [13,14].

To provide an optimal HSV TK activity, substituents with ‘up’ configuration are required. Recent reports indicated that arabinosyl uridine is an excellent candidate for this purpose [12]. As part of our systematic syntheses of radiolabeled thymidine analogs [15–19], we report herein the preparation of 5-[^{125}I]iodoarabinosyl uridine (**1**) (Fig. 1). This synthesis should be able to apply to positron emitter-labeling chemistry accordingly.

2. Methods

*2.1. 1-(β-D-arabinofuranosyl)-5-[^{125}I] iodopyrimidin-2,4(3H)-dione **1***

5-trimethylstannyl arabinosyluridine (**2**) (500 μg) was added to a microcentrifuge tube (1.5 ml) containing $CHCl_3$ (400 μl). Na[^{125}I]I (5×10^4 cpm/10 μl) in NaOH (800 μl, 0.1 N) was added in the above mixture followed by a solution mixture of acetic acid (200 μl) and hydrogen peroxide (30%) with a ratio of 3:1 (v/v). The whole reaction mixture was treated with ultrasonic generator for 1 min and applied onto HPLC for analysis. Agilent HPLC was equipped with a sample loop (20 μl), an analytical column [4.6×150-mm ZORBAR Eclipse XDB-C8 (5-μm material)], and UV detector setting at 260 nm. The fractions collected from UV detector were counted for liquid scintillation on a 96-well microtiterplate reader (Plate CHAMELEON). Time delay between UV signal and radioactivity was calibrated in the chromatogram. The eluent



Scheme 1. Proposed chelating effects of stannyl arabinosyl uridine (not for ribosyl analog).

used was 0.5 mM Na_3PO_4/H_3PO_4 (pH 5.7), with a flow rate of 1 ml/min. More than 99% of the radioactivity in radiochromatogram was obtained as a single peak, corresponding to that of compound **1** (t_R=14.47 min). No further radioactivity was detected between 20 and 40 min when MeOH set as isocratic auxiliary component from 0% to 30%.

3. Results and discussion

One of the most efficient methods of radiohalogenation of compound **1** is via compound **2** by halodestannylation. Synthesis of compound **2** from commercial uridine can be achieved in 32% yield according to referential methods in total six steps [20–34]. It was found that compound **2** was particularly labile. Such an unusual instability for 2′-fluoro moieties has not been addressed in the recent report for synthesis of FIAU. It is hypothesized that the 2′-hydroxyl might participate in the formation of a six-ligand coordination through a chelating effect (Scheme 1), via coordination of π orbital to a tin group followed by a migration of electrons, being eventually substituted by water molecules. To further verify this mechanism, the ribosyl analog with 2′-hydroxyl toward 'down' configuration was prepared, and the stannyl group was found to be very stable in the whole process. These observations indicated that the formation of the six ligand-coordinated complex, facilitated by the geometry of the ligands, might be thermodynamically unfavored.

In contrast to the water-involved destannylation, compound **2** was relatively stable in aprotic solvents, for example, $CHCl_3$, the common cosolvent used for radioiodolabeling [20]. Indeed, the radioiodination of the tin compound **2** was efficient and mild. Approximately 99% of radioactive Na[^{125}I]I was successfully converted into compound **1**. There is no need to prepare the fresh tin compound before the radiolabeling experiment since compound **2** can be stored at −20°C for 3 months without serious decomposition. Bioactivity assay in HSV-1 TK transduced cell models and radiolabeling with Na[^{124}I]I for further applications to image TK-transduced tumor cells are currently in progress.

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