Antimitotic Agents: Ring Analogues and Derivatives of Ethyl [(S)-5-Amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-b]pyrazin-7-yl]carbamate

Carroll Temple, Jr.* and Gregory A. Rener

Organic Chemistry Research Laboratories, Southern Research Institute, P.O. Box 55305, Birmingham, Alabama 35255-5305

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The synthesis of ring analogues and derivatives of the *S* isomer of ethyl [5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl]carbamate, (*S*)-1, a potent antimitotic agent with anticancer activity, was directed toward the determination of the contribution of several structural features of this compound to biological activity. Replacement of the 5-amino with a 5(6*H*)-oxo group and either transposing the 6-ring nitrogen to or incorporation of a ring nitrogen at the 8-position caused a significant decrease in in vitro activity and destroyed in vivo activity. Although in vivo cytotoxicity was reduced, in vivo activity at higher doses relative to (*S*)-1 was retained by replacement of the 5-amino group with hydrogen and by expansion of the 1,2-dihydropyrazine to give a dihydro-1,4-diazepine ring.

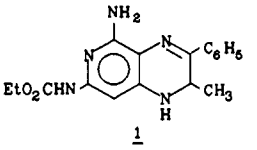
The 1,2-dihydropyrido[3,4-*b*]pyrazines have been identified as antimitotic agents with anticancer activity. With one of the most active compounds, ethyl [(*R*,*S*)-5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl]-carbamate (1), the *S*-isomer was significantly more potent than the *R*-isomer both in vitro and in vivo (Chart I).[1] In this report we describe our work on alterations of the structure of 1 at the 5-amino group and in the pyridine ring while retaining the *S*-configuration at the methyl position of the pyrazine ring. In addition an analogue was prepared in which the pyrazine ring was expanded to give a seven-membered ring.

Chemistry

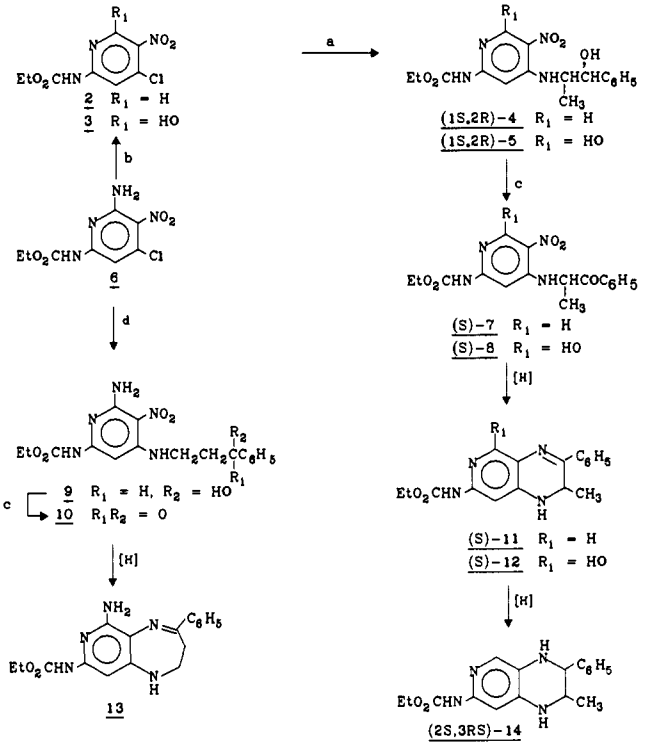
In the conversion of **6**[2] to **3** with isoamyl nitrite in the presence of aqueous H_2SO_4 as previously reported,[3] a minor product was identified as the deaminated pyridine **2** (Scheme I). Reaction of **2** and **3**, respectively, with (1*R*,2*S*)-(−)-norephedrine in refluxing ethanol in the presence of Et_3N afforded the pyridine alcohols (1*S*,2*R*)-**4** and (1*S*,2*R*)-**5**. The oxidation of (1*S*,2*R*)-**4** to give (*S*)-**7** was effected with CrO_3–pyridine as previously described for related compounds.[1] The hydrogenation of (*S*)-**7** in DMAC over Raney nickel gave (*S*)-**11**, whereas, hydrogenation in HOAc resulted in over-reduction to give a mixture of (*S*)-**11** and (2*S*,3*RS*)-**14**, which were separated by flash chromatography. For the oxidation of (1*S*,2*R*)-**5** to give (*S*)-**8**, excess pyridine was used to dissolve the 2-pyridinone. Also the hydrogenation of (*S*)-**8** to give (*S*)-**12** in DMAC required a second addition of Raney nickel to force the reaction to completion.

The preparation of the 1,4-diazepine analogue **13** was carried out by amination of **6** with 3-amino-1-phenylpropanol[4] to give **9**, oxidation of **9** with CrO_3 to give **10**, and hydrogenation of **10** over Raney nickel. Similar procedures were used for the conversion of **18**[2] via (1*S*,2*R*)-**20** and (*S*)-**21** to give (*S*)-**24**, and **19** via (1*S*,2*R*)-**22** and (*S*)-**23** to give (*S*)-**25** (Scheme II). The 4-chloropyrimidine **19** resulted from the reaction of the known 4,6-dichloropyrimidine **15**[5] with sodium azide to give **16**, reduction of the azido group to give **17**, which was followed by nitration of

Chart I



Scheme I [a]



[a] (a) (1*R*,2*S*)-(−)-$C_6H_5CH(OH)CH(NH_2)CH_3$; (b) isoamyl nitrite, H_2SO_4; (c) CrO_3; (d) $C_6H_5CH(OH)CH_2CH_2NH_2$.

(1) 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.

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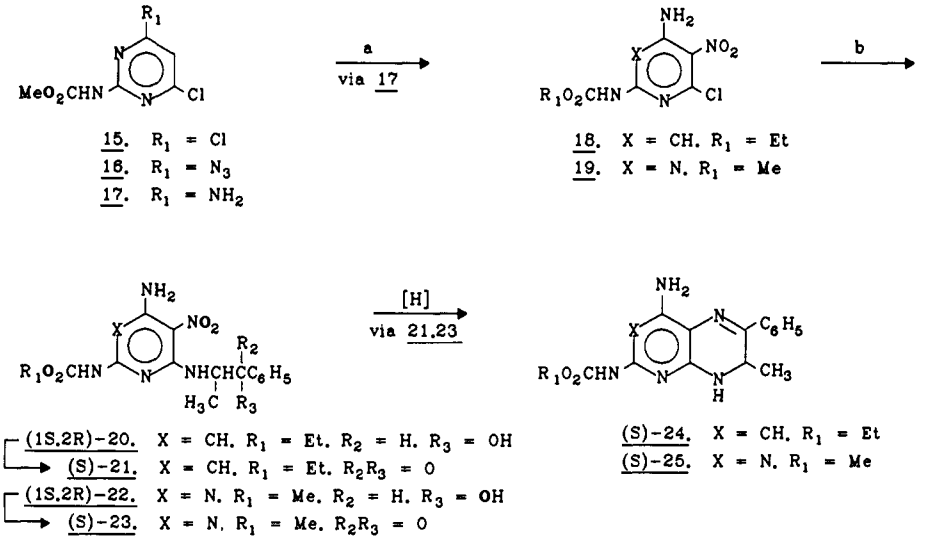
Scheme II [a]



[a] (a) HNO_3–H_2SO_4; (b) (1*R*,2*S*)-(−)-$C_6H_5CH(OH)CH(NH_2)CH_3$; (c) CrO_3.

Table I. Properties of Compounds

compound	reaction solvent	reaction time, h	yield, %	mp, °C	mass spectrum,[a] $(M+1)^+$	^1H NMR spectrum,[a] selected peaks, δ	formula	anal.
2	*a*	–	8.7	195–7	246	8.11 s (3-CH); 9.04 s (6-CH)	$C_8H_8N_3O_4Cl$	C,H,N
(1*S*,2*R*)-4	EtOH–Et_3N	8	65	198–201	361	7.46 s (3-CH); 8.89 s (6-CH)[b]	$C_{17}H_{20}N_4O_5$·0.1EtOAc	C,H,N
(1*S*,2*R*)-5	EtOH–Et_3N	20	19	245–8[c]	377	5.97 s (3-CH)	$C_{17}H_{20}N_4O_6$	H,N;C[d]
(*S*)-7	CH_2Cl_2–C_5H_5N	1.5	97	218–20[c]	359	5.59 m (C*H*NH); 9.03 d (CHN*H*)	$C_{17}H_{18}N_4O_5$	C,H,N
(*S*)-8	CH_2Cl_2–C_5H_5N[a]	2	39	>300[e]	375	5.52 m (C*H*NH); 9.91 d (CHN*H*)[f]	$C_{17}H_{18}N_4O_6$·0.4AcOH·0.8H_2O	C,H,N
9	EtOH–Et_3N	15	83	174–6	376	1.97 m (CHCH_2); 3.31 m (CH_2NH); 9.21 t (CH$_2$N*H*)	$C_{17}H_{21}N_5O_5$	C,H,N
10	CH_2Cl_2–C_5H_5N[g]	2	78	186–8[h]	374	3.48 t (CH_2CO); 3.59 m (CH_2NH); 9.10 t (CH$_2$N*H*)	$C_{17}H_{18}N_5O_5$	C,H,N
(*S*)-11	DMAC	22	63	>300[i]	311	4.94 m (C*H*NH); 7.35 br s (CHN*H*)	$C_{17}H_{18}N_4O_2$·0.2H_2O	C,H,N
(*S*)-12	DMAC	22[j]	55	>300[i]	327	4.80 m (C*H*NH); 7.51 br s(CHN*H*)[k]	$C_{17}H_{18}N_4O_3$·0.6EtOH	C,H,N
13	DMAC	51.5[a]	42	168–71[l]	326	3.10 m (3-CH_2); 3.39 m (2-CH_2); 6.87 t (NH)	$C_{17}H_{19}N_5O_2$	C,H,N
(*S*)-14	HOAc	1.3	20	>300[m]	313	3.62 m (2-CH); 4.32 br s (3-CH)	$C_{17}H_{20}N_4O_2$	C,H,N
16	*a*	*a*	53	165–70[n]	229	6.91 s (5-CH)[o]	$C_6H_8N_6ClO_2$·0.05C_6H_6·0.3H_2O	C,H,N
17	EtOH[a]	3[a]	97	200–3[h]	203	6.12 s (5-CH)	$C_8H_7N_4ClO_2$·0.1H_2O	C,H,N
19	*a*	–	51	197–9	248	3.67 s (OCH_3); 10.72 s (NHCO)	$C_8H_6N_5ClO_4$	C,H,N
(1*S*,2*R*)-20	EtOH–Et_3N[p]	22	68	215[c]	376	4.55 m (C*H*NH); 9.21 d (CHN*H*)[q]	$C_{17}H_{21}N_5O_5$·0.25$CHCl_3$	C,H,N
(*S*)-21	CH_2Cl_2–C_5H_5N	1.5	38	149–51	374	5.95 m (C*H*NH); 9.42 d (CHN*H*)	$C_{17}H_{19}N_5O_5$	C,H,N
(1*S*,2*R*)-22	THF–Et_3N	21[r]	81	132–4	363	4.56 m (C*H*NH); 9.41 d (CHN*H*)	$C_{16}H_{18}N_6O_5$	C,H,N
(*S*)-23	CH_2Cl_2–C_5H_5N	1	90	165[s]	361	5.90 m (C*H*NH); 9.74 d (CHN*H*)[f]	$C_{16}H_{16}N_6O_5$·0.5CH_3OH	C,H,N
(*S*)-24	EtOH	6.5[u]	70[a]	>300[v]	326	4.82 m (C*H*NH); 6.40 d (CHN*H*)[k]	$C_{17}H_{19}N_5O_2$·0.3EtOH·0.2H_2O	C,H,N
(*S*)-25	EtOH	3.5[w]	49[a]	>300[m]	313	4.93 m (C*H*NH); 7.34 d(CHN*H*)[k,q]	$C_{15}H_{18}N_6O_2$·0.4$CHCl_3$·0.2EtOH	C,H,N

[a] See experimental section. [b] Ethyl acetate observed, δ 1.18 t, 1.99 s. [c] With decomposition. [d] Calcd, 54.25; found 54.68. [e] With decomposition from 190 °C. [f] Acetic acid observed, δ 1.91 s. [g] Starting compound 9 was dissolved in CH_2Cl_2–C_5H_5N (12:1) rather than 100% CH_2Cl_2. [h] With bubbling. [i] With gradual decomposition from 180 °C. [j] Additional Ra-Ni (1.5 times the weight of starting material) was added after 3.5 h. [k] Ethanol observed, δ 1.06 t, 3.45 m, 4.35 t. [l] With foaming. [m] With decomposition from 250 °C. [n] With decomposition from 155 °C. [o] Benzene observed, δ 7.36 s. [p] Used a 1:1 mol ratio of Et_3N:18. [q] Chloroform observed, δ 8.32 s. [r] At room temperature, rather than reflux. [s] With gradual softening with 80 °C. [t] Methanol observed, δ 3.17 d, 4.10 q. [u] Two hours at room temperature followed by 4.5 h at 60 °C. [v] With decomposition from 210 °C. [w] 1.5 h at room temperature followed by 2 h at 60 °C.

17 by the procedure of O'Brien.[6] The physical properties of these compounds are presented in Table I.

Biological Results

The new compounds within the group **2–25** were evaluated for cytotoxicity in cultured lymphoid leukemia L1210 cells.[7] In the pyridine alcohols [(1*S*,2*R*)-**4**, (1*S*,2*R*)-**5**, **9**, (1*S*,2*R*)-**20**, (1*S*,2*R*)-**22**], the most cytotoxic compound was (1*S*,2*R*)-**5** (IC_{50}, 39 μM). None of the pyridine ketones [(*S*)-**7**, (*S*)-**8**, **10**, (*S*)-**21**, (*S*)-**23**] gave an IC_{50} value less than 80 μM. Although the target compounds (*S*)-**11**, (*S*)-**12**, **13**, (2*S*,3*RS*)-**14**, (*S*)-**24**, and (*S*)-**25** were more cytotoxic than the ring-opened precursors, alterations in the bicyclic systems had a pronounced effect on activity relative to (*RS*)-, (*R*)-, and (*S*)-**1** (Table II).

Replacement of the 5-amino group of (*S*)-**1** with the 5(6*H*)-oxo moiety to give (*S*)-**12** resulted in a 49 000-fold decrease in cytotoxicity. The importance of the 5,6-amidino moiety (see below) of (*S*)-**1** to cytotoxicity was supported by replacement of the amino group with hydrogen to give (*S*)-**11**, which showed a 181-fold reduction in cytotoxicity. The tetrahydro derivative (2*S*,3*RS*)-**14** of (*S*)-**11** showed a greater loss in cytotoxicity (862-fold). Also, modifications either in the position or number of nitrogens within the pyridine ring destroyed activity. The 3,4-

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Table II. Biological Activity of Compounds

compound	L1210[a] IC_{50}, nM	L1210[b] $MI_{0.5}$, nM	in vivo antileukemic activity[c] dose (mg/kg)	% ILS[d]
(*S*)-11	17	21	24	90
(*S*)-12	4.4×10^3	1.8×10^3	–	–
13	3.7	–	8[e]	35
(2*S*,3*RS*)-14	81	161	70	65
(*S*)-24	3.9×10^3	6.7×10^3	75	0
(*S*)-25	1.7×10^3	3×10^3	200	0
(*RS*)-1	0.2	0.58	1	71
(*R*)-1	17	27	30	88
(*S*)-1	0.094	0.14	0.5	64
CLC[f]	6.4	21	–	–
VCR[g]	3.4	11	2[h]	85

[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] Compounds were evaluated by the protocols of the National Cancer Institute; ref 9. For the determination of activity, lymphocytic leukemia P388 (10^6 cells) were implanted ip into $CD2F_1$ mice 24 h prior to treatment with drug, which was administered ip daily on days 1–5. [d] Increase in life span at the highest nontoxic dose. [e] Highest dose tested. [f] CLC, colchicine; ref 7. [g] VCR, vincristine; refs 7 and 13. [h] Single dose on day 1.

dihydropyrido[2,3-*b*]pyrazine (*S*)-24, isomeric with (*S*)-1, and the 7,8-dihydropteridine analogue (*S*)-25 inhibited the proliferation of L1210 cells at concentrations 3 orders of magnitude larger than that needed for (*S*)-1. The most potent inhibitor of proliferation prepared in this investigation was the dihydro-1,4-diazepine analogue 13, which exhibited a 39-fold decrease in cytotoxicity relative to (*S*)-1.

As noted in other studies, the correlation of the IC_{50} and $MI_{0.5}$ values for (*S*)-11, (*S*)-12, (2*S*,3*RS*)-14, (*S*)-24, and (*S*)-25 suggested that these agents are inhibiting the polymerization of tubulin.[8]

The target compounds with the exception of (*S*)-12 were evaluated for antitumor activity in mice implanted with lymphocytic leukemia P388.[9] Both (*S*)-24 and (*S*)-25 were inactive, whereas, (*S*)-11, 13, and (2*S*,3*RS*)-14 exhibited activity (% ILS > 25). Relative to (*S*)-1, both (*S*)-11 and (2*S*,3*RS*)-14 were significantly less cytotoxic while retaining the same degree of anticancer activity. However, the anticipated instability of (2*S*,3*RS*)-14 toward oxidation might indicate that activity results from the conversion of this compound to (*S*)-11 during the evaluation.

In conclusion, these and previous studies indicate that the cytotoxicity of (*S*)-1 is reduced by the following modifications: substitution on the 5-amino group[8] and replacement of the latter with either hydrogen or the 5(6*H*)-oxo group; removal of the pyridine ring nitrogen,[10] transposing the 6-ring nitrogen to the 8-position, incorporation of a ring nitrogen at the 8-position; and expansion of the 1,2-dihydropyrazine ring to give the dihydro-1,4-diazepine 13. The overall inactivity of (*S*)-24 suggested that the pyridine ring nitrogen of (*S*)-1 might participate in the binding of the latter to tubulin. The decrease in activity for (*S*)-12 and (*S*)-25 might be attributed to the differential ring basicities of (*S*)-1, (*S*)-12, and (*S*)-25. These results suggest that the 5,6-amidino moiety and a favorable spatial position of the 3-phenyl group of (*S*)-1 contribute to the binding of this agent to tubulin.

As in previous work with similar compounds, there was not a linear relationship between the values for the in vitro activities and the observed % ILS of leukemic mice.[8] Apparently this lack of correlation can be attributed to one or more of the following: differences in drug transport, metabolism, and the efficiency of binding to tubulin. Relative to (*S*)-1 a reduction of cytotoxicity and a retention of in vivo activity was observed for both (*R*)-1 and (*S*)-11. The increase in selectivity of these compounds might be related to the nature of their interaction with tubulin[11] and to their membrane transport properties.[12]

Experimental Section

Melting and decomposition temperatures were determined in capillary tubes in a Mel-Temp apparatus. The ^1H NMR spectra were determined on Me_2SO 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 the fast-atom-bombardment mode to provide the $(M + 1)^+$ molecular ion. 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. Elemental analyses were performed on samples dried under high vacuum over P_2O_5. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical value.

Ethyl [4-Chloro-5-nitropyridin-2-yl]carbamate (2) and Ethyl [4-Chloro-5-nitro-6(1*H*)-oxopyridin-2-yl]carbamate (3). To a stirred, chilled (0–5 °C) solution of 6 (30.0 g, 115 mmol)[2] and isoamyl nitrite (27.0 g, 231 mmol) in *N*,*N*-dimethylacetamide (400 mL) was added dropwise 2 N H_2SO_4 (116 mL, 116 mmol) over 1 h. The resulting mixture was allowed to warm to room temperature over 0.75 h and heated gradually to 100 °C over 0.5 h. After stirring at 100 °C for 1 h, the clear solution was cooled to room temperature (ice–H_2O bath) and H_2O (800 mL) was added slowly. The resulting solid was collected, washed with water, and dried in vacuo (P_2O_5) to afford 20.4 g of a mixture of 2 (minor product) and 3 (major product). This material was extracted with $CHCl_3$ (350 mL), the extract was evaporated to dryness, and the residue was recrystallized from EtOH (150 mL) to afford 2: yield, 2.45 g.

The $CHCl_3$-insoluble material was recrystallized from EtOH (600 mL) to afford 3: yield, 13.5 g (40%).[3]

General Procedure for the Preparation of 4, 5, 9, 20, and 22. Ethyl [6-Amino-4-[(3-hydroxy-3-phenylpropyl)amino]-5-nitropyridin-2-yl]carbamate (9). A hot solution of 6 (5.65 g, 21.7 mmol),[2] 3-amino-1-phenylpropanol[4] (3.60 g, 23.8 mmol), and triethylamine (33 mL) in EtOH (73 mL) was refluxed for 15 h, cooled to room temperature, and evaporated to dryness at reduced pressure. The yellow solid was triturated with H_2O (2 × 50 mL) and recrystallized from EtOH (800 mL) to afford 9: yield, 6.8 g.

The remaining alcohols were prepared from (1*R*,2*S*)-(−)-norephedrine hydrochloride which is commerically available.

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Compound (1*S*,2*R*)-5 was purified by recrystallization from CH_3CN and (1*S*,2*R*)-22 by recrystallization from C_6H_6. Compounds (1*S*,2*R*)-4 and (1*S*,2*R*)-20 were purified by flash chromatography ($CHCl_3$–MeOH, 99:1).

General Procedure for the Preparation of 7, 8, 10, 21, and 23. Methyl [(S)-6-Amino-4-[(1-methyl-2-oxo-2-phenylethyl)-amino]-5-nitropyrimidin-2-yl]carbamate (23). To a stirred solution of dry pyridine (4.11 g, 51.9 mmol) in dry CH_2Cl_2 (120 mL) under N_2 was added CrO_3 (2.59 g, 25.9 mmol), and stirring was continued for 0.5 h. A solution of (1*S*,2*R*)-22 (1.56 g, 4.32 mmol) in dry CH_2Cl_2 (95 mL) was added all at once, and the mixture was stirred for 1 h at room temperature under N_2. The insoluble material was removed by filtration (Celite) and washed with dry CH_2Cl_2 (3 × 75 mL), and the combined filtrate and wash was added to 2-propanol (100 mL) to destroy excess chromium reagent. The resulting mixture was evaporated at reduced pressure to a semisolid residue which was suspended in 2-propanol–toluene and reevaporated to remove pyridine. This residue was dissolved in $CHCl_3$ and applied to a flash column (125 g, $CHCl_3$–EtOAc, gradient, (9:1 → 7:3). The product-containing fractions were evaporated to dryness at reduced pressure, and the residue was dissolved in MeOH, filtered to remove a trace of insoluble material, and evaporated again to afford 23 as an off-white solid: yield, 1.41 g.

Compound (*S*)-7 was purified by elution from a pad of silica gel with $CHCl_3$, while compounds 10 and (*S*)-21 were purified by flash chromatography. Compound 10 was eluted with $CHCl_3$ followed by $CHCl_3$–EtOAc (9:1). Compound 21 was eluted with $CHCl_3$ and then recrystallized from toluene.

Compound (*S*)-8 was prepared in a somewhat different manner: To a stirred solution of dry pyridine (1.89 g, 23.9 mmol) in dry CH_2Cl_2 (50 mL) under N_2 was added CrO_3 (1.19 g, 11.9 mmol), and stirring was continued for 0.5 h. A solution of (1*S*,2*R*)-5 (750 mg, 1.99 mmol) in dry CH_2Cl_2–C_5H_5N (60 mL, 5:1) was added, and the mixture was stirred for 2 h at room temperature under N_2. The insoluble material was removed by filtration (Celite) and extracted with boiling pyridine (200 mL) and boiling 2-propanol (200 mL). The combined filtrate and extracts were evaporated to dryness in vacuo, and toluene was evaporated from the residue to remove pyridine. After flash chromatography ($CHCl_3$–MeOH–HOAc, 95:5:5) this residue gave 940 mg of crude product. A 409-mg sample of this material was recrystallized from HOAc to give (*S*)-8: yield, 71 mg. An additional amount (210 mg) of slightly impure (TLC) product was obtained by flash-chromatographing the remaining 531 mg of the above crude product ($CHCl_3$–MeOH–HOAc 99:1:5 and then EtOAc–HOAc 95:5).

General Procedure for the Preparation of 11, 12, 14, 24, and 25 (see Table I). Ethyl [(*S*)-1,2-Dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (11). A solution of 7 (1.30 g, 3.90 mmol) in *N*,*N*-dimethylacetamide (100 mL) was hydrogenated for 22 h at room temperature and atmospheric pressure in the presence of Ra-Ni (4.2 g, washed 3 times with H_2O and 2 times with EtOH). The catalyst was removed by filtration (Celite), the filtrate was evaporated to dryness in vacuo, and the residue was triturated with EtOH (15 mL, under N_2) to deposit (*S*)-11: yield, 765 mg.

In the preparation of (*S*)-12, the residue from the evaporation of the *N*,*N*-dimethylacetamide was triturated with H_2O and the resulting solid was purified by flash chromatography ($CHCl_3$–EtOH, gradient, 9:1 → 8:2). For (2*S*,3*RS*)-14, the residue from the evaporation of the HOAc was suspended in EtOH–H_2O (2:1), adjusted to pH 8–9 with deoxygenated (N_2) aqueous NaOH (0.1 N) under N_2, and concentrated at reduced pressure to deposit a mixture of (*S*)-11 and (2*S*,3*RS*)-14, which was separated by flash chromatography ($CHCl_3$–MeOH, gradient, 99:1 → 97:3). In the preparation of (*S*)-24, concentration of the reaction filtrate to a small volume deposited a 38% yield of product. Evaporation of the filtrate followed by flash chromatography ($CHCl_3$) gave an additional 32% yield of (*S*)-24. Compound (*S*)-25 was purified by flash chromatography to separate an impurity, which gave a mass ion corresponding to transesterification of the methyl carbamate to the ethyl carbamate.

Ethyl [6-Amino-2,3-dihydro-4-phenyl-1*H*-pyrido[4,3-*b*]-[1,4]diazepin-8-yl]carbamate (13). A solution of 10 (0.525 g, 1.41 mmol) in *N*,*N*-dimethylacetamide (100 mL) was hydrogenated at atmospheric pressure and room temperature in the presence of Ra-Ni (1.5 g, washed 3 times with H_2O and 2 times with EtOH). After 21 h, additional washed Ra-Ni (1.5 g) was added, and stirring was continued for another 28 h at room temperature and 2.5 h at 55 °C. The catalyst was removed by filtration (Celite), and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in deoxygenated (N_2) EtOH (10 mL), and deoxygenated (N_2) H_2O (40 mL) was added to give a milky suspension. The mixture was concentrated at reduced pressure to remove EtOH and acidified under argon to pH 2–3 (1 N HCl). The clear solution was stirred 0.5 h under argon and neutralized to pH 7–8 (1 N NaOH, under argon) to deposit crude 13, which was purified by flash chromatography (50 g, $CHCl_3$–MeOH 98:2) to afford 13: yield, 193 mg.

Methyl [4-Azido-6-chloropyrimidin-2-yl]carbamate (16). To a suspension of 15[5] (17.84 g, 80.5 mmol) in 2-propanol (1.0 L) was added a solution of NaN_3 (5.23 g, 80.5 mmol) in H_2O (55 mL), and the mixture was heated at reflux for 1.5 h and cooled to room temperature. The mixture was concentrated (50–100 mL) at reduced pressure and diluted with water (600 mL) to deposit the crude product, which was recrystallized from benzene (200 mL) to afford 16: yield, 9.76 g.

Methyl [4-Amino-6-chloropyrimidin-2-yl]carbamate (17). A partial solution of 16 (603 mg, 2.64 mmol) in EtOH (75 mL) was hydrogenated for 3 h at room temperature and atmospheric pressure in the presence of Ra-Ni (590 mg, washed three times with H_2O and two times with EtOH) to give a clear supernate. The catalyst was removed by filtration (Celite), and the filtrate was evaporated to dryness at reduced pressure to afford 17: yield, 520 mg.

Methyl [4-Amino-6-chloro-5-nitropyrimidin-2-yl]carbamate (19). To a solution of fuming HNO_3 (2.3 mL, d = 1.50) in concentrated H_2SO_4 (11.5 mL) was added 17 (3.01 g, 14.8 mmol) portionwise, over 0.5 h, while maintaining the temperature at 30–35 °C.[6] The resulting solution was stirred for 1 h at 30–35 °C and added dropwise to crushed ice (~100 g). The pale yellow precipitate was collected by filtration, resuspended in H_2O (150 mL), and neutralized (at 0–5 °C) to pH 8 with 1 N NH_4OH. The precipitate of crude 19 was collected by filtration, washed with water (2 × 50 mL), and purified on a short flash column (100 g, EtOAc) to afford 19 as a white solid: yield, 1.86 g.

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