Purines. XLII.[1] Synthesis and Glycosidic Hydrolysis of 7-Alkyladenosines Leading to an Alternative Synthesis of 7-Alkyladenines

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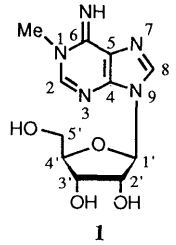
A full account is given of the synthesis and glycosidic hydrolysis of 7-alkyladenosines (4), which established an alternative synthesis of 7-alkyladenines (11). Methylation of N^6-methoxyadenosine (5) with MeI in $AcNMe_2$ at 30 °C for 8 h gave N^6-methoxy-7-methyladenosine, which was isolated in the form of the sulfate [7a (X = 1/2SO$_4$)] in 55% yield. N^6-Methoxy-N^6-methyladenosine (9a) was a by-product in this methylation. Demethoxylation of 7a (X = 1/2SO$_4$) by catalytic hydrogenolysis using hydrogen and Raney Ni catalyst produced, after replacement of the anion with perchlorate ion, 7-methyladenosine perchlorate [4a (X = ClO$_4$)] in a pure and crystalline form. 7-Ethyladenosine perchlorate [4b (X = ClO$_4$)] was also synthesized from N^6-benzyloxyadenosine (6) through a parallel route *via* N^6-benzyloxy-7-ethyladenosine sulfate [8b (X = 1/2SO$_4$)]. On treatment with H_2O at 98—100 °C for 40 min, 4a (X = ClO$_4$) and 4b (X = ClO$_4$) furnished 7-methyladenine (11a) and 7-ethyladenine (11b) in 84% and 55% yields, respectively. Similar hydrolyses of 7a (X = ClO$_4$) and 8b (X = 1/2SO$_4$) gave N^6-methoxy-7-methyladenine (12a) and N^6-benzyloxy-7-ethyladenine (12b), respectively. Catalytic hydrogenolysis of 12b using hydrogen and Raney Ni catalyst afforded 11b in 82% yield. In 0.1 N aqueous HCl at 25 °C, 4a (X = ClO$_4$) and 4b (X = ClO$_4$) were found to undergo glycosidic hydrolysis at rates of 2.22×10^{-3} min^{-1} (half-life 5.2 h) and 1.69×10^{-3} min^{-1} (half-life 6.8 h), respectively. Comparison of these rate constants with those of the other three N^X-methyladenosines (1—3) has revealed that the ease with which depurinylation occurs decreases in going through the series 3- (2) > 7- (4a) ≫ N^6- (3) ≥ 1-methyladenosine (1). On treatment with 1 N aqueous NaOH at 60 °C for 3 h, 4a (X = ClO$_4$) was hydrolyzed to give 11a in 44% yield.

Keywords 7-alkyladenosine; N^6-alkoxyadenosine; regioselective alkylation; hydrogenolytic dealkoxylation; glycosidic hydrolysis; 7-alkyladenine; kinetic study

7-Alkyladenosine (type **4**) is one of the four possible positional isomers of N^X-alkyladenosine. It remained unknown until 1973 when we synthesized 7-methyladenosine sulfate [**4a** (X = 1/2SO$_4$)], although in the form of a hygroscopic solid, from N^6-methoxyadenosine (**5**).[2] In 1974, Singer *et al.*[3] reported that 7-methyl- or 7-ethyladenosine [type **4** with unspecified anion (X^-)] was a by-product of methylation or ethylation of adenosine in neutral aqueous solution. The existence of the 7-methyladenosine structure in transfer ribonucleic acids (tRNAs) of *Bacillus stearothermophilus*[4] and *B. subtilis*[5] as a modified nucleoside component has also been suggested. However, these 7-alkyladenosines still remain poorly characterized, whereas the other three N^X-alkyladenosines, *e.g.*, 1-methyladenosine (**1**),[6] 3-methyladenosine (**2**),[7] and N^6-methyladenosine (**3**),[6] have already been well characterized. In this paper, we present the details of our original procedure for the synthesis of 7-methyladenosine sulfate [**4a** (X = 1/2SO$_4$)] and those of some modifications and improvements introduced into it, which made the corresponding perchlorate [**4a** (X = ClO$_4$)] available in pure, crystalline form. An extension of the modified procedure to the synthesis of 7-ethyladenosine perchlorate [**4b** (X = ClO$_4$)] and the chemical behavior observed for these 7-alkyladenosines are also included. Brief accounts of the results presented here have been published in preliminary form.[2,8]



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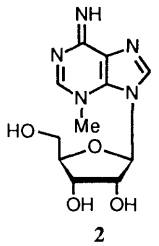

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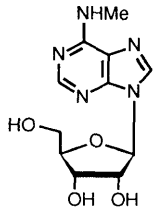

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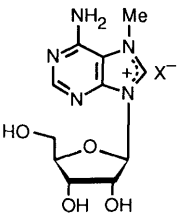

4a

Synthetic Route The synthesis of the first target, 7-methyladenosine (**4a**), was so designed that it becomes a 9-ribosyl version of our previous general synthesis[9] of 7,9-dialkyladeninium salts (**13**), as shown in Chart 1. Thus, N^6-methoxyadenosine (**5**)[10] was treated with MeI in $AcNMe_2$ at 30 °C for 8 h, and methylated products were isolated by means of column chromatography [Amberlite CG-400 (HSO_4^- and/or SO_4^{2-}), H_2O followed by 0.5 N formic acid], giving the 7-methylated product **7a** (X = 1/2SO$_4$)[11] in 55% yield together with the N^6-methyl isomer **9a**·HX (X = HSO$_4$ or 1/2SO$_4$) as a minor product. Hydrogenolysis (Raney Ni/H_2, H_2O, 1 atm, 40 °C, 7 h) of the free base **9a**, which was liberated from **9a**·HX (X = HSO$_4$ or 1/2SO$_4$), afforded N^6-methyladenosine (**3**)[6] in 22—33% overall yield (from **5**). The formation of the N^6-methyl isomer as a by-product was in general agreement with our previous results[9] on alkylation of N^6-alkoxy-9-alkyladenines.

On the other hand, removal of the N^6-methoxy group from the major product **7a** (X = 1/2SO$_4$) was effected by catalytic hydrogenation over 10% Pd–C [60% (v/v) aqueous EtOH, 3.5 atm, room temperature, 36 h] or, more efficiently, over Raney Ni catalyst (H_2O, 1 atm, room temperature, 9 h), producing 7-methyladenosine sulfate [**4a** (X = 1/2SO$_4$)] as a hygroscopic solid. Treatment of crude **4a** (X = 1/2SO$_4$) with $NaClO_4$ in H_2O furnished the corresponding

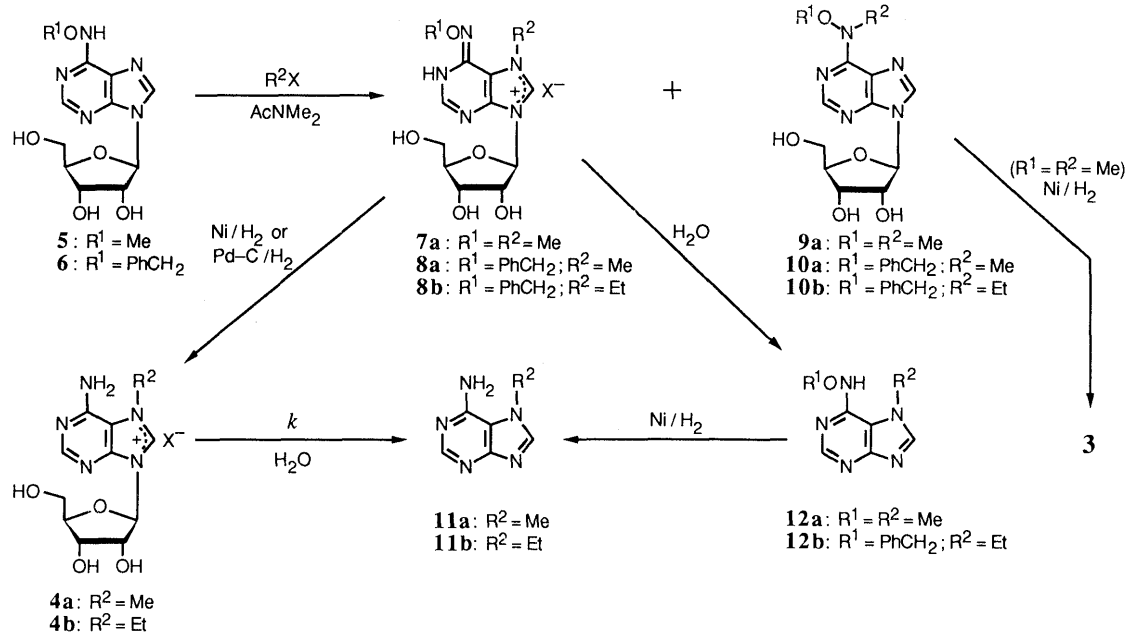

Chart 1

perchlorate [**4a** (X = ClO_4)] in 53% overall yield [from **7a** (X = 1/2SO_4)] in pure, crystalline form. Its ultraviolet (UV) spectrum was similar to those[9] of 7,9-dialkyladeninium salts (**13**). On heating in H_2O at 98—100 °C for 40 min, **4a** (X = ClO_4) gave 7-methyladenine (**11a**)[12] in 84% yield. A similar hydrolysis of **7a** (X = 1/2SO_4) afforded N^6-methoxy-7-methyladenine (**12a**) in 72% yield. Thus, these findings unequivocally established the 7-methyl structures of **7a** (X = 1/2SO_4) and the above salts of **4a**.

It has been demonstrated in our laboratory that the N^6-benzyloxy group is a more favorable control element for preferential 7-alkylation than the N^6-methoxy group.[9] Accordingly, we next tried to alkylate N^6-benzyloxyadenosine (**6**)[10b,d] instead of the N^6-methoxy analogue **5**. On treatment with MeI in $AcNMe_2$ at 30 °C for 5 h, **6** gave N^6-benzyloxy-7-methyladenosine hydriodide [**8a** (X = I)] in 52% yield. A minor product from this reaction was N^6-benzyloxy-N^6-methyladenosine (**10a**), as anticipated, and it was isolated as the perchlorate salt (**10a**·$HClO_4$) in 20% yield. Although this approach failed to improve the yield of the 7-methylated product, it made the isolation of the two products much easier than in the above N^6-methoxy series. Removal of the N^6-benzyloxy group from **8a** (X = I) was then attempted under hydrogenolytic conditions similar to those employed for **7a** (X = 1/2SO_4). However, the poor solubility of **8a** (X = I) in the hydrogenation solvent made the hydrogen uptake so slow that this approach to **4a** utilizing the N^6-benzyloxy group had to be abandoned.

The second target, 7-ethyladenosine (**4b**), was synthesized through a parallel route. Ethylation of **6** with EtI in $AcNMe_2$ at 25 °C for 52 h and isolation of the product by column chromatography [Amberlite CG-400 (HSO_4^- and/or SO_4^{2-}), H_2O] gave N^6-benzyloxy-7-ethyladenosine sulfate [**8b** (X = 1/2SO_4)] in 53% yield. No attempt was made to isolate a by-product presumed to be the N^6-ethyl isomer **10b**. When a product mixture obtained from a separate, similar ethylation of **6** was directly heated, without chromatographic purification, in H_2O at 98—100 °C for 40 min, N^6-benzyloxy-7-ethyladenine (**12b**) was formed in 35% overall yield (from **6**). Catalytic hydrogenolysis of **12b** using hydrogen and Raney Ni catalyst then provided 7-ethyladenine (**11b**)[12] in 82% yield. On the other hand, a similar hydrogenolysis of the perchlorate **8b** (X = ClO_4), which was derived from the above sulfate **8b** (X = 1/2SO_4) in 92% yield, afforded 7-ethyladenosine perchlorate [**4b** (X = ClO_4)] in 53% yield. Direct hydrogenolysis of the sulfate **8b** (X = 1/2SO_4) under similar reaction conditions was also possible, but it furnished, after treatment of the product with aqueous $NaClO_4$, the desired nucleoside **4b** (X = ClO_4) in only 28% yield. Treatment of **4b** (X = ClO_4) with H_2O at 98—100 °C for 40 min liberated the base **11b** in 55% yield.

Finally, benzylation of **6** with $PhCH_2Br$ in $AcNMe_2$ at 30 °C for 24 h produced a complex mixture of products. Two dibenzylated N^6-benzyloxyadenines were among them, and we were unable to isolate the 7-benzylated nucleoside.

Glycosidic Hydrolysis under Acidic Conditions The ready depurinylation (glycosidic hydrolysis) of 7-alkyladenosine salts (**4**) in H_2O, as described in the foregoing section, indicates that their glycosidic bonds should be considerably unstable in aqueous acidic solution. We monitored the glycosidic hydrolyses of 7-methyladenosine perchlorate [**4a** (X = ClO_4)] and 7-ethyladenosine perchlorate [**4b** (X = ClO_4)] to give 7-methyladenine (**11a**) and 7-ethyladenine (**11b**), respectively, in 0.1 N aqueous HCl at 25 °C (Chart 1), determining the unaltered nucleosides by means of high-performance liquid chromatography (HPLC). Both hydrolyses were found to obey pseudo-first-order kinetics with the following rate constants: $k = 2.22 \times 10^{-3}\ min^{-1}$ (half-life 5.2 h) [for **4a** (X = ClO_4)]; $k = 1.69 \times 10^{-3}\ min^{-1}$ (half-life 6.8 h) [for **4b** (X = ClO_4)]. Table I lists the rate constants for the glycosidic hydrolyses of all four possible N^X-methyladenosines in 0.1 N aqueous HCl at various temperatures. Those of 1-methyladenosine (**1**) and N^6-methyladenosine (**3**) were determined in the present study in a manner similar to that described above for **4a, b** (X = ClO_4). It may be seen that the relative ease of depurinylation is in the order of 3-methyl- (**2**) > 7-methyl-

Table I. Rate Constants (k) for the Glycosidic Hydrolyses of N^x-Methyladenosines (**1**—**3**, and **4a**) and 7-Ethyladenosine (**4b**) in 0.1 N Aqueous HCl

Nucleoside	Pseudo-first-order rate constant[a] ($k \times 10^5$, min^{-1}) at			
	80.0 °C	70.0 °C	55.0 °C	25.0 °C
7-Methyladenosine (**4a**)	—	—	—	222
7-Ethyladenosine (**4b**)	—	—	—	169
N^6-Methyladenosine (**3**)	987 (1110)	300	47.3	0.82[b]
3-Methyladenosine (**2**)	—	—	—	4000[c]
1-Methyladenosine (**1**)	724[d] (912)	221 (323)	33.0	0.56[b]

a) The values in parentheses were taken from ref. 13. *b*) Estimated on the basis of the data at 55.0—80.0 °C and Arrhenius equation for reaction rate. *c*) From ref. 7. *d*) The acid hydrolysis of **1** is known[13] to proceed through initial cleavage of the glycosidic bond to form 1-methyladenine, which is then transformed slowly to 5-amino-*N'*-methylimidazole-4-carboxamidine. Under the specified conditions, the first-order rate constant (k') for the latter step was determined to be 52×10^{-5} min^{-1} [lit.[13] $k' = 1.07 \times 10^{-5}$ s^{-1} ($k' = 64.2 \times 10^{-5}$ min^{-1})].

(**4a**) $\gg N^6$-methyl- (**3**) $\geq$ 1-methyladenosine (**1**). The glycosidic bond of **1** has been reported to undergo solvolysis in acidic solution at about the same rate as does adenosine itself.[13] It follows that the introduction of a methyl group into adenosine at the 3- or 7-position makes the glycosidic bond much weaker than that of the parent nucleoside under acidic conditions. Assuming that an A-1 mechanism[13,14] for solvolyses of nucleosides is operating in these glycosidic hydrolyses, we have attributed the accelerated depurinylation of 3-methyladenosine (**2**) to the N^6-protonated structure (even in the weakly alkaline region), in which the exocyclic iminium structure is a very important contributor to the possible resonance hybrid.[7b] In the case of 7-methyladenosine (**4a**) or 7-ethyladenosine (**4b**), the observed instability of the glycosidic bond is probably owing to full-time localization of the positive charge in the imidazole moiety (possibly at the 7-position), since the importance of protonation at the 7-position has been proposed[13,15] for the acid hydrolysis of purine nucleosides. Interestingly, 7-ethyladenosine (**4b**) undergoes depurinylation slightly more slowly than the 7-methyl homologue **4a**, paralleling the observation[16] on 7-alkylguanosines.

Glycosidic Hydrolysis under Alkaline Conditions The chemical instability of the 7,9-dialkyladenine system (type **13**) arises from its imidazolium structure.[1,17] We have reported[1] that 7,9-dialkyladeninium salts (**13**) undergo facile ring-opening under mild alkaline conditions [*e.g.*, 0.5 N aqueous Na_2CO_3 or Amberlite CG-400 (OH^-), room temperature], giving the *trans*-formamidopyrimidine **14** (with carbonyl oxygen *trans* to the pyrimidine ring), which then equilibrates slowly with the *cis*-formamidopyrimidine **16** (with carbonyl oxygen *cis* to the pyrimidine ring), as shown in Chart 2. Treatment of either **13** or **14** with boiling 1 N aqueous NaOH for 60 min is known to produce N^6,7-dialkyladenine (**15**), a rearranged product.[1,17] We expected 7-alkyladenosines (**4**) to behave similarly. On treatment with 0.5 N aqueous Na_2CO_3 or Amberlite CG-400 (OH^-) in H_2O at room temperature, 7-methyladenosine perchlorate [**4a** ($X = ClO_4$)] indeed formed several products as detected by HPLC.[18] However, their structures remained undetermined.

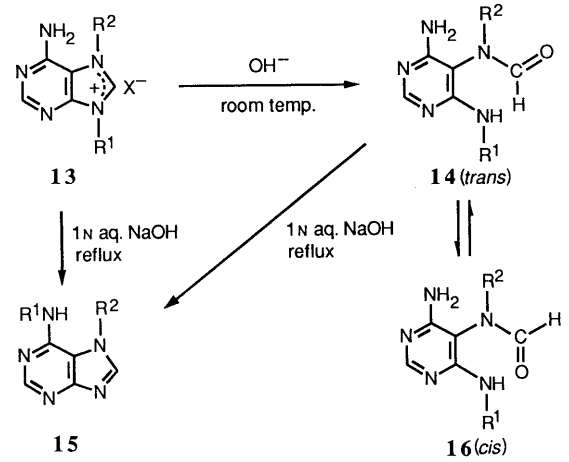

Chart 2

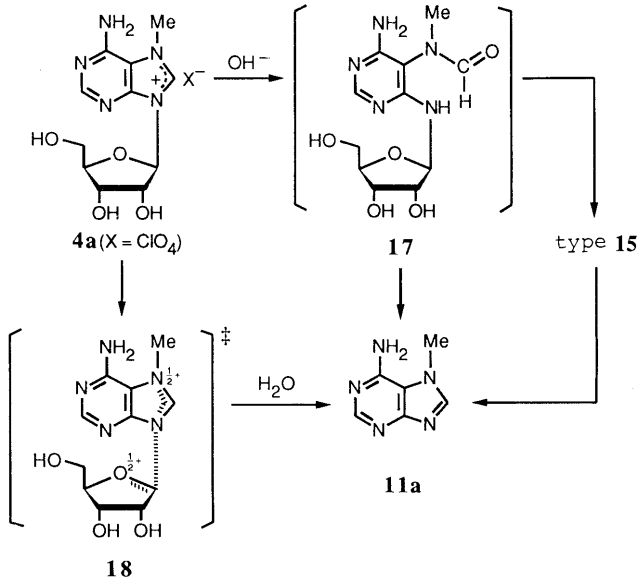

Chart 3

Under more basic and vigorous conditions (1 N aqueous NaOH, 60 °C, 3 h), **4a** ($X = ClO_4$) was hydrolyzed to give 7-methyladenine (**11a**) (Chart 1) in 44% yield, but we were unable to obtain the expected product, 7-methyl-N^6-β-D-ribofuranosyladenine (type **15**). The observed depurinylation in alkaline solution may deserve special mention since nucleosides are generally very resistant to alkaline hydrolysis.[19] In view of the unique structure of **4a** ($X = ClO_4$) in which the nitrogen atoms of the imidazole moiety are activated by full-time possession of the positive charge, the transition structure **18** as in the case of the acid hydrolysis (*vide supra*) may be postulated even in alkaline hydrolysis, as illustrated in Chart 3. The possibility of the alternative pathways **4a** ($X = ClO_4$)$\rightarrow$**17**$\rightarrow$**11a** and/or **4a** ($X = ClO_4$)$\rightarrow$**17**$\rightarrow$type **15**$\rightarrow$**11a** may not be excluded, however, since there are examples to suggest them in the literature.[19a,c,20]

Conclusion

The results described above not only confirm the applicability of our general synthetic method for 7,9-dialkyladeninium salts (**13**)[9] to the synthesis of hitherto

unknown 7-alkyladenosines (type **4**) but also characterize the spectral and chemical features of **4**. The ready glycosidic hydrolysis of **4** also concludes an alternative synthesis of 7-alkyladenines (**11**), which have previously been prepared[12,21] in most cases by inconvenient methods. Moreover, the present kinetic data on depurinylation may also acquire deeper significance because of the importance of the 7- and 3-methyladenosine structures in sequencing deoxyribonucleic acids[3,16,22] and because of the suggestion[4,5] of the existence of the former in tRNAs of bacillary origin.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. Spectra reported herein were recorded on a Hitachi model 323 ultraviolet (UV) spectrophotometer [on solutions in 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)], a JASCO IRA-2 infrared (IR) spectrophotometer, or a JEOL JNM-FX-100 nuclear magnetic resonance (NMR) spectrometer at 25 °C with Me_4Si as an internal standard. Elemental analyses were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, m = multiplet, q = quartet, s = singlet, sh = shoulder, t = triplet.

N^6-Methoxy-7-methyladenosine Sulfate [7a (X = 1/2SO$_4$)] and N^6-Methoxy-N^6-methyladenosine (9a) A mixture of **5**·1/2H_2O[10b] (3.68 g, 12 mmol) and MeI (6.81 g, 48 mmol) in $AcNMe_2$ (24 ml) was stirred at 30 °C for 8 h. The reaction mixture was concentrated *in vacuo* to leave an oil, which was dissolved in H_2O (3 ml). The resulting aqueous solution was applied to a column of Amberlite CG-400 (HSO_4^- and/or SO_4^{2-}) (360 ml), and the column was eluted with H_2O. A 170-ml fraction eluted after the first 180-ml fraction was concentrated *in vacuo*. The residual solid was then washed with EtOH (20 ml) and dried over P_2O_5 at 3 mmHg and room temperature for 24 h to give **7a**·H_2O (X = 1/2SO_4) (2.50 g, 55%) as a colorless solid, mp *ca.* 127 °C (dec.). Recrystallization of the solid by dissolving it in H_2O and adding EtOH to the resulting aqueous solution, followed by drying in the same manner as described above, yielded an analytical sample of **7a**·H_2O (X = 1/2SO_4) as colorless minute needles, mp 128—129 °C (dec.); UV $\lambda_{max}^{H_2O}$ (pH 1) 234 nm (ε 8010), 286 (9000); $\lambda_{max}^{H_2O}$ (pH 7) 235 (7970), 286 (8750); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 3.6—3.85 [2H, m, C(5′)-H's], 3.74 (3H, s, OMe), 3.9—4.25 [2H, br m, C(4′)-H and C(3′)-H], 4.00 [3H, s, N(7)-Me], 4.44 [1H, dd, *J* = 4.4 Hz each, C(2′)-H], 5.84 [1H, d, *J* = 4.4 Hz, C(1′)-H], 7.69 [1H, s, C(2)-H], 9.27 [1H, s, C(8)-H].[23] *Anal.* Calcd for $C_{12}H_{18}N_5O_5 \cdot 1/2SO_4 \cdot H_2O$: C, 38.10; H, 5.33; N, 18.51. Found: C, 37.88; H, 5.31; N, 18.22.

On the other hand, later fractions eluted with 0.5 N formic acid in the above chromatography were combined and concentrated *in vacuo* to leave **9a**·HX (X = HSO_4 or 1/2SO_4) as a glass, which was dissolved in H_2O (20 ml). The resulting solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (15 ml), and the column was eluted with H_2O. The eluate (500 ml) was concentrated *in vacuo* to leave **9a** (1.43 g) as a foam, UV $\lambda_{max}^{95\%\,EtOH}$ 276 nm; $\lambda_{max}^{H_2O}$ (pH 1) 272; $\lambda_{max}^{H_2O}$ (pH 7) 275; $\lambda_{max}^{H_2O}$ (pH 13) 276.

N^6-Benzyloxy-7-methyladenosine Hydriodide [8a (X = I)] and N^6-Benzyloxy-N^6-methyladenosine Perchlorate (10a·HClO$_4$) A mixture of **6**·H_2O[10b] (1.17 g, 3 mmol) and MeI (1.70 g, 12 mmol) in $AcNMe_2$ (6 ml) was stirred at 30 °C for 5 h. The reaction mixture was concentrated *in vacuo*, and the residual oil was dissolved in H_2O (15 ml). The resulting solution was kept in a refrigerator to deposit crystals, which were filtered off, washed with a little H_2O, and dried over P_2O_5 at 3 mmHg and room temperature for 24 h to give **8a**·H_2O (X = I) (829 mg, 52%), mp 91—95 °C (dec.). Recrystallization from H_2O and drying in the same manner as described above furnished an analytical sample of **8a**·H_2O (X = I) as colorless needles, mp 103—108 °C (dec.); UV $\lambda_{max}^{95\%\,EtOH}$ 291 nm (ε 8470); $\lambda_{max}^{H_2O}$ (pH 1) 226 (22700), 286 (10500); $\lambda_{max}^{H_2O}$ (pH 7) 226 (22600), 286 (10200); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 3.55—3.75 [2H, m, C(5′)-H's], 3.75—4.2 [2H, m, C(4′)-H and C(3′)-H], 3.98 [3H, s, N(7)-Me], 4.13 [1H, m, C(2′)-H], 4.65—5.45 [2H, br, C(5′)-OH and C(3′)-OH], 5.10 (2H, m, OC$\underline{H}_2$Ph), 5.45—5.9 [1H, br, C(2′)-OH], 5.90 [1H, d, *J* = 2.9 Hz, C(1′)-H], 7.1—7.5 (5H, m, OCH$_2$$\underline{Ph}$), 7.84 [1H, d, *J* = 3.4 Hz, C(2)-H], 9.40 [1H, s, C(8)-H], 12.17 (1H, dull d, *J* = 3.4 Hz, NH).[23] *Anal.* Calcd for $C_{18}H_{22}IN_5O_5 \cdot H_2O$: C, 40.54; H, 4.54; N, 13.13. Found: C, 40.51; H, 4.25; N, 12.62.

On the other hand, the aqueous filtrate, which was obtained when the crude 7-methylated product was isolated, was neutralized by addition of saturated aqueous $NaHCO_3$ and then extracted with AcOEt (3 × 15 ml). The AcOEt extracts were combined, washed with saturated aqueous NaCl (5 ml), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was then purified by column chromatography [silica gel (55 g), $CHCl_3$–EtOH (10 : 1, v/v)], giving a glass (270 mg). The total amount of the glass was dissolved in MeOH (3 ml), and 70% aqueous $HClO_4$ (120 mg) was added. The precipitate that resulted was filtered off, washed with a little MeOH, and dried to afford **10a**·$HClO_4$ (299 mg, 20% overall yield from **6**·H_2O), mp 151—152 °C. Recrystallization from MeOH yielded an analytical sample of **10a**·$HClO_4$ as colorless prisms, mp 160—161 °C; UV $\lambda_{max}^{95\%\,EtOH}$ 277 nm (ε 20800); $\lambda_{max}^{H_2O}$ (pH 1) 276 (18400); $\lambda_{max}^{H_2O}$ (pH 7) 277 (19500); $\lambda_{max}^{H_2O}$ (pH 13) 277 (20000); NMR (Me_2SO-d_6) δ: 3.4—3.8 [2H, m, C(5′)-H's], 3.70 (3H, s, N^6-Me), 3.99 [1H, m, C(4′)-H], 4.17 [1H, m, C(3′)-H], 4.55 [1H, m, C(2′)-H], 5.18 (2H, s, OC$\underline{H}_2$Ph), 5.99 [1H, d, *J* = 5.4 Hz, C(1′)-H], 7.1—7.7 (5H, m, OCH$_2$$\underline{Ph}$), 8.49 and 8.74 (1H each, s, purine protons). *Anal.* Calcd for $C_{18}H_{21}N_5O_5 \cdot HClO_4$: C, 44.32; H, 4.55; N, 14.36. Found: C, 44.54; H, 4.61; N, 14.08.

N^6-Benzyloxy-7-ethyladenosine Sulfate [8b (X = 1/2SO$_4$)] A mixture of **6**·H_2O[10b] (1.96 g, 5 mmol) and EtI (4.68 g, 30 mmol) in $AcNMe_2$ (10 ml) was stirred at 25 °C for 52 h. The reaction mixture was concentrated *in vacuo*, and the oily residue was dissolved in H_2O (2 ml). The resulting aqueous solution was passed through a column of Amberlite CG-400 (HSO_4^- and/or SO_4^{2-}) (140 ml), and the column was eluted with H_2O. A 300-ml fraction eluted after the first 50-ml fraction was concentrated *in vacuo* to leave a solid, which was washed with acetone (30 ml) and dried over P_2O_5 at 3 mmHg and room temperature for 15 h, giving **8b**·H_2O (X = 1/2SO_4) (1.24 g, 53%), mp 109—110 °C (dec.). Recrystallization of this substance by dissolving it in H_2O and adding acetone to the resulting aqueous solution, followed by drying in the same manner as described above, provided an analytical sample of **8b**·H_2O (X = 1/2SO_4) as colorless prisms, mp 109—110 °C (dec.); UV $\lambda_{max}^{95\%\,EtOH}$ 237 nm (ε 9940), 290 (8460); $\lambda_{max}^{H_2O}$ (pH 1) 232 (9430), 286 (10200); $\lambda_{max}^{H_2O}$ (pH 7) 232 (9340), 286 (10100); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 1.36 [3H, t, *J* = 7 Hz, N(7)-CH$_2$$\underline{Me}$], 3.5—3.8 [2H, m, C(5′)-H's], 3.9—4.2 [2H, m, C(4′)-H and C(3′)-H], 4.34 [2H, q, *J* = 7 Hz, N(7)-C$\underline{H}_2$Me], 4.45 [1H, dd, *J* = 4.4 Hz each, C(2′)-H], 4.98 (2H, s, OC$\underline{H}_2$Ph), 5.83 [1H, d, *J* = 4.4 Hz, C(1′)-H], 7.1—7.5 (5H, m, OCH$_2$$\underline{Ph}$), 7.71 [1H, s, C(2)-H], 9.32 [1H, s, C(8)-H].[23] *Anal.* Calcd for $C_{19}H_{24}N_5O_5 \cdot 1/2SO_4 \cdot H_2O$: C, 48.71; H, 5.59; N, 14.95. Found: C, 48.23; H, 5.41; N, 14.74.

N^6-Benzyloxy-7-ethyladenosine Perchlorate [8b (X = ClO$_4$)] The sulfate **8b**·H_2O (X = 1/2SO_4) (1.03 g, 2.2 mmol) was dissolved in warm H_2O (15 ml), and a solution of $NaClO_4$ (404 mg, 3.3 mmol) in H_2O (1 ml) was added. The resulting mixture was kept in a refrigerator, and the precipitate that resulted was filtered off, washed with cold H_2O (5 ml), and dried to yield **8b** (X = ClO_4) (1.01 g, 92%), mp 141—142 °C. Recrystallization from H_2O gave an analytical sample as colorless needles, mp 142—143 °C[24]; UV $\lambda_{max}^{95\%\,EtOH}$ 235 nm (ε 10000), 290 (8670); $\lambda_{max}^{H_2O}$ (pH 1) 230 (9230), 285 (10000); $\lambda_{max}^{H_2O}$ (pH 7) 231 (9200), 285 (9860); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 1.38 [3H, t, *J* = 7 Hz, N(7)-CH$_2$$\underline{Me}$], 3.5—3.8 [2H, m, C(5′)-H's], 3.9—4.5 [5H, br m, C(4′)-H, C(3′)-H, C(2′)-H, and N(7)-C$\underline{H}_2$Me], 4.9—5.5 [2H, br, C(5′)-OH and C(3′)-OH], 5.10 (2H, s, OC$\underline{H}_2$Ph), 5.6—6.0 [1H, br, C(2′)-OH], 5.90 [1H, d, *J* = 2.7 Hz, C(1′)-H], 7.25—7.5 (5H, m, OCH$_2$$\underline{Ph}$), 7.86 [1H, d, *J* = 3 Hz, C(2)-H], 9.47 [1H, s, C(8)-H], 12.21 (1H, d, *J* = 3 Hz, NH).[23] *Anal.* Calcd for $C_{19}H_{24}ClN_5O_9$: C, 45.47; H, 4.82; N, 13.95. Found: C, 45.19; H, 4.85; N, 13.77.

Hydrogenolysis of 9a Leading to N^6-Methyladenosine (3) A solution of crude **9a** (359 mg), obtained by the above methylation of **5**·1/2H_2O, in H_2O (40 ml) was hydrogenated over Raney Ni W-2 catalyst[25] (0.8 ml) at atmospheric pressure and 40 °C for 7 h. The catalyst was removed by filtration and washed with H_2O (15 ml). The filtrate and washings were combined and concentrated *in vacuo*, leaving a glass. The glass was crystallized from MeOH to give **3** (190 mg, 22% overall yield from **5**·1/2H_2O) as colorless needles, mp 211—213 °C. This sample was identical (by comparison of the IR spectrum and paper partition chromatographic behavior[26]) with authentic **3**.[27]

In a separate run using a crude sample of **9a**, which was isolated from another batch of products from the above methylation of **5**·1/2H_2O, the overall yield of **3** from **5**·1/2H_2O was 33%.

7-Methyladenosine Perchlorate [4a (X = ClO$_4$)] A solution of **7a**·H_2O (X = 1/2SO_4) (1.89 g, 5 mmol) in H_2O (100 ml) was hydrogenated over Raney Ni W-2 catalyst[25] (5 ml) at atmospheric pressure and room temperature for 9 h. The catalyst was filtered off and washed with H_2O (60 ml). The filtrate and washings were combined and concentrated *in*

vacuo to leave **4a** (X = 1/2SO_4) (1.53 g) as a hygroscopic solid. The solid was dissolved in H_2O (3 ml), and a solution of $NaClO_4$ (900 mg, 7.35 mmol) in H_2O (1 ml) was added. The resulting mixture was kept in a refrigerator, and the precipitate that resulted was filtered off, washed with a little H_2O, and dried over P_2O_5 at 3 mmHg and room temperature for 24 h, giving **4a**·1/2H_2O (X = ClO_4) [1.03 g, 53% overall yield from **7a**·H_2O (X = 1/2SO_4)], mp *ca.* 120 °C (dec.). Recrystallization from warm H_2O (below 40 °C) and drying in the same manner as described above produced an analytical sample of **4a**·1/2H_2O (X = ClO_4) as colorless plates, mp *ca.* 120 °C (dec.)[28]; UV $\lambda_{max}^{95\%\,EtOH}$ 272 nm (ε 10100); $\lambda_{max}^{H_2O}$ (pH 1) 271 (12900); $\lambda_{max}^{H_2O}$ (pH 7) 271 (12800); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 3.6—3.8 [2H, br m, C(5′)-H's], 3.9—4.3 [2H, br m, C(4′)-H and C(3′)-H], 4.18 [3H, s, N(7)-Me], 4.35—4.5 [1H, m, C(2′)-H], 6.04 [1H, d, *J* = 3.3 Hz, C(1′)-H], 8.01 (2H, dull, NH_2), 8.44 [1H, s, C(2)-H], 9.67 [1H, s, C(8)-H].[23] *Anal.* Calcd for $C_{11}H_{16}ClN_5O_8 \cdot 1/2H_2O$: C, 33.81; H, 4.39; N, 17.92. Found; C, 33.58; H, 4.42; N, 18.04.

7-Ethyladenosine Perchlorate [4b (X = ClO_4)] i) From **8b** (X = 1/2SO_4): A solution of **8b**·H_2O (X = 1/2SO_4) (468 mg, 1 mmol) in H_2O (25 ml) was hydrogenated over Raney Ni W-2 catalyst[25] (1.5 ml) at atmospheric pressure and room temperature for 19 h. The catalyst was filtered off and washed with H_2O (9 ml). The filtrate and washings were combined and concentrated *in vacuo* to leave **4b** (X = 1/2SO_4) as a glass. The glass was dissolved in H_2O (0.5 ml), and a solution of $NaClO_4$ (184 mg, 1.5 mmol) in H_2O (0.5 ml) was added. The resulting mixture was kept in a refrigerator, and the precipitate that resulted was filtered off, washed with a small amount of cold H_2O, and dried over P_2O_5 at 3 mmHg and room temperature for 24 h, furnishing **4b**·H_2O (X = ClO_4) [117 mg, 28% overall yield from **8b**·H_2O (X = 1/2SO_4)], mp 111—113 °C (dec.). Recrystallization from warm H_2O (below 40 °C) and drying in the same manner as described above produced an analytical sample of **4b**·H_2O (X = ClO_4) as colorless prisms, mp 115—117 °C (dec.)[28]; UV $\lambda_{max}^{95\%\,EtOH}$ 272 nm (ε 11300); $\lambda_{max}^{H_2O}$ (pH 1) 270 (13100); $\lambda_{max}^{H_2O}$ (pH 7) 271 (13100); $\lambda_{max}^{H_2O}$ (pH 13) unstable; NMR (Me_2SO-d_6) δ: 1.48 [3H, t, *J* = 7.1 Hz, N(7)-CH_2Me], 3.6—3.85 [2H, br m, C(5′)-H's], 3.9—4.3 [2H, br m, C(4′)-H and C(3′)-H], 4.48 [1H, br m, C(2′)-H], 4.59 [2H, q, *J* = 7.1 Hz N(7)-C$\underline{H}_2$Me], 5.0—5.5 (2H, br, OH's), 5.6—5.95 (1H, br, OH), 6.06 [1H, d, *J* = 2.7 Hz, C(1′)-H], 8.02 (2H, br, NH_2), 8.47 [1H, s, C(2)-H], 9.80 [1H, s, C(8)-H].[23] *Anal.* Calcd for $C_{12}H_{18}ClN_5O_8 \cdot H_2O$: C, 34.83; H, 4.87; N, 16.93. Found: C, 34.92; H, 4.57; N, 17.09.

ii) From **8b** (X = ClO_4): A solution of **8b** (X = ClO_4) (1.00 g, 1.99 mmol) in H_2O (150 ml) was hydrogenated over Raney Ni W-2 catalyst[25] (2 ml) at atmospheric pressure and room temperature for 18 h. The catalyst was removed by filtration and washed with H_2O (30 ml). The filtrate and washings were combined and concentrated *in vacuo*. The residual solid was washed with EtOH (5 ml) and dried over P_2O_5 at 3 mmHg and room temperature for 24 h, giving **4b**·H_2O (X = ClO_4) (440 mg, 53%), mp 109—111 °C (dec.). This sample was identical [by comparison of the IR spectrum and thin-layer chromatographic (TLC) mobility] with the one obtained by method (i).

N^6-Methoxy-7-methyladenine (12a) A solution of **7a**·H_2O (X = 1/2SO_4) (454 mg, 1.2 mmol) in H_2O (8 ml) was heated at 98—100 °C for 40 min. The reaction mixture was concentrated *in vacuo*, and the residual solid was recrystallized from H_2O (1 ml) to give colorless needles (217 mg) presumed to be the sulfate of **12a**. These crystals were dissolved in H_2O (10 ml), and the resulting solution was passed through a column of Amberlite IRA-402 (HCO_3^-) (3 ml). The column was eluted with H_2O, and the eluate (100 ml) was concentrated *in vacuo* to leave **12a** (154 mg, 72%) as a colorless solid, mp 230—231 °C (dec.). Recrystallization from EtOH furnished an analytical sample as colorless prisms, mp 234—235 °C (dec.); UV $\lambda_{max}^{95\%\,EtOH}$ 277 nm (ε 13400); $\lambda_{max}^{H_2O}$ (pH 1) 228 (6800), 278 (10400); $\lambda_{max}^{H_2O}$ (pH 7) 275 (13800); $\lambda_{max}^{H_2O}$ (pH 13) 296 (13200); NMR (Me_2SO-d_6) δ: 3.78 (3H, s, NMe or OMe), 3.86 (3H, s, OMe or NMe), 7.52 [1H, slightly dull s, C(2)-H],[29] 7.85 [1H, s, C(8)-H], 11.15 (1H, br, NH). *Anal.* Calcd for $C_7H_9N_5O$: C, 46.92; H, 5.06; N, 39.09. Found: C, 46.67; H, 5.22; N, 39.01.

N^6-Benzyloxy-7-ethyladenine (12b) A mixture of **6**·H_2O[10b] (1.57 g, 4 mmol) and EtI (3.74 g, 24 mmol) in $AcNMe_2$ (8 ml) was stirred at 30 °C for 48 h. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in H_2O (25 ml) containing a small amount of $NaHSO_3$. The resulting solution was heated at 98—100 °C for 40 min. After cooling, the precipitate that resulted was filtered off, washed with a small amount of cold H_2O, and then dissolved in H_2O (50 ml). The resulting aqueous solution was made alkaline by addition of saturated aqueous $NaHCO_3$ and cooled to deposit a colorless solid. The solid was filtered off, washed with a little H_2O, and dried to afford **12b** (373 mg, 35% overall yield from **6**·H_2O), mp 155—160 °C. Recrystallization from 30% (v/v) aqueous EtOH yielded an analytical sample as colorless needles, mp 166 °C (sintered at 159 °C); UV $\lambda_{max}^{95\%\,EtOH}$ 277 nm (ε 14800); $\lambda_{max}^{H_2O}$ (pH 1) 225 (sh) (7900), 279 (11300); $\lambda_{max}^{H_2O}$ (pH 7) 276 (15000); $\lambda_{max}^{H_2O}$ (pH 13) 298 (14100); NMR (Me_2SO-d_6) δ: 1.28 [3H, t, *J* = 7.1 Hz, N(7)-CH_2Me], 4.18 [2H, q, *J* = 7.1 Hz, N(7)-C$\underline{H}_2$Me], 5.01 (2H, s, OC$\underline{H}_2$Ph), 7.1—7.6 (5H, m, OCH_2Ph), 7.51 [1H, d, *J* = 3.4 Hz, C(2)-H],[29] 7.89 [1H, s, C(8)-H], 11.15 (1H, br, NH). *Anal.* Calcd for $C_{14}H_{15}N_5O$: C, 62.44; H, 5.61; N, 26.01. Found: C, 62.41; H, 5.67; N, 26.24.

7-Methyladenine (11a) i) By Hydrolysis of **4a**·1/2H_2O (X = ClO_4) in Hot H_2O: A solution of **4a**·1/2H_2O (X = ClO_4) (156 mg, 0.4 mmol) in H_2O (2 ml) was heated at 98—100 °C for 40 min. The reaction mixture was concentrated *in vacuo* to leave a solid. The solid was dissolved in hot H_2O (1 ml), and the resulting aqueous solution was made alkaline with concentrated aqueous NH_3. After cooling, the precipitate that resulted was filtered off, washed with a small amount of cold H_2O, and dried to give **11a** (50 mg, 84%) as colorless needles, mp > 300 °C. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **11a**.[12]

ii) By Hydrolysis of **4a**·1/2H_2O (X = ClO_4) with 1 N Aqueous NaOH: A solution of **4a**·1/2H_2O (X = ClO_4) (78 mg, 0.2 mmol) in 1 N aqueous NaOH (1 ml) was heated at 60 °C for 3 h. After cooling, the precipitate that resulted was filtered off, washed with H_2O, and dried to give **11a** (13 mg, 44%), mp > 300 °C. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **11a**.[12]

7-Ethyladenine (11b) i) From **12b**: A solution of **12b** (200 mg, 0.74 mmol) in MeOH (15 ml) was hydrogenated over Raney Ni W-2 catalyst[25] (0.5 ml) at atmospheric pressure and 45 °C for 20 h. The catalyst was removed by filtration and washed with MeOH (10 ml). The filtrate and washings were combined and concentrated *in vacuo*. The residual solid was washed with AcOEt (3 ml) and dried to give **11b** (99 mg, 82%), mp 251—252 °C (dec.). Recrystallization from 1-butanol produced a pure sample as colorless prisms, mp 258—259 °C (dec.); UV $\lambda_{max}^{95\%\,EtOH}$ 272 nm (ε 9800), 282 (sh) (6500); $\lambda_{max}^{H_2O}$ (pH 1) 273 (13600); $\lambda_{max}^{H_2O}$ (pH 7) 270 (10300), 280 (sh) (6700); $\lambda_{max}^{H_2O}$ (pH 13) 270 (10300), 280 (sh) (6700). *Anal.* Calcd for $C_7H_9N_5$: C, 51.52; H, 5.56; N, 42.92. Found: C, 51.62; H, 5.66; N, 42.68. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **11b**.[12]

ii) From **4b**·H_2O (X = ClO_4): A solution of **4b**·H_2O (X = ClO_4) (166 mg, 0.4 mmol) in H_2O (2 ml) was heated at 98—100 °C for 40 min. The reaction mixture was passed through a column of Amberlite IRA-402 (HCO_3^-) (2.7 ml), and the column was eluted with H_2O. The eluate (50 ml) was concentrated to dryness *in vacuo*, and the residue was chromatographed on a 6-g alumina column using AcOEt–EtOH (5 : 1, v/v) as the eluent, furnishing **11b** (36 mg, 55%), mp 258—259 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC behavior) with authentic **11b**.[12]

Kinetic Procedure for Acid Hydrolyses of the Nucleosides 1, 3, 4a, and 4b The nucleosides **1**,[27] **3**,[27] **4a**·1/2H_2O (X = ClO_4), and **4b**·H_2O (X = ClO_4) were separately dissolved, at 1.1×10^{-3}—1.4×10^{-3} M concentration, in 0.1 N aqueous HCl, and the resulting solutions were kept at 25.0 °C, 55.0 °C, 70.0 °C, or 80.0 °C in a thermoregulated constant-temperature bath (accurate to ±0.05 °C). At intervals, aliquots (1 ml) were withdrawn and diluted by a factor of 10 with the following high-performance liquid chromatography (HPLC) solvents. Small portions (14 μl) of the diluted solutions were then analyzed by means of HPLC. The HPLC analyses were carried out on a Waters ALC/GPC 204 liquid chromatograph by using a μBondapak C_{18} column [0.05 M KH_2PO_4–MeOH (90 : 10, v/v) and 1.5—1.7 ml/min for **4a**·1/2H_2O (X = ClO_4) and **4b**·H_2O (X = ClO_4); 0.1 M KH_2PO_4–MeOH (85 : 15, v/v) and 1.5 ml/min for **3**; 0.025 M Na_2HPO_4–MeOH (70 : 30, v/v) and 1.2 ml/min for **1**], and the peak heights of the nucleosides, located by using a UV absorbance detector operated at 254 nm, were determined. Concentrations of the unaltered substrates in the reaction mixtures were then estimated from calibration curves which had been obtained with nucleoside solutions of known concentration. All hydrolyses were followed for at least two half-lives with at least six measurements, and good pseudo-first-order kinetics were obtained in all cases. The results are summarized in Table I.

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