Synthesis and Evaluation of Mono-, Di-, and Trifluoroethenyl-GABA Derivatives as GABA-T Inhibitors

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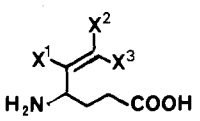
The syntheses of four derivatives of γ-vinyl-GABA, in which vinylic hydrogen atoms were replaced by fluorine, are described. With use of 5-ethenyl-2-pyrrolidinone as starting material, the *E* and *Z* isomers of 4-amino-6-fluoro-5-hexenoic acid were prepared. The 6,6-difluoro and 5,6,6-trifluoro analogues could be synthesized from 4-oxobutanoic acid *tert*-butyl ester and (2,2-difluoroethenyl)- and (trifluoroethenyl)lithium correspondingly. The compounds were tested as inhibitors of GABA-T, and their in vitro and in vivo biochemistry is reported. The most active derivative was (*Z*)-4-amino-6-fluoro-5-hexenoic acid; the structure–activity relationship in the series is discussed.

γ-Vinyl-GABA (4-amino-5-hexenoic acid, 1) is a very selective enzyme-activated inhibitor of GABA-transaminase in mammalian brain.[1] The compound increases brain GABA levels in rodents,[2] has anticonvulsant properties in animal models,[3] and holds promise as a new antiepileptic drug in humans.[4] The postulated mechanism of inhibition of GABA-T by the GABA derivative 1 is depicted in Scheme I. The GABA analogue must be recognized as a substrate by GABA-T and form a Schiff base with pyridoxal phosphate to generate after de- and reprotonation a Michael acceptor, which can then alkylate the enzyme.

In order to increase the electrophilicity of the intermediate Michael acceptor, we considered substitution at the double bond of γ-vinyl-GABA (1) by electronegative groups, such as fluorine atoms. Antecedents to this approach are reported, e.g., for the inhibition of γ-cystathionase by β-(trifluoromethyl)alanine.[5a] As illustrated in Scheme II, both the substrate and the inhibitor yield a Michael acceptor. The one that is activated by two fluorine atoms leads to enzyme inhibition, whereas the one derived from the substrate undergoes normal transformations. Similar differences in reactivity had been reported earlier by Silverman and Abeles for the inhibition of pyridoxal phosphate dependent enzymes by mono- and polyhaloalanines.[5b]

This motivated us to synthesize the γ-vinyl-GABA derivatives **2–5**, analogues of 1 with different fluorine substitution patterns at the double bond, and we have studied their interaction with GABA-T both in vitro and in vivo.



	X^1	X^2	X^3
1	H	H	H
2	F	H	H
3	H	H	F
4	H	F	F
5	F	F	F

Results and Discussion

Chemistry. For the preparation of the target compounds **2–5**, a convenient synthetic entry to the fluorinated allylamines **6**, hitherto not reported in the literature,[6] had to be developed, and three reaction schemes were envisaged.

As potential precursors we considered the alcohols or ethers **7**[7] (Scheme III) with the premise that at a later stage of the synthesis the OR′ group could be transformed into an amine functionality. Another possibility, especially convenient for the synthesis of *mono*fluorinated allylamines **6**, exploits the accessibility of allylamines **8**. It involves installing the fluorine substitution pattern by addition of F–Y to the double bond and subsequent elimination of H–Y.[8] The third suggested approach, related to the sequence via the halogenated allyl alcohols/ethers **7**, is via the fluorinated allyl halides **9**. However, the scarcity of literature references to these compounds[9] and several unsuccessful attempts in our laboratory to prepare the halides **9** from the corresponding olefins $X_2C{=}CXCH_2R$ discouraged us from investigating this route any further.

In the synthesis of the monofluorinated derivatives **2** and **3**, a synthetic route via allylamines **8** was used (Scheme IV). 5-Ethenyl-2-pyrrolidinone[10] (**10**) was reacted with HF/pyridine and *N*-bromosuccinimide in Et_2O[8] to afford a 1:3 mixture of positional isomers of (bromofluoroethyl)pyrrolidinones **11** and **12**. Treatment of this mixture

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(4) Rimmer, E. M.; Richens, A. *Lancet* **1984**, 189.
(5) (a) Alston, T. A.; Muramatsu, H.; Ueda, T.; Bright, H. J. *FEBS Lett.* **1981**, *128*, 293. (b) Silverman, R. B.; Abeles, R. H. *Biochemistry* **1976**, *21*, 4718.
(6) Described is a 3-(2,2-difluorovinyl)-1,2-diazetidine derivative: Putnam, R. E.; Anderson, J. L.; Sharkey, W. H. *J. Am. Chem. Soc.* **1961**, *83*, 386.
(7) (a) $F_2C{=}CFCHOHR$: Normant, J. F.; Foulon, J. P.; Masure, D.; Sauvêtre, R.; Villeras, J. *Synthesis* **1975**, 122. Tarrant, P.; Johncook, P.; Savory, J. *J. Org. Chem.* **1963**, *28*, 839. Sterlin, R. N.; Yatsenko, R. D.; Knunyants, I. L. *Khim. Nauka Prom.* **1958**, *3*, 540; *Chem. Abstr.* **1959**, *53*, 4195. (b) $F_2C{=}CHCHOHR$: Sauvêtre, R.; Normant, J. F. *Tetrahedron Lett.* **1981**, *22*, 957. (c) $F_2C{=}CHCHORR$: Tronchet, J. M. J.; Bonenfant, A.-P. *Helv. Chim. Acta* **1980**, *63*, 1644; *Carbohydr. Res.* **1981**, *93*, 205, and prior work by these authors cited therein. Kiehs, K.; Schwarz, G. U.; Adolphi, H. (BASF A.-G.) S. African Pat. 7 906 502, Dec 31, 1980; *Chem. Abstr.* **1981**, *95*, 37123e. (d) $HFC{=}CFCHOHR$: Sauvêtre, R.; Masure, D.; Chuit, C.; Normant, J. F. *Synthesis* **1978**, 128. (e) $H_2C{=}CFCHOHR$: Bessiere, Y.; Savary, D. N.-H.; Schlosser, M. *Helv. Chim. Acta* **1977**, *60*, 1739.
(8) Olah, G. A.; Welch, J. T.; Vankar, Y. D.; Nojima, M.; Kevekes, I.; Olah, J. A. *J. Org. Chem.* **1979**, *44*, 3872. Bowers, A.; Ibanez, L. C.; Denot, E. *J. Am. Chem. Soc.* **1968**, *90*, 947. Starber, S.; Zupan, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 925. Boche, G.; Fährmann, U. *Chem. Ber.* **1981**, *114*, 4005.
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(10) Gittos, M.; Letertre, G. J. (Merrell Toraude S.A.) Belg. Pat. 873 766, May 16, 1979; *Chem. Abstr.* **1980**, *91*, 9149n.

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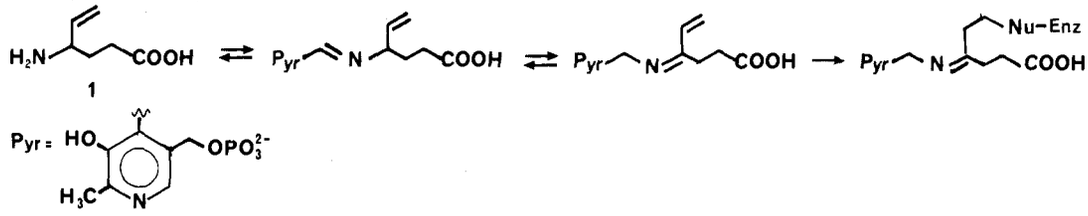
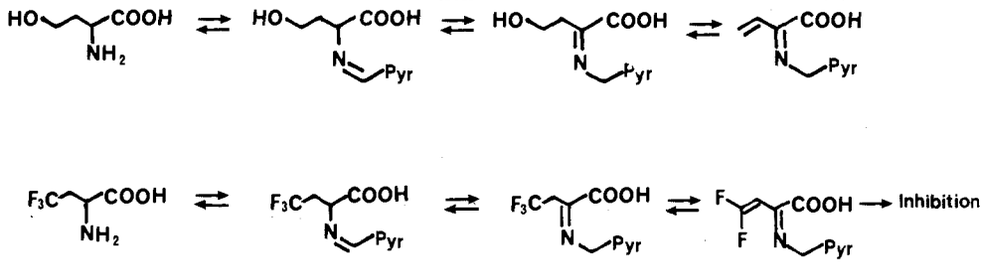
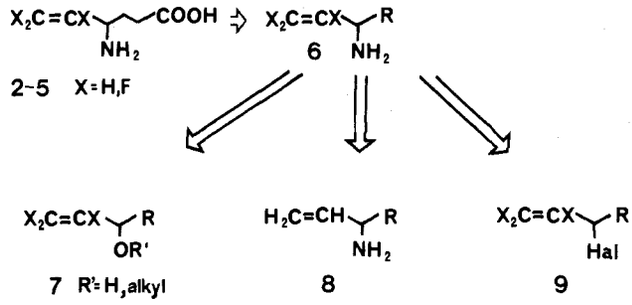
Scheme I. Postulated Mechanism of GABA-T Inhibition by γ-Vinyl-GABA (1)



Scheme II. Cysthathionase: Mechanism of Action and Inhibition



Scheme III. Retrosynthesis of Compounds 2–5



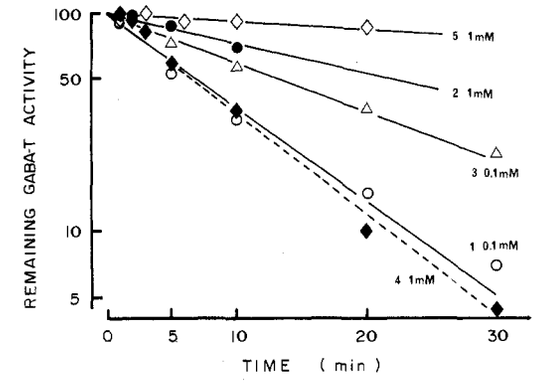
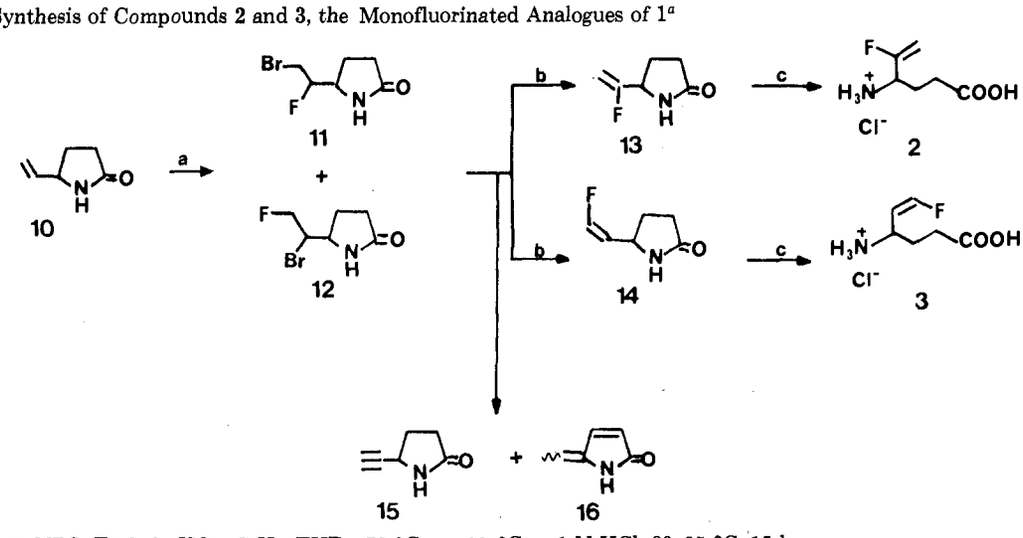


Figure 1. Time-dependent inhibition of GABA-T by 1 and its fluorinated analogues 2–5. Partially purified GABA-T was incubated at 37 °C as described in the Experimental Section with the different compounds at the indicated concentrations. Aliquots were withdrawn at the indicated time points and assayed for residual GABA-T activity.

with base to eliminate the elements of HBr afforded the (fluoroethenyl)pyrrolidinones **13** and **14**, and some experimentation showed that potassium *tert*-butoxide in THF at −30 °C is the best condition for this transformation (~50% yield). The use of potassium *tert*-butoxide at higher temperatures or 1,8-diazabicyclo[5.4.0]undec-7-ene at 0 °C gave rise to further elimination of HF, yielding the ethynylpyrrolidinone **15** together with the doubly unsaturated derivative **16**. The mixture of fluoroethenyl compounds **13** and **14** could be separated by chromatography on silica gel. Acid hydrolysis of the pure isomers then gave our target molecules 4-amino-5-fluoro-5-hexenoic acid (**2**) and (*Z*)-4-amino-6-fluoro-5-hexenoic acid (**3**) as hydrochloride salts.

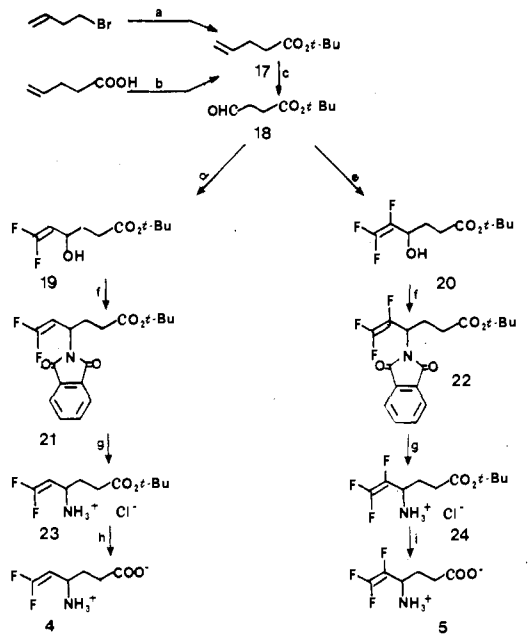
Access to the difluoro- and trifluoroethenyl analogues **4** and **5** was gained via the corresponding fluoroethenyl alcohols **7** (R′ = H), which in turn were obtained by ad-

Scheme IV. Synthesis of Compounds 2 and 3, the Monofluorinated Analogues of 1[a]



[a] a, HF/C_5H_5N, NBS, Et_2O; b, KO-*t*-C_4H_9, THF, −78 °C → −20 °C; c, 1 N HCl, 80–85 °C, 15 h.

Scheme V. Synthesis of the Di- and Trifluoro-Substituted Derivatives 4 and 5[a]



[a] a, Mg, $(t\text{-}C_4H_9OCO)_2O$; b, Ac-O-t-C_4H_9, $HClO_4$; c, O_3, $(CH_3)_2S$; d, $CF_2{=}CH_2$, *sec*-BuLi, −105 °C; e, $CF_2{=}CFH$, *n*-BuLi, −100 °C; f, PhtNH, $P(C_6H_5)_3$, DEAD, THF; g, H_2NNH_2, HCl; h, 1 N HCl, 3 days, NaOH; i, 1 N HCl, 3 days, Et_3N.

dition of (2,2-difluoroethenyl)-[7b] or (trifluoroethenyl)lithium[7a,11] to the aldehyde 4-oxobutanoic acid *tert*-butyl ester (18). This approach could in principle also be used for the synthesis of the monofluorinated derivatives, but was discarded due to the reported[12] instability of the requisite (monofluoroethenyl)lithium reagents.

The aldehyde ester 18 was obtained in two steps from 4-bromobutene (Scheme V). Reaction of 4-butenylmagnesium bromide with di-*tert*-butyl dicarbonate afforded the *tert*-butyl ester 17[14] of allylacetic acid; ozonolysis then, following a procedure described in the literature for a close analogue,[15] gave the aldehyde 18. Reaction of this aldehyde with (2,2-difluoroethenyl)lithium (from 1,1-difluoroethene)[7b] at −105 °C allowed to obtain, after quenching at −50 °C, the fluorinated allyl alcohol 19. The amine functionality was then introduced by reaction with phthalimide in the presence of triphenylphosphine and diethyl azodicarboxylate,[16] and the protected amine 21 was obtained. Hydrazinolysis followed by mild acid treatment gave the ester hydrochloride 23, which on further acid hydrolysis afforded the target molecule, 4-amino-6,6-difluoro-5-hexenoic acid (4), isolated as the hydrochloride salt.

The aldehyde ester 18 also served as starting material for the synthesis of 4-amino-5,6,6-trifluoro-5-hexenoic acid hydrochloride (5). The sequence resembles the one described above for the preparation of the difluoroethenyl analogue 4, with the exception that (trifluoroethenyl)-lithium (from trifluoroethene)[11] was used as the nucleophile in the addition to the aldehyde 18 to give the trifluoroethenyl alcohol ester 20. Subsequent transformation to the protected amine derivative 22 and removal of the protection group to obtain the amine ester hydrochloride 24 and further the target compound 5 followed the path established in the above described sequence toward the analogue 4 (Scheme V). The trifluorovinyl derivative 5 could be purified via its zwitterionic form by addition of Et_3N to the hydrochloride salt.

Table I. pK_a Values of the Amines in Compounds 1–5 and γ-Ethynyl-GABA

compd	1	2	3	4	5	γ-ethynyl-GABA
pK_a	9.5	8.32	9.35	9.32	7.87	8.2

Table II. In Vivo Activity of Vinyl-GABA (1) and the Fluorinated Analogues 2–5[a]

compd	dose, mmol/kg	GABA-T, mmol/h	GABA, μmol/g
1	1.9	29.3 ± 0.5[b]	9.1 ± 0.6[b]
2	1.9	59.7 ± 2.9[b]	3.6 ± 0.1[b]
3	1.9	38.3 ± 1.5[b]	9.8 ± 0.3[b]
4	1.9	42.4 ± 0.7	2.8 ± 0.7
5	3.8	69.4 ± 1.4	2.7 ± 0.2
control		72.5 ± 0.5	2.9 ± 0.2

[a] Brain GABA-T activity and brain GABA levels were measured 6 h after ip administration of drugs. All values are the means ± SEM of five animals, except for control, which represents the mean of 15 animals done on different days. [b] $p < 0.05$ compared to control Student's t test.

In Vitro Biochemistry. 4-Aminobutyrate aminotransferase (GABA-T, E.C. 2.6.1.9) was partially purified from pig brain as described for rabbit brain.[17] When GABA-T was incubated with the fluoroethenyl GABA derivatives 2–5, there was a time-dependent loss of enzyme activity. In Figure 1, the rates of enzyme inhibition at a given concentration of compounds 2–5 are compared. γ-Vinyl-GABA (1) (0.1 mM) was added for reference. The least active inhibitor in this series is the trifluoro derivative 5, followed by the "endo" monosubstituted compound 2. The rate of inhibition of GABA-T by 1 mM difluoro compound 4 is identical with that by the nonfluorinated derivative 1 at a 10 times lower concentration. The "exo" monofluoro derivative 3 inhibits GABA-T at half the rate of γ-vinyl-GABA (1) at 0.1 mM and thus is the most active compound in the series. The inhibition by the mono- and difluoroethenyl compounds 3 and 4 was not reversed by dialysis.[18] The 5-fluoro compound 2 and the trifluoroethenyl derivative 5 showed no competitive inhibition of GABA-T at 1 mM, even at 10 times lower concentrations of GABA. None of the compounds formed a significant amount of glutamate when incubated with GABA-T at a concentration of 1 mM in the presence of radiolabeled α-ketoglutarate and pyridoxal phosphate. As compounds 2 and 5 did not inhibit GABA-T competitively, they

(11) Drakesmith, F. G.; Richardson, R. D.; Stewart, O. J.; Tarrant, P. *J. Org. Chem.* **1968**, *33*, 286.
(12) The synthesis of (1-fluorovinyl)lithium from vinyl fluoride via 1-bromo-1-fluoroethane is described to be accompanied by flashes, minor explosions, and carbon deposition;[11] (2-fluorovinyl)lithium is not described in the literature.
(13) Herrmann, J. L.; Kieczykowski, G. R.; Romanet, R. F.; Wepplo, P. J.; Schlessinger, R. H. *Tetrahedron Lett.* **1973**, 4711.
(14) (a) Shishido, K.; Kazama, Y.; Kodama, H.; Nozaki, H. *J. Am. Chem. Soc.* **1959**, *81*, 5817. (b) Rathke, M. W.; Lindert, A. *J. Am. Chem. Soc.* **1971**, *93*, 2318; BASF Brit. Pat. 1 265 173, March 1, 1972; *Chem. Abstr.* **1972**, *76*, 154425*y*; French Pat. 2 012 256, March 13, 1968; *Chem. Abstr.* **1970**, *73*, 110464. The olefin ester 17 can also be obtained directly from allylacetic acid by esterification with *tert*-butyl acetate/$HClO_4$; see Experimental Section.
(15) Winter, M.; Näf, F.; Furrer, A.; Pickenhagen, W.; Giersch, W.; Meister, A.; Willhalm, B.; Thommen, W.; Ohloff, G. *Helv. Chim. Acta* **1979**, *62*, 135.
(16) Mitsunobu, O.; Wada, M.; Sano, T. *J. Am. Chem. Soc.* **1972**, *94*, 679. Wada, M.; Sano, T.; Mitsunobu, O. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 2833.
(17) John, R. A.; Fowler, L. J. *Biochem. J.* **1976**, *155*, 645.
(18) This was not checked for 2 and 5 as the inhibition was less marked.

probably have a poor affinity for the enzyme. Steric hindrance we believe is not involved. Fluorine substitution however has a dramatic effect on the pK_a value (Table I) and also on the nucleophilicity of these amines. One could conclude that compounds **2** and **5** do not form a Schiff base with the pyridoxal phosphate in the enzyme active site. However, γ-ethynyl-GABA (pK_a = 8.2) is a potent irreversible inhibitor of GABA-T, so that the low basicity of the 5-fluoro compound **2** and the trifluoro derivative **5** probably is not the only reason for their poor activity as GABA-T inhibitors.

In Vivo Biochemistry. The fluoroethenyl GABA derivatives **2–5** were administered to male mice at a given dose. The animals were killed 6 h later, and brain GABA-T activity were measured as described in the Experimental Section. γ-Vinyl-GABA (**1**) was included for comparison. The results are summarized in Table II.

The in vivo effects confirm the activity described in vitro. The trifluoro compound **5** at a dose of 3.9 mmol/kg did not produce a statistically significant change of GABA-T activity nor an elevation of brain GABA levels. The 5-fluoro compound **2** at half this dose did inhibit GABA-T by 18%, resulting in a small but significant increase of GABA levels. A surprising result was obtained for the difluoro derivative **4**, which caused a 42% decrease of GABA-T activity with **no** increase in brain GABA levels and had no effect on glutamic acid decarboxylase activity either in vitro or in vivo. A blockade of GABA synthesis by **4** can be ruled out as this compound did not inhibit GAD in vitro and GAD activity was not decreased in vivo. A possible explanation is that a metabolite of **4** inhibits GAD competitively. The 6-fluoro compound **3** is as potent as γ-vinyl-GABA (**1**) at elevating brain GABA levels, despite a slightly lesser inhibition of GABA-T.

In summary, only the "exo" monofluoroethenyl GABA analogue **3** has an activity that would warrant further investigation.

Experimental Section

For a detailed description of the experimental techniques, refer to the Experimental Section of our earlier work.[19]

^{19}F NMR (84.67 MHz) spectra were recorded on a Bruker WH 80 instrument and the data are reported in ppm upfield from $CFCl_3$ (external reference). The ^1H NMR (200 MHz) spectra were registered on a Bruker WP 200 SY instrument.

Trifluoro- and 1,1-difluoroethene were purchased from PCR Research Chemicals Inc., Gainesville, FL, and used as such. HF/pyridine was bought from Aldrich, as was *n*-butyllithium in hexane. *sec*-Butyllithium in isopentane was purchased from Metallgesellschaft AG, Frankfurt, a.M. All solvents used in the metalation reactions were dried thoroughly. THF was distilled from KOH and then from $LiAlH_4/(C_6H_5)_3CH$ or $Na/(C_6H_5)_2CO$ and Et_2O from $LiAlH_4$ and pentane from P_2O_5.

All other chemicals were used without any further purification of the commercially available material.

5-(1(2)-Bromo-2(1)-fluoroethyl)-2-pyrrolidinone (**11**, **12**). To a solution of 10.7 g (0.06 mol) of *N*-bromosuccinimide in 60 mL of anhydrous Et_2O was added HF/pyridine (70%, 60 mL). The stirred mixture was cooled in an ice bath. A solution of 6.67 g (0.06 mol) of 5-ethenyl-2-pyrrolidinone (**10**)[10] in Et_2O (6 mL) was added slowly. The reaction mixture was allowed to warm to room temperature in about 30 min, stirred for an additional hour, and then poured into a solution of 150 g of K_2CO_3 in water/ice (500 mL). Continuous extraction with Et_2O (1 L) during 6 h, drying the ether layer over Na_2SO_4, and removal of the solvents at reduced pressure (rotatory evaporator, 20 Torr, 30 °C) left an oil, which was bulb-to-bulb distilled twice to give 7.8 g (62%) of a 1:3 mixture of (bromofluoroethyl)pyrrolidinones **11** [^1H NMR ($CDCl_3$) δ 4.0 (m, 2, FCHCHN), 3.57 (dd, 1, J_{FH} = 21 Hz, J_{HH} = 6 Hz, $BrCH_2$), 2.3 (m, 4, CH_2CH_2)] and **12** [^1H NMR ($CDCl_3$) δ 4.67 (dd, 2, J_{FH} = 51 Hz, J_{HH} = 5 Hz, FCH_2), 4.0 (m, 2, BrCHCHN), 2.3 (m, 4, CH_2CH_2)], used in the further reaction sequence without purification.

5-(1-Fluoroethenyl)-2-pyrrolidinone (**13**). To a stirred solution of 6.5 g (0.031 mol) of the mixture of (bromofluoroethyl)pyrrolidinones **11**/**12** (obtained as described above as a 1:3 mixture, namely, 7.75 mmol of **11** and 23.25 mmol of **12**) in THF (40 mL) kept under nitrogen at −78 °C was added dropwise 13.9 g (0.124 mol) of potassium *tert*-butoxide, dissolved in 90 mL of THF. The mixture was allowed to warm to −20 °C, stirred for 1 h at −20 °C, and cooled again to −78 °C, and 5.58 g (5.3 mL, 0.093 mol) of AcOH was added. Adding Et_2O completed the formation of a precipitate, which was filtered off, and the filtrate was concentrated under reduced pressure (rotatory evaporator, 20 Torr, 50 °C) to give 2.0 g (50%) of a mixture of (fluoroethenyl)pyrrolidinones **13** and **14** as an oil. These two fluorinated olefins were separated by means of column chromatography (silica gel, 230–400 mesh, 300 g, AcOEt). Fractions with the compound that corresponded to R_f 0.12 were combined and flash evaporated to yield the 1-fluoroethenyl derivative **13**, contaminated with 5-ethynyl-2-pyrrolidinone (**15**). Final purification was achieved by HPLC on a Partisil M9 10/50 ODS-5 column [eluant: water/CH_3OH (10:1), 5 mL/min]. The product-containing fractions were combined and flash evaporated to give 0.42 g (42% from **11**) of 5-(1-fluoroethenyl)-2-pyrrolidinone (**13**): ^1H NMR ($CDCl_3$) δ 4.67 (dd, 1, J_{FH} = 26 Hz, J_{HH} = 3 Hz, (Z)-CF=C*H*H), 4.47 (dd, 1, J_{FH} = 40 Hz, J_{HH} = 3 Hz, (E)-CF=CH*H*), 4.3 (m, 1, NCH), 2.3 (m, 4, CH_2CH_2); IR ($CHCl_3$) 3440, 3300, 1700 (C=CF and carbonyl), 1410; DCIMS, *m/e* (relative intensity) 259 (2 M + H, 30), 239 (25), 130 (M + H, 100), 110 (50).

(*Z*)-(2-Fluoroethenyl)-2-pyrrolidinone (**14**). From the chromatography described in the preparation of compound **13**, fractions that contained the 2-fluoroethenyl derivative **14** (R_f 0.15) were combined and flash evaporated to give 1.35 g (45% from **12**) of (*Z*)-5-(2-fluoroethenyl)-2-pyrrolidinone (**14**): ^1H NMR ($CDCl_3$) δ 6.50 (dd, 1, J_{FH} = 84 Hz, J_{HH} = 4 Hz, CFH), 4.95 (ddd, 1, J_{FH} = 40 Hz, J_{HH} = 9 Hz, J_{HH} = 4 Hz, CH=CF), 4.5 (m, 1, NCH), 2.2 (m, 4, CH_2CH_2); IR ($CHCl_3$) 3440, 3200, 1690 (HC=CHF and carbonyl); DCIMS, *m/e* (relative intensity) 259 (2 M + H, 10), 130 (100), 110 (5).

4-Amino-5-fluoro-5-hexenoic Acid Hydrochloride (**2**). A solution of 0.30 g (2.4 mmol) of (fluoroethenyl)pyrrolidinone **13** in 3.6 mL of 1 N HCl was heated to 80–85 °C (bath temperatures) for 15 h and concentrated under reduced pressure, and the residue was coevaporated with i-C_3H_7OH to yield crude 4-amino-5-fluoro-5-hexenoic acid hydrochloride (**2**). Recrystallization from i-C_3H_7OH/Et_2O gave 0.15 g (35%) of pure **2**: mp 113 °C; ^1H NMR (200 MHz) (D_2O) δ 5.05 (dd, 1, J_{FH} = 20 Hz, J_{HH} = 4 Hz, (Z)-CF=C*H*H), 4.87 (dd, 1, J_{FH} = 52 Hz, J_{HH} = 4 Hz, (E)-CF=CH*H*), 4.06 (dt, 1, J_{FH} = 23 Hz, J_{HH} = 8 Hz, NCH), 2.45 (m, 2, CH_2CO), 2.08 (m, 2, CH_2); ^{19}F NMR (H_2O) −112.4 (ddd, J_{FH} = 22 Hz, J_{FH} = 52 Hz, J_{FH} = 20 Hz); IR (KBr) 3420, 3000, 1720 (carbonyl), 1680 ($CF=CH_2$), 1620, 1400 cm^{-1}; DCIMS (TFA derivative), *m/e* (relative intensity) 261 (M + CH_5, 20), 244 (M + H, 15), 226 (100), 206 (30). Anal. ($C_6H_{10}FNO_2 \cdot HCl$) C, H, N.

(*Z*)-4-Amino-6-fluoro-5-hexenoic Acid Hydrochloride (**3**). A solution of 1.5 g (0.0116 mol) of (fluoroethenyl)pyrrolidinone **14** in 17.4 mL of 1 N HCl was warmed in an oil bath (95 °C) for 15 h. Solvents were removed under reduced pressure, and the residue was coevaporated with i-C_3H_7OH to afford the crude acid **3**, a semisolid material. Trituration with Et_2O afforded a white solid, which was recrystallized twice from EtOH (95%)/Et_2O to give analytically pure (*Z*)-4-amino-6-fluoro-5-hexenoic acid hydrochloride (**3**) (0.9 g, 42%): mp 178 °C; ^1H NMR (200 MHz) (D_2O) δ 6.84 (dd, 1, J_{FH} = 83 Hz, J_{HH} = 5 Hz, CHF), 4.99 (ddd, 1, J_{FH} = 40 Hz, J_{HH} = 10 Hz, J_{HH} = 5 Hz, CF=CH), 4.33 (dt, 1, J = 10 Hz, J = 5 Hz, CHN), 2.44 (m, 2, CH_2CO), 2.11 and 1.87 [2 m, (1:1), 2, CH_2); ^{19}F NMR (H_2O) −118 (dd, J_{FH} = 83 Hz, J_{FH} = 40 Hz); IR (KBr) 3410, 2940, 1710 (carbonyl), 1680 (CH=CFH), 1610, 1480, 1405, 1230, 1215, 1180 cm^{-1}; DCIMS (TFA derivative), *m/e* (relative intensity) 244 (M + H, 10), 227 (30), 226 (90), 130 (100), 115 (100). Anal. ($C_6H_{10}FNO_2 \cdot HCl$) C, H, N.

4-Pentenoic Acid *tert*-Butyl Ester (**17**). **(a) From 4-Bromobutene.** To 2.4 g (0.1 mol) of magnesium in 50 mL of Et_2O under nitrogen was added dropwise a solution of 13.5 g (10.2 mL,

(19) Kolb, M.; Danzin, C.; Barth, J.; Claverie, N. *J. Med. Chem.* **1982**, *25*, 550.

0.1 mol) of 4-bromobutene in 150 mL of Et_2O at a rate that kept the reaction mixture at reflux. After complete addition, the mixture was refluxed for an additional hour, cooled in an ice bath, and added to a cooled ethereal solution (ice bath) of di-*tert*-butyl dicarbonate (43.6 g, 0.2 mol in 50 mL of Et_2O). This mixture was then refluxed for 1 h and then treated with concentrated aqueous NH_4Cl to obtain a solution, which was extracted with Et_2O (2 × 250 mL). The combined organic layers were washed with water (3 × 300 mL) and brine (200 mL), dried over Na_2SO_4, and then distilled. The solvent was collected in a forerun; 4-pentenoic acid *tert*-butyl ester (17) distilled at 53–56 °C (18 Torr) [lit.[14] bp 68–69 °C (26 Torr)]: yield 9.3 g (59%); ^1H NMR ($CDCl_3$) δ 6.0–5.5 (m, 1, CH=C), 5.1, 5.0, and 4.8 (3 m, 2, C=CH_2), 2.3 (br d, 4, CH_2CH_2), 1.40 (s, 9, 3 CH_3).

(b) From Allylacetic Acid. A solution of 24.5 g (25 mL, 0.245 mol) of allylacetic acid in 500 mL of acetic acid *tert*-butyl ester and 4 drops of concentrated $HClO_4$ was kept at room temperature for 14 h, neutralized by addition of aqueous Na_2CO_3, and concentrated (20 Torr) to obtain a semisolid mixture. Et_2O (500 mL) and more aqueous Na_2CO_3 (~500 mL) were added, layers were separated, and the aqueous layer was extracted with Et_2O (2 × 500 mL). The combined organic layers were dried (Na_2CO_3) and fractionated to give 20 g (52%) of 4-pentenoic acid *tert*-butyl ester (17): bp 51–53 °C (20 Torr) [lit.[14] bp 68–69 °C (26 Torr)].

4-Oxobutanoic Acid *tert*-Butyl Ester (18). A 4.04-g (0.026 mol) sample of the olefin ester 17 was ozonized in CH_2Cl_2 (50 mL, by analogy with a procedure described in the literature[15] for the synthesis of the ethyl ester analogue), the reaction quenched with triphenylphosphine, and 4-oxobutanoic acid *tert*-butyl ester (18) isolated by bulb-to-bulb distillation: 3.3 g (81%); bp 95–105 °C (15 Torr) (see also ref 13); ^1H NMR ($CDCl_3$) δ 9.93 (s, 1, CHO), 2.4 (m, 4, CH_2CH_2), 1.46 (s, 9, 3 CH_3); IR ($CHCl_3$) 2980, 2930, 2820, 1730, 1715 (ester and aldehyde) cm^{-1}.

6,6-Difluoro-4-hydroxy-5-hexenoic Acid *tert*-Butyl Ester (19). To a stirred solution of about 0.035 mol of 1,1-difluoroethylene in THF/pentane (40 mL, 4:1), obtained by condensing ~800 mL of 1,1-difluoroethylene into the cooled solvent mixture (−110 °C, MeOH/liquid N_2 cooling bath) under nitrogen at −100 to −110 °C, was added slowly 40 mL of *sec*-butylithium (0.87 M in isopentane, 35 mmol). The slightly colored solution was stirred at −105 °C for 10 min, 2.37 g (15 mmol) of the aldehyde ester 18 was added, and the reaction mixture was allowed to warm to −50 °C. A 35-mL sample of 1 N H_2SO_4 was added, the mixture was poured into Et_2O (200 mL), the layers were separated, and the aqueous layer was extracted with Et_2O (100 mL). The combined ethereal layers were washed twice with water (2 × 100 mL) and concentrated aqueous $NaHCO_3$ (100 mL) and twice with brine (2 × 100 mL). After drying (Na_2SO_4), the organic layer was flash evaporated (20 Torr, bath temperature ~30 °C) and the residual oil distilled [bulb-to-bulb distillation, bp 135–145 °C (0.05 Torr)] to give 1.8 g (55%) of 6,6-difluoro-4-hydroxy-5-hexenoic acid *tert*-butyl ester (19): ^1H NMR ($CDCl_3$) δ 4.4 (m, 2, F_2C=CHCHN), 2.3 and 1.7 (2 m, 4, CH_2CH_2), 1.43 (s, 9, 3 CH_3); IR ($CHCl_3$) 3600, 3430, 2980, 2920, 1735 (CF_2=CH), 1720 (ester), 1370, 1150, 920, 900, 750 cm^{-1}.

4-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-6,6-difluoro-5-hexenoic Acid *tert*-Butyl Ester (21). In a manner analoguous to the preparation of the trifluoroethenyl compound **22** described below, 2.29 g (0.0103 mol) of the difluoro analogue **19** was reacted with 1.1 equiv of triphenylphosphine, 1.1 equiv of 1,3-dihydro-1,3-dioxo-2*H*-isoindole, and 1.2 equiv of diethyl azodicarboxylate in THF to afford after chromatography 1.39 g (39%) of 4-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-6,6-difluoro-5-hexenoic acid *tert*-butyl ester (21): ^1H NMR ($CDCl_3$) δ 7.80 (m, 4, aryl H), 5.2–4.6 (m, 2, F_2C=CHCHN), 2.3 (m, 4, CH_2CH_2), 1.43 (s, 9, 3 CH_3); IR ($CHCl_3$) 2980, 2930, 1770 (carbonyl), 1735 (CF_2=CH), 1720 (carbonyl), 1390, 1370, 1150, 920, 900 cm^{-1}; DCIMS, *m/e* (relative intensity) 352 (M + 1, 10), 296 (100), 278 (50).

4-Amino-6,6-difluoro-5-hexenoic Acid *tert*-Butyl Ester Hydrochloride (23). Hydrazinolysis of 1.46 g (4.16 mmol) of the difluoroethenyl phthalimide **21** in methanol under the conditions described below for the preparation of the trifluoro analogue **24** (except that the final recrystallization was effected in *i*-C_3H_7OH/pentane) gave 0.57 g (53%) of 4-amino-6,6-difluoro-5-hexenoic acid *tert*-butyl ester hydrochloride (**23**): ^1H NMR ($CDCl_3$) δ 4.8–3.9 (m, 2, F_2C=CH-CHN), 2.3 (m, 4, CH_2CH_2), 1.40 (s, 9, 3 CH_3); IR ($CHCl_3$) 3400, 2980, 1745 ($C-F_2$=CH), 1720 (ester), 1370, 1315, 1160, 920, 900 cm^{-1}; DCIMS, *m/e* (relative intensity) 166 (M + 1, 3), 148 (100), 129 (6), 108 (10), 102 (25).

4-Amino-6,6-difluoro-5-hexenoic Acid (4). A solution of 0.49 g (1.9 mmol) of the difluoroethenyl amine ester **23** in 3.8 mL of 1 N HCl was stirred at ambient temperature for 36 h. The mixture was concentrated under reduced pressure (rotatory evaporator, 20 Torr, 30 °C), the residue triturated with 5 mL of *i*-C_3H_7OH and filtered, and the filtrate concentrated again under reduced pressure. The treatment with *i*-C_3H_7OH was repeated twice to afford a white solid, which then was dissolved in 8 mL of *i*-C_3H_7OH and treated with 2 N NaOH to adjust the pH at 6.5–7.0. A precipitate was formed, which was removed by filtration. The filtrate was concentrated under reduced pressure (rotatory evaporator, 20 Torr, 30 °C) and the residue recrystallized from water/*i*-C_3H_7OH (0.5 + 15 mL) by addition of Et_2O (1–2 mL) to yield 0.115 g (37%) of 4-amino-6,6-difluoro-5-hexenoic acid (**4**): ^1H NMR (200 MHz) (D_2O) δ 4.55 (ddd, 1, J_{HH} = 11 Hz, J_{FH} = 2 Hz, J_{FH} = 23 Hz, F_2C=CH), 4.10 (dddt, 1, J_{HH} = 11 Hz, J_{HH} = 7 Hz, J_{FH} = 2 Hz, J_{FH} = Hz, CHN), 2.25 (m, 2, CH_2CO), 2.1 and 1.9 [2 m (1:1), 2, CH_2]; ^{19}F NMR (D_2O) −79.7 (dd, J_{FF} = 30 Hz, J_{FH} = 2 Hz, (E)-C=C*F*F), −80.4 (dd, J_{FF} = 30 Hz, J_{FH} = 23 Hz, (Z)-C=CF*F*); DCIMS, *m/e* (relative intensity) 222 (M + H, 50) 166 (100), 149 (80), TFA derivative, *m/e* (relative intensity) 279 (M + CH_5, 15), 262 (M + H, 10), 244 (5), 224 (10), 188 (20), 148 (100). Anal. ($C_6H_9F_2NO_2$) C, H, N.

5,6,6-Trifluoro-4-hydroxy-5-hexenoic Acid *tert*-Butyl Ester (20). To a stirred solution of about 0.02 mol of trifluoroethylene in THF/Et_2O/pentane (48 mL, 4:1:1), obtained by condensing ~600 mL of trifluoroethylene into the solvent at −100 °C (MeOH/liquid N_2 cooling bath), was added under nitrogen at −100 °C 9.4 mL (0.018 mol) of *n*-butyllithium in hexane (1.88 M). The slightly yellow solution was stirred for 10 min at −100 °C, and 2.8 g (0.018 mol) of the aldehyde ester 18 dissolved in 4 mL of pentane was added. The reaction mixture was then allowed to warm to −50 °C, when water (10 mL) and then 1 N HCl (20–25 mL) were added. The mixture was poured into Et_2O (200 mL), the layers were separated, and the aqueous layer was extracted with Et_2O (200 mL). The combined organic layers were washed twice with water (2 × 200 mL) and concentrated aqueous $NaHCO_3$ (100 mL) and twice with brine (2 × 100 mL) and then dried over Na_2SO_4. Removal of the solvents under reduced pressure (rotatory evaporator, 20 Torr, 30–40 °C) yielded crude ester **20**, which was purified by bulb-to-bulb distillation to afford 3.4 g (80%) of 5,6,6-trifluoro-4-hydroxy-5-hexenoic acid *tert*-butyl ester (**20**): bp 130–135 °C (0.05 Torr); ^1H NMR ($CDCl_3$) δ 4.47 (dm, 1, *J* = 26 Hz, CHO), 3.1 (m, 1, OH), 2.7–1.8 (m, 4, CH_2CH_2), 1.47 (s, 9, 3 CH_3); IR ($CHCl_3$) 3600 (OH), 3420 (OH), 2980, 2930, 1785 (CF_2=CF), 1715 (ester), 1370, 1310, 1255, 1150, 920, 895, 750, 710, 650 cm^{-1}.

4-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-5,6,6-trifluoro-5-hexenoic Acid *tert*-Butyl Ester (22). To a mixture of 3.4 g (0.014 mol) of the trifluoroethenyl ester **20**, 4.0 g (0.015 mol) of triphenylphosphine, and 2.2 g (0.015 mol) of 1,3-dihydro-1,3-dioxo-2*H*-isoindole in 30 mL of THF under nitrogen was added 2.93 g (0.0168 mol) of diethyl azodicarboxylate in 4 mL of THF. The reaction was exothermic and an orange solution was obtained, which was stirred for 14 h. Addition of pentane (70 mL) precipitated an oil. The upper layer was isolated by decantation and concentrated under reduced pressure (rotatory evaporator, 20 Torr, 30 °C) to afford crude phthalimido ester **22** as an oil, which was chromatographed on silica gel [70–230 mesh, 110 g, $CHCl_3$/hexane (1:1)]. The product-containing fractions were combined and the solvents removed (rotatory evaporator, 20 Torr, 30 °C) to yield 2.9 g (56%) of 4-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-5,6,6-trifluoro-5-hexenoic acid *tert*-butyl ester (**22**): ^1H NMR ($CDCl_3$) δ 7.8 (m, 4, aryl H), 5.2 (dm, 1, *J* = 24 Hz, CHN), 2.4 (m, 4, CH_2CH_2), 1.43 (s, 9, 3 CH_3); IR ($CHCl_3$) 2980, 2930, 1785 (CF_2=CF), 1715 (carbonyl), 1370, 1150 cm^{-1}.

4-Amino-5,6,6-trifluoro-5-hexenoic Acid *tert*-Butyl Ester (24) Hydrochloride. A mixture of 1.5 g (0.004 mol) of the trifluoroethenyl phthalimide **22** and 0.3 g (0.006 mol) of hydrazine hydrate in 15 mL of CH_3OH was stirred for 2 h at ambient temperature. HCl (1 N) (8 mL) was added, and stirring was continued for 2 days. The precipitate formed was removed by

filtration, and the filtrate was concentrated under reduced pressure (rotatory evaporator, 20 Torr, 30 °C). The semisolid residue was taken up into 5 ml of i-C_3H_7OH, the mixture filtered, and the filtrate concentrated again (rotatory evaporator, 20 Torr, 30 °C). The treatment with i-C_3H_7OH was repeated. The resulting oil was dissolved in 4 mL of Et_2O. Addition of pentane precipitated 4-amino-5,6,6-trifluoro-5-hexenoic acid *tert*-butyl ester (**24**) hydrochloride, which was recrystallized from ethyl acetate/pentane to afford 0.6 g (63%) of **24**: ^1H NMR ($CDCl_3$) δ 4.4 (dm, 1, J = 26 Hz, CH=N), 2.4 (m, 4, CH_2CH_2), 1.40 (s, 9, 3 CH_3).

4-Amino-5,6,6-trifluoro-5-hexenoic Acid (5). A solution of 0.6 g (0.0025 mol) of the trifluoropropenyl amine derivative **24** in 1 N HCl (5 mL) was stirred until all of the ester was consumed, about 3 days as judged from TLC [silica gel, $CHCl_3$/$HNEt_2$ (20:1)]. The reaction mixture was filtered, and the solvents were removed under reduced pressure (rotatory evaporator, 20 Torr, 30 °C). The residue was dissolved in i-C_3H_7OH, concentrated again, and then taken up in 5 mL of i-C_3H_7OH. Addition of Et_3N to adjust the pH at 6.5–7.0, followed by Et_2O (3–4 volumes) afforded a precipitate, which was filtered off. The filtrate was concentrated (20 Torr, 30 °C) and a white solid was obtained. To a solution of this solid in water/i-C_3H_7OH (10 mL, 1:10) was added Et_2O to precipitate 4-amino-5,6,6-trifluoro-5-hexenoic acid (**5**), which could then be recrystallized from 1 to 2 mL of water by adding i-C_3H_7OH (~15 mL) and then Et_2O (10 mL): yield 0.16 g (35%); ^1H NMR (CD_3OD) δ 4.23 (dm, 1, J = 28 Hz, CHN), 2.4–2.0 (m, 4, CH_2CH_2); IR (KBr) 3360, 1790 (CF=CF_2), 1550, 1410, 1320, 1090 cm^{-1}; ^{13}C NMR (D_2O) 180.7 (COOH), 48.3 (d, J = 22 Hz, CN), 33.4, 26.5 ppm; ^{19}F NMR (CF_3CO_2H) −97.8 (dd, J_{FF} = 68 Hz, J_{FF} = 34 Hz, (Z)-CF=C*F*F), −116.0 (ddd, J_{FF} = 116 Hz, J_{FF} = 68 Hz, J_{FH} = 3 Hz, (E)-CF=C*F*F), −190.9 (ddd, J_{FF} = 116 Hz, J_{FF} = 34 Hz, J_{FH} = 28 Hz, C*F*=CF_2) ppm; DCIMS (TFA derivative), m/e (relative intensity) 297 (M + CH_5, 4), 280 (M + 1, 4), 262 (60), 242 (10), 206 (6), 166 (100). Anal. ($C_6H_8F_3NO_2$) C, H, N.

Enzyme Preparations and Biochemical Assays. GABA-T was prepared from fresh pig brain following the procedure described by Fowler and John[17] through the second ammonium sulfate precipitation. Specific activity was 2 μmol mg of protein)$^{-1}$ h^{-1}. Succinic semialdehyde dehydrogenase (SSADH) was obtained from guinea pig kidneys following the method of Pitts et al.[20] through the second ammonium sulfate precipitation.

The enzyme tests were done at 37 °C in spectrophotometric cuvettes containing sodium pyrophosphate (0.1 M), mercaptoethanol (30 mM), NAD (1 mM), α-ketoglutarate (5 mM), GABA (6 mM), and SSADH in an amount so that GABA transamination was rate-limiting (final volume was 2 mL). The reaction was started by adding GABA-T. GABA-T activity was determined by the rate of NADH formation followed for 10 min at a wavelength of 340 nm. For competitive inhibition, GABA-T was not preincubated with the inhibitor and GABA concentration was varied from 0.1 to 1 mM, all other constituents being fixed. To detect irreversible inhibition, the enzyme was incubated with the different inhibitors in pyrophosphate buffer (pH 8.6) in the presence of pyridoxal phosphate. At given times, aliquots were withdrawn and remaining GABA-T activity was tested in the spectrophotometric assay by coupling GABA-T with succinic semialdehyde dehydrogenase.[1] Competitive inhibition was measured by adding 1 mM compounds **2–5** to the assay medium, the GABA concentration being varied from 0.1 to 1 mM and α-ketoglutarate being fixed at 0.5 mM.

Substrate activity of the different compounds was measured as follows. Ten microliters of GABA-T preparations, which contained 0.1 mg of protein, was added to 2 mL of the following incubation medium: 0.1 M pyrophosphate (pH 8.6), GABA or the fluoroethenyl derivatives **2–5** (1 mM), 2-keto[2-^{14}C]glutarate (5 mM, sp act. 0.037 μCi/μmol), and mercaptoethanol (1 mM). Two hundred microliter aliquots were withdrawn at different time points, and the radioactive glutamate was separated by ion-exchange chromatography (Dowex 50) and counted. Under these conditions the production of glutamate from GABA was linear for 40 min and corresponded to 16% transformation of GABA.

In Vivo Biochemistry. The drugs were given as aqueous solutions to mice of Swiss Albino Strain (20–25 g). The animals were housed five per cage with free access to water and food under a 12-h day–night cycling system. The animals were killed by decapitation 6 h after drug administration. Brains were removed, split sagitally, and frozen until processed. Half of the brain was homogenized in 10 mL of 0.2 N $HClO_4$ and the supernatant used for GABA determination (automatic amino acid analyzer). The other half was homogenized in 9 volumes of the following buffer: KH_2PO_4 (10 mM, pH 6.8), pyridoxal phosphate (0.1 mM), EDTA (1 mM), glutathion (0.1 mM), triton X 100 (0.13%), and glycerol (20%). GABA-T activity was measured by the coupled spectrophotometric assay.[2]

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Registry No. **2**, 105457-53-2; **2**·HCl, 105457-54-3; **3**, 105457-55-4; **3**·HCl, 105457-56-5; **4**, 105457-57-6; **5**, 105457-58-7; **10**, 7529-16-0; **11**, 105457-59-8; **12**, 105457-60-1; **13**, 105457-61-2; **14**, 105457-62-3; **15**, 105457-63-4; **16**, 14300-85-7; **17**, 32400-25-2; **18**, 51534-77-1; **19**, 105457-64-5; **20**, 105457-65-6; **21**, 105457-66-7; **22**, 105457-67-8; **23**, 105457-68-9; **24**, 105457-69-0; F_2C=CH_2, 75-38-7; F_2C=CFH, 85-41-6; GABA-T, 9037-67-6; Br$(CH_2)_2$CH=CH_2, 5162-44-7; t-BuO_2COCO_2Bu-t, 24424-99-5; H_2C=CH$(CH_2)_2CO_2H$, 591-80-0; H_3CCO_2Bu-t, 540-88-5; 1,3-dihydro-1,3-dioxo-2*H*-isoindole, 85-41-6.

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