Purines. XXXIV. 1) 3-Methyladenosine and 3-Methyl-2′-deoxyadenosine: Their Synthesis, Glycosidic Hydrolysis, and Ring Fission

TOZO FUJII*, TOHRU SAITO, and TSUYOSHI NAKASAKA
Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan. Received March 8, 1989

The first syntheses of 3-methyladenosine (3a) and 3-methyl-2′-deoxyadenosine (3b), both in the form of the p-toluenesulfonate salt, have been achieved through two parallel 6-step routes starting from adenosine (5a) and 2′-deoxyadenosine (5b), which are based on the “fission and reclosure” technology for modification of the adenine ring. These 3-methyladenine nucleosides (3a, b·TsOH) were very unstable under not only acidic but also alkaline conditions. At pH 1 and 25 °C, 3a·TsOH (half-life 17 min) underwent glycosidic hydrolysis (depurinylatation) some thousand times faster than did adenosine (5a) itself. At pH 3.34 and 25 °C, the 2′-deoxyribosyl analogue 3b·TsOH (half-life 2.7 min) was depurinylated 370 times more rapidly than the ribosyl analogue 3a·TsOH (half-life 1010 min). The glycosidic bond of the imidazole 2′-deoxyriboside 11b, an intermediate for the present synthesis of 3b·TsOH, was also hydrolyzed easily at pH 1 and room temperature. In H2O at pH 8.32 and 25 °C, 3a·TsOH readily underwent ring opening in the pyrimidine moiety and came to equilibrium with the (N-methylformamido)imidazole derivative 10a, which was further deformedylated under more alkaline conditions. The ring opening was ca. 30 times as fast as that of 1-methyladenosine (17). In H2O at pH 8.98 and 25 °C, the 2′-deoxyribosyl analogue 3b·TsOH underwent not only similar ring opening but also glycosidic hydrolysis competitively. The possible factors responsible for the high reactivity of 3a·TsOH are discussed on the basis of its X-ray crystal structure.

Keywords 3-methyladenine nucleoside; ring fission–reclosure synthesis; hydrolysis; amidine formamido cyclization; glycosidic hydrolysis; depurinylatation; alkaline hydrolysis; ring-chain equilibrium; kinetic study; high-performance liquid chromatography

Among the 11 possible types of N1,N7-disubstitution in the adenine system (1) is 3,9-dialkyladenines (type 2), 3-substituted adenines (type 3a), 3-methyl-2′-deoxyadenosine (3b), and so forth. The 3-substituted adenosine family constitutes one of the four possible groups of positional isomers of N7-monosubstituted adenosines, and they have been prepared as modified cyclonucleoside derivatives with 3,41 extra N6-substituents (e.g., 4) or as N6,N7-dimethyl derivatives3 modified in the sugar moiety.6 Although 3-methyladenosine (3a) is the simplest member in this family, it was unknown at the time when the present study was undertaken, whereas the other three N7-methyladenosines [x = 1 (17), 6 (19) or 7 (7,8)] had already been synthesized. The third exemplar, 3-methyl-2′-deoxyadenosine (3b), has been assumed to occur as a part structure in methylated deoxyribonucleic acid (DNA) molecules.9 As far as DNA sequencing by the original Maxam–Gilbert method10 is concerned, dimethyl sulfate methylates the 2′-deoxyguanosines in DNA at the 7-position and the 2′-deoxyadenosines at the 3-position, rendering the glycosidic bond of the methylated families labile to hydrolysis on heating at neutral pH. Whereas the methylation of the latter is considerably slower than that of the former, release of the methylated purine base by hydrolysis is considerably faster from the 3-methyl-2′-deoxyadenosines in methylated DNA than from the 7-methyl-2′-deoxyguanosines. This forms a basis for distinguishing between the adenines and guanines in DNA.9,10 Because of such extraordinary instability of the glycosidic bond in the 3-methyl-2′-deoxyadenosine structure (3b) at the polynucleotide level, it is of prime importance to study this part structure at the nucleoside level. We thus investigated the syntheses and stability of 3-methyladenosine (3a) and 3-methyl-2′-deoxyadenosine (3b).

Brief accounts of the results recorded here have been published in preliminary form.11

Synthetic Routes
The target 3-methyladenine nucleosides 3a and 3b would be most directly accessible from adenosine (5a) and 2′-deoxyadenosine (5b) by methylation if the sugar group at the 9-position could orient the incoming methyl group to the 3-position, as in the cases of N6,N7-dimethyladenosine40 and its 2′,3′,5′-tri-O-benzoyl or 2′,3′,5′-tri-O-benzyl derivative40 as well as the 2′-deoxyadenosines in DNA. However, such a one-step route is not feasible since methylation of 5a (or its 2′,3′-O-isopropylidene derivative) and 5b themselves occurs preferentially at the 1-position.7,12 For the synthesis of the desired compounds 3a and 3b, therefore, a circuitous route had to be arranged, and we tried to extend our previous general synthetic route28,29 to 3,9-dialkyladenine (2) from 9-alkyladenine to

© 1989 Pharmaceutical Society of Japan
cover the nucleoside level.

The synthesis of the first target 3a started with methylation of the formamidomimidazole 8a, which was prepared from adenosine 5a through the N-oxide 6a and 1-benzylxoyadenosine perchlorate 7a according to the previously reported procedures. The methylation of 8a was effected with Mel in HCONMe2 in the presence of anhydrous K2CO3 at room temperature for 9 h, giving the N-methylformamido derivative 11a in 86% yield. The succeeding steps were removal of the N′-benzoxoxy group from 11a and cyclization of the resulting amide derivative to reach 3a. In the previous synthesis of 3,9-dimethyladenine hydrochloride (2·HCl, R1 = R2 = Me), the corresponding steps were catalytic hydrogenolysis in H2O containing 1 mol eq. of HCl (Raney Ni/H2, 1 atm, room temp.) and subsequent treatment of the product with Et3N in EtOH. When similar reaction conditions were applied to 11a, the desired conversions seemed to have occurred, but the cyclized product 3a·HCl could not be isolated in crystalline form. This difficulty was overcome by the use of p-toluenesulfonic acid (TsOH) instead of HCl for the hydrogenolysis step. Thus, 11a was hydrolyzed in the presence of Raney Ni catalyst and hydrogen (1 atm, room temp., 70 min) in H2O containing 1 mol eq. of TsOH, and crude 10a·TsOH that resulted was treated with a little Et3N in MeOH at room temperature for 48 h, producing the target nucleoside 3a in the form of the crystalline salt 3a·TsOH in 53% yield (from 11a). The correctness of the assigned structure 3a·TsOH was supported by elemental analysis, the above self-consistent reaction sequence through which it was formed, and its ultraviolet (UV) and nuclear magnetic resonance (NMR) spectra. The UV spectra in 95% aqueous EtOH (λmax 272 nm ε 16500) and in H2O at pH 3 (λmax 270 (17400) (slightly unstable)), 7 (λmax 270 (17400)), and 13 (unstable) were similar to those2h,8 of 3,9-dimethyladenine hydrochloride (2·HCl, R1 = R2 = Me) or perchlorate (2·HClO4, R1 = R2 = Me). The 1H-NMR spectrum in Me2SO-d6 exhibited a three-proton singlet at δ 4.19 [N(3)-Me], two one-proton singlets at 8.59 and 8.74 (purine protons), and two one-proton singlets at 9.12 and 9.21 (NH2), which resembled those observed2c for 3,9-dimethyladenine perchlorate (2·HClO4, R1 = R2 = Me), besides signals attributable to the protons in the ribosyl and p-toluenesulfonate moieties. Finally, the whole structure of 3a·TsOH, especially its exocyclic iminium character, has recently been confirmed by means of X-ray crystallographic analysis.16a

The second target 3-methyl-2′-deoxyadenosine (3b) was then prepared in the form of the p-toluenesulfonate salt 3b·TsOH through a parallel sequence of conversions starting from 2′-deoxyadenosine (5b). Oxidation of 5b with monoperothphonic acid to give the N-oxide 6b was carried out according to the procedure reported by Klenow and Frederiksen.17a In general agreement with previous results,13a,15,17b the reaction of 6b with PhCH2Br in AcNMe2 (room temp., 4 h) and treatment of the benzylated product with NaClO4 gave the 1-benzoxoxy derivative 7b in 85% yield. The perchlorate 7b was converted into the free base by the use of Amberlite IRA-402 (HCO3⁻), and the base was treated with H2O at 3−4°C for 8 d to afford the formamidomimidazole 8b in 70% yield. On methylation with Mel and anhydrous K2CO3 in HCONMe2 at room temperature for 5 h, 8b furnished the N-methylformamido derivative 11b (70% yield), which was hydrogenolyzed in H2O with Raney Ni catalyst and hydrogen at room temperature in the presence of 1 mol eq. of TsOH. The crude 10b·TsOH that formed was then treated with a little Et3N in MeOH at −18°C for 3−9 d to give the desired compound 3b·TsOH in 22% yield (from 11b). The UV spectrum of 3b·TsOH in H2O at pH 7 (λmax 271 nm ε 16900) (slightly unstable) was closely similar to that of 3a·TsOH, but those in H2O at pH 1 and 13 rapidly and that in 95% aqueous EtOH slowly changed the shape of their curves during measurement. The 1H-NMR spectrum in Me2SO-d6.
[selected peaks: δ 2.28 (3H, s, MeC₆H₄SO₃), 4.19 [3H, s, N(3)-Me], 8.63 and 8.71 (1H each, s, purine protons), 9.15 and 9.23 (1H each, br s, = NH₂)] was also similar to that of 3α·TsOH, supporting the correctness of the assigned structure 3β·TsOH.

Glycosidic Hydrolysis of 3-Methyladenine Nucleosides

Remarkable was the instability of the 3-methyladenine nucleosides thus synthesized. On treatment with 0.1 N aqueous HCl (pH ca. 1) at 27°C for 1 h, 3α·TsOH underwent glycosidic hydrolysis (depurinylation) to give 3-methyladenine (9) in 92% yield (Chart 1). We monitored this depurinylation at 25°C, determining the unaltered nucleoside by means of high-performance liquid chromatography (HPLC), and obtained a pseudo-first-order rate constant of 4.00 × 10⁻² min⁻¹ and a half-life of 17 min (Table 1). Adenosine (5α) itself was virtually stable under the same reaction conditions for at least 3 h, but the rate constant for its depurinylation at pH 1 and 37°C was estimated to be 2.16 × 10⁻⁵ min⁻¹ (half-life 22 d) using data reported by Venner. It thus became clear that the introduction of a methyl group into adenosine (5α) at the 3-position made glycosidic hydrolysis some thousand times faster under acidic conditions. The 2-deoxyribosyl analogue 3β·TsOH was even more susceptible to solvolysis; it afforded 9 in 60% yield on treatment with H₂O at pH 3.34 and 20°C for 45 min and in 99% yield on treatment with boiling MeOH for 30 min.

The kinetic study was then extended to include the glycosidic hydrolyses of both 3-methyladenine nucleosides 3α·TsOH and 3β·TsOH in H₂O at various pH’s with a view to comparing their stabilities. It may be seen from Table 1 that the 2-deoxyribosyl analogue 3β·TsOH is very unstable even at pH 7.00 and 37°C, and its rate of hydrolysis at low pH increases with increasing hydronium ion concentration: at pH 3.34 and 25°C it hydrolyzes 370 times more rapidly than does the ribosyl analogue 3α·TsOH, being in general agreement with the fact that 2‘-deoxyadenosine (5β) itself

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction conditions</th>
<th>Pseudo-first-order rate constant (min⁻¹)</th>
<th>Half-life (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyalted DNA</td>
<td>pH</td>
<td>Temp. (°C)</td>
<td>4.81 × 10⁻⁴</td>
</tr>
<tr>
<td>3-Methyl-2‘-deoxyadenosine p-toluene sulfonate (3β·TsOH)</td>
<td>7.0</td>
<td>37</td>
<td>1.01 × 10⁻²</td>
</tr>
<tr>
<td>3-Methyladenosine p-toluene sulfonate (3α·TsOH)</td>
<td>7.00</td>
<td>37</td>
<td>1.99 × 10⁻²</td>
</tr>
<tr>
<td>3-Methyladenosine p-toluene sulfonate (3α·TsOH)</td>
<td>5.00</td>
<td>37</td>
<td>3.91 × 10⁻²</td>
</tr>
<tr>
<td>3-Methyladenosine p-toluene sulfonate (3α·TsOH)</td>
<td>3.34</td>
<td>25</td>
<td>2.54 × 10⁻¹</td>
</tr>
<tr>
<td>Adenosine (5α)</td>
<td>pH</td>
<td>Temp. (°C)</td>
<td>6.87 × 10⁻⁴</td>
</tr>
<tr>
<td>1¹</td>
<td>7.00</td>
<td>25</td>
<td>4.00 × 10⁻²</td>
</tr>
<tr>
<td>3α·H⁺, Z=OH</td>
<td>1</td>
<td>37</td>
<td>2.16 × 10⁻⁵</td>
</tr>
<tr>
<td>3β·H⁺, Z=OH</td>
<td>7.0</td>
<td>37</td>
<td>1.01 × 10⁻²</td>
</tr>
<tr>
<td>3α·2H⁺, Z=OH</td>
<td>5.00</td>
<td>37</td>
<td>1.99 × 10⁻²</td>
</tr>
<tr>
<td>3β·2H⁺, Z=OH</td>
<td>3.34</td>
<td>25</td>
<td>2.54 × 10⁻¹</td>
</tr>
</tbody>
</table>

Table 1. Rate Constants for the Glycosidic Hydrolyses of Adenine Nucleosides and for the Release of 3-Methyladenine (9) from Methylated DNA in H₂O

---

*α) Taken from ref. 24. *b) Data from the hydrolysis in 0.1 N aqueous HCl. *c) Estimated by using data from ref. 18.
undergoes glycosidic hydrolysis about 1000 times faster than does adenosine (5a) itself.\textsuperscript{19}

Why do the 3-methyladenine nucleosides 3a·TsOH and 3b·TsOH undergo depurination so readily? A key to the solution of this question may be obtained from the recently reported X-ray crystal structure of 3a·TsOH.\textsuperscript{16} In the crystal, this salt has an $N^6$-protonated structure in which the exocyclic iminium structure, as shown in formula 3a·TsOH (Chart 1), is a very important contributor to the possible resonance hybrid. The adenine moiety is almost planar, and the $N(9)$–C(1') bond is almost coplanar with the adenine ring. However, the $N(3)$-methyl group is displaced rather significantly from planarity, and the endocyclic and exocyclic angles at $N(3)$, C(4), and $N(9)$ notably depart from those of the usual adenine systems, being in favor of keeping the $N(3)$-methyl and $N(9)$-ribofuranoside groups away from each other. The ribose moiety is in the C(2')-endo (E) puckering conformation and in the high anti (part of syn) conformation with the torsion angle O(1')–C(1')–N(9)–C(4), $\chi = -72.3^\circ$ respect with the adenine moiety. Assuming 3a·TsOH maintains its exocyclic iminium structure even in solution and an A-1 mechanism\textsuperscript{19,20} for solvolyses of nucleosides is operating in its hydrolysis, transition structures 12a and 13a derived from the mono- (3a·H$^+$) and diprotonated (3a·2H$^+$) forms may be considered, as shown in Chart 2. Since the basicity of 3,9-dimethyladenine (2, R$^1$ = R$^2$ = Me) has been shown to be considerably high,\textsuperscript{3a,3c} 3a·TsOH probably exists almost completely in the monoprotonated form (3a·H$^+$) even at pH 7. This view may be supported by the structural analogy between 3a·H$^+$ and the "para-quinonoidal" resonance structure (14) of protonated 1,4-dihydro-4-imino-1-methylpyrimidine whose $pK_a$ is 12.22.\textsuperscript{21} The above X-ray structure suggests that the $N(3)$-methyl group does not exert much of a steric repulsion with the ribosyl moiety, being in agreement with the recent result of molecular mechanics calculations by Czarnik's group.\textsuperscript{22} However, the unusual geometry of the adenine moiety described above may cause the free energy of the starting structure (3a·H$^+$) to be raised, whereas the transition structure (12a) may be stabilized by resonance, thus permitting the activation energy to be lower.

At low pH's the diprotonated species 3a·2H$^+$ should be favored with increasing hydronium ion concentration. The UV spectra of 3,9-dimethyladenine hydrochloride (2·HCl, R$^1$ = R$^2$ = Me)\textsuperscript{22a,22c} or perchlorate (2·HClO$_4$, R$^1$ = R$^2$ = Me)\textsuperscript{22b} in H$_2$O at pH 7 [where it is considered to exist almost completely in the monoprotonated form (vide supra)] and at pH 1 are very similar, but not superimposable on each other. This is also the case for 3a·TsOH, suggesting the existence of the diprotonated species (3a·2H$^+$) at low pH's. The second site of protonation is considered to be N(7), as proposed for the acid-catalyzed solvolysis of protonated adenine nucleosides,\textsuperscript{19a,23} since the nitrogens in the pyrimidine moiety must be less basic owing to the exocyclic iminium structure. Assuming the geometry of the adenine moiety in 3a·2H$^+$ to be similar to that in 3a·H$^+$, the accelerated depurination of 3a·TsOH at low pH's may be explained as in the case of the monoprotonated species.

The observed 370-fold rate acceleration of the 2-deoxyribosyl analogue 3b·TsOH compared to the ribosyl analogue 3a·TsOH is attributable to the absence of the C(2')-OH group, which in a parallel A-1 pathway (Chart 2) inhibits its inductive effect the slow N(9)–C(1') bond cleavage of a protonated nucleoside that results in a C(1') carbocation.\textsuperscript{19,20a} Interestingly, the 3',5'-cyclonucleoside 15 has recently been found to undergo glycosidic hydrolysis 29000 times more slowly than does 3a·TsOH at 25°C in 0.1 N aqueous HCl.\textsuperscript{22} This surprising stability has been explained in terms of (1) the presence of an electron-withdrawing immonium group in 15 at the 5'-position and (2) the generation of an "anti-Bredt transition structure",\textsuperscript{22}

Further interest in the glycosidic hydrolysis stems from the comparison of the rate of depurinylation of 3b·TsOH with that\textsuperscript{24} of methylated DNA which includes 3b as a part structure. It may be seen from Table I that at pH 5.0 and 7.0 methylated DNA releases 3-methyladenine (9) ca. 40 times more slowly than does 3-methyl-2'-deoxyadenosine p-toluenesulfonate (3b·TsOH). This order of the depurinylation rate is coincident with that reported\textsuperscript{25} for the release of 7-methylguanine in the 7-methyl-2'-deoxyguanosine series [the nucleoside level (half-life at pH 7.4 and 37°C, 5.9 h)\textsuperscript{25a} > the nucleotide level (23 h)\textsuperscript{25b} > the DNA level (69 h)\textsuperscript{25b}]. It is suggested that the effect of the negative charge in the phosphate moiety\textsuperscript{25a,25b} and higher-order structure of the molecule on the glycosidic hydrolysis should be taken into account at the DNA level.

**Ring Fission of 3-Methyladenine Nucleosides**

Another notable feature of the chemical behavior of 3a·TsOH and 3b·TsOH was that they were also unstable
under basic conditions, as in the cases of 3,9-dialkyladenine salts (2-HX)\(^{2,21}\), 3,5'-cycloadenosine derivatives (e.g., 4)\(^{2,2,22,27}\) and N\(^6\),N\(^\prime\)-3-trimethyladenosine derivatives.\(^{2,5}\) On treatment with Amberlite CG-400 (OH\(^-\)) in H\(_2\)O at room temperature, 3a-TsOH underwent ring opening in the adenine moiety, and the methylaminoimidazole 16a was isolated in the form of the dihydrochloride (16a·2HCl) (87\%) yield, paralleling the previous results\(^{2,21}\) from a similar treatment of 3,9-dimethyladenine hydrochloride (2·HCl, R\(^1\)=R\(^2\)=Me). In view of the low electron density at the 2-position of 3a·TsOH, the observed conversion may be rationalized in terms of an initial nucleophilic attack of hydroxide ion at the 2-position and succeeding steps proceeding through ring opening to form 10a and decomplexation, as delineated in Chart 3. In 0.1 M aqueous NaHCO\(_3\), the UV spectra of both 3a·TsOH and 10a·TsOH separately changed with time through the same isosbestic point at 253 nm, converging on an identical spectrum. This indicated the existence of equilibrium between the purine 3a and the imidazole 10a under weakly alkaline conditions, as in the cases of 3,9-dialkyladenines (2)\(^{2,20}\). We then followed UV spectrophotometrically the time-courses of the ring opening of 3a·TsOH and of cyclization of 10a·TsOH in 0.1 M aqueous NaHCO\(_3\) (pH 8.32) at 25°C, and the pseudo-first-order rate constants and equilibrium constant for the reversible reaction system (Chart 3) were determined to be \(k=6.62 \times 10^{-3}\) min\(^{-1}\), \(k'=6.88 \times 10^{-3}\) min\(^{-1}\), and \(K=k/k'=0.96\). Isomeric with 3a is 1-methyladenosine (17), which is known to give N\(^6\)-methyladenosine (19) under basic conditions through Dimroth rearrangement.\(^{7,12}\) The rate (\(k_{\text{obsd}}\)) of this rearrangement in H\(_2\)O at pH 8.32 and 25°C is calculated to be \(2.11 \times 10^{-4}\) min\(^{-1}\) on the basis of the rate law reported by Macon and Wolfenden.\(^{28}\) In view of its reaction mechanism involving a rate-determining initial ring opening (17→18→17') and a subsequent fast ring closure (18→19) (Chart 4),\(^{12,28,29}\) the rate of the ring opening corresponding to that of 3a·TsOH may be regarded as that (\(k_{\text{obsd}}\)) of the rearrangement itself. This leads to the conclusion that ring opening of 3a·TsOH in H\(_2\)O at pH 8.32 and 25°C is ca. 30 times as fast as that of the 1-methyl isomer 17.

We next monitored the reaction of the 2-deoxyribosyl analogue 3b·TsOH in H\(_2\)O at pH 8.98 and 25°C by means of HPLC. The results revealed that 3b·TsOH was slowly converted into 3-methyladenine (9) in 45 h, during which time the temporary formation of the monocycle 10b was observed. The appearance of 10b during the reaction is attributable to the attainment of equilibrium with the starting material, being in agreement with the ring opening of the ribosyl analogue 3a·TsOH described above. The observed glycosidic cleavage in alkaline solution to produce 3-methyladenine (9) was quite notable since nucleosides are generally very resistant to alkaline hydrolysis.\(^{29,30}\) In view of the unique structure of 3b·TsOH in which the base moiety is protonated even at pH 8.98, the formation of 9 is assumed to be a result of the glycosidic hydrolysis of 3b·TsOH, and the possibility of the alternative pathway 10b→20→9 (Chart 5) may be excluded.

Although loss of 3-methyladenine (9) from methylated DNA in vivo could be explained in terms of chemical depurination alone, active enzymatic excision has also
been suggested.\textsuperscript{24} This led to the isolations of 3-methyladenine-DNA glycosylase in partially purified form from both bacterial and mammalian sources.\textsuperscript{31} The enzymatic release of 3-methyladenine (9) from methylated DNA has been reported\textsuperscript{21,25} to be markedly dependent on the secondary structure of the DNA: double-stranded DNA is an effective substrate, whereas single-stranded DNA is only attacked at a very slow rate, but a short alkali-treatment has usually been applied to cause separation of the methylated DNA strands.\textsuperscript{21,25} Considering the instability of the 3-methyl-2'-deoxyadenosine part structure in the DNA under alkaline conditions as inferred from the observations at the nucleoside level, the latter half of the above conclusion appears to be unsound.

Glycosidic Hydrolysis of Imidazole Nucleosides

The glycosidic bond of the imidazole 2-deoxyriboside 11b was also unstable in aqueous acidic solution. On treatment with 0.1 N aqueous HCl at room temperature for 3.5 h, 11b furnished the aglycone 21 in 61\% yield. The ribosyl analogue 11a was found to be stable under the same conditions for at least 12 h. The structure of 21\textsuperscript{32} was confirmed by its cyclization in ethanolic HCl at room temperature to produce N'-benzoyl-3-methyladenine (22)\textsuperscript{33} and by its hydrolysis (Raney Ni/H\textsubscript{2}, 50\% aqueous EtOH, 1 atm, 30\°C, 3 h) and spontaneous cyclization to yield 3-methyladenine (9). It has been shown that the glycosidic bond of 1-\beta-D-ribofuranosylimidazole is resistant to acid hydrolysis, but introduction of an amino group into the 5-position facilitates the hydrolysis.\textsuperscript{23a,30a} The ready glycosidic hydrolysis observed for 11b may be attributed to electron-withdrawing effects of the formamido and the N'-benzoylamidino groups in the protonated form and to the 2-deoxyribosyl structure.

Conclusion

The syntheses of the 3-methyladenine nucleosides 3a-TsOH and 3b-TsOH have now become possible through two parallel six-step routes starting from adenosine (5a) and 2'-deoxyadenosine (5b), respectively, as shown in Chart 1. This emphasizes the synthetic utility of our "fission and reclosure" technology\textsuperscript{24} for modification of the adenine ring. These new nucleosides are very unstable under not only acidic but also basic conditions. At pH 1 and 25\°C, 3-methyladenosine p-toluene sulfonate (3a-TsOH) (half-life 17 min) undergoes glycosidic hydrolysis (depurination) some thousand times faster than does adenosine (5a) itself. This accelerated depurination may be attributed to the N'-protonated structure (even in the weakly alkaline region) of 3a-TsOH in which the exocyclic iminium structure is a very important contributor to the possible resonance hybrid and to the unusual geometry of the adenine moiety, as indicated by its X-ray crystal structure.\textsuperscript{16} The 2-deoxyribosyl analogue 3b-TsOH is depurinated at pH 3.34 and 25\°C (half-life 2.7 min) 370 times more rapidly than the ribosyl analogue 3a-TsOH and at pH 5.0 or 7.0 at 37\°C ca. 40 times more rapidly than the corresponding structure in methylated DNA. The glycosidic bond of the imidazole 2-deoxyriboside 11b, an intermediate for the present synthesis of 3b-TsOH, is also hydrolyzed easily at pH 1 and room temperature.

In H\textsubscript{2}O at pH 8.32 and 25\°C, the ribosyl analogue 3a-TsOH easily opens its adenine ring and comes to equilibrium with the (N-methylformamido)imidazole 10a, which is deformylated under more basic conditions to afford 16a. The ring opening is ca. 30 times as fast as that (17 → 18) of 1-methyladenosine (17). The 2-deoxyribosyl analogue 3b-TsOH undergoes not only similar ring opening in H\textsubscript{2}O at pH 8.98 and 25\°C, equilibrating with the monocyte 10b, but also competitive glycosidic hydrolysis to give 3-methyladenine (9) in 45 h.

It is hoped that the present findings concerning the extraordinary instability of the 3-methyladenine nucleosides (3a,b-TsOH), especially that of the 3-methyl-2'-deoxyadenosine structure (3b) under alkaline conditions, will be of help in reaching a better understanding of the nature of the corresponding structure at the polynucleotide level when taken into account in chemical and biochemical studies of methylated DNA.

Experimental

General Notes

All melting points were determined by using a Yamato MP-1 capillary melting point apparatus and are corrected. Paper partition chromatographies (PPC) were developed as described in ref. 2c. High-performance liquid chromatographic (HPLC) analyses were carried out on a Waters ALC/GPC 204 liquid chromatograph. See refs. 1 and 2c for details of other instrumentation and measurements. 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, s = singlet, sh = shoulder, t = triplet.

1-Benzoyl-2'-deoxyadenosine Perchlorate (7b) A mixture of 2'-deoxyadenosine 1-oxide (6b)\textsuperscript{7c} (5.89 g, 22 mmol) and PbH\textsubscript{2}Br (18.8 g, 110 mmol) in AcNMe\textsubscript{2} (50 ml) was stirred at room temperature for 4 h. The resulting solution was diluted with ether (300 ml) to deposit an oil, which was separated from the ethereal layer by decantation, washed with ether (2 × 60 ml), and then dissolved in warm H\textsubscript{2}O (80 ml). The aqueous solution was washed with benzene (2 × 40 ml) and kept, after addition of a solution of NaClO\textsubscript{4} (13.47 g, 110 mmol) in H\textsubscript{2}O (20 ml), in a refrigerator overnight. The precipitate that resulted was filtered off, washed with a little H\textsubscript{2}O, and dried to give 7b (8.61 g, 85\%); mp 143.5–144.5\°C (dec.). Recrystallization from EtOH yielded an analytical sample as colorless minute prisms, mp 143.5–144.5\°C (dec.). UV \textit{λ}\textsubscript{max}\textsuperscript{1H}OH 258 nm (ε 12700), 263 (sh) (11900); \textit{λ}\textsubscript{max}\textsuperscript{pH7} (pH 7) 259 (13100); \textit{λ}\textsubscript{max}\textsuperscript{pH1} (pH 1) 259 (13200), 262 (sh) (13200); NMR (Me\textsubscript{4}SO-d\textsubscript{4}) \textit{δ} 5.41 (2H, s, OCH\textsubscript{3}Ph), 6.38 [1H, dd, J = 6.6 Hz each, C(1')-H], 7.40–7.70 (5H,
br. OCH$_2$Ph). 8.76 and 8.94 (1H each, s, purine protons). Anal. Calcld for C$_{11}$H$_{11}$N$_2$O$_3$•H$_2$O: C, 44.60; H, 4.40; N, 15.30. Found: C, 44.36; H, 4.31; N, 15.32.

N-**Benzyloxy-1-(2-deoxy-β-D-ribofuranosyl)-5-formamido-1H-imidazole-4-carboxamide (8B) A solution of 7b (4.12 g, 9 mmol) in H$_2$O (400 mL) was adjusted to pH 1.75 (Column of Amberlite IRA-402 (2.0 × 36 mm) and eluted with 1.36 M HCl, and then concentrated to a volume of ca. 80 mL. The residual solution was kept in a refrigerator at 3–4°C for 8 d and then concentrated to dryness in vacuo to leave a colorless glass. Purification of this substance by column chromatography [silica gel (30 g), CHCl$_3$:EtOH (7: 1, v/v), followed by column chromatography with CHCl$_3$] afforded 8b (2.41 g, 70%) as a colorless solid, m.p. 137–139°C (dec.). The solid was recrystallized from AcOEt and dried over P$_2$O$_5$ at 2 mmHg and 5°C for 20 h to give an analytical sample of 8b 1/4H$_2$O as colorless needles, m.p. 138–139°C (dec.). M; m/z: 375 (M+); UV $\lambda_{max}$ (pH 1) 253 mm (c 8600), $\lambda_{max}$ (pH 7) 250 (sh) (7200). $\lambda_{max}$ (pH 1) 253 (12400). NMR (Me$_2$SO-d$_6$): $\delta$ 4.87 and 4.93 (2H each, OCH$_2$Ph), 5.50–5.70 (2H, br, NH$_2$), 5.68 and 5.87 (2H, dd each, J = 6 Hz each, C(1′)-H), 7.28–7.40 (5H, m, OCH$_2$Ph), 7.94 [1H, s, C(2′)-H], 8.03 [0.9, H, J = 10.7 Hz, HCONJ (trans)], 8.20 [0.9, H, J = 4.0 Hz, HCONJ (cis)], 9.42 [0.5, H, J = 10.7 Hz, HCONJ (trans)], 9.66 [0.5, H, J = 4.0 Hz, HCONJ (cis)].* Anal. Calcld for C$_{13}$H$_{13}$N$_2$O$_4$: C, 53.75; H, 5.70; N, 14.56; N$_2$, 3.76.

N-**Benzyloxy-5-(N-methylformamido)-1-(D-ribofuranosyl)-1H-imidazole-4-carboxamide (11a) A mixture of 8a (5.87 g, 15 mmol) and anhydrous K$_2$CO$_3$ (3.11 g, 22.5 mmol) in HCONMe$_2$ (95 mL) was stirred at room temperature for 1 h. A solution of Me$_2$N (2.56 g, 18 mmol) in HCONMe$_2$ (5 mL) was then added, and the resulting mixture was stirred at room temperature for 1 h. The reaction was concentrated in vacuo to leave a yellowish brown jelly, which was triturated under nitrogen and then treated with 20 mL of sodium methoxide (2.0 M) and stirred at room temperature for 1 h. The residue was collected by filtration, washed with cold H$_2$O (15 mL), and dried to give 11a (4.61 g, 76%), mp 154–156°C. The filtrate was treated with AcOEt (3 × 50 mL), and the AcOEt extracts were washed with saturated aqueous NaCl (20 mL), dried over anhydrous MgSO$_4$, and concentrated in vacuo. The residue was crystallized from CHCl$_3$ to afford a second crop of 11a (594 mg, 10%), as a colorless solid, mp 154–156°C. The total yield was 5.20% (86%). For analysis, crude 11a was recrystallized from AcOEt to furnish colorless prisms, mp 160–161°C (dec.); [α]$_{D}$ $^{27.7}$ (c 1.01, MeOH); UV $\lambda_{max}$ (MeOH) $\delta$ 260 nm (sh) (6600), 270 nm (sh) (7800), 275 nm (sh) (12400), 300 nm (sh) (23000), 310 nm (sh) (25000). Anal. Calcld for C$_{13}$H$_{12}$N$_2$O$_4$: C, 51.75; H, 4.45; N, 15.36.

N-**Benzyloxy-1-(2-deoxy-β-D-ribofuranosyl)-5-(N-methylformamido)-1H-imidazole-4-carboxamide (11b) A mixture of 8b (2.63 g, 0.66 mmol) and anhydrous K$_2$CO$_3$ (145 mg, 1.05 mmol) in HCONMe$_2$ (5 mL) was stirred at room temperature for 1 h, and then Mel (120 mg, 0.84 mmol) was added. After having been stirred at room temperature for 5 h, the reaction mixture was concentrated in vacuo to leave a yellowish brown jelly, which was extracted with boiling AcOEt (3 × 15 mL). Concentration of the AcOEt extracts under reduced pressure left a colorless glass. This was triturated with AcOEt (30 mL) under cooling and in a refrigerator overnight. The precipitate that resulted was filtered off, washed with a little AcOEt, and dried to give a first crop (134 mg, 50%) of 11b as a colorless powder, mp 139–140°C (dec.). The filtrate and washings were combined and concentrated in vacuo, and the residue was purified on thin-layer chromatography (TLC) [silica gel, CHCl$_3$:EtOH (8: 1, v/v)], and PPC analyses, which indicated that 11a was completely stable over a period of at least 12 h.

**N-**Benzyloxy-1-(2-deoxy-β-D-ribofuranosyl)-5-(N-methylformamido)-1H-imidazole-4-carboxamide (11b) A mixture of 8b (2.63 g, 0.66 mmol) and anhydrous K$_2$CO$_3$ (145 mg, 1.05 mmol) in HCONMe$_2$ (5 mL) was stirred at room temperature for 1 h, and then Mel (120 mg, 0.84 mmol) was added. After having been stirred at room temperature for 5 h, the reaction mixture was concentrated in vacuo to leave a yellowish brown jelly, which was extracted with boiling AcOEt (3 × 15 mL). Concentration of the AcOEt extracts under reduced pressure left a colorless glass. This was triturated with AcOEt (30 mL) under cooling and in a refrigerator overnight. The precipitate that resulted was filtered off, washed with a little AcOEt, and dried to give a first crop (134 mg, 50%) of 11b as a colorless powder, mp 139–140°C (dec.). The filtrate and washings were combined and concentrated in vacuo, and the residue was purified on thin-layer chromatography (TLC) [silica gel (16 g), CHCl$_3$:EtOH (7: 1, v/v)], and PPC analyses, which indicated that 11a was completely stable over a period of at least 12 h.

Acid Hydrolysis of 3a TsoH Leading to 3-Methyladenine (9) A solution of 3a TsoH (363 mg, 0.8 mmol) in 0.1 n aqueous HCl (80 mL) was allowed to stand at 27°C for 1 h. The reaction mixture was concentrated to dryness in vacuo to leave a solid, which was dissolved in warm H$_2$O (0.5 mL). The aqueous solution was made alkaline by addition of conc. aqueous NaOH, and the colorless crystals that deposited were filtered off, washed with a small amount of cold H$_2$O, and dried to give 9 (109 mg, 92%), mp 295–297°C (dec.). Recrystallization from H$_2$O yielded a pure sample as colorless needles, mp > 300°C. This sample was identical by
Acidic Hydrolysis of 3b-TsOH Leading to 3-Methyladenine (9) A solution of 3b-TsOH (17.0 mg, 0.0309 mmol) in 0.2 M acetic buffer (pH 3.34 at 25°C) (1 ml) was kept at 20°C for 45 min. The reaction mixture was concentrated to dryness in vacuo and the residue was extracted by methanolic HCl solution. The ethanolic extract was washed with water, and the aqueous layer was concentrated to a colorless solid. Recrystallization of the solid from chloroform-1.5% methyl acetate (3 mg, 86%) yielded colorless needles, mp > 300°C. This sample was identical (by comparison of the IR spectrum and PCC and TLC mobilities) with authentic 3-Methyladenine (9).

Conversion of 3a-TsOH into 5-(Methylamino)-1-beta-D-ribofuranosyl-1H-imidazole-4-carboxamide (16a) A solution of 3a-TsOH (453 mg, 1 mmol) in H2O (20 ml) was passed through a column of Amberlite CG-400 (OH-) (13 ml) and the column was eluted with H2O. The eluate (100 ml) was concentrated to dryness in vacuo to leave a colorless glass (274 mg).

The glass was dissolved in MeOH (10 ml) and a solution of conc. aqueous HCl (200 mg) in MeOH (2 ml) was added. The resulting mixture was concentrated to dryness in vacuo to give 16a-2HCl·H2O (316 mg, 87%) as a colorless solid, mp 102°C (dec.). Recrystallization of the solid was effected by dissolving it in MeOH and adding ether to the methanolic solution. An analytical sample (dried over P2O5 at 2 mmHg and room temperature for 48 h) as colorless prisms, mp 99—102°C (dec.); UV (2% MeOH) λmax (nm) (log ε) 287 (7.49), 260 (1.75), 260 (2.29), 208 (2.29), 192 (4.60), 166 (4.29) (SH, NMe, H2O, and H2O). 182 (1.78, 205 (1.78), 165 (4.12) (4H, protonated amine protons). Anal. Calcd for C31H31N9O2·2HCl·H2O·C, 33.16; H, 5.84; N, 19.33. Found: C, 33.45; H, 5.97; N, 19.48.

Hydrolysis of 3b-TsOH at pH 8.98 Reaction of 3b-TsOH in 0.1 M carbonate buffer [pH 8.98 and ionic strength 0.5 (KCl)] at 25°C was monitored by means of HPLC analysis in a manner similar to that described below in the kinetic procedure for acidic hydrolysis of 3b-TsOH. The results are summarized in the text.

N-Benzoyl-5-(N-methylformamido)-1H-imidazole-4-carboxamide (21) A solution of 11b (195 mg, 0.50 mmol) in 0.1 n aqueous HCl (20 ml) was stirred at room temperature for 3.5 h. The reaction mixture was neutralized with saturated aqueous NaHCO3 and extracted with CHCl3 (5 x 10 ml). The CHCl3 extracts were washed with saturated aqueous NaCl (10 ml), dried over anhydrous MgSO4, and concentrated in vacuo to leave a glass. The glass was purified by preparative TLC (silica gel, CHCl3·EtOH·H2O (8:1.1.v/v/v) to give 21 (83 mg, 61%), as a colorless glass, MS m/z: 273 (M+); UV λmax (MeOH) 230 (log ε 3.93), 214 (3.71), 202 (3.71) (minor) and 3.71 (minor) (SH, each, HCONMe, 2.94 (2H, s, OC(OH)2), 5.70 (minor) and 5.98 (major) (2H, br, NH2), 7.30—7.40 (5H, 5H, H2O), 7.63 (1H, s, C2-H), 8.16 (1H, s, JCONMe), 12.48 (1H, br. N-H)(29).

Conversion of 21 into N-Benzyl-3-aminomethyl-22 A solution of 21 (10 mg) in 10% (v/v) ethanolic HCl (3 ml) was allowed to stand at room temperature for 72 h. The precipitate that resulted was filtered off and dissolved in H2O (ca. 0.5 ml). The aqueous solution was neutralized with 5% aqueous NH4 and concentrated to dryness in vacuo. Recrystallization of the residue from 30% (v/v) aqueous EtOH gave 22 as colorless prisms, mp 180—181°C This sample was identical (by melting point test and comparison of the IR spectrum and PCC and TLC mobilities) with authentic 22.33

Conversion of 21 into 3-Methyladenine (9) A solution of 21 (35 mg, 0.13 mmol) in 50% (v/v) aqueous EtOH (3 ml) was hydrogenated over a small amount of Raney Ni W-2 catalyst35 at atmospheric pressure and 30°C for 3 h. The catalyst was filtered off by filtration and washed with H2O (3 ml). The combined filtrate and washings were concentrated to dryness in vacuo to leave 9 (16 mg, 84%) as a colorless solid, mp 295—296°C (dec.). Recrystallization from H2O gave a pure sample as colorless needles, mp > 300°C. This sample was identical (by comparison of the IR spectrum and PCC and TLC mobilities) with authentic 3-Methyladenine (9).

Kinetic Procedure i) Glycric Acid Hydrolysis of 3a-TsOH in 0.1 N Aqueous HCl: The substrate (3a-TsOH) was dissolved, at a concentration of 1.3 × 10−3 M, in 0.1 N aqueous HCl kept at 25 ± 0.05°C in a thermostatically controlled, constant-temperature bath. At intervals, aliquots (1 ml) of the reaction solution were withdrawn and diluted with 0.05 M CH3PO4·MeOH (90:10, v/v) by a factor of 10. Small aliquots of diluted solutions were then analyzed by means of HPLC. The HPLC analyses were carried out on a µBondapak C18 column (0.05 mm x 5 mm) with 2 ml/min flow rate, the peak height of the substrate, located by using a UV absorbance detector operated at 254 nm, was determined.

Concentration of the unaltered substrate in the reaction mixture was then estimated from a calibration curve which had been obtained with substrate solutions of known concentration, and the decrease of the concentration of the substrate was found to obey pseudo-first-order kinetics. The results are summarized in the text and Table 1.

ii) Glycosidic Hydrolysis of 3a-TsOH and 3b-TsOH at pH 3.34, 5.00, and 7.00: Buffer solutions used for these hydrolyses were 0.2 M HCl—NaOAc (pH 3.34 at 25°C), 0.2 M AcO—NaOAc (pH 5.00 at 37°C), and 0.2 M K2HPO4—NaHPO4 (pH 7.00 at 37°C). The substrates were separately dissolved in one of the buffer solutions at 2.0 × 10−5 M, and the resulting solutions were kept at 25 ± 0.05°C or at 37 ± 0.05°C in a thermostatically controlled-constant temperature bath. Decrease of the substrate in each solution was followed by means of HPLC in a manner similar to that described above under item (i) except that MeOH:0.05 M K2HPO4—NaHPO4 (pH 6.0) (10/90, v/v) was used for 10-fold dilution of the reaction mixture and for elution of the HPLC column. In all cases, good pseudo-first-order kinetics were obtained. The results are listed in Table 1.

iii) Equilibrium between 3a-TsOH and 10a-TsOH at pH 8.32: The ring opening reaction of 3a-TsOH and cyclization of 10a-TsOH in 0.1 M aqueous NaHCO3 (pH 8.32) at 25°C were separately followed by UV spectrophotometry in a manner similar to that reported previously for the 3.9-dialkyladenine (type 2) series. The results are given in the text.

Acknowledgment Financial support provided by the Ministry of Education, Science and Culture, Japan in the form of a Grant-in-Aid for Scientific Research (B-56470117) and by the Japan Research Foundation for Optically Active Compounds is gratefully acknowledged.


32) In regard to the tautomeric form in the imidazole ring, we prefer to assign tentatively the 5-(N-methylformamido)-1H-imidazole structure by analogy with the previously reported 5-formamido analogue [T. Fujii, T. Saito, and T. Itaya, Chem. Pharm. Bull., 19, 1731 (1971)].


35) The observed complexity of the proton signals is interpretable in terms of cis-trans isomerism of the formamido moiety, as we have experienced in similar structures in similar structures.

