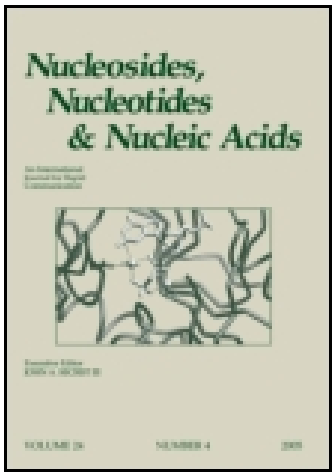

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CHEMICAL AND ENZYMATIC SYNTHESIS OF 4′-THIO-β-D-ARABINOFURANOSYLCYTOSINE MONOPHOSPHATE AND TRIPHOSPHATE

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CHEMICAL AND ENZYMATIC SYNTHESIS OF 4′-THIO-β-D-ARABINOFURANOSYLCYTOSINE MONOPHOSPHATE AND TRIPHOSPHATE

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▫ *N^4-Acetyl-1-(2, 3-di-O-acetyl-4-thio-β-D-arabinofuranosyl)cytosine (2) was synthesized in three steps from 1-(4-thio-β-D-arabinofuranosyl)cytosine (1). The reaction of this partially blocked 4′-thio-ara-C derivative 2 with 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one gave the 5′-phosphitylate derivative 3, which on reaction with pyrophosphate gave the 5′-nucleosidylcyclotriphosphite 4. Product 4 was then oxidized with iodine/pyridine/water and deblocked with concentrated ammonium hydroxide to provide the desired 4′-thio-ara-C-5′-triphosphate 5. This triphosphate 5 was converted to 4′-thio-ara-C -5′-monophosphate 6 by treatment with snake venom phosphodiesterase I. The details of the synthesis, purification, and characterization of both nucleotides are described.*

INTRODUCTION

In an ongoing search for new nucleoside analogues with selective anticancer activity, a series of 4′-thionucleosides has been synthesized in our laboratories.[1] One of these compounds, 4′-thio-ara-C, which was originally synthesized by Whistler et al.[2,3] in the early seventies, was subsequently resynthesized by us in a more efficient manner.[4] This nucleoside has shown very good anticancer activity in a broad spectrum of human tumor xenografts including colon, non-small cell lung, prostate, renal, breast, and pancreatic cancers.[4] This compound is currently being evaluated in phase I clinical trials. In order for us to study its mechanism of action and other pharmacological properties, we needed to synthesize the monophosphate and the triphosphate of 4′-thio-ara-C. Because of the inherent

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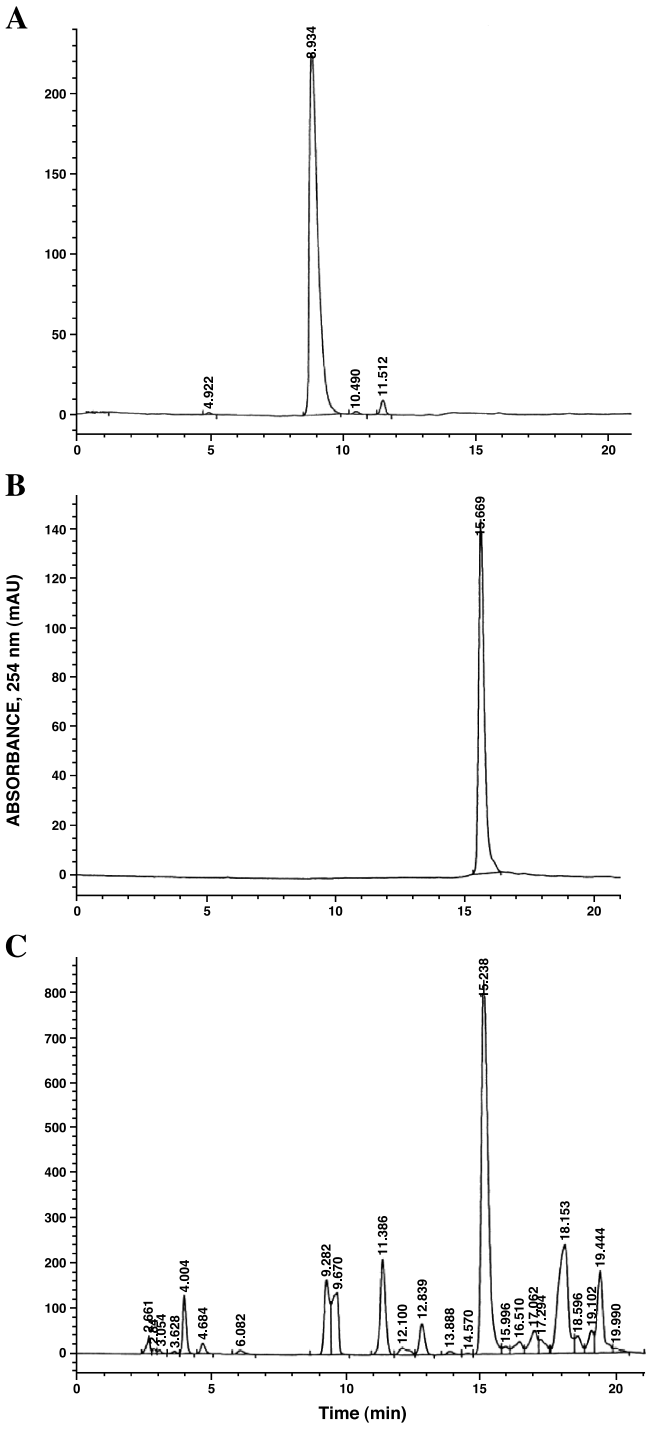

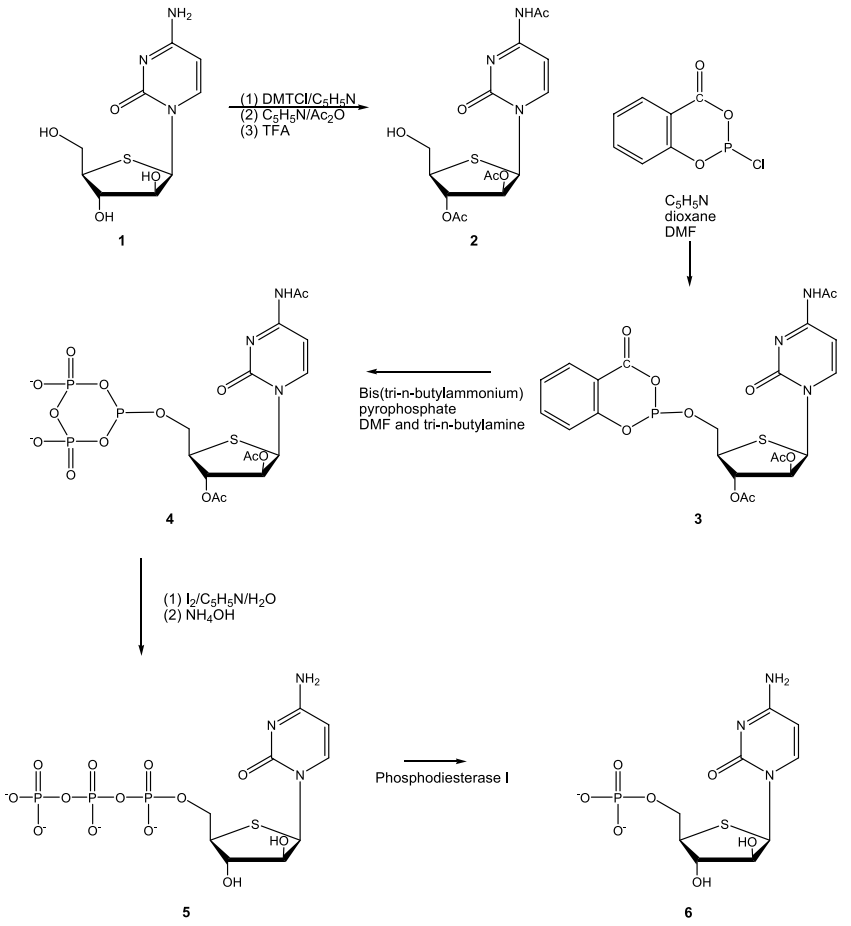

SCHEME 1

difficulty associated with the stability of dichloridates of this class of compounds,[5] we were unable to synthesize the monophosphate or the triphosphate of 4′-thio-ara-C by traditional methods.[6,7] Previously, Ludwig and Eckstein reported a new method for the synthesis of 5′-*O*-(1-thiotriphosphates) and 5′-triphosphates using 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one.[8] The use of this multifunctional reagent was found to give good yields of 5′-*O*-(1-thiotriphosphates) and 5′-triphosphates. Since this reagent was commercially available, we decided to use this approach in the synthesis of 4′-thionucleotides. The details of these syntheses are reported below.

FIGURE 1 HPLC Assays: (A) purified 4′-*S*-araCMP; (B) purified 4′-*S*-araCTP; (C) crude 4′-*S*-araCTP. Elution conditions: column, Waters μBondapak C18, 10 μm; eluent A, 0.01 M $NH_4H_2PO_4$ and .005 M PIC A (tetrabutylammonium phosphate) ion-pairing reagent (pH 5.0); eluent B, MeOH; elution, 20-min linear gradient from 10 to 40% eluent B (chromatogram A) or 20–50% eluent B (chromatograms B & C) at a flow rate of 1 mL/min.

CHEMISTRY

The synthesis of N^4-acetyl-2′, 3′-di-*O*-acetyl-4′-thio-ara-C **2** was carried out from 4′-thio-ara-C **1** in a one-flask sequence of blocking 5′ with dimethoxytrityl followed by acetylation with acetic anhydride in pyridine and subsequent detritylation with trifluoroacetic acid. After silica gel column chromatography of the evaporated crude mixture, a portion of this appropriately blocked nucleoside **2** (0.1 mmol) was dissolved in anhydrous pyridine (0.1 mL) and anhydrous dioxane (0.3 mL). A freshly prepared 1 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one in anhydrous dioxane (0.1 mL) was then added to the well stirred solution of **2**. After 10 min, a well-mixed solution of 0.5 M *bis*(tri-n-butylammonium)pyrophosphate in anhydrous DMF (0.3 mL) and tri-n-butylamine (0.1 mL) was quickly injected, and the reaction mixture was stirred at room temperature for 10 min. A solution of 1% iodine in pyridine/water (98/2, v/v) (2 mL) was then added, and the mixture was stirred for 15 min. Excess iodine was destroyed by adding a few drops of a 5% aqueous solution of sodium bisulfite, and the reaction solution was evaporated to dryness. The resulting residue was dissolved in water (10 mL) and allowed to stand at room temperature for 30 min before concentrated ammonium hydroxide (20 mL) was added. After 3 h, the solution was evaporated to dryness, and the residue was dissolved in water. This solution was applied to an ion-exchange column (16 cm × 2 cm i.d.) containing 5 grams of DEAE Sephadex A-25 in the bicarbonate form. The column was eluted with a linear gradient of 0.01–1.0 M triethylammonium bicarbonate buffer (TEAB) at 4°C with UV monitoring at 254 nm. Product containing fractions were pooled, frozen, and lyophilized to give **5** (41 mg) as the triethylammonium salt hydrate. A portion of **5** (9 mg) was treated with snake venom phosphodiesterase I (22 units) for 46 h at room temperature. The resulting monophosphate **6** was purified as for **5** except using 750 mg of DEAE Sephadex (6 cm × 1 cm i.d. column) and a linear gradient of 0.01–0.35 M TEAB. Monophosphate **6** (4 mg) was also isolated as the triethylammonium salt hydrate. The structures and purities of nucleotides **5** and **6** were verified by HRMS (ESI-TOF), ^{1}H NMR, ^{31}P NMR, and HPLC.

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