THE 7-*N*-OXIDES OF PURINES RELATED TO NUCLEIC ACIDS: THEIR CHEMISTRY, SYNTHESIS, AND BIOLOGICAL EVALUATION†

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Abstract — Recent advances in the chemistry, synthesis, and biological evaluation of the 7-*N*-oxides of purines related to nucleic acids are reviewed. The 7-*N*-oxides covered are those of guanine (**1**), adenine (**2**), and hypoxanthine (**3**) and of related compounds such as 6-mercaptopurine (6-MP) (**72**), the 6-thioxo analogue of **3**, and 6-methylthiopurine, a simple model for azathioprine (**78**), which were all unknown until recently.

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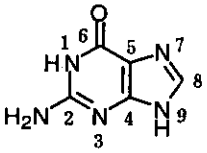
†Dedicated to Emeritus Professor Dr. Shigeru Oae (University of Tsukuba) on the occasion of his 77th birthday.

I. Introduction

Guanine (**1**) and adenine (**2**) are important fundamental biomolecules related to DNA's and RNA's. Hypoxanthine (**3**) is also a biologically significant oxopurine, which occurs in the animal body during the breakdown of nucleic acids and in the plant kingdom as well.[1] It also occurs as the 9-β-D-ribofuranoside inosine and the related nucleotide inosine 5'-phosphate, the former having been identified[2] as a minor component of more than 30 species of tRNA and the latter being an important precursor in the *de novo* biosynthesis of purine nucleotides such as adenosine 5'-phosphate and guanosine 5'-phosphate.[1b,3]

Because these three purines carry four endocyclic nitrogen atoms, four kinds of mono-*N*-oxide should be theoretically possible for each. Among the four possible isomeric *N*-oxides[4] in each case, the 1-, 3-, and 9-*N*-oxides have been prepared by chemical synthesis: 1-hydroxyguanine,[5] guanine 3-*N*-oxide,[6,7] 9-hydroxyguanine,[8] adenine 1-oxide (**41**),[9] adenine 3-oxide,[10] 9-hydroxyadenine,[11] 1-hydroxyhypoxanthine,[12] hypoxanthine 3-oxide,[10,13] and 9-hydroxyhypoxanthine.[8] The remaining 7-*N*-oxide isomers became known only recently, and the definite advances in the chemistry, synthesis, and biological evaluation of the 7-*N*-oxides of these purines emphasize the need for the present review, which covers the literature through the end of 1995.



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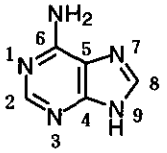

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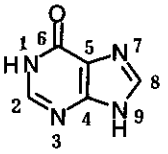

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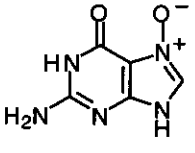

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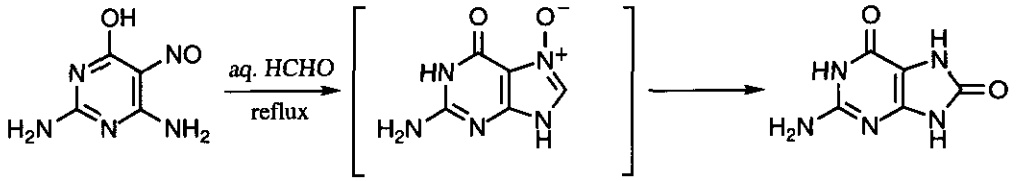
II. Occurrence

In 1985, three research groups[14–16] independently reported the isolation of guanine 7-oxide (**4**) from the culture broths of certain *Streptomyces* species (ATCC 39364;[14] *S. purpurascens* A-347;[15] and No. 3780[16]), together with its observed antitumor,[14–17] antimicrobial,[16] and antiviral[18] activities. The chemical structure of this antibiotic was established as **4** on the basis of elemental[14,15b,16] and spectral[15b,16] analyses; its chemical behavior;[16] and the X-ray molecular structures of the hydrobromide salt (monohydrate),[14] the free base (dihydrate),[15b] and the pentamethylated derivative (**17**).[16] Thus, **4** has so far been a unique purine *N*-oxide shown to occur in nature.

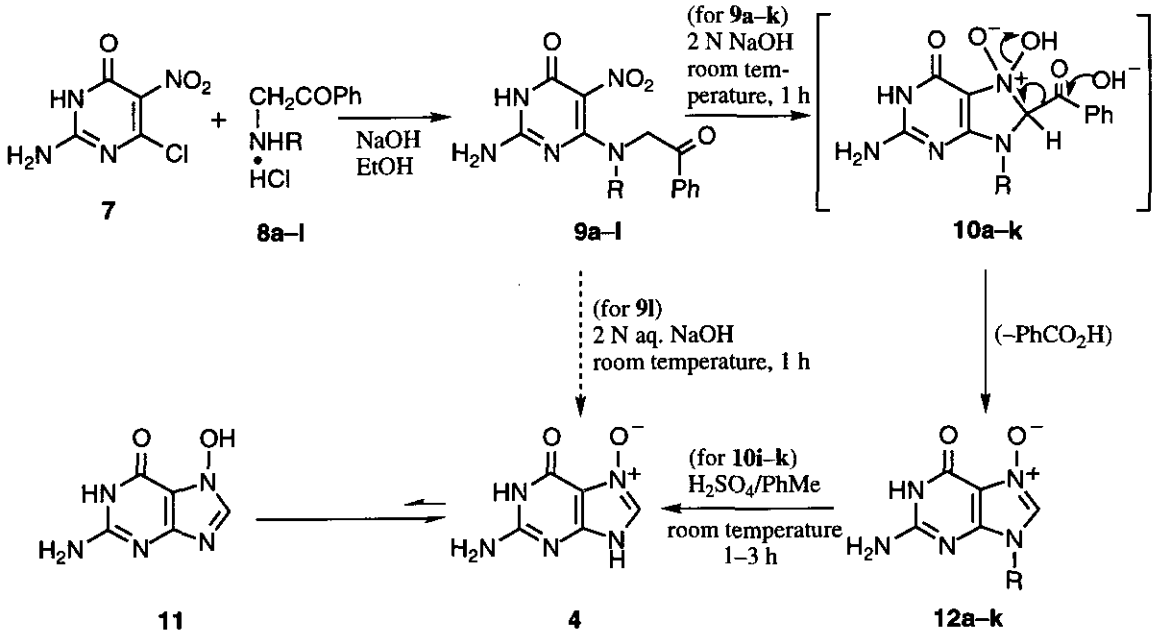
III. Chemistry and Synthesis

A. Guanine 7-Oxide



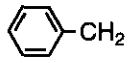
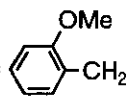
5 4 6

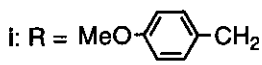
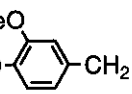
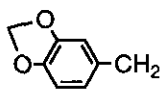
Scheme 1



7 8a–l 9a–l 10a–k 11 4 12a–k

a: R = Me **b**: R = $MeCH_2CH_2$ **c**: R = CH_2=CH–CH_2 **d**: R = 

e: R =  **f**: R = $HO(CH_2)_4$ **g**: R =  **h**: R = 

i: R =  **j**: R =  **k**: R =  **l**: R = H

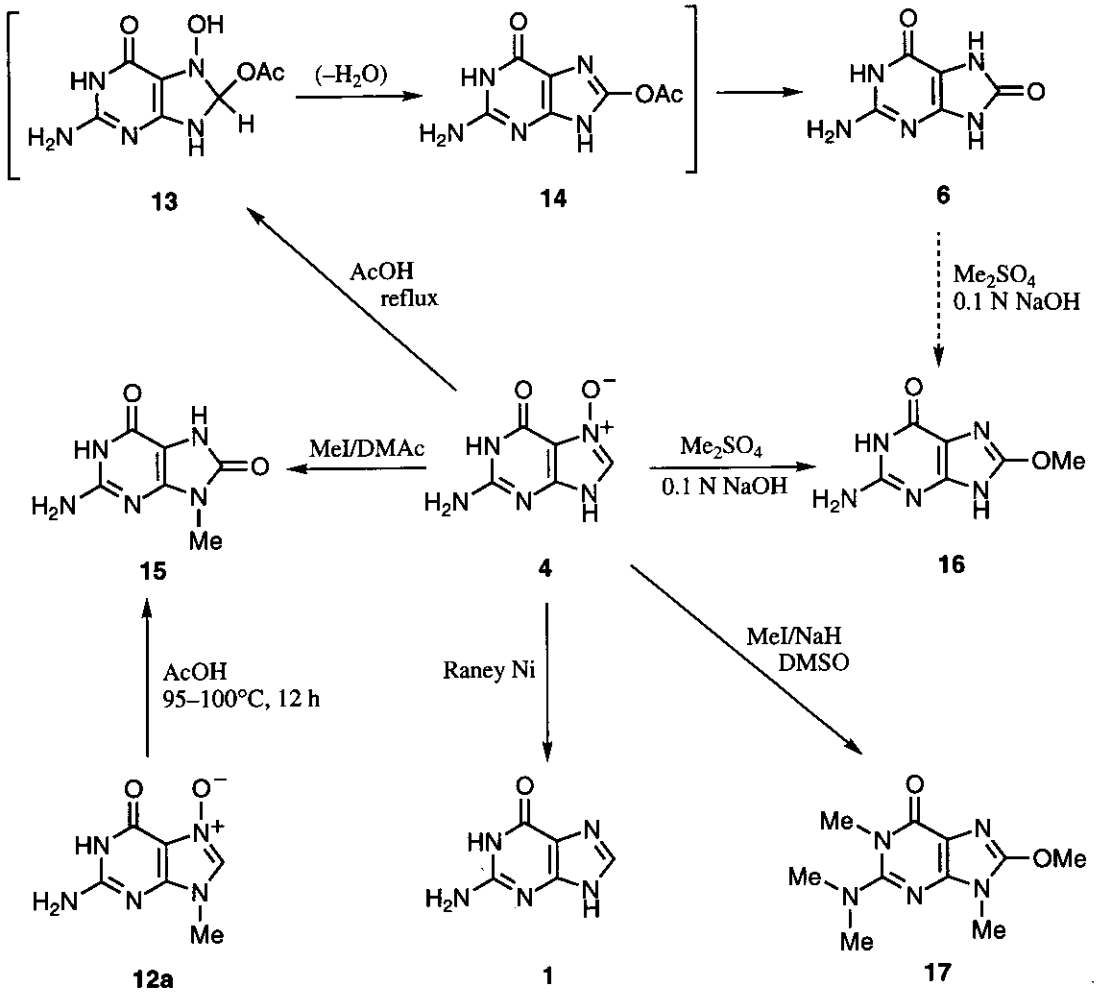
Scheme 2

Direct oxidation of guanine (**1**) with peroxytrifluoroacetic acid has been shown to produce the 3-*N*-oxide,[6] not the 7-*N*-oxide (**4**) as once thought.[19] In a preliminary experiment, Brown's group[20] obtained 8-hydroxyguanine (**6**) from the reaction of 2,4-diamino-5-nitroso-6-hydroxypyrimidine (**5**) with formalin and merely mentioned that it may be possible to obtain the presumed intermediate, guanine 7-*N*-oxide (**4**) (Scheme 1).

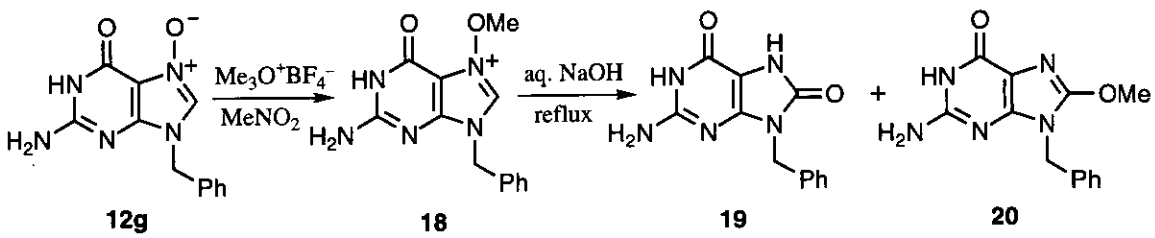
The first chemical synthesis of **4** was accomplished by Fujii and co-workers[21] *via* a newly devised "phenacylamine route", as delineated in Scheme 2. The route started from coupling of 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone (**7**) with appropriate *N*-substituted phenacylamines, generated *in situ* from the corresponding hydrochlorides (**8a–l**) and 1 N aqueous NaOH, giving the 6-phenacylamino-4-pyrimidinones (**9a–l**). On treatment with 2 N aqueous NaOH at room temperature for 10–60 min, the nitropyrimidinones (**9a–k**) cyclized *via* **10a–k** to provide the 9-substituted guanine 7-oxides (**12a–k**), with elimination of benzoic acid. A similar alkali-treatment of **9l** failed to yield guanine 7-oxide (**4**). However, removal of the 9-(arylmethyl) group from **12i–k** was effected with conc. H_2SO_4 at room temperature for 1–3 h in the presence of toluene, producing the target *N*-oxide (**4**). Application of the same procedure to the unmodified benzyl analogue (**12g**) or the allyl analogue (**12c**) failed to give the desired product (**4**).

As regards the problem of the tautomeric forms of guanine 7-*N*-oxide in the solid state, the N(7)-oxide form (**4**) has been preferred by Kern *et al.*[14] on the basis of the X-ray crystal structure of the corresponding hydrobromide salt monohydrate. On the other hand, Kitahara *et al.*[15b] have proposed the N(7)-OH form (**11**) on the basis of the result of an X-ray analysis of a single crystal of the dihydrate of the free base grown in 15% tetrahydrofuran–2 M NH_4OH. In solution, the two forms may coexist at equilibrium,[16] and a uv spectroscopic approach, together with three pK_a values (2.6, 5.8, and 9.5) reported[16] for the free base, may suggest that the neutral species of guanine 7-*N*-oxide has a considerable proportion of the N(7)-oxide structure in H_2O (Scheme 2).[21b]

Scheme 3 summarizes the chemical behavior of **4**. On reduction with Raney Ni, **4** produced guanine (**1**) quantitatively.[15b] Treatment of **4** with refluxing AcOH gave 8-hydroxyguanine (**6**).[16] The formation of **6** from **4** may be explained by assuming **13** and **14** as the intermediates.[22] Permethylation of **4** with MeI in dimethyl sulfoxide (DMSO) in the presence of NaH yielded the pentamethyl derivative (**17**) as the main product.[16] Treatment of **4** with MeI in *N*,*N*-dimethylacetamide (DMAc) at 25–40°C for 72 h gave 9-methyl-8-hydroxyguanine (**15**) (29% yield), which was identical with a sample prepared from **12a** in 80% yield by treatment with hot AcOH.[22] On the other hand, treatment of **4** with dimethyl sulfate in 0.1 N aqueous NaOH furnished 8-methoxyguanine (**16**) (60% yield), which was identical with a sample obtained in 94% yield from 8-methoxyguanosine by glycosidic hydrolysis with *p*-TsOH/AcOH (60–65°C, 40 min).[22] Methylation of **6** with dimethyl sulfate in 0.1 N aqueous NaOH failed to give **16**.[22] Kitahara *et*

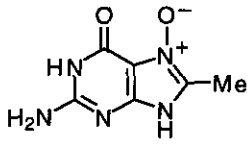


Scheme 3



Scheme 4

al.[23] also observed similar replacement reactions at the 8-position during their attempted chemical modifications of **4**. Werbovetz and Macdonald[24] reported that methylation of 9-benzylguanine 7-oxide (**12g**) with trimethyloxonium tetrafluoroborate in $MeNO_2$ furnished 9-benzyl-7-methoxyguanine salt (**18**) and that **18** was converted to a mixture of 9-benzyl-8-hydroxyguanine (**19**) and 9-benzyl-8-methoxyguanine (**20**) upon treatment with refluxing aqueous NaOH (Scheme 4).[25]



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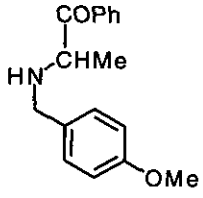

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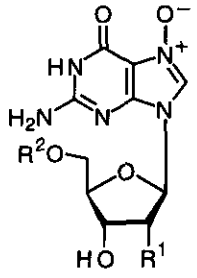
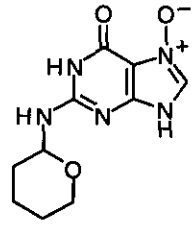


23: R^1 = OH; R^2 = H
24: R^1 = H; R^2 = H
25: R^1 = OH; R^2 = $P(O)(ONa)_2$

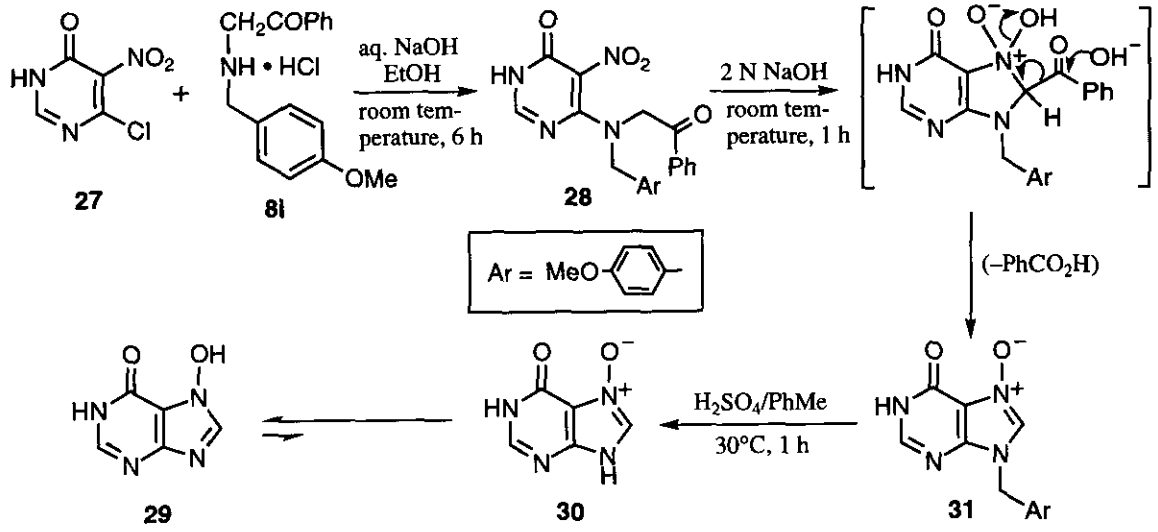


26

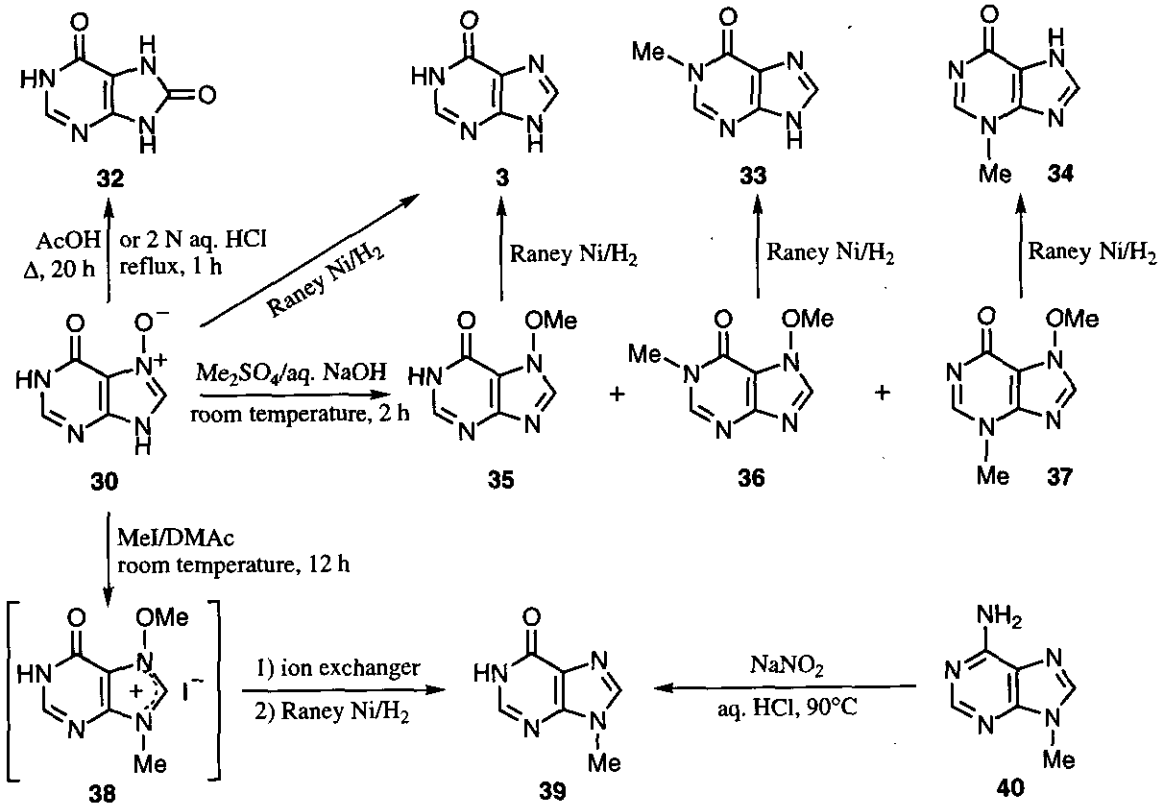
Several derivatives of **4** have been prepared for biological evaluation. Fujii and co-workers[26] synthesized 8-methylguanine 7-oxide (**21**), a model for C(8)-blocked derivatives of **4**, *via* an α-methylphenacylamine version of the "phenacylamine route" (Scheme 2), which started from condensation of **7** with α-(4-methoxybenzylamino)propiophenone (**22**) and proceeded through cyclization of the resulting phenacylaminopyrimidinone and removal of the 4-methoxybenzyl group. Nishii *et al.*[27] prepared guanosine 7-oxide (**23**) from **4** and ribose 1-phosphate by utilizing purine nucleoside phosphorylase from *Bacillus subtilis* PCI 219. Kitahara *et al.*[28] prepared **23** or the 2'-deoxy analogue (**24**) from **4** and ribose 1-phosphate or deoxyribose 1-phosphate using purine nucleoside phosphorylase from bovine spleen. The same group[23] also reported enzymatic conversion of **4** into guanosine 7-oxide 5'-monophosphate disodium salt (**25**) and chemical conversion of **4** into the *N*^{2}-tetrahydropyranyl derivative (**26**).

B. HYPOXANTHINE 7-*N*-OXIDE

Hypoxanthine 7-*N*-oxide (**30**) was not known until 1988, when Fujii's group[29] achieved its chemical synthesis by extending the "phenacylamine route" (Section III, A) to cover the synthesis of this new purine 7-*N*-oxide at the hypoxanthine level. The first step for the synthesis of **30** was coupling of *N*-(4-methoxybenzyl)phenacylamine, generated *in situ* from the corresponding hydrochloride (**8i**) and 1 N aqueous NaOH, with 6-chloro-5-nitro-4(3*H*)-pyrimidinone (**27**), which was effected in EtOH at room temperature for 6 h to furnish the phenacylaminopyrimidinone (**28**) (Scheme 5). On treatment with 2 N aqueous NaOH at room temperature for 1 h, **28** gave the *N*-oxide (**31**) and benzoic acid as well. Removal of the 4-



Scheme 5



Scheme 6

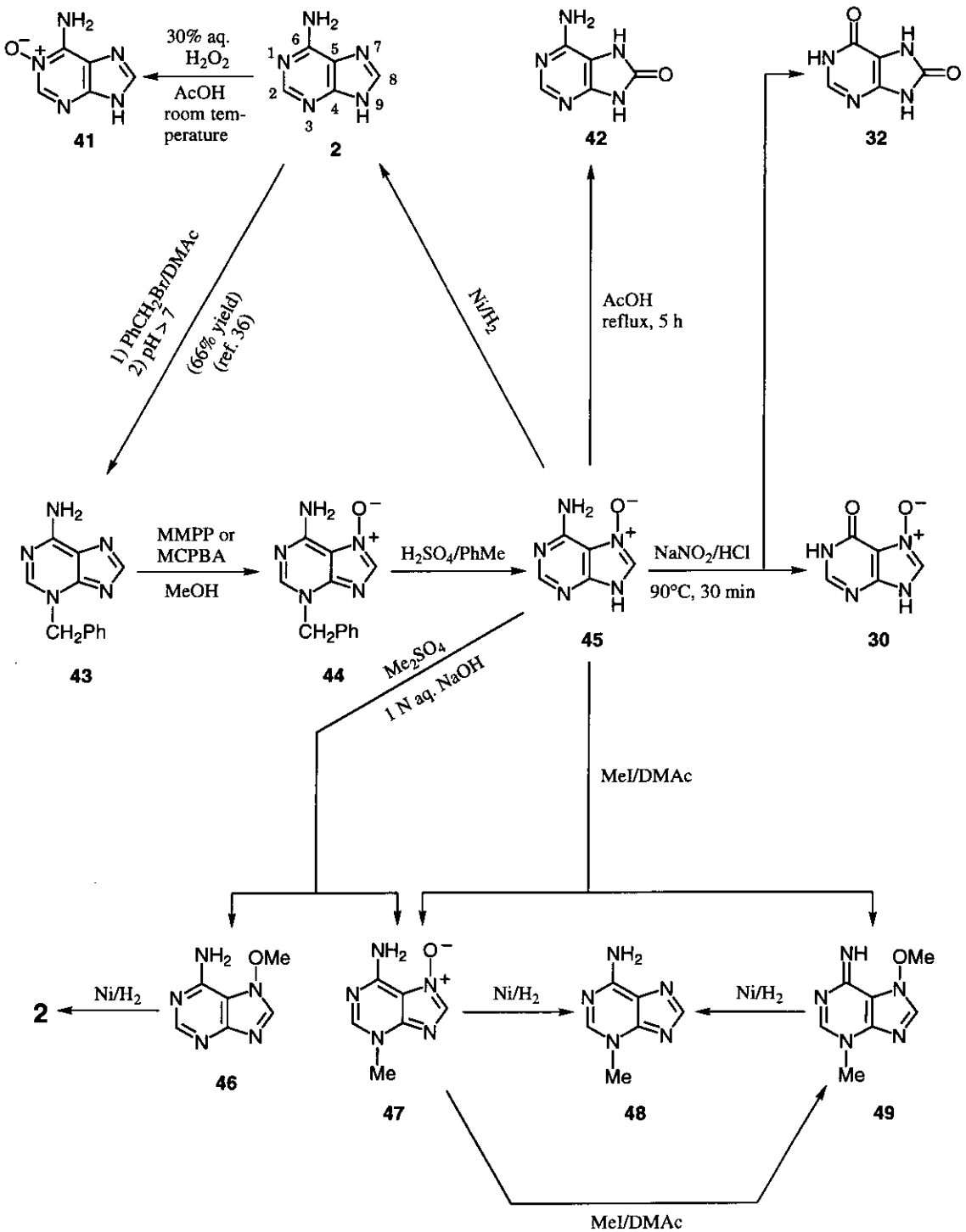
methoxybenzyl group was then carried out with 90% aqueous H_2SO_4 at 30°C for 1 h in the presence of toluene, affording the target 7-*N*-oxide (**30**). Three pK_a values of <1.4 (basic) (for protonated form ⇌ neutral form), 5.02 (acidic) (for neutral form ⇌ monoanion), and 10.23 (acidic) (for monoanion ⇌ dianion) have been obtained spectrophotometrically for **30**,[29b] and a uv spectroscopic approach has suggested that the neutral form of **30** exists in H_2O mainly as the N(7)-OH tautomer (**29**).[29b]

The chemical properties of **30** are illustrated in Scheme 6.[29] On hydrogenolysis using Raney Ni catalyst and H_2 in H_2O, **30** produced hypoxanthine (**3**). Treatment of **30** with hot AcOH for 20 h or with boiling 2 N aqueous HCl for 1 h gave 6,8-dioxopurine (**32**). The apparent migration of the oxygen function from N(7) to C(8) in this case is analogous to that observed for guanine 7-oxide (**4**) (Section III, A). Methylation of **30** with dimethyl sulfate in 0.2 N aqueous NaOH at room temperature afforded 7-methoxyhypoxanthine (**35**) (28% yield), 7-methoxy-1-methylhypoxanthine (**36**) (7%), and 7-methoxy-3-methylhypoxanthine (**37**) (3%). The locations of the methyl groups were established by reductive demethoxylations of **35**, **36**, and **37** (Raney Ni/H_2), which led to the formation of hypoxanthine (**3**), 1-methylhypoxanthine (**33**), and 3-methylhypoxanthine (**34**), respectively. On the other hand, methylation of **30** with MeI in DMAc at room temperature in the absence of alkali gave a complex mixture of products presumed to contain 7-methoxy-9-methyl derivative (**38**), and the mixture yielded 9-methylhypoxanthine (**39**) when subjected to hydrogenolysis (Raney Ni/H_2) after removal of iodide ion by the use of Dowex 50W-X8 (H^+). The compound (**39**) was identical with a sample prepared from 9-methyladenine (**40**) by deamination with $NaNO_2$ in aqueous HCl at 90°C.

C. Adenine 7-Oxide

Adenine (**2**) has a bicyclic ring system consisting of a 4-aminopyrimidine and an imidazole ring in juxtaposition.[30] On treatment with 30% aqueous H_2O_2 in AcOH at room temperature, it undergoes *N*-oxidation preferentially at the 1-position to produce adenine 1-oxide (**41**) in good yield (Scheme 7).[9,31] This regioselectivity appears to reflect the generalization[32] that on *N*-oxidation pyrimidine compounds form only mono-*N*-oxides, whereas imidazoles are resistant to *N*-oxidation.

In 1968, however, Rhaese[33] claimed that treatment of **2** with 0.1 M H_2O_2 in 0.01 M phosphate buffer (pH 7.0) at 37°C for 5 days afforded adenine 7-oxide (**45**) (isolated as a monohydrate sensitive to uv light) in 5% yield without any detectable formation of the N(1)-oxide (**41**). He further claimed that the N(7)-oxide (**45**) was among the products of X-ray irradiation of **2** in 0.05 M phosphate buffer (pH 7.0).[33] Later on, these results were reportedly reproduced by Yamamoto,[34] who further asserted that **45** bound noncovalently to urease, an SH protein, in an experiment using a sample of **45** prepared by the method of Rhaese. This unusual regioselectivity of *N*-oxidation of **2** was so striking as to appear questionable. Moreover, the


30% aq. H_2O_2
AcOH
room temperature
41
2
42
32
1) $PhCH_2Br$/DMAc
2) pH > 7
(66% yield)
(ref. 36)
Ni/H_2
AcOH
reflux, 5 h
MMPP or
MCPBA
MeOH
H_2SO_4/PhMe
$NaNO_2$/HCl
90°C, 30 min
43
44
45
30
Me_2SO_4
1 N aq. NaOH
MeI/DMAc
Ni/H_2
2
46
47
48
49
MeI/DMAc

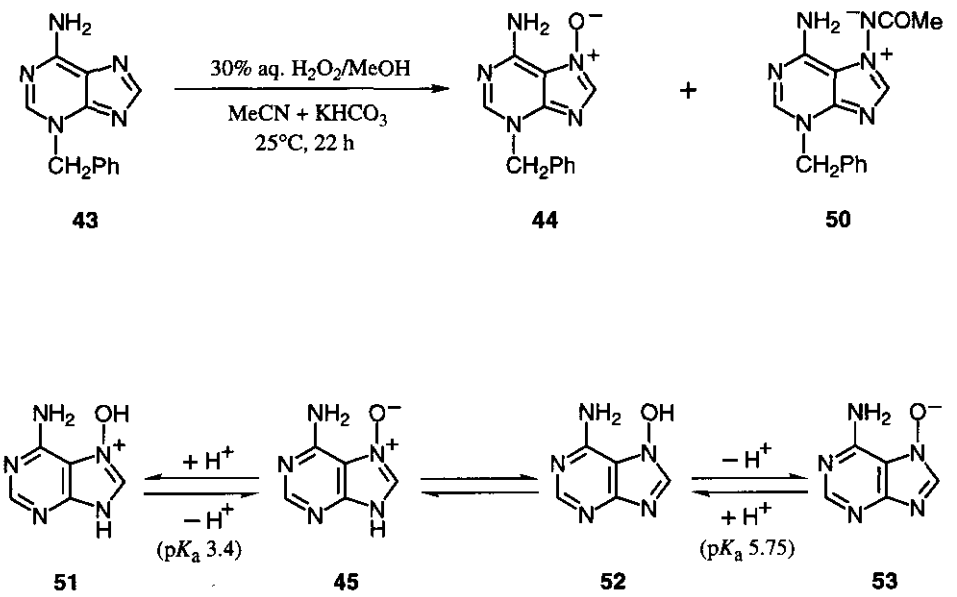
Scheme 7

chemical and spectroscopic evidence adduced by both authors appeared insufficient to allow definite assignment of the N(7)-oxide structure to their samples, which they thought to be the new *N*-oxide (**45**).

Fujii and co-workers[35] reexamined the H_2O_2/buffer oxidation procedure[33] of Rhaese for **2**, but completely failed to reproduce his results; they were unable to obtain any *N*-oxide from **2**. This led them to design a three-step route for the synthesis of adenine 7-oxide (**45**) from adenine (**2**) (Scheme 7).[35] Treatment of 3-benzyladenine (**43**), easily obtainable from **2** according to the literature procedure,[36] with magnesium monoperoxyphthalate hexahydrate (MMPP·$6H_2O$) in MeOH at 30°C for 20 h or with *m*-chloroperoxybenzoic acid (MCPBA) in MeOH–1 M acetate buffer (pH 5.0) (1 : 1, v/v) at 30°C for 15 h gave 3-benzyladenine 7-oxide (**44**) in 40% or 24% yield, respectively. The use of 30% aqueous H_2O_2 in AcOH at room temperature or MCPBA in AcOH at 30°C as the oxidizing agent was found to be ineffective. On treatment with conc. H_2SO_4 at 35°C in the presence of toluene for 3 h, **44** furnished the desired compound, adenine 7-oxide (**45**), in 55% yield. Characterization of **45** as the N(7)-oxide was readily achieved by measurement of its uv spectrum, which was different from those of the three known isomeric *N*-oxides, and by its chemical reactions including deamination and methylation, as shown in Scheme 7. In addition, the location of the oxygen function in **44** and **45** was confirmed by X-ray crystallographic analysis.[35b]

Fujii's group[35b] further found that treatment of **43** with a large excess of 30% aqueous H_2O_2 in MeOH in the presence of MeCN and $KHCO_3$ at 25°C for 22 h produced the N(7)-oxide (**44**) and 7-acetamido-3-benzyladenine (**50**) in 12% and 1% yields, respectively, together with 28% recovery of **43**. They determined pK_a values of **45** spectrophotometrically in H_2O at 30°C, obtaining two values of 3.4 (basic) [for protonated form (**51**) $\rightleftharpoons$ neutral form] and 5.75 (acidic) [for neutral form $\rightleftharpoons$ monoanion (**53**)] (Scheme 8).[35b] A uv spectroscopic approach suggested that the neutral species of **45** exists in H_2O as an equilibrated mixture of the N(7)-oxide (**45**) and N(7)-OH (**52**) tautomers.[35b] As in the case of 3-benzyladenine (**43**) described above, 3-methyladenine (**48**) and 3-ethyladenine (**54**) underwent peroxycarboxylic acid oxidation at N(7), giving **47** and **55** in 13–25% yields.[37a] Treatment of **44**, **47**, and **55** with alkyl halide (R^2X) in DMAc at 30°C afforded the corresponding 7-alkoxy derivatives (**56**) (81–91% yields), which furnished 3-alkyl-8-hydroxyadenines (**57**) in 26–50% yields on treatment with 0.1 N aqueous NaOH at room temperature (Scheme 9).[37a] Treatment of **56** (X = ClO_4) with 1 M R^3ONa in R^3OH (R^3 = Me, Et, or $PhCH_2$) at room temperature gave 8-alkoxy-3-alkyladenines (**58**) in 28–97% yields, and hydrolysis of **58** (R^3 = Me) with boiling 1 N aqueous HCl or hydrogenolysis (Pd–C/H_2) of **58** (R^3 = $PhCH_2$) provided **57** in 73–88% yields.[37b]

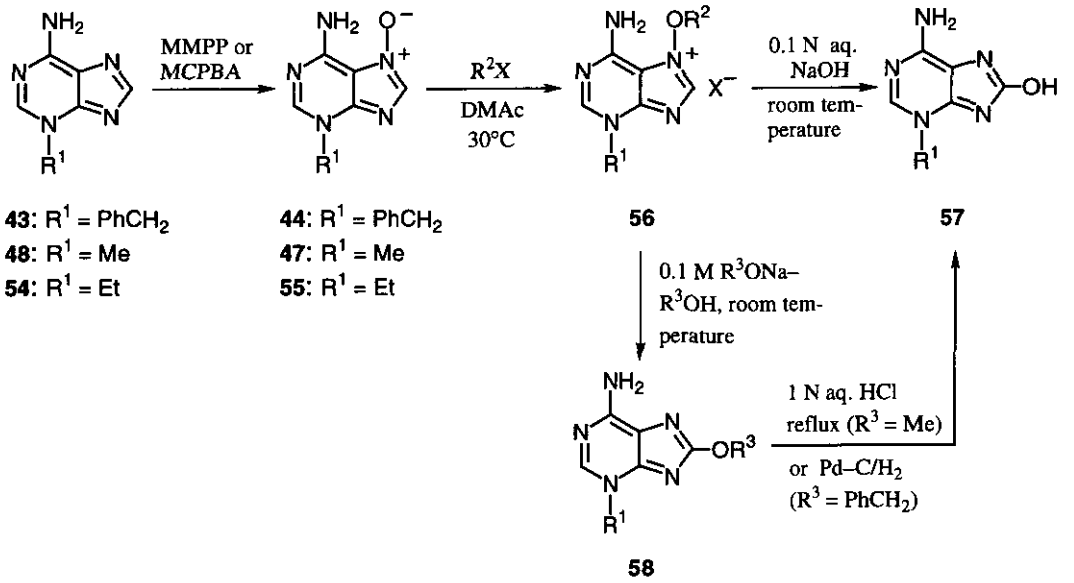
Much information has been accumulated concerning the regioselectivity in *N*-oxidation of N^x-benzyladenines. Oxidation of 9-benzyladenine (**59**) with peroxyacetic acid gives the N(1)-oxide (**63**) in 69% yield;[38] that of 9-benzyladenine-*2-d* (**60**) with MCPBA gives the corresponding N(1)-oxide (**64**) in 71%



43 44 50

51 45 52 53

Scheme 8

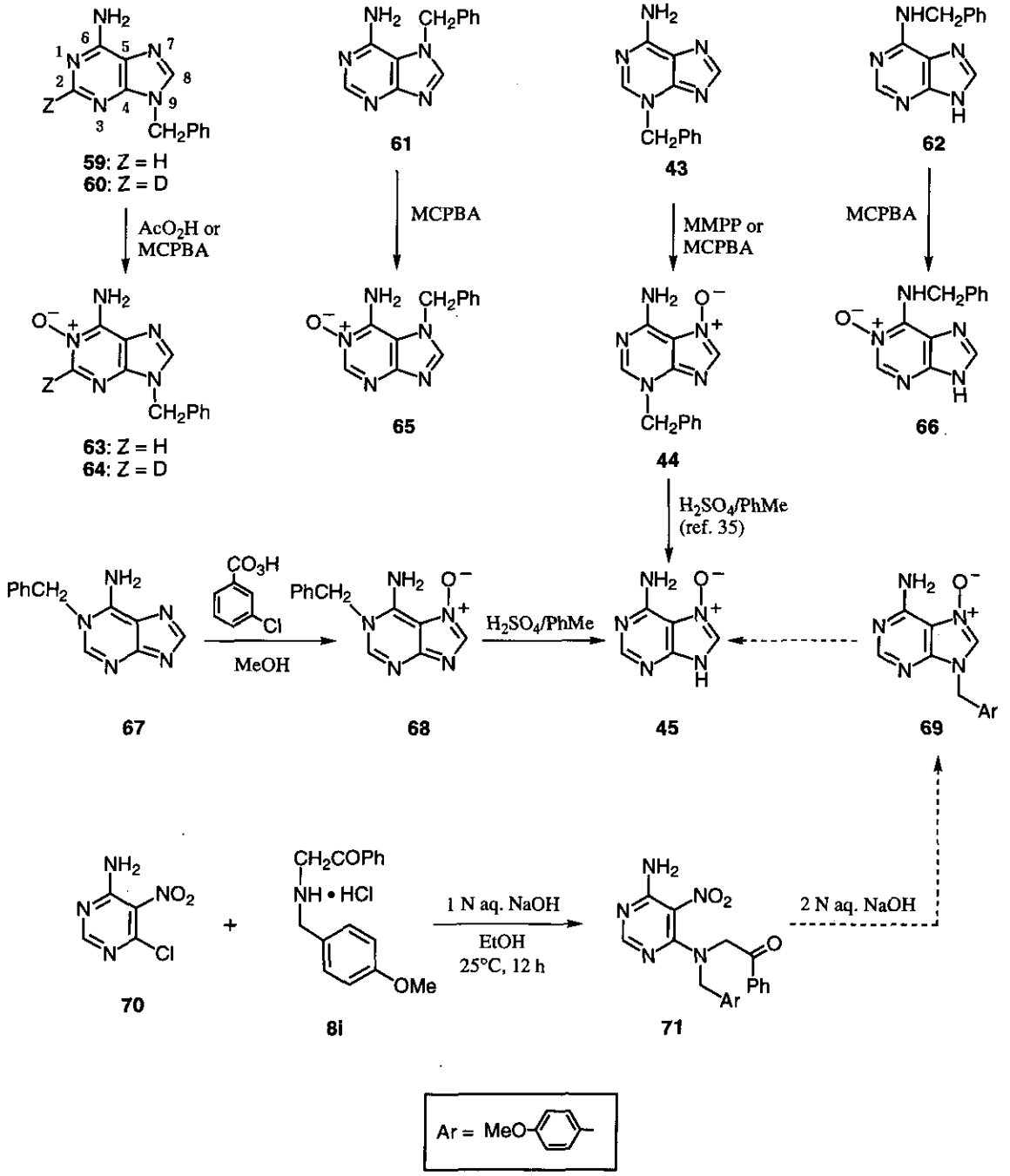


43: R^1 = $PhCH_2$
48: R^1 = Me
54: R^1 = Et

44: R^1 = $PhCH_2$
47: R^1 = Me
55: R^1 = Et

56 57 58

Scheme 9


59: Z = H
60: Z = D
AcO2H or MCPBA
63: Z = H
64: Z = D
61
MCPBA
65
43
MMPP or MCPBA
44
62
MCPBA
66
H2SO4/PhMe (ref. 35)
67
MeOH
68
H2SO4/PhMe
45
69
70
8i
1 N aq. NaOH
EtOH
25°C, 12 h
71
2 N aq. NaOH
Ar = MeO

Scheme 10

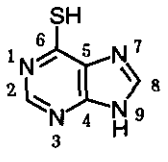
yield;[31] oxidation of 7-benzyladenine (**61**) with MCPBA affords the N(1)-oxide (**65**) in 76% yield;[39] oxidation of 3-benzyladenine (**43**) with MMPP or with MCPBA or with aqueous H_2O_2/MeCN/$KHCO_3$ provides the N(7)-oxide (**44**) in 40% or 24% or 12% yield, respectively (*vide supra*);[35] and oxidation of N^6-benzyladenine (**62**) with MCPBA[40] or with trifluoroperoxyacetic acid[41] furnishes the N(1)-oxide (**66**) (35% yield) or the N(3)-oxide (4%) and N(7)-oxide (4%), respectively. Fujii's group[42] treated 1-benzyladenine (**67**), the remaining positional isomer, with MCPBA in MeOH or in MeOH–0.5 M phosphate buffer (pH 6.6) at 30°C and obtained 1-benzyladenine 7-oxide (**68**) as the main product (Scheme 10). Nonreductive debenzylation of **68** with H_2SO_4/toluene gave adenine 7-oxide (**45**) in 63% yield. The structure of **68** was unequivocally established by an X-ray crystallographic analysis. Thus, the reaction sequence **67** → **68** → **45** afforded an alternative synthesis of **45**.[42]

Yet another synthetic approach to **45** would be an extension of the "phenacylamine route" (Section III, A), as shown in Scheme 10. Fujii's group[42] obtained **71** in 67% yield from 4-amino-6-chloro-5-nitropyrimidine (**70**) and **8i**. However, treatment of **71** with 2 N aqueous NaOH in MeOH at room temperature for 2 h gave a mixture of many products, from which they were unable to isolate the cyclized product (**69**), even if it were present. This led them to abandon the "phenacylamine route" approach.

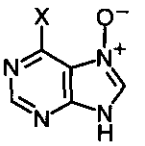
IV. Related Compounds

A. 6-MERCAPTOPURINE 7-*N*-OXIDE

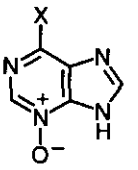
6-Mercaptopurine (6-MP) (**72**), the 6-thioxo analogue of hypoxanthine (**3**), is an antileukemic agent of longstanding clinical usefulness.[43] Among the four possible *N*-oxides of 6-MP, only the N(3)-oxide (**75**) has so far been obtained. It has been synthesized from 6-chloropurine 3-oxide (**76**) and ammonium dithiocarbamate[44] or from 7-aminothiazolo[5,4-*d*]pyrimidine 6-*N*-oxide (**77**) by rearrangement,[45] and a comparison of the activities of the *N*-oxide (**75**) with the parent 6-MP has been made in several biological systems.[45] Fujii *et al.*[46] reported the first synthesis of 6-mercaptopurine 7-*N*-oxide (**73**), in which they adopted a dichloropyrimidine variant (Scheme 11) of their favorite "phenacylamine route" (Section III, A and B). The synthesis of **73** started with the condensation of *N*-(4-methoxybenzyl)phenacylamine, generated from its hydrochloride salt (**8i**), with 4,6-dichloro-5-nitropyrimidine (**83**) in $CHCl_3$ at 0–5°C for 1 h to give the phenacylaminopyrimidine (**79**). Successive treatments of **79** with thiourea, conc. aqueous NH_3, and 2 N aqueous NaOH afforded the *N*-oxide (**80**). Removal of the 4-methoxybenzyl group from **80** was effected in a mixture of conc. H_2SO_4 and toluene at 23°C for 2 h, giving the target compound (**73**). On treatment with sodium dithionite in boiling 50% aqueous MeOH for 1.5 h, **73** produced 6-MP (**72**). The ^{1}H nmr spectrum of **73** indicated that 6-mercaptopurine 7-*N*-oxide exists in Me_2SO-d_6 in the 6-thioxo-1*H*-



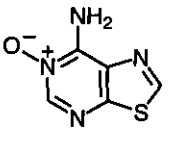
72



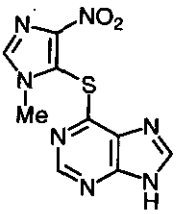
73: X = SH
74: X = SMe



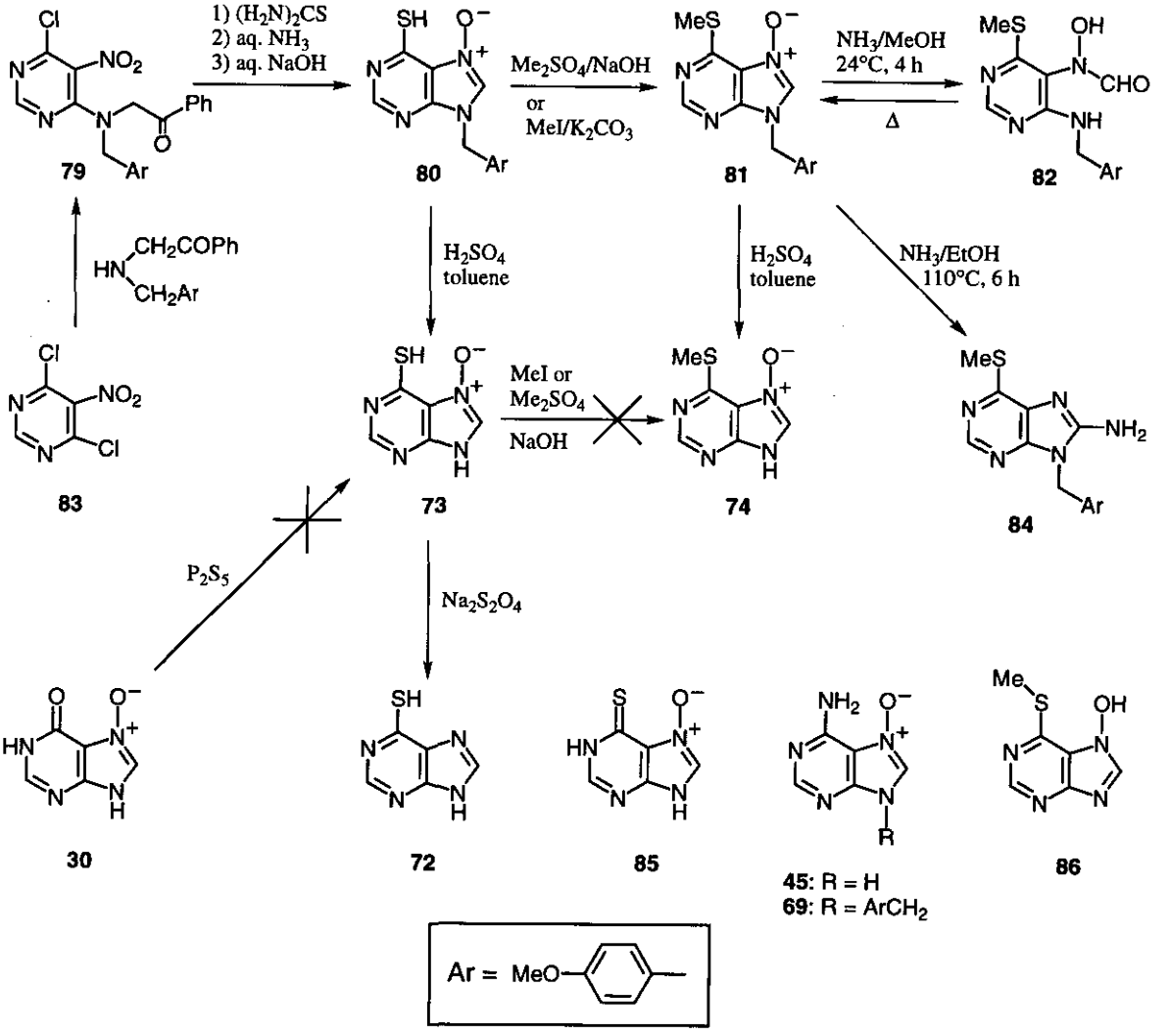
75: X = SH
76: X = Cl



77



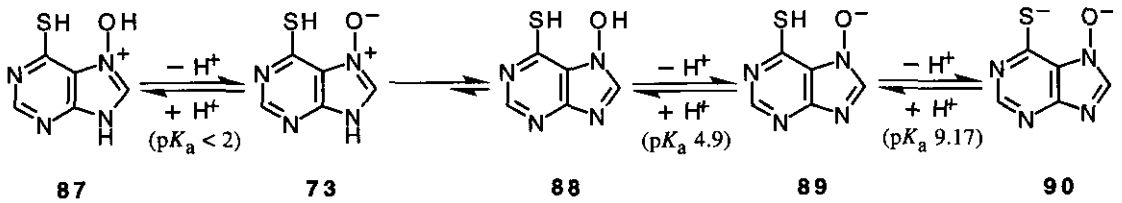
78



79 **80** **81** **82**
83 **73** **74** **84**
30 **72** **85** **45**: R = H **69**: R = $ArCH_2$ **86**

Scheme 11

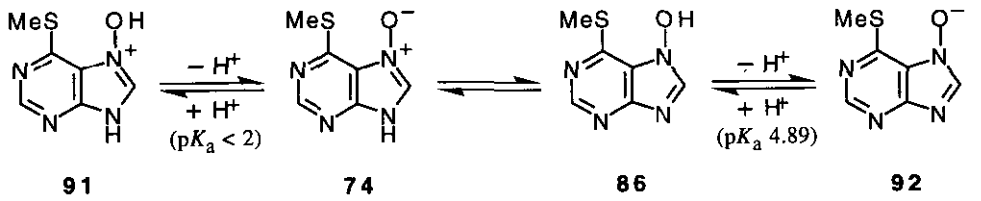
purine form (**85**) rather than the C(6)-SH form (**73**). In an alternative synthetic approach to **73**, Fujii *et al.*[46] heated hypoxanthine 7-*N*-oxide (**30**) with P_2S_5 in boiling pyridine. However, they were unable to obtain **73**, but isolated a compound inferred to be 8-mercaptohypoxanthine. They obtained three pK_a values for **73** spectrophotometrically in H_2O at 30°C, as shown in Scheme 12, and a uv spectroscopic approach suggested the overwhelming predominance of the N(7)-OH tautomer (**88**) over the N(7)-oxide tautomer (**73**) in the neutral species of 6-mercaptopurine 7-*N*-oxide in H_2O.[46b]



Scheme 12

B. 6-METHYLTHIOPURINE 7-*N*-OXIDE

Azathioprine (Imuran®) (**78**), the *S*-(1-methyl-4-nitro-1*H*-imidazol-5-yl) derivative of 6-MP (**72**), is an immunosuppressive agent of longstanding clinical usefulness.[43] It acts as a pro-drug for 6-MP.[43a] Fujii *et al.*[46] reported the first synthesis of 6-methylthiopurine 7-*N*-oxide (**74**), a simple model for the 7-*N*-oxide of azathioprine (**78**). For the synthesis of **74**, they methylated the precursor (**80**) for **73** with dimethyl sulfate in a mixture of 1 N aqueous NaOH and MeOH at room temperature or with MeI and K_2CO_3 in MeOH at room temperature, obtaining the 6-methylthio derivative (**81**) (Scheme 11). Nonreductive debenzylation of **81** with conc. H_2SO_4 in the presence of toluene at 25°C for 1 h gave the desired *N*-oxide (**74**). On the other hand, direct methylation of **73** with MeI or dimethyl sulfate in a mixture of MeOH and 1 N aqueous NaOH resulted in the formation of a mixture of many products, from which they were unable to obtain the *S*-methyl derivative (**74**). The location of the oxygen function in **73**, **74**, and **80** was confirmed by X-ray crystallographic analysis of **74**·H_2O, which was shown to exist in the N(7)-OH form (**86**).



Scheme 13

Fujii *et al.*[46b] also determined two pK_a values for **74** spectrophotometrically in H_2O at 30°C, as shown in Scheme 13, and a uv spectroscopic approach suggested that the neutral species of 6-methylthiopurine 7-*N*-oxide exists in H_2O as an equilibrated mixture of the N(7)-oxide (**74**) and the N(7)-OH (**86**) tautomers.

In an attempt to develop an alternative synthetic route to adenine 7-oxide (**45**), Fujii *et al.*[46] examined amination of **74** under a variety of reaction conditions (Scheme 11). However, all attempts resulted in the recovery of **74**, suggesting the inertness of the C(6)-SMe group in the anionic species (**92**). On the other hand, treatment of the N(9)-arylmethyl derivative (**81**) with 16% methanolic NH_3 at 24°C for 4 h gave an unstable crude compound inferred to be the ring-opened product (**82**), which reverted to **81** on heating in boiling EtOH for 30 min.[46] Treatment of **81** with saturated ethanolic NH_3 in an autoclave at 110°C for 6 h afforded the C(8)-amino derivative (**84**).[46] In either case, the desired adenine derivative (**69**) could not be obtained.

V. Biological Activity

Guanine 7-oxide (**4**) exhibited excellent activity in mice that were inoculated either intraperitoneally or subcutaneously with L1210 leukemia cells.[14,15a] It caused pronounced growth inhibition of several murine and human cell lines *in vitro*.[17] For the L1210 lymphoblastic leukemia, 50% inhibition was obtained below 1 μM.[17] It also inhibited Yoshida sarcoma and L5178Y leukemia cells in culture at IC_{50}'s of 1.65 and 2.40 μg/ml, respectively.[16] The intraperitoneal administration of this antibiotic showed a life-prolongation effect on mice bearing P388 leukemia, and the activity at a dose of 6.0 mg/kg/day was almost comparable to that observed with mitomycin C at a dose of 1.0 mg/kg/day.[16] The *N*-oxide (**4**) also showed a dose-dependent inhibition of the growth of Ehrlich solid carcinoma in mice *via* oral administration.[16] Guanine 7-oxide (**4**) has no activity against *Staphylococcus aureus* 209P, *Bacillus subtilis* PCI 219, *Escherichia coli* NIHJ, *Pseudomonas aeruginosa* IFO 3445, *Micrococcus luteus* PCI 1001, *Candida albicans* 3147, and *Saccharomyces cerevisiae* at a concentration of 100 μg/ml.[15a] Nishii *et al.*[16] reported that **4** was inhibitory to *Candida albicans* but inactive against Gram-positive and Gram-negative bacteria and *Trichophyton* species. Moderate antiviral activity of **4** was demonstrated against DNA and RNA viruses derived from salmonids.[18] The LD_{50} of **4** in mice was determined to be 40–80 mg/kg (by single intraperitoneal administration)[15a] or 53 mg/kg (by intraperitoneal administration).[16] Jackson *et al.*[17] reported that the *N*-oxide (**4**) is converted within sensitive cells into guanosine 7-oxide 5'-triphosphate and this results in inhibition of cellular protein synthesis. Nishii *et al.*[27] reported that the antimicrobial activity of guanosine 7-oxide (**23**) was very weak but it inhibited L5178Y mouse leukemia cells in culture at an IC_{50} of 0.60 μg/ml; intraperitoneal administration of **23** showed a life-prolongation effect on mice bearing P388 leukemia; and **23** showed a dose-dependent inhibition of the growth of Ehrlich solid carcinoma in mice. Kitahara *et al.*[28]

reported the antitumor activities of **23** and the 2'-deoxy analogue (**24**) against mouse leukemia L1210 cells. They also reported the biological activities of the nucleotide analogue (**25**) and the N^2-tetrahydropyranyl derivative (**26**).[23]

In the *in vitro* bioassay of antileukemic activity against murine L5178Y cells, Fujii and co-workers[21b] found that none of the 9-substituted guanine 7-oxide (**12a–k**) was more effective than the parent, natural *N*-oxide (**4**). Within this series, however, the benzyl analogues (**24g–k**) with or without alkoxy functions were more cytotoxic, with IC_{50}'s of 13.0–48.0 μg/ml, than the alkyl analogues (**12a–f**). 8-Methylguanine 7-oxide (**21**), 9-(4-methoxybenzyl)-8-methylguanine 7-oxide, and 9-(4-methoxy-3-sulfobenzyl)-8-methylguanine 7-oxide showed only weak antileukemic activity and no antimicrobial activity.[26]

Hypoxanthine 7-*N*-oxide (**30**) was weakly cytotoxic, with IC_{50} of 100 μg/ml, in the *in vitro* bioassay of antileukemic activity against murine L5178Y cells, and it did not show any antimicrobial activity even at *1000 μg/ml.*[29b] *None of its 9-(4-methoxybenzyl) derivative* (**31**) *and the* 7-methoxy derivatives (**35**, **36**, and **37**) was found to be antileukemic or antimicrobial.[29b] In a similar bioassay, 6-mercaptopurine 7-*N*-oxide (**73**) and its 9-(4-methoxybenzyl) derivative (**80**) were less effective than the parent 6-MP (**72**), but slightly more cytotoxic than hypoxanthine 7-*N*-oxide (**30**); 6-methylthiopurine 7-*N*-oxide (**74**) and adenine 7-oxide (**45**) were inactive at 50 μg/ml concentration.[46b]

In the tobacco callus bioassay of cytokinin activity, each of N^6-benzyladenine 1-oxide (**66**), N^6-benzyladenine 3-oxide, and N^6-benzyladenine 7-oxide was active at 4 μM concentration, being less active than the parent synthetic cytokinin (**62**) by a factor of 40.[41]

REFERENCES AND NOTES

1. For pertinent reviews, see (a) J. H. Lister, 'Fused Pyrimidines. Part II: Purines,' ed. by D. J. Brown, Wiley–Interscience, New York, 1971, pp. 250–251; (b) G. Shaw, 'Rodd's Chemistry of Carbon Compounds,' 2nd ed., Vol. IV, Part L, ed. by S. Coffey, Elsevier Scientific Publishing Co., Amsterdam, 1980, pp. 31–32; (c) Atta-ur-Rahman and M. I. Choudhary, 'The Alkaloids,' Vol. 38, ed. by A. Brossi, Academic Press, New York, 1990, pp. 256–257.
2. For a review, see M. Sprinzl, T. Hartmann, J. Weber, J. Blank, and R. Zeidler, *Nucleic Acids Res.*, 1989, **17**, r1.
3. For reviews, see (a) Ref. 1a, pp. 112–113; (b) G. Shaw, 'Comprehensive Heterocyclic Chemistry,' Vol. 5, ed. by K. T. Potts, Pergamon Press, Oxford, 1984, p. 505 and pp. 598–599.
4. The designation "*N*-oxide" used in this review follows that adopted in ref. 7, footnote 15.
5. A. A. Watson, S. C. Nesnow, and G. B. Brown, *J. Org. Chem.*, 1973, **38**, 3046.
6. U. Wölcke and G. B. Brown, *J. Org. Chem.*, 1969, **34**, 978.

7. J. C. Parham, T. G. Winn, and G. B. Brown, *J. Org. Chem.*, 1971, **36**, 2639.
8. A. A. Watson, *J. Org. Chem.*, 1974, **39**, 2911.
9. (a) M. A. Stevens, D. I. Magrath, H. W. Smith, and G. B. Brown, *J. Am. Chem. Soc.*, 1958, **80**, 2755; (b) M. A. Stevens and G. B. Brown, *ibid.*, 1958, **80**, 2759; (c) T. Fujii and T. Itaya, *Tetrahedron*, 1971, **27**, 351.
10. I. Scheinfeld, J. C. Parham, S. Murphy, and G. B. Brown, *J. Org. Chem.*, 1969, **34**, 2153.
11. A. A. Watson, *J. Org. Chem.*, 1977, **42**, 1610.
12. (a) E. C. Taylor, C. C. Cheng, and O. Vogl, *J. Org. Chem.*, 1959, **24**, 2019; (b) J. C. Parham, J. Fissekis, and G. B. Brown, *ibid.*, 1966, **31**, 966; (c) H. Kawashima, T. Meguro, and I. Kumashiro, *Bull. Chem. Soc. Jpn.*, 1966, **39**, 633.
13. H. Kawashima and I. Kumashiro, *Bull. Chem. Soc. Jpn.*, 1969, **42**, 750.
14. D. L. Kern, G. C. Hokanson, J. C. French, and N. K. Dally, *J. Antibiot.*, 1985, **38**, 572.
15. (a) M. Kitahara, K. Ishii, Y. Kumada, T. Shiraishi, T. Furuta, T. Miwa, H. Kawaharada, and K. Watanabe, *J. Antibiot.*, 1985, **38**, 972; (b) M. Kitahara, K. Ishii, H. Kawaharada, K. Watanabe, T. Suga, T. Hirata, and S. Nakamura, *ibid.*, 1985, **38**, 977.
16. M. Nishii, J. Inagaki, F. Nohara, K. Isono, H. Kusakabe, K. Kobayashi, T. Sakurai, S. Koshimura, S. K. Sethi, and J. A. McCloskey, *J. Antibiot.*, 1985, **38**, 1440.
17. R. C. Jackson, T. J. Boritzki, J. A. Besserer, K. L. Hamelehle, J. L. Shillis, W. R. Leopold, and D. W. Fry, *Adv. Enzyme Regul.*, 1987, **26**, 301.
18. (a) M. Hasobe, M. Saneyoshi, and K. Isono, *J. Antibiot.*, 1985, **38**, 1581; (b) *Idem*, *ibid.*, 1986, **39**, 1291.
19. T. J. Delia and G. B. Brown, *J. Org. Chem.*, 1966, **31**, 178.
20. G. Zvilichovsky and G. B. Brown, *J. Org. Chem.*, 1972, **37**, 1871.
21. (a) F. Nohara, M. Nishii, K. Ogawa, K. Isono, M. Ubukata, T. Fujii, T. Itaya, and T. Saito, *Tetrahedron Lett.*, 1987, **28**, 1287; (b) K. Ogawa, M. Nishii, J. Inagaki, F. Nohara, T. Saito, T. Itaya, and T. Fujii, *Chem. Pharm. Bull.*, 1992, **40**, 343.
22. K. Ogawa, Ph. D. Dissertation, Kanazawa University, May 1994, pp. 29–31.
23. M. Kitahara, K. Shinjyo, M. Fukae, K. Hosoe, T. Shiraishi, K. Ishii, K. Watanabe, and H. Kawaharada, *J. Antibiot.*, 1990, **43**, 352.
24. K. A. Werbovetz and T. L. Macdonald, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 2323.
25. For a discussion of similar activation of C(8), see W. Adam and A. Treiber, *J. Am. Chem. Soc.*, 1995, **117**, 2686.

26. K. Ogawa, M. Nishii, J. Inagaki, F. Nohara, T. Saito, T. Itaya, and T. Fujii, *Chem. Pharm. Bull.*, 1992, **40**, 1315.

27. M. Nishii, Y. Fujihara, J. Inagaki, K. Ogawa, F. Nohara, M. Ubukata, and K. Isono, *Agric. Biol. Chem.*, 1986, **50**, 2697.

28. M. Kitahara, K. Ishii, T. Okazaki, T. Hidaka, and K. Watanabe, *J. Antibiot.*, 1986, **39**, 1288.

29. (a) K. Ogawa, T. Saito, F. Nohara, M. Nishii, T. Itaya, and T. Fujii, *Heterocycles*, 1988, **27**, 885; (b) K. Ogawa, M. Nishii, F. Nohara, T. Saito, T. Itaya, and T. Fujii, *Chem. Pharm. Bull.*, 1992, **40**, 612.

30. Ref. 1a, Chapter 1.

31. For a similar *N*-oxidation of adenine-*2-d* to form adenine-*2-d* 1-oxide, see T. Fujii, T. Saito, K. Kizu, H. Hayashibara, Y. Kumazawa, S. Nakajima, and T. Fujisawa, *Chem. Pharm. Bull.*, 1991, **39**, 301.

32. E. Ochiai, 'Aromatic Amine Oxides,' Elsevier, Amsterdam, 1967, pp. 50–51.

33. H.-J. Rhaese, *Biochim. Biophys. Acta*, 1968, **166**, 311.

34. O. Yamamoto, *J. Radiat. Res.*, 1980, **21**, 239.

35. (a) T. Fujii, K. Ogawa, T. Saito, K. Kobayashi, and T. Itaya, *Heterocycles*, 1994, **38**, 477; (b) T. Fujii, K. Ogawa, T. Saito, K. Kobayashi, T. Itaya, T. Date, and K. Okamura, *Chem. Pharm. Bull.*, 1995, **43**, 53.

36. T. Fujii, G. C. Walker, N. J. Leonard, D. C. DeLong, and K. Gerzon, *J. Med. Chem.*, 1979, **22**, 125.

37. (a) T. Fujii, Y. Takada, K. Ogawa, and T. Itaya, Abstracts of Papers, Part 2, 115th Annual Meeting of Pharmaceutical Society of Japan, Sendai, March 1995, p. 73; (b) T. Fujii, Y. Takada, and T. Itaya, Abstracts of Papers, Part 2, 116th Annual Meeting of Pharmaceutical Society of Japan, Kanazawa, March 1996, p. 139.

38. T. Fujii, C. C. Wu, and T. Itaya, *Chem. Pharm. Bull.*, 1971, **19**, 1368.

39. (a) K. Ogawa, T. Saito, T. Itaya, and T. Fujii, *Heterocycles*, 1994, **38**, 253; (b) T. Fujii, K. Ogawa, T. Saito, and T. Itaya, *Chem. Pharm. Bull.*, 1995, **43**, 328.

40. (a) T. Fujii, K. Ogawa, and T. Itaya, *Heterocycles*, 1994, **37**, 219; (b) T. Itaya, K. Ogawa, Y. Takada, and T. Fujii, *Chem. Pharm. Bull.*, 1996, **44**, in press.

41. T. Fujii, Y. Takada, K. Ogawa, T. Itaya, and S. Matsubara, *Chem. Pharm. Bull.*, 1995, **43**, 325.

42. T. Fujii, K. Ogawa, T. Saito, T. Itaya, T. Date, and K. Okamura, *Chem. Pharm. Bull.*, 1995, **43**, 321.

43. (a) G. B. Elion, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 870; (b) T. A. Krenitsky, W. W. Hall, J. L. Selph, J. F. Truax, and R. Vinegar, *J. Med. Chem.*, 1989, **32**, 1471.

44. A. Giner-Sorolla, *J. Heterocycl. Chem.*, 1971, **8**, 651.

45. G. B. Brown, G. Levin, S. Murphy, A. Sele, H. C. Reilly, G. S. Tarnowski, F. A. Schmid, M. N. Teller, and C. C. Stock, *J. Med. Chem.*, 1965, **8**, 190.

46. (a) K. Ogawa, T. Itaya, and T. Fujii, *Heterocycles*, 1994, **38**, 1225; (b) T. Fujii, K. Ogawa, T. Itaya, T. Date, J. Inagaki, and F. Nohara, *Chem. Pharm. Bull.*, 1995, **43**, 408.

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