9as·oxalate, 136864-61-4; 9at, 133675-42-0; 9at·oxalate, 136864-62-5; 9au, 138697-46-8; 9av, 138722-05-1; 9aw, 114432-81-4; 9aw·oxalate, 114432-82-5; 9b, 114432-53-0; 9c, 114432-64-3; 9d, 114448-94-1; 9d·oxalate, 114448-95-2; 9e, 114432-62-1; 9e·oxalate, 114432-63-2; 9f, 114432-65-4; 9g, 114448-96-3; 9g·oxalate, 114448-97-4; 9h, 114432-39-2; 9h·oxalate, 114432-40-5; 9i, 114432-66-5; 9j, 138697-46-8; 9k, 114432-41-6; 9l, 138697-47-9; 9l·HCl, 114432-67-6; 9m, 114432-54-1; 9m·oxalate, 114432-55-2; 9n, 114432-60-9; 9n·oxalate, 114432-61-0; 9o, 138697-48-0; 9o·HCl, 114432-56-3; 9p, 114432-15-4; 9p·HCl, 114432-57-4; 9q, 138697-49-1; 9r, 114432-93-8; 9r·HCl, 138697-59-3; 9s, 138697-50-4; 9s·HCl, 114432-78-9; 9t, 138697-51-5; 9t·HCl, 114432-73-4; 9u, 114432-74-5; 9u·oxalate (2:1), 114432-75-6; 9v, 114432-48-3; 9v·HCl, 114448-93-0; 9w, 114432-71-2; 9w·HCl, 138697-60-6; 9x, 114432-79-0; 9x·oxalate, 114432-80-3; 9y, 114432-14-3; 9z, 138697-52-6; bromoacetone, 598-31-2; bromomethyl ethyl ketone, 816-40-0; bromomethyl propyl ketone, 817-71-0; bromomethyl cyclopropyl ketone, 69267-75-0; bromomethyl butyl ketone, 26818-07-5; bromomethyl *tert*-butyl ketone, 5469-26-1; bromomethyl cyclohexyl ketone, 56077-28-2; bromomethyl phenyl ketone, 70-11-1; 1-chloro-3-(dibutylamino)propane, 36421-15-5; 1,3-dibromopropane, 109-64-8; *N*-methyl-*N*-(3,4-dimethoxyphenethyl)amine, 3490-06-0; 1-chloro-3-(diethylamino)propane, 104-77-8; 1-chloro-3-(dipropylamino)propane, 39743-36-7; 1-chloro-3-(dimethylamino)propane, 109-54-6; 1-chloro-3-[*N*-methyl-*N*-(3,4-dimethoxybenzyl)]propane, 138697-63-9.

Antimitotic Agents. Chiral Isomers of Ethyl [5-Amino-1,2-dihydro-3-(4-hydroxyphenyl)-2-methylpyrido[3,4-*b*]pyrazin-7-yl]carbamate

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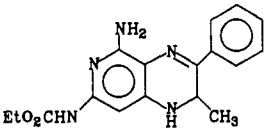
Metabolism studies with ethyl [5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (1) in mice were reported previously to give a hydroxylated metabolite, which was methylated to give a methoxy derivative. The metabolite and its derivative were considered to be 4-(substituted)phenyl compounds, which have been confirmed by the synthesis of the [1,2-dihydro-3-(4-hydroxyphenyl)- and [1,2-dihydro-3-(4-methoxyphenyl)pyrido[3,4-*b*]-pyrazin-7-yl]carbamates (17 and 16). Both the *S*- and *R*-isomers of 17 are active in several biological systems, but the *S*-isomer is more potent then the *R*-isomer. The difference in activity between the *S*- and *R*-isomers of 17 is similar with that observed for *S*- and *R*-isomers of 1. As model reactions, several O-substituted derivatives were prepared by alkylation of (*RS*)-17 with benzyl chloride and condensation of (*RS*)-17 with butyl isocyanate and (*S*)-17 with 2-chloroethyl isocyanate.

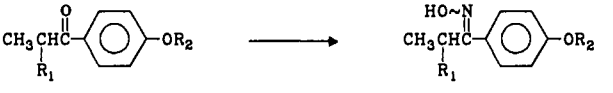
A new type of antimitotic agent, ethyl [5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (1), has shown good in vivo activity against several murine tumors including leukemia sublines resistant to most of the agents in clinical use (Chart I).[1] The *S*-isomer of 1 has entered phase I clinical trials. Metabolism studies with 1 in mice gave urinary products in which one of the major metabolites resulted from hydroxylation.[2] Treatment of the metabolite with diazomethane afforded a methylated derivative, which suggested that hydroxylation of 1 had occurred in the phenyl ring. To confirm the structure of this metabolite and its methyl derivative, methods were developed for the synthesis of the 4-hydroxyphenyl (17) and 4-methoxyphenyl (16) congeners of 1 (Scheme II). In other work the *S*-isomer of 1 exhibited greater potency than the *R*-isomer in several in vitro and in vivo test systems.[3,4] To investigate the possibility that the differences in potency of (*S*)-1 and (*R*)-1 were related to metabolic hydroxylation, the *S*- and *R*-isomers of 17

Chart I



1

Scheme I



2, R_1 = Br, R_2 = CH_3
3, R_1 = PhtN, R_2 = CH_3
4, R_1 = Br, R_2 = H
5, R_1 = N_3, R_2 = H
6, R_1 = PhtN, R_2 = CH_3
7, R_1 = H_2N, R_2 = CH_3
8, R_1 = N_3, R_2 = H
9, R_1 = H_2N, R_2 = H

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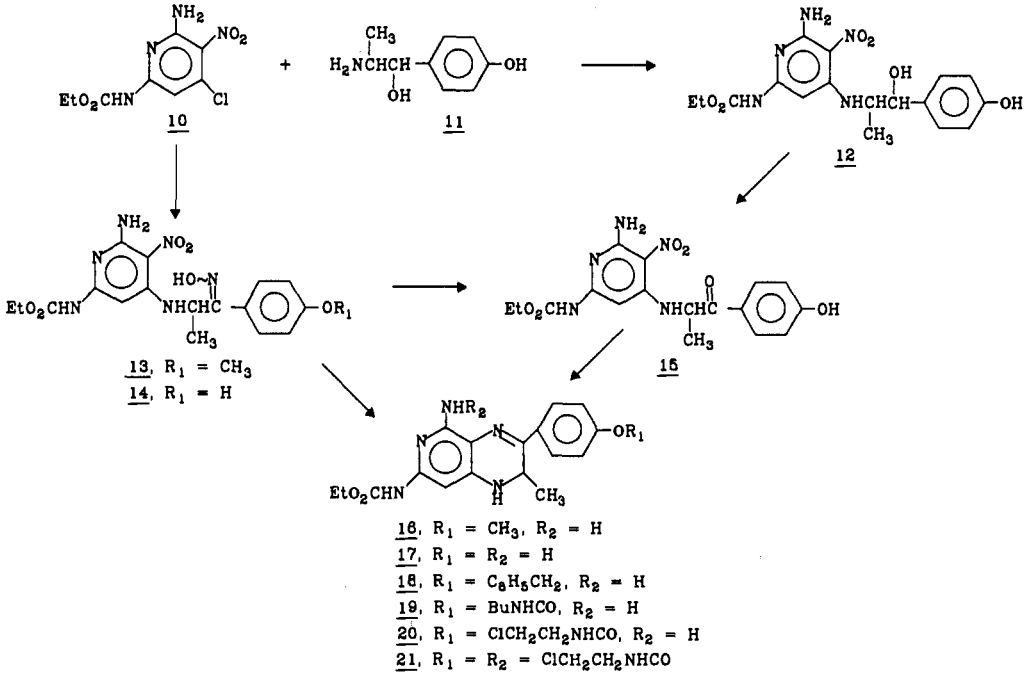
0022-2623/92/1835-0988$03.00/0

Scheme II



were also prepared. Several O-substituted phenyl derivatives of 17 and (*S*)-17 were also prepared and evaluated for biological activity.

Chemistry

The preparation of 17 was attempted by procedures previously developed in our laboratory.[5,6] Reaction of 4[7] with potassium phthalimide, however, gave a complex mixture which was apparently caused by the unprotected hydroxy group of the phenyl ring (Scheme I). This result prompted the initial preparation of the 4-methoxyphenyl compound 16. Alkylation of potassium phthalimide with 2[7] gave 3, which was converted with hydroxylamine to the oxime 6. The phthaloyl blocking group of 6 was cleaved with hydrazine to give 7, which was isolated as a mixture of oxime isomers. The interaction of 7 and 10 provided 13, which was hydrogenated in the presence of Raney nickel to give 16, presumably formed by the intramolecular cyclization of a 5-amino intermediate (Scheme II). The synthesis of 17 required the preparation of the α-amino ketone oxime 9. The latter was prepared by reaction of 4 with sodium azide to give 5, treatment of 5 with hydroxylamine to give the oxime 8, and reduction of the azido group of 8 to afford 9. Alkylation of 9 with 10 gave 14, which was hydrolyzed with acid to give the ketone 15. Catalytic hydrogenation of 15 proceeded presumably via a 5-aminopyridine intermediate to give the target compound 17. HPLC experiments established that 17 was identical with the metabolite formed from 1 in mice and that 16 was identical with the methylated derivative of the metabolite.

The synthesis of the *S*- and *R*-isomers of 17 required the development of a new approach. In a trial reaction, racemic 4-hydroxynorephedrine (11) was reacted with 10 to give racemic 12. The success of this reaction led to the separation of the enantiomers of 11. Racemic 4-hydroxynorephedrine (11) was converted to the D-(−)-tartrate and L-(+)-tartrate, respectively. Four recrystallizations of the D-tartrate from 2-propanol-H_2O (10:1) provided a product, (1*R*,2*S*)-11·tartrate, which gave the reported optical rotation for the pure diastereomer.[8] Similarly, five recrystallizations of the L-tartrate gave a product, (1*S*,2*R*)-11·tartrate with an equal but opposite rotation.

Reaction of 10 with (1*R*,2*S*)-11·D-tartrate gave (1*S*,2*R*)-12, which was followed by oxidation of the alcohol group with the CrO_3–pyridine reagent to give the ketone (*S*)-15 (Scheme II). The structure of (*S*)-15 was confirmed by condensation with hydroxylamine to give (*S*)-14. No difficulties were encountered in the hydrogenation of (*S*)-15 to give (*S*)-17. These procedures were also used in the reaction of 10 with (1*S*,2*R*)-11·L-tartrate to give (1*R*,2*S*)-12, which was converted to (*R*)-17 via (*R*)-15.

The *S*- and *R*-isomers of 17 were separated by HPLC with a protein-bound silica column (Enantiopak), which allowed the determination of the enantiomeric purity of (*S*)-17 (92% ee) and (*R*)-17 (98% ee). To establish that no racemization had occurred during the synthesis, the free base of racemic 11 was reacted with (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate. The reaction mixture containing the two diastereomers was resolved by HPLC using a Spherisorb column.[9] Similarly, treatment of the free bases of (1*R*,2*S*)-11 and (1*S*,2*R*)-11 indicated that the former was obtained in 90% ee and the latter in 99% ee,

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Table I. Properties of Compounds

compd	yield, %	mp, °C	$[\alpha]^{25}_D$, deg[a]	mass spectra[b]	^{1}H NMR spectra:[b] selected peaks, δ	formula	anal.
5	62	79–84		192 (M + 1)$^+$	5.05 q (2-CH)	$C_9H_9N_3O_2$	C,H,N
7	~35[c]	143–5		195 (M + 1)$^+$	1.15 d (1′-CH_3CH), 1.06 d (1′-CH_3CH)[d,e]	$C_{10}H_{14}N_2O_2 \cdot 0.25H_2O$	C,H,N
8	78	oil		206 (M)$^+$	5.36 q (1′-CH), 4.55 q (1′-CH)[d,e]	$C_9H_{10}N_4O_2 \cdot 0.3H_2O$	C,H,N
9	~91[f]	143–7		181 (M + 1)$^+$	4.30 q (1′-CH), 3.77 q (1′-CH)[e]		
(1*R*,2*S*)-11[g]	73	179–82 dec	−34.9 [*c*, 0.8/H_2O][h]		3.31 m (2′-CH), 4.80 d (1′-CH)[d]	$C_{13}H_{19}NO_8 \cdot H_2O$	C,H,N
(1*S*,2*R*)-11[i]	54	183–4 dec	+34.8 [*c*, 1.0/H_2O]		3.31 m (2′-CH), 4.80 d (1′-CH)[d]	$C_{13}H_{19}NO_8 \cdot 1.1H_2O$	C,H,N
13	57	99–116		419 (M + 1)$^+$	5.12 quin (1′-CH), 4.63 quin (1′-CH)[e,j]	$C_{18}H_{22}N_6O_6 \cdot 0.5CH_3CH_2OH$	C,H,N
(*RS*)-14	60[f]	173–5		405 (M + 1)$^+$	5.07 quin (1′-CH), 4.61 quin (1′-CH)[e,k]	$C_{17}H_{20}N_6O_6 \cdot 0.3CH_3CH_2O_2CCH_3$	C,H,N
(*S*)-14 (A)[b]	88[l]	>160 dec	+103 [*c*, 0.9/MeOH]	405 (M + 1)$^+$	4.60 quin (1′-CH)[d,e]	$C_{17}H_{20}N_6O_6 \cdot 0.5H_2O$	C,H,N
(*S*)-14 (B)[b]		>160 dec	+182 [*c*, 0.3/MeOH]	405 (M + 1)$^+$	5.07 quin (1′-CH) 4.60 quin (1′-CH)[d,m]	$C_{17}H_{20}N_6O_6 \cdot 0.9H_2O \cdot 0.1CH_3OH$	C,H,N
12	53	140–5 dec		392 (M + 1)$^+$	3.66 m (1′-CH), 4.76 t (2′-CH)	$C_{17}H_{21}N_5O_6$	C,H,N
(1*S*,2*R*)-12	65	>125 dec	+149 [*c*, 1.2/MeOH]	392 (M + 1)$^+$	3.66 m (1′-CH), 4.76 t (2′-CH)[d]	$C_{17}H_{21}N_5O_6 \cdot 0.4H_2O$	C,H,N
(1*R*,2*S*)-12	61	>125 dec	+154 [*c*, 0.8/MeOH]	392 (M + 1)$^+$	3.66 m (1′-CH), 4.76 t (2′-CH)[d]	$C_{17}H_{21}N_5O_6 \cdot 0.5H_2O$	C,H,N
RS-15	71	209–11 dec		390 (M + 1)$^+$	5.31 quin (1′-CH), 9.79 d (4-NH)[j]	$C_{17}H_{19}N_5O_6 \cdot 0.2CH_3CH_2OH$	C,H,N
S-15	26	197 dec	+18.0 [*c*, 1.2/dioxane]	390 (M + 1)$^+$	5.32 quin (1′-CH), 9.80 d (4-NH)[d]	$C_{17}H_{19}N_5O_6 \cdot 0.5H_2O$	C,H,N
R-15	22	190 dec	−21.4 [*c*, 1.0/dioxane]	390 (M + 1)$^+$	5.31 quin (1′-CH), 9.79 d (4-NH)[d,j]	$C_{17}H_{19}N_5O_6 \cdot 0.3H_2O \cdot 0.2CH_3CH_2OH$	C,H,N
16	80	135–9[n]		356 (M + 1)$^+$	4.77 m (2-CH), 6.88 d (1-NH)[d]	$C_{18}H_{21}N_5O_3 \cdot 0.3H_2O$	C,H,N
RS-17	97	>140 dec		341 (M)$^+$	4.72 m (2-CH), 6.88 br (1-NH)[d,j]	$C_{17}H_{19}N_5O_3 \cdot 0.8H_2O \cdot 0.2CH_3CH_2OH$	C,H,N
S-17	58	>135 dec	−538 [*c*, 0.7/MeOH][g]	341 (M)$^+$	4.73 m (2-CH), 6.86 br (1-NH)[d,j]	$C_{17}H_{19}N_5O_3 \cdot 0.5H_2O \cdot 0.3CH_3CH_2OH$	C,H,N
R-17	94	>135 dec	+665 [*c*, 1.0/MeOH][i]	342 (M + 1)$^+$	4.72 m (2-CH), 6.84 br (1-NH)[d,j]	$C_{17}H_{19}N_5O_3 \cdot 0.1H_2O \cdot 0.4CH_3CH_2OH$	C,H,N
18	34	>175 dec		432 (M + 1)$^+$	4.77 m (2-CH), 6.89 d (1-NH)[o]	$C_{24}H_{25}N_5O_3 \cdot 0.25CH_3(CH_2)_4CH_3$	C,H,N
19	20	130–5 dec		441 (M + 1)$^+$	4.80 m (2-CH), 6.95 d (1-NH)[d,m]	$C_{22}H_{28}N_6O_4 \cdot 0.3H_2O \cdot 0.1CH_3OH$	C,H,N
S-20	40	>220 dec		447 (M + 1)$^+$	4.80 m (2-CH), 6.96 br (1-NH)[p]	$C_{20}H_{23}ClN_6O_4 \cdot 0.3CHCl_3$	C,H,N
S-21	30	>195 dec		552 (M + 1)$^+$	4.94 m (2-CH), 7.49 br (1-NH)[p]	$C_{23}H_{27}Cl_2N_7O_5 \cdot 0.2CHCl_3$	C,H,N[q]

[a] Sample weights were corrected to correspond to anhydrous material. [b] See Experimental Section. [c] Overall crude yield from 4. [d] H_2O observed, δ 3.32. [e] Ratio of oxime isomers: 9, 93:7; 10, 5:6; 11, 7:6; 16, 1:3; 17, 1:0 (another crop showed both isomers); 18 (A), 0:1; 18 (B), 9:1. [f] Crude yield. [g] 90% ee. [h] Reference 9; $[\alpha]^{25}_D$ −34° [*c*, 1.93/H_2O]. [i] 98% ee. [j] EtOH observed, δ 1.06 t, 3.45 q. [k] EtOAc observed, δ 1.17 t, 1.99 s, 4.02 q. [l] Overall crude yield. [m] CH_3OH observed, δ 3.17 s. [n] Presoftening from 109 °C. [o] Hexane observed, δ 0.86 m, 1.22 m. [p] $CHCl_3$ observed, δ 8.32 s. [q] N: calcd, 17.01; found, 16.52.

which are in good agreement with that found for (*S*)-17 and (*R*)-17.

The O-alkylation of 17 to give 18 was effected with the benzyl chloride–NaH combination in DMSO in the absence of oxygen. The reaction of 17 with butyl isocyanate in CH_2Cl_2 gave a low yield of 19 and similarly, reaction of (*S*)-17 with 2-chloroethyl isocyanate gave a mixture of (*S*)-20 and (*S*)-21, which were separated by column chromatography.

Physical properties of compounds are presented in Table I.

Biological Evaluation

The 1,2-dihydropyrido[3,4-*b*]pyrazines prepared in this study were evaluated for in vitro activity (cytotoxicity and mitotic inhibition)[10] against cultured lymphoid leukemia L1210 cells and for in vivo activity against lymphocytic leukemia P388 in mice (Table II).[11] In the 3-phenyl series (1), the *S*-isomer appeared to be slightly more potent than the *RS*-mixture, whereas the *R*-isomer was considerably less potent than either (*RS*)-1 or (*S*)-1.[3,4] The results with the 3-(4-hydroxyphenyl) series (17) appear to parallel the results observed with 1. Comparison of (*RS*)-17 with (*RS*)-1, (*S*)-17 with (*S*)-1, and (*R*)-17 with (*R*)-1 showed that activity of the same order of magnitude was observed for each pair. This data indicated that activity was retained in the hydroxylated metabolite of 1 and that differences in potency between (*S*)-1 and (*R*)-1 cannot be attributed to either metabolic activation or deactivation.

The O-substituted derivatives of the 4-hydroxyphenyl compounds (*RS*)-17 and (*S*)-17 exhibited different degrees of activity. The 4-methoxyphenyl compound 16 showed the same order of activity as was observed for (*RS*)-17 and (*S*)-17. In contrast, the benzyl ether 18 showed a decrease in cytotoxicity (>14-fold) and antimitotic activity (>15-fold) to cultured cells and gave a greater increase in life

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Table II. Biological Data

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]
(*RS*)-1	0.2	0.58	1	71
(*S*)-1	0.09	0.14	0.5	64
(*R*)-1	17.0	27.0	30	88
16	0.48	0.42	1	69
(*RS*)-17	0.22	0.47	0.5	55
(*S*)-17	0.18	0.30	0.22	58
(*R*)-17	32[e]		25	77[e]
18	7	30	1	120
19	0.47		0.25	120[f]
(*S*)-**20**	0.043		1	90
(*S*)-**21**	570			

[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] Increase in life span at the highest nontoxic dose tested. [e] Average of two determinations. [f] Toxic at a dose of 1 mg/kg; when repeated at the 0.25 mg/kg dose, 1/6 45th day survivor.

span (~2-fold) in mice at about the same dose relative to **16**, (*RS*)-**17**, and (*S*)-**17**. In addition, relative to (*R*)-**1** and (*R*)-**17** in which similar IC_{50} values were observed, **18** was active in vivo at a lower dose (>25-fold). In contrast, the phenyl carbamates **19** and (*S*)-**20** showed similar or greater in vitro activity, and based on the 2-fold increase in % ILS, greater selectivity in vivo relative to (*RS*)-**17** and (*S*)-**17**. As indicated by the IC_{50} value, substitution on the 5-amino group of (*S*)-**20** to give (*S*)-**21** reduced activity significantly. Previous results with the ethyl [1,2-dihydropyrido[3,4-*b*]pyrazin-7-yl]carbamates indicated that bulky substituents in the carbamate moiety,[12] substitution on the 5-amino group,[3] or substitution in place of the 3-phenyl group[13] caused a reduction in biological activity. In contrast, the results with **18**, **19**, and (*S*)-**20** showed that substituents in the para position of the 3-phenyl ring either maintained or increased in vivo activity. In addition, the pyridine intermediates **12**–**15** showed activity, although these compounds were considerably less potent than the pyrido[3,4-*b*]pyrazines (e.g. **17**). Structure–activity relationships in a large number of pyridine compounds are currently undergoing investigation.

Experimental Section

Melting and decomposition temperatures were determined in capillary tubes in a Mel-Temp apparatus. The ^1H NMR spectra were determined in DMSO-d_6 solutions with either a Varian XL-100-15 or a Nicolet NT300NB spectrometer with tetramethylsilane as internal standard. Optical rotations (±2%) were measured with a Perkin-Elmer Model 241 automatic polarimeter. 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 reations was followed by thin-layer chromatography (TLC) on plates of silica gel from Analtech, Inc. HPLC chromatograms were determined on an ALC-242 liquid chromatograph equipped with an UV detector (254 nm) and a M-6000 pump. 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.

Identification of the Primary Mouse Metabolite of (*RS*)-1. The urinary metabolites of (*RS*)-**1** were deglucuronided and separated as previously described.[2] Chromatograms were determined on a Waters high-pressure liquid chromatograph equipped with a U6K injector, μBondapak C-18 column, two Model 6000A pumps, Model 440 UV detector, and Model 660 gradient programmer. Gradient elutions were accomplished at 1 mL/min with Waters Program 6 (0% solvent B, intial, to 100% solvent B, final, in 15 mins) using CH_3CN–H_2O (1% CH_3CN, A; 40% CH_3CN, B) containing 26 mM NH_4OAc. Peaks were detected at 365 nm, and the UV spectra of peaks were determined with a Hewlett-Packard Model 1040A spectrophotometer.

Under these conditions (*RS*)-**17** and the metabolite [MS, *m/e* 342 $(M + 1)^+$] gave retention times of 26.0 and 26.1 min, respectively. The UV spectra of (*RS*)-**17** and the metabolite showed peaks at 223 (br), 268, and 378 (br) nm and were essentially superimposable. Methylation (CH_2N_2) of the metabolite gave a product with the same retention time (29 min) as **16** (isocratic elution, 25% CH_3CN containing 0.2% NH_4OAc). The UV spectra [221 (br), 269, 380 (br) nm] of the methylated metabolite and **16** were essentially superimposable.

2-Amino-4′-methoxypropiophenone Oxime (7). To a stirred suspension of potassium phthalimide (50.3 g, 271 mmol) in *N*,*N*-dimethylacetamide (250 mL) at 0 °C was added **2**[7] (60 g, 250 mmol). After stirring for 0.5 h at 0 °C and 8 h at room temperature, the reaction mixture was poured into H_2O (1500 mL), and the clear supernate was decanted from the resulting gummy precipitate. This residue was dissolved in $CHCl_3$ (600 mL), and the solution was washed with 0.5 N NaOH (100 mL) and H_2O (100 mL). The organic layer was dried (Na_2SO_4) and evaporated to give an oil. The latter was dried in vacuo (P_2O_5) and stirred overnight in Et_2O (200 mL) to precipitate crude 4′-methoxy-2-phthalimidopropiophenone (**3**) yield 42.8 g (56%).

A suspension of crude **3** (41.7 g) and hydroxylamine hydrochloride (18.7 g, 270 mmol) in 4:1 ethanol–pyridine (650 mL) was refluxed for 4 h, and the resulting clear solution was evaporated under high vacuum to give an amber oil. This oil was stirred for 2 h with H_2O (200 mL), and the water was removed by decantation. The wash was repeated (200 mL, stirred for 18 h) to give a solid, which was collected, washed with water (200 mL), and dried in vacuo (P_2O_5) to give crude **6**: yield, 39.6 g.

A nearly clear solution of crude **6** (10.0 g) in warm (70 °C) EtOH (600 mL) was treated dropwise with a solution of anhydrous hydrazine (1.19 mL, 37.3 mmol) in EtOH (18 mL), and the resulting solution was stirred at 45 °C for 21 h. The reaction mixture was cooled to 0 °C, treated with 1 N HCl (36 mL), and stirred for 1 h at 0–5 °C. The precipitate of phthalic acid hydrazide was removed by filtration and washed with 1:1 EtOH–H_2O (40 mL), and the combined filtrate and wash were evaporated in vacuo to give an off-white solid. This residue was extracted with warm (60 °C) H_2O (135 mL), and the insoluble material was removed by filtration. The filtrate was concentrated in vacuo to 50 mL and adjusted to pH 2.5 with 1 N HCl. A small amount of insoluble material was removed by filtration, and the clear filtrate was adjusted to pH 10–11 with 1 N NaOH to give a gummy semisolid precipitate (1.5 g) of crude **7**, which was collected by filtration. The clear filtrate was allowed to stand for 2 h at room temperature to deposit the analytical sample of **7**: yield, 266 mg. An additional amount (2.5 g) of crude **7** was obtained by evaporation to dryness of the neutralized filtrate and extraction of the resulting residue with THF (190 mL) containing methanolic ammonia (6 mL, saturated at 20 °C).

2-Amino-4′-hydroxypropiophenone Oxime (9). A solution of sodium azide (398 mg, 6.12 mmol) in deoxygenated (N_2) H_2O (2 mL) was added to a stirred solution of **4**[7] (1.23 g, 5.37 mmol) in deoxygenated (N_2) MeOH (20 mL), and the resulting solution was stirred at room temperature for 16 h. After removal of MeOH at reduced pressure, the mixture was diluted with water (75 mL) and extracted with Et_2O (2 × 100 mL). The combined organic layers were dried (Na_2SO_4) and evaporated to give an oil, which solidified on drying in vacuo (P_2O_5). The off-white solid was

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triturated with water (100 mL), collected by filtration, and dried in vacuo (P_2O_5) to afford **5**: yield, 640 mg.

A solution of **5** (505 mg, 2.64 mmol), hydroxylamine hydrochloride (385 mg, 5.54 mmol), and pyridine (2.5 mL, 31 mmol) in EtOH (10 mL) was heated at reflux for 6 h and concentrated under high vacuum to give an oil. This residue was extracted with Et_2O (3 × 100 mL), and the combined extracts were evaporated at reduced pressure to afford **8** as a colorless oil: yield, 438 mg.

A solution of crude **8** (5.38 g) from another preparation in EtOH (260 mL) was hydrogenated at atmospheric pressure in the presence of Raney nickel (6.0 g, weighed wet, washed 3 × H_2O and 3 × EtOH). At 1-h intervals, the system was evacuated and recharged with fresh hydrogen. After 5 h, the catalyst was removed by filtration (Celite), the amber-orange filtrate was evaporated at reduced pressure, and the resulting pale-pink solid was dried in vacuo (P_2O_5) to give **9**: yield, 4.3 g. The crude material was used without further purification.

4-Hydroxynorephedrine Tartrates (11). A mixture of racemic 4-hydroxynorephedrine (19.0 g, 114 mmol) and D-(−)-tartaric acid (17.5 g, 117 mmol) in H_2O (14 mL) was prepared as previously described.[8] The salt was collected by filtration, washed with 2-propanol (150 mL) and Et_2O, and recrystallized four times from 2-propanol–H_2O (10:1) to give (1*R*,2*S*)-**11**·D-tartrate: yield, 13.2 g (73%). A small portion of this salt was dissolved in H_2O, treated with an equivalent amount of 1 N NaOH, and evaporated to dryness in vacuo. This residue was extracted with hot EtOAc, the extract was evaporated to dryness, and the free base of (1*R*,2*S*)-**11** was reacted with (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (98%) in MeCN at 50 °C for 0.5 h. HPLC chromatograms [5μ Spherisorb ODS1 column, isocratic with 0.02 M $NH_4H_2PO_4$–MeCN (65:35)] of the reaction solution indicated the presence of (1*R*,2*S*)-**11** and (1*S*,2*R*)-**11** in about a 95:5 ratio.[9]

Similarly, the salt from racemic 4-hydroxynorephedrine (17.6 g, 105 mmol) and L-(+)-tartaric acid (16.6 g, 111 mmol) was recrystallized five times from 2-propanol–H_2O (10:1) to give (1*S*,2*R*)-**11**·L-tartrate: yield, 9.0 g (54%). Reaction of the free base with (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (98%) and determination of the HPLC chromatograms of the reaction solution as described above indicated the presence of (1*S*,2*R*)-**11** and (1*R*,2*S*)-**11** in a 99:1 ratio.

Typical Procedure for the Preparation of 4-[(2-Oxoethyl)amino]pyridine Oximes. Ethyl [6-Amino-4-[[2-(4-methoxyphenyl)-1-methyl-2-oxoethyl]amino]-5-nitropyridin-2-yl]carbamate Oxime (13). A hot solution of crude **7** (3.85 g), **10** (4.13 g, 15.9 mmol), and triethylamine (2.00 g, 19.8 mmol) in EtOH (85 mL) was refluxed for 11.5 h and evaporated to dryness at reduced pressure. The resulting yellow foam was triturated with water (100 mL) to give a solid, which was collected, washed with water (100 mL), and dissolved in EtOH (75 mL) at room temperature. After chilling at −20 °C overnight, the clear supernate was decanted from a slightly impure precipitate (TLC) of **13**: yield, 3.99 g (57%). The filtrate was chilled at −78 °C to deposit pure **13**: yield, 1.60 g (23%).

Similarly, **ethyl [6-amino-4-[[2-(4-hydroxyphenyl)-1-methyl-2-oxoethyl]amino]-5-nitropyridin-2-yl]carbamate oxime (14)** was prepared by refluxing crude **9** (3.96 g), **10** (5.79 g, 22.2 mmol), and triethylamine (3.07 mL, 2.23 g, 22.2 mmol) in 2-propanol (130 mL) for 6 h. Recrystallization from EtOAc afforded **14**: yield 1.49 g.

A second crop (3.85 g, 43%) of slightly impure **14** was obtained by evaporation of the ethyl acetate filtrate and trituration of the residue with Et_2O (150 mL).

Ethyl [(*S*)-6-Amino-4-[[2-(4-hydroxyphenyl)-1-methyl-2-oxoethyl]amino]-5-nitropyridin-2-yl]carbamate Oxime [(*S*)-14]. A solution of (*S*)-**15** (1.03 g, 2.64 mmol, 90% ee), pyridine (3.5 mL), and hydroxylamine hydrochloride (365 mg, 5.26 mmol) in EtOH (25 mL) was heated for 6 h at reflux, cooled to room temperature, and evaporated to dryness at reduced pressure. The residue was triturated overnight with H_2O (50 mL), and the resulting solid was collected by filtration: yield 0.94 g (88%). A portion of this product was purified by flash chromatography (130 g, $CHCl_3$–MeOH, 98:2) to give one of the oxime isomers in pure form (A). Other fractions were combined to give the other oxime isomer (B) which contained about 10% of A.

General Procedure for the Preparation of 4-[(2-Hydroxyethyl)amino]pyridines. Ethyl [(1*S*,2*R*)-6-Amino-4-[[2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino]-5-nitropyridin-2-yl]carbamate [(1*S*,2*R*)-12]. A hot solution of (1*R*,2*S*)-**11**·D-tartrate (1.02 g, 3.05 mmol, contaminated with 5% of (1*S*,2*R*-isomer), **10** (0.621 g, 2.38 mmol), and triethylamine (1.18 mL, 0.857 g, 8.48 mmol) in EtOH (10 mL) was refluxed for 21 h, cooled to room temperature, and added dropwise to H_2O (75 mL). The resulting precipitate was collected by filtration, dried in vacuo (P_2O_5), and purified by flash chromatography (125 g, $CHCl_3$–MeOH, 97:3). The resulting product was triturated with H_2O to afford (1*S*,2*R*)-**12** (90% ee) as a yellow glass: yield, 602 mg.

Ethyl [(1*R*,2*S*)-6-amino-4-[[2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino]-5-nitropyridin-2-yl]carbamate (1*R*,2*S*)-12 was similarly prepared from (1*S*,2*R*)-**11**·L-tartrate (8.68 g, 25.7 mmol, contaminated with less than 1% of the 1R,2S-isomer), and the product was isolated in two crops by recrystallization from MeOH: yield, 5.11 g.

Ethyl [6-amino-4-[[2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino]-5-nitropyridin-2-yl]carbamate (racemic 12) was prepared by a similar procedure from a solution of racemic **11**·HCl (2.05 g, 10.1 mmol), 1 N NaOH (10.1 mL), **10** (2.00 g, 7.67 mmol), and triethylamine (1.01 g, 10.1 mmol) in EtOH (25 mL) by refluxing for 6 h. The solid resulting from the H_2O trituration was recrystallized from EtOH to afford the product: yield, 1.60 g.

Ethyl [6-Amino-4-[[2-(4-hydroxyphenyl)-1-methyl-2-oxoethyl]amino]-5-nitropyridin-2-yl]carbamate (15). A solution of **14** (3.76 g, 9.30 mmol) in dioxane (80 mL) and 1 N HCl (80 mL) was heated at 45 °C for 24 h. The solution was cooled and adjusted to pH 5 with 1 N NaOH. After most of the dioxane was removed at reduced pressure, the mixture was neutralized to pH 7. The clear supernate was decanted from the semisolid residue, which was recrystallized from EtOH (50 mL) to afford **15** as a yellow solid: yield, 2.56 g.

Ethyl [(*S*)-6-Amino-4-[[2-(4-hydroxyphenyl)-1-methyl-2-oxoethyl]amino]-5-nitropyridin-2-yl]carbamate [(*S*)-15]. Dry pyridine (235 mL) was treated at 0–5 °C with CrO_3 (7.05 g, 70.5 mmol), and the suspension was stirred for 0.4 h in the ice bath, after which time a solution of (1*S*,2*R*)-**12** (4.71 g, 12.0 mmol, contaminated with 5% of 1*R*,2*S*-**12**) in dry pyridine (210 mL) was added. The ice bath was removed, stirring was continued for 2 h, and the reaction mixture was poured through a pad of silica gel 60 (100 g). The pad was washed with pyridine (250 mL) and EtOAc (400 mL), and the combined eluate was evaporated to dryness. The resulting semisolid was triturated with water, collected by filtration, and extracted with boiling EtOH (4 × 250 mL). The combined extracts were evaporated to dryness, and the residue was dissolved in EtOAc and poured through a pad of silica gel 60 (50 g, eluted with EtOAc) to remove residual Cr salts. The residue from the evaporation of the eluate was purified by flash chromatography (560 g, $CHCl_3$–MeOH, 98:2). The product fractions were combined and evaporated to dryness at reduced pressure, and the resulting residue was triturated with water to afford (*S*)-**15** (90% ee): yield, 1.21 g.

Similarly, **ethyl [(*R*)-6-amino-4-[[2-(4-hydroxyphenyl)-1-methyl-2-oxoethyl]amino]-5-nitropyridin-2-yl]carbamate (*R*-15)** was prepared from (1*R*,2*S*)-**12** (2.08 g, 5.31 mmol): yield, 0.46 g.

Ethyl [5-Amino-1,2-dihydro-3-(4-methoxyphenyl)-2-methylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (16). A solution of **13** (1.01 g, 2.29 mmol) in EtOH (50 mL) was stirred under 1 atm H_2 in the presence of Raney nickel (3 g, weighed wet, washed 2 × H_2O and 2 × EtOH) for 3 h at room temperature and 2 h at 55 °C to give a total H_2 uptake of 9.34 mmol. The catalyst was removed by filtration (Celite), and the yellow-amber filtrate was evaporated to dryness at reduced pressure. The residue was dissolved in hot EtOH (20 mL, under N_2), cooled to room temperature, and diluted dropwise with deoxygenated (N_2) H_2O (85 mL) to deposit **16** as a light yellow solid: yield, 654 mg.

Ethyl [(*S*)-5-Amino-1,2-dihydro-3-(4-hydroxyphenyl)-2-methylpyrido[3,4-*b*]pyrazin-7-yl]carbamate [(*S*)-17]. A solution of crude (*S*)-**15** (1.05 g, contaminated with 5% of (*R*)-**15**) in EtOH (150 mL) was stirred under 1 atm H_2 in the presence of Raney nickel (4 g, weighed wet, washed 3 × H_2O and 2 × EtOH) for 4.5 h at 60 °C. The catalyst was removed by filtration (Celite), the filtrate was evaporated to dryness at reduced pressure, and

the residue was purified by flash chromatography (120 g, $CHCl_3$–MeOH, 97:3). The product-containing fractions were evaporated to dryness, dissolved in EtOH, filtered, and reevaporated to afford (*S*)-17 (90% ee) as a yellow foam: yield, 534 mg.

Ethyl [5-amino-1,2-dihydro-3-(4-hydroxyphenyl)-2-methylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (17) was prepared in the same manner from 15 (0.50 g, 1.3 mmol), but the reaction filtrate was evaporated to dryness at reduced pressure to provide analytically pure product: yield, 431 mg. HPLC [Enantiopak column, isocratic 95:5 0.05 M NaH_2PO_4 (0.1 M NaCl)–2-propanol] indicated a 48:52 mixture of *R* and *S* isomers.

Similarly, **ethyl [(*R*)-5-amino-1,2-dihydro-3-(4-hydroxyphenyl)-2-methylpyrido[3,4-*b*]pyrazin-7-yl]carbamate [(*R*)-17]** was prepared from (*R*)-15 (380 mg, 0.98 mmol) and isolated in pure form by evaporation of the reaction filtrate: yield, 312 mg.

Ethyl [5-Amino-3-[4-(benzyloxy)phenyl]-1,2-dihydro-2-methylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (18). To a stirred suspension of NaH (13.5 mg of 60% oil dispersion, washed 1× hexane) in deoxygenated (N_2) DMSO was added 17 (101 mg, 0.30 mmol). After stirring 0.2 h, the nearly-clear amber solution was treated with benzyl chloride (36 mg, 0.29 mmol), stirred 18 h under N_2, and evaporated to dryness. The residue was triturated with deoxygenated (N_2) H_2O (10 mL) to give a solid, which was purified by flash chromatography (45 g, $CHCl_3$–MeOH, 99:1) followed by recrystallization from EtOAc–hexane to afford 18 as a pale yellow solid: yield, 44 mg.

Ethyl [(*S*)-5-Amino-3-[4-[[[(2-chloroethyl)amino]carbonyl]oxy]phenyl]-1,2-dihydro-2-methylpyrido[3,4-*b*]pyrazin-7-yl]carbamate [(*S*)-20] and Ethyl [(*S*)-5-[[[(2-Chloroethyl)amino]carbonyl]amino]-3-[4-[[[(2-chloroethyl)amino]carbonyl]oxy]phenyl]-1,2-dihydro-2-methylpyrido[3,4-*b*]pyrazin-7-yl]carbamate [(*S*)-21]. To a partial solution of (*S*)-17·0.3EtOH·0.5H_2O (115 mg, 0.316 mmol, contaminated with 5% of (*R*)-17) in dry CH_2Cl_2 (25 mL) under N_2 was added 2-chloroethyl isocyanate (61 mg, 0.57 mmol), and the suspension was stirred for 20 h at room temperature under N_2. The resulting nearly-clear solution was evaporated to dryness (N_2), the residue was dissolved in EtOH (20 mL), and the solution was stirred for 0.5 h and reevaporated. The residue was purified by column chromatography (55 g, $CHCl_3$–MeOH, 99:1) to afford (*S*)-21 (90% ee): yield, 52 mg. Further development of the above column ($CHCl_3$–MeOH, 99:1) afforded (*S*)-20 (90% ee): yield, 56 mg.

Similarly, **ethyl [5-amino-3-[4-[[(butylamino)carbonyl]oxy]phenyl]-1,2-dihydro-2-methylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (19)** was prepared by stirring 17·0.2EtOH·0.8H_2O (101 mg, 0.277 mmol) and *n*-butyl isocyanate (41 mg, 0.41 mmol) in dry CH_2Cl_2 (25 mL) for 144 h at room temperature. Workup with EtOH and flash chromatography (20 g, $CHCl_3$–MeOH, 98:2) afforded 19: yield, 24.7 mg.

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