for providing pharmacological data through the Antiepileptic Drug Development Program, National Institute of Health.

Supplementary Material Available: Complete anticonvulsant and toxicity screening data for all compounds submitted to the National Institute of Health's Antiepileptic Drug Development (ADD) Program protocol is available from the authors.

Registry No. 1, 1121-89-7; **4a**, 97938-45-9; **4b**, 24866-79-3; **4c**, 24866-80-6; **4d**, 124482-59-3; **4e**, 124482-60-6; **4f**, 124482-61-7; **4g**, 124482-62-8; **4h**, 124482-63-9; **4i**, 124482-64-0; **4j**, 124482-65-1; **4k**, 124482-66-2; **4l**, 124482-67-3; **4m**, 51180-38-2; **4n**, 51180-39-3; **5a**, 124482-68-4; **5b**, 124482-69-5; **5c**, 124482-70-8; **5d**, 124482-71-9; **5e**, 124482-72-0; **5f**, 124482-73-1; **5g**, 124482-74-2; **5h**, 124482-75-3; $PhCH_2Br$, 100-39-0; p-$ClC_6H_4CH_2Br$, 622-95-7; p-$MeOC_6H_4CH_2Br$, 2746-25-0; o-$BrC_6H_4CH_2Br$, 3433-80-5; p-$BrC_6H_4CH_2Br$, 589-15-1; p-$IC_6H_4CH_2Br$, 16004-15-2; p-$CF_3C_6H_4CH_2Br$, 402-49-3; 2,4-$Cl_2C_6H_3CH_2Br$, 94-99-5; 3,4-$Cl_2C_6H_3CH_2Br$, 18880-04-1; p-$FC_6H_4CH_2Br$, 459-46-1; p-$PhC_6H_4CH_2Br$, 2567-29-5; p-$TMSC_6H_4CH_2Br$, 17903-42-3; p-$PrOC_6H_4CH_2Br$, 2606-58-8; p-i-$PrOC_6H_4CH_2Br$, 72729-52-3; 2,2′,4,4′-tetrachlorostilbene, 6271-50-7.

Selective Inhibition of γ-Aminobutyric Acid Aminotransferase by (3*R*,4*R*),(3*S*,4*S*)- and (3*R*,4*S*),(3*S*,4*R*)-4-Amino-5-fluoro-3-phenylpentanoic Acids

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(3*R*,4*R*),(3*S*,4*S*)- and (3*R*,4*S*),(3*S*,4*R*)-4-amino-5-fluoro-3-phenylpentanoic acid (**1a** and **1b**) were synthesized and studied as selective inactivators of γ-aminobutyric acid (GABA) aminotransferase. Neither compound caused time-dependent inactivation of the enzyme. Neither compound underwent enzyme-catalyzed transamination nor was fluoride ion eliminated from either compound by the enzyme. No 3-phenyllevulinic acid, the product of elimination of HF followed by enamine hydrolysis, was detected. However, both **1a** and **1b** were competitive reversible inhibitors of GABA aminotransferase; the K_i for **1a** was smaller than the K_m for GABA. These results suggest that **1a** and **1b** bind to the active site of GABA aminotransferase, but γ-proton removal does not occur. Whereas (*S*)-4-amino-5-fluoropentanoic acid (AFPA) is a potent inhibitor of L-glutamic acid decarboxylase (GAD), neither **1a** nor **1b** at concentrations 40 times the K_i of AFPA caused any detectable competitive inhibition of GAD. Therefore, the incorporation of a phenyl substituent at the 3-position of AFPA confirms selective inhibition of GABA aminotransferase over GAD.

The concentrations of the excitatory and inhibitory neurotransmitters L-glutamate and γ-aminobutyric acid (GABA),[1] respectively, are regulated in the central nervous system (CNS) principally by two PLP-dependent enzymes, L-glutamic acid decarboxylase, the enzyme that catalyzes the conversion of L-glutamate to GABA and GABA aminotransferase, the enzyme that degrades GABA to succinic semialdehyde.[2] Convulsive states have been observed in systems where GABA is prevented from functioning normally[3,4] either by the lowering of its concentration in the CNS below a certain level[5] or by blocking its effect.[6] An increase in GABA levels above the threshold limit usually results in protection against these seizures.[3,4,7] The simplicity of administering GABA directly as an anticonvulsant agent is complicated by the fact that it does not permeate through the blood–brain barrier, i.e., a membrane that surrounds the capillaries of the circulatory system in the brain and protects it from passive diffusion of undesirable compounds from the circulating blood. Although GABA aminotransferase is present in cerebral blood vessel endothelial cells,[8,9] inhibition of its activity does not lead to permeability of GABA into the brain.[10] Therefore, GABA degradation at the blood–brain barrier is not the mechanism for the inability of GABA to cross it; presumably, the lipophobicity is the primary mechanism.

Since GABA administration is ineffective for increasing the GABA concentration in the brain, an alternative approach would be administration of a more lipophilic compound that crosses the blood–brain barrier and then once inside the brain selectively inhibits GABA aminotransferase. This would block the degradation of GABA, and provided that inhibition of L-glutamate decarboxylase, the enzyme that catalyzes the biosynthesis of GABA, does not occur, GABA levels should rise, leading to an anticonvulsant effect. This approach has been shown to be effective; various in vitro inhibitors of GABA aminotransferase increase whole-brain GABA levels in vivo and possess anticonvulsant properties.[7,11-14]

γ-Vinyl-GABA (vigabatrin) is a GABA aminotransferase inactivator that is used clinically for the treatment of ep-

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(1) Abbreviations GABA, γ-aminobutyric acid; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; (*S*)-AFPA, (*S*)-4-amino-5-fluoropentanoic acid.

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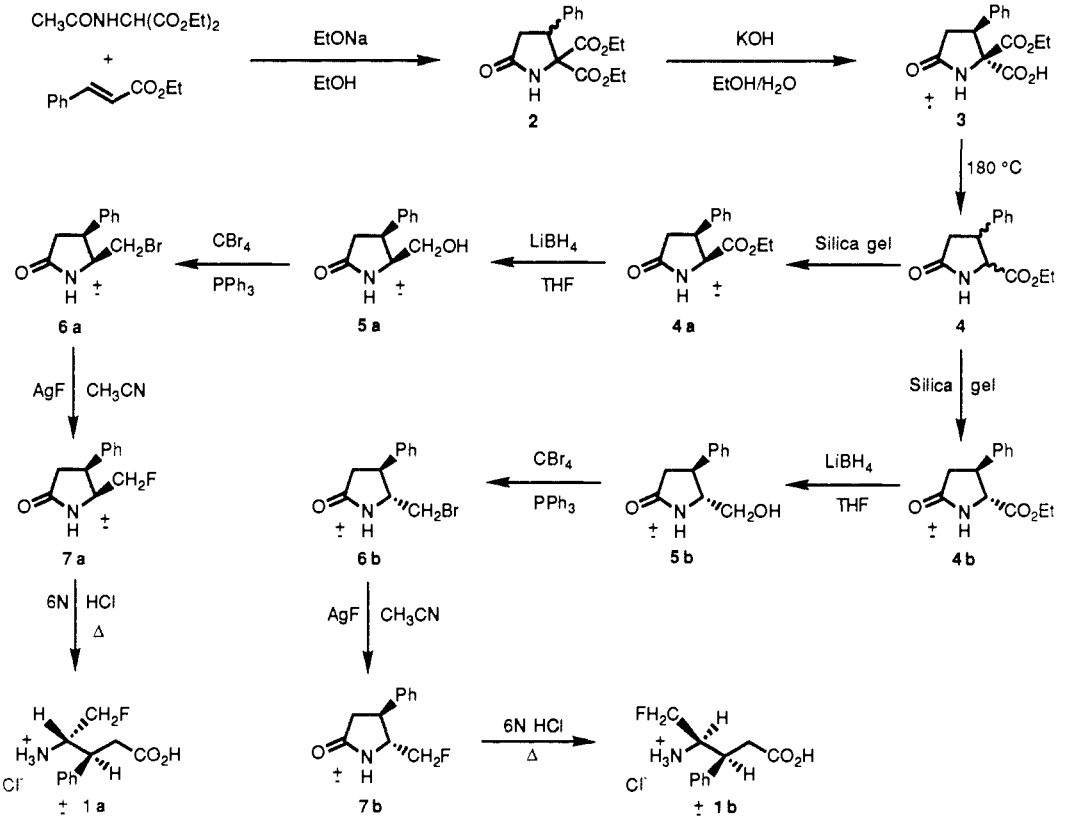
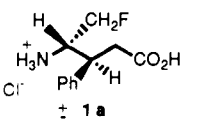
Scheme I. Synthetic Pathway to 1a and 1b



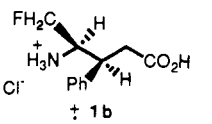
ilepsy.[15] The time course of the anticonvulsant activity was shown to correspond to that of the increase in brain GABA levels.[16,17] However, the increase in the *whole-brain* GABA levels was not the determining factor for protection against seizures; the GABA concentrations had to increase at the nerve terminals of the substantia nigra in order for anticonvulsant activity to be observed.[18-20]

Several years ago we reported the in vitro[21,22] and in vivo[23,24] inactivation of GABA aminotransferase by (*S*)-4-amino-5-fluoropentanoic acid ((*S*)-AFPA), a mechanism-based enzyme inactivator.[25] The in vitro mechanism of inactivation of GABA aminotransferase by (*S*)-AFPA was studied in detail.[22,26] Also, it was shown to be capable of crossing the blood–brain barrier, of inactivating brain GABA aminotransferase, and of raising the whole brain GABA concentration.[23,24] Although it was not very effective against picrotoxin-induced seizures,[22] it did exhibit anticonvulsant activity against bicuculline-, metrazole-, and electroshock-induced seizures and was found to be 2–3 times more potent than the anticonvulsant drug γ-vinyl-GABA.[27] However, it was quite toxic and also was a potent competitive reversible inhibitor of L-glutamate decarboxylase, having a K_i value 10 times lower than the K_m for L-glutamate.[24]

More recently, we have found that there is an unexpected cavity in the GABA binding site of GABA aminotransferase capable of accommodating a phenyl or 4-chlorophenyl substituent at the 3-position of GABA and that these 3-aryl-GABA analogues are substrates for GABA aminotransferase.[28] Attachment of a 3-phenyl substituent to (*S*)-AFPA was expected to result in a compound which not only retains the mechanism-based inactivator properties of (*S*)-AFPA but also possesses selectivity of binding for GABA aminotransferase, since it is unlikely that L-glutamate decarboxylase or other PLP-dependent enzymes also would have a cavity that could accommodate a phenyl substituent. Furthermore, the lipophilicity of a 3-phenyl group should increase the ability of the inactivator to cross the blood–brain barrier. Since both (*R*)- and (*S*)-3-phenyl-GABA bind to the active site of GABA aminotransferase,[28] (3*R*,4*R*),(3*S*,4*S*)- and (3R,4S),(3S,4R)-4-amino-5-fluoro-3-phenylpentanoic acid (**1a** and **1b**) were



± 1a



± 1b

synthesized and studied as selective mechanism-based inactivators of GABA aminotransferase. Both of these compounds are potent, competitive, reversible inhibitors

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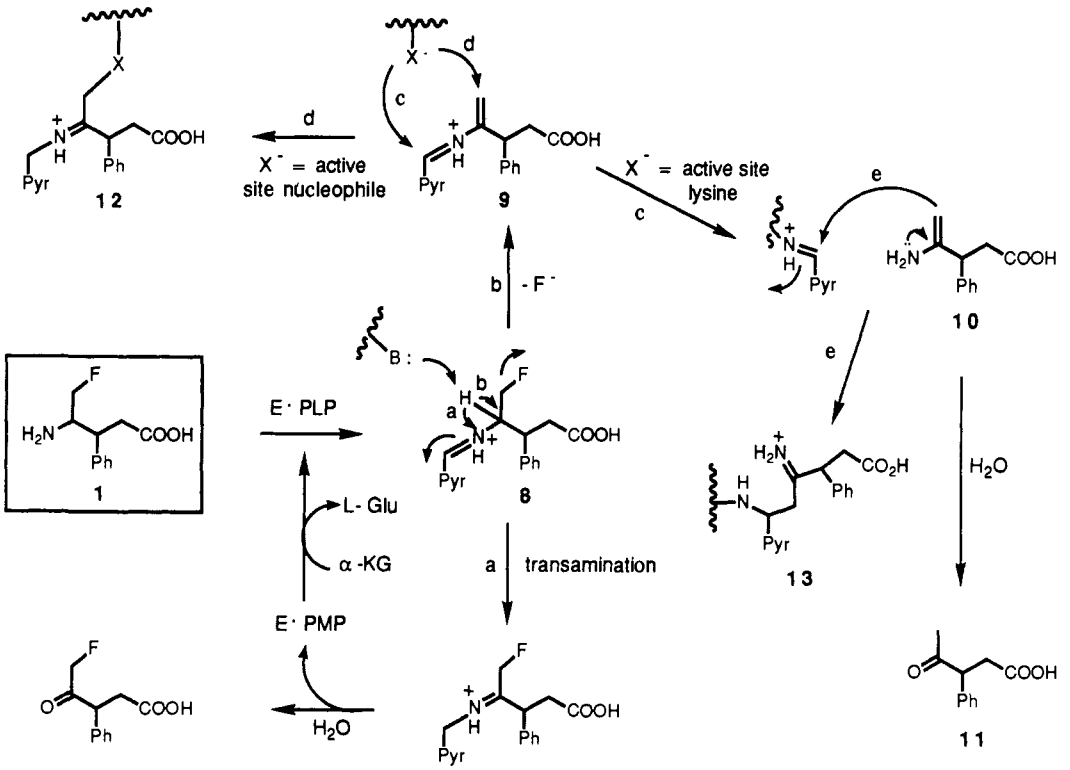
Scheme II. Possible Metabolic Pathways for the Reactions of **1a** and **1b** with GABA Aminotransferase[a]



[a] α-KG is α-ketoglutarate; Pyr is the pyridine ring of either PLP or PMP.

of pig brain GABA aminotransferase; however, it appears that γ-proton removal does not occur, and therefore, neither **1a** nor **1b** inactivates the enzyme.

Results and Discussion

Chemistry. The syntheses of **1a** and **1b** are summarized in Scheme I. The stereochemistry of the racemates was determined after the initial diastereomer separation and purification (**4a** and **4b**). The cis (**4a**) and trans (**4b**) monoesters have distinctive NMR profiles and can be differentiated easily from one another. As reported earlier,[29] the *trans*-ethyl ester methylene resonances appear downfield (by 0.5 ppm) from the corresponding resonances for the cis isomer. Also, the methylene protons adjacent to the lactam carbonyl appear as an apparent doublet in **4a**, but as a doublet of doublets in **4b**. The X-ray crystal structure of **4b** was determined (Figure 1) in order to establish unequivocally the trans relationship of the ester and phenyl substituents. The completion of the synthesis of **1a** and **1b** follows the same general pathway as that used for the synthesis of (*S*)-AFPA.[30]

Enzymology. Incubation of GABA aminotransferase with either **1a** or **1b** resulted in no time-dependent loss of enzyme activity, even at 19 mM concentration of **1a** or 4 mM **1b** for 24 h. There are several possible explanations for this observation (Scheme II): (1) exclusive transamination from **8** could occur (pathway a); (2) elimination of the fluorine from **8** could occur (pathway b) as in the case of (*S*)-AFPA, but the resulting enamine Schiff base with PLP that would form (**9**) subsequently may be converted (pathway c) to a product (**10**) which is released and hydrolyzed to **11** without inactivation; (3) nonproductive active-site binding of **1** may occur; or (4) no active site interaction may be involved at all. The fact that no enzyme inactivation occurred indicates that neither pathway

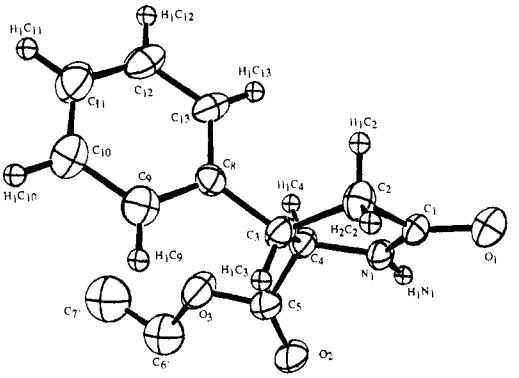


Figure 1. ORTEP Drawing of the X-ray Crystal Structure of **4b**.

d to give **12** nor pathway e to give **13** occurs.

Pathway a was monitored by the conversion of [^{14}C]-α-ketoglutarate to [^{14}C]-L-glutamate. When **1a** or **1b** was incubated with GABA aminotransferase in the presence of [^{14}C]-α-ketoglutarate, *no* [^{14}C]-L-glutamate was produced. This experiment excludes pathway a, which also is a nonoperational pathway in the case of the parent compound, (*S*)-AFPA.[22]

The other metabolic pathway for **1a** and **1b** after Schiff base formation with the active site PLP is elimination of fluoride ion (pathway b, Scheme II). In the case of (*S*)-AFPA, an average of one fluoride ion is eliminated per inactivation event.[22] (*S,E*)-4-amino-5-fluoro-2-pentenoic acid (i.e., 2,3-dehydro-(*S*)-AFPA) undergoes elimination of fluoride ion an average of five times for every inactivation event.[31] Enzyme-catalyzed fluoride ion release was monitored with a specific fluoride ion electrode;[22,32] however, it was found that for **1a** and **1b**, fluoride ions were

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released in a nonenzymatic control. Since there did not appear to be any more fluoride ions released in the enzymatic reaction than in the nonenzymatic control reaction, it may be assumed that relatively few or no elimination events occur enzymatically.

Because of the uncertainty regarding fluoride ion release, another approach was taken to determine if elimination of fluoride ion occurs. If **9** (Scheme II) is generated, its only apparent fate, since inactivation does not occur, would be release as **10** (pathway c) followed by hydrolysis to 3-phenyllevulinic acid (**11**). The enzymatic formation of **11** was monitored by HPLC with the use of a sample of **11** synthesized by the method of Bruderer et al.[33] If 0.35 equiv of **11** per enzyme molecule were formed, it would have been detected; none was detected. These results suggest that **1** is not turned over by either pathway a or b.

Consequently, it was of interest to determine if **1** binds to the active site at all. Lineweaver–Burk analysis (data not shown) of the effect of **1a** and **1b** on GABA aminotransferase indicated that both compounds are competitive reversible inhibitors with K_i (**1a**) = 1.0 mM and K_i (**1b**) = 8.0 mM against GABA, which has a K_m = 1.4 mM. Since both compounds bind to the active site but do not undergo transamination or elimination, it suggests that γ-proton removal does not occur. A similar phenomenon was observed with the 3-phenyl GABA analogues.[28] Whereas (*S*)-3-phenyl GABA was a substrate for GABA aminotransferase, the *R* isomer was only a competitive inhibitor. When substituents are at both the 3- and the 4-positions, as is the case with **1a** and **1b**, they may fit into the active site cavity, but in so doing, the active site base that normally abstracts the γ-proton may become misoriented so that its distance from the γ-proton surpasses its limit of effectiveness, leading to nonproductive binding. It also is relevant that the V_{max} for GABA is 56 times larger than that for 3-phenyl-GABA, even though the K_m value for (*R*,*S*)-3-phenyl-GABA is only 4 times larger than that for GABA.[28]

One of the principal purposes for incorporating a large substituent into the GABA backbone was to evoke selectively of binding to GABA aminotransferase in preference to L-glutamate decarboxylase, the enzyme that catalyzes the biosynthesis of GABA. It was shown previously[24] that (*S*)-AFPA is a potent competitive inhibitor of L-glutamate decarboxylase with a K_i = 0.071 mM (the K_m for L-glutamate is 0.74 mM, i.e., 10 times higher). First, it was found, as is the case with (*S*)-AFPA, that neither **1a** nor **1b** produces any time-dependent inactivation of L-glutamate decarboxylase at concentrations of 2.3 and 2.8 mM, respectively, for 10 h. At a concentration of 2 mM **1a** or **1b** there also is *no* detectable competitive reversible inhibition of L-glutamate decarboxylase. This suggests that the presence of a 3-phenyl substituent on the GABA backbone greatly reduces the affinity of the molecule for the active site of L-glutamate decarboxylase, presumably for steric reasons. This observation can be used to great advantage in the design of selective inhibitors of GABA aminotransferase when inhibition of L-glutamate decarboxylase must be kept to a minimum, for example, in the design of potential anticonvulsant agents that act by GABA aminotransferase inhibition. It is obvious that phenyl is not the appropriate substituent for this purpose because of its adverse effect on the ability of GABA aminotransferase to remove the γ-proton of the 3-phenyl-substituted inactivators. We are currently attaching other substituents to GABA in order to optimize the selectively effect and to assist in the further design of potent and selective GABA aminotransferase inhibitors.

Experimental Section

General Methods. All nonaqueous reactions were carried out under an atmosphere of argon. TLC was run on silica gel 60 F-254 pre-coated plastic plates from Merck. Flash column chromatography was carried out with Merck silica gel 60 (230–400 mesh). ^{1}H NMR spectra were recorded on either a Varian EM 390 A or a Varian XLA 400 spectrometer at 90 MHz and 400 MHz, respectively. Chemical shifts were reported as δ values in parts per million relative to tetramethylsilane (δ 0.00) used as an internal standard. For samples run in D_2O, the HOD resonance was arbitrarily set at δ 4.662. IR spectra were recorded on a Perkin-Elmer 283 infrared spectrometer. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. High-resolution mass spectra were recorded by the Midwest Center for Mass Spectrometry, Lincoln, NE. Elemental combustion analyses were performed by either Galbraith Laboratories, Inc., Knoxville, TN or by Microtech Laboratories, Skokie, IL. GABA aminotransferase activity was measured, as described under Enzymes and Assays, spectrophotometrically on a Perkin-Elmer Lambda 1 Spectrophotometer equipped with a constant-temperature cuvette holder. Radioactivity was measured in a Beckman LS-3133 scintillation counter with the use of 10 mL of 3a70B scintillation fluid (Research Products International). [U-^{14}C]Toluene (4.0×10^5 dpm/mL), obtained from New England Nuclear, was used as an internal standard. Protein concentrations were determined with BCA protein assay reagent from Pierce Chemical Co. with bovine serum albumin as a standard. High-performance liquid chromatography was performed with C_{18} silica gel columns (Beckman Ultrasphere ODS 5 μm, 4.6 mm and 10 mm × 25 cm; Rainin Dynamax 8 μm, 4.6 mm and 21.4 mm × 25 cm) on a Beckman system consisting of a controller (Model 421A), a variable-wavelength detector (Model 163), and two solvent delivery pumps (Model 110B).

Reagents. All HPLC solvents (B&J) were filtered and degassed before use. The in-house distilled water was further purified by passage through a deionizer (Contininental Water Conditioning Corp.) before use. Tetrahydrofuran and diethyl ether were dilled under nitrogen from sodium metal with sodium benzophenone ketyl used as an indicator. Ethyl acetate and hexane (predried over sulfuric acid) were distilled from anhydrous potassium carbonate. All other reagents and solvents were reagent grade, unless otherwise noted. Dowex 50 W (H^+ form) exchange resin, potassium pyrophosphate, α-ketoglutarate, $NADP^+$, β-mercaptoethanol, pyridoxal phosphate, and GABA were purchased from Sigma Chemical Co. Mono- and dibasic potassium phosphate were products of Mallinckrodt. L-[1-^{14}C]Glutamate (58 mCi/mmol) and [5-^{14}C]-α-ketoglutarate (24.2 mCi/mmol) were purchased from Amersham Radiochemicals while [U-^{14}C]-α-ketoglutarate (240 mCi/mmol) was procured from ICN Biomedicals, Inc. All other reagents were purchased from Aldrich Chemical Co.

X-ray Crystallography. Crystals of *trans*-4-phenyl-5-carbethoxy-2-pyrrolidinone **4b** ($C_{13}H_{15}NO_3$, FW = 233.3) were obtained upon recrystallization from acetone/hexane. A colorless crystal of dimensions 0.44 mm × 0.3 mm × 0.2 mm was used for structural determination. A monoclinic cell was determined with a = 11.017 (2) Å, b = 7.275 (1) Å, c = 15.631 (2) Å, β = 91.75 (1)°, V = 1252.2 Å^3, Z = 4, space group = $P2_1/n$, and D_{calcd} = 1.237 g/mL. All measurements were carried out on an Enraf-Nonius CAD 4 diffractometer at −140 °C using MoKα radiation (λ = 0.71073 Å). Unique reflections (2203) were measured in the $\omega/2\theta$ scan mode to a maximum 2θ of 50°. Of these, 926 had an intensity $I < 3\sigma(I)$ and were thus termed unobserved. No absorption correction was applied (μ = 0.8 cm^{-1}). Six standard reflections were measured during the data collection with no significant intensity variation. All calculations were performed on a VAX 11/730 computer with the SDP crystallographic software package.[34] The structure was

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solved with the use of MULTAN[34] and DIRDIF programs.[35]

The full-matrix least-squares refinement with anisotropic thermal parameters for all non-hydrogen atoms resulted in a final $R = 0.068$ and $R_\omega = 0.099$. H atoms were located in idealized positions as fixed contributors to the structure factors. The largest peak height in the final difference Fourier synthesis was determined to be 0.41 (3) e/Å^3. Neutral atomic scattering factors were taken from *International Tables for X-Ray Crystallography*.[36]

(±)-5,5-Dicarbethoxy-4-phenyl-2-pyrrolidinone (2). The procedure of Zymalkowski and Pachaly[29] was modified for the synthesis of **2**. To a suspension of diethyl acetamidomalonate (50 g, 230 mmol) in 80 mL of absolute ethanol was added 80 mL of a 21% solution of sodium ethoxide (210 mmol) in ethanol. The mixture was stirred at ambient temperature for 30 min, after which ethyl cinnamate (46 mL, 270 mmol) was syringed in over a period of 15 min. The resulting dark red solution was stirred at ambient temperature for 2 h and then at reflux for 14 h. After being cooled, the solution was acidified with glacial acetic acid, diluted with $CHCl_3$ (300 mL), and washed with H_2O (4 × 150 mL). After being dried ($MgSO_4$), the organic solvent was evaporated to give a yellow paste. Addition of cold ether to the paste resulted in the precipitation of a white solid (33.5 g, 48%): mp 96–99 °C (lit.[29] mp 98–100 °C); TLC (silica gel; ether) $R_f = 0.31$; NMR ($CDCl_3$) δ 0.87 (t, 3 H), 1.3 (t, 3 H), 2.57 (dd, 1 H), 2.9 (dd, 1 H), 3.73 (m, 2 H), 4.27 (q, 2 H), 4.38 (m, 1 H), 6.62 (br s, 1 H), 7.3 (s, 5 H).

(±)-*cis*-5-Carbethoxy-*trans*-5-carboxy-4-phenyl-2-pyrrolidinone (3). A solution of KOH (2.59 g, 46 mmol) in H_2O (34 mL) was added to **2** (5.35 g, 17.5 mmol) dissolved in ethanol (110 mL), and the mixture was stirred for 10 h at ambient temperature. The ethanol was evaporated and just enough H_2O was added to dissolve the precipitated white solid. Ether (50 mL) was added to the solution and the aqueous layer was acidified with 2 N HCl. The resulting precipitate was washed with H_2O to give **3** as a fluffy, white solid (4.37 g, 90%): mp 145–147 °C; NMR (acetone-d_6/TFA) δ 0.92 (t, 3 H), 2.28 (m, CD_3COCD_2H), 2.97 (dd, 1 H), 3.28 (dd, 1 H), 3.83 (m, 2 H), 4.55 (dd, 1 H), 7.37 (s, 5 H), 8.6 (br s, 1 H). Anal. ($C_{14}H_{15}NO_5 \cdot 0.13H_2O$) C, H, N.

(±)-*cis*/*trans*-5-Carbethoxy-4-phenyl-2-pyrrolidinone (4). Compound **3** (5.02 g, 18 mmol) was heated in an oil bath at a temperature of 180 °C until gas evolution had ceased (6 min). After being cooled the brown solid was dissolved in $CHCl_3$ (30 mL), the solution was washed with 0.05 N KOH (2 × 10 mL) and dried ($MgSO_4$), and the solvent was evaporated to give a white solid (3.6 g, 86%). The ratio of cis to trans isomers was determined to be 2:1, respectively, by means of the NMR spectrum of the mixture. Silica gel chromatography (3:1 ethyl acetate/hexane) was used to separate the diastereomers.

(±)-*cis*-5-Carbethoxy-4-phenyl-2-pyrrolidinone (4a). Recrystallization of **4a**, obtained after chromatography as described above, from acetone/hexane gave a white solid: mp 138–140 °C (lit.[29] mp 139–141 °C); TLC [silica gel; hexane (1)/EtOAc (3)] $R_f = 0.17$; NMR (400 MHz, $CDCl_3$) δ 0.844 (t, 3 H), 2.761 (d, 2 H), 3.697 (dq, 1 H, J = 7.2 and 10.8 Hz), 3.81 (dq, 1 H, J = 10.4 and 7.2 Hz), 3.987 (dt, 1 H, J = 8 and 7.6 Hz), 4.555 (d, 1 H, J = 8 Hz), 6.04 (br s, 1 H), 7.27 (m, 5 H).

(±)-*trans*-5-Carbethoxy-4-phenyl-2-pyrrolidinone (4b). Recrystallization from acetone/hexane of **4b**, obtained after chromatography as described above, afforded white crystals; mp 94–95 °C (lit.[29] mp 94–95 °C); TLC [silica gel; hexane (1)/ethyl acetate (3)] $R_f = 0.24$; NMR (400 MHz, $CDCl_3$) δ 1.261 (t, 3 H), 2.54 (dd, 1 H, J = 17.2 and 6.8 Hz), 2.87 (dd, 1 H, J = 17.2 and 9.6 Hz), 3.73 (m, 1 H), 4.22 (m, 3 H), 6.0 (br s, 1 H), 7.33 (m, 5 H).

(±)-*cis*-5-(Hydroxymethyl)-4-phenyl-2-pyrrolidinone (5a). To a solution of **4a** (4.5 g, 19.3 mmol) in THF (80 mL) was added $LiBH_4$ (1 M solution in THF, 48 mL, 48 mmol)[37] dropwise over a period of 10 min. The mixture was stirred at ambient temperature for 28 h, cooled in an ice bath and quenched with 2 N HCl. A white solid was obtained upon removal of the solvent. This solid was reevaporated from MeOH (3 × 30 mL) and then was chromatographed on silica gel eluting with $CHCl_3$ containing 5% MeOH, giving **5a** (3.28 g, 89%). The product was obtained as white crystals after recrystallization from $CHCl_3$/hexane; mp 146–148 °C; TLC [silica gel; ethyl acetate (7)/acetone (3)] R_f = 0.17; ^{1}H NMR ($CDCl_3$) δ 2.37–3.03 (m, 2 H), 3.22 (m, 2 H), 3.57–4.33 (m, 3 H), 7.23 (d, 5 H), 7.6 (s, 1 H); IR (KBr) 3400 (m), 3240 (m), 1665 (s) cm^{-1}. Anal. ($C_{11}H_{13}NO_2$) C, H, N.

(±)-*trans*-5-(Hydroxymethyl)-4-phenyl-2-pyrrolidinone (5b). The synthesis of **5b** was the same as that for **5a** except that after the reaction mixture was quenched with 2 N HCl, water was added to dissolve all of the salts, the solution was extracted with $CHCl_3$ (4 × 15 mL), the organic extract was dried ($MgSO_4$), and the solvent was evaporated to give a residue which was reevaporated from MeOH. Recrystallization from CH_2Cl_2/hexane–ether(1:1) gave **5b** (1.0 g, 81%) as white crystals: mp 106–107 °C; TLC [silica gel; ethyl acetate (7)/acetone (3)] $R_f = 0.16$; ^{1}H NMR ($CDCl_3$) δ 2.67 (m, 2 H), 3.13–4.13 (m, 5 H), 7.27 (s, 5 H), 7.43 (br, s, 1 H). Anal. ($C_{11}H_{13}NO_2$) C, H, N.

(±)-*cis*-5-(Bromomethyl)-4-phenyl-2-pyrrolidinone (6a). The reaction mixture, consisting of polymer-bound triphenylphosphine (15 g, 46 mmol) and **5a** (2.4 g, 12.6 mmol) in acetonitrile (200 mL) was cooled in an ice bath, and a solution of carbon tetrabromide (16.4 g, 49.5 mmol) in acetonitrile (50 mL) was added dropwise to it. The cold bath was removed, and the mixture was heated to reflux for 45 h. Upon cooling, the polymer was removed by filtration and the filtrate was concentrated in vacuo to give a brown oil. Silica gel chromatography (hexane/ethyl acetate (1:1)) yielded **6a** as a white solid (2.69 g, 84%): mp 91–93 °C; TLC [silica gel; ethyl acetate (7)/acetone (3)] $R_f = 0.4$; ^{1}H NMR ($CDCl_3$) δ 2.77 (d, 2 H), 3.0 (d, 2 H), 3.88 (q, 1 H), 4.24 (q, 1 H), 7.33 (m, 6 H); IR (KBr) 3220 (m), 1705 (s), 1260 (m) cm^{-1}. Anal. ($C_{11}H_{12}BrNO$) C, H, Br, N.

(±)-*trans*-5-(Bromomethyl)-4-phenyl-2-pyrrolidinone (6b). Triphenylphosphine (7.2 g, 27.5 mmol) and **5b** (3.5 g, 18.3 mmol) were dissolved in acetonitrile (150 mL), and the solution was cooled in an ice bath. A solution of carbon tetrabromide (9.23 g, 27.3 mmol) in acetonitrile (50 mL) was added dropwise to the reaction mixture, which was then stirred at ambient temperature for 23 h. Evaporation of the solvent gave a brown residue, which was chromatographed on silica gel (3:1 ethyl acetate/hexane) to give **6b**. The solid was recrystallized from $CHCl_3$/hexane to give **6b** as white needles (2.8 g, 61%); mp 93–94 °C; TLC [silica gel; ethyl acetate (7)/acetone (3)] $R_f = 0.43$; ^{1}H NMR ($CDCl_3$) δ 2.55 (dd, 1 H), 2.92 (dd, 1 H), 3.43 (m, 3 H), 3.95 (m, 1 H), 6.86 (br s, 1 H), 7.32 (s, 5 H); IR (KBr) 3200 (m), 1700 (s), 1255 (m) cm^{-1}. Anal. ($C_{11}H_{12}BrNO$) C, H, Br, N.

(±)-*cis*-5-(Fluoromethyl)-4-phenyl-2-pyrrolidinone (7a). Silver fluoride (0.4 g, 3.15 mmol) and **6a** (0.26 g, 1 mmol) were weighed directly into an aluminum foil covered flask under argon. Acetonitrile (40 mL) was syringed in, and the reaction mixture was stirred at ambient temperature for 40 h. The reaction mixture was filtered through a pad of Celite and silica gel, and the filtrate was concentrated in vacuo to give a yellow residue. Silica gel chromatography with ethyl acetate/hexane (3:1) and recrystallization from CH_2Cl_2/hexane–ether (1:1) gave **7a** as off-white crystals: mp 123–126 °C; TLC [silica gel; ethyl acetate (7)/acetone (3)] $R_f = 0.35$; ^{1}H NMR ($CDCl_3$) δ 2.74 (m, 2 H), 3.57–4.47 (m, 4 H), 6.82 (br s, 1 H), 7.31 (m, 5 H); high-resolution mass spectrum ($C_{11}H_{12}FNO$) m/z 193.0903 (dev 0.2 ppm).

(±)-*trans*-5-(Fluoromethyl)-4-phenyl-2-pyrrolidinone (7b). The synthesis of **7b** was the same as that for **7a** except that the reaction mixture was refluxed for 48 h. Upon cooling, 25 mL of Dowex 50 W (H^+ form) resin in acetonitrile was added to the flask, and this mixture was stirred slowly for 30 min. The Dowex resin was removed by filtration through a pad of Celite, and the filtrate was concentrated to give a yellow oil. Chromatography on silica gel (3:1 ethyl acetate/hexane) and recrystallization from CH_2Cl_2/hexane–ether (1:1) gave white needles of **7b** (0.64 g, 84%); mp 91–92 °C; TLC [silica gel; ethyl acetate (7)/acetone (3)] R_f = 0.39; ^{1}H NMR ($CDCl_3$) δ 2.7 (m, 2 H), 3.3 (m, 1 H), 3.83 (m, 1 H), 4.47 (dm, 2 H, J = 46 Hz), 7.03 (br s, 1 H), 7.3 (s, 5 H). Anal. ($C_{11}H_{12}FNO$) C, H, F, N.

(3*S*,4*S*),(3*R*,4*R*)-4-Amino-5-fluoro-3-phenylpentanoic Acid Hydrochloride (1a). Compound **7a** (60 mg, 0.31 mmol) was refluxed in 6 N HCl (15 mL) for 9.5 h, and then the solvent was

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evaporated. The resulting yellow oil was taken up in H_2O and washed with $CHCl_3$. The aqueous layer was applied to a Dowex 50 W (H^+ form) column (4 cm × 0.5 cm), and the column was washed sequentially with ethanol/water (1:1), H_2O, and 2 N HCl. The ninhydrin-positive fractions were combined, and the solvent was removed to give a thick, colorless paste which could not be solidified. All recrystallization and precipitation efforts were fruitless. TLC [silica gel; 1-butanol (12)/H_2O (5)/acetic acid (3)], R_f = 0.67 (ninhydrin positive); ^1H NMR (400 MHz, D_2O) δ 2.695 (m, 1 H), 2.835 (m, 1 H), 3.329 (m, 1 H), 3.75 (m, 1 H), 4.662 (HOD), 4.70 (d m, 2 H, J = 46.4 Hz), 7.249 (m, 5 H); ^{13}C NMR (400 MHz D_2O, external ref 1,4-dioxane at 67.6 ppm) δ 37.801, 41.580, 55.924, 82.124 (d, J_{CF} = 212.6 Hz); 129.291, 129.707, 130.564, 137.976, 176.035; high-resolution fast atom bombardment spectrometry (FABS) (m/z + H) for $C_{11}H_{14}FNO_2$ = 212.1080 (dev −3.2 ppm). Anal. [$C_{11}H_{14}NO_2F$·Hcl·1.6H_2O] C, H, N.

A white solid (182 mg) was obtained when the above reaction was carried out on a larger scale. However, the product contained an impurity as observed by HPLC. The desired product (t_R = 6.8 min) was obtained as an oil when separated from the impurity (t_R = 16.8 min) by preparative reversed-phase (C_{18}) HPLC with MeOH (29.95%)/H_2O (69.95%)/TFA (0.1%) as the eluant. The ^1H NMR spectrum and retention time were identical with those of the above sample.

(3*S*,4*R*),(3*R*,4*S*)-4-Amino-5-fluoro-3-phenylpentanoic Acid Hydrochloride (1b). Compound **7b** (96 mg, 0.497 mmol) was refluxed in 6 N HCl (16 mL) for 12.5 h, and then after being cooled, H_2O was added and the solution was extracted with $CHCl_3$ (5 × 10 mL). The organic layer yielded unhydrolyzed starting material (**7b**). The aqueous layer was concentrated to give a white solid (91 mg, 74%; 94% based on recovered starting material), which was recrystallized from methanol/ethyl acetate to give a white solid: mp 172–174 °C; TLC [silica gel; 1-butanol (12)/H_2O (5)/CH_3CO_2H (3)] R_f = 0.57 (ninhydrin positive); ^1H NMR (400 MHz, DMSO d_6) δ 2.505 (s, CD_3SOCD_2H), 2.704 (m, 1 H), 3.011 (m, 1 H), 3.357 (br s, H_2O), 3.417 (m, 1 H), 3.741 (m, 1 H), 4.069 (dm, 1 H, J = 46.4 Hz), 4.528 (dm, 1 H, J = 47.2 Hz), 7.303 (m, 5 H), 8.4–10 (br s, 4 H). Anal. ($C_{11}H_{14}FNO_2$·HCl) C, H, Cl, F, N.

Enzymes and Assays. Pig brain GABA aminotransferase, Gabase, succinic semialdehyde dehydrogenase, and L-glutamic acid decarboxylase were obtained and assayed as described previously.[22,24]

Inhibition of GABA Aminotransferase by 1a and 1b. Pig brain GABA aminotransferase (0.006 unit for **1a** and 0.011 unit for **1b**) was incubated at 25 °C with 5 mM α-ketoglutarate, 1 mM $NADP^+$, 5 mM β-mercaptoethanol, excess succinic semialdehyde dehydrogenase, and varying amounts of **1a** or **1b** and GABA in 50 mM potassium phosphate buffer at pH 8.5. Initial rates were measured spectrophotometrically, and kinetic parameters were derived from Lineweaver–Burk plots with seven different concentrations of GABA for every concentration of **1a** and **1b**.

Time-Dependent Inactivation of GABA Aminotransferase by 1a and 1b. Incubation mixtures were set up consisting of GABA aminotransferase (0.018 unit), 2 mM α-ketoglutarate, and a range of **1a** from 0 to 19 mM and of **1b** from 0 to 4 mM in 40 mM potassium pyrophosphate buffer (pH 8.5) at 25 °C. Periodically, 30-μL aliquots were withdrawn and assayed spectrophotometrically in a total volume of 500 μL for enzyme activity remaining.

Inhibition of L-Glutamic Acid Decarboxylase by 1a and 1b. L-Glutamic acid decarboxylase was incubated with 1.4 mM PLP, 2.4 mM β-mercaptoethanol, [1-^{14}C]-L-glutamate (concentrations varied between 0.1 and 0.5 mM), and varying amounts of **1a** or **1b** (0, 0.12, and 0.71 mM for **1a**; 0, 0.1, and 0.44 mM for **1b**) in 100 mL of potassium phosphate buffer at pH 7.2. The vials were incubated at 37 °C for a period of 1 h with gentle shaking. [^{14}C]Carbon dioxide that was evolved was absorbed by 100 μL of an 8% solution of KOH.[24] The reaction was quenched by the addition of 200 μL of 6 M H_2SO_4. The amount of [^{14}C]CO_2 produced could be related to enzyme activity by comparison with the amount of [^{14}C]CO_2 produced by the enzyme in the control, to which no inhibitor was added.

Time-Dependent Inhibition of L-Glutamic Acid Decarboxylase by 1a and 1b. L-Glutamic acid decarboxylase was incubated with **1a** (2.83 mM) or **1b** (2.28 mM), 0.35 mM PLP, and 5 mM β-mercaptoethanol in 87.5 mM potassium phosphate buffer at pH 7.2. At various time intervals 1.8-mL aliquots were removed and assayed with 0.1 mL of 50 mM [1-^{14}C]-L-glutamate.[24]

GABA Aminotransferase Catalyzed Transamination of 1a and 1b. Transamination of **1a** or **1b** was investigated by measuring the conversion of [5-^{14}C]-α-ketoglutarate to [5-^{14}C]-glutamate at various concentrations of the potential substrate as described previously.[38]

Attempted Detection of 3-Phenyllevulinic Acid (11). GABA aminotransferase (2.71 units) was incubated with **1a** (6.2 mM) or **1b** (3.5 mM), 0.7 mM α-ketoglutarate, and 5 mM β-mercaptoethanol in 50 mM potassium pyrophosphate buffer at pH 8.5. Aliquots were removed and injected into an HPLC equipped with a Beckman Ultrasphere ODS 5 μm (4.6 mm × 25 cm) C_{18} column. At a flow rate of 1 mL/min, **1a** had a t_R of 6.73 min, **1b** had a t_R of 13.38 min, and **11** had a t_R of 83 min with MeOH/H_2O/TFA in a ratio of 24.95:74.95:0.1 as the eluent. Compound **11** had a t_R of 10.13 min and **1a** and **1b** eluted in the void volume under eluent conditions of MeOH/H_2O/TFA in a ratio of 49.95:49.95:0.1, respectively.

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Registry No. **1a**, 124687-35-0; **1a** (free base), 124687-37-2; **1b**, 124687-36-1; **1b** (free base), 124687-38-3; **2**, 109838-76-8; **3**, 124687-28-1; **4a**, 124815-47-0; **4b**, 124815-48-1; **5a**, 124687-29-2; **5b**, 124687-30-5; **6a**, 124687-31-6; **6b**, 124687-32-7; **7a**, 124687-33-8; **7b**, 124687-34-9; GABA aminotransferase, 9037-67-6; $CH_3CONHCH(CO_2Et)_2$, 1068-90-2; $PhCH{=}CHCO_2Et$, 103-36-6; L-glutamic acid decarboxylase, 9024-58-2.

Supplementary Material Available: X-ray data (coordinates, anisotropic temperature factors, distances, and angles) for compound **4b** (4 pages) is available. Ordering information is given on any current masthead page.

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