

Purines. LXI.¹⁾ An Attempted Synthesis of 2'-Deoxy-7-methyladenosine: Glycosidic Hydrolyses of the *N*⁶-Methoxy Derivative and 2'-Deoxy-*N*^x-methyladenosines

Tozo FUJII,* Tohru SAITO, and Kimiko IGUCHI

Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan.

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In an attempt to synthesize 2'-deoxy-7-methyladenosine (**5b**), 2'-deoxy-*N*⁶-methoxyadenosine (**13b**) was treated with MeI in AcNMe₂ at 0°C for 7 h to give the 2'-deoxy-*N*⁶-methoxy-7-methyladenosine salt (**14b**), which was unstable and easily underwent glycosidic hydrolysis in H₂O at 16–18°C to form *N*⁶-methoxy-7-methyladenine (**15**). On account of such instability, hydrogenolysis of **14b** in H₂O using hydrogen and Raney Ni catalyst failed to afford the desired nucleoside (**5b**). 2'-Deoxy-*N*⁶-methyladenosine (**2b**), 2'-deoxy-1-methyladenosine (**3b**), and **14b** were found to undergo glycosidic hydrolysis in 0.1N aqueous HCl at 25°C at rates of $7.92 \times 10^{-3} \text{ min}^{-1}$ (half-life 87.5 min), $5.02 \times 10^{-3} \text{ min}^{-1}$ (half-life 138 min), and $2.31 \times 10^{-2} \text{ min}^{-1}$ (half-life 30.0 min), respectively, while the rate in the case of **5b** was roughly estimated to be *ca.* 2 min^{-1} (half-life 0.35 min).

Keywords 2'-deoxyadenosine *N*-methyl isomer; glycosidic hydrolysis; kinetic study; nucleoside *N*-methoxy; nucleoside regioselective *N*-methylation

For the adenosine nucleosides adenosine (**1a**) and 2'-deoxyadenosine (**1b**), four types of *N*^x-monomethyl substitution are possible in principle. In the adenosine series, *N*⁶-methyladenosine (**2a**),²⁾ 1-methyladenosine (**3a**),²⁾ 3-methyladenosine (**4a**),³⁾ and 7-methyladenosine (**5a**)⁴⁾ have all been synthesized in the form of free base and/or salt, and kinetic data on their glycosidic hydrolyses (depurinations) under acidic conditions (Chart 1) have been obtained.^{3–5)} In the 2'-deoxyadenosine series, however, only three 2'-deoxy-*N*^x-methyladenosines, *i.e.*, 2'-deoxy-*N*⁶-methyladenosine (**2b**),²⁾ 2'-deoxy-1-methyladenosine (**3b**),²⁾ and 2'-deoxy-3-methyladenosine (**4b**),³⁾ have been obtained in the form of free base and/or salt. The remaining positional isomer, 2'-deoxy-7-methyladenosine (**5b**), has been assumed to occur, although to a slight extent, as a very unstable partial structure in methylated

DNA molecules,⁶⁾ but this *N*(7)-methylated nucleoside itself still remains to be synthesized and characterized. This paper describes the results of our efforts directed toward the synthesis of **5b**. A comparative study of the depurinations of isomeric 2'-deoxy-*N*^x-methyladenosines is also presented.

In designing a synthetic route to the target nucleoside **5b**, our previous synthesis of 7-methyladenosine perchlorate [**5a** (X = ClO₄)]⁴⁾ from adenosine 1-oxide through *N*⁶-methoxyadenosine was taken as a guide, generating a 2'-deoxy version as shown in Chart 2. Thus, oxidation of 2'-deoxyadenosine (**1b**) with *m*-chloroperoxybenzoic acid (MCPBA) in MeOH at room temperature for 5 h gave the 1-oxide **11b** in 90% yield. This *N*-oxide had previously been obtained from **1b** by oxidation with monoperoxyphthalic acid in H₂O (pH 5, room temp., 2 h)⁷⁾ or

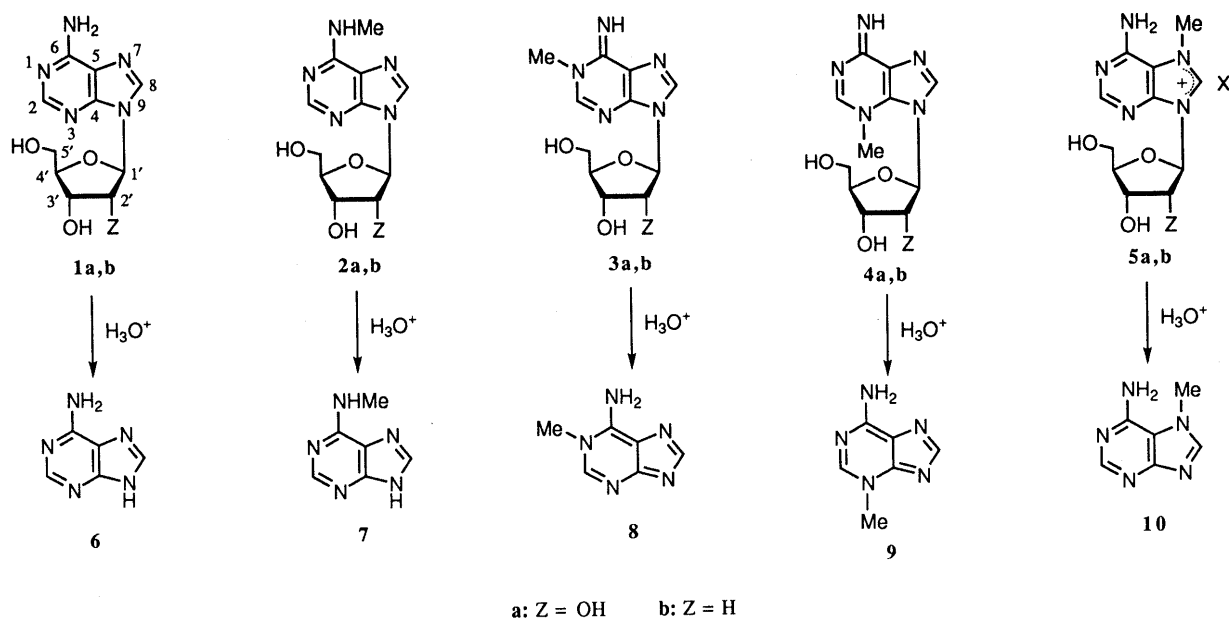


Chart 1

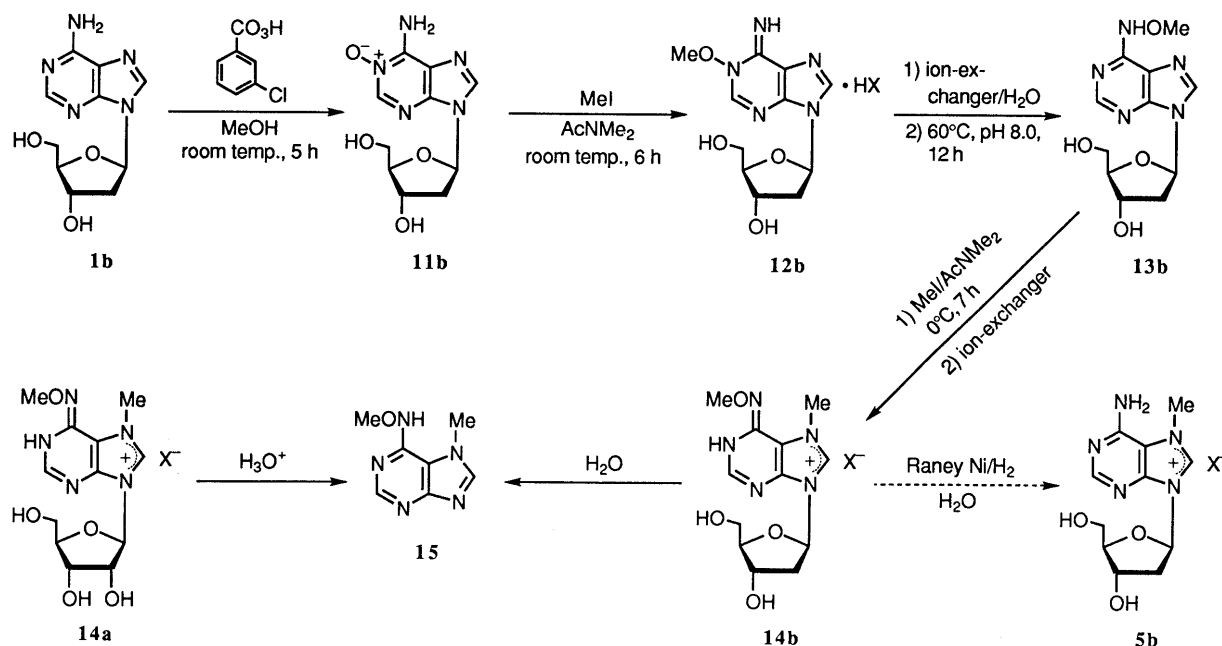


Chart 2

with MCPBA in 30–60% aqueous dioxane (room temp., 3 h)⁸⁾ in 64–70% or 74–75% yield, respectively.

Conversion of **11b** into 2'-deoxy-*N*⁶-methoxyadenosine (**13b**) was effected according to the procedure of Saneyoshi *et al.*,^{8b)} but with some modification. Methylation of **11b** with MeI in AcNMe₂ at room temperature for 6 h yielded 2'-deoxy-1-methoxyadenosine hydriodide [**12b** (X=I)], which was then treated with Amberlite IRA-402 (HCO₃⁻) in H₂O. The resulting aqueous solution of the free nucleoside was heated at pH 8.0 and 60°C for 12 h, affording 2'-deoxy-*N*⁶-methoxyadenosine (**13b**)^{8b)} in 60% yield. In the adenosine series, methylation of *N*⁶-methoxyadenosine with MeI in AcNMe₂ at 30°C for 8 h was reported to provide the 7-methylated product in 55% yield, together with the *N*⁶-methyl isomer as a by-product.⁴⁾ Application of similar methylation conditions to the *N*⁶-benzyloxy analogue^{8b)} of **13b**, but at room temperature, resulted in the formation of a complicated mixture of many products. This is most likely due to the extreme instability of the glycosidic bond in the 2'-deoxyadenosine series, suggesting that the methylation of **13b** and work-up of the reaction mixture should be carried out at low temperature.

Thus, methylation of **13b** with a large excess of MeI in AcNMe₂ at 0°C for 7 h, work-up of the reaction mixture (including column chromatography [Amberlite CG-400 (HSO₄⁻ and/or SO₄²⁻), H₂O] of methylated products) in a cold chamber kept at 4–7°C, and freeze-drying of the aqueous eluates were carefully conducted, and we were able to isolate 2'-deoxy-*N*⁶-methoxy-7-methyladenosine sulfate [**14b** (X=HSO₄ or 1/2SO₄)] as a yellowish solid, mp 65–98°C (dec.). The solid was so unstable that purification by recrystallization was difficult, and therefore it had to be stored in a freezer for further investigation. The UV [in 95% aqueous EtOH or in H₂O (pH 1, 7, or 13)] and ¹H-NMR (in Me₂SO-*d*₆) spectra of **14b** (X=HSO₄ or 1/2SO₄) at 25°C were useless for char-

acterization because they changed so rapidly. However, the ¹H-NMR spectrum taken in CD₃OD–D₂O (2:1, v/v) at –10°C was sufficiently stable, showing a great similarity to that (obtained under similar conditions) of the known ribosyl analogue **14a** (X=1/2SO₄)⁴⁾ except for the signals arising from the sugar moiety. This finding, together with the observation that treatment of **14b** (X=HSO₄ or 1/2SO₄) with H₂O at 16–18°C produced *N*⁶-methoxy-7-methyladenine (**15**),⁴⁾ supported the correctness of the structure of the methylated product [**14b** (X=HSO₄ or 1/2SO₄)].⁹⁾

In an attempt to obtain the target nucleoside (**5b**), **14b** (X=HSO₄ or 1/2SO₄) was hydrogenated in H₂O over Raney Ni catalyst at atmospheric pressure and room temperature, as in the case⁴⁾ of conversion of **14a** (X=1/2SO₄) into **5a**. However, the reaction mixture was found to contain at least both the aglycones **15** and **10**, and we were unable to secure the desired, demethoxylated nucleoside itself. Thus, the glycosidic bond of **5b** appeared to be so labile that this approach to **5b** had to be abandoned.

In view of the unavailability of the ultimate nucleoside **5b**, we tried to estimate its depurinylation rate from those of the penultimate nucleoside **14b** and related nucleosides. We first monitored the depurinylation of **14b** (X=HSO₄ or 1/2SO₄) to give *N*⁶-methoxy-7-methyladenine (**15**) in H₂O (Chart 2) at various pH's and 25°C, determining the unaltered nucleoside or the newly formed aglycone by means of UV spectrophotometry or HPLC analysis. In all cases, the depurinylation was found to obey pseudo-first-order kinetics, as shown in Table I. The kinetic study was then extended to include the depurinations **14a** (X=1/2SO₄)⁴⁾→**15**, 2'-deoxy-*N*⁶-methyladenosine (**2b**)²⁾→*N*⁶-methyladenine (**7**),²⁾ and 2'-deoxy-1-methyladenosine (**3b**)²⁾→1-methyladenine (**8**)²⁾ in 0.1N aqueous HCl (pH ca. 1) at various temperatures. The results are assembled in Tables I and II, together with those reported previously

TABLE I. Rate Constants for the Glycosidic Hydrolyses of *N*⁶-Methoxy-7-methyladenine Nucleosides

| Substrate | Reaction conditions | | Pseudo-first-order rate constant (min ⁻¹) | |
|---|---------------------|------------|---|---------------------------|
| | pH | Temp. (°C) | UV spectrometry | HPLC analysis |
| 2'-Deoxy- <i>N</i> ⁶ -methoxy-7-methyladenosine [14b (X = HSO ₄ or 1/2SO ₄)] | ca. 1 ^{a)} | 25.0 | 2.74 × 10 ⁻² | 2.31 × 10 ⁻² |
| | 3.00 | 25.0 | 4.61 × 10 ⁻³ | — ^{b)} |
| | 6.00 | 25.0 | 4.34 × 10 ⁻³ | — ^{b)} |
| | 7.00 | 25.0 | 4.41 × 10 ⁻³ | 4.23 × 10 ⁻³ |
| <i>N</i> ⁶ -Methoxy-7-methyladenosine [14a (X = 1/2SO ₄)] | ca. 1 ^{a)} | 70.0 | — ^{b)} | 1.29 × 10 ⁻² |
| | ca. 1 ^{a)} | 60.0 | — ^{b)} | 3.99 × 10 ⁻³ |
| | ca. 1 ^{a)} | 40.0 | — ^{b)} | 2.48 × 10 ⁻⁴ |
| | ca. 1 ^{a)} | 25.0 | — ^{b)} | 2.56 × 10 ^{-5c)} |

a) In 0.1 N aqueous HCl. b) Not determined. c) Estimated on the basis of the data at 40.0–70.0 °C and the Arrhenius equation for reaction rate.

TABLE II. Rate Constants (*k*) for the Glycosidic Hydrolyses of 2'-Deoxy-*N*^x-methyladenosines, *N*^x-Methyladenosines, and Related Nucleosides in 0.1 N Aqueous HCl at 25.0 °C

| Depurinylation | Pseudo-first-order rate constant (<i>k</i> × 10 ⁵ , min ⁻¹) | Half-life (min) |
|--|---|-----------------|
| 2'-Deoxyadenosine (1b)→ 6 | 1690 ^{a)} | 41.0 |
| 2'-Deoxy- <i>N</i> ⁶ -methyladenosine (2b)→ 7 | 792 | 87.5 |
| 2'-Deoxy-1-methyladenosine (3b)→ 8 | 502 | 138 |
| 2'-Deoxy-3-methyladenosine (4b)→ 9 | 25400 (pH 3.34) ^{b)} | 2.73 |
| 2'-Deoxy-7-methyladenosine (5b)→ 10 | 200000 ^{c)} | 0.35 |
| 2'-Deoxy- <i>N</i> ⁶ -methoxy-7-methyladenosine (14b)→ 15 | 2310 ^{d)} | 30.0 |
| <i>N</i> ⁶ -Methyladenosine (2a)→ 7 | 0.82 ^{e)} | 84500 |
| 1-Methyladenosine (3a)→ 8 | 0.56 ^{e)} | 124000 |
| 3-Methyladenosine (4a)→ 9 | 4000 ^{b)} | 17.3 |
| | 68.7 (pH 3.34) ^{b)} | 1010 |
| 7-Methyladenosine (5a)→ 10 | 222 ^{e)} | 312 |
| <i>N</i> ⁶ -Methoxy-7-methyladenosine (14a)→ 15 | 2.56 ^{d)} | 27100 |

a) Taken from ref. 5. b) Taken from ref. 3. c) Estimated value (see the text). d) Taken from Table I. e) Taken from ref. 4.

for related nucleosides.

Comparison of the rate constants in Table I reveals that the 2-deoxyribose analogue **14b** (X = HSO₄ or 1/2SO₄) hydrolyzes in 0.1 N aqueous HCl at 25 °C *ca.* 900 times more rapidly than does the ribosyl analogue **14a** (X = 1/2SO₄), being in general agreement with the fact that 2'-deoxyadenosine (**1b**) itself undergoes depurinylation [to form adenine (**6**)] about 1000 times faster than does adenosine (**1a**) itself.^{5,10} As in the cases¹¹ of 7-methylguanosine, 2'-deoxy-7-methylguanosine, and 2'-deoxy-1,7-dimethylguanosine, the depurinylation rate of **14b** (X = HSO₄ or 1/2SO₄) is insensitive to pH in the pH range of 3–7, but increases with increasing hydronium ion concentration at low pH. Assuming that an A-1 mechanism^{5,10–12} for solvolyses of nucleosides is operating in this depurinylation, a mechanism similar to that⁴ proposed for the depurinylation of **5a** may be considered. The accelerated depurinylation of **14b** (X = HSO₄ or 1/2SO₄) at low pH suggests the importance of the protonated form (dication) [**14b**·H⁺ (X = HSO₄ or

1/2SO₄)]¹³ in transition structures (Chart 3).

It may be seen from Table II that in 0.1 N aqueous HCl at 25 °C 7-methyladenosine (**5a**) undergoes depurinylation *ca.* 87 times faster than does the *N*⁶-methoxy derivative **14a**. The retardation of depurinylation caused by introduction of a methoxy group at the exocyclic nitrogen atom may be explained in terms of alteration of the tautomeric form⁹ and the electron-withdrawing effect of the *N*⁶-methoxy group, which should reduce the basicity of the pyrimidine moiety and hence the population of the dication (type **14b**·H⁺). This view may be supported by comparison of the p*K*_a values reported for 9-methyladenine (**16**) [p*K*_a 3.88 ± 0.01 in H₂O at 25 °C],¹⁴ *N*⁶-methoxy-9-methyladenine (**17**) [p*K*_a 3.06 ± 0.05 and 11.26 ± 0.07 (in H₂O at 20 °C)],¹⁵ and *N*⁶,9-dimethyladenine (**18**) [p*K*_a 4.02 ± 0.03 (in H₂O at 20 °C)].¹⁶ Assuming that a similar relation holds for the rate constants for the depurinations of the 2-deoxyribose analogues **5b** and **14b** in 0.1 N aqueous HCl at 25 °C, the rate for **5b** was estimated at *ca.* 2 min⁻¹ (half-life 0.35 min) using the data [*k* = 2.31 × 10⁻² min⁻¹ (half-life 30.0 min)] obtained with **14b** (X = HSO₄ or 1/2SO₄).

It may also be seen from Table II that 2'-deoxy-*N*^x-methyladenosines undergo depurinylation 370–970 times as fast as the corresponding ribosyl analogues. The observed rate acceleration of the 2-deoxyribose analogues is attributable to the absence of the C(2')-OH group, which, in a parallel A-1 pathway (as in Chart 3), inhibits by its inductive effect the slow N(9)–C(1') bond cleavage of a protonated or positively charged nucleoside that results in a C(1') carbocation.^{5,10,11} Although a direct comparison of the depurinylation rates for the 3-methyl and 7-methyl derivatives in the 2'-deoxyadenosine series cannot be made owing to the lack of pertinent data, the relative ease of depurinylation is in the order of 3-methyl- (**4b**), 7-methyl- (**5b**) ≫ unsubstituted (**1b**) > *N*⁶-methyl- (**2b**) ≥ 1-methyl-2'-deoxyadenosine (**3b**). The extraordinary instability of the glycosidic bond of the 3-methyl derivative has been explained in our previous paper,³ and that of the 7-methyl derivative may be explained in a manner similar to that⁴ applied to the ribosyl analogue **5a**. It has been reported that methylated DNA releases 7-methyladenine (**10**) *in vivo* or *in vitro* relatively fast at pH 7.2 and 37 °C (half-life 2.6 ± 0.2 h or 2.8 ± 0.3 h, respectively).^{6d} However, the lack of pertinent data at present makes it difficult to compare the de-

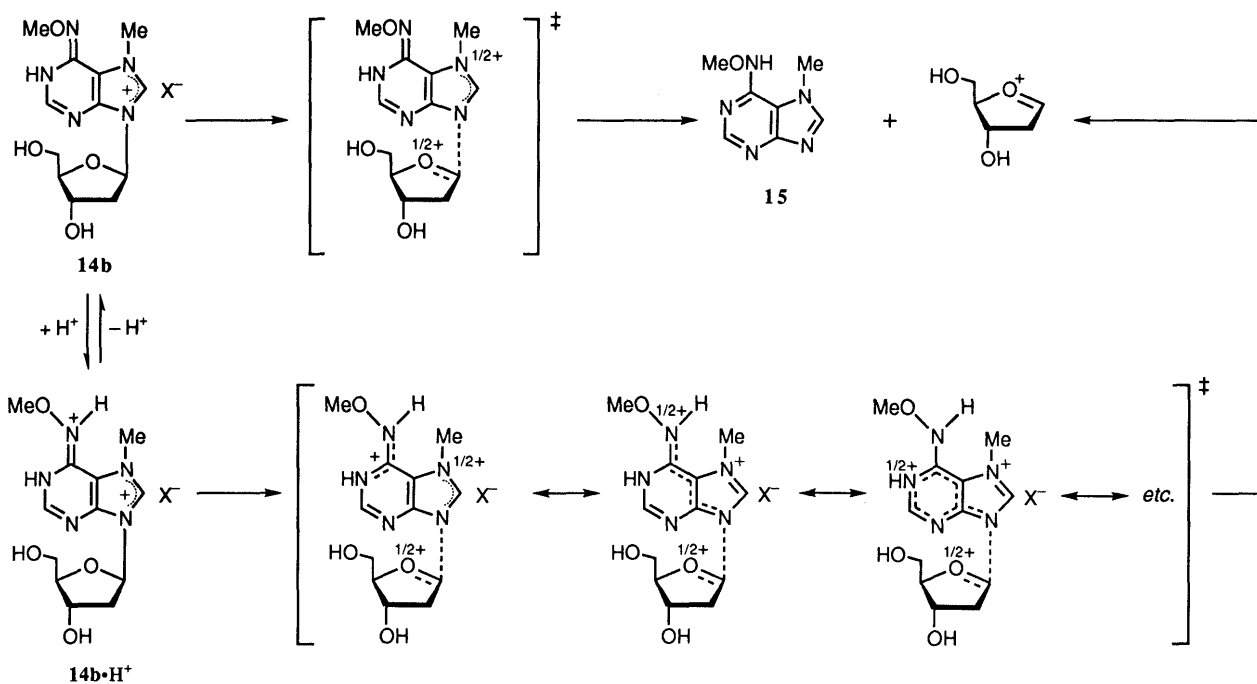
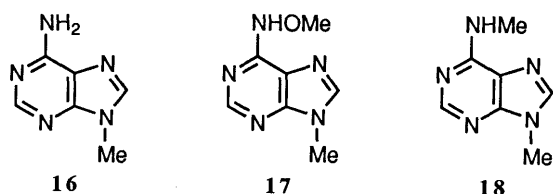


Chart 3



purinylation rates for the 2'-deoxy-7-methyladenosine structures at the nucleoside and the DNA levels.

In conclusion, an attempt to synthesize 2'-deoxy-7-methyladenosine (**5b**), via a route parallel to that employed for the synthesis of the ribosyl analogue **5a**, has been unsuccessful on account of the extraordinary instability of the target nucleoside. However, the easiness of depurinylation of **5b** in 0.1 N aqueous HCl at 25 °C has been estimated from the kinetic data on depurinylation of the *N*⁶-methoxy derivative **14b** (X = HSO₄ or 1/2SO₄) and the corresponding ribosyl analogues. It is hoped that these findings will improve our understanding of the nature of the 2'-deoxy-7-methyladenosine structure at the polynucleotide level in chemical and biochemical studies of methylated DNA's.

Experimental

General Notes All melting points were taken on a Yamato MP-1 capillary melting point apparatus and are corrected. See ref. 4 for details of instrumentation and measurements. The solvents used for measurements of UV spectra were 95% (v/v) aqueous EtOH, 0.1 N aqueous HCl (pH 1), 0.005 M phosphate buffer (pH 7), and 0.1 N aqueous NaOH (pH 13). Elemental analyses and MS measurements were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br=broad, d=doublet, m= multiplet, s=singlet, t=triplet.

2'-Deoxyadenosine 1-Oxide (11b) A solution of 2'-deoxyadenosine (**1b**) (20.0 g, 79.6 mmol) and *m*-chloroperoxybenzoic acid (of 80% purity) (35.0 g, 162 mmol) in MeOH (2000 ml) was stirred at room temperature for 5 h. The colorless precipitate that resulted was collected by filtration, washed with a little MeOH, and dried to give a first crop (17.10 g) of

11b, mp ca. 235 °C (dec.) [lit.^{8b}) mp 228–229 °C (dec.)]. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **11b**.^{7a)} The filtrate and washings, obtained when the first crop of **11b** was isolated, were combined and concentrated *in vacuo* to a volume of ca. 1000 ml, and ether (ca. 1000 ml) was added. The resulting mixture was kept at room temperature overnight, and the colorless solid that deposited was collected by filtration to give a second crop (2.13 g) of **11b**, mp ca. 235 °C (dec.). The total yield of **11b** was 19.23 g (90%).

2'-Deoxy-*N*⁶-methoxyadenosine (13b) A mixture of **11b** (5.00 g, 18.7 mmol) and MeI (13.3 g, 93.7 mmol) in AcNMe₂ (50 ml) was stirred at room temperature for 6 h. The resulting solution was mixed with ether (250 ml) to deposit **12b** (X=I) as a colorless solid, which was filtered off, washed with ether (2 × 50 ml), and then dissolved in H₂O (50 ml). The aqueous solution was passed through a column of Amberlite IRA-402 (HCO₃⁻) (30 ml), and the column was eluted with H₂O (250 ml). The aqueous eluates were combined, brought to pH 8.0 by addition of Et₃N, stirred at 60 °C for 12 h, during which time the pH of the solution was maintained at 8.0 by occasional addition of Et₃N, and then kept in a refrigerator overnight. The pale orange needles that deposited were filtered off and recrystallized from H₂O, giving a first crop (3.02 g) of **13b**·2H₂O as colorless prisms, mp 132–135 °C (lit.^{8b}) mp 123–125 °C for **13b**·2/3H₂O); MS *m/z*: 281 (M⁺); UV λ_{max}^{95% aq. EtOH} 267 nm (ε 13300); λ_{max}^{H₂O} (pH 1) 267 (15600); λ_{max}^{H₂O} (pH 7) 268 (14900); λ_{max}^{H₂O} (pH 13) 283 (11800); ¹H-NMR (Me₂SO-*d*₆) δ: 2.12–2.76 [2H, m, C(2')-H₂], 3.55 [2H, m, C(5')-H₂], 3.76 (3H, s, OMe), 3.85 [1H, m, C(4')-H], 4.36 [1H, m, C(3')-H], 5.00 [1H, m, C(5')-OH], 5.30 [1H, d, *J*=4 Hz, C(3')-OH], 6.23 [1H, dull t, *J*=7 Hz, C(1')-H], 7.58 [1H, dull s, C(2)-H], 8.08 [1H, dull s, C(8)-H], 11.22 (1H, br, NH). This sample was hygroscopic, and its elemental analysis, though showing poor reproducibility, suggested that it contained ca. 2 molar eq of H₂O. In addition, the above UV spectral data were very similar to those¹⁷⁾ reported for *N*⁶-methoxyadenosine.

The mother liquor, obtained when the crude product was filtered off, was concentrated *in vacuo*, and the residue was recrystallized from H₂O to afford a second crop (536 mg) of **13b**·2H₂O, mp 130–133 °C. The total yield of **13b**·2H₂O was 3.56 g (60%).

2'-Deoxy-*N*⁶-methoxy-7-methyladenosine Sulfate [14b (X=HSO₄ or 1/2SO₄)] The following procedure was conducted in a cold chamber kept at 4–7 °C. A mixture of **13b**·2H₂O (1.00 g, 3.15 mmol) and MeI (37.8 g, 266 mmol) in AcNMe₂ (29 ml) was stirred at 0 °C in an ice bath for 7 h. The reaction mixture was mixed with ether-hexane (3:1, v/v) (500 ml) to deposit an oil, which was separated from the supernatant by decantation, washed with ether-hexane (3:1, v/v) (2 × 100 ml), and then dissolved in H₂O (ca. 20 ml). The aqueous solution was passed through

a column of Amberlite CG-400 (HSO_4^- and/or SO_4^{2-}) (212 ml), and the column was eluted with H_2O . A 120-ml fraction eluted after the first 75-ml fraction was freeze-dried to leave **14b** ($\text{X}=\text{HSO}_4$ or $1/2\text{SO}_4$) (205 mg) as an unstable, yellowish solid, mp 65–98 °C (dec.); UV, unstable in 95% aqueous EtOH and in H_2O (pH 1, 7, or 13) at 25 °C; $^1\text{H-NMR}$ [$\text{CD}_3\text{OD}-\text{D}_2\text{O}$ (2:1, v/v)] (at –10 °C) δ : 2.67 [2H, t, $J=6$ Hz, C(2)- H_2], 3.92 (3H, s, OMe), 4.11 [3H, s, N(7)-Me], 6.43 [1H, t, $J=6$ Hz, C(1)-H], 7.84 [1H, s, C(2)-H], 9.25 [1H, s, C(8)-H]. This sample was stored at –80 °C in a freezer for further investigation.

Hydrolysis of 14b ($\text{X}=\text{HSO}_4$ or $1/2\text{SO}_4$) in H_2O Leading to N^6 -Methoxy-7-methyladenine (15) A solution of **14b** ($\text{X}=\text{HSO}_4$ or $1/2\text{SO}_4$) (38 mg) in H_2O (1 ml) was kept at 16–18 °C for 46 h. The reaction mixture was applied to a column of Amberlite IRA-402 (HCO_3^-) (1 ml), and the column was eluted with H_2O (15 ml). The eluate was concentrated *in vacuo*, and the residue was dried and then purified by preparative TLC [alumina, CHCl_3 -EtOH (15:1, v/v)] to give crude **15** (13.4 mg) as a colorless solid. Recrystallization of the solid from EtOH furnished a pure sample of **15** as faintly yellowish prisms, mp 232–234 °C (dec.) [lit.⁴⁾ mp 234–235 °C (dec.)]. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **15**.⁴⁾

Kinetic Procedure i) Glycosidic Hydrolysis of **14b** ($\text{X}=\text{HSO}_4$ or $1/2\text{SO}_4$): The glycosidic hydrolysis of **14b** ($\text{X}=\text{HSO}_4$ or $1/2\text{SO}_4$) to form **15**, as shown in Chart 2, in 0.1 N aqueous HCl or in aqueous solution at various pH's and ionic strength 0.5 at 25 °C was followed by UV spectrophotometry or HPLC analysis. Buffer solutions employed for kinetic runs were 0.1 M $\text{HCO}_2\text{H}-\text{HCO}_2\text{Na}$ (pH 3.00 at 25 °C) and 0.1 M $\text{NaH}_2\text{PO}_4-\text{Na}_2\text{HPO}_4$ (pH 6.00 or 7.00 at 25 °C), and were brought to ionic strength 0.5 with KCl.

In the UV spectrophotometric method, the substrate (5.664–6.003 mg) was dissolved in 0.1 N aqueous HCl (30 ml) or in a buffer solution (30 ml), and the resulting solution was kept at 25.0 °C in a thermoregulated constant-temperature bath (accurate to ± 0.05 °C). At intervals, an aliquot (2 ml) was withdrawn and diluted by a factor of 10 with 0.3 M $\text{NaH}_2\text{PO}_4-\text{Na}_2\text{HPO}_4$ (1:1), and the optical density of the solution at 275 nm was determined at room temperature against a blank buffer solution. All glycosidic hydrolyses were followed through at least two half-lives with at least six determinations, and good pseudo-first-order kinetics were obtained in all cases. The results are included in Table I.

In the HPLC method, the substrate (14.79–15.13 mg) was dissolved in 0.1 N aqueous HCl (25 ml) or in the buffer solutions (25 ml each), and the resulting solution was kept at 25.0 °C as in the case of the above UV spectrophotometric method. At intervals, an aliquot (1 ml) was withdrawn and diluted by a factor of 11 with the following HPLC solvent. A small portion (15 μl) of the diluted solution was then analyzed by means of HPLC. The HPLC analysis was carried out on a Waters ALC/GPC 204 liquid chromatograph by using a $\mu\text{Bondapak C}_{18}$ column [$\text{MeOH}-0.05$ M KH_2PO_4 (15:85, v/v), 1.5 ml/min], and the peak height of the product (**15**), located by using a UV absorbance detector operated at 254 nm, was determined. The concentration of **15** in the reaction mixture was then estimated from a calibration curve which had been obtained with solutions of authentic **15**⁴⁾ of known concentration. Other procedures were similar to those in the above UV spectrophotometric method, and the results are included in Table I.

ii) Glycosidic Hydrolysis of **14a** ($\text{X}=1/2\text{SO}_4$): The glycosidic hydrolysis of **14a** ($\text{X}=1/2\text{SO}_4$)⁴⁾ in 0.1 N aqueous HCl at 70.0 °C, 60.0 °C, or 40.0 °C was monitored by HPLC analysis in a manner similar to that described above for **14b** ($\text{X}=\text{HSO}_4$ or $1/2\text{SO}_4$). The results are assembled in Table

I. iii) Glycosidic Hydrolyses of **2b** and **3b** Leading to **7** and **8**, Respectively: The glycosidic hydrolyses of **2b**²⁾ and **3b**·*p*-TsOH· $1/2\text{H}_2\text{O}$ ²⁾ in 0.1 N aqueous HCl at 25.0 °C were monitored by HPLC analysis in a manner similar to that described above for **14b** ($\text{X}=\text{HSO}_4$ or $1/2\text{SO}_4$), except that the solvents employed for HPLC analysis were $\text{MeOH}-0.1$ M KH_2PO_4 (15:85, v/v) (1.7 ml/min) for **2b/7** and $\text{MeOH}-0.025$ M Na_2HPO_4 (30:70, v/v) (1.2 ml/min) for **3b/8**. The results are incorporated into Table II.

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