(d, 1 H, J = 10.7 Hz, H-2'). Anal. ($C_{17}H_{24}O_2$) C, H.

The 1*E*,3*E*-isomer **17** was obtained in traces as a solid: IR (KBr) 1670, 1600, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.11-1.84 (m, 10 H, cyclohexane), 1.42 (s, 6 H, $(CH_3)_2$), 1.99 (s, 3 H, CH_3C=), 2.15 (m, 1 H, CHC=), 5.53 (s, 1 H, furan), 6.12 (dd, 1 H, J = 15.2 and 7.05 Hz, H-4'), 6.36 (dd, 1 H, J = 15.2 and 11.2 Hz, H-3'), 7.03 (d, 1 H, J = 11.2 Hz, H-2').

Inhibition of L1210, FM3A, Molt/4F, and MT-4 Cell Proliferation. All assays were performed in flat-bottomed Microtests III plates (96 wells) (Falcon) as previously described.[18,19] Briefly, the cells were suspended in growth medium and added to the microplate wells at a density of 5×10^4 L1210, FM3A, or Molt/4F cells/well (200 mL) or 6.25×10^4 MT-4 cells/well in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 48 h (L1210 cells) or 72 h (other cell lines) at 37 °C in a humidified, CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter (Coulter Electronics Ltd., Harpenden, Herts, U.K.). The IC_{50} was defined as the concentration of compound that reduced the number of viable cells by 50%.

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(18) Balzarini, J.; De Clercq, E.; Torrence, P. F.; Mertens, M. P.; Park, J. S.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. Role of thimidine kinase in the inhibitory activity of 5-substituted-2'-deoxyuridines on the growth of human and murine tumor cell lines. *Biochem. Pharmacol.* **1982**, *31*, 1089-1095.

(19) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. Thymidylate synthetase as target enzyme for the inhibitory activity of 5-substituted-2'-deoxyuridines on mouse Leukemia L 1210 cell growth. *Mol. Pharmacol.* **1981**, *19*, 321-330.

Antimitotic Agents. Alterations at the 2,3-Positions of Ethyl (5-Amino-1,2-dihydropyrido[3,4-*b*]pyrazin-7-yl)carbamates

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The reaction of ethyl (6-amino-4-chloro-5-nitropyridin-2-yl)carbamate (**2**) with α-amino ketone oximes gave 4-[(2-oxoethyl)amino]pyridine oximes **3**, which were reductively cyclized to give a series of ethyl (1,2-dihydropyrido[3,4-*b*]pyrazin-7-yl)carbamates (**6**). In another approach, α-nitro ketones, α-oximino ketones, and α-nitro alcohols were reduced to give α-amino alcohols, which were reacted with **2** to give 4-[(2-hydroxyethyl)amino]pyridines (**5**). Oxidation of these alcohols with the chromium trioxide–pyridine reagent gave the corresponding ketones (**4**), which were also reductively cyclized to give **6**. Structure–activity relationship studies indicated that alterations at the 2- and 3-positions of the pyrazine ring of **6** had a significant effect on cytotoxicity and the inhibition of mitosis in cultured lymphoid leukemia L1210 cells. Compounds that exhibited in vitro cytotoxicities at less than 1 nM showed the same level of in vivo activity, whereas the less potent compounds showed wide variations in their in vivo activity.

A number of the 1,2-dihydropyrido[3,4-*b*]pyrazines inhibit the proliferation of cultured L1210 cells at nanomolar concentrations and exhibit activity in mice against lymphocytic leukemia P388.[1-3] Previous work has shown that these types of compounds interact with tubulin and compete with colchicine for binding to tubulin.[4] The correlation of cytotoxicity with antimitotic activity indicated that the compounds prepared in this study also compete with colchicine for binding to tubulin. This mode of action is thought to cause the accumulation of cells at mitosis with both cultured cells and ascites cells in vivo. Previous work indicated that oxidation of the 1,2-dihydro moiety of the pyrazine ring to give the corresponding heteroaromatic ring system and increasing the basicity at the 1-position by the preparation of 1-aminoimidazo[4,5-*c*]pyridines (3-deaza-9-aminopurines) and 2-aminopyrido[3,4-*b*]pyrazines either decreased or destroyed activity.[5,6] In contrast, a methyl group at the 2-position of the pyrazine ring and the presence of electron-donating substituents in the 3-phenyl group either increased or maintained activity.[1] Because

(1) Wheeler, G. P.; Bowdon, B. J.; Temple, C., Jr.; Adamson, D. J.; Webster, J. Biological Effects and Structure–Activity Relationships of 1,2-Dihydropyrido[3,4-*b*]pyrazines. *Cancer Res.* **1983**, *43*, 3567-3575.

(2) Temple, C., Jr.; Wheeler, G. P.; Elliott, R. D.; Rose, J. D.; Comber, R. N.; Montgomery, J. A. 1,2-Dihydropyrido[3,4-*b*]-pyrazines: Structure–Activity Relationships. *J. Med. Chem.* **1983**, *26*, 91-95.

(3) Temple, C., Jr.; Wheeler, G. P.; Elliott, R. D.; Rose, J. D.; Kussner, C. L.; Comber, R. N.; Montgomery, J. A. New Anticancer Agents: Synthesis of 1,2-Dihydropyrido[3,4-*b*]pyrazines (1-Deaza-7,8-dihydropteridines). *J. Med. Chem.* **1982**, *25*, 1045-1050.

(4) Bowdon, B. J.; Waud, W. R.; Wheeler, G. P.; Hain, R.; Dansby, L.; Temple, C., Jr. Comparison of 1,2-Dihydropyrido[3,4-*b*]-pyrazines (1-Deaza-7,8-dihydropteridines) with Several Other Inhibitors of Mitosis. *Cancer Res.* **1987**, *47*, 1621.

(5) Temple, C., Jr. Antimitotic Agents: Synthesis of Imidazo-[4,5-*c*]pyridin-6-ylcarbamates and Imidazo[4,5-*b*]pyridin-5-ylcarbamates. *J. Med. Chem.* **1990**, *33*, 656-661.

(6) Temple, C., Jr.; Rener, G. A. Potential Antimitotic Agents. Synthesis of Some Ethyl Benzopyrazin-7-ylcarbamates, Ethyl Pyrido[3,4-*b*]pyrazin-7-ylcarbamates, and Ethyl Pyrido[3,4-*e*]-*as*-triazin-7-ylcarbamates. *J. Med. Chem.* **1990**, *33*, 3044-3050.

(7) Balenonic, K.; Cerar, D.; Filipovic, L. Synthesis of Aminoalkylglyoxal Derivatives. II. Aminoalkylglyoxal Derivatives of L-Alanine, β-Alanine and L-Tyrosine. *J. Org. Chem.* **1953**, *18*, 868-871 and references therein.

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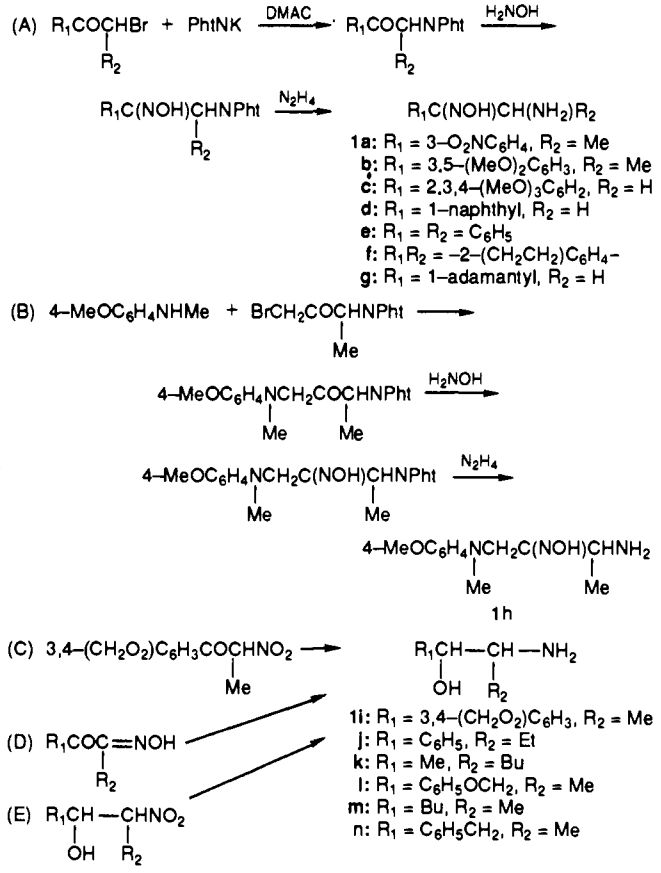
Scheme I



of the unusual biological activity that was observed for these agents, which structurally resemble the pteridine antifolates, additional compounds were prepared for structure–activity relationship (SAR) studies. Biological data for some of the target compounds have been published and are presented in this report for comparison with unreported data.[1]

Chemistry

The α-amino ketone oxime (**1a–h**) and α-amino alcohol (**1i–n**) side chains that were required for the synthesis of the target 1,2-dihydropyrido[3,4-*b*]pyrazines **6** were prepared by a variety of methods. Reaction of α-halo ketones with potassium phthalimide gave α-phthalimido ketones, which were converted to oximes with hydroxylamine prior to removal of the phthaloyl group with hydrazine to give the desired α-amino ketone oximes **1a–g** (eq A, Scheme I).[2,3] In a variation of this approach, 1-bromo-3-phthalimidobutan-2-one was reacted with *N*-methyl-*p*-anisidine to give the intermediate α,α′-diamino ketone (eq B, Scheme I). The latter was converted to the corresponding oxime followed by removal of the phthaloyl blocking group to afford α,α′-diamino ketone oxime **1h**.

Three routes were investigated for the preparation of α-amino alcohols. Reaction of *N*-piperonylimidazole with nitroethane in DMSO in the presence of NaH gave the α-nitro ketone, which was hydrogenated over palladium to provide a poor overall yield of **1i** (eq C, Scheme I).[8,9] A second method, the reduction of α-oximino ketones with $LiAlH_4$, gave good overall yields of 2-amino-1-phenylbutanol (**1j**)[10] and 3-amino-2-heptanol (**1k**) (eq D, Scheme I). Another excellent method for the preparation of α-amino alcohols resulted from the condensation of aliphatic and aromatic aldehydes with excess nitroethane in the presence of alumina-supported potassium fluoride[11] followed by reduction of the intermediate α-nitro alcohols with zinc and sulfuric acid.[12] These transformations are fast and afforded acceptable yields of the α-amino alcohols **1l–m** (eq E, Scheme I). The properties of the new compounds are listed in Table I.

Table I. α-Amino Ketone Oximes and α-Amino Alcohols

$R_1C(NOH)CH(NH_2)R_2$ **1a–h** $R_1CH(OH)CH(NH_2)R_2$ **1i–n**

compd	% yield[a]	mp, °C	mass spectrum,[b] *m/e*	formula	anal.
1a	43	130–4	209 $(M)^+$	$C_9H_{11}N_3O_3$	C, H, N
1b	26	83–90	224 $(M)^+$	$C_{11}H_{16}N_3O_3 \cdot 0.16CH_3CH_2OH$[c]	C, H, N
1c	22	124–30		$C_{11}H_{16}N_2O_4$	C, H, N
1d	53	105–18		$C_{12}H_{12}N_2O \cdot 0.4H_2O$	C, H, N
1e	27	90–100[d]	226 $(M)^+$	$C_{14}H_{14}N_2O \cdot 0.6H_2O$	C, H, N
1f	12[e]	109–11	–	–	–
1g	25	146–51	208 $(M)^+$	$C_{12}H_{20}N_2O \cdot 0.6H_2O$	C, H, N
1h	62	149–52[f]		$C_{12}H_{19}N_3O_2 \cdot HCl \cdot 0.22H_2O$	C, H, N
1i	7	172–5	195 $(M)^+$	$C_{10}H_{13}NO_3 \cdot 1.4HCl$	C, H, N
1k	80[e]	oil	132 $(M+1)^+$	–	–
1l	21	85–103	182 $(M+1)^+$	$C_{10}H_{15}NO_2$	C, H, N
1m	29	oil	132 $(M+1)^+$	$C_7H_{17}NO \cdot 0.3H_2O$	C, N, H[g]
1n	77[e]	oil	–	–	–

[a] Overall yield from α-halo ketone or commercially available reagent. [b] See the introduction to the Experimental Section. [c] ^1H NMR spectrum showed CH_3CH_2OH (δ, 1.06, 3.45). [d] Resolidified and remelted at 125–8 °C. [e] Crude product (TLC) used directly. [f] Decomposition. [g] Calcd, 12.98; found, 12.55.

The alkylation of α-amino ketone oximes (**1a–h**) with 4-chloro-5-nitropyridine **2** gave ketone oximes **3** (Table II), which were hydrogenated over Raney nickel to give directly target compounds **6** (Table III).[2,3] In the second route, α-amino alcohols (**1i–n**) were reacted with **2** to give alcohols **5** (Table II). Oxidation of **5** with the chromium trioxide–pyridine reagent afforded ketones **4**, which also underwent reductive cyclization to **6** when hydrogenated in the presence of Raney nickel.[13] In the synthesis of **6e**, oxime **3e** was hydrolyzed to ketone **4e** under acidic conditions prior to reductive cyclization. No difficulties were encountered in the simultaneous reduction of the two nitro groups of **3a** to give the desired 3-(3-aminophenyl) analogue **6o**. The latter was acylated with 4-azido[^{14}CO]-

(8) Crumbie, R. L.; Nimitz, J. S.; Mosher, H. S. α-Nitro Ketones and Esters from Acylimidazoles. *J. Org. Chem.* **1982**, *47*, 4040–4045.
(9) Baker, D. C.; Putt, S. R. C-Alkylation of Nitromethane. A Synthetic Route to α-Nitroketones. *Synthesis* **1978**, 478–479.
(10) Oppong-Boachie, F. K. Non-Catalytic Reduction of α-Oximinoketones and α-Oximinoalcohols to *erythro*-2-Amino-1-Arylalkan-1-ols. *Bull. Soc. Chem. Belg.* **1985**, *94*, 293–298.
(11) Melot, J.-M.; Texier-Boullet, F.; Fucaud, A. Preparation and Oxidation of α-Nitro Alcohols with Supported Reagents. *Tetrahedron Lett.* **1986**, *27*, 493–496; Yamawaki, J.; Kawate, T.; Ando, T.; Hanafusa, T. Potassium Fluoride on Alumina. An Efficient Solid Base for Elimination, Addition, and Condensation. *Bull. Soc. Chem. Jpn.* **1983**, *56*, 1885–1886.
(12) Hoover, F. W.; Hass, H. B. Synthesis of 2-Amino-1-phenyl-1-propanol and Its Methylated Derivatives. *J. Org. Chem.* **1947**, *12*, 506–509.
(13) Temple, C., Jr.; Rener, G. A. New Anticancer Agents: Chiral Isomers of Ethyl 5-Amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazine-7-ylcarbamate. *J. Med. Chem.* **1989**, *32*, 2089–2092.

Table II. Ethyl [4-(Substituted amino)-6-amino-5-nitropyridin-2-yl]carbamates (3–5)

compd	reaction time, h	% yield	mp, °C	mass[a] spectrum, *m/e*	formula	anal.
3a	21[b]	68	171–5[c]	433 $(M)^+$	$C_{17}H_{19}N_7O_7$	C, H, N
3b	4	56	203–4	448 $(M)^+$	$C_{19}H_{24}N_6O_7$	C, H, N
3c	1.5	50	208–10	464 $(M)^+$	$C_{19}H_{24}N_6O_3$	C, H, N
3d	1.5	27	206–9	424 $(M)^+$	$C_{20}H_{20}N_6O_5 \cdot 0.2H_2O$	C, H, N
3e	2.5	33	~140[d]	450 $(M)^+$	$C_{22}H_{22}N_6O_5 \cdot 1.5HCl$	C, H, N
3f	4	37	234–6	400 $(M)^+$	$C_{18}H_{20}N_6O_5 \cdot 0.2CH_3CH_2OH$[e]	C, H, N
3g	1	65	213–5	432 $(M)^+$	$C_{20}H_{28}N_6O_5 \cdot 0.15HCl$	C, H, N
3h	16	93	>80[c,f]	462 $(M+1)^+$	$C_{20}H_{27}N_7O_6 \cdot H_2O \cdot 2HCl$	C, H, N
4e	*g*	56	193–4	435 $(M)^+$	$C_{22}H_{21}N_5O_5 \cdot 0.5H_2O$	C, H, N
4i	1	89	197–8[d]	418 $(M+1)^+$	$C_{18}H_{19}N_5O_7 \cdot 0.2H_2O$	C, H, N
4j	1	79	165–7	388 $(M+1)^+$	$C_{18}H_{21}N_5O_5$	C, H, N
4k	2	68	164–6	354 $(M+1)^+$	$C_{15}H_{23}N_5O_5 \cdot 0.2H_2O$	C, H, N
4l	1	51	137–9	404 $(M+1)^+$	$C_{18}H_{21}N_5O_6$	C, H, N
4m	1.5	60	85–6	354 $(M+1)^+$	$C_{15}H_{23}N_5O_5 \cdot 0.2H_2O$	C, H, N
4n	2	45	152–4[h]	388 $(M+1)^+$	$C_{18}H_{21}N_5O_5 \cdot 0.1CH_3(CH_2)_5CH_3$[e] $\cdot 0.3H_2O$	C, H, N
5i	43	57	>215[c]	419 $(M)^+$	$C_{18}H_{21}N_5O_7 \cdot 0.2CHCl_3$[e] $\cdot H_2O$	C, H, N
5j	24	72	182–4	390 $(M+1)^+$	$C_{18}H_{23}N_5O_5$	C, H, N
5k	23	46	124–9	356 $(M+1)^+$	$C_{15}H_{25}N_5O_5$	C, H, N
5l	23	77	155–9	406 $(M+1)^+$	$C_{18}H_{23}N_5O_6 \cdot 0.1H_2O$	C, H, N
5m	21	81	75–9	356 $(M+1)^+$	$C_{15}H_{25}N_5O_5 \cdot 0.3H_2O$	C, H, N
5n	26	40	*i*	390 $(M+1)^+$	$C_{18}H_{23}N_5O_5$	C, H, N

[a] See the introduction to the Experimental Section. [b] Reaction solvent, 2-propanol. [c] With foaming. [d] With decomposition. [e] ^1H NMR spectrum showed ethanol, δ 1.06, 3.45 (**3f**); heptane, δ 0.85, 1.24 (**4n**); $CHCl_3$, δ 8.31 (**5i**). [f] Determined on free base. [g] See the Experimental Section. [h] With softening at 65 °C and crystallization at 75 °C. [i] Indefinite with gradual softening from 65 °C.

Table III. Ethyl (5-Amino-1,2-dihydropyrido[3,4-*b*]pyrazin-7-yl)carbamates

compd	reaction solvent	reaction time, h	% yield	mp, °C	mass[a] spectrum, *m/e*	^1H NMR[b] 2-CH_2CH_2, δ	formula	anal.
6b	HOAc	14	86	241–6[c]	385 $(M)^+$	5.07 m	$C_{19}H_{23}N_5O_4 \cdot HCl$	C, H, N
6c	HOAc	14	61	190–3	401 $(M)^+$	4.17 s	$C_{19}H_{23}N_5O_5$	C, H, N
6d	HOAc	11	59	163–5	361 $(M)^+$	4.34 s[d]	$C_{20}H_{19}N_5O_2 \cdot 0.4CH_3CO_2H \cdot 0.2HCl$	C, H, N
6e	EtOH	24	74[e]	242–7[c]	387 $(M)^+$	6.19 s[d]	$C_{22}H_{21}N_5O_2 \cdot CH_3CH_2OH \cdot 1.5HCl$	C, H, N
6f	HOAc	8	96	266–9	337 $(M)^+$	4.48 m	$C_{18}H_{19}N_5O_2 \cdot HCl$	C, H, N
6g	HOAc	12	46	261–71[c]	369 $(M)^+$	4.10 s	$C_{20}H_{27}N_5O_2 \cdot H_2O \cdot 1.3HCl$	C, H, N
6h	EtOH	66	31	>70[c]	398 $(M)^+$	4.05 m	$C_{20}H_{26}N_6O_3 \cdot 0.4H_2O$	C, H, N
6i	dioxane	10[f]	65	>259[c]	370 $(M+1)^+$	7.74 m[d]	$C_{18}H_{19}N_5O_4 \cdot 0.3CH_3CH_2OH$	C, H, N
6j	dioxane	10[f]	61	>270[c]	340 $(M+1)^+$	4.64 m	$C_{18}H_{21}N_5O_2 \cdot 0.2H_2O$	C, H, N
6k	EtOH	6[g]	46	152–5[h]	306 $(M+1)^+$	3.95 br	$C_{15}H_{23}N_5O_2 \cdot 0.5H_2O$	C, H, N
6l	EtOH	5.5	80	169–71[c]	356 $(M+1)^+$	4.16 m[d]	$C_{18}H_{21}N_5O_3 \cdot 0.2CH_3CH_2OH$	C, H, N
6m	EtOH	5[i]	47	>300[j]	306 $(M+1)^+$	3.94 m	$C_{15}H_{23}N_5O_2$	C, H, N
6n	EtOH	3.5[k]	77	168–71[h]	340 $(M+1)^+$	3.87 m	$C_{18}H_{21}N_5O_2$	C, H, N
6o	EtOH	3.5[k]	36	280[c]	341 $(M+1)^+$	4.66 m[d]	$C_{17}H_{20}N_6O_2 \cdot 0.2CH_3CH_2OH$	C, H, N
6p	–	*l*	56[m]	>130[c,n]	486 $(M+1)^+$	4.78 m[d]	$C_{24}H_{23}N_9O_3 \cdot 0.3CHCl_3$	C, H, N

[a] See the introduction to Experimental Section. [b] Chemical shift determined in Me_2SO-d_6. [c] Decomposition. [d] ^1H NMR spectrum showed acetic acid, δ 1.92 (**6d**); ethanol, δ 1.06, 3.45 (**6e,i,l,o**); $CHCl_3$, 8.31 (**6p**). [e] From ketone **4e**. [f] One hour at room temperature followed by 9 **h** at 60 °C. [g] Two hours at room temperature followed by heating at 60 °C for 4 h. [h] With foaming. [i] One and one-half hours at room temperature followed by heating at 60 °C for 3.5 h. [j] With gradual decomposition from 230 °C. [k] One and one-half hours at room temperature followed by heating at 60 °C for 2 h. [l] See the Experimental Section. [m] Overall yield from 4-aminobenzoic acid. [n] Nonradioactive.

benzoyl chloride[14] to give **6p**, which contains both radio- and photoaffinity labels.

Discussion

The target compounds were evaluated for cytotoxicity and antimitotic activity in cultured lymphoid leukemia L1210 cells[4] and for antitumor activity in mice implanted with lymphocytic P388 cells.[15]

The 3-aminophenyl compound **6o** and nonradioactive 3-(4-azidobenzoyl)aminophenyl derivative **6p** were active in these assays (Table IV). Although the in vitro activity of **6p** was reduced relative to that of **6o**, experiments were performed to determine the degree of binding of radiolabeled **6p** to tubulin. The formation of a covalent complex was attempted by exposure of [^{14}C]-**6p** to ultraviolet light (>300 nm) in the presence of pig brain tubulin.[4] The experiments were unsuccessful in that only minimal amounts of ^{14}C-label were covalently bound to the tubulin (e.g., 0.02 mol of [^{14}C]-**6p**/mol of tubulin).

In other studies directed toward the determination of the nature of binding of these agents to tubulin, MM2[16] in Macromodel[17] showed that the pyridine, pyrazine, and phenyl rings of **6q** were essentially coplanar for the conformation of minimum energy. In the 2-methyl derivative **6r**, the pyrazine ring was nonplanar with a pucker of about 7°. In addition the phenyl ring was rotated away from the

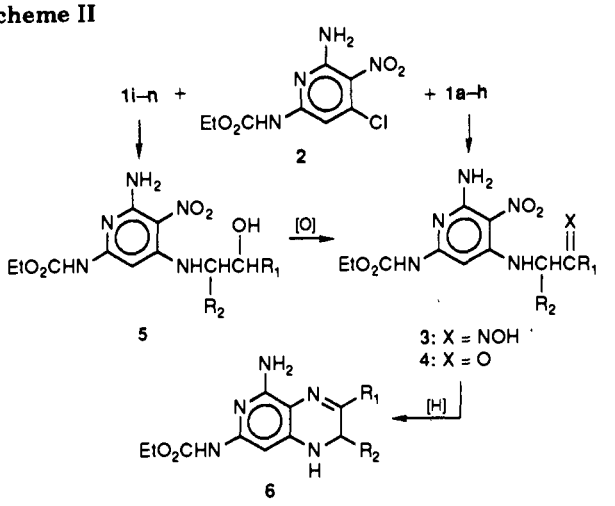
(14) Hixson, S. H.; Hixson, S. S. *p*-Azidophenacyl Bromide, a Versatile Photolabile Bifunctional Reagent. Reaction with Glyceraldehyde-3-phosphate Dehydrogenase. *Biochemistry* **1975**, *14*, 4251–4254.

(15) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems. *Cancer Chemother. Rep. Part 3* **1972**, *3*, 9.

(16) Allinger, N. L. Conformational Analysis. 130. MM2. A Hydrocarbon Force Field Utilizing V_1 and V_2 Torsional Terms. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134.

(17) Still, W. C.; MacPherson, L. J.; Harada, T.; Callahan, J. F.; Rheingold, A. L. Macrocycles in the Construction of Acyclic Stereochemistry. *Tetrahedron* **1984**, *40*, 2775–2281.

Scheme II



a: R_1 = 3–$O_2NC_6H_4$, R_2 = Me (no 6)
b: R_1 = 3,5–$(MeO)_2C_6H_3$, R_2 = Me
c: R_1 = 2,3,4–$(MeO)_3C_6H_2$, R_2 = H
d: R_1 = 1–naphthyl, R_2 = H
e: R_1 = R_2 = C_6H_5
f: R_1R_2 = –2–$(CH_2CH_2)C_6H_4$–
g: R_1 = 1–adamantyl, R_2 = H
h: R_1 = 4–$MeOC_6H_4N(Me)CH_2$, R_2 = Me
i: R_1 = 3,4–$(CH_2O_2)C_6H_3$, R_2 = Me
j: R_1 = C_6H_5, R_2 = Et
k: R_1 = Me, R_2 = Bu
l: R_1 = $C_6H_5OCH_2$, R_2 = Me
m: R_1 = Bu, R_2 = Me
n: R_1 = $C_6H_5CH_2$, R_2 = Me
o: R_1 = 3–$H_2NC_6H_4$, R_2 = Me
p: R_1 = 3–(4–$N_3C_6H_4CO)NHC_6H_4$, R_2 = Me
q: R_1 = C_6H_5, R_2 = H
r: R_1 = C_6H_5, R_2 = Me
s: R_1 = 4–$MeOC_6H_4N(Me)CH_2$, R_2 = Me
t: R_1 = 4–$MeOC_6H_4$, R_2 = Me
u: R_1 = 3,4,5–$(MeO)_3C_6H_2$, R_2 = H
v: R_1 = 2–naphthyl, R_2 = H

2-methyl group to give a dihedral angle of 22.4° between the phenyl ring and the pyrazine ring. The greater potency of **6r** relative to **6q** suggested that the binding of **6r** to tubulin might be facilitated by the nonplanar conformation of the three rings. Of interest, the efficient binding of combretastatin A4 and amphethinile to the colchicine-binding site(s) of tubulin has been attributed, in part, to the angular bicyclic structures of these agents.[18]

The above discussion suggests that the phenyl ring might assume a preferred conformation on binding with tubulin. In contrast to the differences in the in vitro activities of **6q** and **6r**, substitution of a methyl group at the 2-position of 3-(anilinomethyl) compound **6s** to give **6h** had no pronounced effect on in vitro activity. Presumably the presence or absence of a 2-methyl group has no effect on the conformation of the phenyl ring because of the aminomethyl linkage between the phenyl and pyrazine rings. The effects of other alterations at the 2-position of the 3-phenyl series were variable. 2-Ethyl derivative **6j** exhibited activity similar to that of **6r**, whereas, activity was reduced in both 2-phenyl compound **6e** and compound **6f** in which the 2-positions of both the pyrazine and phenyl rings were linked with an ethylene bridge. Presumably the modifications in **6e** and **6f** afforded an unfavorable conformation of the 3-phenyl ring.

In previous SAR studies, the presence of electron-withdrawing groups in the 3-phenyl ring reduced activity, whereas electron-donating groups either maintained or slightly increased activity. Relative to the activity observed with **6r** and **6t**, no significant change in in vivo activity was observed for the derivatives in which the phenyl ring was substituted with 3-amino (**6o**), 3,5-dimethoxy (**6b**), and 3,4-methylenedioxy (**6i**) groups. Of interest, 3-(2,3,4-trimethoxy)phenyl compound **6c** was more potent than 3-(3,4,5-trimethoxyphenyl) compound **6u** in vitro, but both compounds gave about the same increase in life span in vivo. In contrast a significant difference in activity was observed for the 1- and 2-naphthyl compounds **6d** and **6v**. Both were less active in vitro than **6q** and only the 2-naphthyl derivative **6v** showed in vivo activity.

Also, alterations in the linkage between the pyrazine and phenyl rings afforded compounds (**6h,l,n**) that were less cytotoxic than **6r** in vitro. These compounds gave similar increases in life span in vivo although the derivatives with spacer groups required higher doses than **6r**. In other studies the effect on activity of replacement of the 3-phenyl group with an aliphatic moiety was investigated. Both the 3-methyl (**6k**) and 3-butyl (**6m**) compounds were less active than **6r** and showed only minimal activity in vivo, whereas, 3-(1-adamantyl) compound **6g** was completely inactive.

In summary, these results confirm previous work in which the cytotoxicity and the mitotic index values are correlated, but these values are poorly correlated with the increase in life span.[1] Although a similar increase in life span (%ILS) was observed for the most cytotoxic compounds (groups **6b,i** and **6j,o,r,t**), wide variations between cytotoxicity and %ILS were observed for the less potent compounds (groups of **6f,h,q**; **6e,s,v**; **6c,m**; **6d,p**; **6k,l,n,u**). Under the conditions of these evaluations, overall activity was decreased by a spacer group between the pyrazine and phenyl rings (**6h,l,n,s**), by bulky groups in either the pyrazine or phenyl rings (**6d,p**), by replacement of the 3-aryl

Table IV. Biological Activity of Compounds

compd	L1210 IC_{50},[a] nM	L1210 $MI_{0.5}$,[b] nM	P388:[c] 10^6 tumor cell implant (ip) dose,[d] mg/kg	%ILS[e]
6b	2.8	5.6	16	60
6c	11	13	25	76
6d	14.5	9.5	10	0
6e	8.5	19	6	30
6f	4.7	25.5	15	3
6g	500	1100	100	0
6h	5.5	10.5	48	53
6i	1.3		1	63
6j	0.46		0.5	77
6k	66	210	50	30
6l	50		60	77
6m	10.3		50	27
6n	46	59	50	65
6o	0.21	0.2	1.5	71
6p	17	9.2	6	63
6q	4.7	2.8	2[f]	51
6r	0.2	0.58	1	71
6s	7.9	15	3[f]	37[g]
6t	0.48	0.42	1	69
6u	78	~350	100	63
6v	6.0	17	10	66

[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 (number 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] Treatment was ip on days 1–5. [e] Increase in life span at the highest nontoxic dose for each agent, which was evaluated over a range of doses. [f] Schedule: days 1–9. [g] Activity was greater on a single-dose schedule (ref 3).

(18) McGown, A. T.; Fox, B. W. Structural and Biochemical Comparison of the Anti-mitotic Agents Colchicine, Combretastatin A-4 and Amphethimide. *Anti-Cancer Drug Des.* **1989**, *3*, 249–254.

group with an aliphatic group (**6g,k,m**), and by a fixed orientation of the phenyl ring (**6f**). Activity was either maintained or increased by a 2-alkyl group (**6j,r**) and by electron-donating substituents on the 3-aryl group (**6b,-i,o,t**). The participation of the 3-substituent of these agents in their interaction with tubulin would require that the aryl binding domain be narrow in width and somewhat open in length.

Experimental Section

Melting and decomposition temperatures were determined in capillary tubes in a Mel-Temp apparatus. The ^1H 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. The α-halo ketone precursors either were commercially available (desyl chloride, 1-adamantyl bromomethyl ketone) or were prepared by literature procedures (2-bromo-3′-nitropropiophenone,[19] 2-bromo-3′,5′-dimethoxypropiophenone;[20] 2-bromo-2′,3′,4′-trimethoxyacetophenone,[21] 2-bromo-3,4-dihydro-1(2*H*)-naphthalenone,[22] bromomethyl 1-naphthyl ketone,[23] and racemic 1-bromo-3-phthalimidobutan-2-one.[7] For the α-amino alcohols, 2-amino-1-phenylbutanol (**1j**) was prepared by the reported method,[10] and the others were prepared from readily available reagents as described below. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical value.

Typical Procedures for the Preparation of α-Amino Ketone Oximes (1a–g, Table I). I. 2-Amino-3′-nitropropiophenone (1a). To a stirred suspension of potassium phthalimide (4.90 g, 26.5 mmol) in *N,N*-dimethylacetamide (25 mL) at 0 °C was added 2-bromo-3′-nitropropiophenone (6.20 g, 24.0 mmol).[19] After stirring for 0.5 h at 0 °C and 2 h at room temperature, the nearly clear reaction mixture was poured into H_2O (150 mL) to give a gummy precipitate. This residue was separated by decantation and dissolved in $CHCl_3$ (250 mL), and the resulting solution was washed with 0.5 N NaOH (60 mL) and H_2O (50 mL). The organic layer was dried (Na_2SO_4) and evaporated to give a yellow oil, which solidified on drying in vacuo (P_2O_5) to give crude 3′-nitro-2-phthalimidopropiophenone: yield, 7.2 g.

Complete reaction of potassium phthalimide with 2-bromo-3,4-dihydro-1(2*H*)-naphthalenone and desyl chloride required warming at 50 °C for 6 h.

A suspension of crude 3′-nitro-2-phthalimidopropiophenone (7.2 g) and hydroxylamine hydrochloride (3.1 g, 44 mmol) in 4:1 ethanol–pyridine (135 mL) was refluxed for 5 h, and the clear solution was evaporated under high vacuum to give a semisolid residue. The latter was dissolved in hot EtOH (20 mL) and diluted with hot H_2O (225 mL) to deposit an oil. A solution of the oil in EtOH (25 mL) deposited 3′-nitro-2-phthalimidopropiophenone oxime contaminated with phthalimide (~10%): yield, 5.28 g; MS-FAB *m/e* 340 $(M + 1)^+$.

Many of the conversions of the ketones to the oximes required a reflux period of 10–20 h as determined by TLC.

A solution of crude 3′-nitro-2-phthalimidopropiophenone oxime (2.94 g) in warm (70 °C) EtOH (120 mL) was treated dropwise with a solution of anhydrous hydrazine (0.294 g, 10.5 mmol) in EtOH (5 mL) and the resulting solution was stirred at 40 °C for 21 h. The reaction mixture was cooled to 5 °C, treated with 1 N HCl (9.9 mL), and stirred for 1 h at 0–5 °C. The precipitate of phthalic acid hydrazide was removed by filtration, the residue was washed with 1:1 EtOH–H_2O (12 mL), and the combined filtrate and wash were evaporated in vacuo to give an off-white semisolid. This residue was extracted with warm H_2O, and the extract was cooled to 0–5 °C and adjusted to pH 10 with 1 N NaOH. The resulting white precipitate of **1a** was collected by filtration, washed with H_2O, and dried in vacuo over P_2O_5: yield, 638 mg. An additional amount (920 mg, 51%) of slightly impure product was obtained by extraction of the basic filtrate with EtOAc (3 × 75 mL).

II. 3-Amino-1-[*N*-(4-methoxyphenyl)-*N*-methylamino]-butan-2-one Oxime (1h). A solution of racemic 1-bromo-3-phthalimidobutan-2-one (8.50 g, 28.8 mmol)[7] and *N*-methyl-*p*-anisidine (3.95 g, 28.8 mmol) in DMF (100 mL) containing $NaHCO_3$ (2.42 g, 28.8 mmol) was heated with stirring at 60 °C for 4 h. The reaction mixture was cooled to room temperature and diluted dropwise with H_2O (115 mL) over 30 min. The resulting mixture was cooled to 15 °C and the precipitate was collected by filtration, washed with 1:1 H_2O–DMF (10 mL) and H_2O (50 mL), and dried in vacuo (P_2O_5). A mixture of the resulting crude 1-[*N*-(4-methoxyphenyl)-*N*-methylamino]butan-2-one (9.57 g, ~94% yield) and hydroxylamine hydrochloride (2.83 g, 40.8 mmol) in 1:1 EtOAc–pyridine (110 mL) was refluxed with stirring for 5 h and evaporated to dryness in vacuo. The residue was washed with cold H_2O (3 × 25 mL), and then triturated with cold EtOH (40 mL) to crystallize the butan-2-one oxime: yield, 8.26 g (~83%). To the crude oxime in EtOH (330 mL) at 70 °C was added over 20 min a solution of 95% hydrazine (0.834 g, 24.8 mmol) in EtOH (20 mL) and the reaction temperature was lowered to 45 °C and maintained at this temperature for 16 h. The reaction mixture was cooled in an ice bath and treated with 1 N HCl (25 mL). The precipitate was removed by filtration and washed with 1:1 H_2O–EtOH (30 mL), and the combined filtrate and wash were evaporated to dryness in vacuo. This residue was extracted with warm H_2O (3 × 40 mL), and the combined extract was adjusted to pH 10.5 with 1 N NaOH and extracted with EtOAc (2 × 200 mL, Na_2SO_4). The combined extract was evaporated to dryness; the residue was suspended in 9:1 Et_2O–EtOH (100 mL) and acidified with ethanolic HCl to precipitate the hydrochloride of **1h**: yield, 4.96 g.

Typical Procedures for the Preparation of α-Amino Alcohols (1i–n, Table I). I. 2-Amino-1-[3′,4′-(methylenedioxy)phenyl]-1-propanol (1i). Solid NaH (2.11 g, 52.8 mmol of 60% oil dispersion, twice washed with hexane) was treated with dry DMSO (80 mL), followed by the dropwise addition of dry nitroethane (15.5 g, 206 mmol). A solution of piperonylimidazole (prepared from 8.10 g of piperonyl chloride and 5.98 g of imidazole)[8] in dry DMSO (80 mL) was added dropwise to the nitroethane solution, and the thick mixture was stirred for 18 h at room temperature. The reaction mixture poured into a solution of HOAc (2.91 g, 48.4 mmol) in ice–water (200 mL), which was extracted with CH_2Cl_2 (4 × 400 mL). The combined extracts were washed with H_2O (3 × 400 mL) and saturated NaCl (700 mL), dried ($MgSO_4$), and evaporated at reduced pressure to give 4.90 g of semisolid residue. The residue was extracted with $CHCl_3$ (40 mL), and piperonylic acid (1.25 g) was removed by filtration. The filtrate was purified by flash chromatography ($CHCl_3$, 150 g silica gel) to afford the nitro ketone as a pale-yellow oil: yield, 2.10 g (21%); MS-EI *m/e* 223 $(M)^+$.

A solution of the nitro ketone (490 mg, 2.20 mmol) in EtOH (24 mL) containing concentrated HCl (0.52 mL) was hydrogenated at atmospheric pressure in the presence of 10% Pd/C (107 mg). After stirring for 20 h, more catalyst (200 mg) was added and stirring was continued for another 4 h, resulting in a total uptake of 71% of the theoretical amount. The catalyst was removed by

(19) Baker, J. W. Reactions of ω-Substituted Acetophenone Derivatives. Part II. The Mechanism of the Interaction of ω-Halogenoacetophenones with Primary and Tertiary Bases. *J. Chem. Soc.* **1932**, 1148–1157.

(20) Frei, H.; Schmid, H. *Justus Liebigs Ann. Chem.* **1957**, *603*, 169–177.

(21) Horton, W. J.; Thompson, G. Seven-membered Ring Compounds. VII. 7-Acetamidobenzosuberone and α-Amino-γ-3,4,5-trimethoxyphenylbutyric Acid. *J. Am. Chem. Soc.* **1954**, *76*, 1909–1911.

(22) Barfknecht, C. F.; Rusterholz, D. B.; Parsons, J. A. Inhibition of Prolactin by Ergoline Congeners. *J. Med. Chem.* **1974**, *17*, 308–312.

(23) Shevchuk, M. I.; Dombrovskii, A. V. The Preparation of α-Monobromomethyl Aryl Ketones by Bromination of Methyl Aryl Ketones in Dioxane. *J. Gen. Chem. USSR (Engl. Transl.)* **1963**, *33*, 1118–1119.

filtration (Celite), and the filtrate was evaporated at reduced pressure to give a colorless oil. A solution of this residue in 1 N ethanolic HCl (5 mL) was diluted dropwise with Et_2O (75 mL) to deposit **1i**·HCl as a white solid: yield, 176 mg (32%); mp 172–5 °C dec; MS-EI *m/e* 195 $(M)^+$. In another experiment (with 1.55 g of nitro ketone), the use of a greater amount of catalyst (1.55 g) and acid (1.64 mL of concentrated HCl plus 6.5 mL of 10.6 N ethanolic HCl) resulted in the formation of NH_4Cl as a contaminant (C, H, N, NMR).

II. 3-Amino-2-heptanol (1k). The procedure of Oppong-Boachie[10] for the synthesis of 2-amino-1-phenylbutanol (**1j**) was followed. To a stirred suspension of $LiAlH_4$ under N_2 (3.33 g, 87.7 mmol) in anhydrous Et_2O (70 mL) was added dropwise over 0.5 h a solution of 3-oximino-2-heptanone (6.19 g, 43.2 mmol)[24] in anhydrous Et_2O (70 mL). The refluxing reaction mixture was heated for an additional 1 h, diluted with Et_2O (100 mL), cooled to 0–5 °C, and then treated dropwise with H_2O (70 mL). After 0.5 h at 0–5 °C, the organic layer was separated, dried ($MgSO_4$), and treated with excess ethanolic HCl (8.0 mL of 7 N) to deposit an oil. The solvents were evaporated at reduced pressure, and the residue was dried in vacuo (P_2O_5) to afford the crude hydrochloride of **1k** as an oil: yield, 5.78 g.

III. Compounds 1l–n: 3-Amino-1-phenoxy-2-butanol (1l). To a stirred solution of 1:1 phenoxyacetaldehyde–benzyl alcohol (4.43 g, 32.5 mmol) in nitroethane (24 mL) was added KF–Al_2O_3[11] (18.1 g), and the resulting suspension was stirred for 1 h at room temperature. The reaction mixture was diluted with Et_2O (100 mL), the catalyst was removed by filtration and washed with Et_2O, and the combined filtrate and wash were evaporated at reduced pressure to remove Et_2O and under high vacuum to remove excess nitroethane. The resulting crude nitro alcohol was dissolved in 95% EtOH (50 mL) containing the zinc dust (13.9 g), followed by the dropwise addition over 0.5 h of a solution of concentrated H_2SO_4 (13 mL) in H_2O (53 mL). During the addition, the temperature was maintained at 45–55 °C with the aid of an ice bath. After stirring for 18 h at room temperature, the precipitate was removed by filtration, and washed with EtOH (2 × 50 mL) and H_2O (2 × 50 mL). The combined filtrate and washes were concentrated at reduced pressure to remove most of the EtOH, and the resulting suspension was washed with Et_2O (300 mL) to remove nonbasic impurities. The aqueous layer was made strongly basic with 50% NaOH and extracted with Et_2O (2 × 250 mL). The combined organic layers were dried and evaporated to a semisolid, which was purified by column chromatography (150 g, $CHCl_3$–MeOH, gradient, 25% → 100% MeOH) to afford slightly impure **1l** as a white solid: yield, 920 mg. Additional fractions were pooled to afford the analytical sample: yield, 287 mg.

Crude **1n** was used directly without purification.

Typical Procedure for the Preparation of 4-[(2-Oxoethyl)amino]pyridine Oximes (3, Table II). Ethyl [6-Amino-4-[[2-(3-nitrophenyl)-1-methyl-2-oxoethyl]amino]-5-nitropyridin-2-yl]carbamate Oxime (3a). A hot solution of crude **1a** (6.4 g), **2** (6.35 g, 24.4 mmol), and triethylamine (2.46 g, 24.4 mmol) in 2-propanol (100 mL) was refluxed for 23 h and evaporated to dryness at reduced pressure. The resulting yellow oil was washed by decantation with warm (80 °C) H_2O (100 mL) and recrystallized from EtOH to afford **3a**: yield, 6.68 g.

The remaining oximes were prepared in ethanol. Most of the oximes precipitated during the reaction and were collected by filtration and triturated with Et_2O (**3b,d**) or EtOH (**3c,f,g**). Compound **3e** was washed with 1 N HCl to remove unreacted **1e**. Purification of **3h** was effected by column chromatography (silica gel, $CHCl_3$). A solution of **3h** in $CHCl_3$ was treated with ethanolic HCl followed by Et_2O to give the hydrochloride.

Ethyl [6-Amino-4-[(1,2-diphenyl-2-oxoethyl)amino]-5-nitropyridin-2-yl]carbamate (4e). A solution of **3e**·EtOH·0.56HCl (0.60 g, 1.2 mmol) in 1:1 1 N HCl–dioxane (22 mL) was stirred at 45 °C for 72 h. The precipitate was collected by filtration and dissolved in EtOH and the solution was neutralized with Et_3N to afford the product: yield, 0.29 g.

Typical Procedure for Oxidation of 4-[(2-Hydroxyethyl)amino]pyridines (5i–n) to the Corresponding Ketones (4i–n, Table II). Ethyl [6-Amino-4-[(3-oxohept-2-yl)amino]-5-nitropyridin-2-yl]carbamate (4m). To a solution of dry pyridine (8.7 mL, 108 mmol) in dry CH_2Cl_2 (225 mL) was added CrO_3 (5.49 g, 54.9 mmol), and the mixture was stirred for 0.5 h to give a dark red solution. To this solution was added a solution of **5m** (2.87 g, 8.07 mmol) in dry CH_2Cl_2 (175 mL) and the mixture was stirred 1.5 h at room temperature. After filtration (Celite), the insoluble gummy residue was extracted with CH_2Cl_2 (100 mL), and the combined filtrate and wash were evaporated at reduced pressure. Toluene (100 mL) was evaporated from the residue to remove pyridine. This semisolid was dried in vacuo and extracted with $CHCl_3$ (100 mL) and the concentrated extract was purified by flash chromatography (125 g, $CHCl_3$) to afford **4m** as a yellow foam: yield, 1.70 g.

In the purification of **4n**, the product from the column remained a gum after trituration with heptane.

Typical Procedure for the Preparation of 4-[(2-Hydroxyethyl)amino]pyridines (5, Table II). Ethyl [6-Amino-4-[(1-ethyl-2-hydroxy-2-phenylethyl)amino]-5-nitropyridin-2-yl]carbamate (5j). A solution of **2** (5.21 g, 20.0 mmol), **1j** (4.71 g, 23.1 mmol),[10] and Et_3N (23.3 g, 231 mmol) in EtOH (75 mL) was refluxed for 24 h and evaporated at reduced pressure to give a semisolid residue. After washing with water (120 mL), the residue was dissolved in warm EtOH (50 mL) and added slowly to ice-cold water (450 mL) to give a yellow precipitate, which was recrystallized from EtOH (110 mL) to afford **5j**: yield, 4.94 g (63%).

Compound **5m** was purified by flash chromatography ($CHCl_3$ followed by 98:2 $CHCl_3$–MeOH).

Typical Procedure for the Preparation of 1,2-Dihydropyrido[3,4-*b*]pyrazin-7-ylcarbamates from Ketone Oximes (3a–d,f–h) or Ketones (4e,i–o).

Ethyl [5-Amino-1,2-dihydro-2-methyl-3-(phenylmethyl)pyrido[3,4-*b*]pyrazin-7-yl]carbamate (6n). A suspension of **4n** (480 mg, 1.19 mmol) in EtOH (75 mL) was hydrogenated at atmospheric pressure in the presence of Raney Ni (1.5 g, washed 3 × H_2O and 2 × EtOH). After 1.5 h at room temperature, the mixture was heated at 60 °C for 2 h, which resulted in a total uptake of 4.1 mmol of H_2. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The semisolid residue was dried in vacuo (P_2O_5) and triturated with EtOH (10 mL) to afford **6n** as a pale yellow solid: yield, 312 mg.

Compound **6k** was purified by trituration with EtOAc, **6l** by recrystallization from EtOH, and **6m** by reprecipitation from EtOH with H_2O.

Ethyl [5-Amino-3-[3-[(4-azido[^{14}CO]benzoyl)amino]-phenyl]-1,2-dihydro-2-methylpyrido[3,4-*b*]pyrazin-7-yl]-carbamate (6p). To a solution of **6o**·0.56$CHCl_3$ (180 mg, 0.44 mmol) and triethylamine (50 mg, 0.50 mmol) in dry CH_2Cl_2 (50 mL) was added a solution of *p*-azido[^{14}CO]benzoyl chloride (87 mg, 0.48 mmol)[14] in dry CH_2Cl_2 (10 mL). The deoxygenated (N_2) solution was allowed to stand under N_2 at 4 °C for 18 h and evaporated to dryness with a stream of N_2, and the residue was purified by trituration with water (10 mL) followed by column chromatography (37 g silica gel, $CHCl_3$–MeOH 99:1, deoxygenated) to afford **6p** as a glassy yellow solid: yield, 153 mg (61%); specific activity, ~5 μCi/mg. A similar procedure was used for the preparation of nonlabeled **6p**.

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(24) Ferris, A. F. α-Oximino Ketones. III. A New Synthesis of α-Amino Acids. *J. Org. Chem.* **1959**, *24*, 1726–1730.