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Purines. LXX.[1] An Extension of the "Phenacylamine Route" to the Syntheses of the 7-*N*-Oxides of 6-Mercaptopurine and 6-Methylthiopurine, and Antileukemic Activity of Some Purine *N*-Oxides

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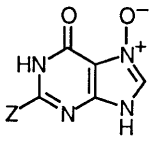
A full account is given of the first syntheses of 6-mercaptopurine 7-*N*-oxide (4) and 6-methylthiopurine 7-*N*-oxide (5). The synthesis of 4 followed a "phenacylamine route", which started from condensation of 4,6-dichloro-5-nitropyrimidine (15) with *N*-(4-methoxybenzyl)phenacylamine to form the phenacylaminopyrimidine derivative (11) and proceeded through conversion into the mercapto derivative, intramolecular cyclization between the NO_2 nitrogen atom and the phenacyl carbanion to give 6-mercapto-9-(4-methoxybenzyl)purine 7-*N*-oxide (12), and removal of the 4-methoxybenzyl group. *S*-Methylation of 12 and removal of the 4-methoxybenzyl group afforded 5. The location of the oxygen function in 4, 5, and 12 was confirmed by X-ray crystallographic analysis of 5 · H_2O, which was shown to exist in the N(7)-OH form (19). A UV spectroscopic approach suggested that the neutral species of 4 exists in H_2O as the N(7)-OH tautomer (21), whereas that of 5 exists as an equilibrated mixture of the N(7)-oxide (5) and the N(7)-OH (19) tautomers. In the *in vitro* bioassay of antileukemic activity against murine L5178Y cells, the *N*-oxides 4 and 12 were found to be weakly cytotoxic.

Key words 6-mercaptopurine 7-*N*-oxide; 6-methylthiopurine 7-*N*-oxide; synthesis; X-ray analysis; cyclization nitro–phenacylamino; antileukemic activity

Among the theoretically possible isomeric *N*-oxides of purines related to nucleic acids, the N(7)-oxides of guanine, hypoxanthine, and adenine were not known until recently.[2] It was only in 1985 that guanine 7-oxide (**1**) was reported as an antitumor antibiotic from the culture broths of certain *Streptomyces* species by three independent research groups.[3-5] This led us to accomplish not only the chemical synthesis of **1**,[6] but also those of 8-methylguanine 7-oxide,[7] hypoxanthine 7-*N*-oxide (**2**),[8,9] and adenine 7-oxide (**3**)[10] for the first time. In a sense, this is also the case for 6-mercaptopurine (6-MP) (**6**), the 6-thioxo analogue of hypoxanthine.



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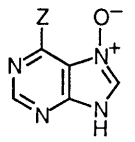

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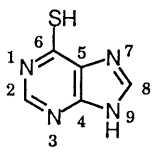

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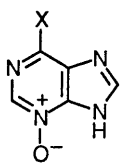

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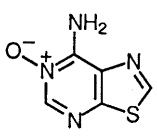

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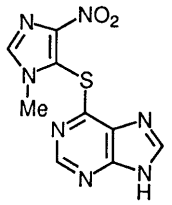

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6-MP (**6**) and azathioprine (Imuran®) (**10**), the *S*-(1-methyl-4-nitro-1*H*-imidazol-5-yl) derivative of 6-MP, are antileukemic and immunosuppressive agents, respectively, of longstanding clinical usefulness.[11] The latter agent acts as a pro-drug for 6-MP.[11a] Among the four possible *N*-oxides of 6-MP, only the N(3)-oxide (**7**) has so far been obtained. It has been synthesized from 6-chloropurine 3-oxide (**8**) and ammonium dithiocarbamate[12] or from 7-aminothiazolo[5,4-*d*]pyrimidine 6-*N*-oxide (**9**) by rearrangement,[13] and a comparison of the activities of the *N*-oxide **7** with the parent 6-MP has been made in several biological systems.[13] In the present work, we tried to synthesize 6-mercaptopurine 7-*N*-oxide (**4**) and its *S*-methyl derivative (**5**), a simple model for the 7-*N*-oxide of azathioprine (**10**), and to evaluate their antileukemic activities. In view of the convertibility of the 6-methylthio group into a 6-amino group in purine compounds by displacement, the synthesis of **5** might afford an alternative access to adenine 7-oxide (**3**). A brief account of a part of the results reported here has been published in a preliminary form.[14]

Of the various strategies conceivable for the syntheses of the target *N*-oxides **4** and **5**, direct *N*-oxidations of 6-MP and its *S*-methyl derivative would offer the shortest synthetic routes. However, they require protection of the sulfur atom from oxidation, a favorable regioselectivity in *N*-oxidation, and selective deprotection of the sulfur atom, all of which appeared to be difficult to achieve. We therefore decided to test first a dichloropyrimidine variant of our favorite "phenacylamine route", which has worked well for the syntheses of guanine 7-oxide (**1**),[6] its 8-methyl derivative,[7] and hypoxanthine 7-*N*-oxide (**2**).[8]

The synthesis of 6-mercaptopurine 7-*N*-oxide (**4**) started

This paper is dedicated to Professor Tohru Hino on the occasion of his retirement from Chiba University in March 1994.

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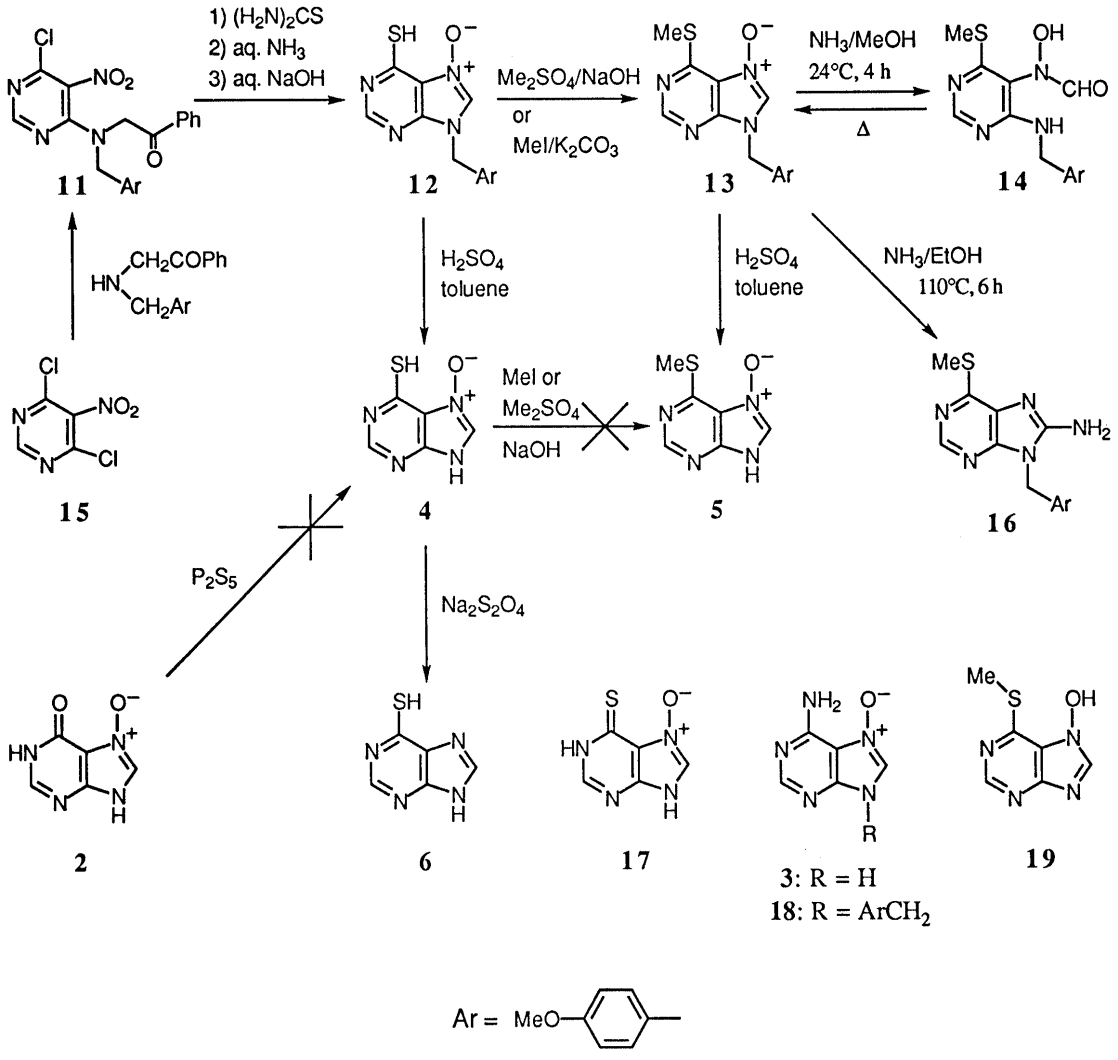

Chart 1

with the condensation of *N*-(4-methoxybenzyl)phenacylamine, generated from its hydrochloride salt,[6] with 4,6-dichloro-5-nitropyrimidine (**15**) in $CHCl_3$ at 0—5 °C for 1 h to give the phenacylaminopyrimidine **11** in 75% yield (Chart 1). Compound **11** was then treated successively with thiourea (boiling EtOH, 5 min), conc. aqueous NH_3, and 2 N aqueous NaOH (0 °C, 5 min), affording the *N*-oxide **12** in 76% yield. Characterization of **12** as the N(7)-oxide was readily achieved by elemental analysis, measurements of its MS [*m/z*: 288 (M^+), 272 ($M^+ - 16$)], UV spectrum [$\lambda_{max}^{H_2O}$ (pH 7) 223 nm (ε 25800),[15] 330 (18800)], and ^{1}H-NMR spectrum in Me_2SO-d_6 [δ 8.19 (C(2)-H), 9.03 (C(8)-H)], and the above and the following self-consistent reaction sequences. Removal of the 4-methoxybenzyl group from **12** was effected in a mixture of conc. H_2SO_4 and toluene at 23 °C for 2 h,[16] and the first target, 6-mercaptopurine 7-*N*-oxide (**4**),[17] was obtained in 75% yield. On treatment with sodium dithionite in boiling 50% (v/v) aqueous MeOH for 1.5 h, **4** furnished 6-MP (**6**) in 33% yield. The correctness of the location of the oxygen function in **4** was supported by a two-step conversion of the precursor **12** into the *S*-methyl derivative **5** (*vide infra*). The ^{1}H-NMR spectrum of **4** in Me_2SO-d_6 exhibited a one-proton doublet (J = 3.5 Hz) at δ 8.10 [C(2)-H] and a one-proton singlet at δ 8.54 [C(8)-H]. This indicated that 6-mercaptopurine 7-*N*-oxide exists in Me_2SO-d_6 in the 6-thioxo-1*H*-purine form (**17**) rather than the C(6)-SH form (**4**).

In an alternative synthetic approach to **4**, hypoxanthine 7-*N*-oxide (**2**)[8] was heated with P_2S_5 in boiling pyridine for 3 h. However, we were unable to obtain **4**, but isolated a compound inferred to be 8-mercaptohypoxanthine in 20% yield.

For the synthesis of the second target (**5**), the precursor **12** for **4** was methylated with dimethyl sulfate in a mixture of 1 N aqueous NaOH and MeOH at room temperature for 1 h, giving the 6-methylthio derivative **13** in 52% yield. Methylation of **12** with methyl iodide (K_2CO_3/MeOH, room temp., 1 h) also produced **13** in 63% yield. Treatment of **13** with conc. H_2SO_4 in the presence of toluene at 25 °C for 1 h afforded the desired *N*-oxide **5** in 90% yield.[16] On the other hand, direct methylation of **4** with methyl iodide 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 we were unable to obtain the *S*-methyl derivative **5**.

On recrystallization from MeOH–H_2O (3 : 1, v/v), **5** gave a monohydrate, which was subjected to X-ray diffraction analysis in order to make a definitive identification and to examine its tautomeric form in the solid state. The final atomic coordinates and equivalent isotropic or isotropic thermal parameters of the atoms are listed in Table I. The

bond lengths and angles are given in Tables II and III, respectively, and a computer-generated drawing of the final X-ray model, together with the atomic numbering scheme employed for the X-ray crystallographic data, is presented in Fig. 1. It may be seen that in the solid state the monohydrate of 6-methylthiopurine 7-*N*-oxide exists in the N(7)-OH form (**19**) rather than the N(7)-oxide form (**5**).

In approaching the problem of the tautomeric form of the *N*-oxide moieties of **4** and **5** in H_2O, their acid dissociation constants in H_2O at 30 °C were determined spectrophotometrically. The pK_a values for **4** were <2 (basic) [for protonated form (**20**)⇌neutral form],[17] 4.9 (acidic) [for neutral form⇌monoanion (**22**)],[17] and 9.17 (acidic) [for monoanion (**22**)⇌dianion (**23**)[17]; those for **5** were <2 (basic) [for protonated form (**24**)⇌neutral form] and 4.89 (acidic) [for neutral form⇌monoanion (**25**)] (Chart 2). The strong UV absorption of purine *N*-oxides in the 215—240 nm region is considered to be due to ≻N→O or the enol anion ≻N–O⁻.[15] Al-

TABLE I. Final Atomic Coordinates and Equivalent Isotropic or Isotropic Thermal Parameters for Atoms of **19**·H_2O with Estimated S.D.'s in Parentheses

Atom	*x*	*y*	*z*	B_{eq} (Å²)
C(1A)	0.3918 (1)	0.0250 (2)	0.6903 (2)	2.29 (1)
N(2A)	0.3671 (1)	−0.1034 (2)	0.6128 (2)	2.77 (1)
C(3A)	0.3319 (1)	−0.0679 (3)	0.4958 (2)	3.10 (1)
N(4A)	0.3173 (1)	0.0803 (2)	0.4434 (2)	2.88 (1)
C(5A)	0.3431 (1)	0.2081 (3)	0.5215 (2)	2.40 (1)
C(6A)	0.3806 (1)	0.1848 (2)	0.6447 (2)	2.13 (1)
N(7A)	0.3960 (1)	0.3438 (2)	0.6915 (2)	2.36 (0)
C(8A)	0.3696 (1)	0.4512 (3)	0.5996 (2)	2.78 (1)
N(9A)	0.3360 (1)	0.3760 (2)	0.4943 (1)	2.67 (1)
S(10A)	0.43449 (4)	−0.00488 (6)	0.84228 (4)	2.82 (0)
C(11A)	0.4359 (2)	−0.2285 (3)	0.8525 (2)	3.64 (1)
O(12A)	0.4376 (1)	0.3849 (2)	0.8124 (1)	2.95 (0)
O(1B)	0.3095 (1)	0.5982 (2)	0.3166 (2)	3.41 (1)
H(3)	0.316 (2)	−0.157 (4)	0.448 (3)	0.5
H(8)	0.374 (2)	0.566 (4)	0.611 (3)	1.1
H(12)	0.396 (2)	0.391 (4)	0.821 (3)	2.8
H(11a)	0.383 (1)	−0.277 (3)	0.788 (2)	2.2
H(11b)	0.457 (2)	−0.259 (4)	0.939 (3)	3.2
H(11c)	0.478 (2)	−0.276 (5)	0.837 (4)	5.7
H(21a)	0.269 (1)	0.579 (4)	0.252 (2)	2.0
H(21b)	0.309 (2)	0.514 (4)	0.360 (3)	3.2

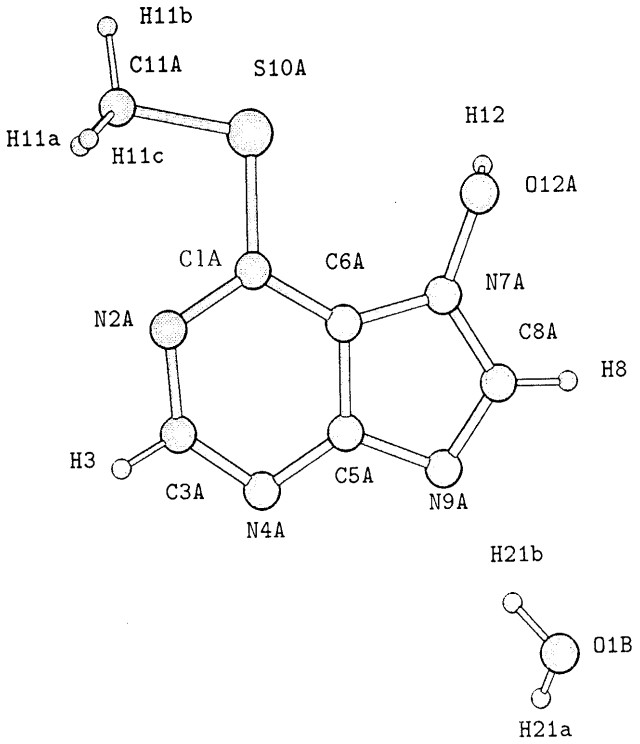

Fig. 1. A Parallel View of the Molecular Structure of **19**·H_2O and the Numbering Scheme Employed for X-ray Crystallographic Data

TABLE II. Bond Lengths in **19**·H_2O

Bond	Length[a] (Å)	Bond	Length[a] (Å)	Bond	Length[a] (Å)
C(1A)–N(2A)	1.343 (3)	C(5A)–C(6A)	1.392 (3)	S(10A)–C(11A)	1.796 (3)
C(1A)–C(6A)	1.385 (3)	C(5A)–N(9A)	1.381 (3)	C(11A)–H(11a)	0.929 (19)
C(1A)–S(10A)	1.739 (3)	C(6A)–N(7A)	1.375 (3)	C(11A)–H(11b)	1.026 (44)
N(2A)–C(3A)	1.343 (3)	N(7A)–C(8A)	1.348 (3)	C(11A)–H(11c)	1.062 (57)
C(3A)–N(4A)	1.328 (3)	N(7A)–O(12A)	1.372 (3)	O(12A)–H(12)	0.908 (45)
C(3A)–H(3)	0.889 (32)	C(8A)–N(9A)	1.319 (3)	O(1B)–H(21a)	0.743 (19)
N(4A)–C(5A)	1.341 (3)	C(8A)–H(8)	0.926 (33)	O(1B)–H(21b)	0.912 (36)

a) Estimated S.D.'s are given in parentheses for the least significant digits.

TABLE III. Bond Angles in **19**·H_2O

Bond	Angle[a] (°)	Bond	Angle[a] (°)	Bond	Angle[a] (°)
N(2A)–C(1A)–C(6A)	117.7 (2)	C(6A)–C(5A)–N(9A)	110.8 (2)	C(5A)–N(9A)–C(8A)	104.2 (2)
N(2A)–C(1A)–S(10A)	122.0 (2)	C(1A)–C(6A)–C(5A)	120.0 (2)	C(1A)–S(10A)–C(11A)	101.9 (1)
C(6A)–C(1A)–S(10A)	120.3 (2)	C(1A)–C(6A)–N(7A)	135.5 (2)	S(10A)–C(11A)–H(11a)	113.1 (14)
C(1A)–N(2A)–C(3A)	117.8 (2)	C(5A)–C(6A)–N(7A)	104.5 (2)	S(10A)–C(11A)–H(11b)	107.9 (18)
N(2A)–C(3A)–N(4A)	128.8 (2)	C(6A)–N(7A)–C(8A)	107.6 (2)	S(10A)–C(11A)–H(11c)	108.5 (24)
N(2A)–C(3A)–H(3)	114.4 (22)	C(6)–N(7A)–O(12A)	126.1 (2)	H(11a)–C(11A)–H(11b)	114.4 (28)
N(4A)–C(3A)–H(3)	116.8 (22)	C(8A)–N(7A)–O(12A)	126.2 (2)	H(11a)–C(11A)–H(11c)	100.9 (28)
C(3A)–N(4A)–C(5A)	113.3 (2)	N(7A)–C(8A)–N(9A)	113.1 (2)	H(11b)–C(11A)–H(11c)	111.8 (31)
N(4A)–C(5A)–C(6A)	122.5 (2)	N(7A)–C(8A)–H(8)	121.9 (25)	N(7A)–O(12A)–H(12)	107.7 (17)
N(4A)–C(5A)–N(9A)	126.8 (2)	N(9A)–C(8A)–H(8)	125.1 (25)	H(21a)–O(1B)–H(21b)	101.5 (29)

a) Estimated S.D.'s are given in parentheses for the least significant digits.

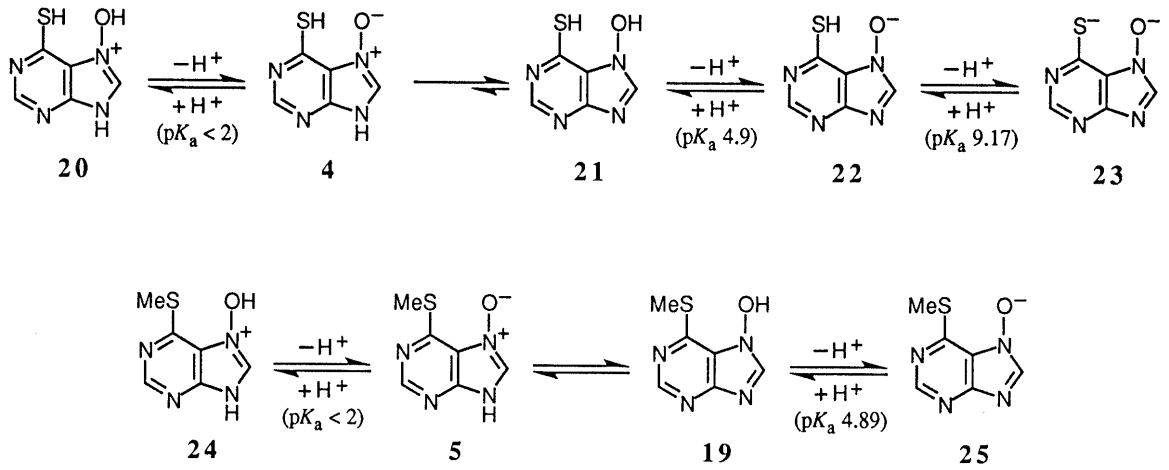

Chart 2

though a fairly strong absorption band (sh) (ε 11700) was observed at 230 nm in the UV spectrum of **4** in H_2O at pH 7, it may be regarded as that arising from the monoanionic species (**22**) in view of the above pK_a values. The spectrum in H_2O at pH 3.0 should roughly reflect that of the neutral species, and it exhibited no maximum absorption in the 230 nm region. This is suggestive of the overwhelming predominance of the N(7)-OH tautomer (**21**) over the N(7)-oxide tautomer (**4**) in the neutral species of 6-mercaptopurine 7-*N*-oxide in H_2O.

As regards the tautomeric form of 6-methylthiopurine 7-*N*-oxide, its neutral species UV spectrum in H_2O at pH 3.0 exhibited two absorption bands at 223 nm (ε 12200) and 297 nm (ε 13200). On the other hand, the neutral species spectrum of 9-(4-methoxybenzyl)-6-methylthiopurine 7-oxide (**13**), a fixed model for the N(7)-oxide tautomer (**5**), showed a strong absorption at 226 nm (ε 30000). Comparison of the molecular absorptivity of the band in the 230 nm region in the former spectrum with that of the latter spectrum suggests that the neutral species of 6-methylthiopurine 7-*N*-oxide exists in H_2O as an equilibrated mixture of the N(7)-oxide (**5**) and the N(7)-OH (**19**) tautomers.

Now that the *S*-methyl derivatives **5** and **13** had become available, we checked the feasibility of an alternative synthetic route to adenine 7-oxide (**3**)[10] from either of them. Amination of **5** with saturated methanolic NH_3 or conc. aqueous NH_3 was examined under a variety of reaction conditions (Chart 1). However, all attempts resulted in the recovery of **5**, suggesting the inertness of the C(6)-SMe group in the anionic species **25**. On the other hand, treatment of the N(9)-arylmethyl derivagive **13** with 16% methanolic NH_3 at 24 °C for 4 h gave an unstable crude compound inferred to be the ring-opened product **14**, which reverted to **13** on heating in boiling EtOH for 30 min. Treatment of **13** with saturated ethanolic NH_3 in an autoclave at 110 °C for 6 h afforded the C(8)-amino derivative **16** in 18% yield. In either case, the desired adenine derivative **18** could not be obtained, and the observed reactivity of **13** at the C(8) atom toward nucleophiles is interpretable in terms of the N(7)-oxide structure.

Finally, in view of the significant antileukemic activities of 6-MP (**6**)[11] and guanine 7-oxide (**1**)[3-5,18] we evaluated some of the above new *N*-oxides and the previously reported *N*-oxides, such as adenine 7-oxide (**3**),[10] 1-benzyladenine 7-oxide,[19] 3-benzyladenine 7-oxide,[10] 7-benzyladenine 1-oxide,[1,20] and 7-benzyladenine 3-oxide,[1] for cytotoxicity to murine L5178Y leukemia cell line *in vitro*. It may be seen from Table IV that most of these compounds were inactive at 50 μg/ml concentration except for **4** and **12**. The *N*-oxides **4** and **12** were less effective than the parent 6-MP (**6**), but slightly more cytotoxic than hypoxanthine 7-*N*-oxide (**2**).

TABLE IV. Antileukemic Activity of Purine *N*-Oxides against Murine L5178Y Cells in Culture

Compound	% inhibition (at 50 μg/ml)	IC_{50}[a] (μg/ml)
6-Mercaptopurine (6-MP) (**6**·H_2O)	94	0.045
Guanine 7-oxide (**1**·1/3H_2O)	—[b]	1.10[c]
Hypoxanthine 7-*N*-oxide (**2**)	47	100[d]
6-Mercaptopurine 7-*N*-oxide (**4**)	67	7.2
6-Methylthiopurine 7-*N*-oxide (**5**)	−12	—[b]
6-Mercapto-9-(4-methoxybenzyl)purine 7-*N*-oxide (**12**·2/5H_2O)	92	>10
Adenine 7-oxide (**3**)	31	—[b]
1-Benzyladenine 7-oxide[e]	−13	—[b]
3-Benzyladenine 7-oxide[e]	27	—[b]
7-Benzyladenine 1-oxide	6	—[b]
7-Benzyladenine 3-oxide[e]	0	—[b]

a) The IC_{50} is defined as the concentration of a test compound required to inhibit cell growth by 50%. *b*) Not determined. *c*) Taken from ref. 6*b*. *d*) Taken from ref. 8*b*. *e*) As the monohydrate.

In conclusion, the present work has established multistep synthetic routes to the hitherto unknown 7-*N*-oxides at the 6-MP level. This success enlarges the usefulness of our "phenacylamine route"[6-8] for the synthesis of purine 7-*N*-oxides. The *N*-oxides **4** and **12** have been found to show only weak antileukemic activity.

Experimental

General Notes All melting points were determined by using a Yamato MP-1 or a Büchi model 530 capillary melting point apparatus and are corrected. Chromatography and measurements of spectra and acid dissociation constants were carried out as described previously.[10b] Elemental analyses and MS measurements were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, d = doublet, m = multiplet, s = singlet, sh = shoulder, t = triplet.

4-Chloro-6-[(4-methoxybenzyl)(2-oxo-2-phenylethyl)amino]-5-nitropyrimidine (11) 2-[(4-Methoxybenzyl)amino]-1-phenylethanone hydrochloride[6] (17.51 g, 60.0 mmol) was added to 1 N aqueous NaOH (66 ml) in small portions, and the resulting mixture was extracted with $CHCl_3$ (80 ml). The $CHCl_3$ extracts were combined, dried over anhydrous $MgSO_4$, and concentrated to dryness *in vacuo* to leave a yellow oil, which was dissolved in $CHCl_3$ (60 ml). The $CHCl_3$ solution was then stirred at 0—5 °C, 4,6-dichloro-5-nitropyrimidine (**15**) (5.82 g, 30.0 mmol) was added in small portions, and stirring was continued at 0—5 °C for 1 h. The crystals (the phenacylamine hydrochloride) that deposited were removed by filtration and washed successively with $CHCl_3$ (5 × 3 ml) and ether (2 × 3 ml). The filtrate and washings were combined and concentrated *in vacuo*, and the residue was extracted with ether (200 ml). Concentration of the ethereal extracts under reduced pressure left a solid, which was triturated with EtOH (30 ml). The yellow crystals that resulted were filtered off, washed successively with EtOH (5 ml) and ether (5 ml), and dried to give **11** (9.35 g, 75%), mp 101—104 °C. Recrystallization from EtOH yielded an analytical sample of **11** as yellow fine needles, mp 107—109 °C; IR ν_{max}^{Nujol} 1698 cm^{-1} (COAr); ^{1}H-NMR (Me_2SO-d_6) δ: 3.73 (3H, s, OMe), 4.74 (2H, s, C$\underline{H}_2$Ar), 5.09 (2H, s, C$\underline{H}_2$COPh), 6.90 [2H, d, J=8.6 Hz, C(3′)-H and C(5′)-H],[21] 7.27 [2H, d, J=8.6 Hz, C(2′)-H and C(6′)-H],[21] 7.55 (2H, m), 7.69 (1H, m), and 7.95 (2H, m) (COPh), 8.54 [1H, s, C(2)-H]. *Anal.* Calcd for $C_{20}H_{17}ClN_4O_4$: C, 58.19; H, 4.15; N, 13.57. Found: C, 58.33; H, 4.17; N, 13.58.

6-Mercapto-9-(4-methoxybenzyl)purine 7-*N*-Oxide (12) A stirred mixture of **11** (2.064 g, 5.00 mmol) and thiourea (419 mg, 5.50 mmol) in abs. EtOH (25 ml) was heated under reflux for 5 min. The reaction mixture was then cooled to 0 °C, and conc. aqueous NH_3 (14 ml) was added dropwise. After 10 min, 2 N aqueous NaOH (10 ml) was added dropwise at 0 °C, and the mixture was stirred at 0 °C for 5 min. The resulting reddish purple solution was brought to pH 4 by addition of 10% aqueous HCl. The precipitate that resulted was collected by filtration, washed successively with H_2O (3 × 10 ml), MeOH (2 × 10 ml), and ether (2 × 2 ml), and dried to give **12**·2/5H_2O (1.12 g, 76%), mp 156—160 °C (dec.). Recrystallization from 50% (v/v) aqueous MeOH and drying over P_2O_5 at 2 mmHg and 30 °C for 10 h provided an analytical sample of **12**·2/5H_2O as pale yellow needles, mp 161—163 °C (dec.); MS *m/z*: 288 (M^+), 272 (M^+-16); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 221 nm (ε 25100), 333 (16700); $\lambda_{max}^{H_2O}$ (pH 1) 224 (19100), 330 (18300); $\lambda_{max}^{H_2O}$ (pH 7) 223 (25800), 330 (18800); $\lambda_{max}^{H_2O}$ (pH 13) 225 (26300), 329 (18700); ^{1}H-NMR (Me_2SO-d_6) (unstable) δ: 3.73 (3H, s, OMe), 5.27 (2H, s, C$\underline{H}_2$Ar), 6.92 [2H, d, J=8.6 Hz, C(3′)-H and C(5′)-H],[21] 7.33 [2H, d, J=8.6 Hz, C(2′)-H and C(6′)-H],[21] 8.19 [1H, s, C(2)-H], 9.03 [1H, s, C(8)-H], 13.7 (1H, br, SH). *Anal.* Calcd for $C_{13}H_{12}N_4O_2S$·2/5H_2O: C, 52.84; H, 4.37; N, 18.96. Found: C, 52.88; H, 4.33; N, 19.02.

6-Mercaptopurine 7-*N*-Oxide (4) A suspension of **12**·2/5H_2O (1.07 g, 3.62 mmol) in toluene (14 ml) was stirred at 23 °C, and conc. H_2SO_4 (7.2 g, 72 mmol) was added dropwise. The resulting mixture was stirred vigorously at 23 °C for 2 h. The toluene layer was removed from the reaction mixture by decantation, and the dark brown, oily residue was triturated with ether (30 ml). The insoluble yellow solid that resulted was filtered off, washed with ether (3 × 5 ml), and then dissolved in H_2O (20 ml). On standing, the aqueous solution deposited pale yellow granules, which were filtered off, washed successively with H_2O (2 × 1 ml), MeOH (2 × 1 ml), and ether (2 × 1 ml), and dried to give **4** (455 mg, 75%), mp >300 °C (dec.). Recrystallization from 50% (v/v) aqueous MeOH afforded an analytical sample of **4** as yellow needles, mp >300 °C (dec.); pK_a (in H_2O at 30 °C and ionic strength 1.0): <2, 4.9, and 9.17±0.03; MS *m/z*: 168 (M^+), 152 (M^+-16); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 235 nm (ε 18300), 329 (19800); $\lambda_{max}^{H_2O}$ (pH 1) 329 (19800); $\lambda_{max}^{H_2O}$ [pH 3.0 [in glycine–HCl buffer (ionic strength 1.0)]] 329 (19600); $\lambda_{max}^{H_2O}$ (pH 7) 230 (sh) (11700), 291 (sh) (8000), 319 (sh) (12100), 333 (13100); $\lambda_{max}^{H_2O}$ (pH 13) 228 (17200), 291 (6800), 329 (13400); ^{1}H-NMR (Me_2SO-d_6) δ: 8.10 [1H, d, J=3.5 Hz, C(2)-H], 8.54 [1H, s, C(8)-H], 12.40 and 13.61 (1H each, br, NH's). *Anal.* Calcd for $C_5H_4N_4OS$: C, 35.71; H, 2.40; N, 33.31. Found: C, 35.68; H, 2.49; N, 33.02.

Deoxygenation of 4 to Form 6-Mercaptopurine (6) A stirred solution of **4** (75 mg, 0.45 mmol) in 50% (v/v) aqueous MeOH (10 ml) was heated under reflux, and sodium dithionite (of 82% purity) (1.4 g, 6.8 mmol) was added in small portions over a period of 1 h. Stirring and heating were continued for a further 1.5 h, and the reaction mixture was concentrated *in vacuo* to a volume of *ca.* 3 ml and then cooled in an ice bath. The yellow solid that deposited was filtered off, washed successively with small amounts of H_2O, EtOH, and ether, and dried to give **6**·H_2O (25 mg, 33%[22]), mp >300 °C. This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **6**·H_2O.

Reaction of Hypoxanthine 7-*N*-Oxide (2) with P_2S_5 A mixture of P_2S_5 (340 mg, 1.53 mmol) and pyridine (3 ml) was stirred at 60 °C, and **2**[8] (304 mg, 2 mmol) was added in one portion. The resulting mixture was heated under reflux for 3 h and then concentrated *in vacuo* to leave a yellow solid. The solid was heated in H_2O (5 ml) under reflux for 1 h, and the resulting aqueous solution was kept at room temperature overnight. The pale greenish solid that deposited was filtered off and dissolved in 5% aqueous NH_3 (*ca.* 10 ml). After treatment with activated charcoal powder, the ammoniacal solution was neutralized with AcOH. The pale greenish solid that deposited was filtered off and recrystallized from H_2O to give a compound (69.9 mg, 20%) inferred to be 8-mercaptohypoxanthine, mp >300 °C. Further recrystallization from H_2O and drying over P_2O_5 at 2 mmHg and 75 °C for 10 h yielded an analytical sample as pale greenish prisms, mp >300 °C; MS *m/z*: 168 (M^+); UV $\lambda_{max}^{H_2O}$ (pH 1) 233 nm (ε 9700), 289 (26200); $\lambda_{max}^{H_2O}$ (pH 7) 234 (13700), 292 (23000); $\lambda_{max}^{H_2O}$ (pH 13) 232 (23600), 289 (24100); ^{1}H-NMR (Me_2SO-d_6) δ: 7.96 (1H, s, purine proton), 12.59, 13.02, and 13.16 (1H each, br, NH's). *Anal.* Calcd for $C_5H_4N_4OS$·1/4H_2O: C, 34.78; H, 2.63; N, 32.45. Found: C, 34.75; H, 2.56; N, 32.45.

The UV spectra of this sample in H_2O at pH 1, 7, and 13 were similar to those [$\lambda_{max}^{H_2O}$ (pH 1) 234 nm (ε 8700), 290 (27200)[23a]; $\lambda_{max}^{H_2O}$ (pH 1) 233.5 (9300), 289 (24100)[23b]; $\lambda_{max}^{H_2O}$ (pH 7) 234 (12900), 291 (19100)[23b]; $\lambda_{max}^{H_2O}$ (pH 13) 232 (21500), 289 (21800)[23b]] reported for 8-mercaptohypoxanthine.

9-(4-Methoxybenzyl)-6-methylthiopurine 7-Oxide (13) i) By Methylation of **12** with Me_2SO_4/NaOH: A solution of **12**·2/5H_2O (828 mg, 2.80 mmol) in a mixture of 1 N aqueous NaOH (2.8 ml) and MeOH (16.6 ml) was stirred at room temperature, and dimethyl sulfate (372 mg, 2.80 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 1 h and then concentrated *in vacuo*. The residue was triturated with H_2O (5 ml), and the insoluble pale yellow solid that resulted was filtered off, washed successively with EtOH (2 × 1 ml) and ether (2 × 1 ml), and recrystallized from EtOH (with recourse to decoloration by activated charcoal powder) to give **13** (443 mg, 52%) as colorless needles, mp 195—203 °C (dec.). Further recrystallization from EtOH yielded an analytical sample as colorless needles, mp 195—205 °C (dec.); MS *m/z*: 302 (M^+); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 228 nm (ε 29100), 282 (10700), 318 (8700); $\lambda_{max}^{H_2O}$ (pH 1) 225 (25600), 302 (15900); $\lambda_{max}^{H_2O}$ (pH 7) 226 (30000), 289 (10500), 311 (11400); $\lambda_{max}^{H_2O}$ (pH 13) unstable; ^{1}H-NMR (Me_2SO-d_6) (unstable) δ: 2.57 (3H, s, SMe), 3.72 (3H, s, OMe), 5.31 (2H, s, C$\underline{H}_2$Ar), 6.91 [2H, d, J=8.6 Hz, C(3′)-H and C(5′)-H],[21] 7.34 [2H, d, J=8.6 Hz, C(2′)-H and C(6′)-H],[21] 8.77 and 9.09 (1H each, s, purine protons). *Anal.* Calcd for $C_{14}H_{14}N_4O_2S$: C, 55.62; H, 4.67; N, 18.53. Found: C, 55.66; H, 4.58; N, 18.57.

ii) By Methylation of **12** with MeI/K_2CO_3: A mixture of **12**·2/5H_2O (828 mg, 2.80 mmol) and anhydrous K_2CO_3 (386 mg, 2.79 mmol) in MeOH (16.6 ml) was stirred at room temperature for 30 min, and then methyl iodide (397 mg, 2.80 mmol) was added in one portion. The resulting solution was stirred at room temperature for 1 h. The colorless crystals that deposited were filtered off, washed successively with H_2O (2 × 1 ml), EtOH (1 ml), and ether (1 ml), and dried to afford **13** (532 mg, 63%), mp 195—203 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC mobility) with the one obtained by method (i).

6-Methylthiopurine 7-*N*-Oxide (5) A suspension of **13** (333 mg, 1.10 mmol) in toluene (3 ml) was stirred at 25 °C, and conc. H_2SO_4 (1.65 g, 16.5 mmol) was added dropwise. The resulting mixture was stirred vigorously at 25 °C for 1 h and then poured onto ice (10 g). The aqueous mixture was washed with toluene (3 ml), and the toluene layer was separated from the aqueous layer and washed with H_2O (2 ml). The aqueous washings and the above aqueous layer were combined, brought to pH 7 by addition of 2 N aqueous NaOH, concentrated *in vacuo* to a volume of *ca.* 4 ml, and then brought to pH 5 with 1 N aqueous HCl. The colorless solid that deposited was filtered off, washed successively with H_2O (1 ml), EtOH (1 ml), and ether (1 ml), and dried to furnish **5** (181 mg, 90%), mp 216—218 °C (dec.). Recrystallization from 50% (v/v) aqueous MeOH and drying over P_2O_5 at 2 mmHg and 50 °C for 10 h yielded an analytical sample of **5** as colorless prisms, mp 220—223 °C (dec.); pK_a (in H_2O at 30 °C and ionic strength 1.0): <2 and 4.89±0.02; MS *m/z*: 182 (M^+), 166 (M^+-16); UV $\lambda_{max}^{95\%\,aq.\,EtOH}$ 222 nm (ε 11900), 262 (sh) (4600), 297 (13300); $\lambda_{max}^{H_2O}$ (pH 1) 224 (10100), 306 (14200); $\lambda_{max}^{H_2O}$ [pH 3.0 [in glycine–HCl buffer (ionic strength 1.0)]] 223 (12200), 297

(13200); $\lambda_{max}^{H_2O}$ (pH 7) 230 (18100), 260 (7700), 280 (8800), 318 (5800); $\lambda_{max}^{H_2O}$ (pH 13) 230 (18100), 260 (7700), 280 (8800), 318 (5800); ^{1}H-NMR (Me_2SO-d_6) δ: 2.66 (3H, s, SMe), 8.69 and 8.75 (1H each, s, purine protons), 12.70 [1H, s, N(9)-H or N(7)-OH]. *Anal.* Calcd for $C_6H_6N_4OS$: C, 39.55; H, 3.32; N, 30.75. Found: C, 39.43; H, 3.27; N, 30.56.

Reaction of 13 with Methanolic Ammonia A suspension of **13** (100 mg, 0.331 mmol) in 16% methanolic NH_3 (10 ml) was stirred at 24 °C for 4 h, during which time it turned into a clear solution. The colorless solid that deposited was collected by filtration, washed successively with EtOH (1 ml) and ether (1 ml), and dried to yield a first crop (41 mg) of a compound inferred to be 5-(*N*-hydroxyformamido)-4-[(4-methoxybenzyl)amino]-6-methylthiopyrimidine (**14**), mp 154—155 °C (dec.); ^{1}H-NMR (Me_2SO-d_6) (unstable) δ: 2.45 (3H, s, SMe), 3.71 (3H, s, OMe), 4.52 (2H, d, J=5.9 Hz, NHC$\underline{H}_2$Ar), 6.87 [2H, d, J=8.5 Hz, C(3′)-H and C(5′)-H],[21] 7.24 [2H, d, J=8.5 Hz, C(2′)-H and C(6′)-H],[21] 7.88 [1H, s, C(2)-H], 8.13 (1H, t, J=5.9 Hz, N$\underline{H}$CH$_2$Ar), 8.31 (1H, s, NCHO). The above filtrate and washings were combined and concentrated *in vacuo*, and the residue was triturated with EtOH (2 ml). The insoluble colorless solid that resulted was filtered off, washed successively with EtOH (0.5 ml) and ether (2 × 0.5 ml), and dried to give a second crop (45 mg) of **14**, mp 155—156 °C (dec.). The total yield of **14** was 86 mg (81%). The crude **14** was so unstable that an attempted recrystallization from EtOH gave a mixture of **13** and **14**; heating the crude **14** (10 mg) in boiling EtOH (2 ml) for 30 min and concentration of the resulting solution under reduced pressure left **13** as a pale yellow solid, which was identical (by comparison of the IR and ^{1}H-NMR spectra and TLC mobility) with an authentic sample.

8-Amino-9-(4-methoxybenzyl)-6-methylthiopurine (16) A solution of **13** (303 mg, 1.00 mmol) in saturated ethanolic NH_3 (20 ml) was heated in an autoclave at 110 °C for 6 h. The reaction mixture was concentrated *in vacuo* to leave a reddish brown oil. Purification of the oil by flash chromatography[24] [silica gel, $CHCl_3$ followed by $CHCl_3$–MeOH (20 : 1, v/v)] afforded **16** (55 mg, 18%) as a colorless solid, mp 180—181 °C. Recrystallization from MeOH yielded an analytical sample of **16** as colorless prisms, mp 180—181 °C; MS *m/z*: 301 (M^+); UV $\lambda_{max}^{95\%\ aq.\ EtOH}$ 229 nm (ε 26500), 302 (18500); $\lambda_{max}^{H_2O}$ (pH 1) 228 (23300), 297 (21200); $\lambda_{max}^{H_2O}$ (pH 7) 227 (22800), 303 (18000); $\lambda_{max}^{H_2O}$ (pH 13) 227 (22700), 303 (17700); IR ν_{max}^{Nujol} cm^{-1}: 3340, 3220 (NH_2); ^{1}H-NMR (Me_2SO-d_6) δ: 2.58 (3H, s, SMe), 3.70 (3H, s, OMe), 5.18 (2H, s, C$\underline{H}_2$Ar), 6.87 [2H, d, J=8.9 Hz, C(3′)-H and C(5′)-H],[21] 7.19 (2H, s, NH_2), 7.23 [2H, d, J=8.9 Hz, C(2′)-H and C(6′)-H],[21] 8.39 [1H, s, C(2)-H]. *Anal.* Calcd for $C_{14}H_{15}N_5OS$: C, 55.80; H, 5.02; N, 23.24. Found: C, 55.88; H, 5.00; N, 23.00.

X-ray Structure Determination of 6-Methylthiopurine 7-*N*-Oxide (5) For X-ray diffraction analysis, an analytical sample of **5** was recrystallized from MeOH–H_2O (3 : 1, v/v) to give colorless transparent prisms of the monohydrate (**19**·H_2O). A crystal measuring 0.40 × 0.20 × 0.10 mm was selected from among them and used for all data collection. Unit cell constants and intensity data were obtained with a Rigaku AFC-5R automatic diffractometer using graphite-monochromated Cu $K\alpha$ radiation (λ = 1.5418 Å). The unit cell dimensions were determined from angular settings of 46 2θ-values in the range of 85—90°, affording the following crystal data: a = 19.680(2) Å; b = 8.011(2) Å; c = 14.041(2) Å; α = 90.00(0)°; β = 129.72(7)°; γ = 90.00(0)°; U = 1702.8(6) Å^3; space group $C2/c$; Z=8; D_x = 1.562 g/cm^3; $F(000)$ = 832; μ(Cu $K\alpha$) = 3.105 mm^{-1}. Out of 1261 unique reflections ($0° \leq 2\theta \leq 120°$) measured by using the $\omega/2\theta$ scan technique at a rate of 16°/min, 1150 without $|F_{obs}| = 0$ were considered unique and observed. No absorption corrections were applied.

The structure was solved by a direct method using the program SHELXS-86[25] and the difference Fourier method. Refinement of atomic parameters was carried out using the full-matrix least-squares method with anisotropic temperature factors. All hydrogen atoms were clearly located on difference Fourier maps and refined with isotropic temperature factors. Throughout the refinement, the function $\Sigma w(|F_o| - |F_c|)^2$ was minimized, and the weight used during the final refinement stage was $w = 1/(\sigma|F_o|^2 + 0.003|F_o|^2)$; the final R value, 0.0400 (R_w = 0.0610). The atomic scattering factors were taken from the literature.[26] The final atomic positions and equivalent isotropic or isotropic thermal parameters for all atoms are listed in Table I. The bond lengths and angles are given in Tables II and III, respectively. A computer-generated,[27] parallel view of the structure of **19**·H_2O is presented in Fig. 1.

Bioassay Procedure Compounds **3**[10]—**6** and **12**, 1-benzyladenine 7-oxide,[19] 3-benzyladenine 7-oxide,[10] 7-benzyladenine 1-oxide,[1,20] and 7-benzyladenine 3-oxide[1] were subjected to *in vitro* bioassay of antileukemic activity against murine L5178Y cells in a manner similar to that described previously.[6b] The results are given in Table IV.

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