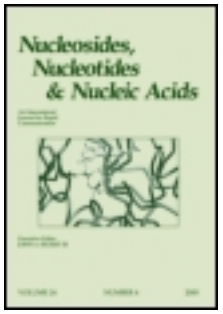

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A Newly Devised Method for the Debenzylation of N ^{6}-Benzyladenosines. A Convenient Synthesis of [6-^{15} N]-Labeled Adenosines

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A NEWLY DEVISED METHOD FOR THE DEBENZYLATION OF N^6-BENZYLADENOSINES. A CONVENIENT SYNTHESIS OF [6-^{15}N]-LABELED ADENOSINES §

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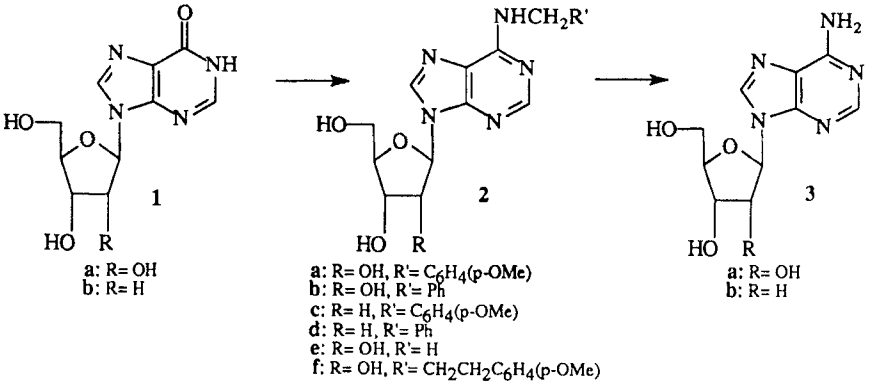
Abstract: [6-^{15}N]-Labeled adenosine was conveniently prepared from inosine (**1a**) by the silylation-benzylamination of **1a** and subsequent oxidative debenzylation with ammonium peroxydisulfate in a pH 7.2 buffer solution.

NMR studies employing oligonucleotides regio-selectively labeled with ^{15}N provide valuable information regarding nucleic acid structures, nucleic acids binding with drugs, and nucleotide-protein interactions. [1] The potential utility of the ^{15}N-labeled oligonucleotides has led to considerable interest in the development of synthetic routes to the required ^{15}N-labeled nucleosides. The N_1- and N^6-positions of the adenine ring are good candidates for the ^{15}N-labeling because they can form hydrogen bonds with suitable donors or acceptors in the nucleic acids, drug, and proteins. [2] On this line, many efforts have been made to establish the preparative methods for [1-^{15}N]- and [6-^{15}N]-labeled adenosines. As a result, a first synthesis of the [1-^{15}N]-labeled adenosines has been accomplished *via* N_1-benzylation of the corresponding [6-^{15}N]-labeled adenosines and subsequent Dimroth rearrangement. [3] The synthetic methods used for the [6-^{15}N]-labeled adenosines are i) direct amination of 6-chloropurine nucleosides by ^{15}N-enriched ammonia at high temperature in a sealed tube, [4] ii) enzymatic coupling of [6-^{15}N]-labeled adenine with an appropriate sugar, [2b] or iii) nucleophilic substitution on the 6-position in the 6-chloropurine nucleosides or 6-*O*-benzenesulfonylinosines with ^{15}N-enriched benzylamine followed by debenzylation which is carried out by means of RuO_2-$NaIO_4$ oxidation and subsequent ammonolysis. [3,5] Among these methods, the last one was advantageous for a large scale preparation of the [6-^{15}N]-labeled adenosines compared to the other methods in

§ Dedicated to professor M. Ikehara on the occasion of his 70th birthday.



Scheme 1

view of the reaction conditions employed and of the use of liquid ^{15}N-source. This method, however, required the protection of the hydroxyl groups in the sugar moiety during the reactions and the two-steps operation for the debenzylation, causing decrease of the overall yield.

In a previous work, [6] we have documented the oxidative demethylation of N^6-monomethyladenosine derivatives involving a single-electron transfer process under UV-irradiation in the presence of a heterocyclic *N*-oxide. This result let us to examine the oxidation of N^6-benzyladenosines (*cf.* **2a-d**) with a single-electron oxidant in aqueous medium to develop an alternative route for the debenzylation.

In this paper, we describe a newly devised method for the debenzyltion of **2a-d** which involves oxidation with ammonium peroxydisulfate [(NH4)2S2O8] under mild conditions. The present method is based on the chemical reactivities of cation radical species of **2** and provides a simple and high-yield procedure for the [6-^{15}N]-labeled adenosines from inosines (**1**) without protecting the sugar moiety.

The N^6-benzyladenosine derivatives **2a-d** were prepared easily by the reaction of **1** with appropriate benzylamines according to the procedure previously reported, [7] *e.g.*, heating of a mixture of inosine (**1a**) and three equimolar amounts of (*p*-methoxybenzyl)-amine in hexamethyldisilazane containing a catalytic amount of ammonium sulfate at 140 °C under argon resulted in the formation of N^6-(*p*-methoxybenzyl)adenosine (**2a**) in 89% yield.

In order to remove the benzyl group from **2a-d**, some single-electron oxidants such as ferric perchlorate, ceric ammonium nitrate, and (NH4)2S2O8 were examined under various conditions. Among them, employment of (NH4)2S2O8 as an oxidant in a neutral buffer

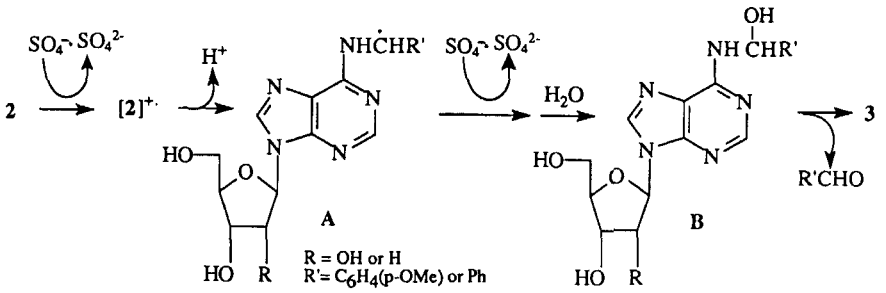
solution resulted in the most smooth debenzylation of **2a-d** to give adenosines (**3**), *i.e.*, when **2a** was treated with two equimolar amounts of $(NH_4)_2S_2O_8$ in pH 7.2 1 M phosphate buffer at 80 °C for 2 h, adenosine (**3a**) was obtained almost quantitatively. Analogous results were obtained in the cases of **2b-d** to give the corresponding adenosines **3a,b**. Some efforts to search for a metal-ion catalyst for carrying out the $(NH_4)_2S_2O_8$-oxidation of **2a** under milder conditions and other oxidants (*e.g.*, lead tetraacetate, iodosylbenzene diacetate, and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone) were unsuccessful. Peroxydisulfate ion ($S_2O_8^{2-}$) generates double the molar quantity of sulfate anion radical ($SO_4^{-\cdot}$), a very strong single-electron oxidant, under the conditions employed. [8] Thus, the possible reaction sequence for the debenzylation is outlined as shown in Scheme 2.

The oxidation of **2** by the generated $SO_4^{-\cdot}$ gives a cation radical of **2**, ($[2]^{+\cdot}$), which releases a proton from its benzyl methylene to give a benzyl radical (**A**). Further single-electron oxidation of **A** by another $SO_4^{-\cdot}$ and subsequent trapping of the resulting cation by water provide an unisolable aminal intermediate (**B**). Elimination of benzaldehydes from **B** results in the formation of **3** as an ultimate product.

Two possible pathways are considered in the oxidative debenzylation of **2**, *i.e.*, one is *via* the single-electron oxidation of the adenine ring and another is *via* that of the benzene ring because the oxidation potential of the adenine ring is very close to that of the benzyl moiety [E^{ox}_p= 1.54 V *vs* SCE for **2a** and **2b**; 1.65 for 2',3',5'-*O*-triacetyladenosine; 1.46 for *p*-methoxybenzylamine; 1.7 for benzylamine in MeCN]. To distinguish between two pathways, further experiments were carried out by employment of N^6-monomethyladenosine (**2e**) and N^6-(*p*-methoxyphenethyl)adenosine (**2f**) as substrates. When **2e** and **2f** were treated with $(NH_4)_2S_2O_8$ under conditions similar to the case of **2a**, the formation of **3a** was observed, respectively, though their efficiency were lower than the case of **2a**. In these reactions, other products were not detected. These facts indicate that the single-electron oxidation of **2a-d** appears to occur preferentially in the adenine ring rather than the benzene ring.

The present conversion of **1** to **3** was applied to the synthesis of [6-^{15}N]-labeled adenosine. Treatment of **1a** with ^{15}N-enriched benzylamine under the thermal conditions analogous to those employed for the preparation of **2b** followed by chromatographic purification allowed isolation of [6-^{15}N]-labeled N^6-benzyladenosine which was converted readily into [6-^{15}N]-labeled adenosine [4] by the $(NH_4)_2S_2O_8$-oxidation.

This synthetic methodology is in principle applicable to the preparation of [6-^{15}N]-labeled 2'-deoxyadenosine [2b,3,5] from **1b** and of [4-^{15}N]-labeled cytidines [2b,9] from uridines.



Scheme 2

Experimental:

Melting points were determined on a Yanagimoto micro hot-stage apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were determined at 270 MHz with a JEOL JNX GX-270 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to the standard chemical shift of the solvent (DMSO-d_6). Ultraviolet (UV) spectra were recorded [λ_{max} nm (ε x 10^{-3})] on a Shimadzu-260 spectrophotometer and infrared spectra (IR) with a Perkin Elmer 1650 FT-IR spectrometer. Elemental analyses were carried out in the Microanalytical Center of our university. Thin-layer chromatographic (TLC) analyses were performed on Silica gel 60 F-254 plates (Merck Art. 5715, 0.25 mm thick) and TLC-scanning was carried out with a Shimadzu CS-9000 dual-wavelength flying-spot scanner (detector: 260 nm). Mass spectral data were obtained on a JEOL JMS-D 300 machine operating at 70 eV. Rotary evaporation was carried out under reduced pressure with the bath temperature below 35 °C unless otherwise specified. Column chromatographic separation was accomplished on silica gel (Wakogel *C*-300). Polarographic analyses were carried out at ambient temperature under argon with a Yanaco Polarographic Analyzer P-1100 using tetra-*n*-butylammonium perchlorate as a supporting electrolyte and dry MeCN as a solvent.

N^6-Monomethyladenosine (**2e**) was prepared by using the Dimroth rearrangement of N_1-methyladenosine according to the procedure reported. [10] ^{15}N-Labeled benzylamine was prepared by the $LiAlH_4$-reduction of ^{15}N-enriched benzamide (99 atom % ^{15}N, Isotec Inc.). [11]

N^6-Benzyladenosines (2a-d): According to the Vorbruggen's procedure, [7] **2a-d** were prepared from inosines (**1a,b**). As a typical example, a mixture of inosine (**1a**) (268

mg, 1 mmole) and (*p*-methoxybenzyl)amine (0.39 ml, 3 mmole) in hexamethyldisilazane (0.84 ml, 4 mmole) containing ammonium sulfate (13 mg, 0.1 mmol) was heated at 140 °C under argon for 36 h. After treatment of the reaction mixture with MeOH (20 ml) containing a small amount of aqueous tetrabutylammonium fluoride at room temperature for 30 min. The solution was evaporated, adsorbed onto silica gel (5 g), and chromatographed on a column (3 x 30 cm) using silica gel (15 g, 300 mesh). Elution of the column with $CHCl_3$-MeOH (50 : 1, v/v) gave N^6-(*p*-methoxybenzyl)adenosine (**2a**), after evaporation of the solvent and recrystallized from MeOH.

The structures of the products **2a-d** were confirmed by microanalytical and spectral data described below.

Compound **2a**: 89%; m.p. 143-144 °C (MeOH)(lit [12]: m.p. 146-147 °C, without description of the spectral data); UV (MeOH): 270 (22.5); Mass (*m/z*): 387 (M^+, 22%), 255 (94), 136 (28), and 121 (100); IR (KBr): 3326 and 1633 cm^{-1}; ^1H NMR: 3.6-3.9 (2H, m, 5'-2H), 3.78 (3H, s, OMe), 4.04 (1H, br s, 4'-H), 4.22 (1H, br s, 3'-H), 4.6-4.8 (3H, m, 2'-H and NHCH_2), 5.27 (1H, br d, *J*= 4.9 Hz, OH), 5.47 (1H, br d, *J*= 4.4 Hz, OH), 5.53 (1H, br d, *J*= 6.3 Hz, OH), 5.98 (1H, d, *J*= 6.3 Hz, 1'-H), 6.93 and 7.35 (each 2H, each d, *J*= 8.3 Hz, Ph-H), 8.28 (1H, s, 2-H), 8.4 (1H, br, NH), and 8.44 (1H, s, 8-H).

Anal. Calcd. for $C_{18}H_{21}N_5O_5$: C, 55.80; H, 5.46; N, 18.08. Found: C, 55.58; H, 5.49; N, 17.99.

N^6-Benzyladenosine (**2b**): 95%; m.p. 185 °C (MeOH) (lit.: m.p. 184-185 °C [7]; 186-187 °C [13]).

N^6-(*p*-Methoxybenzyl)-2'-deoxyadenosine (**2c**): 84%; m.p. 162 °C (hexane-EtOH); UV (MeOH): 271 (20.7); Mass (*m/z*): 371 (M^+, 11%), 255 (73), 136 (38), and 121 (100); IR (KBr): 3336 and 1623 cm^{-1}; ^1H NMR: 2.3 and 2.8 (each 1H, m, 2'-2H), 3.59-3.74 (2H, m, 5'-2H), 3.77 (3H, s, OMe), 3.96 (1H, d, *J*= 2.4 Hz, 4'-H), 4.48 (1H, d, *J*= 1.5 Hz, 3'-H), 4.7 (2H, br s, NHCH_2), 5.3 and 5.4 (each 1H, each br s, 2 OH), 6.43 (1H, br t, *J*= 7 Hz, 1'-H), 6.93 and 7.34 (each 2H, each d, *J*= 8.3 Hz, Ph-H), 8.27 (1H, s, 2-H), 8.4 (1H, br, NH), and 8.43 (1H, s, 8-H).

Anal. Calcd. for $C_{18}H_{21}N_5O_4$: C, 58.21: H, 5.70; N, 18.86. Found: C, 58.00; H, 5.66; N, 18.65.

N^6-Benzyl-2'-deoxyadenosine (**2d**): 92%; m.p. 175-176 °C (hexane-EtOH) (lit. [13] m.p. 175.5-176.5 °C).

In a similar manner, [6-^{15}N]-labeled **2b** was prepared by employment of the ^{15}N-enriched benzylamine in place of benzylamine. The structure of the labeled product was supported by its microanalytical and spectral data, *e.g.*, the presence of one atom of ^{15}N in this compound was seen in the molecular ion (*m/z* : 358) for the [6-^{15}N]-labeled **2b**. The

^{15}N-content of this compound was estimated to be 99% by mass spectroscopy. In the ^{1}H NMR spectrum, the amino proton of this compound appeared at δ 8.44 as doublet signals with the expected large ^{15}N-^{1}H coupling of 91.3 Hz.

N^6-(*p*-Methoxyphenethyl)adenosine (2f): According to the procedure described above, **2f** was prepared by the thermal reaction of **1a** with (*p*-methoxyphenethyl)amine followed by column chromatographic purification, m.p. 199 °C (EtOH) (lit. [14] m.p. 195-196 °C, without description of the spectral data); UV (MeOH): 268 (20.0); Mass (*m/z*): 401 (M^+, 11%), 267 (36), 148 (100), and 135 (82); IR (KBr): 3384 and 1621 cm^{-1}; ^{1}H NMR: 2.82 (2H, m, CH_2CH_2Ph), 3.4-3.8 (4H, m, $NHCH_2CH_2$ and 5'-2H), 3.70 (3H, s, OMe), 3.95 (1H, d, *J*= 3.4 Hz, 4'-H), 4.13 (1H, m, 3'-H), 4.60 (1H, dd, *J*= 5.8 and 11.2 Hz, 2'-H), 5.17 (1H, br d, *J*= 4.4 Hz, OH), 5.43 (2H, br, 2 OH), 5.87 (1H, d, *J*= 6.3 Hz, 1'-H), 6.83 and 7.15 (each 2H, each d, *J*= 8 Hz, PhH), 7.89 (1H, br, NH), 8.22 (1H, s, 2-H), and 8.34 (1H, s, 8-H).

Anal. Calcd. for $C_{19}H_{23}N_5O_5$: C, 56.85; H, 5.78; N, 17.45. Found: C, 56.81; H, 5.91; N, 17.16.

Debenzylation of 2a-d: A solution of **2a** (3.9 mg, 0.01 mmole) in pH 7.2 1 M phosphate buffer (1.0 ml)-MeCN (0.5 ml) was stirred at 80 °C in the presence of $(NH_4)_2S_2O_8$ (4.6 mg, 0.02 mmole), $(NH_4)_2Ce(NO_3)_6$ (11.0 mg, 0.02 mmole), or ferric perchlorate (9.3 mg, 0.02 mmole). TLC-densitometric analyses of the mixtures after stirring for 1.5 h showed the formation of adenosine (**3a**) and the recovery of **2a** unchanged. The yields of **3a** in these reactions were as follows: 93% for $(NH_4)_2S_2O_8$; 1.2% for $(NH_4)_2Ce(NO_3)_6$; 0% (not detected) for ferric perchlorate.

When the $(NH_4)_2S_2O_8$-oxidation of **2a** (0.1 mmole) was carried out at 37 °C in the presence of a metal ionic catalyst (CuCl, AgCl, $FeSO_4$, or $MnCl_2$; 0.02 mmole) for 20 h, the formation of **3a** was observed. The yields of **3a** in these reactions were as follows: 27% for CuCl; 26% for AgCl; 10% for $FeSO_4$; 6% for $MnCl_2$ (by TLC-densitometry).

A mixture of **2a** (38.7 mg, 0.1 mmole) and $(NH_4)_2S_2O_8$ (45.6 mg, 0.2 mmole) was heated in pH 7.2 1 M phosphate buffer (1.0 ml)-MeCN (0.5 ml) at 80 °C for 2 h. TLC analysis of the mixture showed the completion of the reaction and the presence of a sole product. After removal of the solvent under reduced pressure, the resulting residue was subjected to column chromatography [$CHCl_3$-MeOH (20 : 1, v/v)] to isolate adenosine (**3a**) (95%) which was identical in every respect with an authentic sample.

Under analogous conditions, the oxidation of **2b-d** with $(NH_4)_2S_2O_8$ was carried out to give **3a** or **3b**. The yields of **3a,b** were as follows: 89% (**3a** from **2b**); 95% (**3b** from **2c**); 88% (**3b** from **2d**).

An analogous result was obtained in the debenzylation of the [6-^{15}N]-labeled **2b** to give the desired [6-^{15}N]-labeled **3a**, [4] of which amino protons appeared at δ 7.31 as doublet signals with a coupling of 89.9 Hz in the ^{1}H NMR spectrum.

Comparative Experiments in the $(NH_4)_2S_2O_8$ Oxidation of 2a, 2e, and 2f: A solution of **2e** or **2f** (0.01 mmole) in pH 7.2 1 M phosphate buffer (1.0 ml)-MeCN (0.5 ml) containing $(NH_4)_2S_2O_8$ (2.3 mg, 0.01 mmole) was heated at 80 °C for 1 h. TLC analyses of the reaction mixtures showed the formation of **3a** in 1% (from **2e**) and 11% (from **2f**) yields, respectively, and no formation of detectable amounts of other products. Under the analogous conditions, **2a** was converted into **3a** in 25% yield. The structure of **3a** was confirmed by spectral comparison with the authentic sample after chromatographic separation.

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