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# NUCLEOSIDES AND NUCLEOTIDES. 142. AN ALTERNATIVE SYNTHESIS OF 9-(5,6-DIDEOXY-β-D-*RIBO*-HEX-5-YNOFURANOSYL)ADENINE AND ITS ANTIVIRAL ACTIVITY[1]

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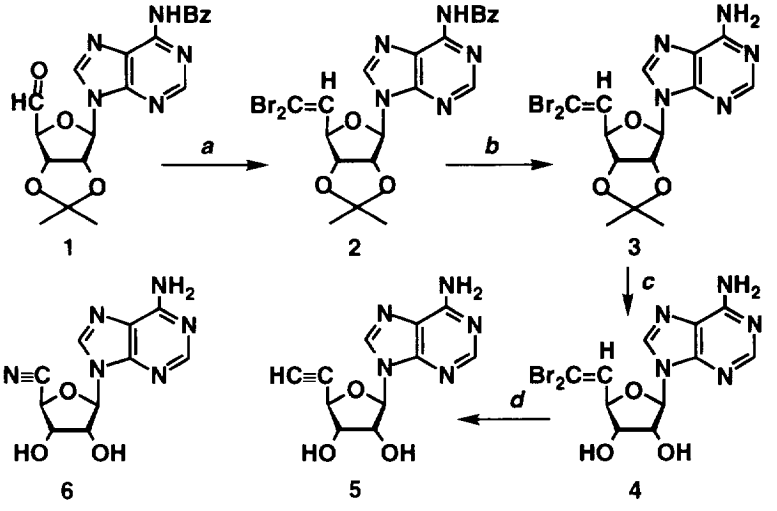
**Abstract**: An alternative synthesis of 9-(5,6-dideoxy-β-D-*ribo*-hex-5-ynofuranosyl)adenine (**5**), which is known to be a potent, irreversible, and time-dependent inhibitor of AdoHcy hydrolase, has been accomplished. Compound **5** had a potent antiviral activity against influenza A and B viruses as well as vesicular stomatitis virus. Since the usual inhibitors of AdoHcy hydrolase, such as neplanocin A, are inactive on both influenza viruses, the mechanism of action of **5** would be different from that of known AdoHcy hydrolase inhibitors.

Nucleoside analogues having an alkynyl group at the base or sugar moieties have interesting biological activities. We have reported that 2-alkynyladenosines showed anti-anaphylaxis[2] and hypotensive[3] activities in rats due to agonist activity to A2 adenosine receptors, and 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide had both a potent antitumor[4] and antiviral[5] activities *in vitro* as well as *in vivo*. 5-Ethynyl-2'-deoxyuridine 5'-monophosphate and 4'-ethynylthymidine are known as potent inhibitors of thymidylate synthetase[6] and the thymidine kinase encoded herpes simplex virus type 1,[7] respectively. Thus, an acetylenic bond attached in nucleosides brings a variety of biological activities due to its compact shape, electronic structure, and rigidity.

Recently, 9-(5,6-dideoxy-β-D-*ribo*-hex-5-ynofuranosyl)adenine (**5**) was synthesized and evaluated as a time-dependent and irreversible inhibitor ($K_i$ = 173 nM)[8] of bovine *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase, which catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine. Inhibition of AdoHcy hydrolase causes accumulation of AdoHcy, which is a highly potent product inhibitor of *S*-adenosylmethionine (AdoMet)-dependent methyltransferases.[9] Although a close correlation between the inhibitory activity to AdoHcy hydrolase and antiviral activity against vesicular stomatitis virus (VSV) has been reported by Cools and De Clercq,[10] antiviral activity of **5** has not been reported. As a part of our program of the synthesis of nucleosides bearing alkynyl groups in the base or sugar moiety, we describe an alternative synthesis of **5** and its antiviral activity against VSV, human cytomegalovirus (HCMV), and influenza A and B viruses.

We adopted Corey's method[11] for the synthesis of **5** starting from **1**.[12] Treatment of **1** with $CBr_4$ and $Ph_3P$ in the presence of Zn dust in $CH_2Cl_2$ gave **2**. Due to difficulty in separating **2** and the resulting triphenylphosphine oxide, the mixture was treated with $NH_3$/MeOH, and **3** was obtained as a crystalline in 41% yield from **1**. Dehydrobromination of **3** with BuLi in THF at -78 °C afforded the corresponding ethynyl derivative in low yield along with a large amount of adenine. Removal of the isopropylidene group in **3** was done with aqueous 90% $CF_3CO_2H$, giving **4** in a quantitative yield. Subsequently, **4** was converted into **5**[13] in 78% yield by treatment with 10 equiv. of BuLi in THF at -78 °C.

**Scheme I**[a]




[a] a) $CBr_4$, $Ph_3P$, Zn, $CH_2Cl_2$, room temperature, 45 h; b) $NH_3$/MeOH (saturated at 0 °C), room temperature, overnight; c) aqueous 90% $CF_3CO_2H$, room temperature, 0.5 h; d) BuLi (10 eq.), THF, -78 °C, 4 h.

Antiviral activity of **5** was compared with that of the intermediate **4** and a structurally related cyano derivative **6**[14] against VSV, HCMV, and influenza A and B viruses. As control compounds, neplanocin A, one of the most potent AdoHcy hydrolase inhibitor,[9] was selected and ribavirin was also selected as a broad spectrum anti-RNA virus nucleoside, the mechanism of which is as an IMP dehydrogenase inhibitor.[15] The results are summarized in Table I. As expected, **5** showed a potent anti-VSV activity with an $EC_{50}$ value of 0.14 μg/mL and this potency is comparable to that of neplanocin A. Although **6** is structurally akin to **5**, the activity of **6** was almost 10 times less potent than **5**. The intermediate **4** and ribavirin were not active up to 10 μg/mL. Compound **6** had inhibitory activity against HCMV with an $EC_{50}$ value of 1.4 μg/mL, which is less active than neplanocin A, but is about 6 times more active than **5**. Quite interestingly, **5** showed a potent inhibitory activity against influenza A and B viruses and is 7-50 times more potent than ribavirin. It should be

noted that **5** was not cytotoxic to the confluent cells such as MDCK cells up to 50 μg/mL that were used in the antiviral assays. On the other hand, neplanocin A did not show any antiviral activity against all the influenza viruses tested.

**Table I.** Inhibitory Effects of **5** and Its Analogues on Replication of Several Viruses[a]

| Compds | $EC_{50}$ (μg/mL) VSV[b] | HCMV[b] | influenza A[c] ($H_1N_1$, Fu 88) | influenza B[c] (Fu 275) | influenza B[c] (singapore) |
|---|---|---|---|---|---|
| **4** | >10 | >10 | NT[d] | 32 | NT[d] |
| **5** | 0.14 | 8.2 | 0.38 | 1.8 | 0.9 |
| **6** | 1.2 | 1.4 | NT[d] | 10 | NT[d] |
| **neplanocin A** | 0.38 | 0.20 | >20 | >20 | >20 |
| **ribavirin** | >10 | >10 | 19.6 | 36.9 | 6.7 |

[a]Antiviral assay was done by previously reported method.[16] [b]50% plaque reduction dose. Geometric mean of two to three independent experiments. [c]Concentration required to reduce virus-induced cytopathogenicity by 50%. Geometric mean of two independent experiments. [d]Not tested.

AdoHcy hydrolase inhibitors are particularly active against VSV and HCMV,[17,18] but inactive againt influenza A and B viruses,[19] which is in accord with the antiviral results on neplancin A in our evaluation system. These results suggest that the antiviral mechanism of action of **5** may be different from that of the usual AdoHcy hydrolase inhibitors such as neplanocin A. Further detailed studies of antiviral activity and its mechanism of action against influenza viruses are currently under way in our laboratory.

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