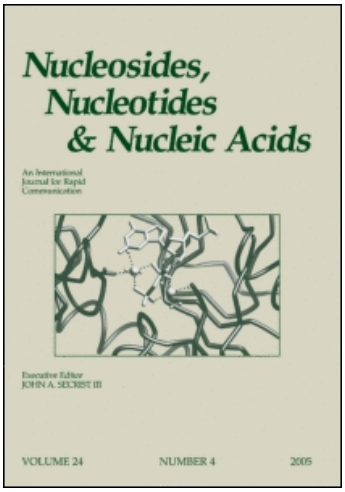

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FORMATION OF 2′-DEOXY-2-NITROADENOSINES BY REACTION OF 2′-DEOXYADENOSINES WITH COPPER(II) NITRATE/ACETIC ANHYDRIDE

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ABSTRACT

Nitration of 9-substituted [ethyl, $(Ac)_2$-2′-deoxyribosyl, $(Ac)_3$-ribosyl] N^6-acetyladenine derivatives with $Cu(NO_3)_2 \cdot 3H_2O/Ac_2O$ was examined. Nitration proceeded at the 2-position, although the yield was low. Removal of the acetyl groups gave 2′-deoxy-2-nitroadenosine derivatives.

INTRODUCTION

Nitric oxide (NO) is involved in important physiological functions such as vasodilation[1] and neurotransmission.[2] However, it can have adverse effects: NO forms harmful peroxynitrite ($ONOO^-$) by reaction with superoxide, and it has been reported that peroxynitrite reacts with the guanine moiety of DNA to form 8-nitroguanine and 8-oxoguanine,[3] the latter of which is considered to be a DNA modification responsible for mutation/cancer induction.[4] The adenine moiety of DNA also forms 8-oxoadenine and 2-hydroxyadenine by treatment with oxidizing agents.[5–6] Nitrated adenine derivatives, however, had not been described until a recent report by

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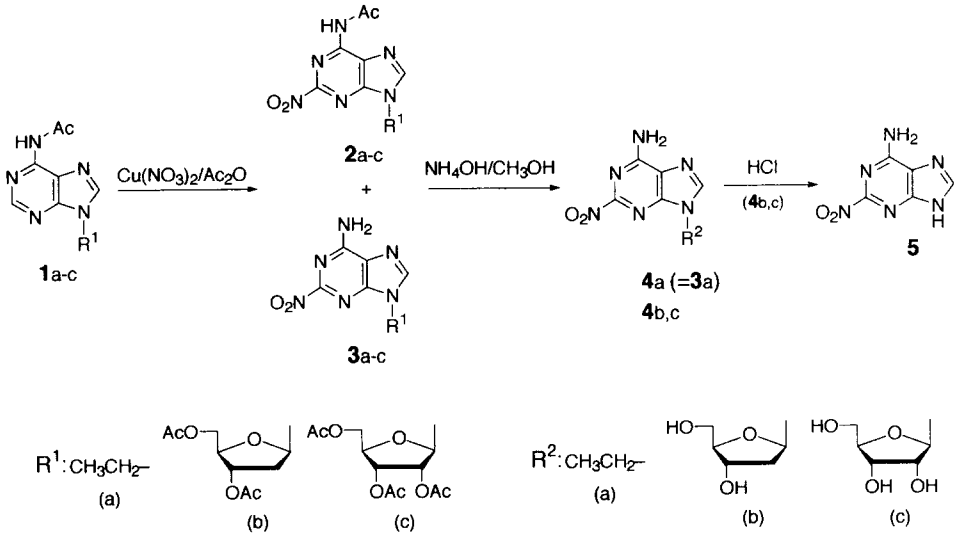
Koomen's group who synthesized 2-nitroadenosine via 6-chloro-2-nitropurineriboside derivatives that were prepared by the nitration of 6-chloropurine derivative with tetrabutylammonium nitrate/trifluoroacetic anhydride (TBAN/TFAA).[7] These investigators also obtained 2-nitroadenosine by nitration of N^6,N^6,$O^{2'}$,$O^{3'}$,$O^{5'}$-pentaacetyladenosine with TBAN/TFAA and subsequent deacetylation, however, the yield was low due to a side reaction in the course of the deacetylation.[7] Except for their study, direct nitration of adenine derivatives has been unreported, although many studies have been published on direct nitration of uracil derivatives using a variety of nitrating agents; $NO_2{}^+BF_4{}^-$,[8] NO_2OCOCF_3,[9–10] NO_2-O_3,[11] and $Cu(NO_3)_2{\cdot}3H_2O/Ac_2O$.[12] Sodum et al. reported quite recently on the reaction products of 2′-deoxyadenosine with peroxynitrite. They showed that the main product was 2′-deoxy-8-oxoadenosine, however, the minor one was only suggested to be some nitrated derivative.[13] In this study, we examined the preparation of nitrated 2′-deoxyadenosine derivatives. Since the glycosidic bond of 2′-deoxyadenosine is less stable than that of adenosine against acid, milder conditions that cause low yield of nitration product are required for the preparation of 2′-deoxy-2-nitroadenosine. Reaction of N^6,$O^{3'}$,$O^{5'}$-triacetyl-2′-deoxyadenosine with $Cu(NO_3)_2{\cdot}3H_2O/Ac_2O$ afforded 2-nitro derivatives and subsequent removal of the acetyl groups gave 2′-deoxy-2-nitroadenosine.

RESULTS AND DISCUSSION

Nitration of 9-substituted N^6-acetyladenine derivative (**1**) (Sch. 1) was carried out with $Cu(NO_3)_2{\cdot}3H_2O/Ac_2O$. The reaction gave a small yield of



Scheme 1. Syntheses of 2′-deoxy-2-nitroadenosine derivatives.

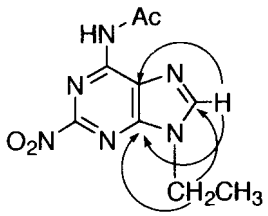


Figure 1. HMBC correlations of compound **2a**.

products (**2** and **3**) with unreacted starting material. The products were separated by preparative thin layer chromatography (PLC) (silica gel). The yield of **2** was slightly larger than that of **3** and the total yield of **2** and **3** was $7 \sim 33\%$. The mass spectrum of **2a** showed that it has a mono nitrated structure. The NMR spectrum of **2a** suggested that the position of the nitro group is C2 or C8. The correct position of the nitro group was finally determined to be C2 by means of HMBC correlations (Fig. 1). From these results, the structure of **2a** was determined to be N^6-acetyl-9-ethyl-2-nitroadenine. Similarly, the structures of **2b**,**c** were determined to be 2-nitroadenine derivatives. Deacetylation of **2a**–**c** with $NH_4OH/MeOH$ gave the corresponding 9-ethyl-2-nitroadenine (**4a**), 2′-deoxy-2-nitroadenosine (**4b**) and 2-nitroadenosine (**4c**), respectively. Compound **4** has a characteristic UV absorption spectrum with a λmax at a long wavelength due to the effect of the nitro group (Fig. 2). In the course of deacetylation, 2-methoxyadenine derivatives were obtained as a by-product by replacement of the nitro group of **4** with the methoxy group as reported.[7] The yield of 2-methoxyadenine derivatives was less than 25%. Structures of **3a**–**c** were determined to be N^6-deacetylated **2a**–**c** from spectral data. Deacetylation of **3b**,**c** with $NH_4OH/MeOH$ also gave **4b**,**c**. When adenine derivatives without the N^6-acetyl group were employed as substrate, no reaction proceeded within the $Cu(NO_3)_2{\cdot}3H_2O/Ac_2O$ system. This indicates that radical nitration mechanisms may be involved.[7] It has been reported that 2′-deoxy-8-nitroguanosine is very unstable and decomposes to 8-nitroguanine within a few minutes.[3,13] The stability of **4b** was then examined using HPLC. Compound **4b** was fairly stable and no decomposition was observed under treatment with aqueous solution (pH 6.8) at 60°C for 4 h. Acid treatment (0.1 N HCl at 60°C for 40 min) of **4b** gave 2-nitroadenine (**5**). When N^6-acetyladenine was allowed to react with $Cu(NO_3)_2{\cdot}3H_2O/Ac_2O$, the reaction did not give N^6-acetyl-2-nitroadenine but gave other products (unstable ring-nitrogen-mononitrated N^6-acetyladenine and stable N^6-acetyl-2(8)-nitro-8(2)-oxoadenine) and their structures were deduced from NMR and mass spectroscopic data. When 9-ethylguanine was allowed to react with $Cu(NO_3)_2{\cdot}3H_2O/Ac_2O$, the reaction did not give a nitro derivative but gave instead *N*-ethylparabanic acid.

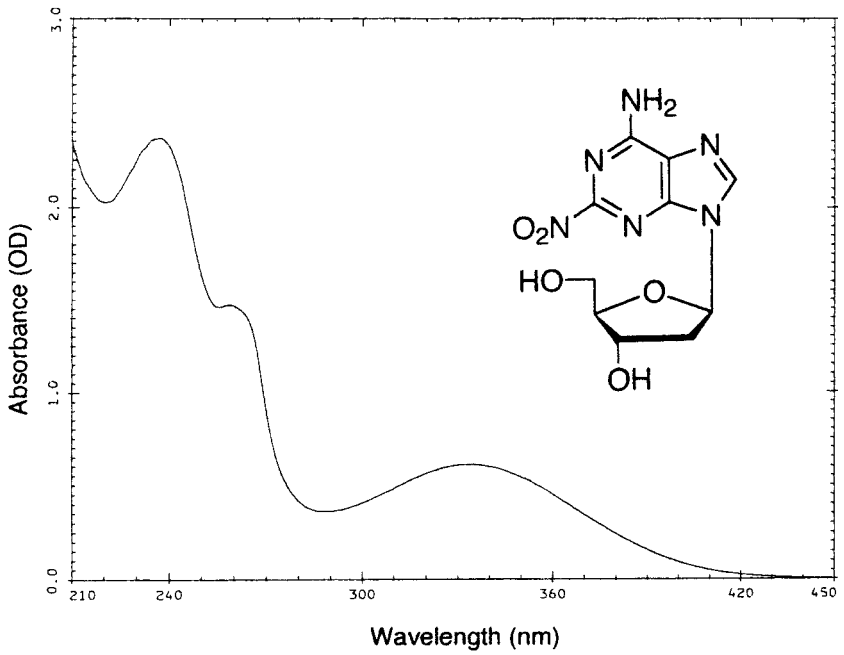


Figure 2. UV absorption spectrum of compound **4b** at pH 6.8.

EXPERIMENTAL SECTION

Materials and Methods

^1H and ^{13}C NMR spectra were recorded on a JEOL EX 270, GSX 400, or ALPHA 500 spectrometer, and chemical shifts were expressed in ppm using Me_4Si as the internal standard. EI and FAB mass spectra were obtained with a JEOL JMS-DX300 spectrometer. ESI mass spectra were obtained with a Micromass Quattro II spectrometer. HPLC analyses were carried out using a Shimadzu LC-10AD apparatus equipped with an SPD-M6A, a photodiode array UV detector. The Merck LiChrosphere 100 RP-18e, 4×250 mm column used was eluted at a flow rate of 0.6 mL/min with a 0.15 M phosphate buffer (pH 7.0)–MeOH system. Melting points were measured with a Yanagimoto micro melting point apparatus and are uncorrected. Silica gel 60 PF_{254} (Merck) was used for preparative thin-layer chromatography (PLC).

Nitration of N^6-Acetyladenine Derivatives (**1**) with $Cu(NO_3)_2 \cdot 3H_2O/Ac_2O$

As a general practice, the reaction of **1c** which showed the highest yield was described. $Cu(NO_3)_2 \cdot 3H_2O$ (800 mg, 3.32 mmol) was dissolved in 6 mL of

Ac_2O. After 15 min, **1c** (200 mg, 0.46 mmol) was added and the mixture was left at room temperature for 22 h. MeOH (80 mL) was then added and the mixture was evaporated to dryness. Water was added to the residue and the products were extracted with AcOEt. Separation and purification of the products were carried out by PLC (silica gel, AcOEt). The yields of **2c** and **3c** were 41 mg (19%) and 29 mg (14%), respectively, and **1c** was recovered in a 20 mg (10%) yield. In the case of **1b**, separation of the products was carried out by PLC (silica gel, AcOEt:MeOH = 40:1), and the yields of **2b** and **3b** were 3.4% and 3.3%, respectively, and the recovery of starting material was 11%. A similar procedure was employed for the reaction of **1a** and products **2a** and **3a** were separated by PLC (silica gel, AcOEt) in total yield 19%.

***N*⁶-Acetyl-9-ethyl-2-nitroadenine (2a).** Colorless needles (MeOH-AcOEt), mp 194–196°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.49 (t, 3H, $J = 7.3$ Hz, CH_2CH_3), 2.32 (s, 3H, $COCH_3$), 4.34 (q, 2H, CH_2CH_3), 8.80 (s, 1H, 8-H), 11.28 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 14.8 (CH_2CH_3), 24.4 ($COCH_3$), 39.1 (CH_2CH_3), 125.2 (5-C), 147.5 (8-C), 149.6 (6-C), 151.8 (4-C), 153.6 (2-C), 168.9 ($COCH_3$); EI-MS: m/z 250 (M^+), 208 ($M^+ - Ac + H$), 162 ($M^+ - Ac + H - NO_2$); HRMS calcd for $C_9H_{10}N_6O_3$: 250.0814, found 250.0814.

***N*⁶,*O*³′,*O*⁵′-Triacetyl-2′-deoxy-2-nitroadenosine (2b).** Colorless solid; ^{1}H NMR ($CDCl_3$): δ 2.10, 2.17 (each s, each 3H, 3′-, 5′-$OCOCH_3$), 2.77 (m, 1H, 2′-H), 2.78 (s, 3H, N-$COCH_3$), 2.89 (m, 1H, 2″-H), 4.42 (m, 3H, 4′-H, 5′-H, 5″-H), 5.45 (m, 1H, 3′-H), 6.55 (dd, 1H, $J = 6.1$, 7.6 Hz, 1′-H), 8.45 (s, 1H, 8-H), 8.94 (bs, 1H, NH); EI-MS: m/z 422 (M^+); HRMS calcd for $C_{16}H_{18}N_6O_8$: 422.1185, found 422.1183.

***N*⁶,*O*²′,*O*³′,*O*⁵′-Tetraacetyl-2-nitroadenosine (2c).** Colorless solid; ^{1}H NMR ($CDCl_3$): δ 2.10, 2.14, 2.20 (each s, each 3H, 2′-, 3′-, 5′-$OCOCH_3$), 2.79 (s, 3H, N-$COCH_3$), 4.47 (m, 2H, 4′-H, 5′-H), 4.53 (m, 1H, 5″-H), 5.62 (dd, 1H, $J = 4.3$, 5.5 Hz, 3′-H), 5.78 (m, 1H, 2′-H), 6.30 (d, $J = 5.8$ Hz, 1′-H), 8.41 (s, 1H, 8-H), 8.90 (bs, 1H, NH): EI-MS: m/z 480 (M^+); HRMS calcd for $C_{18}H_{20}N_6O_{10}$: 480.1241, found 480.1241.

9-Ethyl-2-nitroadenine (3a = 4a). Yellow powder (H_2O), mp 280°C (decomp); ^{1}H NMR (DMSO-d_6): δ 1.44 (t, 3H, $J = 7.3$ Hz, CH_2CH_3), 4.23 (q, 2H, CH_2CH_3), 8.16 (bs, 2H, NH_2), 8.44 (s, 1H, 8-H); EI-MS: m/z 208 (M^+); HRMS calcd for $C_7H_8N_6O_2$: 208.0708, found 208.0704; UV λmax (nm) (pH 6.8) 245, 268 (sh), 346.

***O*³′,*O*⁵′-*D*iacetyl-2′-deoxy-2-nitroadenosine (3b).** Colorless solid; ^{1}H NMR ($CDCl_3$): δ 2.10, 2.16 (each s, each 3H, 3′-, 5′-$OCOCH_3$), 2.74 (m, 1H, 2′-H), 2.88 (m, 1H, 2″-H), 4.39 (m, 2H, 5′-H, 5″-H), 4.42 (m, 1H, H-4′),

5.44 (m, 1H, 3′-H), 6.48 (bs, 2H, NH_2), 6.49 (dd, 1H, $J = 5.9$, 7.6 Hz, 1′-H), 8.24 (s, 1H, 8-H); EI-MS: m/z 380 (M^+); HRMS calcd for $C_{14}H_{16}N_6O_7$: 380.1082, found 380.1082.

***O*$^{2'}$,*O*$^{3'}$,*O*$^{5'}$-Triacetyl-2-nitroadenosine (3c).** Colorless solid; ^{1}H NMR ($CDCl_3$): δ 2.10, 2.13, 2.18 (each s, each 3H, 2′-, 3′-, 5′-$OCOCH_3$), 4.47 (m, 2H, 4′-H, 5′-H), 4.50 (m, 1H, 5″-H), 5.64 (dd, 1H, $J = 4.6$, 5.5 Hz, 3′-H), 5.77 (m, 1H, 2′-H), 6.24 (d, $J = 5.2$ Hz, 1′-H), 6.27 (bs, 2H, NH_2), 8.17 (s, 1H, 8-H); EI-MS: m/z 438 (M^+); HRMS calcd for $C_{16}H_{18}N_6O_9$: 438.1134, found 438.1137.

Preparation of 2-Nitroadenine Derivatives (**4**)

Compound **2a**, **2b**, **2c**, **3b** or **3c** was treated with conc. NH_4OH/MeOH (1:3) at 50°C for 30 min and the product was separated and purified by PLC. The yield of **4** was about 70%.

2′-Deoxy-2-nitroadenosine (4b). Pale yellow powder; ^{1}H NMR (D_2O): δ 2.60 (ddd, 1H, $J = 3.7$, 6.4, 14.0 Hz, 2′-H), 2.87 (m, 1H, 2″-H), 3.83 (dd, 1H, $J = 4.6$, 12.5 Hz, 5′-H), 3.88 (dd, 1H, $J = 3.4$, 12.5 Hz, 5″-H), 4.19 (m, 1H, 4′-H), 4.69 (dd, $J = 3.4$, 6.1 Hz, 3′-H), 6.50 (t, $J = 6.7$ Hz, 1′-H), 8.49 (s, 1H, 8-H); ESI-MS: positive ion mode, m/z 296.95 $(M + H)^+$, negative ion mode, m/z 294.98 $(M - H)^-$; UV: λmax (nm) (pH 6.8) 236, 262 (sh), 334.

2-Nitroadenosine (4c).[7] Pale yellow powder; ^{1}H NMR (CD_3OD): δ 3.79, 3.90 (each m, each 1H, 5′-H, 5″-H), 4.15 (m, 1H, 4′-H), 4.36 (dd, 1H, $J = 3.7$, 4.9 Hz, 3′-H), 4.69 (m, 1H, 2′-H), 6.03 (d, 1H, $J = 5.5$ Hz, 1′-H), 8.53 (s, 1H, 8-H); ESI-MS: positive ion mode, m/z 312.91 $(M + H)^+$, negative ion mode, m/z 310.98 $(M - H)^-$; UV: λmax (nm) (pH 6.8) 236, 261 (sh), 334.

2-Methoxyadenosine.[7] Yield <25%; ^{1}H NMR (DMSO-d_6): δ 3.53. 3.64 (each m, each 1H, 5′-H, 5″-H), 3.82 (s, 3H, CH_3), 3.93 (m, 1H, 4′-H), 4.15 (dd, 1H, $J = 3.4$, 4.9 Hz, 3′-H), 4.62 (m, 1H, 2′-H), 5.78 (d, $J = 6.1$ Hz, 1′-H), 7.31 (bs, 2H, NH_2), 8.14 (s, 1H, 8-H); FAB-MS m/z 298 $(M + H)^+$.

Preparation of 2-Nitroadenine (**5**)

Acid hydrolysis of **4b** and **4c** was carried out in 0.1N HCl at 60°C for 40 min and in 1N HCl at 90°C for 1h, respectively. Yellow precipitates of **5** which appeared after cooling the reaction mixture in almost quantitative yield were collected.

2-Nitroadenine (5). mp >300°C; ^{1}H NMR (DMSO-d_6): δ 8.05 (bs, 2H, NH_2), 8.40 (s, 1H, 8-H); EI-MS m/z 180 (M^+), 134 ($M^+ - NO_2$); HRMS

calcd for $C_5H_4N_6O_2$: 180.0396, found 180.0397. UV: λmax (nm) (pH 6.8) 235, 262 (sh), 280 (sh), 334.

Reaction of N^6-Acetyladenine with $Cu(NO_3)_2{\cdot}3H_2O/Ac_2O$

N^6-Acetyladenine was allowed to react with $Cu(NO_3)_2{\cdot}3H_2O/Ac_2O$ as described for the nitration of N^6-acetyladenine derivatives (**1**). Products were separated by PLC ($CHCl_3$:MeOH = 9:1).

Ring-nitrogen-mono-nitrated adenine. Yield 3.1%; ^{1}H NMR (DMSO-d_6): δ 7.73 (bs, 2H, NH_2), 8.33 and 8.97 (each s, each 1H, 2-H and 8-H); EI-MS: m/z 180 (M^+); HRMS calcd for $C_5H_4N_6O_2$: 180.0395, found 180.0401. The position (1, 3, 7 or 9) of the N-nitro group could not be deduced.

***N*^{6}-Acetyl-2(8)-nitro-8(2)-oxoadenine.** Yield 2.1%; ^{1}H NMR (DMSO-d_6): δ 2.15 (s, 3H, CH_3), 11.22 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 23.0 (CH_3), 115.0, 136.5, 152.0, 152.6, 152.8, 169.5; EI-MS: m/z 238 (M^+), 196 ($M^+ - C_2H_2O$), 150 ($M^+ - C_2H_2O - NO_2$); HRMS calcd for $C_7H_6N_6O_4$: 238.0450, found 238.0455.

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