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Facile Synthesis of 2′,5′-Dideoxy-, 2′,3′-Dideoxy- and 3′-Deoxy-1, N^6-ethenoadenosine Nucleosides

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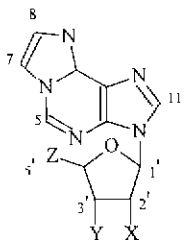
Facile synthetic methods of 2′,5′-dideoxy-, 2′,3′-dideoxy- and 3′-deoxy-1,N^6-ethenoadenosine nucleosides by either an enzymatic dideoxyribosyl transfer reaction or a simple chemical reaction were proposed. The synthetic products were isolated and purified by preparative HPLC and their structures were confirmed by ^{1}H NMR (500 MHz) and FAB-MS including high resolution mass measurement. These modified nucleoside analogs have not been reported yet. Therefore, these modified nucleoside analogs are of potential value to be studied further for biological activity such as anticancer or antiviral.

Key words: nucleoside phosphorylase, 2′,5′-dideoxy-1,N^6-ethenoadenosine, 2′,3′-dideoxy-1, N^6-ethenoadenosine, 3′-deoxy-1,N^6-ethenoadenosine

INTRODUCTION

Several purine nucleoside analogs have been used extensively as antibiotic, anticancer and antiviral agents [1-3]. Traditionally both natural and unnatural such nucleosides have been prepared by various chemical methods [4]. However, the steps of chemical productions are long and complicated, and undesirable by-products are formed. Enzymatic synthesis using nucleoside phosphorylases therefore has been considered as an alternative method for developing and screening several new derivatives that have high potential of biological activity. The substrate specificities and synthetic applications of nucleoside phosphorylases (purine nucleoside phosphorylase and thymidine phosphorylase) toward modified purine, pyrimidine and ribose derivatives have been studied intensively [5]. Several nucleoside *N*-transfer reactions via those phosphate esters of sugar was transribosylation [6], trans-2′-deoxyribosylation [7], transarabinosylation [8], trans-aminoribosylation [9], and trans-2′,3′-dideoxyribosylation [10, 11] and trans-*α*-*L*-2′,3′-dideoxyribosylation [12] by nucleoside phosphorylases. The results of recent studies toward trans-2′,5′-dideoxyribosylation in author's laboratory [13,14] and Fathi *et al.* [15] showed the versatility of the synthesis of 2′,5′-dideoxy ribosyl modified nucleosides by enzymatic dideoxyribosyl transfer reaction. We further applied this simple method to obtain a novel derivative, 2′,5′-dideoxy-1,N^6- ethenoadenosine (1a), which might have significant biological activity. Facile chemical synthesis of 2′,3′- dideoxy-1,N^6-ethenoadenosine (1b) and 3′-deoxy-1,N^6-ethenoadenosine (1c) were also tried as alternative methods.



	X	Y	Z
1a	H	OH	CH_3
1b	H	H	CH_2OH
1c	OH	H	CH_2OH

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MATERIALS AND METHODS

General

All reagents and solvents were of analytical reagent quality. 2′,5′-Dideoxythymidine, 1,N^6-ethenoadenine, 3′-deoxyadenosine, thymidine phosphorylase (EC 2.4.2.4.) and purine nucleoside phosphorylase (EC 2.4.2.1.) were purchased from Sigma Chemical Co. (St. Louis, MO). 2′,3′-Dideoxyadenosine was purchased from Fairfield Research Chemical Co. (Fairfield, NJ). Chloroacetaldehyde was obtained from Aldrich Chemical Co. (Milwaukee, WI). ^{1}H NMR spectra were obtained at ambient temperature at 500 MHz with a Varian VXR-500 spectrometer (Palo Alto, CA). The DMSO-d_5 peak in the NMR solvent (DMSO-d_6) was used as the internal reference for all the ^{1}H NMR spectra and was referenced at 2.49 ppm relative to TMS. The fast atom bombardment mass spectrometry (FAB-MS) spectral data were obtained using DTT/DTE (dithiothreitol : dithioerythreitol = 3 : 1) as the sample matrix on a Kratos MS-50 high resolution mass spectrometer (Chestnut Ridge, NY). Analytical HPLC utilized an Alltech (Deerfield, IL) Econosphere RP-C18 (150×4.6 mm, 3 μm) column eluted at 1 mL/min with a linear gradient of 4~10% CH_3CN in 50 mM $HCOONH_4$ for 5 min. Preparative HPLC was done on an Alltech Econosphere RP-C18 (250×22.5 mm, 10 μm) column eluted at 5 mL/min isocratically with a mobile phase of 10~15% CH_3CN and 1% dimethyl formamide in water. Waters 440 UV detector (Millipore Co., Bedford, MA) was used for sample detection at 254 nm and 280 nm. The HPLC samples were filtered through Centrifree Filters (Amicon Division, W. R. Grace & Co., Beverly, MA) to prevent column contamination by high molecular weight impurities like proteins. The mobile phase were filtered through 0.2 μm Millipore filters and degassed prior to use. Cytotoxicity against human

solid tumor cell line, HT-29 human colon adenocarcinoma, was assayed by the microculture tetrazolium assay method [16] at the Purdue Cancer Center Cytotoxicity Laboratory. Adriamycin was used for antitumor cytotoxicity control.

Enzymatic Synthesis of 2′,5′-Dideoxy-1,N^6-ethenoadenosine

A suspension of 80 mg (0.5 mmol) of 1,N^6-ethenoadenine(3) and 22.6 mg (0.1 mmol) of 2′,5′-dideoxythymidine(2) in 15 mL of 5 mM sodium phosphate buffer, pH 7.4 was prepared. To this suspension was added 20 units of thymidine phosphorylase and 20 units of purine nucleoside phosphorylase. After 5 days at room temperature with stirring, the reaction mixture was freeze-dried. The dried mixture was redissolved in 3 mL of water and then followed by filtration to remove excess insoluble ethenoadenine. The mixture was filtered through an Amicon filter to remove the enzymes. The filtrate was loaded on a preparative HPLC column (10% CH_3CN in water, isocratic, 3 mL/min) by consecutive injection of 1.0 mL each. The fractions corresponding to the 2′,5′-dideoxy-1,N^6-ethenoadenosine were collected, combined and finally freeze-dried, yielding 14.7 mg (0.057 mmol, 57%) of 2′,5′-dideoxy-1,N^6-ethenoadenosine(1a) as a white powder : mp 147 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.285 (H5, s, 1H), δ 8.485 (H11, s, 1H), δ 8.066 (H7, d, 1H, $^3J_{7,8}$ = 1.5 Hz) δ 7.549 (H8, d, $^3J_{8,7}$ = 1.5 Hz) δ 6.421 (H1′, t, 1H, $^3J_{1',2'\beta} = {}^3J_{1',2'\alpha}$ = 6.7 Hz) δ 4.226 (H3′, dt, 1H, $^3J_{3',2'\beta}$ = 6.7 Hz, $^3J_{3',2'\alpha} = {}^3J_{3',4'}$ = 4.3 Hz) δ 3.932 (H4′, qd, 1H, $^3J_{4',5'}$ = 6.4 Hz, $^3J_{4',3'}$ = 4.3 Hz) δ 2.832 (H2′β, dt, 1H, $^2J_{2'\beta,2'\alpha}$ = 13.3 Hz, $^3J_{2'\beta,1'} = {}^3J_{2'\beta,3'}$ = 6.7 Hz) δ 2.352 (H2′α, ddd, 1H, $^2J_{2'\alpha,2'\beta}$ = 13.3 Hz, $^3J_{2'\alpha,1'}$ = 6.7 Hz, $^3J_{2'\alpha,3'}$ = 6.7 Hz) δ 1.271 (H5′, d, 3H, $^3J_{5',4'}$ = 6.4 Hz); FAB-MS m/z (relative intensity) 260.1151 (260.114, calc. for $C_{12}H_{14}N_5O_2$; MH^+, 73) and 160 (BH^+, 100).

Chemical Synthesis of 2′,3′-Dideoxy-1,N^6-ethenoadenosine

To a solution of 63 mg (0.25 mmol) of 2′,3′-dideoxyadenosine in 2.5 mL of 50 mM ammonium formate buffer, pH 6.0, 0.9 mL of 50% chloroacetaldehyde solution (5.0 mmol) was added. After 30 hr at 20 ℃, the reaction mixture was loaded on a preparative HPLC column (10% CH_3CN in water, isocratic, 3 mL/min) by consecutive injection of 1.0 mL each. The fractions corresponding to the 2′,3′-dideoxy-1,N^6-ethenoadenosine were collected, combined and finally freeze- dried, yielding 24.0 mg (0.093 mmol, 37%) of 2′,3′- dideoxy-1,N^6-ethenoadenosine(1b) as a white powder : mp 93 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.274 (H5, s, 1H), δ 8.548 (H11, s, 1H), δ 8.063 (H7, d, 1H, $^3J_{7,8}$ = 1.4 Hz) δ 7.541 (H8, d, $^3J_{8,7}$ = 1.4 Hz) δ 6.370 (H1′, dd, 1H, $^3J_{1',2'\beta}$ = 6.8 Hz, $^3J_{1',2'\alpha}$ = 3.2 Hz) δ 4.972 (H5′OH, t, 1H, $^3J_{5'OH,5'} = {}^3J_{5'OH,5''}$ = 5.6 Hz) δ 4.141 (H4′, ddt, 1H, $^3J_{4',3'\beta}$ = 8.4 Hz, $^3J_{4'3'\alpha}$ = 6.5 Hz, $^3J_{4',5'\alpha} = {}^3J_{4',5''}$ = 4.4 Hz) δ 3.639 (H5′, ddd, 1H, $^2J_{5',5''}$ = 11.8 Hz, $^3J_{5',5'OH}$ = 5.6 Hz, $^3J_{5'4'}$ = 4.4 Hz) δ 3.526 (H5″, ddd, 1H, $^2J_{5'',5'}$ = 11.8 Hz, $^3J_{5'',5'OH}$ = 5.6 Hz, $^3J_{5''4'}$ = 4.4 Hz) δ 2.4-2.5 (H3′β, H3′α, m, 2H, δ 2.0-2.1 (H2′β, H2′α, m, 2H) ; FAB-MS m/z (rela- tive intensity) 260.1145 (260.1148, calc. for $C_{12}H_{14}N_5O_2$; MH^+, 58) and 160 (BH^+, 100).

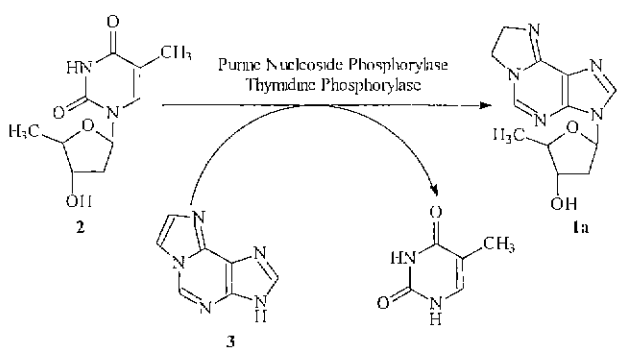
Chemical Synthesis of 3′-Deoxy-1,N^6-ethenoadenosine

To a solution of 6.3 mg (25 μmole) of 3′-deoxyadenosine in 0.5 mL of 50 mM ammonium formate buffer, pH 5.0, 0.1 mL of 50% chloroacetaldehyde solution (0.5 mmole) was added. The mixture was heated for 10 min at 90℃ water bath and then immediately cooled down to room temperature. The reaction mixture was loaded on a preparative HPLC column (10% CH_3CN in water, isocratic, 3 mL/min). The fraction corresponding to 3′-deoxy-1,N^6-ethenoadenosine was collected, combined and finally freeze-dried, yielding 3.1 mg (12 μmole, 48%) of 3′-deoxy-1,N^6-ethenoadenosine(1c) as a white powder : mp 147℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.293 (H5, s, 1H), δ 8.563 (H11, s, 1H), δ 8.070 (H7, d, 1H, $^3J_{7,8}$ = 1.5 Hz) δ 7.555 (H8, d, $^3J_{8,7}$ = 1.5 Hz) δ 6.035 (H1′, d, 1H, $^3J_{1',2'}$ = 1.7 Hz) δ 4.563 (H2′, ddd, 1H, ^{3}J2′,3′β = 5.4 Hz, $^3J_{2',3'\alpha}$ = 2.3 Hz, $^3J_{2',1'}$ = 1.7 Hz) δ 4.399 (H4′, ddt, 1H, $^3J_{4',3'\beta}$ = 9.5 Hz, $^3J_{4',3'\alpha}$ = 5.9 Hz, $^3J_{4',5'} = {}^3J_{4',5''}$ = 3.7 Hz) δ 3.726 (H5″, d(broad), 1H, $^2J_{5'',5'}$ = 12.5 Hz) δ 3.555 (H5′, d(broad), 1H, $^2J_{5',5''}$ = 12.5 Hz) δ 2.247 (H3′β, ddd, 1H, $^2J_{3'\beta,3'\alpha}$ = 13.2 Hz, $^3J_{3'\beta,4'}$ = 9.5 Hz, $^3J_{3'\beta,2'}$ = 5.4 Hz) δ 1.910 (H3′α, ddd, 1H, $^2J_{3'\alpha,3'\beta}$ = 13.2 Hz, $^3J_{3'\alpha,4'}$ = 5.9 Hz, $^3J_{3'\alpha,2'}$ = 6.7 Hz); FAB-MS m/z (relative intensity) 276.1092 (276.1096, calc. for $C_{12}H_{14}N_5O_3$; MH^+, 85) and 160 (BH^+, 100).

RESULTS AND DISCUSSION

Enzymatic Synthesis of 2′,5′-Dideoxy-1,N^6-ethenoadenosine

2′,5′-Dideoxy-1,N^6-ethenoadenosine was synthesized from 2′,5′-dideoxythymidine(2) and 1,N^6-ethenoadenine (3) through the coupled enzymatic dideoxyribosyl transfer reaction catalyzed by coupled enzyme system of purine nucleoside phosphorylase and thymidine phosphorylase. The phosphorolysis of a 2′,5′-dideoxythymidine (2) is firstly catalyzed by thymidine phosphorylase to form 2′,5′-dideoxyribose-1-phosphate and followed by the synthesis of the desired product (1a) from the 2′,5′-dideoxyribose-1-phosphate and 1,N^6-ethenoadenine base (3) by purine nucleoside phosphorylase. The net result of this coupled enzymatic reaction is the transfer of a 2′,5′-dideoxyribosyl moiety from a pyrimidine nucleoside (2) to 1,N^6-ethenoadenine base (3). Selection of pyrimidine nucleoside (2′,5′-dideoxythymidine, 2) as a dideoxyribosyl donor instead of purine derivatives such as 2′,5′-dideoxyadenosine is based on the knowledge that pyrimidine derivatives generally favor phosphorolytic



cleavage more than purine derivatives, that is, pyrimidine derivatives are therefore more efficient dideoxyribosyl donor [17]. Chemical synthesis of 2′,5′-dideoxy-1,N^6-ethenoadenosine from the reaction of 2′,5′-dideoxyadenosine with chloroacetaldehyde through ring formation was not tried because 2′,5′-dideoxyadenosine is much rarer than the corresponding 2′,5′-dideoxythymidine.

Chemical Synthesis of 2′,3′-Dideoxy- and 3′-Deoxy-1,N^6-ethenoadenosine

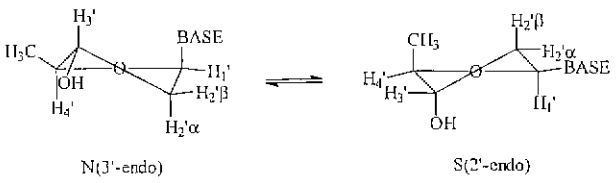
Enzymatic synthesis of 2′,3′-dideoxy-1,N^6-ethenoadenosine(1b) was attempted using 1,N^6-ethenoadenine (3) as the dideoxyribosyl acceptor and either 2′,3′-dideoxythymidine or 2′,3′-dideoxyadenosine as the dideoxyribosyl donor. However no detectable amount of product was found after 24 hr reaction at room temperature in 5 mM sodium phosphate buffer, pH 7.4. It could be ascribed to the lower activity of nucleoside phosphorylase toward 2′,3′-dideoxyribosyl moiety and 1,N^6-ethenoadenosine moiety. Although a large amount of enzymes may be utilized to overcome this lower activity problem, simple chemical synthesis of 2′,3′-dideoxy-1,N^6-ethenoadenosine (1b) from mixing 2′,3′-dideoxyadenosine and chloroacetaldehyde in room temperature for 30 hr was another alternative. In the case of 3′-deoxy-1,N^6-ethenoadenosine (1c) synthesis, 3′-deoxyadenosine and chloroacetaldehyde was reacted for 10 min at elevated temperature (90℃). The products were isolated by preparative HPLC and structures of the products were confirmed by FAB-MS and ^{1}H NMR spectral analysis.

Conformational Analysis of Modified Ribose Rings

It has been known that the conformation of sugar rings (i.e., 2′,5′-dideoxyribosyl, 2′,3′-dideoxyribosyl or 3′-deoxyribosyl) is not frozen in a fixed conformation but exists in a dynamic equilibrium between two conformers, C2′-endo (*S*) and C3′-endo (*N*), in solution [18]. Because the vicinal coupling constants ($^3J_{H1',H2'\beta}$, $^3J_{H3',H4'}$) show a larger variance from the *N* to *S* conformer, Davis and Danyluk [19] proposed the use of following formula to estimate the percentage of the 3′-endo conformer (*N*) in the equilibrium mixture.

$$\%\,N = 100\ {}^3J_{H3',H4'} / ({}^3JH1',H2'\beta\ +\ {}^3J_{H3',H4'})$$

From the observation of coupling constants ($^3J_{H1',H2'\beta}$, $^3J_{H3',H4'}$), it could be concluded that 61% of 2′,5′-dideoxy-1,N^6-ethenoadenosine exists as the *S* conformer in DMSO solution. The percentage of *S* conformer for 2′,5′-dideoxy-1,N^6-ethenoadenosine is very similar to those for 2′,5′-dideoxy-5-fluorourine, 2′-deoxyuridine, and thymidine [13]. This similarity in conformation may partially account for its ready trans-2′,5′-dideoxyribosylation by nucleoside phosphorylases despite of the fact that it is not a natural substrate for these enzymes. The percentage of *S* conformer for 2′,3′-dideoxy-1,N^6-ethenoadenosine (45% *S* in DMSO solution) is somewhat lower than that of 2′,5′-dideoxy-1,N^6-ethenoadenosine. These results are in agreement with the previous studies on the dideoxyribose conformation of 2′,3′-dideoxyadenosine by Ludemann *et al.* [20] that the 5′-CH_2OH group has minimum hinderance in the *N* state. Comparatively, 3′-deoxy-1,N^6-ethenoadenosine exists mainly in the *N* conformer (85% *N* in DMSO solution). It means that introduction of a hydroxyl group at the 2′ *α* position of 2′,3′-dideoxy-1,N^6-ethenoadenosine significantly stabilized the *N*-state by minimizing the steric interaction between the base atoms and the 2′ *α* hydroxyl group [20].



Biological Activity of Ribose Modified 1,N^6-Ethenoadenosine Derivatives

Ribose-modified 1,N^6-ethenoadenosine derivatives were tested for *in vitro* antitumor activities toward human colon adenocarcinoma (HT-29) cell line. The ED_{50} of 2′,5′-dideoxy-1,N^6-ethenoadenosine, 2′,3′-dideoxy-1,N^6-ethenoadenosine, and 3′-deoxy-1,N^6-ethenoadenosine was over 39 μM, 39 μM and 197 μM, respectively. In terms of expected biological activity, 2′,5′-dideoxy-1,N^6-ethenoadenosine might be metabolically cleaved by purine nucleoside phosphorylase to its nucleosides which could interfere with nucleotide biosynthesis and/or the function of deaminases. Many of 2′,3′-dideoxynucleosides have been shown to block the infectivity of HIV *in vitro*, and some 2′,3′-dideoxyadenosine derivatives are currently under *in vivo* evaluations of its anti-HIV efficacy [21]. 2′,3′-Dideoxy-1,N^6-ethenoadenosine as a close analog of 2′,3′-dideoxyadenosine may be successfully phosphorylated to yield corresponding 5′-triphosphate by cellular enzymes, and compete with the binding of normal nucleotides to the retroviral reverse transcriptase and/or be incorporation into the viral DNA chain, resulting in DNA-chain termination due to the lack of the 3′-hydroxy group. In case of 3′-deoxy-1,N^6-ethenoadenosine, it may be activated to the corresponding 5′-triphosphate by the same manner, and act as a competitive inhibitor for viral reverse transcription due to the lack of the 3′-hydroxyl group. Because the biological activities of 2′,5′-dideoxy-1, N^6-ethenoadenosine, 2′,3′-dideoxy-1,N^6-ethenoadenosine and 3′-deoxy-1,N^6-ethenoadenosine have not been reported yet, further studies may be necessary.

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