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A Practical Preparation of (6'R)-6'-C-Methylenplanocin A (RMNPA), a Potent Antiviral Agent, and the Determination of Its 6'-Configuration. Diastereoselective Deamination by Adenosine Deaminase

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We previously synthesized (6'R)- and (6'S)-6'-C-methylenplanocin A's (2a and 2b, respectively), and found that one of them has a potent antiviral activity, though its 6'-configuration has not been confirmed. This report describes the determination of the 6'-configuration and practical preparation of the antivirally active diastereomer. The 6'-configuration of the active diastereomer was determined as R by the modified Mosher's method as well as by synthesizing 2b from the known cyclopentenone derivative 10. A practical method for preparing the 6'R-diastereomer was developed by using diastereoselective deamination with Ado deaminase as the key step. Treatment of the diastereomeric mixture of 2a and 2b, which was prepared via an addition reaction of Me2Al with the 6'-formyl derivative 3, with Ado deaminase from calf intestine, deaminated 2b selectively to give the corresponding (6'S)-inosine congener 5, and left the desired 2a not deaminated. After silica gel column chromatography, 2a was obtained in a pure form.

Key words  S'-adenosylhomocysteine hydrolase; neplanocin A; antiviral agent; adenosine deaminase; modified Mosher's method

Although RNA viruses such as influenza, parainfluenza, respiratory syncytial, and measles viruses often cause serious diseases, efficient vaccines for these viruses have not been developed. Consequently, antiviral drugs effective against these pathogenic viruses are required.

In recent years, much attention has been focused on the broad-spectrum anti-RNA-viral activity of S'-adenosylhomocysteine (AdoHcy) hydrolase inhibitors.2-4) AdoHcy hydrolase is responsible for the hydrolysis of AdoHcy to adenosine (Ado) and t-homocysteine (Hcy),5,3) and is a key enzyme in transmethylation reactions using S'-adenosyl-t-methionine (AdoMet) as a methyl donor.2) Because such transmethylation reactions are involved in the maturation of viral mRNAs and are critical in the virus replicative cycle, inhibitors of AdoHcy hydrolase are assumed to achieve their broad-spectrum antiviral activity due to the inhibition of transmethylation reactions.2-4) In fact, a close correlation has been found between the antiviral activity of a series of Ado analogues and their inhibitory effects on AdoHcy hydrolase.5)

Neplanocin A (NPA, I, Chart 1),6) a carbocyclic nucleoside antibiotic, which is one of the most potent AdoHcy hydrolase inhibitors, has a notable anti-RNA-viral effect in vitro;6) however, it also has a toxic effect on the host cells.8) The mechanism of action of NPA has been extensively explored8,9), the cytopathic effect could be attributed mainly to phosphorylation of the primary hydroxyl group at the 6'-position (the 6'-position of NPA corresponds to the 5'-position of Ado) by Ado kinase and subsequent metabolism by cellular enzymes,8) while the antiviral effect would be due to the inhibition of AdoHcy hydrolase via suppression of virus mRNA maturation.9) NPA is also known to be rapidly deaminated by Ado deaminase to the chemotherapeutically inactive inosine congener,10,11) which would reduce the therapeutic potency of NPA. Based on these results, extensive chemical modifications of NPA have been done to develop efficient antiviral agents.11,12) We have chosen the 6'-moiety of NPA as the target site for modifications because of its important role in interactions with these enzymes, namely AdoHcy hydrolase, Ado deaminase, and Ado kinase, and we have prepared various 6'-modified derivatives of NPA.13a-e) We had synthesized a diastereomeric mixture of (6'R)- and (6'S)-6'-C-methyl-NPA (2a and 2b, respectively), which was obtained by an addition reaction of Me2Al with the 6'-formyl derivative of NPA (3) and subsequent removal of the protecting groups.1a) The two individual diastereomers were separated, and it was found that the major diastereomer, which has the 6'R-configuration as described below, has significant antiviral activity while the minor one (6'S-diastereomer) is inactive.11) However, only a small quantity of the active diastereomer was obtained, because it was purified by reverse-phase HPLC. Accordingly, a better method to obtain the active diastereomer is needed for further evaluation of this antiviral agent.

Here, we describe a practical method for preparing the active diastereomer by using diastereoselective enzymatic deamination as a key step, and the determination of its 6'-configuration.

Chart 1

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Determination of the 6'-Configuration of 6'-C-Methylneplanocin A’s  Determination of the 6'-configuration was investigated by the modified Mosher’s method, which is very effective for determining the absolute configuration of secondary hydroxyl groups of organic compounds.13) Treatment of the major diastereomer 2a with HClO₄/aceto- tone system gave the 2',3'-O-isopropylidene derivative 6, the N⁰-amino function of which was protected with a benzoyl group by the usual method to give 7. Treatments of 7 with (R)- and (S)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl) in the presence of 4-dimethylaminopyridine (DMAP) in MeCN afforded the corresponding (R)- and (S)-MTPA esters, 8 and 9, respectively (Chart 3). The δδ (δ₂₃−δ₁₆) values obtained from the 1H-NMR spectra are shown in Fig. 1a; all of the protons of the cyclopentene ring moiety have δδ < 0 values and the 6'-C-methyl protons have the δδ > 0 value of +0.085 ppm.14) This result was applied to the model to determine the absolute configurations of the secondary alcohols shown in Fig. 1b, in which the protons with δδ < 0 must be located on the left side of the MTPA plane and the protons with δδ > 0 on the right side.13) Thus, the 6'-configuration of the major diastereomer was suggested to be R.

The result from the modified Mosher’s method was confirmed by synthesizing the 6'S-diastereomer 2b (SMNPA) from a tosyoxyxycyclopentenyl derivative 11 (Chart 4). Compound 11 was prepared from the known cyclopente-none derivative 10, and its stereochemistry was previously confirmed based on X-ray crystallographic analysis of the 3-deazaadenine derivative 13, which was derived from 11.11) The tosyoxyxycyclopentenyl derivative 11 was treated with sodium in the presence of NaH and 15-crown-5 in DMF at 80°C to give the desired (6'S)-6'-C-methylneplanocin A derivative 12 in 64% yield. Removal of the protecting groups was done by treating 12 with HCl/MeOH to furnish (6'S)-6'-C-methylneplanocin A (2b, SMNPA), which gave spectral data in accord with those of the minor diastereomer previously obtained by HPLC.
Therefore, it was demonstrated that the diastereomer with significant antiviral activity has the $6'R$-configuration. The determination of the configuration by the modified Mosher's method has been done based on the $\Delta\delta$ values of several nonequivalent protons on each side of the MTPA plane. Although, in this case, only the 7-methyl protons were located on the right side on the MTPA plane, the modified Mosher's method indicated the correct $6'$-configuration.

Practical Method for Preparing RMNPA Because we previously recognized that the biologically inactive diastereomer was deaminated by Ado deaminase but the active one was resistant to deamination, development of a practical method for preparing RMNPA, the biologically active diastereomer, was planned based on this different susceptibility.

First the susceptibility of RMNPA and SMNPA to Ado deaminase from calf intestine, which is commercially available, was investigated in detail. The diastereomeric mixture (0.5 mmol) in 50 mm Tris–HCl buffer (pH 7.2, 500 μl) was treated with the Ado deaminase (0.4 units) at 25°C. The reaction was monitored by HPLC and the result is shown in Fig. 2. Under these conditions, SMNPA was deaminated effectively but RMNPA was significantly resistant to the enzymatic deamination: after 6 h, SMNPA was almost entirely converted to the corresponding iminosine congener 5, while 93% of RMNPA remained without having been deaminated. Therefore, it was considered that after treatment of the diastereomeric mixture of 2a and 2b with the Ado deaminase at 25°C, RMNPA would be isolated readily in a pure form. A solution of the diastereomeric mixture of RMNPA and SMNPA (4 mmol), which was synthesized from neplanocin A via the addition reaction of Me₃Al with 3, and calf intestine Ado deaminase (660 units) in 50 mm Tris–HCl buffer (pH 7.2, 80 ml) was stirred at 25°C for 17 h. After the usual silica gel flash column chromatography, RMNPA was obtained in a pure form in 73% yield together with the deaminated (6'S)-6'-C-methylinosine congener 5 in 25% yield.

Our result suggested that the three-dimensional structure around the 6'-moiety of NPA and its analogues is important for the compounds to be recognized as substrates by Ado deaminase.

This method can provide enough RMNPA for further biological evaluation. Thus, the therapeutic potential of RMNPA as an antiviral drug in vitro as well as in vivo can be further pursued.

Experimental
Melting points were measured on a Yanagimoto MP-3 micro melting point apparatus and are uncorrected. NMR spectra were recorded with a JEOL FX-270, or a GCX-400 spectrometer with tetramethylsilane as an internal standard. Mass spectra were measured on a JEOL SX-102 spectrometer. High-resolution mass spectra were measured on a JMS DX-303 spectrometer. Thin-layer chromatography was done on Merck precoated plates 60F 254. Flash chromatography was conducted with Merck silica gel 9385. NPA was prepared according to a reported method.

(6'R)-2',3'O-Isopropyliden-6'-C-methylneplanocin A (6) A mixture of the major 6'-diastereomer 2a (14 mg, 0.051 mmol) and 70% HClO₄ (10 μl) in acetone (3 ml) was stirred at room temperature for 2 h. The reaction mixture was neutralized with 0.8 M NaHCO₃ and the insoluble salt was filtered off. The filtrate was evaporated, and the residue was purified by column chromatography (silica gel, CHCl₃-MeOH, 20:1, followed by 10:1) to give 6 (15 mg, 94%) as a white powder. $^1$H-NMR (DMSO-$d_6$, $D_2$O) δ: 7.33, 7.11 (each s, each 1H, H-2, 8), 4.89 (m, 1H, H-5), 4.69 (d, 1H, J = 19.2 Hz, H-1'), 4.62 (d, 1H, J = 5.5 Hz, H-3'), 3.87 (d, 1H, J = 5.5 Hz, H-2'), 3.68 (q, 1H, J = 6.6 Hz, H-6'), 0.60 (s, each 1H, iso-propyl-CH$_3$ x 2), 0.57 (d, 3H, J = 6.6 Hz, 7'-CH$_3$), 2.48 (m, 3H, FAB-MS m/z: 422 (MH$^+$)).

(6'R)-N$^2$-Benzyly-2',3'O-Isopropyliden-6'-C-methylneplanocin A (7) Trimethylsilyl (TMSCl) (40 μl, 0.31 mmol) was added to a solution of
6 (16 mg, 0.050 mmol) in pyridine (1 ml), and the mixture was stirred at room temperature for 30 min, then BrCl (14 ml, 1.5 mmol) was added, and the whole was further stirred at room temperature for 1.5 h. To this mixture, H$_2$O (200 ml) was added at 0°C, and the whole was stirred. After 5 min, 28% NH$_4$NO$_3$ (200 ml) was added, and the mixture was stirred at room temperature for 40 min. The resulting mixture was evaporated, and the residue was partitioned between CHCl$_3$ and brine. The organic phase was dried (Na$_2$SO$_4$), evaporated, and purified by column chromatography (silica gel; CHCl$_3$-MeOH, 20:1) to give 7 as an oil (17 mg, 80%). $^{1}H$-NMR (CDCl$_3$) δ 9.46 (brs, 1H, N$_3$H), 8.80, 7.93 (each s, each 1H, H-2, 8), 8.07–7.42 (m, 5H, Ph), 6.17 (brs, 1H, OH), 5.79 (m, 1H, H-5), 5.64 (d, 1H, J = 5.9 Hz, H-3), 4.78 (d, 1H, J = 5.9 Hz, H-2), 4.70 (q, 1H, J = 6.6 Hz, H-6), 1.51 (1H, each s, each 3H, isopropyl-CH$_2$), 1.49 (3H, J = 6.6 Hz, 7-CH$_3$). FAB-MS m/z: 422.1847 (Calcd for C$_{23}$H$_24$N$_8$O$_4$: 422.1828).

(R)- and (S)-MTPA Esters (8 and 9) (+)-(R)- or (−)-(S)-MTPAC (6, 0.017 mmol) was added to a solution of 7 (4.0 mg, 0.0055 mmol) and DMAP (9.0 mg, 0.074 mmol) in MeCN (0.5 ml), and the resulting mixture was stirred at room temperature for 24 h. After addition of MeOH (100 µl), the mixture was evaporated, and the residue was partitioned between CHCl$_3$ and 0.5N HCl. The organic phase was washed with H$_2$O (3×200 µl), and the residue was purified by column chromatography (silica gel; CHCl$_3$-MeOH, 120:1) to give the corresponding (R)-MTPA ester 8 or (S)-MTPA ester 9 as an oil. The (R)-MTPA ester 8 (yield 50%): $^{1}H$-NMR (CDCl$_3$, 400 MHz) δ 9.01 (brs, 1H, N$_3$H), 8.772 (s, 1H, H-2), 7.868 (s, 1H, H-8), 8.04–7.37 (m, 10H, Ph), 5.897 (m, 1H, H-6), 5.715 (brs, 1H, H-5), 5.599 (brs, 1H, H-1), 5.473 (d, 1H, J = 5.4 Hz, H-3), 4.792 (d, 1H, J = 5.4 Hz, H-2), 3.546 (d, 3H, J = 1.0 Hz, OMe), 1.585 (d, 3H, J = 6.5 Hz, 7-CH$_3$). FAB-MS m/z: 638.2229 (Calcd for C$_{28}$H$_{35}$F$_4$N$_8$O$_{13}$: 638.2266).

(S)-MTPA ester 9 (yield 66%): $^{1}H$-NMR (CDCl$_3$, 400 MHz) δ 9.05 (brs, 1H, N$_3$H), 8.773 (s, 1H, H-2), 7.825 (s, 1H, H-8), 8.04–7.37 (m, 10H, Ph), 5.935 (m, 1H, H-6), 5.524 (brs, 1H, H-5), 5.524 (brs, 1H, H-1), 5.363 (d, 1H, J = 5.4 Hz, H-3), 4.740 (d, 1H, J = 5.4 Hz, H-2), 3.587 (d, 3H, J = 1.0 Hz, OMe), 1.632 (s, 3H, J = 6.6 Hz, 7-CH$_3$), 1.493 (1H, each s, each 3H, isopropyl-CH$_2$). FAB-MS m/z: 638.2205 (Calcd for C$_{28}$H$_{35}$F$_4$N$_8$O$_{13}$: 638.2266).

(6'S)-2',3'-O-Isopropylidene-6'-methoxy-6'-methylhexanefuranocin A (12) A suspension of adenine (68 mg, 0.50 mmol), 15-crown-5 (50 µl, 0.25 mmol) and NaH (50% in oil, 24 mg, 0.50 mmol) in dimethyl formamide (DMF) (2.5 ml) was stirred at room temperature under an argon atmosphere for 1.5 h. Then, a solution of 7 (54 mg, 0.24 mmol) in DMF (0.5 ml) was added, and the mixture was stirred at 80°C for 2 h. The resulting mixture was cooled to room temperature, and the solvent was removed. The residue was taken up in EtOAc (50 ml), and the insoluble material was filtered off. The filtrate was washed with brine (10 ml), filtered through Whatman 15PS filter paper, and evaporated. The residue was purified by flash chromatography (silica gel, CHCl$_3$-MeOH-25% NH$_4$OAc = 30:1:1) to give 12 (55 mg, 64%) as a solid. $^{1}H$ NMR (CDCl$_3$) δ 8.38, 7.86 (each s, each 1H, H-2, 8), 5.78 (brs, 1H, H-5), 5.32 (3H, 3H, NH$_2$, and H-1), 5.46 (d, 1H, J = 5.6 Hz, H-3), 4.77–4.46 (6H, 3H, OCH$_2$ and H-2), 4.45 (q, 1H, J = 6.6 Hz, H-6), 3.41 (3H, 3H, OMe), 1.49 (3H, J = 6.6 Hz, 6-CH$_3$), 1.48, 1.36 (each s, each 3H, isopropyl-CH$_2$). FAB-MS m/z: 362 (M+H).

14) Although H-2 had a $\Delta\delta$ value of +0.001 ppm, the value was very small and may be an observational error. The small $\Delta\delta$ value of H-2 suggests that these MTPA esters would be in an *anti*-conformation around the glycosyl linkage, because $\Delta\delta$ values are proportional to the distance between the protons and the MTPA moiety (ref. 13).

15) When the enzymatic reaction was done at 35°C, considerable deamination of RMNPA was observed.