1,2-Dihydropyrido[3,4-*b*]pyrazines: Structure-Activity Relationships

Carroll Temple, Jr.,* Glynn P. Wheeler, Robert D. Elliott, Jerry D. Rose, Robert N. Comber, and John A. Montgomery

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35255. *Received May 17, 1982*

Certain derivatives containing the 1,2-dihydropyrido[3,4-*b*]pyrazine (1-deaza-7,8-dihydropteridine) ring system are active against experimental neoplasms in mice. The mechanism of action of these agents has been attributed to the accumulation of cells at mitosis. Identification of the structural features that are necessary for activity was accomplished by evaluation of modified 1-deazapteridines and ring and ring-opened analogues. Relative to ethyl 4-amino-1-deaza-7,8-dihydro-6-[(*N*-methylanilino)methyl]pteridine-2-carbamate (**11**) and the corresponding 6-phenyl compound (**12**), no antitumor activity was observed with 7,8-dihydropteridines, 3-deaza-7,8-dihydropteridines, and the corresponding heteroaromatic compounds. Also, activity was diminished or destroyed when 1-deaza-7,8-dihydropteridines were oxidized to 1-deazapteridines or reduced to 1-deaza-5,6,7,8-tetrahydropteridines. In addition, replacement of the 4-amino group with other substituents destroyed activity. The presence of a 6-substituent containing an aryl group appeared to be necessary for activity, which was increased when a methyl group was substituted at the 7-position.

Recently, we reported that 1,2-dihydropyrido[3,4-*b*]-pyrazines (1-deaza-7,8-dihydropteridines) inhibited the proliferation of cultured cells at low concentration and were active against several experimental neoplasms, including the methotrexate-resistant and vincristine-resistant lines of lymphocytic leukemia P388.[1] These compounds were shown to cause the accumulation of cells at mitosis with both cultured cells and ascites cells in vivo.[2] Since the parent ring structure of these compounds could not be related to any of the known anticancer drugs, an investigation to determine the structural features necessary for activity was initiated. In addition to modification of the functional groups of active 1-deaza-7,8-dihydropteridines, some ring analogues of the latter were examined.

Chemistry. A number of ring analogues, **1–3**, and the corresponding heteroaromatic compounds, **4–6**, have been prepared previously.[3,4] Also, several compounds, **7–9**, have been reported in which the 4-amino group of the active compound **10**[3] has been replaced with other groups.[5,6]

The importance to activity of the 7,8-dihydro moiety of the 1-deaza-7,8-dihydropteridines was initially investigated. Oxidation of a solution of **11**[1] in acetone with permanganate gave the heteroaromatic 1-deazapteridine **22**. A similar procedure was used to oxidize **12**[1] and **13**,[1] respectively, to **23** and **24**. In addition, a solution of **11** in acetonitrile was reduced with sodium cyanoborohydride to give the tetrahydro derivative **29**.

To determine the contribution to activity of the ethoxycarbonyl grouping of **11**, we attempted the preparation of the corresponding 2,4-diamino-1-deaza-7,8-dihydropteridine **14**. The catalytic hydrogenation of **25**[7] over platinum was terminated after the uptake of 1 molar equiv of hydrogen to give **14** as the major product. However, before purification or testing could be carried out, this product underwent air-oxidation to regenerate an appreciable amount of **25**.

To modify the 4-amino group, the dicarbamate **15** was desired. This compound was prepared as previously described, but in the present work was isolated and characterized.[7]

In regard to the 6-position, substituents such as phenyl and anilinomethyl are known to give active compounds, and it was desirable to determine the effect on activity of other groups at this position. Treatment of 1-amino-4-phenyl-2-butanone hydrochloride[8] with hydroxyamine hydrochloride in aqueous ethanol in the presence of sodium acetate gave the corresponding oxime, which was alkylated with ethyl 6-amino-4-chloro-5-nitro-2-pyridinecarbamate (**30**)[3] to give **31**. After hydrolysis of the oxime function of **31**, the resulting ketone **32** was hydrogenated in the presence of Raney nickel to give the 6-phenethyl derivative **16**. Similarly, the 6-(2-naphthyl) compound **17** was prepared via the pyridine intermediates **33** and **34**. To introduce a group at the 7-position, the 7-methyl-6-phenyl compound **18** was prepared via **35** and **36**.

To stabilize the dihydro moiety of the 1-deaza-7,8-dihydropteridines toward air-oxidation and metabolic transformation in vivo, we carried out the synthesis of the 8-methyl derivative **21**. Alkylation of 2-(methylamino)-1-phenylethanol[9] with **30** gave **37**, which was oxidized with chromium(VI) oxide in pyridine to give the ketone **38**. Catalytic hydrogenation of **38** in the presence of Raney nickel gave **21**.

Several monocyclic analogues of **12** were prepared. Catalytic hydrogenation of **40**[11] over Raney nickel gave **41**, which was condensed with benzaldehyde to give **42**. Also, the simultaneous hydrodechlorination and reduction of the nitro group of **30**[3] over palladium on charcoal gave the 5,6-diaminopyridine **43**. The latter was condensed with ethyl benzoylacetate to give **44**.

Biological Evaluation. The biological data for the compounds listed in Tables I and II was accumulated over a period of about 15 years. Some of the compounds were screened for cytotoxicity in the KB and HEP-2 cell culture

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(4) Elliott, R. D.; Temple, Jr., C.; Montgomery, J. A. *J. Org. Chem.* **1970**, *35*, 1676.
(5) Elliott, R. D.; Temple, Jr., C.; Montgomery, J. A. *J. Med. Chem.* **1974**, *17*, 553.
(6) Elliott, R. D.; Temple, Jr., C.; Frye, J. L.; Montgomery, J. A. *J. Med. Chem.* **1975**, *18*, 492.
(7) Elliott, R. D.; Temple, Jr., C.; Montgomery, J. A. *J. Org. Chem.* **1968**, *33*, 533.
(8) Degraw, J.; Isakotellis, P.; Kisliuk, R.; Gaumont, Y. *J. Heterocycl. Chem.* **1971**, *8*, 105.
(9) This compound was prepared by the procedure of McManus and co-workers[10] and purified by silica gel chromatography ($CHCl_3$ to 20% MeOH–$CHCl_3$).
(10) McManus, S. P.; Larson, C. A.; Hearn, R. A. *Synth. Commun.* **1973**, *3*, 177.
(11) Camps, R. *Arch. Pharm. (Weinheim, Ger.)* **1902**, *240*, 350. Curry, H. M.; Mason, J. P. *J. Am. Chem. Soc.* **1951**, *73*, 5043.

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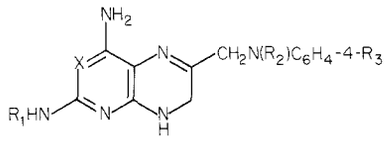
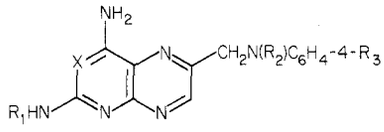
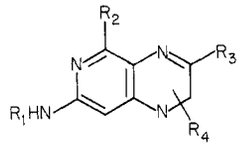


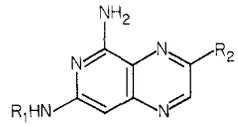
1, X = CH; R_1 = EtO_2C; R_2 = Me; R_3 = MeO_2C
2, X = N; R_1 = H; R_2 = Me; R_3 = MeO_2C
3, X = N; R_1 = R_2 = H; R_3 = EtO_2C

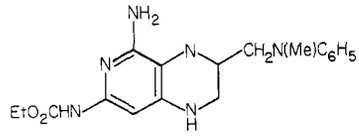


4, X = CH; R_1 = EtO_2C; R_2 = Me; R_3 = MeO_2C
5, X = N; R_1 = H; R_2 = Me; R_3 = MeO_2C
6, X = N; R_1 = R_2 = H; R_3 = EtO_2C



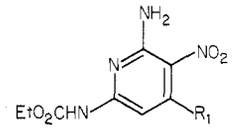
7, R_1 = EtO_2C; R_2 = OH; R_3 = 4-$MeO_2CC_6H_4N(Me)CH_2$; R_4 = 8-H
8, R_1 = H; R_2 = Cl; R_3 = 4-$MeO_2CC_6H_4N(Me)CH_2$; R_4 = 8-H
9, R_1 = H; R_2 = SH; R_3 = 4-$HO_2CC_6H_4N(Me)CH_2$; R_4 = 8-H
10, R_1 = EtO_2C; R_2 = NH_2; R_3 = 4-$MeO_2CC_6H_4N(Me)CH_2$; R_4 = 8-H
11, R_1 = EtO_2C; R_2 = NH_2; R_3 = $C_6H_5N(Me)CH_2$; R_4 = 8-H
12, R_1 = EtO_2C; R_2 = NH_2; R_3 = C_6H_5; R_4 = H
13, R_1 = EtO_2C; R_2 = NH_2; R_3 = 4-$CF_3C_6H_4$; R_4 = 8-H
14, R_1 = H; R_2 = NH_2; R_3 = $C_6H_5N(Me)CH_2$; R_4 = 8-H
15, R_1 = EtO_2C; R_2 = EtO_2CNH; R_3 = $C_6H_5N(Me)CH_2$; R_4 = 8-H
16, R_1 = EtO_2C; R_2 = NH_2; R_3 = $C_6H_5CH_2CH_2$; R_4 = 8-H
17, R_1 = EtO_2C; R_2 = NH_2; R_3 = 2-$C_{10}H_7$; R_4 = 8-H
18, R_1 = EtO_2C; R_2 = NH_2; R_3 = C_6H_5; R_4 = 7-Me
19, R_1 = EtO_2C; R_2 = NH_2; R_3 = 4-$C_6H_5C_6H_4$; R_4 = 8-H
20, R_1 = EtO_2C; R_2 = EtO_2CNH; R_3 = Me; R_4 = 8-H
21, R_1 = EtO_2C; R_2 = NH_2; R_3 = C_6H_5; R_4 = 8-Me



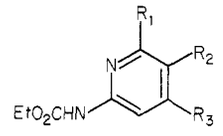


22, R_1 = EtO_2C; R_2 = $C_6H_5N(Me)CH_2$
23, R_1 = EtO_2C; R_2 = C_6H_5
24, R_1 = EtO_2C; R_2 = 4-$CF_3C_6H_4$
25, R_1 = H; R_2 = $C_6H_5N(Me)CH_2$
26, R_1 = EtO_2C; R_2 = $MeO_2CC_6H_4N(Me)CH_2$
27, R_1 = EtO_2C; R_2 = 4-$EtO_2CC_6H_4NHCH_2$
28, R_1 = EtO_2C; R_2 = $C_6H_5N(Me)CO$

29



30, R_1 = Cl
31, R_1 = $C_6H_5(CH_2)_2C(NOH)CH_2NH$
32, R_1 = $C_6H_5(CH_2)_2COCH_2NH$
33, R_1 = 2-$C_{10}H_7C(NOH)CH_2NH$
34, R_1 = 2-$C_{10}H_7COCH_2NH$
35, R_1 = $C_6H_5C(NOH)CH(Me)NH$
36, R_1 = $C_6H_5COCH(Me)NH$
37, R_1 = $C_6H_5CH(OH)CH_2N(Me)$
38, R_1 = $C_6H_5COCH_2N(Me)$



39, R_1 = R_2 = NH_2; R_3 = $C_6H_5CH(OH)CH_2N(Me)$
40, R_1 = R_3 = H; R_2 = NO_2
41, R_1 = R_3 = H; R_2 = NH_2
42, R_1 = R_3 = H; R_2 = $C_6H_5CH{=}N$
43, R_1 = R_2 = NH_2; R_3 = H
44, R_1 = NH_2; R_2 = $C_6H_5C(=CHCO_2Et)NH$; R_3 = H

systems and for antitumor activity against lymphoid leukemia L1210 in mice (Table I). Other compounds were evaluated for inhibition of proliferation of cultured lymphoid leukemia L1210 cells and antitumor activity against lymphocytic leukemia P388 in mice (Table II). Although the cell culture data reported in Tables I and II cannot be compared directly, the overall biological implications are straightforward.

Previously we reported that certain 1-deaza-7,8-dihydropteridines (e.g., **10–12**, Table II) are highly active in cell culture and showed antitumor activity in mice.[1] The effect on activity of modifying the pyridine ring of the 1-deaza-7,8-dihydropteridines was examined by evaulation of dihydro compounds derived from the pteridine ring system and its 3-deaza ring analogue. The 3-deaza-7,8-dihydropteridine **1** showed a high ED_{50} in KB cell culture and no antitumor activity against lymphoid leukemia L1210 in mice (Table I).[3] Similarly, the 2,4-diamino-7,8-dihydropteridine **2**[4] showed a high ED_{50} in KB cell culture. Although the related pteridine **3**[4] exhibited borderline activity in KB cell culture, this compound gave no significant activity against L1210 in mice. The absence of an ethoxycarbonyl moiety on the 2-amino group of **2** and **3** might have contributed to the lack of activity. Nevertheless, antitumor activity appeared to be diminished relative to the 1-deaza-7,8-dihydropteridines, when the 3-N and 1-CH were interchanged and when N replaced the 1-CH.

A number of heteroaromatic bicyclic ring systems were examined for activity. The previously prepared 1-deazapteridines **25** and **26**,[3,7] 3-deazapteridine **4**,[3] and pteridines **5** and **6**[4] were noncytotoxic in cell culture experiments, and **4–6** and **25** were shown also to be inactive against L1210 in mice (Table I). Although **22** showed cytotoxicity in the KB system, this compound was not tested in vivo. However, the related compound **23** showed no antitumor activity and gave a low mitotic index (Table II). Also, both **24** (Table II) and **28** (Table I), products resulting from the oxidation of **11**, showed no significant biological activity. These results demonstrate that the 7,8-dihydro moiety of the 1-deaza-7,8-dihydropteridines

Table I

no.	cell culture cytotoxicity: KB ED_{50},[a] μM	antitumor act.: L1210 10^5 tumor cell implant, ip schedule, days	% ILS (mg/kg)[b]
1	24	1	0 (400)
		1-9	0 (100)
2	21		
3	10	1	8 (25)
		1-9	0 (100)
4	240	1	0 (400)
		1-9	0 (100)
5	19	1	3 (400)
		1-9	2 (100)
6	17	1	0 (400)[c]
		1-9	0 (48)
7	8	1	2 (400)
8	>100		
9	29		
15	1.2[d]	1	46 (300)
		1-9	14 (50)
20	91	2	0 (400)
22	6.7×10^{-1}		
25	>100	2	0 (400)
		1-9	0 (50)
26	19[d]		
27	42[d]		
28	>100	1	0 (400)
29	6×10^{-1}	1	10 (400)

[a] Concentration of agent that inhibits colony formation of KB human epidermoid carcinoma cells by 50% (ref 15). [b] Lymphoid leukemia L1210; increase in life span for the highest nontoxic dose tested (ref 15). [c] Toxic dose. [d] Concentration of agent that inhibits colony formation of human epidermoid carcinoma no. 2 by 50% after 12 days (ref 16).

was necessary for both in vivo and in vitro activity. Further, the tetrahydro derivative **29** showed cytotoxicity in KB cell culture but no activity against L1210 in mice.

The effect on activity of the substituents on the 1-deaza-7,8-dihydropteridine ring was next investigated. As described above in the preparation of 2,4-diamino-7,8-dihydro-1-deazapteridine (**14**), the isolated sample underwent air-oxidation to **25**. Although no information on the contribution to activity of the ethoxycarbonyl group of **11** was obtained, these results indicated that substitution of an acyl group on the 2-amino group stabilized the 1-deaza-7,8-dihydropteridine ring system.

Substitution of the 4-amino group of **11** with an ethoxycarbonyl group gave **15**, which was considerably less active in the HEP-2 cell culture system than **11** in the L1210 system. However, **15** showed good activity against L1210 in mice at high doses (Table II). Replacement of the 4-amino group of **10** with hydroxy, chloro, and mercapto groups gave compounds (**7–9**) that showed no antitumor activity. Thus, the presence of either a 4-amino or 4-acylamino function appeared to be necessary for activity.

As previously described,[1] antitumor activity is retained when the 6-position in the 1-deaza-7,8-dihydropteridines is substituted with an anilinomethyl (**10** and **11**), phenyl (**12**), or 4-biphenyl (**19**) function. Also, the 6-phenethyl (**16**) and 6-(2-naphthyl) (**17**) compounds inhibited the proliferation of L1210 cells at a low concentration and were active against P388 in mice (Table II). In contrast, the previously prepared 6-methyl compound **20** showed no cytotoxicity in cell culture or activity against L1210 in mice (Table I).[12] Thus, the active 1-deaza-7,8-dihydropteridines are substituted at the 6-position with a substituent larger than methyl. Although it would appear that a phenyl moiety in the side chain might be necessary for activity, it is not known if a long-chain alkyl group would also give active compounds.

Unexpectedly, the 7-methyl-6-phenyl compound **18** showed a lower ID_{50} and a higher mitotic index than **12** in L1210 cells. This increase in activity will be explored by the synthesis of additional 6,7-disubstituted derivatives. Although **21** showed significant inhibition of L1210 cells in culture and caused the accumulation of cells in mitosis, this compound was disappointing in that no antitumor activity was observed against P388 in mice on a single dose schedule.

Both of the ring-opened analogues **42** and **44** were inactive against P388 in mice, as was the pyridine intermediate **39** derived from **37**. Apparently, the bicyclic ring system is necessary for activity.

In summary, it has been established that activity in the 1-deaza-7,8-dihydropteridines is diminished or destroyed by (a) addition of N to the 1-position or interchange of the 3-N and 1-CH, (b) oxidation to the corresponding heteroaromatic system, (c) reduction to the corresponding tetrahydro derivative, (d) replacement of the 4-amino group with other substituents, (e) replacement of the aryl moiety at the 6-position with a methyl group, (f) replacement of the 8-NH with NMe, and (g) opening the pyrazine ring. In contrast, activity was increased by the substitution of a methyl group at the 7-position.

Experimental Section

Mass spectra were determined with a Varian MAT 311A spectrometer, and the ^1H NMR spectra were determined with a Varian XL-100-15 spectrometer with tetramethylsilane as an internal reference.

1-Amino-4-phenyl-2-butanone Oxime. A solution of crude 1-amino-4-phenyl-2-butanone hydrochloride (9.84 g, 49.2 mmol),[8] hydroxylamine hydrochloride (6.84 g, 98.4 mmol), and NaOAc·$3H_2O$ (13.4 g, 98.4 mmol) in 50% EtOH (250 mL) was heated at 75–80 °C for 30 min, filtered, treated with a hot solution of picric acid (11.7 g, 51.1 mmol), cooled to 25 °C, filtered, and allowed to stand for 2 days. The crystalline picrate was collected, washed with 2:1 H_2O–EtOH, and dried in vacuo: yield 9.62 g; mp 151 °C (Kofler Heizbank). The mother liquor was evaporated to dryness in vacuo, and the residue was crystallized from hot H_2O (500 mL) to give an additional amount of the picrate: yield 3.62 g; mp 151 °C. A solution of the picrate in 3:1 EtOH–H_2O (400 mL) was treated with washed Bio-Rad AG1-X8 (Cl^-) ion-exchange resin (100 g) and stirred for 18 h. The solution was filtered, and the resin was washed with 3:1 EtOH–H_2O. The filtrate and wash were treated with additional resin (40 g), stirred for 2 h, and filtered. The almost-colorless solution was evaporated to dryness in vacuo, and the residue was reevaporated with EtOH (3 × 200 mL). The residue was stirred with EtOH (100 mL) and filtered, and the precipitate was rinsed with additional EtOH (40 mL). The filtrate and wash were diluted with Et_2O (600 mL) to give a crystalline hydrochloride, which was collected, washed with Et_2O, and dried in vacuo (P_2O_5): yield 5.36 g (50%); mp 193 °C; ^1H NMR (Me_2SO-d_6, 6%, w/v) δ 2.70 (m, CH_2CH_2), 3.52 (s, CH_2N), 7.27 (m, C_6H_5), 8.43 (s, NH_3), 11.25 (s, OH). Anal. ($C_{10}H_{14}N_2O$·HCl·$0.3H_2O$) C, H, N.

Diethyl 1,2-Dihydro-3-[(*N*-methyl-*N*-phenylamino)methyl]pyrido[3,4-*b*]pyrazine-5,7-dicarbamate (15). A suspension of diethyl 4-[[3-(*N*-methyl-*N*-phenylamino)-2-oxopropyl]amino]-3-nitro-2,6-pyridinedicarbamate hemihydrate (2.00 g, 4.14 mmol)[7] in EtOH (200 mL) was hydrogenated at room temperature and atmospheric pressure over Raney nickel catalyst (~5 g) for 18 h. The solution was filtered under N_2, and the filtrate was concentrated in vacuo to about half volume. The precipitate of glossy white crystalline solid was collected by filtration under N_2, washed with ice-cold EtOH, and dried in vacuo over P_2O_5: yield 0.70 g (39.8%); mp 80–83 °C with prior sintering;

(12) Montgomery, J. A.; Wood, N. F. *J. Org. Chem.* **1964**, *29*, 734.

Table II. Biological Data

no.	inhibn of proliferation: L1210 ID_{50},[a] μM	mitotic index[b] 12 h (μM)	mitotic index[b] 24 h (μM)	antitumor activity:[c] P388 10^6 tumor cell implant, ip schedule, days	antitumor activity:[c] P388 10^6 tumor cell implant, ip % ILS (mg/kg)
14	5.8×10^{-3}	0.61 (0.3)		1-9	30 (25)
		0.44 (0.1)			
11	8.4×10^{-3} [d]	0.77 (0.03)	0.80 (0.3)	1	114 (100)
12	4.7×10^{-3} [e]	0.65 (0.03)	0.54 (0.3)	1	55 (12.5)[e]
				1-9	51 (2)
13	1.5×10^{-1}		0.01 (0.3)	1	0 (90)[f]
				1-9	17 (50)
16	1.3×10^{-2}		0.33 (0.3)	1	29 (100)
17	6×10^{-3}	0.60 (0.03)		1-5	66 (10)[g]
				1-5	53 (5)
18	5.1×10^{-4}	0.69 (0.003)		1	54 (10)[h]
				1-5	75 (1)
19	6.1×10^{-3}	0.65 (0.01)		1	36 (25)
		0.42 (0.3)			
21	1.6×10^{-1}	0.68 (0.3)		1	12 (100)
23	$>3 \times 10^{-1}$		0.02 (0.3)	1	0 (200)
24	$>3 \times 10^{-1}$				
39	$>3 \times 10^{-1}$			1	0 (200)
42	$>3 \times 10^{-1}$			1	0 (200)
44	$>3 \times 10^{-1}$		0.03 (0.3)		

[a] Concentration of agent that inhibits proliferation of cultured lymphoid leukemia L1210 cells to 50% control during 48 h (ref 2). [b] Fraction of the cell population of cultured lymphoid leukemia L1210 cells in mitosis (ref 2). [c] Lymphocytic leukemia P388; increase in life span for the highest nontoxic dose tested (ref 15). [d] Average of three determinations. [e] Average of two determinations. [f] Highest dose tested. [g] One 30th day survivor. [h] Toxic by weight change.

^{1}H NMR (Me_2SO-d_6, 5%, w/v) δ 1.20 (t, CH_3), 2.97 (s, CH_3N), 4.00 (m, CH_2), 6.89 (m, 8-CH, C_6H_5, 1-NH), 8.47 and 9.40 (NH). Anal. ($C_{21}H_{26}N_6O_4$) C, H, N.

Ethyl 5-Amino-1,2-dihydro-3-(2-phenylethyl)pyrido[3,4-*b*]pyrazine-7-carbamate (16). A solution of **32** (300 mg, 0.775 mmol) in DMAC (7 mL) was hydrogenated at room temperature and atmospheric pressure in the presence of Raney nickel (890 mg, weighed wet, washed with EtOH) for 20 h to give an H_2 uptake of 58 mL (3.07 mmol). The reaction mixture was filtered under N_2 and evaporated in vacuo at 25 °C. The residual syrup was stirred with H_2O (10 mL) to give a white powder, which was collected, washed with H_2O, and dried in vacuo (P_2O_5): yield 230 mg (88%); mp 163 °C; mass spectrum, *m/e* 339 (M^+); ^{1}H NMR (Me_2SO-d_6, 6%, w/v) δ 1.20 (t, CH_3), 2.50 and 2.90 (2 m, CH_2CH_2), 3.88 (s, CH_2N), 4.08 (q, OCH_2), 5.30 (s, NH_2), 6.41 (s, 8-H, 1-NH), 7.27 (m, C_6H_5), 9.04 (s, CONH). Anal. ($C_{18}H_{21}N_5O_2$) C, H, N.

Ethyl 5-Amino-1,2-dihydro-3-(2-naphthyl)pyrido[3,4-*b*]-pyrazine-7-carbamate (17) was prepared by hydrogenation of **34** (1.9 mmol) in EtOH (1300 mL) by a procedure similar to that described for **15**. Concentration of the filtrate to one-sixth volume gave slightly impure **17**: yield 405 mg (<59%); mp 209–212 °C. To stabilize the product, we acidified the filtrate with concentrated HCl (0.2 mL) and concentrated to give the hydrochloride of **17**: yield 215 mg (25%); mp 251–256 °C dec; mass spectrum, *m/e* 361 (M^+); ^{1}H NMR (Me_2SO-d_6, 5%, w/v) δ 1.06 (t, CH_3CH_2OH), 1.26 (t, 3H, CH_3), 3.45 (q, CH_3CH_2OH), 4.19 (q, 2 H, CH_2), 4.71 (br s, 2 H, 2-CH_2), 6.15 (s, 1 H, 8-CH), 7.46–8.60 (m, 7 H, $C_{10}H_7$). Anal. ($C_{20}H_{19}N_5O_2 \cdot C_2H_5OH \cdot 1.22HCl$) C, H, N.

Ethyl 5-Amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazine-7-carbamate (18) was prepared in 70% yield from **36** by a procedure similar to that described for **15**: mp 233–240 °C dec; mass spectrum, *m/e* 325 (M^+); ^{1}H NMR (Me_2SO-d_6, 5%, w/v) δ 1.06 (t, CH_3OH_2OH), 1.21 (d, 2-CH_3), 1.28 (t, 3 H, CH_3), 3.45 (q, CH_3CH_2OH), 3.76 (H_2O), 4.23 (q, 2 H, CH_2), 5.12 (m, 1 H, 2-CH), 6.22 (s, 1 H, 8-CH), 7.48 and 8.17 (2 m, 5 H, C_6H_5), 7.74 (br s, 2 H, NH_2), 8.84 (br s, 1 H, 1-NH), 1.16 (s, NH). Anal. ($C_{17}H_{19}N_5O_2 \cdot 0.43C_2H_5OH \cdot 0.57H_2O$) C, H, N.

Ethyl 5-Amino-1,2-dihydro-1-methyl-3-phenylpyrido[3,4-*b*]pyrazine-7-carbamate (21). A solution of **38** (0.50 g, 1.3 mmol) in EtOH (300 mL) was hydrogenated in the presence of Raney nickel (~1.5 g, washed with H_2O and EtOH) over 3 h. After filtration, the filtrate was concentrated to 50 mL and cooled to give the product: yield 0.17 g (39%); mp 173–175 °C dec; mass spectrum, *m/e* 325 (M^+); ^{1}H NMR (Me_2SO-d_6, 5%, w/v) δ (t, CH_3CH_2OH), 1.22 (t, 3, CH_3CH_2), 2.85 (s, 3, 1-CH_3), 3.45 (q, CH_3CH_2OH), 4.10 (q, 2, CH_2O), 4.36 (s, 2, 2-CH_2), 5.68 (br s, 2, NH_2), 6.64 (s, 1, 8-CH), 7.72 (m, 5, C_6H_5), 9.26 (br s, 1, NH). Anal. ($C_{17}H_{19}N_5O_2 \cdot 0.14C_2H_5OH$) C, H, N.

Ethyl 5-Amino-3-[(*N*-methyl-*N*-phenylamino)methyl]-pyrido[3,4-*b*]pyrazine-7-carbamate (22) and 5-Amino-7-[(ethoxycarbonyl)amino]-*N*-methyl-*N*-phenylpyrido[3,4-*b*]pyrazine-3-carboxamide (28). A solution of **11** (10.5 g, 29.6 mmol)[1] in acetone (500 mL) was treated dropwise with stirring with a solution of potassium permanganate in acetone (0.27%, 740 mL) until the color of permanganate persisted. The precipitate (MnO_2) was removed by filtration and washed with acetone, and the combined filtrate and wash were evaporated to dryness. The colored residue was dissolved in chloroform (200 mL), and the solution was washed with water (100 mL) and evaporated to dryness. The resulting yellow solid was recrystallized from ethanol to give a mixture of **22** and **28**: yield 5.1 g. These components were separated on a silica gel 60 H (350 g, E. Merck) column developed with a mixture of chloroform–methanol (99:1) at a rate of 40 mL/h. The initial fractions containing **22** were evaporated, and the combined solids were crystallized from ethanol: yield 1.26 g (12%); mp 182–183 °C. Anal. ($C_{18}H_{20}N_6O_2$) C, H, N.

The middle fractions were a mixture of **22** and **28**: yield 0.84 g. The later fractions were essentially pure **28**, which were recrystallized from ethanol: yield 2.00 g (18%); mp 210–211 °C; mass spectrum, *m/e* 366 (M^+). Anal. ($C_{18}H_{18}N_6O_3$) C, H, N.

Ethyl 5-Amino-3-phenylpyrido[3,4-*b*]pyrazine-7-carbamate (23). A solution of **12** (1.00 g, 3.22 mmol)[1] in acetone (500 mL) containing magnesium sulfate (2 g) was treated dropwise with stirring with a solution of potassium permanganate (0.34 g) in acetone (150 mL). After refrigeration for 2 h, the mixture was filtered (Celite), and the filtrate was evaporated to dryness in vacuo. The residue was washed with cold ethanol and dried in vacuo over P_2O_5: yield 0.95 g (96%); mp 204–207 °C. Anal. ($C_{16}H_{15}N_5O_2$) C, H, N.

Ethyl 5-amino-3-[4-(trifluoromethyl)phenyl]pyrido[3,4-*b*]pyrazine-7-carbamate (24) was prepared similarly from **13** (300 mg, 0.792 mmol),[1] magnesium sulfate (0.4 g), and potassium permanganate (83 mg) in acetone (140 mL): yield 56 mg (19%); mp >325 °C dec; mass spectrum, *m/e* 377 (M^+). Anal. ($C_{17}H_{14}F_3N_5O_2$) C, H, N.

Ethyl 5-Amino-1,2,3,4-tetrahydro-3-[(*N*-methyl-*N*-phenylamino)methyl]pyrido[3,4-*b*]pyrazine-7-carbamate (29). A stirred suspension of **11** (100 mg, 0.267 mmol)[1] in CH_3CN (2 mL) under N_2 was treated with $NaBH_3CN$ (45.9 mg, 0.730

mmol). A microsyringe was then used to add acetic acid (0.0243 mL) gradually over a period of 10 min. The suspension was stirred for 2 h under N_2, treated with additional acetic acid (0.0243 mL), stirred for 1 h, diluted with $CHCl_3$ (10 mL), washed with saturated $NaHCO_3$ solution (2 × 5 mL) followed by water, dried over $MgSO_4$, and evaporated to dryness in vacuo. The residue was triturated with Et_2O, collected by filtration, and dried in vacuo (P_2O_5): yield 92 mg (90%); mass spectrum, *m/e* 356 (M^+), 310 (M^+ – EtOH). Anal. ($C_{18}H_{24}N_6O_2 \cdot 0.2H_2O \cdot 0.2CHCl_3$) C, H, N.

Ethyl 6-Amino-4-[(2-oxo-4-phenylbutyl)amino]-5-nitro-2-pyridinecarbamate Oxime (31). A solution of **30** (261 mg, 1.00 mmol), 1-amino-4-phenyl-2-butanone oxime (241 mg, 1.10 mmol), and *N,N*-diisopropylethylamine (0.575 mL, 3.30 mmol) in EtOH (4 mL) was heated at 54 °C for 24 h. The crystalline precipitate was collected by filtration, washed with cold EtOH, and dried in vacuo (P_2O_5): yield 337 mg (84%); mp 187 °C. Anal. ($C_{18}H_{22}N_6O_5$) C, H, N.

Ethyl 6-amino-4-[(2-naphthyl-2-oxoethyl)amino]-5-nitro-2-pyridinecarbamate oxime (33) was prepared similarly in 49% yield when **30** and aminomethyl 2-naphthyl ketone oxime[13] was refluxed in EtOH for 1 h: mp 216–219 °C; mass spectrum, *m/e* 424 (M^+).

Ethyl 6-amino-4-[(1-methyl-2-oxo-2-phenylethyl)amino]-5-nitro-2-pyridinecarbamate oxime (35) was prepared similarly in 31% yield when **30** and 2-aminopropiophenone oxime[14] was refluxed in EtOH for 2 h, mp 176–178 °C. Anal. ($C_{17}H_{20}N_6O_5 \cdot 0.1HCl$) C, H, N.

Ethyl 6-Amino-4-[(2-oxo-4-phenylbutyl)amino]-5-nitro-2-pyridinecarbamate (32). A solution of **31** (6.17 g, 15.4 mmol) in warm dioxane (60 mL) was treated with 1 N HCl (120 mL), stirred at 55 °C for 1 h, and cooled in an ice bath. The precipitated hydrochloride was collected, washed with cold H_2O, then suspended in H_2O (300 mL), and neutralized with 1 N NaOH. The yellow product was collected, washed with H_2O, and dried in vacuo (P_2O_5): yield 5.08 g (83%); mp 155 °C; mass spectrum, *m/e* 387 (M^+). Anal. ($C_{18}H_{21}N_5O_5$) C, H, N.

Ethyl 6-amino-4-[(2-naphthyl-2-oxoethyl)amino]-5-nitro-2-pyridinecarbamate (34) was prepared similarly in 66% yield when **33** was refluxed in the acidic mixture for 4 h: mp 198–199 °C. Anal. ($C_{20}H_{19}N_5O_5 \cdot 0.1C_4H_8O_2$) C, H, N.

Ethyl 6-amino-4-[(1-methyl-2-oxo-2-phenylethyl)amino]-5-nitro-2-pyridinecarbamate (36) was prepared similarly in 98% yield when **35** was refluxed in the acidic mixture for 7 h: mp 168–174 °C. Anal. ($C_{17}H_{19}N_5O_5 \cdot H_2O \cdot 0.33C_4H_8O_2$) C, H, N.

Ethyl 6-amino-4-[*N*-(2-hydroxy-2-phenylethyl)-*N*-methylamino]-5-nitro-2-pyridinecarbamate (37). A solution of **30** (3.40 g, 13.1 mmol), 2-(methylamino)-1-phenylethanol (2.17 g, 14.4 mmol),[9] and triethylamine (1.32 g, 13.1 mmol) in ethanol (75 mL) was refluxed with protection by a drying tube for 2 h and evaporated to dryness in vacuo. The residue was stirred with 1 N HCl for 1 h, followed by neutralization (pH 7) with 1 N NaOH. The product was collected by filtration and used without further purification: yield 4.8 g (98%); mp 108–110 °C; mass spectrum, *m/e* 375 (M^+).

Ethyl 6-amino-4-[*N*-methyl-*N*-(2-oxo-2-phenylethyl)amino]-5-nitro-2-pyridinecarbamate (38). To a solution of pyridine (8.70 g, 110 mmol) in CH_2Cl_2 (131 mL), protected with a drying tube, chromium(VI) oxide (5.52 g, 55.2 mmol) was added with stirring. After 15 min, a solution of **37** (3.45 g, 9.20 mmol) in CH_2Cl_2 (35 mL) was added. After an additional 20 min, the residue was separated by decantation and washed with Et_2O (242 mL). The combined decantate and wash were evaporated to dryness, the residue was dissolved in Et_2O (1700 mL), and the solution was washed with aqueous 5% $NaHCO_3$ (200 mL), H_2O (200 mL), and saturated NaCl solution (200 mL). Concentration of the Et_2O solution to a small volume, followed by cooling in an ice bath, gave the product: yield 2.00 g (58%); mp 139–140 °C. Anal. ($C_{17}H_{19}N_5O_5$) C, H, N.

Ethyl 5,6-Diamino-4-[*N*-(2-hydroxy-2-phenylethyl)-*N*-methylamino]-2-pyridinecarbamate (39). A solution of **37** (0.3 g, 0.8 mmol) in ethanol (90 mL) containing Raney nickel (0.9 g, weighed wet and washed successively with H_2O and ethanol) was hydrogenated at room temperature and atmospheric pressure for 1 h. The catalyst was removed by filtration, and the filtrate was acidified with 1 N HCl (2.5 mL). The resulting solution was concentrated to a small volume in vacuo and diluted with diethyl ether: yield 0.3 g (85%); mp 155–160 °C with foaming; ^1H NMR (Me_2SO-d_6) δ 1.06 (t, CH_3CH_2OH), 1.27 (t, 3 H, OCH_2CH_3), 3.02 (s, 3 H, N-CH_3), 3.0–3.76 [m, 2 H, $N(CH_3)CH_2$, CH_3CH_2OH], 4.22 (q, 2 H, CH_3CH_2O), 5.02 (br d, 1 H, COH), 6.52 (s, 1 H, 3-CH), 7.2–7.6 (m, 5 H, C_6H_5), 7.6–8.3 (br s, NH_2, NH_3^+), 11.01 (s, 1 H, $NHCO_2Et$). Anal. ($C_{17}H_{23}N_5O_3 \cdot 0.09C_2H_5OH \cdot 2.48HCl$) C, H, N.

Ethyl 5-(Benzylidenamino)pyridine-2-carbamate (42). A solution of **40** (0.50 g, 2.2 mmol)[12] in EtOH (75 mL) containing Raney nickel (0.5 g, washed with H_2O and EtOH) was hydrogenated at room temperature and atmospheric pressure. When the theoretical amount of H_2 was absorbed, the catalyst was removed by filtration. The filtrate containing **41** was treated with benzaldehyde (0.73 g, 6.9 mmol), *p*-toluenesulfonic acid (50 mg), and 3Å molecular sieves (10 g). After refluxing for 16 h, the mixture was filtered, and the filtrate was cooled to deposit **42**: yield 0.36 g (61%). For analysis, a portion of this product was recrystallized from ethanol: mp 176–178 °C; ^1H NMR (Me_2SO-d_6, 3%, w/v) δ 1.26 (t, 3, CH_3CH_2), 4.18 (q, 2, CH_2CH_3), 7.76 (m, 7, 3-CH, 4-CH, C_6H_5), 8.27 (s, 1, 6-CH), 8.74 (s, 1, CHC_6H_5). Anal. ($C_{15}H_{15}N_3O_2$) C, H, N.

Ethyl 6-Amino-5-[[1-(ethoxycarbonyl)-2-phenylethen-2-yl]amino]-2-pyridinecarbamate (44). A solution of **30** (1.0 g, 3.9 mmol)[3] in EtOH (60 mL) was hydrogenated in the presence of 10% palladium on charcoal (100 mg) at room temperature and atmospheric pressure for 8 h. The catalyst was removed by filtration, and the filtrate containing **43** was treated with ethyl benzoylacetate (0.74 g) and Et_3N (0.39 g) and refluxed for 103 h. The solvent was removed in vacuo, and the black tarry residue was purified by elution from a silica gel column with $CHCl_3$ to give **44**: yield 0.25 g. This sample was recrystallized twice from EtOH: yield 0.18 g (12%); mass spectrum, *m/e* 370 (M^+); ^1H NMR (Me_2SO-d_6, 5%, w/v) δ 1.20 (m, 6, CH_3), 4.10 (m, 4, CH_2CH_3), 4.94 (s, 1, CHCO), 5.94 (br s, 2, NH_2), 6.66 (m, 2, 3-CH, 4-CH), 7.33 (s, 5, C_6H_5), 9.36 (s, 2, NH). Anal. ($C_{19}H_{22}N_4O_4$) C, H, N.

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(13) Prepared from bromomethyl 2-naphthyl ketone by the hexamethylenetetramine method previously described.[1]

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