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NEW NEPLANOCIN ANALOGUES. III. 6'*R*-CONFIGURATION IS ESSENTIAL FOR THE ANTIVIRAL ACTIVITY OF 6'-*C*-METHYL-3-DEAZANEPLANOCIN A'S [1]

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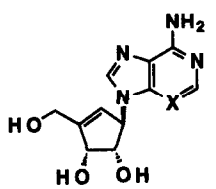
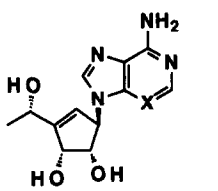
Abstract: (6'*R*)- and (6'*S*)-6'-*C*-methyl-3-deazaneplanocin A's were synthesized from D-ribose as anti-RNA virus agents. Of these compounds, (6'*R*)-6'-*C*-methyl-3-deazaneplanocin A (**4b**) showed the greatest anti-RNA virus activity *in vitro*. It was found that the 6'*R*-configuration was essential for the antiviral activity of 6'-*C*-methylneplanocin A derivatives.

Neplanocin A (NPA, **1**)[2] has a broad-spectrum antivirus activity due to its inhibitory effect on cellular *S*-adenosylhomocysteine (AdoHcy) hydrolase,[3] which regulates biologically important methylation processes.[4] However, NPA itself also showed apparent cytotoxicity to host cells.[5] It has been recognized that the detrimental toxicity of NPA could be derived, for the most part, from phosphorylation of the primary hydroxyl group at its 6'-position by adenosine kinase and subsequent metabolism by cellular enzymes.[5] Consequently, to remove or reduce such side effects, much attention has been focused on chemical modification of NPA.[6]

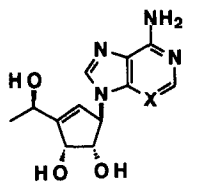
We recently reported the synthesis of (6'*R*)- and (6'*S*)-6'-*C*-methylneplanocin A's (RMNPA **3b** and SMNPA **3a**, respectively) from NPA, which were designed not to be phosphorylated due to steric hindrance of the methyl group but still to have inhibitory effect on AdoHcy hydrolase, and found that one of the diastereomers showed significant antiviral activity against various pathogenic viruses such as parainfluenza, respiratory syncytial, measles, and cytomegalo viruses, without considerable cytotoxicity toward host cells.[7] However, the 6'-configurations of each diastereomer have not been confirmed.



1 (NPA) : X = N
2 : X = CH

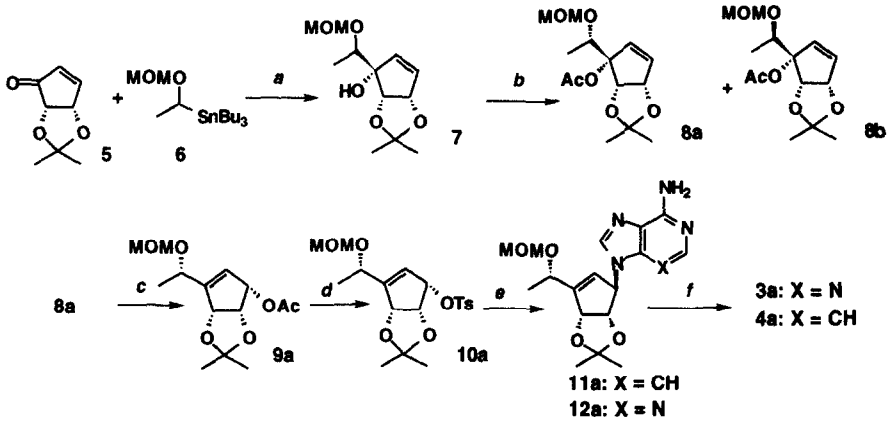


3a (SMNPA) : X = N
4a : X = CH



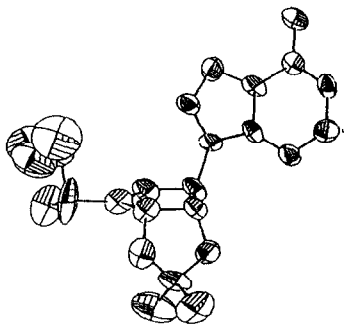
3b (RMNPA) : X = N
4b : X = CH

In this communication, we describe synthesis of (6'*R*)- and (6'*S*)-6'-*C*-methyl-3-deaza-NPA's (**4b** and **4a**, respectively) as another example of non-cytotoxic antiviral agents and identification of the 6'-configurations at the 6'-position of **3** and **4**.



Reagents: a) BuLi/THF b) Ac_2O, DMAP / CH_2Cl_2 c) $PdCl_2(MeCN)_2$, benzoquinone / THF d) i. K_2CO_3 / MeOH ii. TsCl, DMAP / CH_2Cl_2 e) 3-deazaadenine (adenine), NaH, 15-crown-5, DMF f) HCl / MeOH

We used a cyclopentenone derivative **5** as a starting material for the synthesis of target compounds, which could be prepared readily from D-ribose[8] and has been recognized as an efficient synthon for constructing the backbone structure of NPA.[9] Since an organo-tin compound **6**, the synthetic equivalent to the corresponding organo-lithium, was thought to be a suitable reagent for introducing a 1-hydroxyethyl unit to the cyclopentene ring, we synthesized it from acetaldehyde.[10] Therefore, **5** was treated with [(1-methoxymethyloxy)ethyl]lithium, generated from **6** and BuLi, in THF at -78 °C to give the addition product **7** in 90% yield as expected, in a diastereomeric mixture at the α-position of the methoxylmethyloxy group. The tertiary hydroxyl of the diastereomeric mixture **7** was acetylated by treating with Ac_2O/DMAP in CH_2Cl_2 and the product was separated at this stage by silica gel flash chromatography into the major diastereomer **8a** and the minor one **8b** in 53 and 29% yields, respectively. When **8a** was heated under reflux in THF with $PdCl_2(MeCN)_2$ as a catalyst for 18 h in the presence of benzoquinone,[9] a desired rearrangement product **9a** was obtained in 58% yield with a recovery of 9% of **8a**. Compound **9a** was then converted to tosylate **10a** by a standard procedure[9] in 50% yield. An SN2 substitution reaction of compound **10a** at the allylic position was done with a sodium salt of 3-deazaadenine as a nucleophile by heating at 80°C in DMF in the presence of 15-crown-5 ether



X-ray structure of 11a

to give the protected carbocyclic nucleoside **11a** in 50% yield. The configuration of the 6'-position was identified as *S* at this stage from a X-ray crystallographic analysis of **11a**.[11] Deprotection of **11a** by HCl/MeOH gave (6'*S*)-6'-*C*-methyl-3-deazaneplanocin A (**4a**)[12] in high yield.[13] In the same way, (6'*R*)-6'-*C*-methyl-3-deazaneplanocin A (**4b**)[12] was also synthesized from the corresponding cyclopentene derivative **8b**.

A close correlation between inhibitory effects of adenosine analogues on AdoHcy hydrolase and their antiviral potency has been demonstrated, in which activity against vesicular stomatitis virus was used as a index of antiviral effects.[14] Therefore we evaluated **4a** and **4b** together with several reference compounds, for anti-vesicular stomatitis virus activity *in vitro*, as well as cytotoxic effects on the host cells in both growing and stationary phases. The results are summarized in Table 1. (6'*R*)-6'-*C*-Methyl-3-deaza-NPA (**4b**) had significant antiviral activity (ED_{50} = 1.0 μg/mL) against the virus. The potency was somewhat lower than those of NPA (**1**), 3-deaza-NPA (**2**), and RMNPA (**3b**).[13] None of the compounds tested except for NPA showed cytotoxic effects on host cells at a concentration up to 500 μg/mL in stationary phase. However, NPA(**1**), 3-deaza-NPA (**2**), and RMNPA (**3b**) showed evident cytotoxicities toward the host cells in growing phase (IC_{50} = 0.26, 0.55, and 2.2 μg/mL, respectively). Compound **4b** had a much reduced cytotoxicity (IC_{50} = 12 μg/mL) which signified that **4b** had the most excellent selectivity index in the compounds tested. In preliminary experiments, **4b** also showed antiviral effects toward other pathogenic RNA viruses (parainfluenza-3 virus, HA-I C243 strain, ED_{50} = 1.3 μg/mL; measles virus, Sugiyama strain, ED_{50} < 3.7 μg/mL). On the other hand, (6'*S*)-6'-*C*-methyl-3-deaza-NPA (**4a**) as well as the corresponding adenine congener SMNPA (**3a**), was wholly inactive. This demonstrated that the 6'*R*-configuration was essential for the antiviral activity of 6'-*C*-methyl-NPA derivatives.

Table 1. Anti-vesicular stomatitis virus activity and cytotoxicity in Vero cell cultures.[a]

Compound	ED_{50} (μg/mL)[b]	IC_{50} (μg/mL)[c]		Selectivity index[d]
		Growing	Stationary	
1	0.24	0.26	152	1.1
2	0.24	0.55	>500	2.3
3a	>100	>100	>500	--
3b	0.24	2.2	>500	9.2
4a	>100	>100	>500	--
4b	1.0	12	>500	12

[a]Antiviral assay was done by a previously reported method (*Ref.* 7a). [b]Concentration required to inhibit virus-induced cytopathogenicity by 50%. [c]MTT method was used to measure cytotoxicity. [d]Ratio of cytotoxicity, IC_{50} (growing) for antiviral activity, ED_{50}.

In conclusion, (6'*R*)-6'-*C*-methyl-3-deaza-NPA showed a significant anti-RNA virus effect. This compound should be further pursued for its therapeutic potential as an antiviral agent.[15]

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10. Compound **6** was prepared as follows: Acetaldehyde was treated with one equivalent of Bu_3SnLi, prepared from LDA and Bu_3SnH in situ, at -78°C in THF, to give (1-hydroxyethyl)tributyltin. This was treated with chloromethoxymethane and *N,N*-dimethylaniline in dichloromethane at room temperature to afford pure **6**, after it was purified by silica gel column chromatography.
11. Compound **11a** was crystallized from EtOAc. The crystal data were as follows: $C_{18}H_{24}N_4O_4$, M = 360.30, monoclinic; space group $P2_1$, a = 15.62 (2), b = 8.62 (1), c = 6.950 (9) Å, α = 90.02 (7), β = 91.7 (2), γ = 89.98 (6)°, V = 934.6 (7) Å^3, Z = 2, D_x = 1.28 gcm^{-3}, F (000) = 384, μ (Cu$K\alpha$) = 6.72 cm^{-1}. A total of 1572 independent reflections were collected and used for the structure analysis. The final R value was 0.169.
12. Compound **4a** and **4b** exhibited satisfactory analytical and spectral data.
13. Tosylate **10a** was also converted to the corresponding adenine derivative, namely, (6'*S*)-6'-*C*-methylneplanocin A (SMNPA, **3a**), which was identified with the minor diastereomer synthesized previously from NPA. Therefore, it was confirmed that the diastereomer that had significant antiviral effect as well as inhibitory effect on AdoHcy hydrolase was (6'*R*)-6'-*C*-methyl-NPA (RMNPA, **3b**).
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15. Compound **4b** was completely resistant to deamination by calf intestinal adenosine deaminase, though NPA is deaminated rapidly into a biologically inactive inosine congener under the same reaction conditions (data not shown).

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