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

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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 $AcNMe_2$ 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 H_2O at 16—18 °C to form N^6-methoxy-7-methyladenine (15). On account of such instability, hydrogenolysis of 14b in H_2O 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 0.1 N aqueous HCl at 25 °C at rates of 7.92×10^{-3} min^{-1} (half-life 87.5 min), 5.02×10^{-3} min^{-1} (half-life 138 min), and 2.31×10^{-2} 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 adenine 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 (depurinylations) 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 depurinylations 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** ($X = ClO_4$)][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 H_2O (pH 5, room temp., 2 h)[7] or

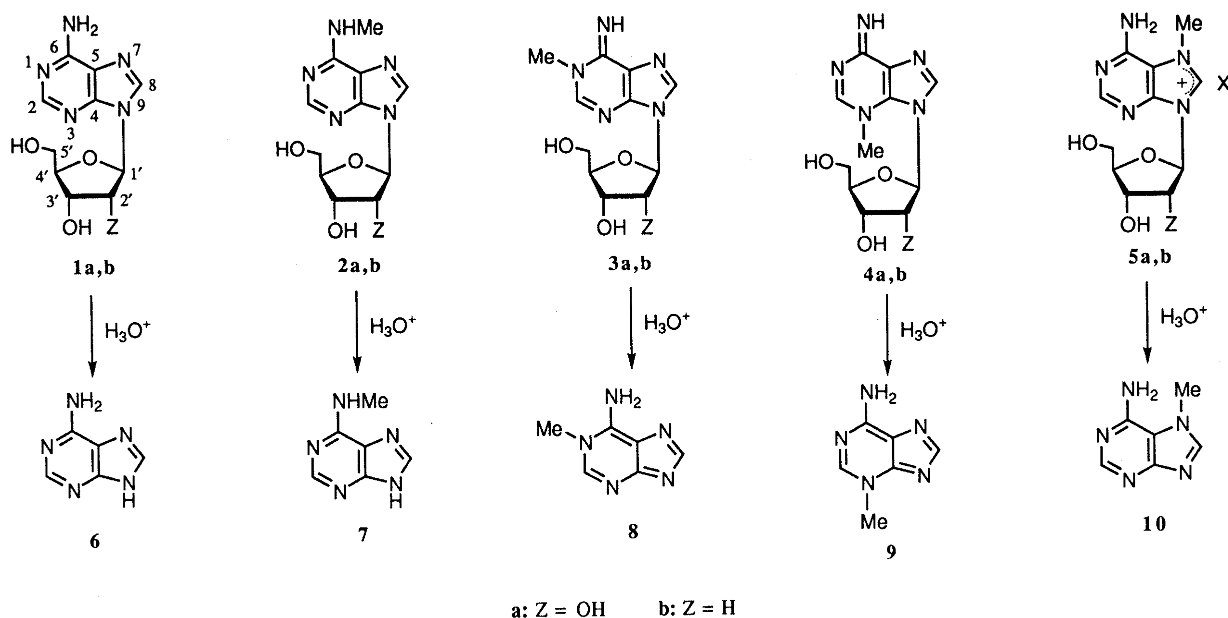

Chart 1

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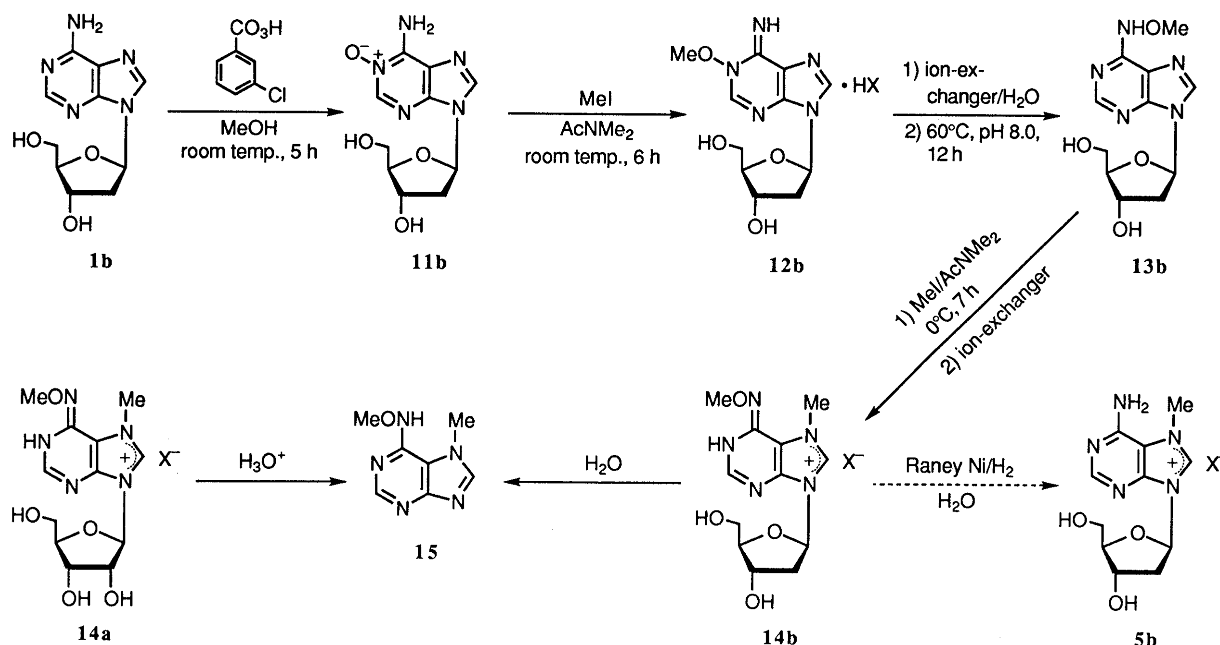

Chart 2

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 $AcNMe_2$ at room temperature for 6 h yielded 2′-deoxy-1-methoxyadenosine hydriodide [**12b** (X = I)], which was then treated with Amberlite IRA-402 (HCO_3^-) in H_2O. 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 $AcNMe_2$ 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 $AcNMe_2$ at 0 °C for 7 h, work-up of the reaction mixture (including column chromatography [Amberlite CG-400 (HSO_4^- and/or SO_4^{2-}), H_2O] 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 = HSO_4 or $1/2SO_4$)] 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 H_2O (pH 1, 7, or 13)] and ^{1}H-NMR (in Me_2SO-d_6) spectra of **14b** (X = HSO_4 or $1/2SO_4$) at 25 °C were useless for characterization because they changed so rapidly. However, the ^{1}H-NMR spectrum taken in CD_3OD–D_2O (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 = $1/2SO_4$)[4] except for the signals arising from the sugar moiety. This finding, together with the observation that treatment of **14b** (X = HSO_4 or $1/2SO_4$) with H_2O at 16—18 °C produced N^6-methoxy-7-methyladenine (**15**),[4] supported the correctness of the structure of the methylated product [**14b** (X = HSO_4 or $1/2SO_4$)].[9]

In an attempt to obtain the target nucleoside (**5b**), **14b** (X = HSO_4 or $1/2SO_4$) was hydrogenated in H_2O over Raney Ni catalyst at atmospheric pressure and room temperature, as in the case[4] of conversion of **14a** (X = $1/2SO_4$) 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 = HSO_4 or $1/2SO_4$) to give N^6-methoxy-7-methyladenine (**15**) in H_2O (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 depurinylations **14a** (X = $1/2SO_4$)[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 0.1 N aqueous HCl (pH *ca.* 1) at various temperatures. The results are assembled in Tables I and II, together with those reported previously

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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-N^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}
N^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^{-5}[c]

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 ($k \times 10^5$, min^{-1})	Half-life (min)
2′-Deoxyadenosine (**1b**)→**6**	1690[a]	41.0
2′-Deoxy-N^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-N^6-methoxy-7-methyladenosine (**14b**)→**15**	2310[d]	30.0
N^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
N^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-deoxyribosyl analogue **14b** (X = HSO_4 or $1/2SO_4$) hydrolyzes in 0.1 N aqueous HCl at 25 °C *ca.* 900 times more rapidly than does the ribosyl analogue **14a** (X = $1/2SO_4$), 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 cases[11] of 7-methylguanosine, 2′-deoxy-7-methylguanosine, and 2′-deoxy-1,7-dimethylguanosine, the depurinylation rate of **14b** (X = HSO_4 or $1/2SO_4$) 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 that[4] proposed for the depurinylation of **5a** may be considered. The accelerated depurinylation of **14b** (X = HSO_4 or $1/2SO_4$) at low pH suggests the importance of the protonated form (dication) [**14b**·H^+ (X = HSO_4 or $1/2SO_4$)][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 form[9] 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 pK_a values reported for 9-methyladenine (**16**) [pK_a 3.88 ± 0.01 in H_2O at 25 °C)],[14] N^6-methoxy-9-methyladenine (**17**) [pK_a 3.06 ± 0.05 and 11.26 ± 0.07 (in H_2O at 20 °C)],[15] and N^6,9-dimethyladenine (**18**) [pK_a 4.02 ± 0.03 (in H_2O at 20 °C)].[16] Assuming that a similar relation holds for the rate constants for the depurinylations of the 2-deoxyribosyl 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 [$k = 2.31 \times 10^{-2}$ min^{-1} (half-life 30.0 min)] obtained with **14b** (X = HSO_4 or $1/2SO_4$).

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-deoxyribosyl 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 that[4] 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-

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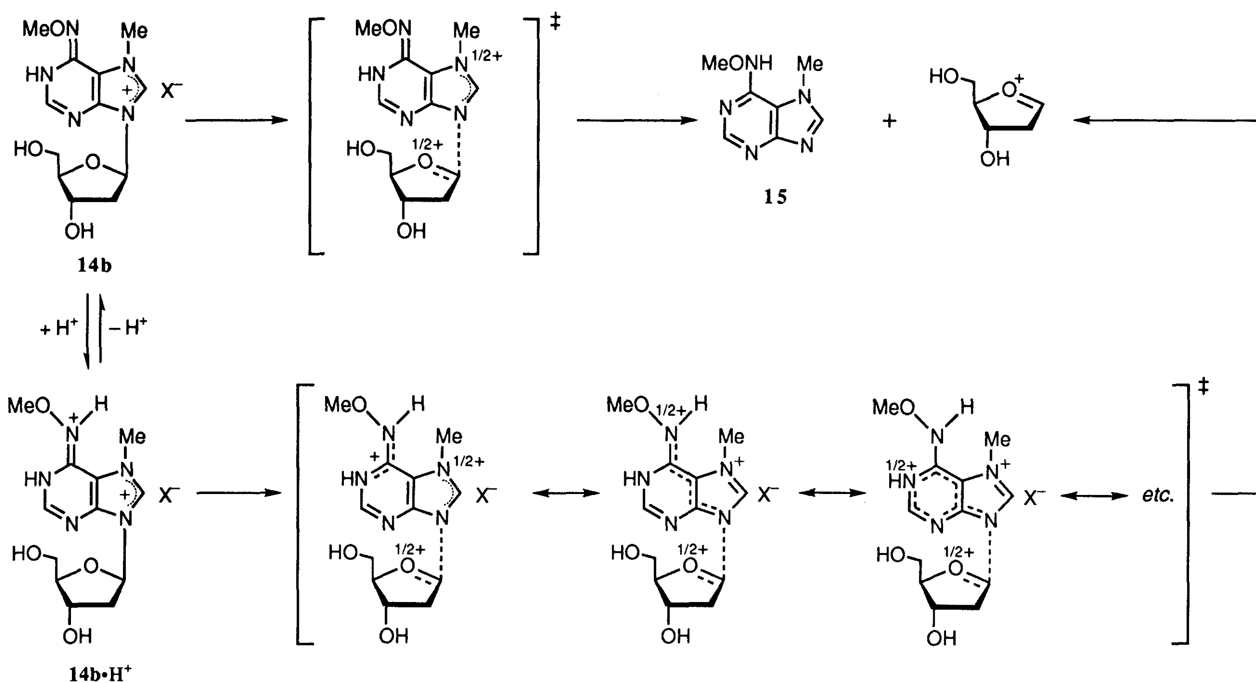
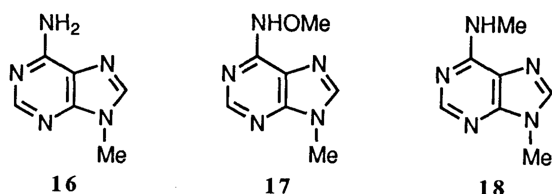


Chart 3



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 = HSO_4 or 1/2SO_4) 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 $AcNMe_2$ (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 H_2O (50 ml). The aqueous solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (30 ml), and the column was eluted with H_2O (250 ml). The aqueous eluates were combined, brought to pH 8.0 by addition of Et_3N, stirred at 60 °C for 12 h, during which time the pH of the solution was maintained at 8.0 by occasional addition of Et_3N, and then kept in a refrigerator overnight. The pale orange needles that deposited were filtered off and recrystallized from H_2O, giving a first crop (3.02 g) of **13b**·$2H_2O$ as colorless prisms, mp 132—135 °C (lit.[8b)] mp 123—125 °C for **13b**·$2/3H_2O$); MS *m/z*: 281 (M^+); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 267 nm (ε 13300); $\lambda_{max}^{H_2O}$ (pH 1) 267 (15600); $\lambda_{max}^{H_2O}$ (pH 7) 268 (14900); $\lambda_{max}^{H_2O}$ (pH 13) 283 (11800); ^{1}H-NMR (Me_2SO-d_6) δ: 2.12—2.76 [2H, m, C(2′)-H_2], 3.55 [2H, m, C(5′)-H_2], 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 H_2O. In addition, the above UV spectral data were very similar to those[17)] 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 H_2O to afford a second crop (536 mg) of **13b**·$2H_2O$, mp 130—133 °C. The total yield of **13b**·$2H_2O$ was 3.56 g (60%).

2′-Deoxy-N^6-methoxy-7-methyladenosine Sulfate [14b (X = HSO_4 or 1/2SO_4)] The following procedure was conducted in a cold chamber kept at 4—7 °C. A mixture of **13b**·$2H_2O$ (1.00 g, 3.15 mmol) and MeI (37.8 g, 266 mmol) in $AcNMe_2$ (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 H_2O (*ca.* 20 ml). The aqueous solution was passed through

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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** ($X=HSO_4$ or $1/2SO_4$) (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}H-NMR [CD_3OD–D_2O (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 ($X=HSO_4$ or $1/2SO_4$) in H_2O Leading to N^6-Methoxy-7-methyladenine (15) A solution of **14b** ($X=HSO_4$ or $1/2SO_4$) (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** ($X=HSO_4$ or $1/2SO_4$): The glycosidic hydrolysis of **14b** ($X=HSO_4$ or $1/2SO_4$) 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 HCO_2H–HCO_2Na (pH 3.00 at 25 °C) and 0.1 M NaH_2PO_4–Na_2HPO_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 NaH_2PO_4–Na_2HPO_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 μBondapak C_{18} column [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** ($X=1/2SO_4$): The glycosidic hydrolysis of **14a** ($X=1/2SO_4$)[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** ($X=HSO_4$ or $1/2SO_4$). 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·$1/2H_2O$[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** ($X=HSO_4$ or $1/2SO_4$), except that the solvents employed for HPLC analysis were MeOH–0.1 M KH_2PO_4 (15 : 85, v/v) (1.7 ml/min) for **2b**/**7** and 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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