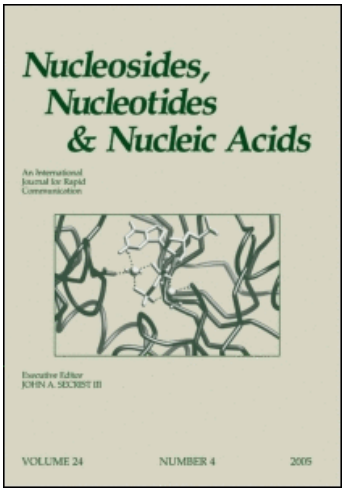

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In Search of New Inhibitors of HIV-1 Replication: Synthesis and Study of 1-(2'-Deoxy-β-D-Ribofuranosyl)-1,2,4-Triazole-3-Carboxamide as a Selective Viral Mutagenic Agent

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IN SEARCH OF NEW INHIBITORS OF HIV-1 REPLICATION: SYNTHESIS AND STUDY OF 1-(2′-DEOXY-β-D-RIBOFURANOSYL)-1,2,4-TRIAZOLE-3-CARBOXAMIDE AS A SELECTIVE VIRAL MUTAGENIC AGENT

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□ *With the emergence of HIV strains resistant or cross-resistant to nearly all antiretroviral regimen, novel therapy approaches have to be considered. As a part of our current work on viral mutagenic compounds, we prepared 1-(2′-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (2′-deoxy-ribavirin) and its 5′-triphosphate derivative. The nucleoside mutagenic activity was evaluated on HIV-1 NL4-3 in CEMx174 cell culture. After 2.5 months, no reduction on HIV-1 viability was observed. On the other hand, in vitro experiments with purified HIV-1 RT demonstrated that the triphosphate analog can be incorporated opposite to several natural nucleosides.*

Keywords HIV-1; nucleoside analogues; deoxy-ribavirin; triphosphate analogue

Significant progress has been accomplished in the discovery of new antiviral agents and therapeutic approaches against human immunodeficiency virus (HIV) infection. However, none of them is fully effective to eradicate the virus, due in part to the high capacity of this virus to mutate. However, recent literature data has shown that the high mutation rate observed with RNA viruses and retroviruses can be transformed into an advantage for the host (for a general review see.[1] These viruses replicate close to the threshold of error catastrophe and a moderate increase in mutation rate during proviral DNA synthesis dramatically reduces their infectivity and viability.

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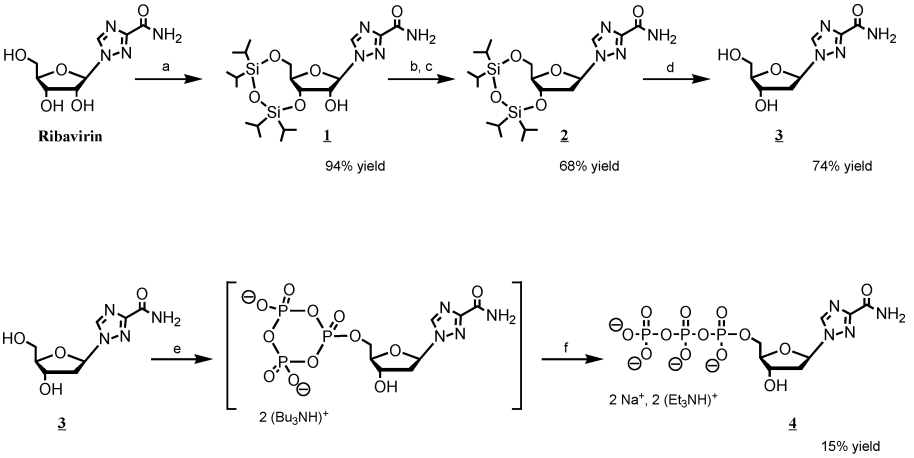
This strategy has been validated against HIV-1 in 1999 with 5-OH-dC[2] and a molecule (SN 1212) is under phase I clinical trials.[3] Within the framework of our research program, we designed and synthesized several compounds that are able to base pair with a maximum of natural nucleosides in order to create hyper-variability in proviral DNA.[4] Based on the antiviral activity reported for ribavirin[5] we decided to evaluate the mutagenic potency of its 2′-deoxy-analog, 1-(2′-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide **3**. Here, we report the preparation of the nucleoside analog **3** and its 5′-triphosphate **4** as well as the preliminary results of their evaluation as mutagenic compounds.

From a synthetic viewpoint, the 1-(2′-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide **3** was prepared by the strategy described in L-series.[6] However, reagents for the radical deoxygenation were slightly modified to improve the global yield from 10.5 to 50% (Scheme 1). In our protocol, the 3′- and 5′-OH of commercially available ribavirin were selectively protected by the bidentate reagent 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-*O*-disiloxane ($TIPSiCl_2$) to give compound **1** in 94% yield. Then, the 2′-hydroxyl group was treated with *O*-phenyl chlorothionoformate. The crude material was deoxygenated with tris(trimethylsilyl)silane in the presence of AIBN in dioxane to give compound **2**. A change of silane reagent ($((Me_3Si)_3SiH$ was used instead of Ph_2SiH_2[6]) increased the yield from 31 to 68% for the two steps. Finally, pure compound **3** was obtained after elimination of the protecting group with triethylamine trihydrofluoride in dichloromethane.



a) $TIPSiCl_2$, Et_3N, Pyridine, DMF, RT, 17h; b) PhOC(S)Cl, DMAP, CH_3CN, RT, 12h; c) $(Me_3Si)_3SiH$, AIBN, dioxane, 80°C, 1h; d) $Et_3N \cdot 3HF$, CH_2Cl_2, RT, 3h; e) $POCl_3$, $PO(OEt)_3$, 0°C, 1h30 then 0.5M bis-tri-n-butylammonium pyrophosphate, DMF, Bu_3N, 0°C, 1 min; f) TEAB 1M pH = 7.5, RT, 10 min.

SCHEME 1 Chemical synthesis of 2′-deoxyribavirin and its 5′-triphosphate derivative.

The triphosphate derivative (dRTP) **4** was prepared without further protection of the 3′-hydroxyl of compound **3** by the "one pot" protocol initially developed by Ludwig[7] and more recently described in an online free access patent.[8] Nucleoside **3** was treated with phosphorous oxychloride in triethyl phosphate for 1 h 30 at 0°C then tributylamine and a 0.5M solution of bis-tri-n-butylammonium pyrophosphate in dry DMF were added. After 1 min, the reaction was quenched with TEAB 1M and the mixture was stirred at room temperature for 10 min. The crude triphosphate was easily purified by HPLC chromatography on C18 Zorbax SB column with TEAB 0.5M as eluant. The final compound was converted to its sodium salt by exchange on Dowex 50WX2 resin.

The 2′-deoxy-ribavirin (dR) was tested for its potency to reduce HIV-1 replication. HIV-1 NL4-3 virions were cultured on CEM × 174 cell lines in the presence or absence of dR. Replication (RT activity) has been monitored every 4 days. Over a period of 2.5 months, we did not detect any difference of virus replication in the presence or absence of 1mM dR. The lack of activity can possibly be attributed to a lack of nucleoside phosphorylation by cellular kinases, or the triphosphate dRTP is not substrate of RT. Alternatively, once incorporated into nascent DNA, deoxyribavirin is not able to induce mutations in viral genome or induces silent mutations. In order to answer the last question, the sequence analysis of genes obtained at the end of the ex vivo experiments is underway. We also used the nucleoside 5′-triphosphate (dRTP) to check its incorporation into the DNA primer strand by purified reverse transcriptase (RT). We found that dRTP is introduced by RT in front of the 4 natural bases with an efficiency such as: dR :dU $\gg$ dR :dG $\cong$ dR :dC > dR :dA. To complete this study, incorporation efficiency of dRTP versus natural dNTP (Kpol/Kd) will be evaluated. We also plan to synthesize oligonucleotides modified with dR in order to determine the incorporation of natural dNTP opposite dR when the modification is placed in the template strand and to measure the melting temperature of duplexes including a mismatch (dR:dU, dR:dC, dR:dG, or dR:dA).

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