Synthesis of Pteridine Derivatives Related to Folic Acid and Methanopterin from Pyrazine-2,3-dicarbonitrile

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Pteridine derivatives related to folic acid and methanopterin were synthesized by two methods. The first synthesis is initiated by the radical substitution of 5-methylpyrazine-2,3-dicarbonitrile (**3**) with the (*N*-acylanilino)alkyl radical to give 6-methyl-5-(*N*-acylanilino)alkylpyrazine-2,3-dicarbonitrile (**9**) and was followed by the substitution of the 2-carbonitrile with methylamine and further conversion to 1-methyl-2-amino-6-(*N*-acylanilino)-alkyl-7-methylpteridin-4(1*H*)-imine **11** by the action of guanidine. The second method is initiated by radical hydroxymethylation of 5-methylpyrazine-2,3-dicarbonitrile (**3**) to give 5-hydroxymethyl-6-methylpyrazine-2,3-dicarbonitrile (**15**), followed by oxidation of the hydroxymethyl group, *N*-phenylimination, and the substitution of the 2-carbonitrile with methylamine to give 6-methyl-2-methylamino-5-(*N*-phenylimino)methenylpyrazine-3-carbonitrile (**18**). The reduction of the imino group and the final cyclization with guanidine gives 2-amino-6-anilinomethyl-1,7-dimethylpteridin-4(1*H*)-imine (**20**).

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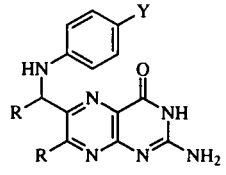
Introduction.

Folic acid (**1**) [1] and methanopterin (**2**) [2], biological C_1-unit mediators [3,4], have a substituent of an anilinoalkyl group at the 6-position of the pteridine ring. Much effort has been made to synthesize pteridine analogues including methotrexate [5], deaza derivatives [6], and other analogues [7] as inhibitors of methyl transferase. Methanopterin has a methyl substituent at the 7-position as well as an anilinoethyl substituent at the 6-position. The methyl group at the 7-position of the pteridine ring may not have an important biochemical function, but it is useful to locate the anilinoalkyl group at the 6-position of the pteridine ring in our synthetic strategy.

We have developed two processes for the synthesis of 2-amino-6-anilinoalkyl-1,7-dimethylpteridin-4(1*H*)-imine from 5-methylpyrazine-2,3-dicarbonitrile (**3**) as the analog of folic acid and methanopterin. The procedures for these syntheses are described in this paper.



1: Folic acid (R = H, Y = CONHCH(COOH)$(CH_2)_2$COOH)

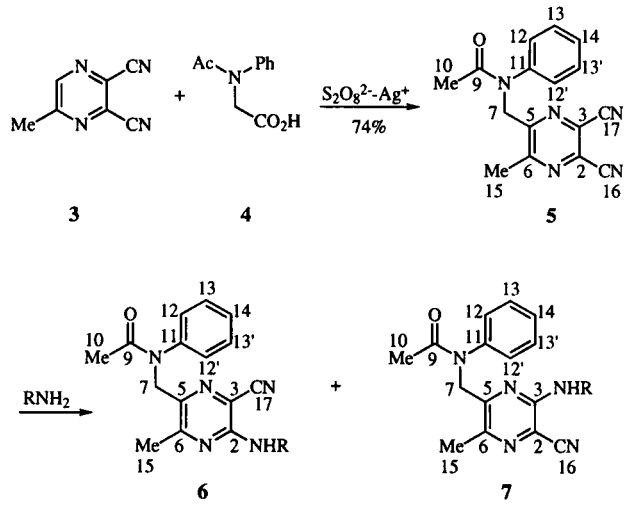
2: Methanopterin (R = Me, Y = $CH_2(CHOH)_3CH_2O$-Ribose-O-(PO_2H)-OCH(COOH)$(CH_2)_2$COOH)

Results and Discussion.

i) Syntheses by Radical Substitution Using the *N*-Acylanilinoalkyl Radical.

5-Methylpyrazine-2,3-carbonitrile (**3**) was prepared from 2-oxopropanal and 2,3-diaminomaleonitrile [8] and subjected to radical alkylation by the *N*-acetylanilinomethyl radical which was generated from *N*-acetyl-2-anilinoacetic acid (**4**) by Minisci's procedure [9] (Scheme 1). This radical alkylation has been well characterized [10] and applied to the alkylation of pyrazines and pteridines [11]. Only one site is available for substitution on the pyrazine ring of **3**. The structure of **5** for the radical alkylation product was confirmed by ^1H nmr signals which support the existence of the isolated methylene and methyl groups on the ring as well as the acetyl and phenyl groups.

Scheme 1



6a, 7a (R = Me) (53%, 0%); **6b, 7b** (R = Et) (29%, 15%); **6c, 7c** (R = Bu) (37%, 24%)

Table 1

^{13}C NMR Chemical Shifts of **5**. **6** and **7**

Compound	C_2	C_3	C_5	C_6	C_7	C_9	C_{10}
5	130.2	130.9	157.0	155.6	52.0	171.0	22.1
6a	138.8	116.0	154.9	155.5	50.7	170.6	22.5
6b	138.8	116.0	154.3	155.5	50.7	170.5	22.5
6c	138.7	116.1	154.5	155.5	50.7	170.6	22.5
7b	115.8	139.8	154.0	152.5	52.2	170.5	22.3
7c	115.9	139.8	154.2	152.5	52.1	170.4	22.3

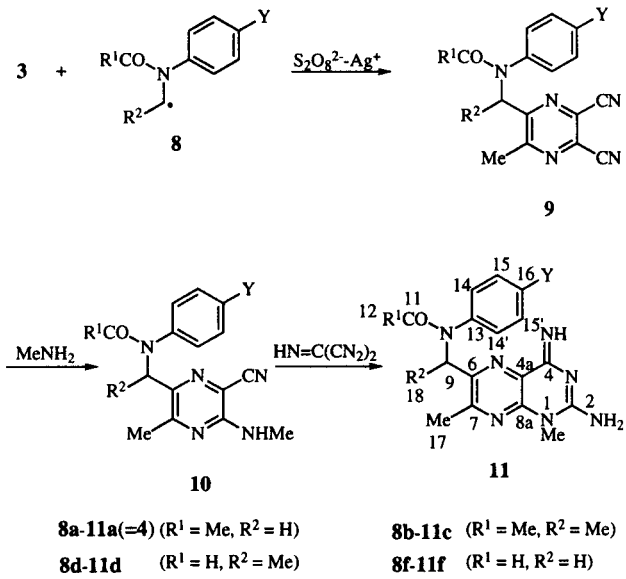
Compound	C_{11} - C_{14}	C_{15}	C_{16}/C_{17}	NR
5	127.9, 128.6 130.0, 142.8	21.8	112.0/113.2	----------
6a	128.0, 128.3 129.6, 143.1	21.8	109.7	27.9
6b	128.0, 128.3 129.6 143.1	21.9	109.4	14.7, 36.0
6c	128.0, 128.3 129.6, 143.1	21.9	109.3	13.7, 20.0, 31.5, 40.8
7b	127.7, 128.0 129.0, 143.6	19.6	110.2	14.7, 36.2
7c	127.7, 128.0 129.6, 143.6	20.0	110.2	13.7, 19.6, 31.4, 41.1

Treatment of **5** with methyl-, ethyl-, and butylamine [12] gave 2-alkylamino-6-methyl-5-(*N*-acylanilino)methylpyrazine-3-carbonitrile (**6**) and 3-alkylamino-6-methyl-5-(*N*-acylanilino)methylpyrazine-2-carbonitrile **7** in the yields shown in Scheme 1. The structures of **6** and **7** were supported by the new ^1H nmr signals due to the alkylamino group in addition to the corresponding signals of **5**. Structures **6** and **7** were discriminated by the comparison of the ^{13}C nmr spectra summarized in Table 1. Though the ^1H nmr spectra led to a somewhat ambiguous conclusion, the independent synthesis of the related compounds settled this problem (see the synthesis of **18** described later). The methyl (15-CH_3) and methylene groups (7-CH_2-) on the pyrazine ring resonate at a higher field when located *para* to the amino group and at a lower field when located *para* to the nitrile group. Methylamine gave a single product **6a** whose structure was easily assigned by the similarity of its ^{13}C nmr spectrum when compared with those of **6b** and **6c**. The reaction of methylamine and ethylamine were carried out under the same conditions, and we have no definite explanation for the higher regioselectivity with methylamine. Preferential formation of the 2-substitution products **6a-6c** rather than the 3-substitution products **7a-7c** must be due to the difference in the inductive effect of the methyl and *N*-acylanilinomethyl group. The *N*-acylanilino group is not directly conjugated to the pyrazine ring, and the electronegative nitrogen modifies the 5-methylene group to be a more electron withdrawing group than the methyl group. This modification of the pyrazine ring makes the *para*-position more susceptible to nucleophilic substitution by amines.

5-Methylpyrazine-2,3-dicarbonitrile (**3**) was treated with (*N*-acetylanilino)methyl **8a**, (*N*-acetyl-*p*-cyanoanilino)methyl **8b**, 1-(*N*-acetylanilino)ethyl **8c**, (*N*-formylanilino)methyl **8d**, (*N*-formyl-*p*-methoxycarbonylanilino)-methyl **8e**, and the 1-(*N*-formylanilino)ethyl radical **8f** from the corresponding carboxylic acids, and the products **9a(=6a)-9f** were obtained (Table 2 and Scheme 2).

Treatment of the products **9** with methylamine gave the methylamination products **10a-10f** which are substitution

Scheme 2



8a-11a(=4) (R^1 = Me, R^2 = H) **8b-11c** (R^1 = Me, R^2 = Me)

8d-11d (R^1 = H, R^2 = Me) **8f-11f** (R^1 = H, R^2 = H)

Table 2

Transformation of 5-Methylpyrazine-2,3-dicarbonitrile (**3**) into 2-Aminopteridin-4(1*H*)imine Derivatives **11**

Starting material	R^1	R^2	Y	Product	Yield/%	Product	Yield/%	Product	Yield/%
8a(=4)	Me	H	H	**9a(=5)**	74	**10a(=6b)**	53	**11a**	57
8b	Me	H	CN	**9b**	47	**10b**	29	**11b** [a]	41
8c	Me	Me	H	**9c**	85	**10c**	41	**11c**	34
8d	H	H	H	**9d**	69	**10d**	53	**11d**	45
8e	H	H	CO_2Me	**9e**	70	**10e**	24	**11e** [a]	76
8f	H	Me	H	**9f**	24	**10f**	56	**11f**	45

[a] Compounds **11b** and **11e**: *N*-acetyl and *N*-formyl groups were cleaved and these products have a 4-cyano- or a 4-methoxycarbonylanilinomethyl substituent on the pteridine ring (see formula).

Table 3

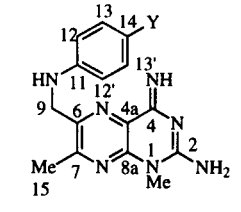
^{13}C NMR Chemical Shifts of Compounds **11** and **14**

Compound	N_1R	C_2	C_4	C_{4a}	C_6	C_7	C_{8a}
11a	29.6	156.8	157.3	129.7	142.8	157.4	148.2
11c	29.5	156.9	157.8	130.2	144.3	157.4	147.9
11d	29.6	156.6	156.7	129.7	140.8	157.3	148.4
11f	29.6	156.9	157.7	129.3	143.5	157.3	148.1
14a	12 2, 37.5	156.3	156.7	129.7	143.0	157.2	147.7
14b	13 7, 20.0 38.8, 42.2	156.5	157.0	120.0	142.9	157.2	147.8

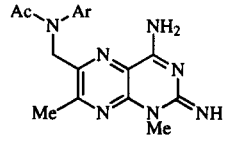
Compound	C_9	C_{11}	$(R^1)_{12}$	C_{13} - C_{16}	C_{17}	$(R^2)_{18}$
11a	50.5	170.4	22.0	120.0, 128.0, 128.2, 141.2	22.6	----
11c	49.9	170.2	22.1	119.5, 128.5, 129.1, 138.8	23.1	17.3
11d	46.8	162.3	--	120.1, 123.7, 127.1, 140.0	22.0	----
11f	49.4	162.4	--	119.7, 128.4, 128.9, 137.5	22.0	17.4
14a	50.6	170.4	22.0	120.0, 128.0, 128.1, 140.9	22.5	----
14b	50.6	170.4	22.0	119.9, 127.9, 128.0, 141.3	22.5	----

products at the 2-position of the pyrazine ring (Scheme 2). The methylamine-substituted products **10a-10f** were reacted with guanidine to give 2-amino-6-(arylamino)-methyl-1,7-dimethylpteridin-4(1*H*)imines **11a-11f** (Scheme 2 and Table 3). The *N*-acetyl and *N*-formyl groups of **10b** and **10e** were cleaved under the strongly basic reaction conditions, and those products **11b** and **11e** are arylaminomethyl derivatives instead of *N*-acyl derivatives (Table 4).

The structural assignments of these products depend mostly on the ^1H nmr, ^{13}C nmr, and mass spectra though these structures cannot be differentiated from the alternative tautomeric structures of compounds **12**. We preferred the structures of products **11** based on the molecular orbital calculations by MOPAC (Version 6.01) [13]. All the PM3, AM1, and MNDO calculations show a larger heat of formation for 2-amino-1-methylpteridin-4(1*H*)-imine than for the 4-amino-1-methyl-2(1*H*)-imine; ΔE = 4.99, 4.48, and 3.46 Kcal/mol by PM3, AM1, and MNDO, respectively. Compounds **11** may exist partly as tautomeric forms of compounds **12** and all of the products



11b (Y = CN); **11e** (Y = CO_2Me)



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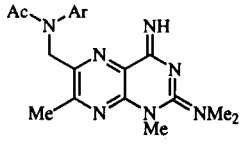

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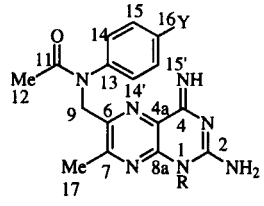


14a (R = Et); **14b** (R = Bu)

Table 4

^{13}C NMR Chemical Shifts of Compounds **11** and **20**

Compound	N_1Me	C_2	C_4	C_{4a}	C_6	C_7	C_{8a}
11b	29.1	155.9	156.7	133.3	140.7	156.7	147.8
11e	29.2	156.1	156.6	130.9	141.1	157.6	147.6
20	29.1	155.8	156.7	128.8	141.8	156.8	148.2

Compound	C_9	C_{11}-C_{14}	C_{15}	Y
11b	44.4	96.4, 112.6, 119.4, 151.6	21.3	120.5
11e	44.5	111.3, 116.5, 119.4, 152.1	21.3	51.3, 166.4
20	45.0	112.6, 116.1, 128.5, 147.7	21.3	--

11 were not obtained as crystalline compounds but as powders. The effort to obtain a dimethylamino derivative **13** from **10a** using 1,1-dimethylguanidine gave only **11a** with preferential elimination of dimethylamine instead of ammonia. The imino group of compounds **11** is rather stable and treatment of **11a** with diluted hydrochloric acid or diluted sulfuric acid (0.5-1.0 mol/l) with heating led to recovery of the starting material in addition to complex degradation products.

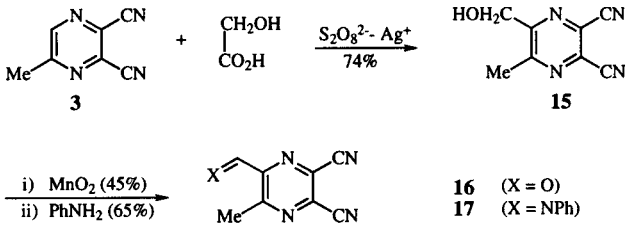
Treatment of the 2-ethylamino- **6b** and 2-butylamino-pyrazine-3-carbonitrile derivatives **6c** with guanidine in the same manner as in the case of 2-methylaminopyrazine-3-carbonitrile (**6a**) gave 2-amino-6-(*N*-acetylanilino)-methyl-1-ethyl-7-methylpteridin-4(1*H*)-imine (**14a**) and 2-amino-6-(*N*-acetylanilino)methyl-1-butyl-7-methylpteridin-4(1*H*)-imine (**14b**) in 43 and 34% yields, respectively. Treatment of 3-ethylamino- **7b** and 3-butylamino-pyrazine-2-carbonitrile derivatives **7c** with guanidine did not give the corresponding pteridine products exclusively. Direct reaction of guanidine with 5-methylpyrazine-2,3-dicarbonitrile (**3**) did not give the expected pteridine product but gave only a complex mixture.

ii) Syntheses by Radical Substitution Using the Hydroxymethyl Radical.

Pyrazine-2,3-dicarbonitrile is susceptible to nucleophilic substitution with amines but 5,6-unsymmetrically substituted pyrazine-2,3-dicarbonitrile gave both 2- and 3-amino derivatives as illustrated by the above-mentioned radical alkylation of 5-methylpyrazine-2,3-dicarbonitrile (**3**) (see Scheme 1). Regiospecific amination at 2-position of **3** is required for the present purpose. The amination site, as described in the previous section, is somewhat selective when one of the substituents at the 5- and 6-position of the pyrazine ring is an electronegative group. We extended this concept to cause a regiospecific amination as a strategy for the synthesis of folic acid and methanopterin analogues.

Radical hydroxymethylation of 5-methylpyrazine-2,3-dicarbonitrile (**3**) to give **15** was carried out by Minisci's procedure [9] using glycolic acid as a hydroxymethyl radical source (Scheme 3). The structure of product **15** was ascertained by the appearance of an ^1H nmr signal due to the hydroxymethyl group with the disappearance of the hydrogen on the pyrazine ring of **3**. Product **15** was transformed into the *N*-phenyliminomethenyl derivative **17** *via*

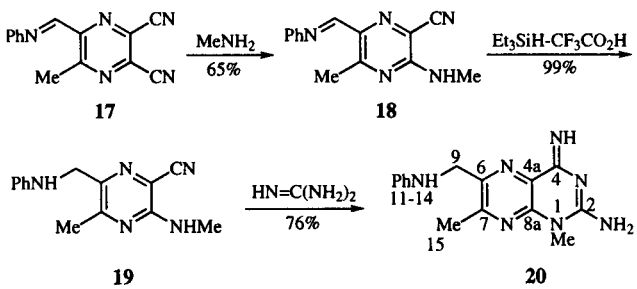
Scheme 3



aldehyde **16** by manganese dioxide oxidation followed by imination with aniline (Scheme 3). Though the yields of these processes are not satisfactory, all the starting materials are readily available and only **17** was obtained after simple procedures. We therefore transformed **17** into the target compounds.

Treatment of the imine-derivative **17** with methylamine gave a single product **18** in 65% yield (Scheme 4). The structure of **18** is supported by the appearance of ^1H nmr signals due to the methylamine moiety in addition to the corresponding signals of the imine derivative **17**.

Scheme 4



Methylamino derivative **18** was also ascertained by the transformation into acetate **6a** by reductive acetylation with zinc-acetic anhydride. In the methyl amination, the nucleophilic substitution takes place regiospecifically at the *para*-position to the imino group. This specificity originates from the strong electron withdrawing effect of the conjugated *N*-phenylimine group. For the same reason, 5-acetyl-6-methylpyrazine-2,3-dicarbonitrile (acetyl instead of the *N*-phenyliminomethenyl group in **17**) behaves in the same manner in the amination to give a single amination product.

After several trials giving unsatisfactory yields, reduction of the phenyliminomethenyl group to an anilinomethyl derivative **19** was achieved in quantitative yield by triethylsilane-trifluoroacetic acid [14]. Treatment of **19** with guanidine gave 1-methyl-6-anilinomethyl-7-methylpteridin-4(1*H*)-imine (**20**) in 76% yield (Scheme 4 and Table 4).

Products **9a-9f** and **20** reported here are the analogues of folic acid and methanopterin, and those are characterized by the modification of the pteridine ring by ^{1}*N*-alkyl substitution and by a carbonyl group instead of a 4-imino group. Those analogues are expected to affect the biological C_1-metabolism and act as enzyme inhibitors.

EXPERIMENTAL

General Methods.

The ^{1}H nmr spectra (90 MHz) were obtained on a Hitachi R-90 spectrometer and the 68 MHz ^{13}C nmr spectra were obtained with a JEOL JNM-EX270 spectrometer. All the nmr measurements were made in deuteriochloroform using TMS as an internal standard unless otherwise mentioned. Chemical shifts (δ) and coupling constants (J) were recorded in ppm and Hz respectively. The ir spectra were obtained with a Perkin-Elmer FT-IR-1600 spectrometer in chloroform unless otherwise mentioned. Conventional mass spectra were measured by a Shimadzu QP-1000 spectrometer at an ionization potential of 70 eV, and high resolution mass spectra were obtained with a JEOL JMS-DX300 spectrometer. Melting points were determined with a Yamato MP-21 apparatus and were uncorrected. The tlc plates for the preparative scale tlc separation of reaction products were made by putting 22 g of silica gel (Merk 60 GF_{254}) on a 20 x 20 cm glass plate. All the spectral measurements and elemental analyses were performed using the equipment at the Materials Characterization Central Laboratory, Waseda University.

Synthesis of 5-Methylpyrazine-2,3-dicarbonitrile.

An aqueous solution (*ca.* 15%) of 2-oxopropanal (15.3 g, 32 mmoles), 3.3 g (31 mmoles) of diaminomaleonitrile, and 33 ml of ethanol were mixed and refluxed for 3 hours. The cooled reaction mixture was concentrated under reduced pressure to remove ethanol. The resulting aqueous solution was extracted with chloroform, and the extract was concentrated after drying over sodium sulfate. The residue thus obtained was subjected to a rough chromatography on silica gel (3.8 x 10 cm column, chloroform) to remove polar impurities. Recrystallization from chloroform gave 5-methylpyrazine-2,3-dicarbonitrile (**3**) [8], (mp 103°) in 61% yield.

Syntheses of 2-(*N*-acylanilino)acetic Acid **8a** and **8c**, 2-(*N*-Acylanilino)propionic Acid **8d** and **8f**, and their *p*-Substituted Anilino Derivatives **8b** and **8e**.

The acids **8a**, **8c**, **8d**, **8f** [15] are known compounds and were prepared as reported. The ^{1}H nmr and ir spectra of these compounds are all consistent with the reported data. 2-(*N*-Acetyl-*p*-cyanoanilino)acetic acid (**8b**) was synthesized by addition of sodium bromoacetate (10.3 g, 76 mmoles) in 95 ml of dry THF to the mixture of *N*-acetyl-*p*-cyanoaniline (6.1 g, 38 mmoles), sodium hydride (76 mmoles), sodium iodide (0.78 g, 5 mmoles), and tris[2-(2-methoxyethoxy)ethyl]amine (2.2 ml) in 150 ml of dry THF. Sodium iodide and the tris-amine were added to accelerate the reaction, The reaction mixture was refluxed for 9 hours, and the cooled mixture was extracted with ether after addition of excess hydrochloric acid (1 mol/l). The dried extract was concentrated and the residue was recrystallized from ethyl acetate to give crystals of **8b** (mp 170-171°) in 65% yield; ^{1}H nmr (90 MHz, DMSO-d_6): 1.91 (3H, s), 4.33 (2H, s), 7.56 (2H, d, J = 8.6), 7.91 (2H, d, J = 8.6); ir: 3505, 3020, 2234, 1729, 1670, 1606 cm^{-1}; ms: m/z (%) 218 (M^+, 2.0), 176 (74), 159 (10), 131 (100), 102 (28).

Anal. Calcd. for $C_{11}H_{10}N_2O_3$: C, 60.55; H, 4.62; N, 12.84. Found: C, 60.58; H, 4.45; N, 12.85.

2-(*N*-Formyl-*p*-methoxycarbonylanilino)acetic acid (**8e**) was prepared by the same procedure as that for **8b** but with refluxing for 4 hours. Recrystallization from ethyl acetate gave **8e** (mp 157-158°) in 72% yield; ^{1}H nmr (90 MHz, DMSO-d_6): 3.85 (2H, s), 4.51 (2H, s), 7.45 (2H, d, J = 8.6), 7.98 (2H, d, J = 8.6, 8.79 (1H, s); ir: 1719, 1685, 1607, 982 cm^{-1}; ms: m/z (%) 237 (M^+, 39), 209 (100), 206 (21), 178 (27), 132 (84).

Anal. Calcd. for $C_{11}H_{11}NO_5$: C, 55.70; H, 4.67; N, 5.90. Found: C, 55.75; H, 4.78; N, 5.89.

Substitution of 5-Methylpyrazine-2,3-dicarbonitrile (**2**) with the (*N*-Acetylanilino)alkyl Radical.

In a two-necked flask were placed 5-methylpyrazine-2,3-dicarbonitrile (**3**) (29 mg, 0.2 mmole), 2-(*N*-acetylanilino)acetic acid (**4** = **8a**) (77 mg, 0.4 mmole), and silver nitrate (6.9 mg, 0.04 mmole), and the system was flushed with argon. The mixture was dissolved with 1 ml of acetonitrile-water (7:3) and warmed to 70-80°, and then ammonium persulfate (110 mg, 0.48 mmole) in 2.6 ml of the same mixed solvent was added during a 10-minute period. The reaction mixture was refluxed for 4 hours and the cooled mixture was extracted with ethyl acetate after neutralization with sodium hydrogen carbonate. The residue from the extract was subjected to a preparative tlc (one plate), developed twice by ethyl acetate-hexane (1:1) and recrystallized from chloroform-hexane to give 43 mg of **5** (=**9a**) (74%). Compound **5** (=**9a**) melted at 151-152°; ^{1}H nmr (90 MHz): 1.98 (3H, s), 2.78 (3H, s), 5.03 (2H, s), 7.43 (5H, m); ir: 2390, 1656, 1595 cm^{-1}; ms: m/z (%) 291 (M^+, 13), 249 (100), 157 (12), 145 (15), 116 (15), 79 (54), 77 (100).

Anal. Calcd. for $C_{16}H_{13}N_5O$: C, 65.97; H, 4.50; N, 24.04. Found: C, 66.15; H, 4.31; H, 23.87.

N-Acetylanilino derivatives **8b** and **8c** and *N*-formylanilino derivatives **8d-8f** were reacted with pyrazinedicarbonitrile **3** by the same procedure as the reaction of **8a** except for the refluxing period and the solvent system for tlc (**8b**, 7 hours, dichloromethane; **8c**, **8e** and **8f**, 9 hours, dichloromethane; **8d**, 6 hours, ethyl acetate-hexane (1:1)), and the products **9b-9f** were obtained in the yields shown in Table 2. Compound **9b** decomposed at 158°; ^{1}H nmr (90 MHz): 2.10 (3H, s), 2.78 (3H, s), 5.00 (2H, s), 7.56 (2H, d, J = 8.6), 7.79 (2H, d, J = 8.6); ir: 2234, 1670, 1606, 1507 cm^{-1}; ms: m/z (%) 316 (M$^+$, 3.3), 274 (71), 176 (6.1), 102 (45).

Anal. Calcd. for $C_{17}H_{12}N_6O$: C, 64.55; H, 3.82; N, 26.57. Found: C, 64.73; H, 3.83; N, 26.65.

Compound **9c** melted at 123°; ^{1}H nmr (90 MHz): 1.22 (3H, d, J = 7.3), 1.77 (3H, s), 2.92 (3H, s), 5.86 (1H, q, J = 7.3), 7.17-7.56 (5H, m); ir: 2236, 1651, 1595, 1533 cm^{-1}; ms: m/z (%) 305 (M$^+$, 4.8), 263 (33), 248 (40), 120 (100), 77 (47).

Anal. Calcd. for $C_{17}H_{15}N_5O$: C, 66.87; H, 4.95; N, 22.93. Found: C, 66.76; H, 4.81; N, 22.95.

Compound **9d** melted at 105°; ^{1}H nmr (90 MHz): 2.83 (3H, s), 5.20 (2H, s), 7.16-7.63 (5H, m), 8.57 (1H, s); ir: 2213, 1675, 1595, 1494 cm^{-1}; ms: m/z (%): 277 (M$^+$, 19), 248 (67), 157 (26), 105 (100), 77 (74).

Anal. Calcd. for $C_{15}H_{11}N_5O$: C, 64.97; H, 4.00; N, 25.26. Found: C, 64.71; H, 3.95; N, 25.51.

Compound **9e** melted at 46°; ^{1}H nmr (90 MHz): 2.82 (3H, s), 3.93 (3H, s), 5.19 (2H, s), 7.31 (2H, d, J = 8.6), 8.11 (2H, d, J = 8.6), 8.64 (1H, s); ir: 2246, 1718, 1682, 1605, 1436 cm^{-1}; ms: m/z (%) 335 (M$^+$, 10), 307 (61), 276 (31), 164 (100); hrms Calcd. for $C_{17}H_{13}N_5O_3$: m/z = 335.1019. Found: m/z = 335.1021.

Compound **9f** decomposed at 137° dec; ^{1}H nmr (90 MHz): 1.43 (3H, d, J = 7.3), 2.90 (3H, s), 5.89 (1H, q, J =7.3), 7.22-7.54 (5H, m), 7.94 (1H, s); ir: 2246, 1669, 1595, 1496 cm^{-1}; ms: m/z (%) 291 (M$^+$, 3.0), 263 (26), 248 (42), 172 (23), 120 (100), 77 (30).

Anal. Calcd. for $C_{16}H_{13}N_5O$: C, 65.97; H, 4.49; N, 24.04. Found: C, 65.72; H, 4.19; N, 23.83.

Synthesis of 2-Methylaminopyrazine-3-carbonitrile Derivatives (**10a**(=**6a**)-**10f**) by Methylamination of Pyrazine-2,3-dicarbonitriles (**9a**(=**5**)-**9f**).

A mixture of 0.4 ml of an aqueous solution of methylamine (4.6 mmoles) and 0.5 ml (5.3 mmoles) of triethylamine in 5 ml of THF was added slowly (30 minutes) to one of the pyrazine-2,3-dicarbonitrile derivatives **9a-9f** (1.4 mmoles) dissolved in 2 ml of THF over a period of 0.5 hour. After further stirring for 5 hours, water was added to the mixture which was then extracted with chloroform. The extract was dried over sodium sulfate, and the residue after evaporation of the solvent was purified by preparative silica gel tlc (two plates) and developed three times with ethyl acetate-hexane (1:1). Recrystallization from chloroform-hexane gave 6-methyl-2-methylamino-5-(*N*-acylanilino)alkylpyrazine-3-carbonitrile **10a-10f** in the yields shown in Table 2.

Compound **10a** melted at 138-140°; ^{1}H nmr (90 MHz): 1.91 (3H, s), 2.50 (3H, s), 3.04 (3H, d, J = 5.0), 4.90 (2H, s), 4.95-5.26 (1H, br s), 7.03-7.47 (5H, m); ir: 3446, 2215, 1646, 1585 cm^{-1}; ms: m/z (%) 295 (M$^+$, 4.3), 253 (65), 161 (87), 120 (57), 106 (26), 93 (57), 77 (65).

Anal. Calcd. for $C_{16}H_{17}N_5O$: C, 65.07; H, 5.80; N, 23.71. Found: C, 64.93; H, 5.73; N, 23.46.

Compound **10b** decomposed at 158°; ^{1}H nmr (90 MHz): 2.03 (3H, s), 2.42 (3H, s), 3.00 (3H, d, J = 4.6), 4.90 (2H, s), 4.94-5.22 (1H, br s), 7.61-7.89 (4H, m); ir: 3433, 2230, 1670, 1606, 1588 cm^{-1}; ms: m/z (%) 320 (M$^+$, 17), 277 (71), 73 (25).

Anal. Calcd. for $C_{17}H_{16}N_6O$: C, 63.74; H, 5.03; N, 26.23. Found: C, 63.62: H, 5.09; N, 26.01.

Compound **10c** melted at 183°; ^{1}H nmr (90 MHz): 1.32 (3H, d, J = 7.5), 1.78 (3H, s), 2.67 (3H, s), 3.07 (3H, d, J = 5.0), 4.88-5.28 (1H, br s), 6.09 (1H, q, J = 7.5), 6.75-7.49 (5H, m); ir: 3446, 2215, 1641, 1580 cm^{-1}; ms: m/z (%) 309 (M$^+$, 19), 266 (45), 175 (100), 149 (94), 93 (25), 77 (22).

Anal. Calcd. for$C_{17}H_{19}N_5O$: C, 66.00; H, 6.19; N, 22.64. Found: C, 66.15; H, 6.18; N, 22.48.

Compound **10d** melted at 152°; ^{1}H nmr (90 MHz): 2.53 (3H, s) 3.04 (3H, d, J = 4.8), 5.15 (2H, s), 4.91-5.21 (1H, br s), 6.99-7.56 (5H, m), 8.53 (1H, s); ir: 3446, 2215, 1672, 1585 cm^{-1}; ms: m/z (%) 281 (M$^+$, 53), 251 (83), 161 (100), 120 (46), 105 (28), 77 (31).

Anal. Calcd. for $C_{15}H_{15}N_5O$: C, 64.04; H, 5.37; N, 24.89. Found: C, 64.02; H, 5.40; N, 25.05.

Compound **10e** melted at 168°; ^{1}H nmr (90 MHz): 2.45 (3H, s), 2.96 (3H, d, J = 5.5), 3.84 (3H, s), 4.97 (2H, s), 4.89-5.24 (1H, br s), 7.21 (2H, d, J = 8.6), 7.97 (2H, d, J = 8.6), 8.56 (1H, s); ir: 3441, 2213, 1712, 1675 1574 cm^{-1}; ms: m/z (%) 339 (M$^+$, 61), 310 (88), 161 (100), 146 (10), 120 (76).

Anal. Calcd. for $C_{17}H_{17}N_5O_3$: C, 60.17; H, 5.05; N, 20.64. Found: C, 59.89; H, 5.09; N, 20.41.

Compound **10f** melted at 214°; ^{1}H nmr (90 MHz): 1.50 (3H, d, J = 7.5), 2.65 (3H, s), 3.06 (3H, d, J = 4.8), 4.92-5.32 (1H, br s), 5.97 (1H, q, J = 7.5), 6.83-7.57 (5H, m), 8.26 (1H, s); ir: 3446, 2215, 1667, 1595 cm^{-1}; ms: m/z (%) 295 (M$^+$, 36), 266 (8.2), 175 (100), 120 (15), 77 (18).

Anal. Calcd. for $C_{16}H_{17}N_5O$: C, 65.07; H, 5.80; N, 23.71. Found: C, 64.84; H, 5.90; N, 23.78.

Alkylamination of 5-(*N*-Acetylanilino)methyl-6-methylpyrazine-2,3-dicarbonitrile (**5**) with Ethyl- and Butylamine.

A mixture of a 0.67 ml of aqueous solution of ethylamine (6.7 mmoles), and 0.26 ml (2.6 mmoles) of triethylamine in 7 ml of THF was added slowly (30 minutes) with 200 mg (0.67 mmole) of 5-(*N*-acetylanilino)methyl-6-methylpyrazine-2,3-dicarbonitrile (**5**) in 2 ml of THF. The reaction mixture was stirred for 5 hours at room temperature and concentrated *in vacuo*.The residue thus obtained was subjected to preparative tlc on silica gel (2 plates) and developed repeatedly 4 times with ethyl acetate-hexane (1:1). In the case of butyl amination, the reaction was carried out essentially by the same method mentioned above but using butylamine itself instead of an aqueous solution. Two isomeric products **6b** and **7b** or **6c** and **7c** were recrystallized from chloroform-hexane to give the products in the yields shown in Table 1.

Compound **6b** melted at 112°; ^{1}H nmr (90 MHz): 1.24 (3H, 3, J = 7.2), 1.91 (3H, s), 2.47 (3H, s), 3.51 (2H, double q, J = 5.6 and 7.2), 4.85 (2H, s), 4.98-5.32 (1H, br s), 6.98-7.59 (5H, m); ir: 3426, 2215, 1651, 1595, 1497 cm^{-1}; ms: m/z (%) 309 (M$^+$, 5.0), 266 (64), 146 (15), 77 (33), 43 (100).

Anal. Calcd. for $C_{17}H_{19}N_5O$: C, 66.00; H, 6.19; N, 22.64. Found: C, 66.06; H, 6.32; N, 22.83.

Compound **7b** was obtained as a non-distillable yellow oil; ^{1}H nmr (90 MHz): 1.23 (3H, t, J = 7.2), 2.06 (3H, s), 2.39 (3H, s), 3.28-3.70 (2H, m), 4.95 (2H, s), 4.99-5.20 (1H, br s), 7.13-7.56

(5H, m); ir: 3436, 2215, 1687, 1594, 1492 cm^{-1}; ms: m/z (%) 309 (M^+, 3.0), 266 (27), 146 (3.0), 77 (32), 43 (100); hrms Calcd. for $C_{17}H_{19}N_5O$: m/z = 309.1592. Found: m/z = 309.1569.

Compound **6c** melted at 125°; ^1H nmr (90 MHz): 0.88 (3H, t, J = 6.3), 1.07-1.71 (4H, m), 1.83 (3H, s), 2.40 (3H, s), 3.19-3.54 (2H, m), 4.81 (2H, s), 4.86-5.11 (lH, br s), 7.04-7.44 (5H, m); ir: 3431, 2213, 1653, 1574, 1486 cm^{-1}; ms: m/z (%) 337 (M^+, 41), 294 (100), 203 (80), 147 (94), 77 (63), 57 (80), 43 (100).

Anal. Calcd. for $C_{19}H_{23}N_5O$: C, 67.63; H, 6.87; N, 20.76. Found: C, 67.93; H, 6.93; N, 21.04.

Compound **7c**, was obtained as a non-distillable yellow oil; ^1H nmr (90 MHz): 0.97 (3H, t, J = 6.3), 1.29-1.79 (4H, m), 2.06 (3H, s), 2.37 (3H, s), 3.24-3.63 (2H, m), 4.95 (2H, s), 5.19-5.60 (1H, br s), 7.18-7.64 (5H, m); ir: 3426, 2213, 1646, 1681, 1494 cm^{-1}; ms: 13 m/z (%) 337 (M^+, 19), 294 (100), 77 (22), 57 (34), 43 (82); hrms Calcd. for $C_{19}H_{23}N_5O$: m/z = 337.1904. Found: m/z = 337.1865.

Syntheses of 2-Aminopteridin-4(1*H*)-imine Derivatives **11a-f** and **14a,b** from 2-Methylaminopyrazine-3-carbonitriles Derivatives **10a-f** and **6b,c**.

A mixture of guanidinium carbonate (90 mg, 0.5 mmole) and sodium methoxide (54 mg, 0.99 mmole) in 2 ml of dry methanol was stirred for 1 hour and one of the compounds **10** (0.66 mmole) dissolved in 4 ml of dry methanol was added. The reaction mixture was refluxed for 6 hours and cooled thoroughly in an ice bath. The resulting precipitates were collected by filtration and recrystallized from methanol to give a yellow powdery solid of one of the compounds **11** in the yields shown in Table 2. Starting with **10b** and **10e** having a cyano or methoxycarbonyl substituent at the *para* position of the (*N*-acylanilino)methyl moiety, the *N*-acyl groups were lost in products **11b** and **11e.**

Product **11a** was obtained as a yellow powder and decomposed at about 195°; ^1H nmr (90 MHz): 1.93 (3H, s), 2.60 (3H, s), 3.63 (3H, s), 5.02 (2H, s), 7.00-7.55 (5H, m); ir: 3515, 3397, 1639, 1603 cm^{-1}; ms: m/z (%) 337 (M^+, 63), 294 (100), 252 (23), 203 (20), 161 (20), 120 (38), 77 (13); hrms Calcd. for $C_{17}H_{19}N_7O$: m/z = 337.1653. Found: m/z = 337.1700.

Product **11b** was obtained as a yellow powder and decomposed at about 200°; ^1H nmr (90 MHz, in DMSO-d_6): 2.59 (3H, s), 3.48 (3H, s), 4.46 (2H, s), 6.93 (2H, d, J = 8.8), 7.53 (2H, d, J = 8.8). Signals due to NH appear at 3.31 together with the signals due to moisture in DMSO-d_6; ir: 3432, 2230, 1606, 1588 cm^{-1}; ms: m/z (%) 320 (M^+, 54), 203 (100), 161 (35), 120 (37); hrms Calcd. for $C_{16}H_{16}N_8$: m/z = 320.1500. Found: m/z = 320.1497.

Product **11c** was obtained as a yellow powder and decomposed at around 152°; ^1H nmr (90 MHz) 1.46 (3H, d, J = 7.0), 1.83 (3H, s), 2.82 (3H, s), 3.65 (3H, s), 6.39 (1H, q, J = 7.0), 6.61-7.49 (5H, m); ir: 3513, 3404, 1646, 1632, 1595 cm^{-1}; ms: m/z (%) 351 (M^+, 48), 308 (42), 217 (100), 175 (31); hrms Calcd. for $C_{18}H_{21}N_7O$: m/z = 351.1809. Found: m/z = 351.1816.

Product **11d** was obtained as a pale orange powder and decomposed at about 194°; ^1H nmr (90 MHz): 2.67 (3H, s), 3.61 (3H, s), 5.12 (2H, s), 7.02-7.52 (5H, m), 8.56 (1H, s); ir: 3518, 1672, 1636, 1595, 1564 cm^{-1}; ms: m/z (%) 323 (M^+, 13), 293 (12), 203 (40), 120 (18), 77 (17); hrms Calcd. for $C_{16}H_{17}N_7O$: m/z = 323.1496. Found: m/z = 323.1519.

Product **11e** was obtained as a yellow powder and decomposed at about 180°; ^1H nmr (90 MHz, DMSO-d_6) 2.60 (3H, s), 3.49 (3H, s), 3.75 (3H, s), 4.47 (2H, s), 6.90 (2H, d, J = 8.8), 7.76 (2H, d, J = 8.8); ir: 3512, 3395, 1712, 1574 cm^{-1}; ms: m/z (%) 353 (M^+, 34), 203 (100), 161 (31), 120 (38); hrms Calcd. for $C_{17}H_{19}N_7O_2$: m/z = 353.1602. Found: m/z = 353.1600.

Product **11f** was obtained as a yellow powder and decomposed at about 101°; ^1H nmr (90 MHz): 2.17 (3H, d, J = 7.0), 2.74 (3H, s), 3.65 (3H, s), 6.22 (1H, q, J = 7.0), 6.75-7.49 (5H, m), 8.28 (1H, s); ir: 3521, 3397, 1661, 1639, 1603 cm^{-1}; ms: m/z (%) 337 (M^+, 72), 308 (6.1), 217 (100), 175 (37), 77 (22); hrms Calcd. for $C_{17}H_{19}N_7O$: m/z = 337.1653. Found: m/z = 337.1671.

Similar treatment of **6b** and **6c** gave **14a** and **14b** respectively.

Product **14a** was obtained as a yellow powder and decomposed at about 148°; ^1H nmr (90 MHz): 1.28 (3H, t, J = 7.3), 1.94 (3H, s), 2.01 (3H, s), 4.34 (2H, q, J = 7.3), 5.01 (2H, s), 6.94-7.59 (5H, m); ir: 3521, 3397, 1639, 1588 cm^{-1}; ms: m/z (%) 351 (M^+, 1.3), 280 (40), 189 (8.9), 149 (25), 129 (30), 97 (36); hrms Calcd. for $C_{18}H_{21}N_7O$: m/z = 351.1809. Found: m/z = 351.1796.

Product **14b** was obtained as a yellow powder and decomposed at about 140°; ^1H nmr (90 MHz) 0.96 (3H, t, J = 6.3), 1.10-1.80 (4H, m), 1.92 (3H, s), 2.58 (3H, s), 3.23 (2H, q, J = 6.3), 5.03 (2H, s), 7.19-7.47 (5H, m); ir: 3513, 3448, 3397, 1653, 1632, 1595 cm^{-1}; ms: m/z (%) 379 (M^+, 1.2), 337 (0.9), 57 (56); hrms Calcd. for $C_{20}H_{25}N_7O$: m/z = 379.2122. Found: m/z = 379.2120.

Radical Hydroxymethylation of 5-Methylpyrazine-2,3-dicarbonitrile (**3**).

A mixture of **3** (1.4 g, 10 mmoles), glycolic acid (2.3 g, 30 mmoles), and silver nitrate (0.34 g, 2 mmoles) in 90 ml of a mixed solvent of acetonitrile-water (7:3) was warmed to 70-80° and added dropwise with 9.6 g (42 mmoles) of ammonium persulfate in 40 ml of acetonitrile-water (7:3) over a 10-minute period. The mixture was refluxed for 5 hours, cooled, and neutralized with aqueous sodium hydrogen carbonate. The residue from the ethyl acetate extraction gave the crude product which was subjected to preparative tlc on silica gel (one plate), and developed twice with ethyl acetate-hexane. The crystals thus obtained were recrystallized from chloroform-hexane to give 43 mg (74%) of 5-(*N*-acetylanilino)methyl-6-methylpyrazine-2,3-dicarbonitrile (**15**).

Compound **15** melted at 102°; ^1H nmr (90 MHz): 2.73 (3H, s), 3.26 (1H, t, J = 5.3), 4.94 (2H, d, J = 5.3); ir: 3518, 2246, 1539 cm^{-1}; ms: m/z (%) 174 (M^+, 44), 145 (48), 128 (10), 117 (44), 76 (67), 57 (100).

Anal. Calcd. for $C_8H_6N_4O$: C, 55.17; H, 3.47; N, 32.17. Found: C, 54.91; H, 3.24; N, 32.22.

Synthesis of 5-(*N*-Phenylimino)methenyl-6-methylpyrazine-2,3-dicarbonitrile (**17**) from **15**.

In a dried reaction vessel was placed 220 mg (2.5 mmoles) of activated manganese dioxide, 44 mg (0.25 mmole) of the hydroxymethylpyrazine derivative **15** and 5 ml of dry dichloromethane, and the mixture was stirred for 22 hours at room temperature. The filtrate of the reaction mixture through celite was concentrated, and the residue was subjected to silica gel chromatography (a 20 x 70 mm column) eluted with dichloromethane. Recrystallization of the crude product from chloroform-hexane gave 5-formyl-6-methylpyrazine-2,3-dicarbonitrile (**16**) in 45% yield.

Compound **16** decomposed at 115-117°; ^{1}H nmr (90 MHz): 3.03 (3H, s), 10.18 (1H, s); ir: 2841, 2236, 1723, 1523 cm^{-1}; ms: m/z (%) 172 (M^+, 58), 143 (54), 117 (26); hrms Calcd. for $C_8H_4N_4O$: m/z = 172.0386. Found: m/z = 172.0412.

The formyl derivative **16** (69 mg, 0.40 mmole) was mixed with 0.33 g of 4A-molecular sieves, 0.1 ml of acetic acid, and 1 ml of dry ethanol. The mixture was then treated with 0.030 ml (0.40 mmole) of aniline dissolved in 1 ml of dry ethanol and stirred for 4 hours. The filtrate of the mixture though celite was concentrated and the residue was subjected to preparative tlc (1 plate) and developed with dichloromethane. Recrystallization of the crude crystal from chloroform gave yellow crystal of **17**.

Compound **17** decomposed at 148-150°; ^{1}H nmr (90 MHz): 3.18 (3H, s), 7.32-7.60 (5H, m), 8.75 (1H. s); ir: 2390, 1523, cm^{-1}; ms: m/z (%) 247 (M^+, 9.1), 104 (12), 77 (26).

Anal. Calcd. for $C_{14}H_9N_5$: C, 68.01; H, 3.67; N, 28.32. Found: C, 67.74; H, 3.56; N, 28.22.

Methylamination of the *N*-Phenyliminomethenylpyrazine Derivative **17**.

Methylamine was extracted with ether from commercially available aqueous solution after the addition of sodium hydroxide solution, and the ether extract was then dried over magnesium sulfate. To a large excess *(ca.* 100 equivalents) of the dried ethereal solution of methylamine was added 0.1 ml (1.1 mmoles) of dried triethylamine and 3 ml of dry THF, and the mixture was treated with 22 mg (0.087 mmole) of **17** in 3 ml of dry THF by slow addition (30 minutes). After stirring for 7 hours at room temperature, water was added to the solution, which was then extracted with dichloromethane. The residue from the extract, after drying and evaporation of the solvent, was subjected to silica gel chromatography (a 17 x 70 mm column) eluted with ethyl acetate-hexane (1:1) to yield the crude product. Recrystallization from chloroform-hexane gave 2-methylaminopyrazine-3-carbonitrile derivative **18** (mp 182-185° dec) in 65% yield; ^{1}H nmr (90 MHz): 2.93 (3H, s), 3.16 (3H, d, J = 4.5), 5.25-5.72 (1H, br s), 7.10-7.53 (5H, m), 8.53 (1H, s); ir: 2400, 1518, 1421 cm^{-1}; ms: m/z (%) 250 (M^+, 100), 235 (16), 173 (17), 77 (23).

Anal. Calcd. for $C_{14}H_{13}N_5$: C, 66.92; H, 5.21; N, 27.67. Found: C, 66.79; H, 5.19; N, 27.84.

Reduction of **18** to 2-Methylamino-5-anilinomethyl-6-methylpyrazine-3-carbonitrile (**19**).

A mixture of the iminopyrazine derivative **18** (0.80 mmole), triethylsilane (0.15 ml, 0.96 mmole) in 3 ml of dry dichloromethane was added slowly with 0.37 ml (4.8 mmoles) of trifluoroacetic acid and the mixture was stirred for 4 hours. After the addition of saturated sodium hydrogen carbonate, the mixture was extracted with ether, and the ether was evaporated after drying to give the crude product. The crude product thus obtained was subjected to preparative tlc (2 plates) and developed with ethyl acetate-hexane (1:1) to give 2-methylamino-5-anilinomethyl-6-methylpyrazine-3-carbonitrile (**19**) (mp 151° chloroform-hexane) in 99% yield; ^{1}H nmr (90 MHz): 2.56 (3H, s), 3.10 (3H, d, J = 4.5), 4.27 (2H, br s), 4.60-4.87 (1H, br s), 4.96-5.30 (1H, br s), 6.58-7.39 (5H, m); ir: 3444, 2218, 1604, 1586 cm^{-1}; ms: m/z (%) 253 (M^+, 39), 161 (91), 106 (21), 77 (26).

Anal. Calcd. for $C_{14}H_{15}N_5$: C, 66.38; H, 5.97; N, 27.65. Found: C, 66.57, H, 5.80; N, 27.53.

Syntheses of 1-Methyl-2-amino-6-anilinomethyl-7-methylpteridin-4(1*H*)-imine (**20**) from 2-Methylaminopyrazine-3-carbonitrile Derivative **19**.

A mixture of guanidinium carbonate (61 mg, 0.34 mmole) and sodium methoxide (18 mg, 0.34 mmole) in 1 ml of dry methanol was stirred for 1 hour at room temperature, and **19** (0.34 mmole) in 2 ml of dry methanol was added dropwise. The reaction mixture was refluxed for 7 hours and cooled throughly in an ice bath to give a precipitate. Collection of the precipitate by filtration and recrystallization from methanol gave product **20** as a yellow powder. Product **20** decomposed at about 180°; ^{1}H nmr (90 MHz, in DMSO-d_6): 2.56 (3H, s), 3.52 (3H, s), 4.36 (2H, d, J = 4.5), 6.47-7.31 (5H, m), 7.44-7.92 (1H, diffused); ir: 3405, 1636, 1600 cm^{-1}; ms: m/z (%) 295 (M^+, 7.0), 294 (35), 203 (100), 106 (41), 77 (40); hrms Calcd. for $C_{15}H_{17}N_7$: m/z = 295.1547. Found: m/z = 295.1552.

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