Queuine Analogues. Their Synthesis and Inhibition of Growth of Mouse L5178Y Cells in Vitro

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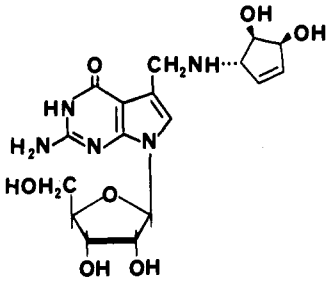
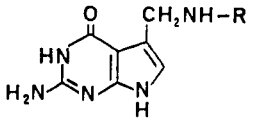
A variety of analogues of queuine (7-[(3*S*,4*R*,5*S*)-4,5-dihydroxycyclopent-1-en-3-ylaminomethyl]-7-deazaguanine), i.e., those with 7-N-substituted aminomethyl side chains and those in which the oxygen function at the 6 position of the 7-deazaguanine ring was replaced by sulfur, were synthesized and tested for ability to act as substrates for tRNA-guanine transglycosylase and for inhibitory effects on growth of mouse L5178Y leukemic cells in vitro. Of the compounds tested, analogues with sulfur at the 6 position of the 7-deazaguanine ring in place of oxygen or with an *N*-*o*-hydroxyphenyl, *N*-*m*-hydroxyphenyl, or iodoacetyl group in the 7-aminomethyl side chain in place of the naturally occurring cyclopentene diol moiety markedly inhibited the growth of cells at concentrations of 1–10 μg/mL, although queuine itself had practically no effect at a concentration of 100 μg/mL.

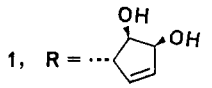
Queuosine (7-[(3*S*,4*R*,5*S*)-4,5-dihydroxy-2-cyclopent-1-en-3-ylaminomethyl]-7-deazaguanosine) (According to the IUPAC nomenclature, this compound is designated as 2-amino-5-[(3*S*,4*R*,5*S*)-4,5-dihydroxycyclopent-1-en-3-ylaminomethyl]-7-[β-D-ribofuranosyl]pyrrolo[2,3-*d*]pyrimidin-4-one.) is a hypermodified nucleoside located at the



Queuosine

first position of the anticodon of $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$ in most prokaryotic and eukaryotic cells.[1] The unique feature of this modified nucleoside with respect to its biosynthesis in tRNA is that the base of queuosine (queuine **1**) or its precursor molecule (7-aminomethyl-7-deazaguanine **2**) is incorporated into precursor tRNA by an exchange reaction with guanine originally located in the precursor tRNA; this exchange reaction is catalyzed by tRNA-guanine transglycosylase.[1]



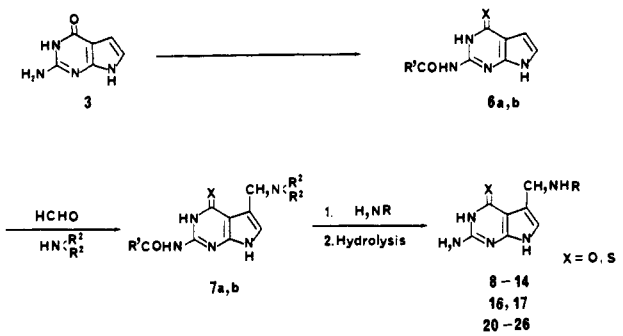


1, R = ···

2, R = – H

Eukaryotic cells, including mammalian cells and those of *Dictyosterium discoideum*, cannot synthesize queuine but take it from outside as a nutrient or from intestinal flora.[1-3] In tumor cells, the insertion of queuine into tRNA is almost always incomplete and undermodified tRNA with guanine in place of queuine is always present.[4,5] Intravenous injection of a large amount of queuine into tumor-bearing mice resulted in complete conversion of tRNA lacking Q to that containing Q in tumor tissues.[6,7] Katze and Beck reported the antitumor activity of queuine against Ehrlich ascites tumor in vivo,[7] but we were unable to detect antitumor activity of normal queuine in mice with L-1210 or S-180 tumors.[6] These previous findings suggested that if some queuine analogue could be incorporated into tRNA, and then affect protein synthesis by changing the codon recognition properties of the tRNA or by cross-linking with some component(s) in the protein-synthesizing machinery, it should be effective as an anticancer reagent.[1]

Scheme I



This consideration prompted us to synthesize a series of queuine analogues with a variety of substituents, e.g., a substituted or unsubstituted acyl, alkyl, phenyl, and cyclopentenyl, attached to the 7-aminomethyl nitrogen and 6-thioqueuine analogues, and to examine their biological properties. This paper reports the syntheses of queuine analogues, their abilities to function as substrates for tRNA-guanine transglycosylase, and their inhibitory effects

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(1) Nishimura, S. In *Progress in Nucleic Acid Research and Molecular Biology*; Cohen, W. E., Ed.; Academic Press: New York, 1983; Vol. 28, 49–73.
(2) Reyniers, J. P.; Pleasants, J. R.; Wostmann, B. S.; Katze, J. R.; Farkas, W. R. *J. Biol. Chem.* **1981**, *256*, 11 591–11 594.
(3) Ott, G.; Kersten, H.; Nishimura, S. *FEBS Lett.* **1982**, *146*, 311–314.
(4) Okada, N.; Shindo-Okada, N.; Sato, S.; Itoh, Y. H.; Oda, K.; Nishimura, S. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4247–4251.
(5) Roe, B. A.; Stankiewicz, A. F.; Rizi, H. L.; Weiss, C.; DiLauro, M. N.; Pike, D.; Chen. C. Y.; Chen, E. Y. *Nucleic Acids Res.* **1979**, *6*, 673–688.
(6) Nishimura, S.; Shindo-Okada, N.; Kasai, H.; Kuchino, Y.; Noguchi, S.; Iigo, M.; Hoshi, A. *Recent Res. Cancer Research* **1983**, *84*, 401–412.
(7) Katze, J. R.; Beck, W. T. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 313–319.

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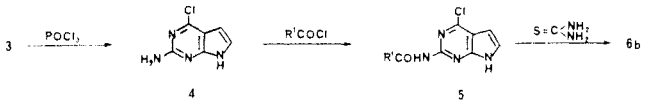
Scheme II



on the growth of mouse L5178Y leukemic cells in vitro.

Chemistry

We synthesized a variety of queuine analogues that differ from queuine not only in the structure of their substituents attached to the 7-aminomethyl nitrogen but also in the polar hetero atom attached to the 6 position of the 7-deazaguanine ring, to determine what effects structural modification would have on biological activity.

Synthesis of queuine (1) has been reported,[8] but the procedure involves so many steps that it was too laborious and time consuming for use in syntheses of analogues in this work. Recently, we developed a new, more efficient method[9] to synthesize queuine (1) and its biosynthetic precursor, preQ^1 base (7-aminomethyl-7-deazaguanine 2). For this method the desired N-substituted aminomethyl side chain is introduced at position 7 of N^2-protected 7-deazaguanine **6a** and 7-deaza-6-thioguanine **6b** by a sequence of three reactions: the Mannich reaction, followed by an amine-exchange reaction using an appropriate amine, and deprotection of the N^2-acyl group leading to 1 or an analogue (Scheme I).

The compounds synthesized are listed in Table I. 7-Deazaguanine (2-aminopyrrolo[2,3-*d*]pyrimidin-4-one, **3**)[10] was used as a common starting material. It was converted to a 2-acylamino compound **6a** followed by reaction with formaldehyde (35% in water), and dialkylamine at 60 °C gave a Mannich base. Under the reaction conditions employed, a disubstituted aminomethyl group was introduced at the desired β-position of the pyrrole nucleus of the pyrrolo[2,3-*d*]pyrimidine ring (position 7 of 7-deazapurine) providing 2-(acylamino)-5-(*N*,*N*-disubstituted (aminomethyl))pyrrolo[2,3-*d*]pyrimidin-4-one **7a** in good yields. Compound **7a** was a key intermediate in the synthesis of queuine analogues. When **7a** was treated with an appropriate amine, e.g., ammonia or a primary or secondary amine, at 75 °C, the disubstituted amino function of **7a** was displaced by the added amine giving 7-deazaguanine derivatives with the corresponding amino function in the 7 side chain. Deacylation of these compounds gave the desired queuine analogues [2-amino-5-(substituted (aminomethyl))pyrrolo[2,3-*d*]pyrimidin-4-ones, **8–14**, **16**, **17**, **20–23**] in high yields. A similar procedure was used to prepare 6-thioqueuine analogues (**24–26**). N^2-Acylated 7-deaza-6-thioguanine (2-(acylamino)pyrrolo[2,3-*d*]pyrimidine-4-thione, **6b**) was prepared from **3** and used in the Mannich reaction in place of **6a** (Scheme I, X = S).

The imino **15** and acyl derivatives **18** and **19** of **2** (preQ$_1$ base) were synthesized by treating **2** with the appropriate reagent. The (methylthio)queuine analogue **27** was synthesized by methylation of the thio function of **25**. Compound **27** exists in a lactim form, which is quite different from the ring structure (lactam form) of the other compounds listed in Table I. All products gave satisfactory results on elemental analysis (C, H, N or C, H, N, S), and their IR and NMR spectra (Table I) were consistent with the proposed structures. The facile route described above proved advantageous from the view point of yield and simplicity and enabled us to obtain sufficient amounts of a variety of analogues of queuine to allow extensive studies on their structure–activity relationships.

Biological Results and Discussion

The queuine analogues synthesized and tested (Table I) are grouped into several classes. The first class, compounds **24**, **25**, **26**, and **27**, are analogues of queuine with a sulfur or S–CH_3 group in place of the oxygen function at position 6 of the 7-deazaguanine ring. The second class, compounds **8–19**, are 7-deazaguanines with a variety of substituents attached to the 7-aminomethyl nitrogen in place of the naturally occurring moiety, cyclopentenediol. Analogues of the third class are stereoisomers of 1 (compounds **20** and **21**) and derivatives of 1 or **21** that have the isopropylidene cyclopentenediol moiety with the same or a different configuration from that of 1 (compounds **22**, **23**). As shown in Table II, all compounds tested except compound **18** could be incorporated into tRNA lacking Q by a reaction catalyzed by rat liver tRNA-guanine transglycosylase. Their relative rates of incorporation varied considerably.

Table II also shows the percent inhibitions of growth of L5178Y cells by the analogues at concentrations of 1–100 μg/mL. It should be noted that native queuine (1) had almost no effect on the growth of L5178Y cells at a concentration of 10 μg/mL but was slightly inhibitory at 100 μg/mL.

Unlike native queuine, several queuine analogues strongly inhibited the growth of L5178Y cells. The most inhibitory analogues were compounds **13**, **14**, **18** and **24–26**. At a concentration of 10 μg/mL, 7-[((*o*- and [((*m*-hydroxyphenyl)amino)methyl]deazaguanine (**13** and **14**) caused more than 50% inhibition of growth. The strongest inhibitor was 7-[((iodoacetyl)amino)methyl]-7-deazaguanine (**18**), which caused 50% inhibition of cell growth at a concentration of 1 μg/mL. Queuine analogues containing an iodinated phenyl group in the 7 side chain (**15** and **16**) were moderately inhibitory. Queuine analogues having a sulfur group in place of the oxygen function at position 6 (**24**, **25**, and **26**) were also strongly inhibitory, causing about 50% inhibition of cell growth at a concentration of 10 μg/mL or less. A 4,5-isopropylidene derivative of 6-thioqueuine, 25, was inhibitory comparable to the 7-iodoacetyl analogue **18**. In contrast, the 6-methylthio analogue **27** was only weakly inhibitory.

Among the other compounds, **8–12**, **17**, **19–23**, analogues with 7-aliphatic side chains and the compound with a cyclopentyl **12** or isobutyryl **19** group were moderately inhibitory (Table II). Other analogues with aliphatic side chains (**8–11** and **17**), those with a stereoisomeric cyclopentenediol moiety (**20** and **21**), and the isopropylidene derivatives of compounds **21** and 1 (**22** and **23**) were less inhibitory even at a concentration of 100 μg/mL and caused scarcely any inhibition at a concentration of 10 μg/mL.

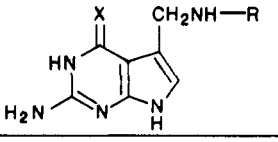
Since almost all queuine analogues tested were found to be good substrates for rat liver tRNA-guanine trans-

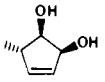
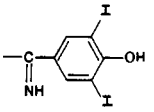
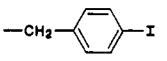
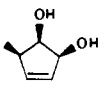
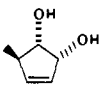
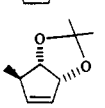
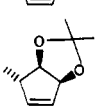
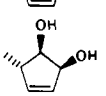
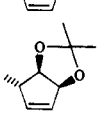
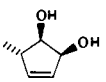
(8) (a) Ohgi, T.; Kondo, T.; Goto, T. *Chem. Lett.* **1979**, 1283, 419–1286. (b) Kondo, T.; Nakasuka, S.; Goto, T. *Chem. Lett.* **1980**, 559–562. (c) Kondo, T.; Ohgi, T.; Goto, T. *Chem. Lett.* **1983**, 419–422.

(9) Akimoto, H.; Hitaka, T.; Imamiya, E.; Nomura, H.; Nishimura, S. *Chem. Pharm. Bull.*, in preparation. Nomura, H.; Akimoto, H. Japan Patent application no. 1983-165760 September 7, 1983 and no. 1982-041910 March 16, 1982. It is known that Mannich reaction of free 7-deazaguanine led to a substitution at position 8 (Seela, F.; Lupke, U. *Chem. Ber.* **1977**, *110*, 1462–1469). In contrast, when N^2-acyl-7-deazaguanine (**6a**) was used in the reaction, the orientation of the substitution changed and a substituted aminomethyl group was introduced at position 7. In fact, the products 1 and **2** obtained by the present method were confirmed to be physicochemically and biochemically identical with the corresponding samples synthesized according to the method reported by Goto et al.[8]

(10) Davoll, J. *J. Chem. Soc.* **1960**, 131–138.

Table I. Physical Constants for 2-Aminopyrrolo[2,3-*d*]pyrimidine Derivatives



compd	X	R	IR ν_{max}, cm^{-1}	NMR, δ	formula	anal.
1	O		3320, 2750, 1680, 1610	4.28–4.60 (2 H, s), 4.50 (2 H, br s), 6.13 (1 H, dd), 6.35 (1 H, m), 7.12 (1 H, s)	$C_{12}H_{15}N_5O_3 \cdot 2HCl \cdot {}^1/_2H_2O$	C, H, N, Cl
2	O	—H	3100, 1670, 1605, 1050	4.32 (2 H, s), 7.12 (1 H, s)	$C_7H_9N_5O \cdot 2HCl \cdot 2H_2O$	C, H, N, Cl
8	O	—$CH_2CH_2CH_3$	3175, 2720, 1675	0.90 (3 H, t), 1.67 (2 H, m), 3.00 (2 H, t), 4.23 (2 H, s), 6.93 (1 H, s)	$C_{10}H_{15}N_5O \cdot 2HCl \cdot H_2O$	C, H, N, Cl
9	O	—CH_2CHCH_2OH (OH)	3300, 1690, 1625, 1050	3.28 (2 H, br d), 3.68 (2 H, br d), 4.43 (2 H, br d), 5.30 (1 H, m), 7.12 (1 H, br d)	$C_{10}H_{15}N_5O_3 \cdot 2HCl \cdot 1{}^1/_2H_2O$	C, H, N, Cl[a]
10	O	—$CH_2CH_2OCH_2CH_2OH$	3325, 1695, 1130, 1070	3.28 (2 H, br t), 3.50–3.77 (2 H × 3, m), 4.23 (2 H, s), 6.83 (1 H, br s)	$C_{11}H_{17}N_5O_3 \cdot 2HCl \cdot {}^1/_2H_2O$	C, H, N, Cl
11	O	—CH_2COOH	3450, 1680, 1655, 1630	4.18 (2 H, s), 4.60 (2 H, s), 7.23 (1 H, s)	$C_9H_{11}N_5O_3 \cdot {}^1/_2H_2O$	C, H, N
12	O		3200, 1680, 1600	1.67–2.40 (8 H, m), 3.67 (1 H, m), 4.30 (2 H, s), 7.00 (1 H, s)	$C_{12}H_{17}N_5O \cdot 2HCl \cdot H_2O$	C, H, N, Cl
13	O		3460, 1675, 1655, 1615	4.20 (2 H, br s), 6.20–6.70 (5 H, m)	$C_{13}H_{13}N_5O_2$	C, H, N
14	O		3400, 1640	4.14 (2 H, br s), 5.78–6.17 (3 H, m), 6.50 (1 H, s), 6.80 (1 H, t)	$C_{13}H_{13}N_5O_2 \cdot {}^1/_2H_2O$	C, H, N
15	O		3370, 1663, 1625, 1460	4.63 (2 H, br s), 6.80 (1 H, s), 7.87 (2 H, s)	$C_{14}H_{12}N_6O_2I_2 \cdot 1{}^1/_2H_2O$	C, H, N
16	O		3400, 1675, 1635, 1585	4.20 (2 H, s), 4.32 (2 H, s), 6.87 (1 H, s), 7.22 (2 H, d), 7.67 (2 H, d)	$C_{14}H_{14}N_5OI$	C, H, N
17	O		3370, 3175–2500, 1680	1.80 (6 H, m), 2.80–3.67 (4 H, m), 4.30 (2 H, q), 7.02 (1 H, s)	$C_{12}H_{17}N_5O \cdot 2HCl \cdot H_2O$	C, H, N, Cl[b]
18	O	—$COCH_2I$	3420, 1670, 1950, 1595	3.80 (2 H, s), 4.38 (2 H, d), 6.60 (1 H, s)	$C_9H_{10}N_5O_2I$	C, H, N
19	O	—$COCH(CH_3)_2$	3420, 1670, 1640, 1600	1.12 (6 H, d), 2.38 (1 H, m), 4.40 (2 H, d), 6.57 (1 H, s)	$C_{11}H_{15}N_5O_2$	C, H, N
20	O		3400, 3220, 1690, 1675	4.04 (2 H, s), 6.03 (1 H, d), 6.23 (1 H, d), 7.03 (1 H, s)	$C_{12}H_{15}N_5O_3 \cdot 2HCl$	C, H, N, Cl
21	O		3425, 3350, 1690, 1670	4.42–4.63 (2 H, m), 4.70 (2 H, s), 6.32 (1 H, dd), 6.52 (1 H, m), 7.30 (1 H, s)	$C_{12}H_{15}N_5O_3 \cdot 2HCl$	C, H, N, Cl
22	O		3470, 2930, 1665, 1620	1.37 (6 H, s), 3.85 (2 H, m), 4.60 (1 H, d), 5.30 (1 H, d), 5.88 (2 H, s), 6.58 (1 H, s)	$C_{15}H_{19}N_5O_3 \cdot CH_3OH$	C, H, N
23	O		2930, 1635, 1545, 1380	1.37 (6 H, s), 3.85 (2 H, m), 4.60 (1 H, d), 5.30 (1 H, d), 5.88 (2 H, s), 6.58 (1 H, s)	$C_{15}H_{19}N_5O_3 \cdot {}^1/_2H_2O$	C, H, N
24	S		2930, 2780, 1690, 1590	4.20–4.60 (2 H, m), 4.47 (2 H, br s), 6.13 (2 H, m), 7.03 (1 H, s)	$C_{12}H_{15}N_5O_2S \cdot 2HCl$	C, H, N, S, Cl
25	S		3330, 1625, 1590, 1570	1.33 (6 H, s), 3.97 (2 H, m), 4.60 (1 H, d), 5.20 (1 H, d), 5.83 (2 H, br s), 6.73 (1 H, s)	$C_{15}H_{19}N_5O_2S \cdot {}^1/_2H_2O$	C, H, N, S, Cl
26	S	—H	2920, 1690, 1595, 1195	4.13 (2 H, br s), 6.90 (1 H, s)	$C_7H_9N_5S \cdot 2HCl$	C, H, N, S, Cl
27	(SCH_3)		3380, 3250, 1640, 1345	2.90 (3 H, s), 4.72 (2 H, br s), 6.22 (1 H, d), 6.43 (1 H, d), 7.43 (1 H, s)	$C_{13}H_{17}N_5O_2S \cdot 2HCl$	C, H, N, S, Cl

[a] C: calcd, 34.01; found, 34.46. [b] H: calcd, 6.26; found, 6.84.

Table II. Inhibition of Growth of L5178Y Cells by Queuine Analogues and Their Ability as Substrates for Rat Liver tRNA-guanine Transglycosylase

compd no.	inhbtn of growth of L5178Y cells, 1 μg/mL	10 μg/mL	100 μg/mL	rate of incorprtn into yeast tRNA,[a] %
1		<10	20	100
8		<10	43	94
9		<10	31	88
10		<10	9	98
11		<10	20	48
12		17	74	80
13	25	53	90	76
14	12	65	79	33
15		31	89	48
16		19	77	98
17		<10	36	65
18	49	87	89	0
19		16	87	36
20		<10	53	70
21		<10	23	120
22		<10	21	100
23		<10	34	55
24	12	54	88	78
25	46	87	94	123
26	<10	36	77	50
27	<10	<10	23	120

[a] Expressed as percent as compared with rate of incorporation of normal queuine.

glycosylase, it is reasonable to assume that they were incorporated into tRNA of L5178Y cells, when added to the tissue culture medium. Therefore, it is possible that inhibition of cell growth by the queuine analogues is due to their incorporation into tRNA. It should be mentioned that the tyrosine tRNA-compound **24** (6-thioqueuine-containing $tRNA^{Tyr}$), isolated from mouse mammary FM3A cells, can efficiently read the amber codon UAG in TMV mRNA, when injected into *Xenopus* oocytes.[11] Since oxygen at position 6 of purine is involved in base-pairing in codon–anticodon interaction, replacement of the oxygen by sulfur in queuine should modify the codon recognition property of tRNA and may thereby result in inhibition of cell growth.

Other queuine analogues that were strongly inhibitory were compounds with a ((hydroxyphenyl)amino)methyl group as a side chain. However, compounds with aliphatic side chain, **8–11**, **18**, and **19** or a cyclopentene ring **12** showed much weaker inhibition. One possible explanation for this structure–inhibition relationship is that the planar structure of the side chain at position 7 of 7-deazaguanine is important for inhibition of cell growth. A side chain attached to the 7-position of 7-deazaguanine should not have any effect on codon recognition of tRNA, since X-ray crystallographic analysis of the 5′-phosphate of Q nucleoside suggested that the cyclopentene diol side chain of Q in tRNA stretches to the outside of the molecule, without interference with the attachment site of mRNA.[12] Thus it is possible that tRNA having such analogues inhibits cell growth by some mechanisms other than alteration of the codon recognition property. Another possibility is that inhibition of the cell growth by queuine analogues has no relation with tRNA, and these analogues function as free molecules to inhibit cell proliferation by another mechanism. In this connection, it is noteworthy that when queuine was injected intravenously into mice, a large amount became loosely bound to protein fractions in a variety of organs.[13] Although compound **18** containing a $COCH_2I$ group instead of cyclopentene diol cannot act as substrate for tRNA-guanine transglycosylase, it strongly inhibited cell growth. Since this queuine analogue contains iodine in the side chain, it may be inhibitory by binding covalently to a cellular component(s) that is important in cell proliferation.

This is the first extensive study on the biological properties of 7-deazaguanine analogues, because difficulties in chemical syntheses of 7-deazaguanine analogues have hampered previous studies. But with our new procedure for synthesis of 7-deazaguanine analogues it will now be possible to examine the biological properties of a variety of 7-deazaguanine analogues. Further studies are needed on the actual mechanism of inhibition of cell growth. We are now testing the antitumor activities of queuine analogues in L1210 or P388 tumor-bearing mice.

Experimental Section

Infrared (IR) spectra were determined in KBr disks with a Hitachi 215 spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained in Me_2SO-d_6 for the free bases or in D_2O for the HCl salts using Varian XL-100-12 and Varian EM-360 instruments: chemical shifts (δ) are reported in ppm downfield from tetramethylsilane used as an internal standard in Me_2SO-d_6 or 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt in D_2O. Where analyses are indicated by symbols of elements, the analytical results for these elements were within ±0.4% of the theoretical values. Pertinent physical data for the compounds are summarized in Table I.

Materials and Methods

Assay of Ability of Queuine Analogues To Act as Substrates of tRNA-Guanine Transglycosylase. The procedure described previously was employed.[14] Namely, 0.5 A_{260} unit of the specified queuine analogue was incubated with [^{3}H]-guanine-labeled yeast tRNA (0.25 A_{260} unit, containing approximately 10 000 cpm of [^{3}H]guanine in the first position of the anticodon, prepared by use of 10 Ci/mmol of [^{3}H]guanine) and partially purified rat liver tRNA-guanine transglycosylase (fraction III) in 0.1 mL of 70 mM Tris-HCl (pH 7.5) at 37 °C for 2 h. The radioactivity remaining in the acid-insoluble fraction was counted. The extents of incorporation of queuine analogues could be measured by counting the decrease of [^{3}H]guanine labeled tRNA due to release of [^{3}H]guanine from tRNA on insertion of the analogue.

Assay of the Inhibitory Effects of Queuine Analogues on Growth of L5178Y Cells. The method used was essentially as described previously.[15] Mouse leukemia L5178Y cells were cultured in MEM medium containing 10% fetal calf serum. The queuine analogues were added at final concentrations of 1–100 μg/mL to the cell culture (4×10^4 cells/mL). Then culture was continued for 3 days, and the number of cells was counted. Percent inhibition of growth was expressed as the number of cells in the presence of a queuine analogue as a percentage of that in its absence. Without queuine analogues, the cell number generally increased to 1×10^6 cells/mL after culture for 3 days.

General Procedure for Preparation of 2-Amino-5-(*N*-substituted (aminomethyl))pyrrolo[2,3-*d*]pyrimidin-4-ones (8–14, 16, 17, 20–23). A suspension of 2-aminopyrrolo[2,3-*d*]-pyrimidin-4-one (**3**, 0.01 mol) in dry pyridine (20 mL) was treated

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with an alkanoyl chloride (0.035 mol) at 85 °C for 30 min with stirring, and then the mixture was evaporated in vacuo. Partial hydrolysis of the resulting semisolid with 8% ammonia in EtOH (12.5 mL) at room temperature for 2 h gave 2-(acylamino)-pyrrolo[2,3-*d*]pyrimidin-4-one (**6a**) in nearly quantitative yield. This 2-acylamino derivative (0.01 mol) was dissolved in 80% aqueous acetic acid (100 mL) and treated with 35% formalin (0.035 mol) and dialkylamine (0.03 mol) at 60 °C for 2 h. The mixture was evaporated in vacuo, and the residue was dissolved in a mixture of MeOH (100 mL) and 2 N HCl (100 mL), heated at 80 °C for 1 h, and then neutralized with 15% aqueous ammonia. After workup, 2-(acylamino)-5-((dialkylamino)methyl)pyrrolo-[2,3-*d*]pyrimidin-4-ones were obtained in 60–90% yield. Finally, the 5-(dialkylamino)methyl compounds (0.01 mol) were dissolved in a mixture of MeOH (25 mL) and tetrahydrofuran (25 mL). The solution was heated with excess of an appropriate monoalkylamine (RNH_2 in Scheme I) or ammonia in a sealed tube at 75 °C for 24 h and then hydrolyzed if necessary, to afford the desired product in 50–80% yield. Physical data on the products are shown in Table I.

General Procedure for Preparation of 2-Amino-5-(aminomethyl)pyrrolo[2,3-*d*]pyrimidine-4-thione Derivatives (24–26). 2-Amino-4-chloropyrrolo[2,3-*d*]pyrimidine (**4**), prepared from 2-aminopyrrolo[2,3-*d*]pyrimidin-4-one (**3**) by treatment with phosphorous oxychloride, was acylated nearly quantitatively with an alkanoyl chloride in dry pyridine by a procedure similar to that described for 2-(acylamino)pyrrolo[2,3-*d*]pyrimidin-4-one (**6a**). The 2-(acylamino)-4-chloro derivative (0.01 mol) obtained was suspended in 2-methoxyethanol (35 mL), treated with thiourea (0.07 mol) with stirring at 100 °C for 2.5 h, and evaporated to dryness in vacuo. After workup, 2-(acylamino)pyrrolo[2,3-*d*]pyrimidine-4-thione (**6b**) was obtained in 70–90% yield (Scheme I). This compound was then converted to the desired products (**24–26**) by a procedure similar to that described in the preceding section.

2-Amino-5-[1-(3,5-diiodo-4-hydroxyphenyl)-1-[(iminomethyl)amino)methyl]]pyrrolo[2,3-*d*]pyrimidin-4-one (15). A solution of **2** (252 mg) in 0.1 M sodium borate buffer (pH 8.5–9.0; 200 mL) and MeOH (50 mL) was treated with methyl 3,5-diiodo-4-hydroxybenzimidate hydrochloride[16] (746 mg) at room temperature for 65 h with stirring. The precipitate formed was collected by centrifugation and washed successively with water and 30% aqueous MeOH. This crude material was purified further by chromatography on a silica gel column with CH_3Cl–MeOH–5% NH_3 in EtOH (70:25:5) as eluent to afford the pure product of **15** (133 mg) as a pale yellow powder.

2-Amino-5-[(3*S*,4*R*,5*S*)-4,5-dihydroxycyclopent-1-en-3-ylaminomethyl]-4-(methylthio)pyrrolo[2,3-*d*]pyrimidine Dihydrochloride (27). A solution of **25** (34.2 mg) in a mixture of MeOH (1.0 mL) and 2 N NaOH (0.1 mL) was treated with methyl iodide (21.3 mg) at room temperature for 5 h with stirring and then evaporated in vacuo. The residue was purified by chromatography on a short silica gel column with MeOH–$CHCl_3$ (1:9) as eluent to yield a pale yellow solid; this was hydrolyzed in a mixture of MeOH (1.0 mL) and 2 N HCl (1.0 mL) at room temperature for 5 h. Evaporation of the solvent in vacuo yielded the desired product (29 mg) as a white powder.

2-Amino-5-((iodoacetamido)methyl)pyrrolo[2,3-*d*]pyrimidin-4-one (18). A methanolic solution (10 mL) of **2** (25.2 mg) was combined with an aqueous solution (1 mL) of K_2CO_3 (55.2 mg) and cooled. A solution of *p*-nitrophenyl iodoacetate in dry tetrahydrofuran (1.0 mL) was added to this mixture with stirring, and the resulting mixture was kept at 0 °C for 30 min. The mixture was evaporated in vacuo, and the residue was purified by chromatography on a silica gel column using 15% MeOH in $CHCl_3$ as eluent to give the desired product (**18**, 15.2 mg) as a white powder.

2-Amino-5-(isobutyramidomethyl)pyrrolo[2,3-*d*]pyrimidin-4-one (19). In a procedure similar to that described above, compound **2** (25.2 mg) was treated with isobutyric anhydride (19.0 mg) to give the desired product (**19**, 17.0 mg) as a white powder.

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