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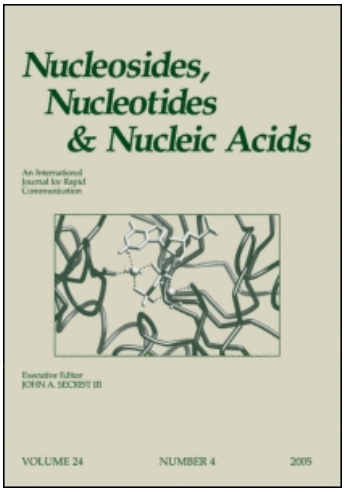

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Synthesis and Primary Evaluation of Novel HIV-1 Inhibitors

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SYNTHESIS AND PRIMARY EVALUATION OF NOVEL HIV-1 INHIBITORS

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□ *The overcoming of antiviral drug resistance is an important challenge in the treatment of HIV-1 infection.*

According to the theory of viral error catastrophe, slightly increasing the mutation rate could exceed the error threshold for viability of a viral population and kill it. Investigation of this mechanism could lead to the discovery of new antiviral agents capable of bypassing viral resistance.

To this aim, we designed several modified nucleosides. We describe here the synthesis and partial evaluation of 8-amido-2′-deoxyadenosine. The supplementary amide group on the base should allow base-pairing with several natural nucleosides, thus creating supplementary mutations that would kill the virus.

Keywords HIV-1; antiviral drug resistance; viral error catastrophe

The overcoming of antiviral drug resistance is an important challenge in the treatment of Human Immunodeficiency Virus type 1 (HIV-1) infection. NRTIs used against HIV are modified nucleosides that lack the 3′-hydroxyl group of the sugar moiety. They are phosphorylated and incorporated into the elongating DNA strand by the viral reverse transcriptase (RT) and stop DNA synthesis. These NRTIs are therefore referred to as "chain terminators." However, due to its exceptionally high mutation rate, HIV-1 develops resistance. An important resistance mechanism simply consists of excising the chain terminating nucleotide incorporated by RT once DNA synthesis is blocked.

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It, therefore, is necessary to investigate new HIV-1 inhibitors that act against the virus with different mechanisms.

HIV-1 and other retroviruses exhibit mutation rates 1,000,000 times greater than host organisms, thus constantly placing them close to the threshold of "error catastrophe." This threshold is the limit above which viral replication stops due to an intolerable number of mutations. Thus, even a slight increase in the mutation rate of the virus should be sufficient to obliterate the viral population.[1]

The goal of this work is to design novel inhibitors, with unmodified sugar moieties, but with modified bases, that will be incorporated into the nascent viral DNA and will create enough supplementary mutations to drive the virus above its error threshold for viability.

This concept has recently been investigated in the context of HIV-1 and could be of great utility in the treatment of AIDS.[2] Mutagenic nucleosides could also be used efficiently in combination with chain terminators. Recent studies showed that HIV strains resistant to nucleoside reverse transcriptase inhibitors exhibited no cross-resistance towards a mutagenic nucleoside analogue.[3]

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CHEMISTRY

Our goal was to design and synthesize novel base-modified nucleoside analogues using commercially available nucleosides. Referring to the structure of ribavirin, which is known to pair equally well with two natural nucleotides,[4] we decided to introduce an amide function on the base, hoping that it will induce extra hydrogen bonds and render the modified nucleoside mutagenic. Based on this strategy several modified nucleosides were designed. Here, we focus on 8-amido-2′-deoxyadenosine, which we synthesized starting with commercially available 2′-deoxyadenosine (Figure 1). The synthetic strategy was inspired by Matsuda's synthesis of 8-amidoadenosine[5] and is based on the insertion of the amide by oxidation of a cyano group.

The bromination of 2′-deoxyadenosine was carried out according to the procedure described by Ben Gaied et al.[6] and resulted in a very high yield 8-bromo-2′deoxyadenosine. Thiolation of (1) also proceeded with excellent yield. The sugar moiety was then protected by acetic anhydride under mild condition (3), and the thiomethyl group was oxidized using potassium permanganate to give the protected 8-methylsulfonyl-2′-deoxyadenosine (4). This reaction was carried out at 0°C and needed close monitoring, since excessive reaction times result in a slight yield drop. Substitution of the sulfonyl group by sodium cyanide was also a smooth reaction and resulted in the protected 8-cyano-2′-deoxyadenosine (5) with a 95% yield. The last step consisted in an alkaline hydrolysis of the cyano group with simultaneous

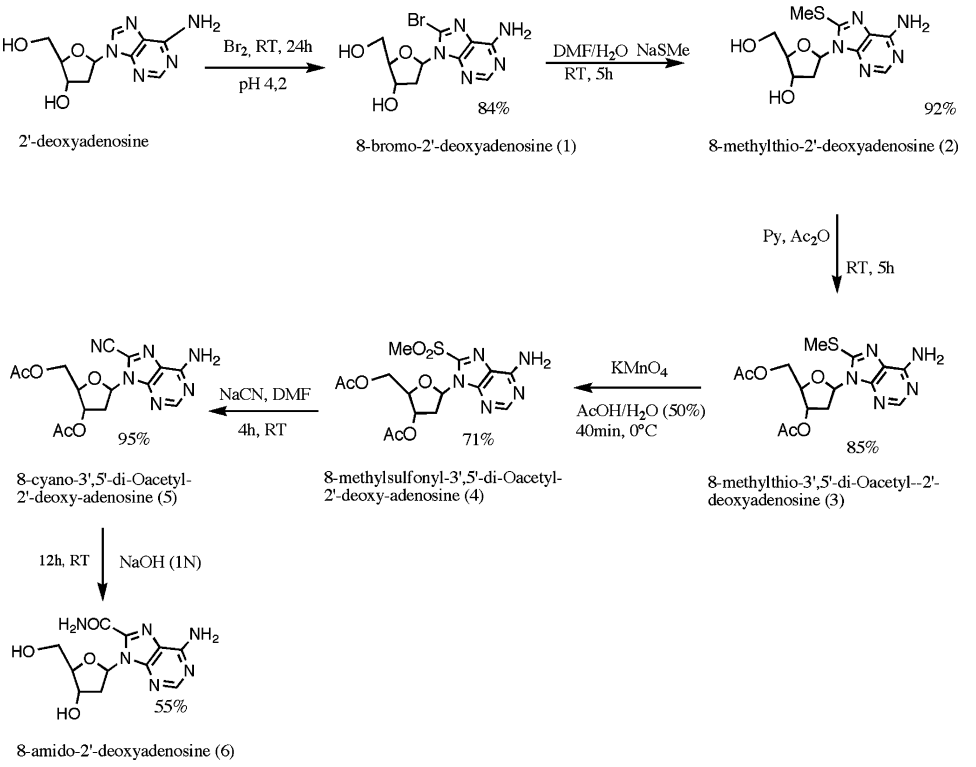

FIGURE 1 Synthetic route to 8-amido-2′-deoxyadenosine.

cleavage of the sugar protecting groups. It was carried out in water, in presence of five equivalents of sodium hydroxide. After purification, 8-amido-2′-deoxyadenosine (6) was obtained as a pure white powder.

The overall yield of this six-step synthesis was 27% (average yield of 80% per step). The poorest yield was that of the last reaction, a result of the difficult purification of the final product, which has very low solubility. Matsuda used a 50/50 mixture of ethanol and water to recristallize 8-amidoadenosine, and we found the use of pure water more efficient for the deoxynucleoside.

The 8-amido-2′-deoxyadenosine-5′-triphosphate derivative was also chemically synthesized, in order to perform in vitro RT incorporation tests (Figure 2).

Perfectly dry conditions and reagents were used to perform this one-pot triphosphate synthesis. The first step consisted in forming the monophosphate using phosphorous oxychloride in triethyl-phosphate. The cyclic triphosphate was then formed using a bis-tri-n-butyl-ammonium-pyrophosphate solution in DMF. The product was hydrolyzed to 8-amido-2′-deoxyadenosine-5′-triphosphate with TEAB.

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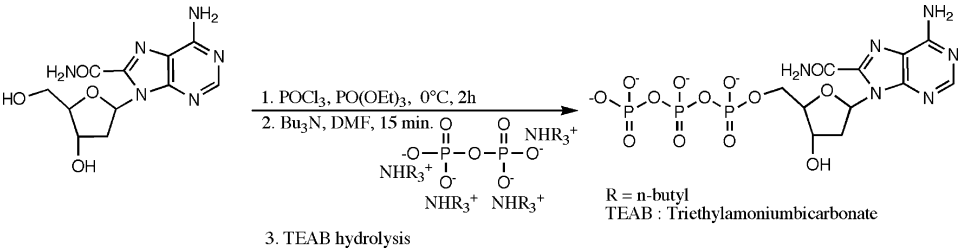

FIGURE 2 Chemical synthesis of 8-amido-2′-deoxyadenosine-5′-triphosphate.

The triphosphate derivative was purified using a C-18 reverse phase preparative chromatography, with a pH 7.5-buffer solution of triethylamine acetate and acetonitrile. Reverse phase chromatography was followed by an ion exchange chromatography to replace ammonium cations by lithium cations. Purity of the product was verified by ^{31}P NMR, which clearly showed 3 phosphorous atoms: (αP: (d, $j_{p\text{-}p} = 18.4$ Hz) -9.41 ppm, βP: (t, $J_{p\text{-}p} = 18.8$ Hz) -19.1 ppm and γP: (d; $J_{p\text{-}p} = 18.8$ Hz) -3.80 ppm). UV spectrum proved the presence of the 8-amido-2′-deoxyadenosine (maximal absorption at 288 nm). Mass spectroscopy was also performed and interestingly displayed 4 peaks corresponding to the triphosphate derivatives with 0, 1, 2, and 3 lithium cations.

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BIOLOGICAL TESTS

8-Amido-2′-deoxyadenosine was tested on MT4 and CEM-SS cells and showed no cytotoxicity at concentrations up to 1.10^{-4} M on noninfected cells. In a preliminary test, antiviral activity was estimated by measuring the RT activity in the cell culture supernatant. Five days after infection of MT4 cells with HIV-1 IIIB and of CEM-SS cells with HIV-1 LAI, 8-amido-2′-deoxyadenosine displayed no significant antiviral activity in this assay at concentrations up to $1\,10^{-4}$ M. These results, however, preclude a mutagenic activity of this compound, as several replication cycles might be required to accumulate lethal mutations. Prolonged in vitro tests involving 10 to 12 sequential passages, followed by the sequencing of the viral genome need to be performed in order to evaluate its mutagenic activity. Moreover, in vitro tests did not tell whether the compound was phosphorylated and did not give any direct information about the new base-pairing possibilities induced by the modified base.

In order to answer these questions, other experiments are in progress. First, we wish to evaluate the base pairing properties of the compound with natural nucleosides. Oligonucleotides bearing the modified nucleoside will be prepared using the phosphoramidite chemistry. Melting temperature (Tm) of duplexes containing different modified nucleoside/natural

nucleoside base pairs will be measured. When compared to the natural duplexes Tm, they will inform us about the base-pairing abilities of the modified base. Second, we will also measure in vitro incorporation the modified nucleoside 5′-triphosphate by RT. These specific tests will help us to understand the possible reason for the failure or success of 8-amido-2′-deoxyadenosine in inhibiting HIV replication.

Several base-modified nucleosides have been designed, in order to investigate the lethal mutagenesis theory. According to this mechanism, HIV-1 replication could be suppressed by a mutagenic nucleoside that drives the virus over its error threshold for viability. Among those nucleosides, 8-amido-2′-deoxyadenosine was readily prepared, starting from commercially available 2′-deoxyadenosine. The six steps of the synthesis proceeded smoothly, offering good yields, even though the last step was limiting, due to the very poor solubility of the final compound. The 5′-triphosphate of the modified nucleoside also was synthesized and purified.

8-amido-2′-deoxyadenosine showed no activity on HIV-1 replication in cell culture, but more tests need to be done, to determine its mutagenic effect, its base pairing possibilities and its incorporation by RT.

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