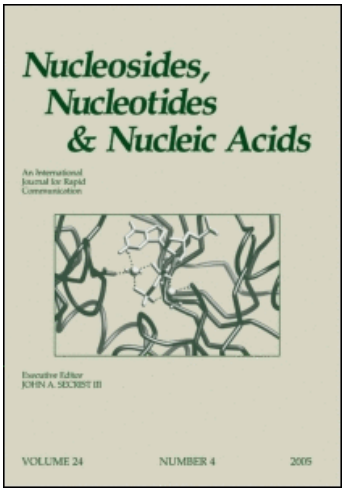

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FURANO- AND PYRROLO [2,3-*D*] PYRIMIDINE NUCLEOSIDES AND THEIR 5′-O-TRIPHOSPATES: SYNTHESIS AND ENZYMATIC ACTIVITY

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□ *A series of bicyclic [2,3-d]furano- and pyrrolopyrimidine ribonucleosides were synthesized and converted chemically into corresponding 5'-O-triphosphates. Substrate properties of the triphosphates toward some RNA and DNA polymerases are reported*

Keywords Furano- and pyrrolopyrimidine ribonucleosides; nucleoside triphosphates; DNA and RNA polymerases

INTRODUCTION

During past decades a large number of nucleoside analogs have been synthesized in the hope of obtaining therapeutically useful agents. Several nucleoside-derived drugs have been approved for the therapy of virus-induced infections caused by HIV, hepatitis B virus, and the family of herpes viruses.

Recently bicyclic furano- and pyrrolopyrimidine 2′-deoxynucleosides were synthesized and shown to be potent and selective inhibitors of some herpes family viruses (varicella-zoster or human cytomegaloviruses) lacking noticeable cytotoxicity.[1a] Herein, we describe the synthesis of new bicyclic [2,3-*d*]furano- and pyrrolopyrimidine ribonucleosides and their 5′-O-triphosphates.

RESULTS AND DISCUSSION

The target furanoribonucleosides (III) were synthesized similarly to,[1a,1b,2] (Scheme 1). The Pd(0)-catalyzed coupling of 5-iodouridine (**I**)

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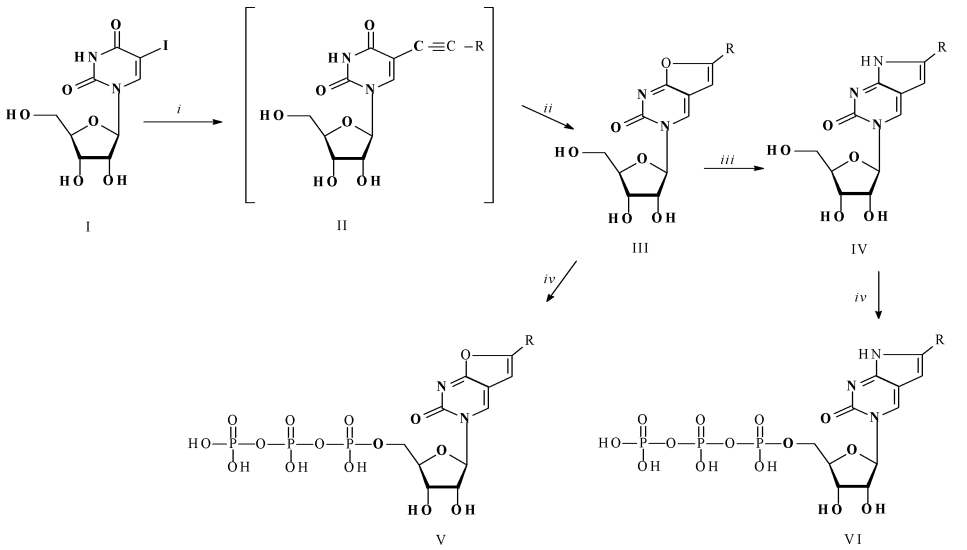

III: R = CH_2OH, CH_2OCH_3, C_5H_{11}, C_6H_{13}, **IV**: R = CH_2OH, C_6H_{13},

V, **VI**: R = C_6H_{13}

SCHEME 1 Syntesis of bicyclic[2,3-*d*]furano- and pyrrolopyrimidine ribonucleosides and 5′-triphosphates. i: CH=CR, Pd[PPh_3]$_4$ CuI, Et_3N, DMF, 20°C, 24 hours or CH=CR, Pd/C, CuI, Et_3N, CH_3CN, 70°C, 1.5 hours. ii: CuI, Et_3N, MeOH, 65°C, 4 hours. iii: 25% aq. NH_3, MeOH, 20°C, 20 hours. iv :1) $POCl_3$, proton sponge, $(EtO)_3PO$, 4°C, 4 hours, 2) $(Bu_3N)_2H_2P_2O_7$, DMF, 20°C, 1 minute, 3) 1 M aq. $(Et_3N)HCO_3$, 20°C, 40 minutes.

with appropriate alkynes resulted in 5-alkynylderivatives (**II**), which underwent intermolecular cyclization in the presence of CuI to give the [2,3-*d*]furanopyrimidines (**III**). We found that the interaction of (**I**) with terminal alkynes in the presence of catalytic amounts of 10% Pd/C and CuI[2] affords higher yields of corresponding derivatives of [2,3-*d*]furopyrimidines (**III**) than they were in the presence of tetrakis(triphenyl phosphine)-palladium (0) and CuI.[1] The reaction of (**III**) with aqueous ammonia afforded pyrrolo[2,3-*d*]pyrimidines (**IV**), which were purified by silica gel chromatography and isolated in 56–83% yields. The treatment of (**III**) with NH_3/MeOH under the conditions described in[3] was less effective and led to lower yields of the target (**IV**). Antiviral properties of the obtained nucleoside analogs are under investigation. According to preliminary data, activity of compounds (**III**) and (**IV**) in hepatitis C virus artificial replicon system were rather low and displayed high cytotoxicities in Huh 7 cell culture. As a result selectivity indexes of compounds (**III**) and (**IV**) were low (1.5–3). The synthesized bicyclic ribonucleosides were neither cytotoxic nor active in Vero cell cultures infected with vaccinia virus.

The synthesized bicyclic nucleosides (**III**) and (**IV**) were triphosphorylated by the T.Kovacs procedure[4] to give the corresponding

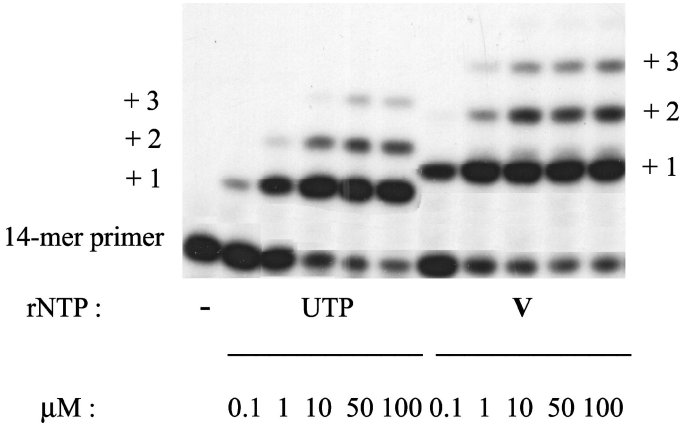

FIGURE 1 Dose-dependent incorporation of the compound **V** into the oligonucleotide 3′-end catalyzed by TDT. Reactions were carried out as described in[7b], using 5′-[^{32}P]-labeled primer CCCAGTCACGACGT$_{OH}$ and TDT (0.5 activity units). Reactions products were analyzed by denaturing 16% PAGE.

5′-O-triphosphates (**V** and **VI**, respectively).[5] Total yields of the synthesized triphosphates achieved 12–17%.

The [2,3-*d*]furanopyrimidine 5′-O-triphosphate (**V**) was tested in extension reactions catalyzed by RNA polymerase of hepatitis C virus,[6] template-independent calf thymus terminal deoxynucleotidyl transferase (TDT)[7] and two template-dependent DNA polymerases: DNA polymerase I (Klenow fragment) and HIV reverse transcriptase (RT). Only TDT and RT recognized the compound (**V**). Figure 1 presents the dose-dependent incorporation of triphosphate (**V**) into the 14-mer primer 3′-end catalyzed by TDT. The affinity of compound (**V**) to TDT was close to that of UTP. The 50% utilization of the oligonucleotide was observed at a concentration of 0.5 μM and both (**V**) and UTP were able to be incorporated into the primer 3′-end several times. As expected, the electrophoretic mobilities of oligonucleotides containing bulky residues differed from those containing UMP fragments. Thus, the [2,3-*d*]furanopyrimidine 5′-O-triphosphate (**V**) was proved to be an effective substrate of TDT.

RT also was able to incorporate both UTP and compound (**V**) into the primer 3′-ends but the efficacies of elongation were by 3–4 orders of magnitude lower if compared with a reference compound dTTP.

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5. 3-(5′-Triphosphoryl-β-D-ribofuranosyl)-6-hexyl-2,3-dihydrofuro-[2,3-*d*]pyrimidin-2-on (**V**): UV (H_2O, pH 6): λ_{max} 244 nm (ε 11900), 331 nm (ε 6500). ^{1}H-NMR (D_2O): 8.77 1H, s, H4), 6.57 (1H, s, H5), 6.12 (1H, d, *J* 2.6, H1′), 4.48–4.35 (5H, m, H2′, H3′, H4′, H5′), 2.75 (2H, dd, *J* 7.1, 7.5, α-CH_2 (C_6H_{13})), 1.73 (2H, m, β-CH_2 (C_6H_{13})), 1.31 (6H, m, 3 × CH_2 (C_6H_{13})), 0.89 (3H, dd, *J* 6.8, 6.5, CH_3) ^{31}P-NMR (D_2O): −10.32 (1P, d, *J* 20.1 Pγ), −10.86 (1P, d, *J* 20.3, Pα), −22.50 (1P, dd, Pβ); 3-(5′-triphosphoryl-β-D-ribofuranosyl)-6-hexyl-2,3-dihydropyrrolo-[2,3-*d*]pyrimidin-2-on (**VI**): UV (H_2O, pH 6): λmax 263 nm (ε 4000), 344 nm (ε 3650). ^{1}H-NMR (D_2O): 8.60(1H, s, H4), 5.80(1H, d *J* 2.9, H1′), 5.73(1H, s, H5), 4.51–4.33 (5H, m, H2′, H3′, H4′, H5′), 2.43 (2H, m, α-CH_2 (C_6H_{13})), 1.48 (2H, m, β-CH_2 (C_6H_{13})), 1.24 (6H, m, 3 × CH_2 (C_6H_{13})), 0.82 (3H, dd, *J* 6.4, 6.8, CH_3); ^{31}P-NMR (D_2O): −10.11 (1P, d, *J* 20.3 Pγ), −10.75 (1P, d, *J* 19.5, Pα), −22.35 (1P, dd, Pβ)}.
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