THE 11 POSITIONAL ISOMERS OF \( N^\alpha,N^\beta \)-DIMETHYLADENINE: THEIR CHEMISTRY, PHYSICOCHEMICAL PROPERTIES, AND BIOLOGICAL ACTIVITIES

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Abstract — Various \( N^\alpha,N^\beta \)-disubstituted adenines are represented by the corresponding 11 possible positional isomers of \( N^\alpha,N^\beta \)-dimethyladenine, namely, \( N^6,N^6 \)- (2), \( N^6,1 \)- (3), \( N^6,3 \)- (4), \( N^6,7 \)- (5), \( N^6,9 \)- (6), 1,3- (7), 1,7- (8), 1,9- (9), 3,7- (10), 3,9- (11), and 7,9-dimethyladenine (12). The chemistry, physicochemical properties, and biological activities of these \( N^\alpha,N^\beta \)-dimethyladenines are reviewed with 513 reference citations.

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I. INTRODUCTION

Structurally unique is adenine (1), an important fundamental biomolecule, in that it carries one exocyclic and four endocyclic nitrogen atoms. Accordingly, five kinds of mono-N-substitution pattern and 11 kinds of di-N-substitution pattern are possible for this heterocycle in principle. Indeed, all these substitution patterns (with a variety of substituents) have been shown to occur in nature as well as by chemical synthesis,\(^1\)\(-^6\) with the exception that genuine 1,3-disubstituted adenines (type \( 7^7 \)) still remain unknown. Quite a recent review article by us has treated the chemistry, physicochemical properties, and biological activities of the five positional isomers of \( N^\alpha \)-methyladenine, the prototypes of mono-N-substituted adenines.\(^8\) The aim of the present article is to review the 11 possible positional isomers of \( N^\alpha,N^\beta \)-dimethyladenine in much the same
sense in order to supplement previous ones\textsuperscript{1–4} by reorganizing (in part) and updating the literature through the early part of 1998. The 11 positional isomers covered are $N^6,N^6$-dimethyladenine (2), $N^6,1$-dimethyladenine (3), $N^6,3$-dimethyladenine (4), $N^6,7$-dimethyladenine (5), $N^6,9$-dimethyladenine (6), 1,3-dimethyladenine (7),\textsuperscript{7} 1,7-dimethyladenine (8), 1,9-dimethyladenine (9), 3,7-dimethyladenine (10), 3,9-dimethyladenine (11), and 7,9-dimethyladenine (12).\textsuperscript{7}

\begin{align*}
\text{1} & \quad \text{2} & \quad \text{3} & \quad \text{4} \\
\text{5} & \quad \text{6} & \quad \text{7} & \quad \text{8} \\
\text{9} & \quad \text{10} & \quad \text{11} & \quad \text{12}
\end{align*}

\textbf{II. $N^6,N^6$-Dimethyladenine}

$N^6,N^6$-Dimethyladenine (2) has been the most frequently investigated isomer among the 11 $N^x,N^y$-dimethyladenines. The occurrence of 2 in epiphytic bacteria (isolated from barley)\textsuperscript{9} and in the headspace and essential oil of the whole plant of \textit{Plectranthus coleoides Marginatus}\textsuperscript{10} has been reported. As the aglycon, 2 is contained in the antibiotic puromycin [6-dimethylamino-9-[3-(p-methoxy-L-phenylalanylamino)-3-deoxy-$\beta$-D-ribofuranosyl]purine], a protein biosynthesis inhibitor (in both bacterial and mammalian cells) produced by \textit{Streptomyces alboniger};\textsuperscript{1–3,5} and in the nucleoside antibiotics A201A, A201C, A201D, and A201E, protein biosynthesis inhibitors produced by \textit{Streptomyces capreolus}.\textsuperscript{5,11} The occurrence of new metabolites containing 2 in spores of \textit{Streptomyces alboniger} has also been reported.\textsuperscript{12} The existence of 2 in the form of 2'-deoxy-$N^6,N^6$-dimethyladenosine structure in DNA of some species of algae,\textsuperscript{13} in the form of $N^6,N^6$-dimethyl-2'-O-methyladenosine structure in mRNA,\textsuperscript{14} and in the form of
\(N^6,N^6\)-dimethyladenosine structure in RNA's\(^{14-31}\) from a number of sources has been known.

\(N^6,N^6\)-Dimethyladenine (2) has been reported to have very weak or no cytokinin activity in certain test systems.\(^{32-38}\) At \(10^{-6}\) M concentration, 2 stimulated proliferation of *Castanea vesca* tissue in vitro;\(^{39}\) it also had a stimulatory effect on *Betula verrucosa*.\(^{39}\) It exhibited cytokinin-like effects on the cambial tissue of forest trees cultivated in vitro, including tissues from willow, poplar, eucalyptus, oak, beech, chestnut, elm, maple, and pine.\(^{40}\) Stimulation of the growth of *Quercus* cambial tissue culture,\(^{41}\) of *Fagus silvatica* cambial tissue culture,\(^{41}\) and of sorghum (*Sorghum bicolor*) primary callus\(^{42}\) and chlorophylls increase in cucumber cotyledons\(^{43}\) by 2 were also reported. *N^6,N^6*-Dimethyladenine (2) has been tested for reversing the abscisic acid-induced inhibition of the germination of lettuce achene;\(^{44}\) for inhibiting the covalent incorporation of exogenous *N^6*-benzyladenine into total RNA of tobacco cells, grown in shaken liquid medium;\(^{45}\) for inhibition of cytokinesis [protein synthesis (\(^3\)H-leucine incorporation)] in *Allium sativum* root meristems;\(^{46}\) for somatic embryogenesis and plant recovery from mature tissues of the olive cultivars “Canino” and “Moraiolo”;\(^{47}\) and for the effect on *in vitro* development of zygotic embryos of taro (*Colocasia esculenta var. antiquorum*).\(^{48}\) A plant senescence-delaying composition containing foliar fertilizers and 2 has been applied for a patent.\(^{49}\)

Investigated also are effects of 2 on the following biological processes: the multiplication of the DNA-phage \(\lambda\) and of the RNA-phage M12 as well as that of the host bacteria *Escherichia coli*;\(^{50}\) growth of lactic acid bacteria in presence of combination with folic acid analogues;\(^{51}\) growth of purine-requiring mutants of *E. coli*, strains W-11 and B-96, and purine biosynthesis;\(^{52}\) inhibition of bulking for activated sludges;\(^{53}\) formation of aerial mycelia and spores of *Streptomyces viridochromogenes*;\(^{54}\) inhibition of germ-tube growth and appressoria formation during primary infection of barley powdery mildew;\(^{55}\) adenine-induced growth inhibition of *Staphylococcus aureus*;\(^{56}\) inhibition of growth of *Tetrahymena pyriformis*;\(^{57}\) inhibition of regeneration of hydra whose tentacles and hypostome have been removed;\(^{58}\) production of triploid eggs and larvae in the Pacific oyster *Crassostrea gigas*, the giant sea scallop *Placopecten magellanicus*, and the blue mussel *Mytilus edulis*;\(^{59}\) tetraploid induction in eggs or embryos of *Mytilus edulis* during early development;\(^{60}\) growth of mouse Sarcoma 180 cells;\(^{61}\) activation and analysis of nondividing cell nuclei for prenatal screening, as a cytostatic factor extract supplement;\(^{62}\) inhibition of apoptosis for treating neurodegenerative diseases;\(^{63}\) inhibition of formylglycinamide ribonucleotide formation in human epidermoid carcinoma in cell culture;\(^{64}\) induction of a persistent stellate morphology in cultured human glioma cells, without affecting the cAMP content;\(^{65}\) adenosine-dependent formation of cAMP in guinea pig cerebral cortical slices;\(^{66}\) inhibition of DNA synthesis in two mammalian cell lines, 3T3 and CHEF/18 fibroblasts;\(^{67}\) DNA synthesis in activated mammalian oocytes;\(^{68}\) the cAMP-binding sites of two high-affinity cAMP-binding proteins
from wheat germ;\textsuperscript{69} the Ca\textsuperscript{2+} binding activity of an Achlya and Blastocladiella glycoprotein;\textsuperscript{70} inhibition of hepatocytic protein degradation;\textsuperscript{71} inhibition of autophagic sequestration and endogenous protein degradation in isolated rat hepatocytes;\textsuperscript{72} protein synthesis and degradation in isolated rat hepatocytes;\textsuperscript{73} and \textit{in vitro} destruction of cyclin from clam embryos.\textsuperscript{74}

Effects on the following enzymes have been reported: nonspecific adenosine deaminase from Taka-diastase;\textsuperscript{75} adenine deaminase of Pseudomonas syoxantha;\textsuperscript{76} barley powdery mildew adenosine deaminase;\textsuperscript{77} extracellular adenine deaminase from Streptomyces sp. J-350P;\textsuperscript{78} human plasma adenosine deaminase;\textsuperscript{2} adenosine nucleosidase (adenosine ribohydrolase, EC 3.2.2.7) from barley leaves;\textsuperscript{80} purine-2'-deoxyribonucleosidase of Crithidia luciliae;\textsuperscript{81} adenine phosphoribosyltransferase from Ehrlich ascites tumor cells\textsuperscript{82} and from rabbit polymorphonuclear leukocyte;\textsuperscript{83} 3'-nucleotidase (3'-ribonucleotide phosphohydrolase, EC 3.1.3.6) from wheat germ;\textsuperscript{84} phosphodiesterase specific for cyclic nucleotides;\textsuperscript{85} modulation of human erythrocyte acid phosphatase activity;\textsuperscript{86} inhibition of human erythrocyte membrane phosphatidylinositol 4-kinase;\textsuperscript{87} specificity for yeast glyceraldehyde-3-phosphate dehydrogenase at the cAMP-binding site;\textsuperscript{88} bovine milk xanthine oxidase and rabbit liver aldehyde oxidase;\textsuperscript{89} and oxidation of NADH by a horseradish peroxidase system.\textsuperscript{90}

As a protein kinase inhibitor, 2 has been used in the study of the following enzymes, biological processes, and those in oocytes: gibberellin-induced elongation, reorientation of cortical microtubules, and change of isoform of tubulin in epicotyl segments of azuki bean (Vigna angularis) seedlings;\textsuperscript{91} Tetrahymena cell division in the presence or absence of okadaic acid;\textsuperscript{92} the programmed rearrangement of cortical skeleton in furrowing Paramecium and the tensegrity model of cytokinesis;\textsuperscript{93} cyclic activation of histone H\textsubscript{1} kinase during sea urchin egg mitotic divisions;\textsuperscript{94} cyclin-dependent kinases from a variety of sources;\textsuperscript{95} chromosome movement and distribution in mitosis and meiosis of grasshopper spermatocytes;\textsuperscript{96} phosphoglycolate removal and end-joining using \textit{Xenopus} egg extracts;\textsuperscript{97} activation of \textit{Xenopus laevis} eggs in the absence of intracellular Ca activity;\textsuperscript{98} the transition to interphase in activated mouse oocytes;\textsuperscript{99} tumor necrosis factor signal transduction in bovine aortic endothelial cells;\textsuperscript{100} treatment of cancer in combination with taxol-type compounds;\textsuperscript{101} cleavage in sea urchin eggs;\textsuperscript{102} triggering meiosis in the starfish \textit{Marthasterias glacialis} and \textit{Asterias rubens} oocytes;\textsuperscript{103,104} starfish oocyte maturation;\textsuperscript{105} germinal vesicle breakdown, M-phase-promoting factor activation, and H\textsubscript{1}-histone kinase activation in \textit{Xenopus} oocytes, induced by either progesterone, M-phase-promoting factor transfer, or okadaic acid microinjection;\textsuperscript{106} the transition to metaphase during the first meiotic cell division of mouse oocytes;\textsuperscript{107} chromatin behavior at different stages of mouse oocyte maturation;\textsuperscript{108} H\textsubscript{1} kinase activity and M-phase-promoting factor activation in cattle and pig oocytes;\textsuperscript{109} \textit{in vitro} maturation of goat oocytes;\textsuperscript{110} activation of mammalian oocytes;\textsuperscript{111,112} meiotic resumption and subsequent development to the blastocyst stage of bovine oocytes;\textsuperscript{113} synchroni-
zation of cell division in eight-cell bovine embryos produced in vitro;\textsuperscript{114} germinal vesicle breakdown of bovine oocytes;\textsuperscript{115} maturation, fertilization, and development of bovine oocytes;\textsuperscript{116} activation of unfertilized eggs of the newt \textit{Cynops pyrrhogaster};\textsuperscript{117} cyclic reorientation of cortical microtubules in bean cell walls;\textsuperscript{118} mouse embryo cleavage arrest and synchronization and subsequent development;\textsuperscript{119} and length of cell cycles and the state of phosphorylation of putative intermediate filament proteins in sea urchin embryos.\textsuperscript{120}

Effects of 2 on the following biological processes have also been investigated: oligogalacturonide-induced cytoplasmic acidification in tobacco cells grown in suspension cultures;\textsuperscript{121} oxygen consumption by mitochondrial preparations from soybean cells;\textsuperscript{122} \textit{p}-coumaric acid disappearance and delayed inhibition of \textit{p}-coumaric acid disappearance in a cell suspension of soybean;\textsuperscript{123} succinate and malate oxidations in mitochondria isolated from fresh potato tuber;\textsuperscript{124} the mutagenic effect of UV light on \textit{Escherichia coli}, radiation resistant strain B/r;\textsuperscript{125} energy-linked amino acid transport systems of \textit{Achlya} (a freshwater mold);\textsuperscript{126} production of an experimental nephrotic syndrome in rats;\textsuperscript{127} induction of nephrotoxicity in mice;\textsuperscript{128} GABA (4-aminobutyric acid) responses and diazepam enhancement of GABA responses, using mouse spinal cord neurons in dissociated cell culture;\textsuperscript{129} binding, to rat brain membranes, of benzodiazepines;\textsuperscript{130} displacement of \textsuperscript{3}H\textit{diazepam binding in rat brain;\textsuperscript{131} regulation by GABA of the displacement of benzodiazepine antagonist binding in rat brain;\textsuperscript{132} the benzodiazepine receptor binding in rat brain;\textsuperscript{133} the aminophylline-resistant relaxation of isolated rabbit coronary artery;\textsuperscript{134} induction of cell elongation in cultured fibroblasts;\textsuperscript{135} uptake of adenosine into human blood platelets;\textsuperscript{136} enucleation of adherent mouse peritoneal exudate cells (macrophages) in combination with centrifugation;\textsuperscript{137} uptake of adenosine by human fibroblast lysosomes;\textsuperscript{138} the cytokinetip, phenotypic, and molecular effects elicited in HL-60 human leukemic cells by a low dose (0.6 \textmu M) of 2;\textsuperscript{139} and an assay for identifying fungicides or other anti-proliferative agents that inhibit mitosis or meiosis.\textsuperscript{140} The use of 2 as a stabilizer for pesticides containing DDVP (phosphoric acid 2,2-dichloroethenyl dimethyl ester)\textsuperscript{141} and a vasodilator composition containing 2\textsuperscript{142} have been applied for patents.

As regards the synthesis of 2, Baker \textit{et al.}\textsuperscript{143a} started from 4-amino-6-dimethylamino-2-methylthiopyrimidine (13) and obtained 2 through 14, 15, and 16 or through 14, 18, and 19 or through 14, 18, and 17,\textsuperscript{143b} as illustrated in Scheme 1. Goldman \textit{et al.}\textsuperscript{144} treated 4,5-diamino-6-dimethylaminopyrimidine (20) with boiling CH(OEt)\textsubscript{3}/Ac\textsubscript{2}O to obtain a mixture of 2 and its 9(or 7)-acetyl derivative (Scheme 2). Recrystallization of the mixture from EtOH afforded the acetyl derivative in 20\% yield, and treatment of the ethanolic filtrate with 1 N aqueous NaOH at 100°C for 5 min afforded 2 in 66\% yield.

In a synthetic approach to 2 from a purine derivative, Elion \textit{et al.}\textsuperscript{145} reached 2 from hypoxanthine (24) via 6-mercaptopurine (21) and 6-methylthiopurine (22), as shown in Scheme 3. The step 22→2 was repeated under similar reaction conditions by Albert and
Scheme 1

Scheme 2

Scheme 3
Brown (20% aqueous Me₂NH, 140°C, 24 h)¹⁴⁶ by Skinner et al.,⁵⁸ and by Okumura et al. (130–135°C, 17 h, 45% yield).³²ᵃ Alternatively, Albert and Brown¹⁴⁶ heated 6-chloropurine (25), obtainable from 2₄ by chlorination with POCl₃, with 15% methanolic Me₂NH at 100°C for 1 h to secure 2.³⁷,¹⁴⁷ Lambe et al.¹⁴⁸ prepared [8-¹⁴C]-2 from [8-¹⁴C]-2₅ by heating with Me₂NH in THF under an N₂ atmosphere at 50°C for 2.5 h. Girgis and Pedersen¹⁴⁹ reported a one-step 26% conversion of 2₄ into 2, in which a mixture of P₂O₅, Me₂NH·HCl, and 2₄ was heated at 150°C for 24 h. A slight modification of this procedure by Motawia et al.¹⁵⁰ improved the yield of 2 to 56%. Yet another synthesis of 2 from a purine derivative includes that of Breshears et al.,¹⁴⁷ who converted 2,6,8-trichloropurine (2₆) into 2 via 2,8-dichloro-6-dimethylaminopurine (2₃), as depicted in Scheme 3.

![Scheme 4](image)

The formation of N⁶,N⁶-dimethyladenine (2) by methylation of DNA¹⁵¹,¹⁵² and RNA¹⁵³–¹⁵⁵ molecules and hydrolysis of the resulting products has been known. Alcoholysis of puromycin with ethanolic HCl has been shown to give 2, O-methyl-L-tyrosine, and 3-amino-3-deoxyribose,¹⁴³ᵃ,¹⁵⁶ᵃ and treatment of a crude sample of O-demethylpuromycin with ethanolic HCl at 80–83°C for 1 h gave 2 as revealed by paper chromatographic analysis.¹⁵⁶ᵇ Treatment of the ribofuranosyl derivative (2₇ or 2₈) in a mixture of AcOH and Ac₂O with 96% sulfuric acid at 20–25°C for 17 h or 18 h was reported to provide 2·₂H₂SO₄ in 92% or 100% yield, respectively (Scheme 4).¹⁵⁷ Hydrolysis of the xylofuranosyl derivative (2₉) in boiling dilute aqueous HCl for 3 h afforded 2, and it was isolated as the picrate in 79% yield.¹⁵⁸ Lambe et al.¹⁴⁸ found that only a small amount of N⁶,N⁶-dimethyladenine arabinoside, a potent inhibitor of varicella-zoster virus replication in vitro, was cleaved to 2 in rats. Preparation of 2 by exchange amination of adenine (1) with Me₂NH in the presence of HCl (Scheme 4) has been applied for a patent.¹⁵⁹
### Table I. N<sup>6</sup>,N<sup>6</sup>-Dimethyladenine (2): Physical and Spectral Characteristics

<table>
<thead>
<tr>
<th>Item</th>
<th>Specification&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>Literature (ref. No.)</th>
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<tr>
<td>Melting point&lt;sup&gt;b)&lt;/sup&gt;</td>
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<tr>
<td>2·HCl</td>
<td>251–253°C (145a, 147); 249–250°C (147)</td>
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<tr>
<td>2·2HCl</td>
<td>225–227°C (decomp) (156a)</td>
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<td>2·2HBr</td>
<td>255–258°C (decomp) (157)</td>
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<td>2·2H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>210–215°C (157)</td>
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<td>2·picrate</td>
<td>247°C (158); 244–245°C (decomp) (143a); 241–242°C (decomp) (144b)</td>
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<tr>
<td>Acid dissociation constant</td>
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<td>basic pK&lt;sub&gt;a&lt;/sub&gt;</td>
<td>3.87 (H&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;c)&lt;/sup&gt; (146, 160, 161, 169); 3.9 (H&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;d)&lt;/sup&gt; (165); 4.3 (H&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;e)&lt;/sup&gt; (164); 4.4 (H&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;f&lt;/sup&gt; (163); 4.45 (H&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;d)&lt;/sup&gt; (164); 4.10 (D&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;c)&lt;/sup&gt; (166)</td>
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<tr>
<td>acidic pK&lt;sub&gt;a&lt;/sub&gt;</td>
<td>9.95 (H&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;d)&lt;/sup&gt; (164); 10 (H&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;e)&lt;/sup&gt; (164); 10.0 (H&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;d)&lt;/sup&gt; (165); 10.1 (H&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;f&lt;/sup&gt; (163); 10.5 (H&lt;sub&gt;2&lt;/sub&gt;O)&lt;sup&gt;c)&lt;/sup&gt; (146, 160, 161, 169); 10.54 [59% (v/v) aq. EtOH]&lt;sup&gt;e)&lt;/sup&gt; (162); 13.0 (DMSO)&lt;sup&gt;c)&lt;/sup&gt; (167); 14.7 (MeOCH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;OH)&lt;sup&gt;c)&lt;/sup&gt; (168)</td>
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<td>Paper chromatography</td>
<td>(16, 19, 21, 22, 25, 26, 29, 30, 146, 151, 153, 170–175)</td>
<td>(12, 18, 20, 24, 155, 156b, 176–179)</td>
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<td>TLC</td>
<td>(16, 19, 21, 22, 25, 26, 29, 30, 146, 151, 153, 170–175)</td>
<td>(12, 18, 20, 24, 155, 156b, 176–179)</td>
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<td>(30, 153, 198)</td>
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<td>UV spectrum</td>
<td>In H&lt;sub&gt;2&lt;/sub&gt;O at various pH’s (21, 22, 145a, 147, 160, 163–165, 181, 206, 207); in methylcyclohexane, dioxane, i-PrOH, or H&lt;sub&gt;2&lt;/sub&gt;O (208)</td>
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<td>Thermal perturbation differential spectrum</td>
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<td>&lt;sup&gt;1&lt;/sup&gt;H NMR spectrum</td>
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<td>&lt;sup&gt;13&lt;/sup&gt;C NMR spectrum</td>
<td>Solid (216); in DMSO–&lt;sup&gt;d&lt;/sup&gt;6 (150, 216, 220, 225, 226)</td>
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<td>Crystal structure</td>
<td>2(\cdot)(2)H_2O (228); 2(\cdot)HCl(\cdot)H_2O (229); 2(\cdot)2HCl (229); 2(\cdot)HNO_3 (230); 2(\cdot)H_2SO_4 (231); (2)(\cdot)2H_2SO_4(\cdot)2H_2O (232); 2(\cdot)CCl_3CO_2H (233); (2)(\cdot)(hydroxyethenetrichloronitrile)(\cdot)1,4-dioxane (234); 2(\cdot)picrate (235)</td>
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<tr>
<td>Ionization potential</td>
<td></td>
<td>(210)</td>
</tr>
<tr>
<td>Singlet and triplet π→π* transition energies</td>
<td></td>
<td>(251)</td>
</tr>
<tr>
<td>Electron density</td>
<td>By the LCAO MO method (219); by the INDO and STO-3G MO methods (252)</td>
<td></td>
</tr>
<tr>
<td>Electronic structure</td>
<td></td>
<td>(210, 253–256)</td>
</tr>
</tbody>
</table>

<sup>a)</sup> With or without reference number(s) in parentheses.  
<sup>b)</sup> Reported for analytical samples, in most cases.  
<sup>c)</sup> Potentiometric.  
<sup>d)</sup> UV spectral.  
<sup>e)</sup> \(^1\)H NMR spectral.  
<sup>f)</sup> For the N(1)-deuterated species.

Included in Table I are the fruits of an additional comprehensive survey of papers describing the physical properties and spectral characteristics of \(N^6,N^6\)-dimethyladenine (2).\(^{160–256}\)

Interactions of 2 with the following substances have been reported: self-association in various aqueous media at 25°C\(^{257}\) and in D_2O;\(^{223}\) H_2O vapor (hydration);\(^{204}\) benzanthracene;\(^{258,259}\) β-cyclodextrin in phosphate buffer (pH 7) at 25°C;\(^{260}\) proflavine;\(^{261}\) the methyl esters of arginine, serine, and methionine in DMSO;\(^{262}\) bovine serum albumin in D_2O;\(^{263}\) an antibody directed toward \(N^6-(\Delta^2\)-isopentenylnyl)adenosine;\(^{264}\) Cu(I) ions in aqueous media;\(^{265}\) \([\text{ImH}]\text{[RuCl}_4\text{Im}_2]\) (Im = imidazole) in MeOH;\(^{266}\) \((4\text{-NO}_2\text{ImH})\text{[RuCl}_4\text{-(5-NO}_2\text{Im)}\text{]}\) in CD_3OD;\(^{267}\) the mixed bridged diene-rhodium(I) complex \([\text{codRh(μ-Cl)-(μ-OAc)}\text{Rh(cod)}]\) (cod = 1,5-cyclooctadiene) in MeOH;\(^{268}\) \([\text{nbdlRh(acac)}]\) (nbdl = norbornadiene) in MeOH, \([\text{CO}_2\text{Rh(acac)}]\) in MeOH, or \([\text{CO}\text{Rh(acac)}\text{(PPh}_3\text{)}]\) in MeOH/CH_2Cl_2;\(^{269}\) the Rh\(^{4+}\) formamidinate complex Rh_2(μ-form)_2(μ-O_2CCF_3)_2(H_2O)_2 (form = \(N,N^\prime\)-di-p-tolylformamidinate anion) in H_2O;\(^{270}\) Re_2Cl_2(ACo)_4 in EtOH containing MeO-
Na$_2$[Re$_2$C$_8$] in EtOH; cis- and trans-[Pt(NH$_3$)$_2$Cl$_2$] in H$_2$O at 37°C; HgCl$_2$ in EtOH/aqueous NaOH; MeHgOH or MeHgCl$_2$/MeHgOH or MeHgN-O$_3$/MeHgOH in boiling H$_2$O or MeHgNO$_3$ in aqueous MeCN.

![Scheme 5](image)

As regards the chemical behavior of 2, Albert and Brown$^{146}$ reported its instability on heating in boiling 10 N aqueous NaOH for 1 h. The high stability of 2 in 25% aqueous TsOH at 100°C for 4 h and in a 1:1 mixture of TFA and formic acid (97–100%) at 200°C for 1.5 h was confirmed by Gordon et al.$^{273}$ and by Lakings et al.$^{184}$ respectively. Anderson and Beauchamp$^{266}$ prepared N$_6$N$_6$-dimethyladenine-8-d (30) from 2 by heating anaerobically in refluxing D$_2$O for 2.5 h (Scheme 5). Kiessling et al.$^{274}$ treated 2 in DMF with $^3$H$_2$ in the presence of Pd black to prepare N$_6$N$_6$-dimethyl-[3H]adenine. McGhee and von Hippel$^{275}$ found that the reaction of 2 with formaldehyde in 0.02 M phosphate buffer (pH 6.95) at 24 ± 1°C came to equilibrium with a product presumed to be 31, and they determined the equilibrium constant to be 11.4 M$^{-1}$. Takiura’s group$^{276}$ reported that the reaction of 2 or N$_6$-methyladenine (94) with glyoxal trimer hydrate in AcOH at 100°C for 6 h did not produce fluorescence, whereas adenine (1), 1-methyladenine (115), 7-methyladenine (159), 9-methyladenine (146), adenosine (76), AMP, ADP, ATP, and 2'-deoxyadenosine gave rise to fluorogenic reactions under similar conditions.

Itaya’s group$^{277}$ reported that methylation of 2 with MeI in AcNM$_2$ in the presence of K$_2$CO$_3$ at rt for 4–9 h gave N$_6$N$_6$,9-trimethyladenine (32a) and N$_6$N$_6$,3-trimethyladenine (33a) in 54% and 14% yields, respectively (Scheme 5);$^{278}$ ethylation with EtI under similar reaction conditions afforded 9-ethyl-N$_6$N$_6$-dimethyladenine (32b) and the 3-ethyl isomer (33b) in 72% and 13% yields, respectively; and benzyla...
in a similar manner furnished 9-benzyl-N^6,N^6-dimethyladenine (32c) and the 3-benzyl isomer (33c) in 65% and 30% yields, respectively. When these alkylation were effected in the absence of K_2CO_3, the regioselectivity in alkylation suffered a complete reversal.277 Methylation at 40°C for 48 h produced, after basification and chromatographic separation of the reaction products, 32a (0.7% yield) and 33a (83%); ethylation at 80°C for 7 h, 32b (1.8%) and 33b (90%); and benzylolation at 40°C for 24 h, 32c (2.8%) and 33c (86%). Miyaki and Shimizu219 found that benzylolation of 2 with PhCH_2Br in AcNMe_2 at 110°C for 7 h gave, after basification and chromatographic separation of the reaction products, 32c (5%) and 33c (66%) and that heating 33c·HBr in DMF at 150°C for 40 h gave, after basification, 32c in 22% yield, indicating the occurrence of N(3)→N(9) benzyl migration under these thermal conditions. Pal and Horton163 methylated 2 with dimethyl sulfate in a mixture of 0.01 M phosphate buffer (pH 7.0) and EtOH at pH 7.0 and rt for 3-4 h and obtained, after chromatographic separation of the products, 32a (8.3% yield), 33a (66%), and N^6,N^6,1-trimethyladenine (22.9%).

Scheme 6

The preferential N(9)-alkylation of 2 in the presence of added base has been adopted by many research groups for the preparation of 9-substituted N^6,N^6-dimethyladenines (type 32). Kelley's group279 obtained 32a from 2 in 36% yield by methylation (MeI/MeONa/DMSO, rt, 1 h); 32b from 2 by ethylation (EtI/Br_2/Me_2SO);280 32 (R = CH_2CH_2Cl) from 2 in 76% yield by alkylation (BrCH_2CH_2Cl/benzene/NaOH, 80°C, 0.5 h). Takemoto and co-workers282 synthesized 34, 35, and 38 from 2, as shown in Scheme 6. They also prepared poly(N^6,N^6-dimethyl-9-vinyladene).282 LaMontagne et al.283 synthesized 9-(3-benzylxylo-2-hydroxypropyl)-N^6,N^6-di-
methyladenine (36) from 2 by alkylation with 4-benzylxoymethyl-2-oxo-1,3-dioxolane in the presence of K$_2$CO$_3$. Imai and Seo$^{284}$ obtained 9-(2-chloro-6-fluorobenzyl)-N$_6$N$_6$-dimethyladenine (37) by treatment of 2 with 2-chloro-6-fluorobenzyl chloride in AcNMe$_2$ in the presence of K$_2$CO$_3$ at 110°C for 6 h. Ramzaeva et al.$^{285}$ employed conditions of phase-transfer catalysis in the system benzene/aqueous NaOH/Bu$_4$N$^+$Br$^-$ for N(9)-alkylation of 2 with 2,6-dichlorobenzyl chloride. Many other compounds of type 32, such as those where the N(9)-R groups are 2,6-dihalogenobenzyl;$^{159,286,287}$ 3-(4-benzamidopiperidino)propyl,$^{288,289}$ 3-[4-(aryloxymethyl)piperidino]propyl,$^{290}$ and 3-(4-aryl-1-piperazinyl)propyl,$^{291}$ have been analogously synthesized from 2 and applied for patents. N$_6$N$_6$-Dimethyladenine (2) was conjugated at N(9) to Sepharose through a 12-atom spacer moiety to yield a matrix for preparation of an affinity column for purification of cytokinin oxidase.$^{292}$

![Scheme 7](image)

Sukhodub et al.$^{293}$ found that treatment of 2 with thio-TEPA (1,1',1"-phosphinothioylidinetrisaziridine) in H$_2$O at 37°C for 3–5 d produced an N$^x$-(2-aminoethyl) de-
rivative, as detected by field ionization MS. Toyota et al.\textsuperscript{294} reported that Mitsunobu reaction of 2 with PhCH$_2$OH gave the N(9)-benzyl derivative (32c) and the N(3)-benzyl isomer (33c) in 64\% and 36\% yields, respectively (Scheme 7), but the N(7)-benzyl isomer was not detectable in the reaction mixture.

Baker et al.\textsuperscript{272} reported condensation of the chloromercuri derivative of 2 with $\alpha$-bromoacetoglucose (39) in boiling xylene for 1 h and deacetylation of the condensed product (26\% yield) with MeONa in boiling MeOH for 45 min to give $N^6,N^6$-dimethyl-"7"-$\beta$-D-glucopyranosyladenine [reported mp 239--241\degree C (decomp)] in 76\% yield. Baker and Schaub\textsuperscript{295} condensed the chloromercuri derivative of 2 with 2,3,5-tri-O-benzoyl-D-xylofuranosyl bromide (41) and debenzoylated the condensation product in a similar manner to obtain a mixture of the "7"- and 9-xylosides (in poor yield), from which the "7"-xyloside could be isolated. In addition, Baker et al.\textsuperscript{157} reported a similar condensation of the chloromercuri derivative of 2 with 2,5-di-O-benzoyl-3-phthalimido-3-deoxy-$\beta$-D-ribofuranosyl chloride (42) to produce a mixture of the corresponding "7"- and 9-nucleosides. Later on, the structures of these $N^6,N^6$-dimethyl-"7"-glycosyladenines were reassigned by Townsend et al.\textsuperscript{170} as the corresponding 3-glycosyl derivatives, on the basis of UV and $^1$H NMR spectral data. Kissman et al.\textsuperscript{296} synthesized $N^6,N^6$-dimethyladenosine (69) by similar condensation of the chloromercuri derivative of 2 with 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride (43) or its tri-O-acetyl analogue (44), followed by debenzoylation or deacetylation with MeONa/MeOH. The attempt to couple 2 with 2,3,4-tri-O-acetyl-6-deoxy-6-nitro-$\alpha$-D-glucopyranosyl bromide (40) in boiling nitromethane in the presence of Hg(CN)$_2$ and anhydrous CaSO$_4$ for 3 h was reported to be unsuccessful.\textsuperscript{297}

\begin{equation}
\text{Pedersen's group}\textsuperscript{298} condensed the trimethylsilylated derivative (50), prepared from 2 by treating with boiling hexamethyldisilazane in the presence of (NH$_4$)$_2$SO$_4$, with the sugar derivative (45) in MeCN in the presence of trimethylsilyl triflate at rt for 3 h to obtain the $\beta$-furanoside derivative (51) and its $\alpha$-anomer (54) in 20\% and 36\% yields, respectively (Scheme 8). The fluorinated sugar derivative (46) similarly reacted with 50

\begin{scheme}
\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {$2$};
\node (b) at (2,0) {$50$};
\node (c) at (2,1) {$51$: X = H}
node (d) at (2,0.5) {$52$: X = F}
node (e) at (2,-0.5) {$53$: X = OMs}

\node (f) at (4,0) {$54$: X = H}
node (g) at (4,0.5) {$55$: X = F}
node (h) at (4,-0.5) {$56$: X = OMs}

\draw[->] (a) -- (b);
\draw[->] (b) -- (c);
\draw[->] (b) -- (d);
\draw[->] (b) -- (e);
\draw[->] (b) -- (f);
\draw[->] (b) -- (g);
\draw[->] (b) -- (h);
\end{tikzpicture}
\end{center}
\textbf{Scheme 8}
\end{scheme}
to give 52 and 55 in 13% and 18% yields, respectively. \(^{298}\) Replacement of 45 or 46 by 47 in these condensations but at -30°C for 2 h resulted in the formation of 53 and 56 in 18% and 21% yields, respectively. \(^{298}\) Similar condensation of 50 with the azido sugar (57) at -30°C to 0°C for 3 h afforded 58 as an anomeric mixture (\(\alpha:\beta = 4:1\)) in 35% yield (Scheme 9). \(^{298}\)

![Scheme 9](image)

Pedersen's group\(^{150}\) also condensed 2 with the phthalimido sugar derivative (48) in a similar manner to obtain the 9-\(\beta\)-furanoside (59) and the 9-\(\alpha\)-furanoside (60) in 25% and 15% yields, respectively (Scheme 10). On the other hand, coupling of the Na salt (61), generated in situ from 2 by treatment with NaH in MeCN, with the glycosyl bromide (49) (\(\alpha:\beta = 1:5\)) in MeCN at rt for 24 h produced the 9-\(\alpha\)-furanoside (60) (34%), the 7-\(\alpha\)-
furanoside (62) (39%), and an unexpected product (63) (4%).

Miyaki and Shimizu condensed 2 with 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide (64) in MeCN at 60–65°C for 18 h to obtain the corresponding 3-furanoside (65) and the 9-furanoside (66) in 36% and 11% yields, respectively (Scheme 11). Although 65 remained unchanged on heating at 100°C for 1.5 h in the presence of HgBr₂, N(3)→N(9) ribosyl migration took place on heating [in AcNMe₂-xylene (1:10, v/v)] at 100°C for 1.5 h and then at 130–140°C for 2 h, producing 66 in 36% yield. One of the applications of this ribosyl migration was demonstrated by transfer of the ribosyl moiety from N⁴-acetyl-2',3',5'-tri-O-acetylcytidine (67) to 2 to prepare N⁶,N⁶-dimethyladenosine (69) via 68, as delineated in Scheme 11.

Zintchenko et al. reported an enzymic transglycosylation from α-D-ribose 1-phosphate to 2 in phosphate buffer (pH 7.0) at 60°C using the cell paste of Escherichia coli BM-11. Enzymic transglycosidations from pyrimidines to 2 have also been reported: from [deoxyribosyl-¹⁴C]thymidine in phosphate buffer (pH 6.0) at 40°C using trans-N-deoxyribosylase (EC 2.4.2.6) from Lactobacillus helveticus; from thymidine in citrate buffer (pH 6.0) at 37°C using the nucleoside deoxyribosyltransferase (EC 2.4.2.6) from Lactobacillus leichmannii, giving 2'-deoxy-N⁶,N⁶-dimethyladenosine in 86% yield; from 3'-deoxythymidine using purified thymidine phosphorylase and purine phosphorylase, giving 2',3'-dideoxy-N⁶,N⁶-dimethyladenosine; from 5'-deoxythymidine in 0.02 M phosphate buffer at 37°C for 3–5 d, affording 2',5'-dideoxy-N⁶,N⁶-dimethyladen-
osine in low yield (in the region of 10%);\textsuperscript{305} from 2'-deoxyuridine in 50 mM aqueous AcONH\textsubscript{4} (pH 5.7) at 37°C for 16 h in the presence of alginate gel-entrapped cells of auxotrophic thymidine-dependent strain of \textit{Escherichia coli}, obtaining 2'-deoxy-N\textsuperscript{6},N\textsuperscript{6}-dimethyladenosine in 70% yield;\textsuperscript{306} from 2',3'-dideoxycytidine using a nucleoside deoxyriboinosyltransferase from \textit{Lactobacillus leichmannii}\textsuperscript{307} in 50 mM citrate buffer (pH 6.2) at 37°C for 48 h, obtaining 2',3'-dideoxy-N\textsuperscript{6},N\textsuperscript{6}-dimethyladenosine in 78% yield;\textsuperscript{308} from 2',3'-dideoxy-3'-fluorouridine in a phosphate buffer containing potassium azide in the presence of purine nucleoside phosphorylase and thymidine phosphorylase immobilized on DEAE cellulose;\textsuperscript{309} and from arabinofuranosyluracil in phosphate buffer at 50°C for 5 d in the presence of uridine phosphorylase and pyrimidine nucleoside phosphorylase, giving 9-(\beta-D-arabinofuranosyl)-N\textsuperscript{6},N\textsuperscript{6}-dimethyladenine.\textsuperscript{310}

\begin{equation}
\text{NaH/DMF/NBS} \\
\text{100°C, 0.5 h} \\
(33\% \text{ yield})
\end{equation}

\begin{equation}
\text{BSTFA Me3SiCl pyridine} \\
\text{100°C, 1 h}
\end{equation}

\begin{equation}
\text{m-CPBA/MeOH} \\
\text{30°C, 20 h} \\
(40\% \text{ yield})
\end{equation}

\begin{equation}
\text{30% aq. Me\textsubscript{2}NH} \\
\text{reflux, 3 h} \\
(65\% \text{ yield})
\end{equation}

\begin{equation}
(\text{C\textsubscript{3}F\textsubscript{7}CO\textsubscript{2})\textsubscript{2} CF\textsubscript{2}C\textsubscript{Cl}C\textsubscript{F}\textsubscript{2}Cl}
\end{equation}

\begin{equation}
\text{30°C, 2 h; then reflux, 1 h} \\
(17.5\% \text{ yield})
\end{equation}

\begin{equation}
\text{H\textsubscript{2}NOSO\textsubscript{2}H KOH/H\textsubscript{2}O} \\
\text{100°C, 72 h}
\end{equation}

\begin{equation}
\text{Scheme 12}
\end{equation}

Kelley \textit{et al.}\textsuperscript{311} reported the bromination of the anion of 2 with NBS in hot DMF to provide 8-bromo-N\textsuperscript{6},N\textsuperscript{6}-dimethyladenine (70), as shown in Scheme 12, which was found to be inactive against influenza A virus. Treatment of the TMS derivative (50), prepared from 2 by silylation with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in the presence of Me3SiCl and pyridine, with bis(heptafluorobutyryl) peroxide in CF\textsubscript{2}ClCFC\textsubscript{2} produced the 8-(heptafluoropropyl) derivative (71) in low yield.\textsuperscript{312} Oxidation of 2 with \textit{m}-CPBA in MeOH at 30°C for 20 h afforded the N(3)-oxide (73) in 40% yield with 23% recovery of 2.\textsuperscript{313} The correctness of the structure of 73 was confirmed by direct comparison with a sample prepared from 6-chloropurine 3-oxide (72)\textsuperscript{314,315} and dimethylamine (Scheme 12).\textsuperscript{313} Amination of 2 with hydroxylamine-O-sulfonic acid in alkaline medium at 100°C for 72 h furnished the 3-amino derivative (74) in 13% yield, together with a small amount of the 9-amino derivative (75).\textsuperscript{193} The reactions of 2 with the OH radical in H\textsubscript{2}O at pH 6-8 and 20°C\textsuperscript{169} and with the
sulfate radical anion (SO\(_4\)^{2-}\) in H\(_2\)O at pH 7\(^{242,316}\) and the electron paramagnetic resonance spectrum as well as the protonation site of one-electron-reduced 2 (generated by X-Ray irradiation in a 9 M LiCl glass at 4 K)\(^{317}\) have been investigated. Morimoto and Tsuda\(^{318}\) reported that the rate of alkaline hydrolysis of p-nitrophenyl acetate in Clark–Lubs’ buffer (pH 8.2) at 25°C was enhanced in the presence of 2. Shelf life-extended films containing 2 as a photographic antifogging agent\(^{319}\) and direct-positive color films containing 2 with wide exposure latitude\(^{320}\) have been applied for patents.

\(N^6,N^6\)-Dimethyladenine (2) was found to demethylate, as indicated by the formation of formaldehyde, by liver microsomal enzymes (obtained from rat, guinea pig, or mouse) in the presence of an NADPH-generating system.\(^{321}\) However, 2 was refractory to mammalian xanthine oxidase.\(^{322}\) It acted as a competitive inhibitor of the extracellular adenine deaminase from \textit{Nocardioides} sp. J-275L.\(^{323}\) Preparation of \textit{Escherichia coli} mutants, resistant to 2, from parental \textit{E. coli} strains H-8311 and H-8285 by chemical mutagenesis and manufacture of L-threonine and L-isoleucine with these mutants have been applied for a patent: The production of the two amino acids was 10–20% higher than did the parental strains.\(^{324}\)

### III. \(N^6,1\)-DIMETHYLADENINE

Wacker and Ebert\(^{325}\) studied the methylation of adenosine (76) with dimethyl sulfate in H\(_2\)O at pH's 6–8, 8.8–9.0, and 13 and obtained a crystalline compound, assumed to be \(N^6,1\)-dimethyladenosine (81), most efficiently from the reaction carried out at pH 8.8–9.0 (Scheme 13). They claimed to have hydrolyzed this dimethylated nucleoside to \(N^6,1\)-dimethyladenine (3) with 2 N aqueous HCl and identified the latter base only by formation of its picrate according to the method of Bredereck et al.\(^{326}\) Robins' group\(^{327}\) methylated \(N^6\)-methyladenosine (78) with MeI in DMSO to obtain 81·HI, which was converted into the free nucleoside (81) (identical with the one which Wacker and Ebert\(^{325}\) had isolated from the direct methylation of 76, as described above). The structure of 81·HI was established by hydrolysis to the base (3), which was in turn prepared by an unambiguous synthesis from 6-benzylthio-1-methylpurine (80) and a saturated solution (at 25°C) of MeNH\(_2\) in EtOH.\(^{327}\) Robins' group explained that the above methylation of 76 by Wacker and Ebert probably proceeded through N(1)-methylation to form 1-methyladenosine (77), Dimroth rearrangement\(^{328}\) of 77 to 78 under alkaline conditions, and N(1)-methylation of 78 to give 81.\(^{327}\) Toraya et al.\(^{329}\) recently repeated the above 78→81→3 route with some modification: methylation of 78 with MeI in AcNMe\(_2\) at rt for 4 d and hydrolysis of 81 with 0.5 N aqueous HCl at 100°C for 1 h.

Methylation of \(N^6,1\)-dimethyladenine (3) with MeI in DMF at 100°C (in a closed vessel) for 10 min was reported to produce \(N^6,1,9\)-trimethyladenine hydriodide (79·HI) in 72.5% yield.\(^{330}\)

The following physical properties and spectral characteristics of \(N^6,1\)-dimethyladenine
(3) have been recorded in the literature: the melting point for the free base (3), mp >300°C;\textsuperscript{327} for the picrate, mp 235°C\textsuperscript{325} or mp 236°C\textsuperscript{327} paper chromatography;\textsuperscript{325,327} TLC;\textsuperscript{329} HPLC;\textsuperscript{329} MS;\textsuperscript{199} UV in H\textsubscript{2}O (at various pH's)\textsuperscript{325,327,329,331} and in a MeOH solution;\textsuperscript{327,331} \textsuperscript{1}H NMR in D\textsubscript{2}O.\textsuperscript{329}

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme13.png}

Scheme 13
\end{center}

\(N^6,1\)-Dimethyladenine (3) was among a series of synthetic purine analogues used for the study of the interactions between lima bean lectin and adenine (1), and the binding affinity of 3 was found to be weak.\textsuperscript{332} Starfish oocytes are naturally arrested at the prophase stage of the first meiotic division and resume meiosis in response to the maturation-inducing hormone 1-methyladenine (115), which is produced and released by the ovarian follicle cells under the influence of a peptide hormone (gonad-stimulating substance) from the radial nerve.\textsuperscript{329,333} Toraya \textit{et al.}\textsuperscript{329} found that the \(N^6\)-methylated derivative (3) still retained oocytes maturation-inducing activity, but to a much lesser extent.

**IV. \(N^6,3\)-DIMETHYLADENINE**

In their synthesis of \(N^6,3\)-dimethyladenine (4), Jones and Robins\textsuperscript{334} treated 6-mercaptopurine (21) with methyl \(p\)-toluenesulfonate in AcNMe\textsubscript{2} at 140°C for 2.5 h and basified a solution of the resulting tosylate salt (82-TsOH) to obtain the free base (82) in ca. 50% yield (Scheme 14). Treatment of 82 with aqueous MeNH\textsubscript{2} in MeOH at rt for 15 h furnished 4 in good yield.
Scheme 14

Alternatively, Fujii’s group prepared 4 from N'-methyl-5(4)-(N-methylformamido)imidazole-4(5)-carboxamidine (83) [see Section VII (Scheme 28)] by cyclization in H2O at rt under alkaline conditions. In yet another synthesis of 4, Fujii’s group methylated 21 with MeI in AcNMe2 to secure 82, which was then treated with MeONH2 in H2O (pH 5.0). Methylation of the resulting N6-methoxy derivative (84) with MeI in AcNMe2 afforded N6-methoxy-1,3-dimethyladeninium iodide (86) (40% yield) and N6-methoxy-N6,3-dimethyladenine (85) (isolated in 36% yield in the form of 85·HClO4). Hydrogenolysis of 85, generated from 85·HClO4 by the use of Amberlite IRA-402 (HCO3−), using Raney Ni catalyst and hydrogen gave 4 in 43% yield (Scheme 14).

Scheme 15

Methylation of N6-methoxy-N6-methyladenine (87), prepared from 6-chloropurine (25) and N,O-dimethylhydroxylamine, with MeI in AcNMe2 was also found to produce 85 (isolated in 67% yield as the perchlorate salt), an immediate precursor for 4, as well as the 9-methylated product (88) (18%) (Scheme 15).

The N6-methoxy-N6,3-dimethyl derivative (85), isolated as the perchlorate (93), was also
among the six products (84, 85, 88, and 90–92) obtained from the reaction of \( N^6 \)-methoxadenine (89) with an excess of \( \text{MeI} \) in \( \text{AcNMe}_2 \) at 40°C for 7 h (Scheme 16).337

![Scheme 16](image)

Direct methylation of \( N^6 \)-methyladenine (94) with an excess of \( \text{MeI} \) in \( \text{AcNMe}_2 \) at 38–42°C for 6 h was found to produce 4 in 82% yield, together with \( N^6,9 \)-dimethyladenine (6) (1.3%), \( N^6,3,7 \)-trimethyladenine (95) (1.8%), and \( N^6,1,9 \)-trimethyladenine (79) (0.3%) (Scheme 17).337

![Scheme 17](image)

The following physical properties and spectral characteristics of \( N^6,3 \)-dimethyladenine (4) have been reported in the literature: the melting point for the free base (4), mp 314–315°C (decomp),334 or mp >300°C;336,337 for 4·HI, mp 241–242°C (decomp);337 TLC;335 MS;199 UV for the free base (4) in H₂O (at various pH's);334,337 and in 95% aqueous EtOH;337 for 4·HI in H₂O (at various pH's) and in 95% aqueous EtOH.337

El'tsov et al.330 reported that methylation of 4 in DMF with an excess of \( \text{MeI} \) at 100°C (in a closed vessel) for 10 min afforded \( N^6,3,9 \)-trimethyladenine hydriodide (96·HI) (16%
yield) and N\textsuperscript{6},3,7-trimethyladenine hydriodide (95·HI) (64%) (Scheme 18). Fujii et al.\textsuperscript{337} found that a similar methylation of 4, but in AcNMe\textsubscript{2} at 38–40°C for 6 h, furnished 96·HI (15% yield) and 95·HClO\textsubscript{4} [29%, after treatment of the primary product (95·HI) with 70% aqueous HClO\textsubscript{4} in EtOH], being in general agreement with the above results obtained by El'tsov et al.\textsuperscript{330} Oxidation of 4 with m-CPBA in a mixture of 50% aqueous MeOH and 1 M phosphate buffer (pH 6.5) at 30°C for 20 h produced the N(7)-oxide (97) in 40% yield, together with 30% recovery of 4 (Scheme 18).\textsuperscript{338} Treatment of 3-methyladenine 7-oxide (99) with one molar equiv. of dimethyl sulfate in 0.1 N aqueous NaOH at rt for 17 h gave the N\textsuperscript{6}-methyl derivative (97) (13% yield) and 8-hydroxy-3-methyladenine (98a) (4%), together with 50% recovery of 99.\textsuperscript{339} In the presence of added MeOH, a similar methylation of 99 at rt for 2.5 h produced 97 (14% yield) and 8-methoxy-3-methyladenine (98b) (11%), together with 57% recovery of 99.\textsuperscript{339}

![Scheme 18](image)

Binding of 4 to lima bean lectin has been measured by a fluorimetric assay based on allosteric enhancement of 1,8-anilinonaphthalenesulfonate binding.\textsuperscript{332}

**V. N\textsuperscript{6},7-DIMETHYLADELINE**

The first synthesis of N\textsuperscript{6},7-dimethyladenine (5), achieved by Prasad and Robins,\textsuperscript{340} started from 1-methyl-4-nitroimidazole-5-carbonitrile (100) and proceeded through 4-amino-1-methylimidazole-5-carboxamide (101), 7-methylhypoxanthine (102), and 6-chloro-7-methylpurine (103), as shown in Scheme 19. Alternatively, Taylor and Loeffler\textsuperscript{341} synthesized 5 from the amino derivative (104)\textsuperscript{340} via the 4-ethoxymethyleneamino derivative (105), cyclization of 105 with MeNH\textsubscript{2} to form 1,7-dimethyladenine (8),
and the Dimroth rearrangement\(^3\) of 8 in boiling H\(_2\)O for 20 h. Fujii's group\(^4\) found that similar treatment of 8 with boiling H\(_2\)O for 9.5 h afforded 5 (63% yield) as well as 1,7-dimethylhypoxanthine (144: \(R^1 = R^2 = Me\)) (3.5%).

\[
\begin{align*}
100 & \xrightarrow{1) \text{concd } H_2SO_4, 2) \text{Raney } Ni/H_2} 101 \\
104 & \xrightarrow{\text{(ref. 340) \ Raney } Ni/H_2, EtOH, 1.9 \text{ atm, } 3.5 \text{ h (86% yield)}} 105 \\
105 & \xrightarrow{\text{MeNH}_2/\text{EtOH, } \Delta, \text{ reflux, 2 h (80% yield)}} 8 \\
8 & \xrightarrow{\text{MeNH}_2/\text{EtOH, } \Delta, \text{ benzene, reflux, 40 min (90% yield)}} 5 \\
5 & \xrightarrow{\text{H}_2O, \text{ reflux, 20 h (100% yield)}} 106
\end{align*}
\]

**Scheme 19**

Treatment of 7,9-dimethyladeninium iodide (12: \(X = I\)) with boiling 1 N aqueous NaOH for 1 h resulted in rearrangement to 5 in 87% yield, and similar treatment of the trans-formamide (106) also gave 5 in 72% yield (Scheme 20) (see also Section XII).\(^3\) Alternatively, cyclization of 106 to 5 (84%) was effected with NaH in AcNMe\(_2\) at rt for 40 min.\(^3\)

\[
\begin{align*}
12 & \xrightarrow{\text{1 N aq. } NaOH, \text{ reflux, 1 h (87% yield)}} 5 \\
5 & \xrightarrow{\text{1 N aq. } NaOH, \text{ reflux, 1 h (72% yield)}} 106 \text{ (trans)} \\
5 & \xrightarrow{\text{or NaH/AcNMe}_2, \text{ rt, 40 min (84% yield)}} 106 \text{ (trans)}
\end{align*}
\]

**Scheme 20**

The following may serve to locate papers describing the physical properties and spectral characteristics of \(N^6,7\)-dimethyladenine (5): the melting point for the free base (5), mp 300°C\(^3\) or 309–310°C\(^4\) or 311°C;\(^1\) MS;\(^1\) UV for the free base (5) in EtOH,\(^3\) or in 95% aqueous EtOH,\(^3\) and in H\(_2\)O (at various pH's);\(^3\) \(1H\) NMR in CDCl\(_3\).\(^3\)

**VI. \(N^6,9\)-DIMETHYLADENINE**

\(N^6,9\)-Dimethyladenine (6) was isolated, together with five organic compounds (1-hexa-
It was among a series of 15 $N^6$-substituted 9-methyladenines assessed as antagonists of A$_2$-adenosine receptor-mediated stimulation of adenylate cyclase in membranes of human platelets and rat PC12 cells and of A$_1$-adenosine receptor-mediated inhibition of adenylate cyclases in membranes of rat fat cells and as inhibitors of binding of $N^6$-[(R)-1-[3H]phenyl-2-propyl]adenosine to A$_1$-adenosine receptors in rat brain membranes. It has been disclosed for determining the viability of tissue with adenosine/adenosine agonist and A$_1$-adenosine receptor antagonist. Methods for prevention and treatment of ischemia-reperfusion and endotoxin-related injury by the use of adenosine and purino receptor antagonists including 6 have been provided.

Scheme 21

The synthesis of 6 by Robins and Lin$^{349}$ started from 4,6-dichloro-5-nitropyrimidine (107) and proceeded through the 6-methylamino derivative (108), the 5-amino derivative (109), and 6-chloro-9-methylpurine (110) or through 4,6-bis(methylamino)-5-nitropyrimidine (111) and 4,6-bis(methylamino)-5-aminopyrimidine (112), as depicted in Scheme 21. Goldner and Carstens$^{350}$ obtained 111 from 107 in 88% yield by amination with boiling ethanolic MeNH$_2$ for 45 min and cyclized 112, prepared by reduction of 111 with Raney Ni catalyst and hydrogen in MeOH, by heating in boiling HCONH$_2$ for 20 min to secure 6 in 49% yield. Brown and Jacobsen$^{351}$ heated 112 on a steam bath with 90% formic acid for 1 h to obtain the 5-formamido derivative (113), which furnished 6 on heating at 250°C until effervescence ceased. Sakata and co-workers$^{352}$ prepared 6 from 6-methylthiopurine (22) through 9-methyl-6-methylthiopurine (114), as shown in Scheme 22. They determined the rate constant for
the reaction of 114 with MeNH₂ in EtOH at 25 ± 1°C to be of very low value (<10⁻⁶ s⁻¹ M⁻¹).³⁵³

Scheme 22

Robins' group³²⁷ methylated 1-methyl adenine (115)⁸ with methyl p-toluenesulfonate in AcNMe₂ at 125°C for 2.5 h to obtain 1,9-dimethyl adenine p-toluenesulfonate [9-HX (X = TsO)], which underwent Dimroth rearrangement³²⁸ to afford 6 when heated in 0.1 N aqueous NaOH for 5 min (Scheme 23). Fujii's group³⁵⁴ methylated 115 with MeI in AcNMe₂ at 60–65°C for 11 h and converted the resulting hydriodide [9-HX (X = I)] into the perchlorate [9-HX (X = ClO₄)] in 27% yield (from 115). The perchlorate was then converted into the free base (9) by treating with Amberlite IRA-402 (HCO₃⁻), and heating of an aqueous solution of 9 under reflux for 3 h gave 6 in 54% yield. The Dimroth rearrangement of 9 to 6 under alkaline conditions was also carried out by Dodin et al.³⁵⁵

N₆,9-Dimethyl adenine-2-d (119) was prepared from 9-methyl adenine-2-d (116) through 1,9-dimethyl adenine-2-d hydriodide (117) and the putative intermediate (118) by a route (Scheme 24) analogous to that employed for the preparation of the unlabeled species (6).³⁵⁶

Scheme 23

Scheme 24
Methylation of adenine (1) with trimethyl phosphate in H$_2$O at pH 10-11 and 60°C for 24 h gave a mixture of six products, from which 6 (10% yield), 3-methyladenine (158) (6%), 9-methyladenine (146) (27%), and 4,6-bis(methylamino)-5-(N-methylformamido)pyrimidine (1%) were isolated.\textsuperscript{357} Methylation of 9-methyladenine (146)\textsuperscript{8} under similar conditions (at pH 9.5-10.0 and 37°C for 24 h) gave 6 (3%) and 1,9-dimethyladenine (9) (2%) with 94% recovery of the starting material.\textsuperscript{357} Shugar's group\textsuperscript{358} found that methylation of 9-methyladenine (146)\textsuperscript{8} with dimethyl sulfate in 0.15 M phosphate buffer (pH 7.5) at pH 7-7.5 for 1.5 h gave 1,9-dimethyladenine (9) (ca. 40% yield) (see also Section IX, Scheme 33), which was quantitatively rearranged to 6 on treatment at pH 13 and 60°C for 20 min. When this methylation was effected under strongly alkaline conditions (in 2 N aqueous KOH for 1.5 h), 6 was obtained in only ca. 2% yield.\textsuperscript{358} As mentioned before (Section IV and Scheme 17), 6 (1.3% yield) was among four products from direct methylation of $N^6$-methyladenine (94) with an excess of MeI in AcNMe$_2$ at 38-42°C for 6 h.\textsuperscript{337}

In a multistep synthesis of 6 by Fujii's group,\textsuperscript{359} 1-methoxy-9-methyladenine (120) obtained from its hydriodide salt (120-HI) was methylated with MeI in AcNMe$_2$ at 50°C for 25 h to give 1-methoxy-$N^6$-9-dimethyladenine hydriodide (121-HI) in 51% yield (Scheme 25). Direct methylation of adenine 1-oxide with MeI in AcNMe$_2$ in the presence of 30% aqueous H$_2$O$_2$ also gave, after treatment of the product with NaClO$_4$, 121-HClO$_4$ but in only 4% yield, together with its ring-opened product, 5-formamido-$N'$-methoxy-$N,1$-dimethylimidazole-4-carboxamidine (126) (16%).\textsuperscript{360} This methylation of adenine 1-oxide

\begin{align*}
&\text{120-HI} \\
&\xrightarrow{\text{HI}} \text{MeO} \\
&\xrightarrow{\text{H}_2\text{O}} \text{120} \\
&\xrightarrow{\text{Me/AcNMe}_2} \text{121} \\
&\xrightarrow{\text{HX}} \text{6}
\end{align*}

\textbf{Scheme 25}
to form the trimethylated product (121-HX) was considered to proceed via 1-methoxyadenine hydriodide, its free base, 120-HI, and 120 in a one-pot manner.\textsuperscript{360} Hydrogenolysis of 121-HClO\textsubscript{4} with 10% Pd-C catalyst and hydrogen and treatment of the product with Amberlite IRA-402 (HCO\textsubscript{3}\textsuperscript{-}) provided 6 in 38% yield.\textsuperscript{361} Alternatively, methylation of N\textsuperscript{6}-methoxy-9-methyladenine (90) (see also Section IV, Scheme 16), prepared from 120-HI by Dimroth rearrangement,\textsuperscript{362} with MeI in AcNMe\textsubscript{2} at 30°C for 7 h furnished N\textsuperscript{6}-methoxy-N\textsuperscript{6},9-dimethyladenine hydriodide (88-HI) in 24% yield, together with N\textsuperscript{6}-methoxy-7,9-dimethyladeninium iodide (91: X = I) (see also Section IV, Scheme 16) in 59% yield.\textsuperscript{336,363} The free base (88) (see also Section IV, Schemes 15 and 16), obtained from 88-HI by the use of Amberlite IRA-402 (HCO\textsubscript{3}\textsuperscript{-}), was then hydrogenolyzed with Raney Ni catalyst and hydrogen to produce 6 in 53% or 95% yield.\textsuperscript{336,363}

For papers describing the physical properties and spectral characteristics of N\textsuperscript{6},9-dimethyladenine (6), the reader is referred to Table II, which includes additional references.\textsuperscript{364-377}

Interactions of 6 with the following substances have been reported: self-association in aqueous solutions\textsuperscript{365,373,378,379} and in D\textsubscript{2}O;\textsuperscript{223,368,374,380} H\textsubscript{2}O vapor (hydration);\textsuperscript{204} butyric acid in CDCl\textsubscript{3} via hydrogen bonding;\textsuperscript{369} 1,3-dimethyluracil in H\textsubscript{2}O\textsuperscript{381} and in D\textsubscript{2}O;\textsuperscript{382-384} 1,4- and 2,4-dimethyluracils in D\textsubscript{2}O;\textsuperscript{384} poly(U) in H\textsubscript{2}O;\textsuperscript{385} poly(5-bromouridylic acid) in H\textsubscript{2}O;\textsuperscript{386} p-cresol in CDCl\textsubscript{3};\textsuperscript{387} diazepam in CDCl\textsubscript{3} and nitrazepam in CDCl\textsubscript{3};\textsuperscript{388} K\textsubscript{2}PtCl\textsubscript{4} in 2.5 N aqueous HCl at 50°C for several hours to give Cl\textsubscript{3}Pt(C\textsubscript{7}H\textsubscript{10}-N\textsubscript{5})\textsubscript{3}H\textsubscript{2}O, which reacted with aqueous NH\textsubscript{3} to produce cis-Cl\textsubscript{2}Pt(C\textsubscript{7}H\textsubscript{9}N\textsubscript{5})(NH\textsubscript{3}).\textsuperscript{364}

\begin{center}
\textbf{Scheme 26}
\end{center}

As regards the chemical behavior of 6, El'tsov \textit{et al.}\textsuperscript{330} found that methylation of 6 with MeI in DMF at 100–105°C for 10 min gave a 51:30:19 mixture of 79-HI, 96-HI, and 122...
### TABLE II. $N^{6,9}$-Dimethyladenine (6): Physical and Spectral Characteristics

<table>
<thead>
<tr>
<th>Item</th>
<th>Specification$^a$</th>
<th>Literature (ref. No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point$^b$</td>
<td>193.5–195°C (350); 193–195°C (357); 190–191°C (349); 185–186°C (351, 354); 181.5–182.5°C (352)</td>
<td>(356)</td>
</tr>
<tr>
<td><strong>6-2-d (119)</strong></td>
<td>184–185°C</td>
<td>(356)</td>
</tr>
<tr>
<td>Acid dissociation constant</td>
<td>basic $pK_a$</td>
<td>4.12 ± 0.03 (H$_2$O at 20°C and ionic strength 0.01$^c$) (351); 4.02 ± 0.03 (H$_2$O at 20°C)$^c$ (354); 3.8 ± 0.05 (0.1 M aq. NaCl)$^c$ (364)</td>
</tr>
<tr>
<td>TLC</td>
<td></td>
<td>(221, 357, 358, 365)</td>
</tr>
<tr>
<td>MS</td>
<td>6-2-d (119)</td>
<td>(356)</td>
</tr>
<tr>
<td>UV spectrum</td>
<td></td>
<td>(345, 366)</td>
</tr>
<tr>
<td>UV photoelectron spectrum</td>
<td></td>
<td>(210)</td>
</tr>
<tr>
<td>IR spectrum</td>
<td></td>
<td>(345, 367$^d$)</td>
</tr>
<tr>
<td>$^1$H NMR spectrum</td>
<td></td>
<td>(345)</td>
</tr>
<tr>
<td>In D$_2$O (223, 368); in CD$_3$OD (221); in MeOH (368); in DMSO-$d_6$ (356); in CDCl$_3$ (221, 352, 356, 369); in CCl$_4$ (368); in liquid NH$_3$ (370)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{13}$C NMR spectrum</td>
<td>6-2-d (119)</td>
<td>(356)</td>
</tr>
<tr>
<td>Crystal structure</td>
<td></td>
<td>(345)</td>
</tr>
<tr>
<td>Dipole moment (in H$_2$O)</td>
<td>6.6 ± 1.1 D (at 0°C), 7.9 ± 0.9 D (20°C), and 7.4 ± 0.6 D (40°C) (373)</td>
<td></td>
</tr>
<tr>
<td>Neutron diffraction</td>
<td>In D$_2$O</td>
<td>(374)</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td>Between CHCl$_3$ and H$_2$O</td>
<td>(375)</td>
</tr>
<tr>
<td>Partial specific volume</td>
<td>0.731 ml/g</td>
<td>(365)</td>
</tr>
<tr>
<td>Potential surface</td>
<td></td>
<td>(247)</td>
</tr>
<tr>
<td>Enthalpy of hydration</td>
<td></td>
<td>(204, 247–249)</td>
</tr>
<tr>
<td>Enthalpy of solution</td>
<td>In H$_2$O</td>
<td>(248, 249)</td>
</tr>
<tr>
<td>Enthalpy of sublimation</td>
<td></td>
<td>(248–250, 376)</td>
</tr>
<tr>
<td>Ionization potential</td>
<td></td>
<td>(210)</td>
</tr>
<tr>
<td>Electronic spectrum</td>
<td>Calculated by the CNDO/OPTIC-2 method</td>
<td>(377)</td>
</tr>
<tr>
<td>Electronic structure</td>
<td></td>
<td>(210)</td>
</tr>
</tbody>
</table>

---

$^a$ With or without reference number(s) in parentheses.

$^b$ Reported for analytical samples, in most cases.

$^c$ UV spectral.

$^d$ In the vapor phase.
(X = I) in 94% yield (Scheme 26). The corresponding N(1)-CD₃, N(3)-CD₃, and N(7)-CD₃ species were similarly obtained by using CD₃I instead of MeI in the above reaction. Fujii’s group isolated 96-HI (17% yield) and 79-HClO₄ (11% yield, after conversion from 79-HI) from the reaction mixture obtained by methylation of 6 with MeI in AcN-Me₂ at 40°C for 6 h. Kos and van der Plaas have reported the reductive removal of the methylamino group from 6 to provide 9-methylpurine (123) in 42% yield, which was effected with sodium in liquid NH₃ for 30 min.

A multistep conversion of 6 into 1,9-dimethyladenine (9), as shown in Scheme 27, has been reported by Fujii’s group. Oxidation of 6 with m-CPBA in EtOH at 35°C for 6 h gave the N(1)-oxide (124) in 62% yield. Alternatively, the N(1)-oxidation was accomplished with 30% aqueous H₂O₂ in AcOH at 55°C for 20 h, but in 23% yield. Later on, Dodin et al. recorded a similar peracetic acid oxidation of 6. Reversion of 124 to 6 (72% yield) was effected by hydrogenolysis using Raney Ni catalyst and hydrogen. The N(1)-oxide (124) underwent methylation almost exclusively at the N(1)-O atom when treated with MeI in AcN-Me₂, resulting in the formation of the 1-methoxy derivative (121-HI) in 89% yield. The location of the third methyl group was established by demethylation with boiling pyridine (or boiling EtOH) leading to the N(1)-oxide (124) and also by catalytic hydrogenolysis of the corresponding perchlorate (121-HClO₄) (Scheme 25) to afford 6. Treatment of the free base (121) with boiling H₂O under mildly alkaline conditions for 70 min provided the isomeric product (125) in 71% yield. Alternatively, this rearrangement was feasible by treating 121 with boiling H₂O (pH 9) for 3 h. On the other hand, treatment of 121 with H₂O at rt for 42 h furnished the monocyclic compound (126) (72% yield), which recylized almost exclusively to 125 on treat-
ment with boiling H₂O for 7.5 h. Catalytic hydrogenolysis of 125 gave, after conversion of the product into a salt form, 1,9-dimethyladenine perchlorate (9·HClO₄) in 71% yield. Thus, the above multistep conversion of 6 into 9 achieved by Fujii's group has demonstrated the usefulness of the MeO group as an easily removable control synthon in the structural transformation reverse to that (9→6) which occurs in the usual Dimroth rearrangement in the adenine series.³²⁸ Heating 6 in boiling 1 N aqueous NaOH for 30 min resulted in 90% recovery of 6, indicating its stability under alkaline conditions.³⁸⁰

VII. 1,3-DIMETHYLADENINE

![Scheme 28](image)

1,3-Dimethyladenine (7)⁷ occurs in nature as the 2-oxo derivative (1,3-dimethylisoguanine) (127), a new purine from the marine sponge *Amphimedon viridis*.³⁹¹ In their methylation study of adenosine (76) using dimethyl sulfate in DMF at 100°C for 2 h, Brookes and Lawley³⁹² isolated, after hydrolysis of the products with boiling 1 N aqueous HCl for 1 h, a dimethyladenine in the form of the sulfate salt for which the structure “1,3-dimethyladeninium sulfate (7: X = HSO₄)” was proposed, although the N⁶,3-methyladenine structure (4·H₂SO₄) was considered as a possibility by these authors. Later on, however, Broom *et al.*³²⁷ established the structure of the above “1,3-
dimethyladenine" to be in reality 3,7-dimethyladenine (10).

Fujii and co-workers\textsuperscript{336} found that methylation of \textit{N\textsuperscript{6}}-methoxy-1-methyladenine (128) with MeI in AcNMe\textsubscript{2} at 30°C for 9 h gave \textit{N\textsuperscript{6}}-methoxy-1,3-dimethyladeninium iodide (86) (44% yield) and \textit{N\textsuperscript{6}}-methoxy-1,9-dimethyladenine (125) (isolated as 125·HClO\textsubscript{4} in 38% yield) (Scheme 28). The \textit{N\textsuperscript{6}}-methoxy-1,3-dimethyl compound (86) was alternatively obtainable from \textit{N\textsuperscript{6}}-methoxy-3-methyladenine (84) by similar methylation (Section IV, Scheme 14).\textsuperscript{336} With the aim of synthesizing a genuine 1,3-dimethyladenine structure, they next tried to remove the \textit{N\textsuperscript{6}}-methoxy group from 86. On catalytic reduction using Raney Ni catalyst and hydrogen (MeOH, 3 atm, 18–20°C, 20 h), 86 produced two 1,2-dihydro derivatives [130 (26% yield) and 129·HI (17%)], instead of the desired product (7: \(X = \text{I}\)) (Scheme 28). Oxidation of 130 with iodine in EtOH at rt for 30 min regenerated 86 (38% yield), which reverted to 130 in 92% yield upon reduction with NaBH\textsubscript{4} in MeOH at rt for 30 min. Further reduction of 130 with Raney Ni catalyst and hydrogen (EtOH, 1 atm, 50°C, 3 h) provided the demethoxy derivative (129) in 71% yield. The difficulty in removing the \textit{N\textsuperscript{6}}-methoxy group without partial saturation of the adeninium ring and the high-yield two-step synthesis of 129 from 86 led them to examine the dehydrogenation of 129 as an alternative route to 7.\textsuperscript{335} Although trials conducted with iodine, sodium nitrite, air, or chloranil for this step all failed, treatment of 129 with DDQ in CHCl\textsubscript{3} at rt for 50 h afforded a dark brown solid presumed to be 7 (\(X = 2,3\)-dichloro-5,6-dicyano-4-hydroxyphenolate). Since the solid was unstable and difficult to purify by recrystallization, conversion into the bromide salt (7: \(X = \text{Br}\)) was attempted by treating it with concd aqueous HBr in MeCN under ice-cooling. However, the product isolated was not the desired salt but the hydrobromide of the ring-opened derivative (83). The hydrobromide (83·HBr) was also found to be unstable in H\textsubscript{2}O at rt at pH 7 or above: It quickly underwent recyclization to give \textit{N\textsuperscript{6},3}-dimethyladenine (4) in 53% yield [based on the dihydro derivative (129) used]. The sequence 7→83→4 thus concluded a Dimroth rearrangement.\textsuperscript{328} Although Fujii's group has been unable to characterize the 1,3-dimethyladenine structure (7) obtained by the DDQ oxidation of 129, the above results indicate its virtual formation and extreme instability. Since the rate of ring opening of 7 could not be measured directly, that of the \textit{N\textsuperscript{6}}-methoxy derivative (86: \(X = \text{ClO}_4^–\) for I) was determined instead.\textsuperscript{335} Treatment of 86 in H\textsubscript{2}O with Amberlite IRA-402 (HCO\textsubscript{3}–) at rt afforded the monocycle (131) in 92% yield. In H\textsubscript{2}O at pH 7.72 (ionic strength 0.5) and 25°C, this ring opening proceeded at a rate of 1.36×10\textsuperscript{-1} min\textsuperscript{-1} (half-life 5.1 min). On the other hand, treatment of 131 with 0.1 N aqueous HCl at 25°C for 21 h gave the recyclized product (86) [isolated as the perchlorate (86: ClO\textsubscript{4}– for I) in 53% yield. The above ring opening of 86 in the pyrimidine moiety was ca. 270 times as fast as that of \textit{N\textsuperscript{6}}-methoxy-3,9-dimethyladenine (168).\textsuperscript{335} Therefore, the genuine 1,3-dimethyladenine structure (7) itself may be regarded as one of the most unstable dimethyladenines in H\textsubscript{2}O under alkaline conditions.\textsuperscript{335,390}

The dihydro derivative (129) was found to give the N(9)-benzylated product (132) when
treated with PhCH₂Br in AcNMe₂ (Scheme 29), and alkaline hydrolysis of 132 afforded 1-benzyl-5-methylaminoimidazole-4-carboxamide (133).³⁹³

\[
\text{129} \xrightarrow{\text{PhCH₂Br/AcNMe₂}} \begin{array} {c}	ext{132} \xrightarrow{1 \text{ N aq. NaOH}} \text{133} \\
1 \text{N} \text{aq. NaOH} & \text{reflux, } 1 \text{ h} & (17\% \text{ yield})
\end{array}
\]

\[\text{Scheme 29}\]

VIII. 1,7-DIMETHYLADENINE

As regards the biological activity of 1,7-dimethyladenine (8), its inability in triggering either stimulation of \[^{86}\text{Rb}^+\] uptake alone or both this elementary event and the integrated process of germinal vesicle breakdown in \textit{Marthasterias glacialis} oocytes have been reported.³⁹⁴ Doreé disclosed that 8 did not show stimulation of \[^{24}\text{Na}^+\] influx in fully grown prophase-blocked starfish oocytes.³⁹⁵ The inability of 8 to replace 1-methyladenine (115) in releasing meiosis inhibition in starfish oocytes has also been known.³⁹⁶

In 1960, Taylor and Loeffler³⁴¹ reported the synthesis of 8 from 4-amino-5-cyano-1-methylimidazole (104) through the 4-ethoxymethyleneamino derivative (105), as described in Section V (Scheme 19).

\[
\text{134} \xrightarrow{\text{MeI/AcNMe₂}} \begin{array} {c}	ext{135} \xrightarrow{1) \text{MeOH}} \text{136} \xrightarrow{2) \text{NaClO}_4} \text{8} \\
18^\circ \text{C}, 20 \text{ h} & (89\% \text{ yield}) & \text{reflux, } 5 \text{ h} & (57\% \text{ yield}) & \text{Raney Ni/H}_2
\end{array}
\]

\[\text{Scheme 30}\]

In an alternative synthesis of 8 (Scheme 30), Fuji’s group³⁹⁷ methylated \(^N^6\)-methoxy-1-methyladenosine (134), obtainable from adenosine (76) in four steps,³³⁶ with MeI in AcNMe₂ to secure the N(7)-methylated product (135) in 89% yield. The 7-methyl derivative (135) was found to be susceptible to solvolysis: It afforded the perchlorate salt (136·HClO₄) of the aglycon in 57% yield when treated with boiling MeOH and then with NaClO₄. The aglycon salt (136·HClO₄) was then subjected to catalytic hydrogenolysis (Raney Ni/H₂, H₂O, 1 atm, 20°C, 5 h), giving the desired compound (8·HClO₄) in 47%
yield [in 7% overall yield from adenosine (76)].

![Chemical structures](image)

**Scheme 31**

In yet another synthesis of 8 (Scheme 31), Fujii's group treated 4-amino-1-methylimidazole-5-carboxamide perchlorate (101·HClO$_4$), which was obtainable from adenosine (1) in four steps [via 3-benzyladenine, 3-benzyl-7-methyladenine hydriodide (137), and 4-benzylamino-1-methylimidazole-5-carboxamide (138)], with POCl$_3$ in DMF below 35°C for ca. 3 h to obtain 4-dimethylaminomethyleneimino-1-methylimidazole-5-carbonitrile (139) in 70% yield, together with small amounts of 7-methylhypoxanthine (102) and a substance inferred to be the N-formyl-5-carboxamide derivative (140). Cyclization of 139 was then effected with MeNH$_2$·HCl in EtOH in the presence of Et$_3$N at rt for 23 h, and the product was isolated in the form of the perchlorate salt, affording 8·HClO$_4$ in 68% yield [in 12% overall yield from adenine (1)].

The following physical properties and spectral characteristics of 1,7-dimethyladenine (8) have been reported in the literature: the melting point for the free base (8), mp 170–171°C; for 8·3/5H$_2$O, mp 163–168°C; for 8·HCl (crude), mp 224–230°C (decomp); for anhydrous 8·HClO$_4$, mp 278–280°C (decomp); for 8·HClO$_4$·1/5H$_2$O, mp 263–264°C (decomp); pK$_a$ ca. 6.5 or 6.50 ± 0.10 (in H$_2$O at 25 ± 0.1°C); for 8·HClO$_4$, pK$_a$ 7.86 ± 0.03 (in H$_2$O at 40°C and ionic strength 0.5); MS; UV for the free base (8) in EtOH and in 0.1 N aqueous HCl, for 8·3/5H$_2$O in 95% aqueous EtOH and in H$_2$O (at pH 1, 7, and 13); for 8·HClO$_4$·1/5H$_2$O in 95% aqueous EtOH and in H$_2$O (at pH 1, 7, and 13); 1H NMR for 8·3/5H$_2$O in DMSO-$d_6$; for 8·HClO$_4$·1/5H$_2$O in DMSO-$d_6$. Probably the most salient feature in the chemical behavior of 1,7-dimethyladenine (8) is that it undergoes Dimroth rearrangement under slightly alkaline conditions, giving N$^6$,7-dimethyladenine (5), and this affords a sound basis for one of the preparative methods for 5, as described above in Section V (Scheme 19). In some cases, the rearrangement reactions of 1,7-dialkyladenines (141) leading to N$^6$,7-dialkyladenines (143)
are accompanied with hydrolytic deaminations to give 1,7-dialkylhypoxanthines (144)\textsuperscript{342} and/or 7-alkylhypoxanthines (145),\textsuperscript{341,342} when effected in boiling H\textsubscript{2}O (Scheme 32). Thus, treatment of 1,7-dimethyladenine (8) (or 141: R\textsubscript{1} = R\textsubscript{2} = Me) with boiling H\textsubscript{2}O for 9.5 h gave N\textsuperscript{6},7-dimethyladenine (5) (or 143: R\textsubscript{1} = R\textsubscript{2} = Me) (63% yield) as well as 1,7-dimethylhypoxanthine (144: R\textsubscript{1} = R\textsubscript{2} = Me) (3.5%).\textsuperscript{342} For the concomitant deamination in the Dimroth rearrangement of 141, Fujii’s group\textsuperscript{342} has proposed possible mechanisms involving hydrolysis of the amidine moiety of the putative intermediate (142) [resulting from hydrolytic fission at the N(1)-C(2) bond of 141] and/or a direct hydrolytic deamination via an addition–elimination at C(6). However, a recent study on the Dimroth rearrangement, hydrolytic deamination, and pyrimidine-ring breakdown of 1-alkoxy-7-alkyladenines suggests that a third mechanism, which proceeds through N(1)-C(6) bond fission, may operate in these deamination reactions.\textsuperscript{400}

![Scheme 32](image)

IX. 1,9-DIMETHYLADENINE

There have been several papers dealing with the biological activity of 1,9-dimethyladenine (9). Dorée and Guerrier\textsuperscript{401} reported that neither 9 nor 1,9-dibenzyladenine inhibited nuclear maturation of the starfish oocytes induced by 1-methyladenine (115). Dorée \textit{et al.}\textsuperscript{103} demonstrated the localization and specificity of 1-methyladenine (115) receptors in eggs of the starfish \textit{Marthasterias glacialis} and \textit{Asterias rubens} and found that 9 (2 \times 10\textsuperscript{-4} M) significantly inhibited the absorption of 115 (1.5 \times 10\textsuperscript{-7}, 5 \times 10\textsuperscript{-8}, and 2 \times 10\textsuperscript{-8} M) but did not affect the initiation of egg meiosis. 1,9-Dimethyladenine (9) was found to be devoid of the ability to replace 115 in triggering meiosis in the starfish oocytes and of the ability to inhibit the 115-dependent induction of meiosis.\textsuperscript{104} Dorée\textsuperscript{395} also reported that 9 did not show stimulation of \textsuperscript{24}Na\textsuperscript{+} influx in fully grown prophase-blocked starfish oocytes. Yoshikuni \textit{et al.}\textsuperscript{402} found that 9 did not inhibit the specific binding of 1-[\textsuperscript{3}H]methyladenine to cortices isolated from full-grown prophase-arrested oocytes of the starfish \textit{Asterina pectinifera}.

As regards the synthesis of 9, methylation of 1-methyladenine (115)\textsuperscript{8} with methyl p-toluenesulfonate in AcNMe\textsubscript{2} to yield 9-TsOH\textsuperscript{327} or with MeI in AcNMe\textsubscript{2} to yield 9-HI (and 9-HClO\textsubscript{4})\textsuperscript{354} is described above in Section VI (Scheme 23). The regioselectivity in
this methylation is in general agreement with preferential N(9)-methylation of 1-benzyladenine. On the basis of the fact that 9-substituted adenines are methylated most easily at N(1), Dubois' group conversely methylated 9-methyladenine (146) with MeI in AcNMe2 and isolated the product (9) in the form of the free base, perchlorate, and hydrochloride (Scheme 33). Analogous routes to 9 from 146 using dimethyl sulfate in H2O at pH 7–7.5 or using trimethyl phosphate in H2O at pH 9.5–10.0 and 37°C and to 1,9-dimethyladenine-2-d hydridi (117) from 9-methyladenine-2-d (116) (Scheme 24) are described above in Section VI.

Beasley and Rasmussen found that methylation of adenine (1) with MeI in DMF at 30°C for 168 h (Scheme 33) gave a product mixture (63% yield), which consisted of 9 (less than 5%), 3-methyladenine (158) (56%), 9-methyladenine (146) (30%), and 7-methyladenine (159) (5–10%). Muravich-Aleksandr et al. reported that methylation of 1 with MeI in DMF at 100°C gave 1-methyladenine hydriodide (115·HI) and 9·HI.

The multistep conversion of N6,9-dimethyladenine (6) into 9·HClO4 through 1-methoxy-N6,9-dimethyladenine hydridi (121·HI) utilizing an N-methoxy group as a control synthon, as described in Section VI (Scheme 27), represents an alternative syn-
thesis of 1,9-dimethyladenine (9).361
References to the physical properties and spectral characteristics of 1,9-dimethyladenine (9) are indicated by number in Table III, with some additions.408–410

**Table III.** 1,9-Dimethyladenine (9): Physical and Spectral Characteristics

<table>
<thead>
<tr>
<th>Item</th>
<th>Specificationa)</th>
<th>Literature (ref. No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point (9)</td>
<td>Free base (9)</td>
<td>Not specified</td>
</tr>
<tr>
<td>9-HCl</td>
<td>Not specified</td>
<td>(399, 408, 409)</td>
</tr>
<tr>
<td>9-HI</td>
<td>277–278°C (decomp) (for a crude sample) (354); &gt;310°C (410)</td>
<td></td>
</tr>
<tr>
<td>9-HClO4</td>
<td>303–304°C (decomp) (354); not specified (399)</td>
<td></td>
</tr>
<tr>
<td>9-TsOH</td>
<td>Not specified</td>
<td>(327)</td>
</tr>
<tr>
<td>9-2-d-HI (117)</td>
<td>298–299°C (decomp)</td>
<td>(356)</td>
</tr>
<tr>
<td>Acid dissociation constant</td>
<td>basic pK&lt;sub&gt;a&lt;/sub&gt;</td>
<td>9.03 ± 0.05 (H₂O at 25 ± 0.1°C)c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.08 ± 0.07 (for 9-HClO₄ in H₂O at 20°C)c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.94 ± 0.05 (for 9-HClO₄ in 0.1 M buffers at 40°C and ionic strength 0.50)c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.96 ± 0.04 or 8.97 ± 0.03 (for 9-HClO₄ in 1/9 M buffers at 40°C and ionic strength 1.0)c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.1 ± 0.05 (in 0.1 N aq. NaCl)c)</td>
</tr>
<tr>
<td>TLC</td>
<td></td>
<td>(358)</td>
</tr>
<tr>
<td>Paper electrophoresis</td>
<td>9-HClO₄</td>
<td>(354)</td>
</tr>
<tr>
<td>UV spectrum</td>
<td></td>
<td>In H₂O and in dioxane</td>
</tr>
<tr>
<td>9-HI</td>
<td></td>
<td>In 0.025 M phosphate buffer containing 10% EtOH (pH 7.1) (410)</td>
</tr>
<tr>
<td>9-HClO₄</td>
<td></td>
<td>In H₂O at various pH’s and in 95% aq. EtOH (354)</td>
</tr>
<tr>
<td>9-TsOH</td>
<td></td>
<td>In H₂O at various pH’s (327)</td>
</tr>
<tr>
<td>9-2-d-HI (117)</td>
<td></td>
<td>In H₂O at various pH’s and in 95% aq. EtOH (356)</td>
</tr>
<tr>
<td>IR spectrum</td>
<td></td>
<td>In CHCl₃</td>
</tr>
<tr>
<td>¹H NMR spectrum</td>
<td></td>
<td>(345)</td>
</tr>
<tr>
<td>9-HClO₄</td>
<td></td>
<td>In DMSO-d₆</td>
</tr>
<tr>
<td>9-2-d-HI (117)</td>
<td></td>
<td>In DMSO-d₆</td>
</tr>
<tr>
<td>¹³C NMR spectrum</td>
<td></td>
<td>(345)</td>
</tr>
<tr>
<td>9-HCl</td>
<td></td>
<td>In H₂O</td>
</tr>
<tr>
<td>Crystal structure</td>
<td></td>
<td>(408)</td>
</tr>
</tbody>
</table>

a) With or without reference number(s) in parentheses.  
b) Reported for analytical samples, in most cases.  
c) UV spectral.

Interactions of 9 with the following substances have been reported: indole-3-acetic acid to form a 1:1 complex; Na[Co(acac)₂(NO₂)₂] in H₂O; 9-HClO₄ with K₂PtCl₄ in
0.1 N aqueous HCl at 22°C for 22 h to give Cl₂Pt(C₇H₁₀N₅)·H₂O, which reacted with aqueous NH₃ to produce cis-Cl₂Pt(C₇H₉N₅)(NH₃). The latter complex was converted into cis-Cl₂Pt(C₇H₁₀N₅)(NH₃) by treatment with 0.2 N aqueous HCl. The chemical behavior of 1,9-dimethyladenine (9) is characterized primarily by the ability to undergo Dimroth rearrangement to give N⁶,9-dimethyladenine (6), which affords a basis for one of the most important methods of preparing 6, as summarized above in Section VI (Schemes 23 and 24).

In a kinetic approach to the mechanistic problem, Fujii's group has found that the rearrangement of 9-substituted 1-alkyladenines (147) to the corresponding N⁶-isomers (149), including that of 9 (or 147: R¹ = R² = Me) to 6 (or 149: R¹ = R² = Me), at 40°C proceeds by a mechanism involving a rate-determining initial ring opening, caused by attack of hydroxide ion on both the protonated (147·H⁺) and the neutral species (147) at the 2-position, and a subsequent fast ring closure of the putative monocyclic intermediates (148) (Scheme 34). This is in general agreement with the mechanism which Macon and Wolfenden proposed for the Dimroth rearrangement of 1-methyladenosine (77) (or 147: R¹ = Me; R² = β-D-ribofuranosyl) to N⁶-methyladenosine (78) (or 149: R¹ = Me; R² = β-D-ribofuranosyl) at 25°C. The hydroxide attack on the protonated species is much faster than that on the neutral species (by a factor of 90–1100), and the former is influenced by the electronic effect of a substituent at the 1-position, whereas the latter is influenced by the steric effect. Interestingly, the electron-withdrawing β-D-ribofuranosyl group at the 9-position accelerates the ring opening of both the protonated and the neutral species.

Kohda's group reported that treatment of 9·HCl with hydrazine monohydrate in MeOH at rt for 36 h gave 6-hydrazino-9-methylpurine (150), 5-amino-1-methyl-4-(4-amino-1,2,4-triazol-3-yl)imidazole (151), and the Dimroth rearrangement product N⁶,9-dimethyladenine (6), as illustrated in Scheme 35.

![Scheme 35](image)

It is of interest to note that 1,9-dimethyladenine (9) occurs in nature in the form of the 8-oxo derivative: In 1985, Cimino et al. reported the isolation of a new purine (156) and known 1-methyladenine (spongopurine) (115), although both only as the acetyl derivative (157 and acetylspongopurine), from the English Channel sponge *Hymeniacidon sanguinea* Grant. While the new acetyl derivative (157) was fully characterized by
means of spectroscopic and X-ray crystallographic analyses, the parent base (156) remained unknown because of the difficulty in separating 156 and 115 from each other at the free base level.415

Scheme 36

Fujii's group416 was able to secure the free base (156) itself by two alternative syntheses starting from 8-bromo-9-methyladenine (152), which was obtainable from 9-methyladenine (146)8 by bromination (Scheme 36). The first route included methylation of 152 with MeI to give the 1-methylated product (155·HI), conversion of 155·HI into the free base (155), and treatment of 155 with AcONa in boiling AcOH to produce 156 (36% yield) and 157 (34%).

The second route included treatment of 152 with boiling 1 N aqueous NaOH and methylation of the resulting 8-oxo derivative (153) with MeI, affording 156 in 63% overall yield (from 146). The rearranged isomer (154) and the N6-acetyl derivative (157) were also synthesized from 156. These synthetic results made it possible to characterize fully 156 itself,416 in advance of the yet unrealized isolation of this substance from natural sources and to compare the reaction rates in the Dimroth rearrangements of 156 (to 154) and related compounds such as 155 (to 8-bromo-N6,9-dimethyladenine) and 1,9-dimethyladenine (9) [to N6,9-dimethyladenine (6)].412b

X. 3,7-DIMETHYLADENINE

In 1964, Robins' group327 reported that methylation of 3-methyladenine (158) with MeI in MeOH containing KOH at rt for 60 h or with dimethyl sulfate in DMF at 100°C for 2 h gave 3,7-dimethyladenine hydriodide (10·HI) or 10·MeOSO3H and that methylation of
7-methyladenine (159) with dimethyl sulfate in DMF at 100°C for 2 h produced 10·MeO-SO₃H, as identified by two-dimensional paper chromatography and UV spectroscopy (Scheme 37).

![Scheme 37](image)

The essentially reciprocal directivity in methylation of 158 and 159 was in line with that in alkylation of 3- and 7-alkyladenines reported by Leonard and Fujii⁴¹⁷ and by Montgomery and Thomas.⁴¹⁸ Fujii's group⁴¹⁷c methylated 158 with Mel in AcNMe₂ at 27°C for 5 h to secure 10·HI in 67% yield. Yamauchi et al.³⁵⁷ methylated 158 with trimethyl phosphate in H₂O at pH 9.5–10.0 at 60°C for 24 h to obtain 10 in 12% yield with 70% recovery of 158. As summarized above in Section VII, a product obtained by methylation of adenosine (76) and previously assigned the structure “1,3-dimethyladenine” by Brookes and Lawley³⁹² was shown to be 3,7-dimethyladenine (10).³²⁷

In yet another synthetic approach (Scheme 37), Fujii's group³³⁶ methylated N⁶-methoxy-7-methyladenine (160) with MeI in AcNMe₂ at 40°C for 2 h to obtain N⁶-methoxy-3,7-dimethyladenine (92) (44% yield) and N⁶-methoxy-7,9-dimethyladeninium iodide (91: X = I) (36%). Hydrogenolysis of 92·HClO₄ using Pd-C catalyst and hydrogen afforded 3,7-dimethyladenine perchlorate (10·HClO₄) in 59% yield. Muravich-Aleksandr et al.⁴⁰⁷ reported that 10·HI and 158·HI were the main products from the reaction of adenine (1) with MeI in DMF at 150°C.

The following physical properties and spectral characteristics of 3,7-dimethyladenine (10) are found in the literature: the melting point for 10·HI, mp >300°C;³²⁷,⁴¹⁷c for 10·HClO₄, mp 308–309°C (decomp);³³⁶ for 10·H₂SO₄, mp (not specified);³²⁷ for 10·MeOSO₃H, mp (not specified);³²⁷ for 10·picrate, mp 256°C;³²⁷ paper chromatography for 10·HI;³²⁷ TLC;³⁵⁷ MS;¹⁹⁹ UV in H₂O (at various pH's),³⁵⁷ for 10·HI in H₂O (at various pH's) and
in MeOH,\textsuperscript{327} for 10-HI in H\textsubscript{2}O (at various pH's) and in 95% aqueous EtOH,\textsuperscript{417c} for 10-HClO\textsubscript{4} in H\textsubscript{2}O (at various pH's) and in 95% aqueous EtOH;\textsuperscript{336} nonfluorescent in H\textsubscript{2}O (pH < 7) at rt;\textsuperscript{419} \textit{\textsuperscript{1}H} NMR for 10-HI in DMSO-\textit{d}\textsubscript{6}.\textsuperscript{417c}

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme38.png}
\end{center}

\begin{center}
\textbf{Scheme 38}
\end{center}

As regards the chemical behavior of 3,7-dimethyladenine (10), Robins' group\textsuperscript{327} observed that treatment of 10-HI with 2 N aqueous NaOH at rt for 4 d changed the UV spectrum to \( \lambda_{\text{max}} \) (pH 11) 267 nm, which was similar to the spectrum of 3,7-dibenzylhypoxanthine, suggesting basic hydrolysis to occur to give the corresponding 3,7-dimethylhypoxanthine (162) (Scheme 38). However, Fujii's group\textsuperscript{390} found that 10-HI gave the ring-opened monocycle (161) as the major product together with a trace amount of 162 under similar reaction conditions, and 161 was isolated in 47\% yield in the form of the perchlorate salt when 10-HI was treated with 1 N aqueous NaOH at 30°C for 7 d; in 0.1 N aqueous NaOH (pH 13), 10-HI was considerably stable at rt. Hydrolysis of 10-HI in 1 N aqueous NaOH at 80°C for 30 min gave 161 (39\% yield) and 162 (1\%), whereas that under reflux for 2 h furnished 161 (49\%) and the monodemethylated monocycle (101) (2\%), but without giving any 162.\textsuperscript{390} Conversion of 161 into theobromine utilizing ethoxycarbonylation has enhanced the usefulness of such ring opening of 10-HI.\textsuperscript{390,420}

\textbf{XI. 3,9-DIMETHYLADENINE}

3,9-Disubstitution in the adenine series has previously been known only in cyclic derivatives\textsuperscript{421} (e. g., 3,5'-cyclo-2',3'-O-isopropylideneadenosine \textit{p-toluenesulfonate}\textsuperscript{421a}), \( N^6,N^6 \)-dialkyl derivatives,\textsuperscript{421b,422} an \( N^6 \)-monomethylated derivative,\textsuperscript{330} or an \( N^6 \)-methyl-8-oxo derivative (\textit{i. e.}, caissarone\textsuperscript{338,423} isolated from the sea anemone \textit{Bunodosoma caissarum} Correa 1964). It is also assumed to occur as a partial structure, in the form of 3-alkyl-2'-deoxyadenosine, in alkylated DNA molecules.\textsuperscript{424} Although the prototype of
this disubstitution is 3,9-dimethyladenine (11), it remained unknown until a general synthetic route to 3,9-dialkyladenine salts, eventually shown to be applicable even to the syntheses of 3-methyladenosine p-toluenesulfonate and 2'-deoxy-3-methyladenosine p-toluenesulfonate was established by Fujii and co-workers.

\[ \text{(11)} \]

\[ \text{MeO} \]

\[ \text{NH} \]

\[ \text{Me} \]

\[ \text{H}_2\text{O} \]

\[ \text{H}_2\text{N} \]

\[ \text{OH} \]

\[ \text{Me} \]

\[ \text{Me} \]

\[ 120 \]

\[ \text{H}_2\text{N} \]

\[ \text{OH} \]

\[ \text{Me} \]

\[ \text{Me} \]

\[ 163 \]

\[ 1) \text{NaH or K}_2\text{CO}_3 \text{ in DMF} \]

\[ 2) \text{MeI \ rt, 1 h} \]

\[ \text{(for 164-HCl)} \]

\[ \text{Raney Ni/H}_2 \]

\[ \text{H}_2\text{O, 1 atm \ rt, 3 h} \]

\[ (66\% \text{ yield}) \]

\[ \text{NH} \cdot \text{HCl} \]

\[ 164 \]

\[ \text{LiAlH}_4/\text{THF} \]

\[ \text{rt, 2.5 h} \]

\[ (74\% \text{ yield}) \]

\[ \text{H}_2\text{N} \]

\[ \text{OH} \]

\[ \text{Me} \]

\[ \text{Me} \]

\[ 165 \text{HCl} \]

\[ \text{(for 167-HCl)} \]

\[ \text{Raney Ni/H}_2 \]

\[ \text{H}_2\text{O, 1 atm \ rt, 3 h} \]

\[ (84\% \text{ yield}) \]

\[ \text{NH} \cdot \text{HCl} \]

\[ 166 \text{HCl} \]

\[ \text{AcOCH(OEt)}_2/\text{DMF} \]

\[ \text{rt, 80 min} \]

\[ (89\% \text{ yield}) \]

\[ \text{NH} \]

\[ \text{Me} \]

\[ \text{Me} \]

\[ 11 \text{HCl} \]

\[ \text{H}_2\text{N} \]

\[ \text{OH} \]

\[ \text{Me} \]

\[ \text{Me} \]

\[ 167 \]

\[ \text{(for 168-HCl)} \]

\[ \text{10\% Pd-C/H}_2 \]

\[ \text{70\% aq. EtOH} \]

\[ \text{1 atm, rt, 7 h} \]

\[ (97\% \text{ yield}) \]

\[ \text{H}^+ \cdot \text{MeOH} \]

\[ \text{reflux, 7-8 h} \]

\[ \text{NH} \cdot \text{HCl} \]

\[ 168 \]

\[ \text{DCO}_2\text{D/MeCN} \]

\[ \text{30\%C, 24 h} \]

\[ (76\% \text{ yield}) \]

\[ \text{NH} \]

\[ \text{Me} \]

\[ \text{Me} \]

\[ 169 \]

\[ \text{(for 169-HCl)} \]

\[ \text{Raney Ni/H}_2 \]

\[ \text{H}_2\text{O, 1 atm \ 21\%C, 4 h} \]

\[ \text{NH} \cdot \text{HCl} \]

\[ 170 \]

\[ \text{Et}_3\text{N/MeOH} \]

\[ \text{reflux, 30 min} \]

\[ \text{or HClO}_4/\text{MeOH} \]

\[ \text{reflux, 7 h} \]

\[ \text{NH} \cdot \text{HCl} \]

\[ 171 \text{HCl} \]

\[ \text{Scheme 39} \]

In reaching 3,9-dimethyladenine (11) (Scheme 39), they reduced the formamidoimidazole derivative (163), the readily isolable ring-opened intermediate in the Dimroth rearrangement of 1-methoxy-9-methyladenine (120), with LiAlH\textsubscript{4} in THF at rt for 2.5 h to obtain the methylamino derivative (167) (74\% yield), which was treated with ethanolic HCl. The resulting hydrochloride (167-HCl) was then treated with ethyl orthoformate at 80°C for 4 h or in MeOH at rt for 40 min, furnishing the cyclized product (168-HCl) in 87\% or 94\% yield, respectively. Hydrogenolysis of 168-HCl or of 168-HClO\textsubscript{4} using Pd-C catalyst and hydrogen in 70\% aqueous EtOH or in EtOH gave 11-HCl or 11-HClO\textsubscript{4} in 61\% or 56\% yield, respectively. Alternatively, hydrogenation of 167 over Raney Ni catalyst in H\textsubscript{2}O containing one molar equiv. of HCl proceeded smoothly at rt, producing the amidine hydrochloride (166-HCl) in 84\% yield. Reaction of 166-HCl
with diethoxymethyl acetate in DMF at rt for 80 min afforded 11·HCl in 89% yield. In an alternative route to 11, methylation of the Na salt of 163, generated in situ from 163 and NaH in DNF at rt, with MeI in DMF at rt for 1 h produced the N-methylformamido derivative (164) in 87% yield. Similar methylation of the K salt, generated in situ from 163 and anhydrous K₂CO₃ in DMF at rt, also gave 164 in 84% yield. Hydrolysis of 164 with boiling 1 N aqueous NaOH for 15 min provided 167 in 97% yield. On the other hand, treatment of 164 with 5% ethanolic HCl at rt for 9 h gave the cyclized product (168·HCl) (47% yield), which was demethoxylated to 11·HCl (61% yield) (vide supra). In an alternative permutation, 164 was converted into the demethoxy derivative (165·HCl) (66% yield) by catalytic hydrogenolysis (Raney Ni/H₂, H₂O containing one molar equiv. of HCl, 1 atm, rt, 3 h). Treatment of 165·HCl with 10% methanolic HCl in boiling MeOH for 8 h or with 70% aqueous HClO₄ in boiling MeOH for 7 h gave 11·HCl or 11·HClO₄ in 73% or 81% yield, respectively. Alternatively, 165·HCl readily cyclized in boiling EtOH in the presence of 0.1 molar equiv. of Et₃N, furnishing 11·HCl in 89% yield.

For the synthesis of 3,9-dimethyladenine-2-d (171), Fujii’s group treated 167 with formic-d acid-d (of over 99% isotopic purity) in MeCN at 30°C for 24 h, securing the deuterioformamido derivative (169) in 76% yield (Scheme 39). Hydrogenolytic demethoxylation of 169 was then effected with Raney Ni catalyst and hydrogen at 1 atm and 21°C in H₂O containing one molar equiv. of HCl for 4 h, and cyclization of the resulting amidine hydrochloride (170) in boiling EtOH containing a little Et₃N for 30 min furnished the desired 2-deuterated species, 3,9-dimethyladenine-2-d hydrochloride (171·HCl), in 48% overall yield (from 169). Alternatively, cyclization of 170 was effected in boiling MeOH in the presence of 70% aqueous HClO₄ for 7 h, giving 171·HClO₄ in 71% overall yield (from 169).

The following physical properties and spectral characteristics of 3,9-dimethyladenine (11) have been recorded in the literature: the melting point for 11·HCl, mp 281–282°C (decomp); for 11·HClO₄, mp 333–334°C (decomp) or mp >300°C; for the bicarbonate salt (11·H₂CO₃), mp 161–162°C (decomp); for 11-2-d·HCl·1/2H₂O (171·HCl·1/2H₂O), mp 285.5–287.5°C (decomp); for 11-2-d·HClO₄ (171·HClO₄), mp >300°C; UV for 11·HCl, for 11·HClO₄, and for 11·H₂CO₃ in H₂O (at various pH’s) and in 95% aqueous EtOH; UV for 11-2-d·HCl·1/2H₂O (171·HCl·1/2H₂O) and for 11-2-d·HClO₄ (171·HClO₄) in H₂O (at various pH’s) and in 95% aqueous EtOH; ¹H NMR (DMSO-d₆) for 11·HCl, ¹H NMR, ¹H NMR, and ¹H NMR for 11-2-d·HCl·1/2H₂O (171·HCl·1/2H₂O), and for 11-2-d·HClO₄ (171·HClO₄);¹H NMR atomic orbital coefficient for the HOMO of 11 and the heat of formation estimated for 11. In an attempt to isolate the free base of 3,9-dimethyladenine (11), Fujii’s group treated an aqueous solution of 11·HCl with Amberlite IRA-402 (HCO₃⁻) at rt. However, the substance isolated from the resulting solution in 97% yield was the bicarbonate salt (11·H₂CO₃), suggesting that the basicity of the free base is considerably high, in con-
trast to the rather low basicity of the \( N^6 \)-methoxy derivative (168) \([pK_a \ 5.09 \pm 0.03 \ (at \ 20^\circ C)]\) for 168-HClO\(_4\). On the other hand, replacement of the ion-exchange resin by Amberlite CG-400 (OH\(^-\)) in the above neutralization resulted in the formation of the methylaminimidazole (166), which was characterized as the hydrochloride (166-HCl) (61% yield). Since the same hydrochloride was obtained from 165-HCl by a similar treatment, the observed conversion of 11-HCl into 166 seemed to proceed through hydrolytic ring opening followed by deformylation, as delineated in Scheme 40.

\[ \begin{align*}
\text{11-HCl} & \quad \text{+ H}_2\text{O} \quad \xrightleftharpoons[k]{k'} \quad \text{165-HCl} \\
\iff & \quad \xrightarrow{\text{Scheme 40}} \\
\text{11-HCl} & \quad \text{+ H}_2\text{O} \quad \xrightleftharpoons[k]{k'} \quad \text{166} 
\end{align*} \]

Scheme 40

It was found that in aqueous NaHCO\(_3\) the UV spectral changes of both 11-HCl and 165 with time went through the same isosbestic point at 256 nm, converging on an identical spectrum. Actually, 3,9-dimethyladenine was isolated in 66% yield as the perchlorate (11-HClO\(_4\)) from a solution of 165 in 0.5 M aqueous NaHCO\(_3\) which had been kept at 25°C for 6 h. All these observations indicated the existence of an equilibrium between 3,9-dimethyladenine (11) and the ring-opened derivative (165) in H\(_2\)O, and this was confirmed by following spectrophotometrically the time-courses of the ring-opening reaction of 11-HCl and of cyclization of 165-HCl in 0.1 M aqueous NaHCO\(_3\) (pH 8.32) at 25°C: The reactions in both directions (Scheme 40) were found to obey pseudo-first-order kinetics \((k = 2.88 \times 10^{-3} \text{ min}^{-1}; k' = 9.63 \times 10^{-3} \text{ min}^{-1}; K_{eq} = k/k' = 0.30)\); the rate and equilibrium constants for the reactions in H\(_2\)O at various pH's (7.50, 8.98, 9.62, and 10.08) and ionic strength 0.5 at 25°C were also determined.\(^{429}\) It is of particular interest to emphasize that among the four possible \( N^x \),9-dimethyladenines \([i.e., \text{the } N^6,9- (6), 1,9- (9), 3,9- (11), \text{and } 7,9\)-dimethyl (12) isomers]\), the 3,9-dimethyl isomer (11) has been found to undergo hydrolytic fission of the adenine ring most rapidly under alkaline conditions (see also Section XII).\(^{390,425c}\)

\[ \begin{align*}
\text{11-HClO}_4 & \quad \xrightarrow{\text{NaBH}_4/\text{MeOH} \quad \text{rt, 30 min} \ (77\% \ \text{yield})} \quad \text{172} \\
\text{171-HClO}_4 & \quad \xrightarrow{\text{NaBH}_4/\text{MeOH} \quad \text{rt, 30 min} \ (73\% \ \text{yield})} \quad \text{173} 
\end{align*} \]

Scheme 41
Fujii's group\textsuperscript{428} reported that treatment of 11·HClO\textsubscript{4} with NaBH\textsubscript{4} in MeOH at rt for 30 min furnished the 1,2-dihydro derivative (172) in 77% yield (Scheme 41); the NaBH\textsubscript{4} reduction of the 2-deuterated species (171·HClO\textsubscript{4}) under similar conditions gave the corresponding 1,2-dihydro derivative (173) in 73% yield.

**XII. 7,9-DIMETHYLADENINE**

7,9-Disubstitution in the adenine series has been known to occur in nature in the form of agelasine (from the sea sponge \textit{Agelas dispers}),\textsuperscript{430} agelasines A–F (from the Okinawan sea sponge \textit{A. nakamurai}),\textsuperscript{431} ageline A (agelasine F\textsuperscript{431c}) and ageline B (from a Pacific sea sponge \textit{Agelas sp.}),\textsuperscript{432} epi-ageline C (from the marine sponge \textit{Agelas mauritiana}),\textsuperscript{433} ageline G (from an Okinawan marine sponge \textit{Agelas sp.}),\textsuperscript{434} and agelasines H and I (from \textit{Agelas sp.} collected at Yap Island),\textsuperscript{68} which all are 9-methyladenines with diterpene or modified diterpene units at the 7-position. The existence of the 7-methyladenosine structure in tRNA's of \textit{Bacillus steaothermophilus}\textsuperscript{435} and \textit{B. subtilis}\textsuperscript{436} as a modified nucleoside component has also been suggested, and 7-methyl- or 7-ethyladenosine has been reported to be a by-product of methylation or ethylation of adenosine (76) in neutral aqueous solution.\textsuperscript{207} Although the prototype of this disubstitution is 7,9-dimethyladeninium salt (12), it remained unknown until 1973 when Fujii's group\textsuperscript{363} reported the first synthesis of 7,9-dimethyladeninium perchlorate (12: X = ClO\textsubscript{4}) (Scheme 42).

\[\text{Scheme 42}\]

The synthesis of 12 (X = ClO\textsubscript{4}) started with methylation of \textit{N}\textsuperscript{6}-methoxy-9-methyladenine (90) with MeI in AcNMe\textsubscript{2} at 30°C for 7 h to give the 7-methyl derivative [91 (X = I)] (59% yield) and the \textit{N}\textsuperscript{6}-methyl derivative (88-HI) (24%).\textsuperscript{336,363} As described above in Section X (Scheme 37), the former product was alternatively obtainable in 36% yield.
from N⁶-methoxy-7-methyladenine (160) by similar methylation. Conversion of 91 (X = I) into the corresponding perchlorate [91 (X = ClO₄)] (83% yield) and subsequent hydrogenolysis of 91 (X = ClO₄) using Pd–C catalyst and hydrogen or hydrogenolysis of 91 (X = I) in H₂O using Raney Ni catalyst and hydrogen gave 12 (X = ClO₄) or 12 (X = I) in 92% or 80% yield, respectively. The permutation 90→91→12 has afforded a firm basis for establishing parallel ones leading to a general synthesis of 7,9-dialkyladeninium salts, to syntheses of 7-methyl- and 7-ethyladenosine perchlorates, and to an attempted synthesis of 2'-deoxy-7-methyladenosine salt.

Raney Ni/H₂, 30% aq. EtOH
1 atm, rt, 22 h (45% yield from 163)

NOMe

\[
\begin{array}{c}
\text{H₂N} \\
\text{Me/DMF} \\
30°C, 41 h
\end{array}
\]

\[
\begin{array}{c}
\text{MeON} \\
\text{MeON} \\
\text{MeON}
\end{array}
\]

\[
\begin{array}{c}
\text{NH₂} \\
\text{Me} \\
\text{Me}
\end{array}
\]

\[
\begin{array}{c}
\text{H₂N} \\
\text{MeON} \\
\text{MeON}
\end{array}
\]

\[
\begin{array}{c}
\text{NH₂} \\
\text{Me} \\
\text{Me}
\end{array}
\]

Scheme 43

In an alternative approach to 7,9-dimethyladeninium salts, Fujii's group methylated the formamidoimidazole (163) in the absence of added base with MeI at 30°C for 41 h (Scheme 43). When a crude product presumed to be the N(3)-methylated derivative [174 (X = I)] was treated with boiling EtOH for 5 h, N⁶-methoxy-7,9-dimethyladeninium iodide [91 (X = I)], the known penultimate intermediate for the synthesis of 12 (X = I or ClO₄) as shown above (Scheme 42), was obtained in 61% overall yield (from 163). Alternatively, hydrogenolysis (Raney Ni/H₂) of crude 174 and spontaneous cyclization
of the resulting demethoxy derivative directly produced 12 (X = I) in 45% yield (from 163). The C(2)-deuterated species (177) of 12 (X = I) was also prepared from N6-methoxy-9-methyladenine-2-d (175) through 176, as illustrated in Scheme 43. In yet another synthetic approach (Scheme 44), Maki's group440 obtained 12 (X = I) from N6-acetyl-9-methyladenine (178) via the 7-methyl derivative (179).

The following physical properties and spectral characteristics of 7,9-dimethyladeninium salt (12) have been reported: the melting point for 12 (X = ClO₄), mp 276–277°C (decomp);336,363 for 12 (X = I), mp 267–268°C (decomp)437a or 274–275°C (decomp)437b or 280–281°C;440b for 12-2-d (X = I) (177), mp 266.5–269.5°C (decomp);343b UV for 12 (X = ClO₄) in H₂O (at various pH's) and in 95% aqueous EtOH;336,363 UV for 12 (X = I) in H₂O (at various pH's),437b in 95% aqueous EtOH,437b and in MeOH;440b for 12-2-d (X = I) (177) in H₂O (at various pH's) and in 95% aqueous EtOH;343b fluorescence emission spectrum for 12 (X = ClO₄);441 1H NMR (DMSO-d₆) for 12 (X = ClO₄),336,363 for 12 (X = I),437b,440b and for 12-2-d (X = I) (177).343b

As regards the chemical behavior of 7,9-dimethyladeninium salt (12), Fujii's group343 found that 12 was unstable under mildly alkaline conditions. On treatment with 0.5 N aqueous Na₂CO₃ at rt for 30 min, 12 (X = I) produced the ring-opened derivative (106)
(with carbonyl oxygen *trans* to the pyrimidine ring) in 56% yield (Scheme 45). Replacement of the inorganic base by Amberlite CG-400 (OH⁻) in the above treatment also afforded 106 in 83% yield. Similar treatment of the C(2)-deuterated species (177) gave the corresponding ring-opened derivative (183) in 51% yield. Under more drastic alkaline conditions, 12 underwent rearrangement:³⁴³ On treatment with boiling 1 N aqueous NaOH for 60 min, 12 (X = I) rearranged to N⁶,7-dimethyladenine (5) in 87% yield. Similar treatment of 106 or treatment of 106 with NaH in AcNMe₂ at rt for 40 min also gave 5 in 72% or 84% yield, respectively.

The ring-opened derivative (106) was also unstable in solution at rt, giving slowly an equilibrated mixture of 106 and its *cis* isomer (180) in H₂O, in D₂O at 25°C, and in DMSO-d₆ at 25°C (Scheme 45), and rate constants (k₁, k₂, and k⁻₂) for the system of reactions that produces 180 (*via* 106) and 5 (*via* 106 and 182) from 12 were determined: The values k₁ = 5.47 × 10⁻³ min⁻¹, k₂ = 1.49 × 10⁻³ min⁻¹, and k⁻₂ = 0.84 × 10⁻³ min⁻¹ were obtained for 12 (X = ClO₄⁻)→106 ⇄180 in H₂O at pH 9.84, 25°C, and ionic strength 0.50.³⁴³

Scheme 46
Fujii’s group further reported that the NaBH₄ reduction of 12 (X = I) in MeOH at rt furnished the 7,8-dihydro derivative (181) in 84% yield. In H₂O at 60°C, 181 slowly decomposed to give the ring-opened derivative (106) in 49% yield. The results of the NaBH₄ reduction of 12 (X = I) is in general agreement with those reported for 7,9-disubstituted purines.

Now that the reaction rates for ring opening of all the four possible Nₓ,9-dimethyl-adenines under alkaline conditions have become available as summarized above, it is possible to make a comparison between them. It may be seen from Scheme 46 that the relative ease with which the adenine ring undergoes hydrolytic fission decreases in the order 3,9- (11) > 7,9- (12) > 1,9- (9) >> N₆,9-dimethyl isomer (6).

Finally, as regards the biological activity of 7,9-dimethyladeninium salt (12), Kobayashi et al. reported that 12 (X = Cl) had little or no inhibitory effect, even at 100 μM, on the pig brain Na⁺,K⁺-ATPase.

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