New Anticancer Agents: Alterations of the Carbamate Group of Ethyl (5-Amino-1,2-dihydro-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamates

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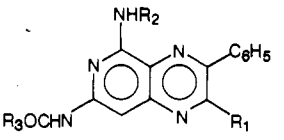
The ethyl (1,2-dihydropyrido[3,4-*b*]pyrazin-7-yl)carbamates have been reported to bind with cellular tubulin, to produce an accumulation of cells at mitosis, and to exhibit cytotoxic activity against experimental neoplasms in mice. Studies on the disposition of ethyl (5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate (**8**) in mice showed that one metabolite was formed by cleavage of the ethyl carbamate moiety. Analogues with alterations in the carbamate group were prepared by transformations at the carbamate of **8**, by reductive cyclization of nitropyridine intermediates, and by hydride reduction of the ring of heteroaromatic compounds. In vitro and in vivo evaluations of analogues indicated that a carbamate group was required for activity. No significant change in activity was observed when ethyl was replaced by methyl. However, activity was reduced when ethyl was replaced with bulky aliphatic groups and when ethoxy was replaced with a methylamino group. Also, the activity of **8** was decreased by acetylation of the 5-amino group and was destroyed by substitution of an amino group at the 8-position.

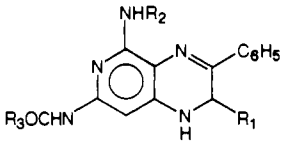
Previous studies on one of the more active 1,2-dihydropyrido[3,4-*b*]pyrazines showed that **8** has broad spectrum activity against experimental tumors including tumors that are resistant to drugs in clinical use.[1] Both **8** and vincristine arrest mitosis, presumably by binding at different sites on tubulin, since combinations of the two drugs are synergistic in mice bearing P388 leukemia.[1] One of the metabolites formed from **8** in vivo resulted from cleavage of the carbamate group to an amino group.[2] To determine the effect of alterations of the carbamate group, compounds were prepared in which this moiety was replaced with an amino group or was completely removed. Additionally, derivatives with a potentially more stable carbonylamino function were prepared either by replacement of the ethoxy group of the carbamate with other substituents or by substitution of an electron-donating amino group adjacent to the carbamate moiety. Also acetamidopyrido[3,4-*b*]pyrazines were prepared and evaluated.

Chemistry. In initial experiments on modification of the carbamate group, the air-sensitive 1,2-dihydropyridopyrazines (e.g., **6**) were considered to be unsuitable as intermediates. One approach was to perform transesterifications on the heteroaromatic pyridopyrazines (e.g., **1**) followed by reduction to give the 1,2-dihydro derivative (Scheme I). The acid-catalyzed reaction of **1** in hot cyclohexanol gave a high yield of **2**, which was reduced in dioxane with $NaBH_4$ on neutral alumina to give **7**. In contrast, reaction of **1** in hot CH_3OH to give **3** was unsuccessful, probably because of the low reaction temperature and a requirement for a prolonged reaction time (see below). The desired methyl carbamate **26** was prepared by procedures previously developed in our laboratory (Scheme II).[3] Although the known bis[carbonyl azide] **13**[4] was expected to rearrange via a diisocyanate intermediate **14** in refluxing CH_3OH to give the dicarbamate **16**, this

Scheme I



1: $R_1 = R_2 = H$; $R_3 = EtO$
2: $R_1 = R_2 = H$; $R_3 = c\text{-}C_6H_{11}O$
3: $R_1 = R_2 = H$; $R_3 = MeO$
4: $R_1 = Me$; $R_2 = H$; $R_3 = EtO$
5: $R_1 = H$; $R_2 = Ac$; $R_3 = EtO$



6: $R_1 = R_2 = H$; $R_3 = EtO$
7: $R_1 = R_2 = H$; $R_3 = c\text{-}C_6H_{11}O$
8: $R_1 = Me$; $R_2 = H$; $R_3 = EtO$
9: $R_1 = Me$; $R_2 = H$, $R_3 = MeNH$
10: $R_1 = Me$; $R_2 = H$; $R_3 = Et(Me)CHO$
11: $R_1 = Me$; $R_2 = H$; $R_3 = C_6H_5CH_2O$
12: $R_1 = H$; $R_2 = Ac$; $R_3 = EtO$

reaction gave a mixture of **16** and the monocarbamate **17**. The latter results from rearrangement of one of the carbonyl azide groups of **13**, whereas, the second underwent displacement by CH_3OH possibly via intermediate **15**. An additional amount of dicarbamate **16** was prepared by treatment of crude **17** with hydrazine to give **18**, nitrosation of **18** to give **19**, and rearrangement of the latter in CH_3OH. The conversion of **16** to **26** via **21–23** followed standard procedures.[3] Treatment of **26** with methanolic hydroxyethanesulfonic acid afforded the corresponding water-soluble salt.

With the observation that substitution of a methyl group at the 2-position of **6** gave a compound that was more resistant to air oxidation at the 1,2-dihydro moiety, the use of **8**[3b] as an intermediate was envisaged (Scheme I). Treatment of **8** with deoxygenated aqueous methylamine in a sealed tube at 105 °C for 3 h afforded directly the methylureido compound **9**. Similarly, treatment of **8** with 2-butanol at 100 °C in the presence of *p*-toluenesulfonic acid for 142 h gave the 1-methylpropyl carbamate **10**. The benzyl carbamate **11** was prepared from **8** and benzyl alcohol in the presence of *p*-toluenesulfonic acid.

To determine the contribution of the carbamate group to activity, both **27** (Scheme II) and **30** (Scheme III) were prepared. Preferential displacement of the 4-chloro group of **20** with an α-aminoketone oxime to give **24** is supported by similar reactions of **20** with other nucleophiles.[5] Treatment of **24** with ethanolic ammonia gave **25**, which was reductively cyclized in the presence of Raney nickel to give **27**.

Previously, the sensitivity to air of a 2-demethyl-1,2-dihydro analogue of **30** prevented its evaluation.[3b] The

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(4) Markees, D. G.; Kidder, G. W. *J. Am. Chem. Soc.* **1956**, *78*, 4130.
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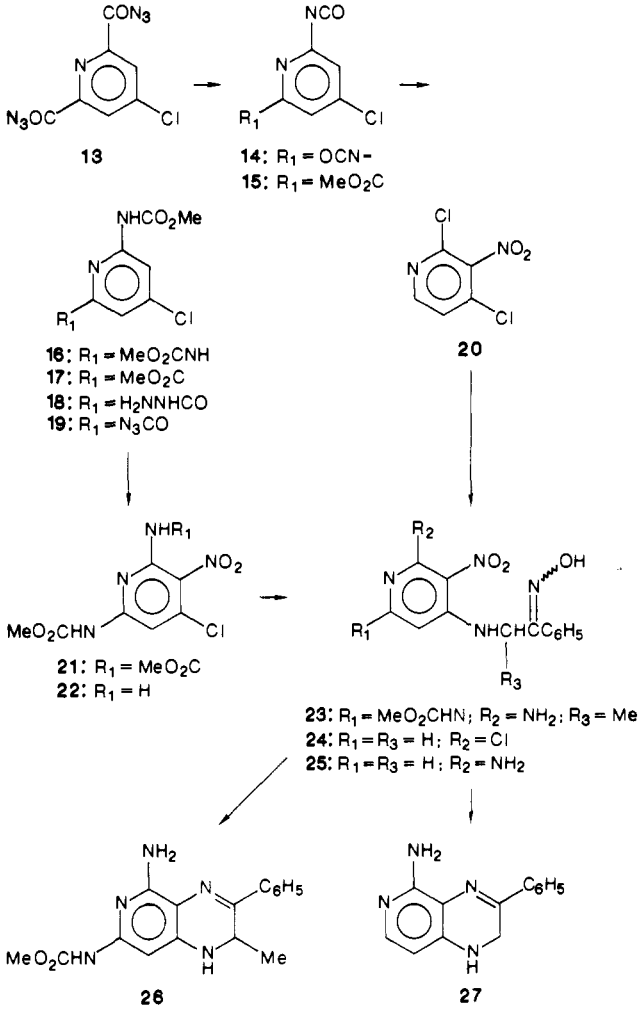
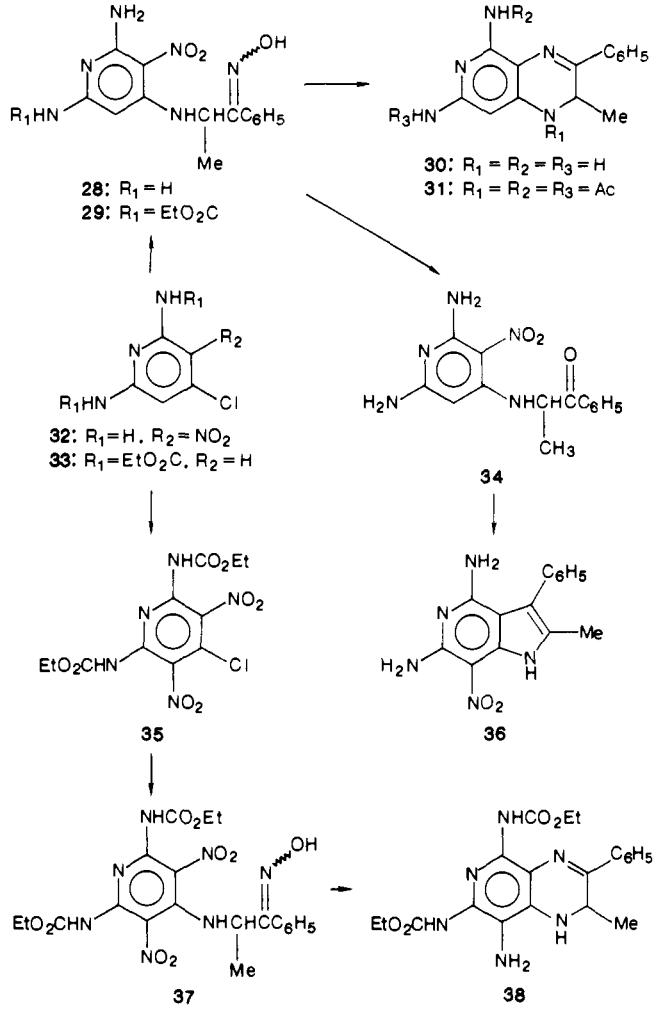
Scheme II



Scheme III



stabilizing effect of a 2-methyl group on the 1,2-dihydro moiety was confirmed by the synthesis of **30**. One approach to **30** was aborted when it was found that either hydride or catalytic reduction of **4** was difficult and led to mixtures of hydrogenated products. Thus, **32**[6] was reacted with an α-aminoketone oxime to give **28**. Also, this product could be prepared in good yield by cleavage of the urethane group of **29** with anhydrous hydrazine at room temperature. Reductive cyclization of **28** in acetic acid in the presence of Raney nickel gave **30**, which was complexed with nickel and was difficult to purify. A better yield of **30** was isolated from the hydrogenation of **28** in *N*,*N*-dimethylacetamide at 60 °C. Another type of difficulty was encountered when the preparation of **30** via the ketone **34** was attempted. Hydrolysis of the oxime group of **28** was effected with aqueous HCl in dioxane to give **34**, which was isolated as the free base. This product was unstable, in that cyclodehydration occurred to give the 3,7-dideazapurine **36** either on recrystallization from EtOH or on silica gel chromatography.

Compound **38** was prepared to determine the effect on activity of substitution of an electron-donating amino group adjacent to the urethane group (Scheme III). Dinitration of **33** gave **35**, which was converted via **37** to **38** by standard procedures.[3] Acetylated derivatives of the pyrido[3,4-*b*]pyrazines were prepared by two routes. Treatment of **1** with acetic anhydride gave **5**, which was reduced with $NaBH_4$ on alumina to give **12**. In the acetylation of **30** with acetic anhydride at 65 °C, the triacetyl product **31** was formed.

Biological Evaluation. The analogues of **8**, compounds **6–12**, **26**, **27**, **30**, **31**, and **35**, were prepared to determine the effect of the carbamate group on biological activity. Compounds **8–11**, **26**, **30**, and **31** contain a chiral center and were tested as racemic mixtures. An improvement in activity might result either from an increase in the stability of the carbamate moiety or from an increase in the tightness of binding of agent to tubulin.[7] The inhibition of proliferation with these compounds, vincristine, and colchicine were compared in vitro against lymphoid leukemia L1210 (Table II).[8] On the basis of the IC_{50} values, selected compounds were evaluated for inhibition of mitosis ($MI_{0.5}$)[8] in cultured L1210 cells and for antileukemic activity (% ILS) in mice implanted with lymphocytic leukemia P388.[9]

Previously, it was reported that substitution of a methyl group at the 2-position of **6** gave a compound (**8**) with increased potency.[1] Substitution for the ethyl group of the carbamate in **8** with methyl to give **26** gave no significant differences in activities, whereas substitution with a 1-

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Table I. Properties of Compounds

compd	yield, %	mp, °C	mass spectra[a]	^{1}H NMR spectra,[a] selected peaks, δ	formula	anal.
2	91	236–8 dec	363 (M^+)	9.53 (2-CH)	$C_{20}H_{21}N_5O_2$	C, H, N
5	74	213–5	351 [$(M + 1)^+$]	2.66 (CH_3CO), 9.42 (2-CH)	$C_{18}H_{17}N_5O_3$	C, H, N
7	41	124–30	365 (M^+)	4.41 (2-CH_2), 6.75 (1-NH)[b]	$C_{20}H_{23}N_5O_2 \cdot H_2O$	C, H, N
9	42	180–5	311 [$(M + 1)^+$]	4.76 (2-CH), 6.89 (1-NH)[b,c]	$C_{16}H_{18}N_6O \cdot 0.5CH_3CH_2OH \cdot 0.7H_2O$	C, H, N
10	58	105–10	354 [$(M + 1)^+$]	4.85 (2-CH), 7.11 (1-NH)[b,d]	$C_{19}H_{23}N_5O_2 \cdot 0.12C_7H_8O_3S \cdot 0.5H_2O$	C, H, N
11	33	185–9	388 [$(M + 1)^+$]	5.11 (2-CH), 8.48 (1-NH)[d]	$C_{22}H_{21}N_5O_2 \cdot C_7H_8O_3S$	C, H, N
12	37	220–5	353 (M^+)	2.32 (CH_3CO), 4.52 (2-CH_2)	$C_{18}H_{19}N_5O_3$	C, H, N
16	~37	228–30			$C_9H_{10}ClN_3O_4$	C, H, N[e]
21	94	199–201	304 (M^+)		$C_9H_9ClN_4O_6$	C, H, N
22	42	192–5	246 (M^+)		$C_7H_7ClN_4O_4$	C, H, N
23	74	168–73	374 (M^+)	1.35,[f] 1.67 (CH_3C)	$C_{16}H_{18}N_6O_5$	C, H, N
24	17[g]	167–8	306 (M^+)	4.61 (CH_2)[b]	$C_{13}H_{11}ClN_4O_3 \cdot 0.2H_2O$	C, H, N
25	34	197–8	287 (M^+)	4.72 (CH_2)[b,c]	$C_{13}H_{13}N_5O_3 \cdot 0.12CH_3CH_2OH \cdot 0.28H_2O$	C, H, N
26	82	92–7		4.83 (2-CH), 6.99 (1-NH)[g,h]	$C_{18}H_{17}N_5O_2 \cdot 0.4CHCl_3 \cdot 0.24CH_3CO_2H$	C, H, N
26	74	indef		5.12 (2-CH), 8.53 (1-NH)[i]	$C_{16}H_{17}N_5O_2 \cdot 1.2HOCH_2CH_2SO_3H \cdot 0.5H_2O$	C, H, N
27	55	>180 dec	224 (M^+)	4.70 (2-CH_2), 8.53 (1-NH)[c]	$C_{13}H_{12}N_4 \cdot 0.2CH_3CH_2OH \cdot 1.74HCl$	C, H, N
28	64; 51	185[j]	317 [$(M + 1)^+$]	1.30,[k] 1.59 (CH_3C)[l]	$C_{14}H_{16}N_6O_3 \cdot 0.3CH_3OH$	C, H, N
30	17; 60	250 dec	253 (M^+)	4.68 (2-CH), 6.51 (1-NH)[g]	$C_{14}H_{15}N_5 \cdot 0.3CH_3CO_2H$	C, H, N
31	7	indef	380 [$(M + 1)^+$]	2.12, 2.26, 2.38 (CH_3CO),[b,h] 5.85 (2-CH)	$C_{20}H_{21}N_5O_3 \cdot 0.8CHCl_3 \cdot 0.2H_2O$	C, H, N
35	100[m]	174–9	377 (M^+)		$C_{11}H_{12}ClN_5O_8$	C, H, N
36	*a*	290–5	284 [$(M + 1)^{+n}$]	2.20 (2-CH_3)[b,o]	$C_{14}H_{13}N_5O_2 \cdot 0.05CH_3CH_2O_2CCH_3 \cdot 0.2H_2O$	C, H, N
37	74	179–81	506 [$(M + 1)^+$]	1.28,[p] 1.60 (CH_3C)	$C_{20}H_{23}N_7O_9$	C, H, N
38	50	>300 dec	413 [$(M + 1)^+$]	5.28 (2-CH), 8.67 (1-NH)[b,c]	$C_{20}H_{24}N_6O_4 \cdot 0.3CH_3CH_2OH \cdot 0.2H_2O$	C, H, N

[a] See Experimental Section. [b] H_2O observed, δ 3.30–3.35. [c] CH_3CH_2OH observed, δ 1.06–1.07, 3.44–3.48. [d] *p*-Toluenesulfonic acid observed, δ 2.29 (CH_3). [e] N: calcd, 16.18; found, 15.67. [f] Major isomer, ratio 3:1. [g] CH_3CO_2H observed, δ 1.92. [h] $CHCl_3$ observed, δ 8.30. [i] $HOCH_2CH_2SO_3H$ observed, δ 2.67, 3.66 (CH_2). [j] Softening from 110 °C. [k] Major isomer, ratio 5:3; method B, ratio 7:1. [l] CH_3OH observed, δ 3.18. [m] Crude yield. [n] Exact mass: calcd, 283.1071; found, 283.107. [o] EtOAc observed, δ 1.16, 1.98, 4.02. [p] Major isomer, ratio 4:1.

Table II. Biological Data—1,2-Dihydropyrido[3,4-*b*]pyrazines

compd	L1210[a] IC_{50}, nM	L1210[b] $MI_{0.5}$, nM	P388[c] 10^6 tumor cell implant, ip, qd 1–5	
			dose, mg/kg	% ILS[d]
6	4.7[e]	2.8	2[f]	51
7	230[e]	260	200[g]	0
8[h]	0.51	1.5	1	75
8[i]	0.54	0.74	0.5	46
9	20		12	0[j]
10	1.2[e]		12	60[k]
11	63			
12	8.6	23	25	49
26	0.54	0.8	1	88
27	2800	>10000	50	0
30	500	850		
31	>100			
38	>300		200	0
VCR[l]	3.4	11	1.5[m]	122
COC[n]	6.4	21		

[a] Nanomolar concentration of agent that inhibits proliferation of cultured lymphoid leukemia L1210 cells to 50% control growth during 48 h. [b] Nanomolar concentration of agent that causes a mitotic index (fraction of cells in mitosis divided by total cells) of 0.5 for cultured lymphoid leukemia L1210 cells during an exposure period of 12 h. [c] Lymphocytic leukemia P388. [d] Increase in life span at the highest nontoxic dose tested. [e] Average of two determinations. [f] Schedule: qd 1–9. [g] Schedule, day 1. [h] Hydrochloride. [i] 2-Hydroxyethanesulfonate. [j] Nontoxic dose. [k] Toxic dose when repeated. A dose of 3 mg/kg gave a % ILS of 14. [l] Vincristine, see ref 1b and 8. [m] Schedule: days 1, 5, 9. [n] Colchicine, see ref 8.

methylpropyl group to give **10** reduced potency both in vitro and in vivo. Further, the benzyl compound **11** was about 100 times less effective than **8** in preventing growth in vitro. This loss in activity can be attributed to a steric effect of the phenyl group of the benzyl moiety. The further loss in activity with a cyclohexyl group (**7**) suggested that steric hindrance decreased the binding of **7** and **11** as well as **10** to tubulin. The requirement of the alkoxycarbonyl moiety of the carbamate for activity was shown by the preparation of the inactive 7-amino compound **30**. Complete removal of the carbamate group (**27**) destroyed activity. Compound **9** was prepared and evaluated to determine if the chemically more stable ureido group in place of the carbamate would increase activity. Although **9** prevented proliferation of L1210 cells at nanomolar concentrations, **9** showed no in vivo activity at a dose 12 times that of **8**. The presence of an acylamino group at the 5-position of the ring (**12**) reduced but did not destroy activity whereas the fully acylated derivative **31** was inactive. Increasing the electron density in the pyridine ring should stabilize the carbamate group, but the 8-amino compound **38** showed no activity. Presumably, a substituent at the 8-position prevents binding to tubulin.

These results demonstrate that effective binding to tubulin requires a carbamate moiety and that tighter binding is observed in carbamates with a straight-chain alkoxy group (**6**, **8**, and **26**). The loss of activity observed with the bulky carbamates (**7** and **11**) is attributed to a decrease in binding of agent to tubulin presumably caused by steric hindrance. However, because of the inactivity of the 7-amino compound **30**, cleavage of the bulky carbamates to amino derivatives cannot be eliminated from consideration. The reduced activities observed with acylated derivatives (**12** and **31**) suggest that the 1-NH and 5-NH_2 group of **8** participate in binding with tubulin.

Experimental Section

Melting and decomposition temperatures were determined in capillary tubes in a Mel-Temp apparatus. The ^{1}H NMR spectra were determined on DMSO-d_6 solutions with either a Varian XL-100-15 or a Nicolet NT300NB spectrometer with tetramethylsilane as internal standard. Mass spectra were taken with a Varian Mat 311A spectrometer operating in either the electron-impact or fast-atom-bombardment mode to provide the M^+ and $(M + 1)^+$ molecular ion, respectively. The progress of reactions was followed by thin-layer chromatography (TLC) on plates of silica gel from Analtech, Inc. Flash chromatography was performed with silica gel 60 (230–400 mesh) from E. Merck. Raney nickel no. 2800 was obtained from Davison Specialty Chemical Co. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical value.

Cyclohexyl (5-Amino-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate (2). A solution of 1 (700 mg, 2.27 mmol)[3a] in cyclohexanol (25 mL) containing *p*-toluenesulfonic acid (50 mg) was heated with stirring at 140 °C for 6 h and evaporated to dryness in vacuo. The residue was stirred with EtOH for 1 h, and the insoluble **2** was collected by filtration and dried under reduced pressure over P_2O_5: yield 751 mg.

Ethyl (5-Acetamido-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate (5). A solution of 1 (715 mg, 2.31 mmol) and 4-

pyrrolidinopyridine (6 mg) in acetic anhydride (20 mL) was heated with stirring at 80 °C for 10 h and cooled to room temperature to deposit the product. The solid was collected by filtration, washed with ethanol, and dried in vacuo over P_2O_5: yield 600 mg.

Cyclohexyl (5-Amino-1,2-dihydro-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate (7). A solution of **2** (350 mg, 0.963 mmol) in dioxane (15 mL) containing $NaBH_4$ on alumina (2 g) was stirred under N_2 for 2 h. The residue was removed by filtration and washed with dioxane (75 mL), and the combined filtrate and wash was acidified (pH ~2) with 1 N HCl. After stirring under N_2 for 1.5 h to destroy the boron adduct, the solution was neutralized with 1 N NaOH to deposit **7**: yield 150 mg.

5-Amino-1,2-dihydro-2-methyl-7-[[(methylamino)carbonyl]amino]-3-phenylpyrido[3,4-*b*]pyrazine (9). A suspension of **8** (100 mg, 0.307 mmol)[3b] in deoxygenated (N_2) aqueous methylamine (~8 M, 15 mL) was heated at 105 °C in a sealed tube under argon for 3 h. The resulting clear yellow solution was lyophilized to give a yellow powder, which was purified by flash chromatography [20 g; $CHCl_3$–MeOH (97:3)] to afford **9** as a yellow foam: yield 40.3 mg.

1-Methylpropyl and Benzyl (5-Amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamates (10 and 11). A solution of **8** (102 mg, 0.313 mmol)[3b] and *p*-toluenesulfonic acid hydrate (11 mg) in deoxygenated (N_2) 2-butanol (15 mL) was heated at 100 °C in a sealed tube under argon for 142 h, and the clear yellow solution was evaporated to dryness in vacuo. The residue was dissolved in EtOH (2 mL), which was diluted with H_2O (25 mL) to precipitate the product: yield 69 mg.

The benzyl carbamate was prepared from **8** (110 mg, 0.340 mmol), *p*-toluenesulfonic acid hydrate (25 mg), and deoxygenated (N_2) benzyl alcohol (17 mL) at 105 °C under argon for 66 h. After evaporation in vacuo of the reaction mixture, trituration of the residue with EtOH (5 mL) afforded the toluene sulfonate of **11**: yield 63 mg.

Ethyl (5-Acetamido-1,2-dihydro-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate (12). A solution of **5** (400 mg, 1.14 mmol) in dioxane (16 mL) containing $NaBH_4$ on alumina (11.4 g) was stirred under N_2 for 2 h. The residue was collected by filtration and washed with acetone (350 mL), and the wash was evaporated in vacuo. The resulting wet residue was stirred with 0.1 M KH_2PO_4 (250 mL) to afford a yellow solid. The combined solids from two identical experiments were recrystallized under N_2 from EtOH: yield 300 mg.

Dimethyl (4-Chloropyridine-2,6-diyl)bis[carbamate] (16). **Caution**: The 4-chloropyridine-2,6-diylcarboxylic acid diazide (**13**) can be explosive when stored as a dry solid. To a solution of (4-chloropyridine-2,6-diyl)bis[carboxylic acid] dihydrazide (251 g, 1.09 mol)[4] in 10% HCl (2155 mL) was added with stirring a solution of $NaNO_2$ (180 g, 2.61 mol) in H_2O (615 mL) over a period of 1.5 h. The reaction mixture was maintained below 15 °C with an ice–salt bath. Vigorous foaming occurred near the end of the addition. The resulting mixture was stirred at room temperature for 1 h, and the dicarboxylic acid diazide (**13**) was collected by filtration, washed well with H_2O (2800 mL), and dried under aspirator pressure until no H_2O dripped from the funnel. This residue was added portionwise to boiling MeOH (3500 mL) with stirring, and the solution was refluxed for 8 h. The resulting mixture was cooled in an ice bath, and crude **16** (109 g) was collected by filtration and recrystallized from MeOH: yield 50.0 g (18%); mp 228–30 °C.

A major byproduct in the solids recovered from evaporation of the recrystallization filtrate (52.4 g) and concentration of the reaction filtrate (73.3 g) to 100 mL was identified (mass spectrum) as crude **17**. The combined solids were dissolved in 95% MeOH (1200 mL) at 60 °C, and the solution was treated with anhydrous hydrazine (66 mL). After 1 h at this temperature, the crude acid hydrazide **18** (87.5 g) was collected by filtration, suspended in 10% HCl (710 mL), and treated with a solution of $NaNO_2$ (32 g) in H_2O (200 mL) to give **19**. Rearrangement of the latter as described above provided an additional amount of pure **16** (32.3 g, 11%) and impure **16** (22 g, 8%).

Dimethyl (4-Chloro-3-nitropyridine-2,6-diyl)bis[carbamate] (21). Solid **16** (9.46 g, 36.6 mmol) was added portionwise to concentrated H_2SO_4 (35 mL) over a period of 1 h while maintaining the temperature below 7 °C with an ice–salt bath. The resulting solution was added with vigorous stirring over 30 min to red fuming HNO_3 (d, 1.6; 95 mL) at 0 °C. This solution was allowed to warm to room temperature and after 105 min was added with stirring to crushed ice (800 mL). The solid that deposited was collected by filtration and resuspended in H_2O (300 mL), and the resulting mixture was cooled in an ice bath while the pH (meter) was adjusted to 8.5 with 10% NaOH. The light yellow solid was collected by filtration and dried in vacuo over P_2O_5: yield 10.4 g.

Methyl (6-Amino-4-chloro-5-nitropyridin-2-yl)carbamate (22). A suspension of **21** (5.0 g, 16 mmol) in Et_3N (50 mL) and H_2O (0.29 mL, 16 mmol) was heated at reflux for 9 h and allowed to stand at room temperature for 16 h to give a yellow solid. The product was collected by filtration, recrystallized from MeOH (100 mL), and dried in vacuo over P_2O_5: yield 1.72 g.

Methyl [6-Amino-4-[(1-methyl-2-oxo-2-phenylethyl)amino]-5-nitropyridin-2-yl]carbamate Oxime (23). A solution of **22** (1.50 g, 6.08 mmol), α-aminopropiophenone oxime (1.25 g, 7.60 mmol), and Et_3N (0.62 g, 6.1 mmol) in 2-propanol (30 mL) was heated at reflux for 21 h and chilled overnight at −20 °C. The yellow solid that deposited was collected by filtration and dried in vacuo (P_2O_5) to give **23** as a 3:1 mixture of oxime isomers: yield 1.68 g.

2-Chloro-3-nitro-4-[(2-oxo-2-phenylethyl)amino]pyridine Oxime (24). A solution of **20** (4.81 g, 24.9 mmol),[5] α-aminoacetophenone oxime (3.74 g, 24.9 mmol), and Et_3N (2.53 g, 25.0 mmol) in MeOH (60 mL) was refluxed with stirring for 2 h and evaporated to dryness in vacuo. This residue was applied to a column (65 × 45 mm) of silica gel H with chloroform and eluted with the same solvent to remove **20** and two unidentified colored bands before collecting the major fraction. After evaporation of this fraction under reduced pressure, the resulting residue was triturated with Et_2O to afford **24**: yield 0.79 g (10%); mp 167–8 °C. A second crop of homogenous **24** (TLC) was isolated from the Et_2O filtrate: yield 0.56 g (7%); mp 157–8 °C. The lower melting point was attributed to a different ratio of *E*:*Z* oxime isomers.

2-Amino-3-nitro-4-[(2-oxo-2-phenylethyl)amino]pyridine Oxime (25). A solution of **24** (472 mg, 1.52 mmol) in ethanolic ammonia (20 mL, saturated at 0 °C) was heated in a stainless steel bomb at 60 °C for 16 h. Most of the ammonia was allowed to evaporate to deposit a solid that was collected by filtration and dried in vacuo over P_2O_5: yield 155 mg.

Methyl (5-Amino-1,2-dihydro-2-methyl-3-phenylpyrido-[3,4-*b*]pyrazin-7-yl)carbamate (26). A solution of **23** (750 mg, 2.00 mmol) in glacial AcOH (40 mL) was hydrogenated at atmospheric pressure in the presence of Raney nickel (2.3 g wet, washed with H_2O and AcOH). The hydrogen uptake was 104% of the theoretical amount after 5 h. The catalyst was removed by filtration (Celite), the filtrate was evaporated to dryness in vacuo, and the residue was dissolved in $CHCl_3$ (60 mL). After washing with deoxygenated (N_2) H_2O (3 × 20 mL), the $CHCl_3$ layer was dried over anhydrous K_2CO_3 and evaporated to dryness in vacuo to afford **26** as a yellow foam: yield 610 mg.

A solution of **26** (470 mg, 1.26 mmol) in MeOH (10 mL) was treated with 0.7 N methanolic 2-hydroxyethanesulfonic acid (1.8 mL), and a trace amount of insoluble material was removed by filtration. The filtrate was evaporated to dryness in vacuo, and the resulting semisolid was triturated with Et_2O, collected by filtration, and dissolved in distilled, deionized H_2O. A small amount of precipitate was removed by filtration, and the filtrate was lyophilized to afford a fluffy light orange solid: yield 443 mg.

5-Amino-1,2-dihydro-3-phenylpyrido[3,4-*b*]pyrazine (27). A solution of **25** (475 mg, 1.50 mmol) in glacial AcOH (75 mL) was hydrogenated at atmospheric pressure in the presence of Raney nickel (0.5 g wet, washed with H_2O and AcOH). The uptake of hydrogen was 131% of the theoretical amount in 5 h. The catalyst was removed by filtration (Celite), the filtrate was evaporated to dryness in vacuo, and the residue was dissolved in EtOH and acidified with 3.6 N ethanolic HCl. This solution was concentrated in vacuo to deposit the hydrochloride: yield 260 mg.

2,6-Diamino-4-[(1-methyl-2-oxo-2-phenylethyl)amino]-5-nitropyridine Oxime (28). Method A. A solution of **29** (1.01 g, 2.60 mmol)[3b] in anhydrous hydrazine (10 mL) was stirred overnight at room temperature and evaporated to dryness in

vacuo. The resulting orange foam was triturated with H_2O (20 mL), collected by filtration, and dissolved in EtOAc. The solution was added to a column of silica gel (100 g) and eluted with EtOAc to give **28** as a 3:5 mixture of oxime isomers: yield 543 mg.

Method B. A solution of **32** (2.95 g, 15.6 mmol),[6] α-aminopropiophenone oxime (2.61 g, 15.9 mmol), and Et_3N (1.61 g, 15.9 mmol) in EtOH (60 mL) and *N,N*-dimethylacetamide (10 mL) was refluxed with stirring for 72 h. The solution was evaporated to dryness, and the brown residue was triturated with EtOH (30 mL) to afford **28** as a yellow solid: yield 2.51 g (~51%). The ^1H NMR spectrum showed the presence of Et_3N resonances as well as a 7:1 ratio of oxime isomers.

5,7-Diamino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazine (30). A solution of **28** (1.0 g, 3.2 mmol) in glacial AcOH (80 mL) was hydrogenated in the presence of Raney nickel (3.0 g, washed with H_2O and AcOH) for 24 h. The hydrogen uptake was 11.3 mmol (118% of theoretical). The catalyst was removed by filtration (Celite), the filtrate was evaporated to dryness, and the reddish brown residue was dissolved in deoxygenated (N_2) H_2O (20 mL). The pH of the solution was adjusted to ca. 10 with deoxygenated (N_2) 1 N NaOH, and the resulting suspension was extracted under N_2 with $CHCl_3$ (3 × 130 mL). The combined extracts were dried (Na_2SO_4) and evaporated to dryness in vacuo. The resulting brownish yellow semisolid was triturated with EtOAc (25 mL) to give **30**: yield 151 mg.

A higher yield of **30** (60%) was obtained when the reduction was carried out in *N,N*-dimethylacetamide at 60 °C, but the ^1H NMR and elemental analysis indicated that the product was slightly contaminated by unidentified hydrocarbon impurities.

1-Acetyl-5,7-diacetamido-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazine (31). A suspension of **30** (102 mg, 0.37 mmol) in deoxygenated (N_2) acetic anhydride (1.5 mL) was heated under N_2 at 65 °C for 1.5 h, and the dark reaction mixture was evaporated to dryness in vacuo. The residue was triturated with deoxygenated (N_2) H_2O (5 mL), collected by filtration, dried in vacuo (P_2O_5), and purified on a short silica gel column [10 g; $CHCl_3$–MeOH (98:2)] to give an amber glass: yield 12.3 mg.

2,6-Diamino-4-[(1-methyl-2-oxo-2-phenylethyl)amino]-3-nitropyridine (34) and 4,6-Diamino-2-methyl-7-nitro-3-phenyl-1*H*-pyrrolo[3,2-*c*]pyridine (36). A solution of **28** (0.50 g, 1.6 mmol) in dioxane (10 mL) and 1 N HCl (10 mL) was heated at 50 °C for 18 h, chilled to 0 °C, and slowly neutralized to pH 7 with 1 N NaOH to precipitate **34**: yield 429 mg (~90%). TLC showed only minor impurities. Recrystallization from EtOH (100 mL) generated a mixture (275 mg) of desired ketone and a byproduct at higher R_f on TLC. About 1 mg of this material in MeOH was applied to a silica gel TLC plate (250 μm), which was developed in $CHCl_3$–MeOH (97:3). The band (**34**) at R_f 0.25 was scraped and extracted with MeOH to obtain a sample for analysis: MS-FAB, m/e 302 $(M + 1)^+$. The byproduct band (**36**) at R_f 0.55 was also scraped and extracted with MeOH to obtain a sample for analysis: MS-FAB, m/e 284 $(M + 1)^+$.

A sample of the mixture (100 mg) was subjected to chromatography on a thick-layer (200-μm) silica gel plate, which was developed twice with $CHCl_3$–MeOH (97:3). This resulted in the complete conversion of **34** to **36**. The silica gel was extracted with tetrahydrofuran and EtOAc, which were evaporated to dryness to afford **36** as an orange solid: yield 85 mg.

Diethyl (4-Chloro-3,5-dinitropyridine-2,6-diyl)bis[carbamate] (35). A solution of **33** (25.0 g, 86.9 mmol)[4] in concentrated sulfuric acid (75 mL) at 0 °C was added slowly with stirring to red fuming nitric acid (d, 1.6; 50 mL) at 0 °C. The solution was stirred at room temperature for 3 h and added to a mixture of ice and H_2O (800 mL). The solid that deposited was collected by filtration and resuspended in ice and H_2O (800 mL). This mixture was adjusted to pH 8 with 10% NaOH, and the insoluble pale yellow solid was collected by filtration, washed with H_2O, and dried in vacuo over P_2O_5: yield 32.7 g (100%); mp ~165 °C. A portion (1.0 g) of the crude product was heated with stirring in EtOH (30 mL). The mixture was cooled to room temperature, and the insoluble material was removed by filtration. The filtrate was heated to boiling and diluted with hot H_2O (30 mL) to deposit **35**: yield 0.63 g.

Diethyl [3,5-Dinitro-4-[(1-methyl-2-oxo-2-phenylethyl)amino]pyridine-2,6-diyl]bis[carbamate] Oxime (37). A solution of **35** (5.75 g, 15.2 mmol), α-aminopropiophenone oxime (2.50 g, 15.2 mmol), and Et_3N (1.52 g, 15.2 mmol) in EtOH (110 mL) was heated at 45 °C for 1 h. After cooling to room temperature, the solid was collected by filtration, washed with EtOH, and dried in vacuo (P_2O_5) to afford **37** as a 1:4 mixture of oxime isomers: yield 5.68 g.

Diethyl (8-Amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazine-5,7-diyl)bis[carbamate] (38). A suspension of **37** (3.00 g, 5.94 mmol) in EtOH (375 mL) and *N,N*-dimethylacetamide (75 mL) was hydrogenated at atmospheric pressure in the presence of Raney nickel (10.0 g wet, washed with H_2O and EtOH). The hydrogen uptake was 39.6 mmol (111% of theoretical) after 1.5 h. The catalyst was removed by filtration (Celite), and the filtrate was evaporated to dryness in vacuo to give a brown semisolid. The residue was dissolved in EtOH (5 mL), which was treated with 1 N ethanolic HCl (6 mL) to deposit the hydrochloride as a yellow solid: yield 1.38 g.

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Registry No. 1, 83269-15-2; **2**, 121572-29-0; **5**, 121572-30-3; **6**, 82585-91-9; **7**, 87607-30-5; **8**, 83269-10-7; **8**·*x*HCl, 121572-62-1; **8** 2-hydroxyethanesulfonate, 121572-63-2; **9**, 121572-31-4; **10**, 121572-32-5; **10** tosylate, 121572-33-6; **11**, 121572-34-7; **11** tosylate, 121572-35-8; **12**, 87607-28-1; **13**, 121572-36-9; **16**, 121572-37-0; **17**, 121572-38-1; **18**, 121572-39-2; **19**, 121572-40-5; **20**, 5975-12-2; **21**, 121572-41-6; **22**, 121572-42-7; (*E*)-**23**, 121572-43-8; (*Z*)-**23**, 121572-57-4; (*E*)-**24**, 121572-44-9; (*Z*)-**24**, 121572-58-5; **25**, 121572-45-0; **26**, 121572-46-1; **26** acetate, 121572-47-2; **26** 2-hydroxyethanesulfonate, 121572-56-3; **27**, 87619-51-0; **27**·*x*HCl, 121572-48-3; (*E*)-**28**, 121572-49-4; (*Z*)-**28**, 121572-61-0; **29**, 83269-20-9; **30**, 121572-50-7; **30** acetate, 121572-51-8; **31**, 121596-27-8; **32**, 40497-64-1; **33**, 63708-78-1; **34**, 121572-52-9; **35**, 121572-53-0; **36**, 121572-54-1; (*E*)-**37**, 121572-55-2; (*Z*)-**37**, 121572-60-9; **38**, 121596-28-9; **38**·*x*HCl, 121572-59-6; (4-chloropyridine-2,6-diyl)bis[carboxylic acid]dihydrazide, 98276-29-0; α-aminopropiophenone oxime, 83269-21-0; α-aminoacetophenone oxime, 82585-31-7.