Fluorinated Conformationally Restricted γ-Aminobutyric Acid Aminotransferase Inhibitors

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Fluorinated Conformationally Restricted γ-Aminobutyric Acid Aminotransferase Inhibitors

Hejun Lu and Richard B. Silverman*

Department of Chemistry, Department of Biochemistry, Molecular Biology, and Cell Biology, and the Center for Drug Discovery and Chemical Biology, Northwestern University, Evanston, Illinois 60208-3113

On the basis of the structures of several potent inhibitor molecules for γ-aminobutryric acid aminotransferase (GABA-AT) that were previously reported, six modified fluorine-containing conformationally restricted analogues were designed, synthesized, and tested as GABA-AT inhibitors. The syntheses of all six molecules followed from a readily synthesized ketone intermediate. Three of the molecules were found to be irreversible inhibitors of GABA-AT with comparable or larger k_{inact}/K_I values than that of vigabatrin, a clinically used antiepilepsy drug, and the other three were reversible inhibitors. A possible mechanism for inactivation by one of the inactivators is proposed.

There are two principal neurotransmitters involved in the regulation of brain neuronal activity: γ-aminobutyric acid (GABA[a]), one of the most widely distributed inhibitory neurotransmitters, and L-glutamic acid, an excitatory neurotransmitter.[1] The GABA concentration is regulated by two pyridoxal 5′-phosphate (PLP)-dependent enzymes, L-glutamic acid decarboxylase (GAD), which catalyzes the conversion of L-glutamate to GABA, and GABA aminotransferase (GABA-AT), which degrades GABA to succinic semialdehyde (SSA) concomitant with the conversion of α-ketoglutarate to L-glutamate.[2] When the concentration of GABA diminishes below a threshold level in the brain, convulsions result;[3] raising the brain GABA levels terminates the seizure.[4] A reduction in the concentrations of GABA and of the enzyme GAD has been implicated not only in the symptoms associated with epilepsy[5,6] but also with several other neurological diseases such as Huntington's chorea,[7,8] Parkinson's disease,[9,10] Alzheimer's disease,[11] and tardive dyskinesia.[12] GABA brain levels cannot be increased by administration of GABA because it does not cross the blood−brain barrier. An approach that has been successful to increase brain GABA levels is the use of a compound that crosses the blood−brain barrier and then inhibits or inactivates GABA-AT, thereby increasing the GABA concentration. Numerous competitive inhibitors of GABA-AT, particularly compounds having a similar backbone structure to GABA,[13] show anticonvulsant activity. A variety of mechanism-based inactivators[14] of GABA-AT[15] also have been shown to be effective anticonvulsant agents. The most effective of the mechanism-based inactivators as an anticonvulsant agent is 4-amino-5-hexenoic acid (**1**, Figure 1; γ-vinyl-GABA),[16] which has the commercial generic name vigabatrin. This compound, which has high potency,[17] has been shown to be an effective treatment for epilepsies that are resistant to other anticonvulsant drugs[18] and currently is prescribed in over 60 countries worldwide, but it was not approved in the U.S. It also was found that vigabatrin

prevents cocaine addiction in rats and baboons.[19] This activity was observed with other forms of addiction, including nicotine addiction[20] and methamphetamine, alcohol, and heroin addictions.[21] Self-administration of cocaine by rats decreased or was prevented by vigabatrin administration in a dose-dependent fashion, without affecting the craving for food.[22] GABA was found to antagonize the extracellular dopamine levels responsible for drug addiction, and by positron emission tomography (PET) in primates, it was shown that vigabatrin, which causes a rise in GABA levels, inhibits these drug-induced dopamine increases.[21] However, because vigabatrin is not approved in the U.S., new GABA-AT inhibitors and inactivators are needed.

Conformationally restricted analogues of GABA are of considerable interest among medicinal chemists.[23] We previously reported the design and synthesis of a series of conformationally restricted analogues of GABA. Among them, (1*R*,4*S*)-4-aminocyclopent-2-enecarboxylic acid (**2**) was shown to be a potent inhibitor and substrate of GABA-AT, but it was not a time-dependent inactivator.[24] (1*S*,3*S*)-3-Amino-4-methylenecyclopentanecarboxylic acid (**3**) was found to inactivate GABA-AT but only in the absence of 2-mercaptoethanol (indicating that a reactive species was produced that was released from the enzyme prior to inactivation), while (1*S*,3*S*)-3-amino-4-difluoromethylenecyclopentanecarboxylic acid (**4**) is a much more potent time-dependent inhibitor of γ-aminobutyric acid aminotransferase, even in the presence of 2-mercaptoethanol (indicating that any reactive species generated are not released prior to inactivation).[25] Compound **4** is 187 times more potent of an inactivator of GABA-AT than vigabatrin, and the corresponding *E*- and *Z*-monofluoro analogues of **3** also inactivate GABA-AT but not with the same potency as **4**. This suggests an important significance for fluorine in the molecules.

Fluorine and fluoroalkyl substitutents have an important electronic effect on the neighboring groups in a molecule and also mimic hydrogen and alkyl groups, respectively.[26] Therefore, the introduction of one or more fluorine-containing groups into molecules alters their physical properties, as well as their biological activities.[27] Fluorinated substrates also can be used as mechanistic probes and inhibitors for obtaining information about the catalytic mechanism of various enzymatic transformations.[28] Consequently, it was thought that other fluorinated conformationally restricted analogues of **2** and **3** should be evaluated for their inhibitory activities. On the basis of that hypothesis, we designed six new fluorine-containing conformationally restricted vigabatrin analogues (**5**−**10**, Figure 2).

* To whom correspondence should be addressed. Address: Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3113. Phone: 847-491-5653. Fax: 847-491-7713. E-mail: agman@chem.northwestern.edu.

[a] Abbreviations: CAN, ceric ammonium nitrate; DBDMH, 1,3-dibromo-5,5-dimethylhydantoin; GABA, γ-aminobutyric acid; GABA-AT, γ-aminobutyric acid aminotransferase; GAD, L-glutamic acid decarboxylase; LiHMDS, lithium hexamethyldisilazide; MFSDA, methyl fluorosulfonyldifluoroacetate; PMBCl, *p*-methoxybenzyl chloride; SSA, succinic semialdehyde dehydrogenase; TBAF, tetrabutylammonium fluoride, TPAP, tetra-*n*-propylammonium perruthenate.
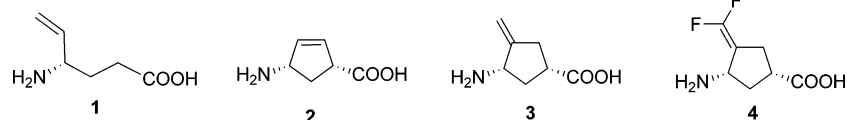
Figure 1. Vigabatrin (**1**) and conformationally restricted analogues.
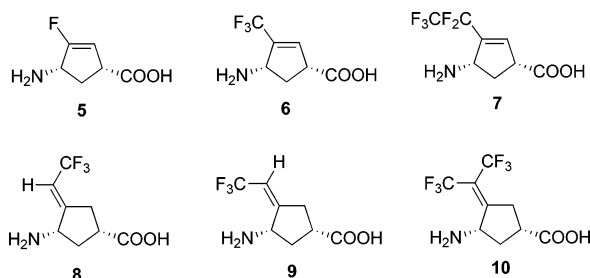
Figure 2. New fluorinated conformationally restricted potential inhibitors and inactivators of GABA-AT.

Scheme 1[a]
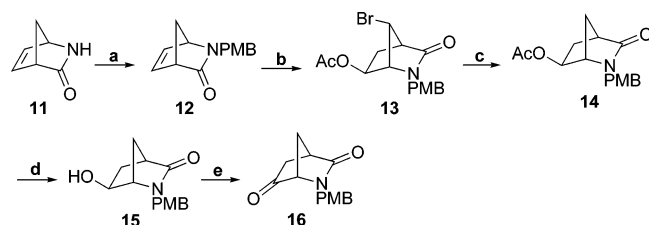
[a] Reagents and conditions: (a) PMBCl, NaH, Bu$_4$NI, DMF, 0 °C to room temp, 2 h, 83%; (b) DBDMH, HOAc, room temp, 20 h, 97%; (c) Bu$_3$SnH, AIBN, PhH, reflux, 12 h, 95%; (d) K$_2$CO$_3$, MeOH, H$_2$O, room temp, 2 h, 87%; (e) NMO, TPAP, DCM, room temp, 24 h, 70%.

Compounds **5**−**7** are C-3 fluorine or fluoroalkyl substituted analogues of **2**, while **8**−**10** are trifluoromethylated analogues of **3** and **4**. The presence of strongly electron-withdrawing fluorine and fluoroalkyl groups also may enhance binding and/or reactivity of these compounds during GABA-AT turnover based on a Michael addition mechanism.[23a] The results of this investigation are reported here.

Results and Discussion

Chemistry. Key intermediate **16** for the synthesis of all of the analogues was synthesized according to a modification of a procedure we reported previously[29] (Scheme 1). Protection of **11** was realized by treatment with *p*-methoxybenzyl chloride (PMBCl) in the presence of sodium hydride in DMF instead of LiHMDS in THF as reported. This revised procedure turned out to be much easier and gave higher yields of product.

The synthesis of **5** was first attempted by a direct halogen exchange reaction of **17**. However, treatment of **17** with (PhSO$_2$)$_2$NF/^{n}BuLi resulted in the *ent*-**12** reduction product as the major product. Conversion of **17** to organotin intermediate **18** followed by fluorine−metal exchange with XeF$_2$ afforded **19** in a satisfactory yield (Scheme 2). Deprotection of **19** with ceric ammonium nitrate (CAN) followed by acidic hydrolysis gave **5**.

Syntheses of target molecules **6** and **7** via elimination of an alcohol or the corresponding tosylated derivative were unsuccessful (Scheme 3). Treatment of **16** with TMSRf (Rf = CF$_3$ or CF$_3$CF$_2$) in the presence of a catalytic amount of TBAF afforded tertiary alcohols **21a** and **21b** in excellent yields.[30] However, direct dehydration of **21a** or **21b** with various dehydration conditions failed to produce the desired products **22a** and **22b**. Activation of **21a** and **21b** with TsCl in the

presence of NaH successfully gave the tosylates **23a** and **23b**, respectively. Unfortunately, attempted elimination reactions of **23a** and **23b** with various bases always resulted in formation of **21a** and **21b**, respectively, with only a trace amount of the desired products detected.

An alternative synthesis of target molecule **6** (Scheme 4) started with iodination of **16** with hydrazine and iodine followed by elimination of one molecule of HI in the presence of potassium *tert*-butoxide to give **17**. Direct trifluoromethylation of **17** with in situ generated CF$_3$Cu from FSO$_2$CF$_2$CO$_2$Me (methyl fluorosulfonyldifluoroacetate, MFSDA) and CuI[31] successfully afforded **22a**. Removal of the PMB group with CAN followed by acidic hydrolysis gave **6**.

Compound **7** was synthesized from **17** using steps similar to those used to prepare **6** (Scheme 5). A first attempt at pentafluoroethylation of **17** with CF$_3$CF$_2$CO$_2$Na/CuI at 140 °C only resulted in decomposition of the substrate. Treatment of **17** with CF$_3$CF$_2$SiMe$_3$/KF/CuI, however, afforded **22b** in good yields.

A Wittig reaction of **16** with CHBr=PPh$_3$, generated in situ from bromomethyltriphenylphosphonium bromide and ^{t}BuOK afforded an *E/Z* mixture of bromomethylenes **25a** and **25b**, which was easily separated by column chromatography on silica gel. The conformations of the double bonds in the two isomers were determined based on NOE experiments. Trifluoromethylation of **25a** and **25b** with CF$_3$Cu under similar conditions used for **17** produced compounds **26a** and **26b**, respectively. Removal of the PMB protecting group with CAN followed by acidic hydrolysis gave **8** and **9** (Scheme 6).

Treatment of **16** with CBr$_4$ and PPh$_3$ in toluene afforded **28**, which was double-trifluoromethylated with in situ generated CF$_3$Cu to give **29**. Removal of the PMB group with CAN, followed by hydrolysis with 4 N HCl(aq) at 75 °C gave **10** (Scheme 7).

It is noteworthy that, compared to the compounds with an exocyclic double bond (**27a**, **27b**, and **30**), the hydrolysis of **20**, **24a**, and **24b**, which have endocyclic double bonds, was found to be much easier. The reaction is usually completed in 1 h when the compounds are treated with 4 N aqueous HCl at 70 °C. Prolonged heating and stirring of these compounds under these conditions resulted in major side reactions. No such side reactions were observed from hydrolysis of **27a**, **27b**, and **30**.

Enzyme Inhibition Results. Compounds **6**, **8**, and **9** showed concentration and time-dependent inhibition of pig brain GABA-AT in the presence of β-mercaptoethanol (Table 1). Compounds **5**, **7**, and **10** showed only weak reversible inhibition of GABA-AT in the presence of β-mercaptoethanol. None of the three reversible inhibitor target molecules was more potent than **2** or **4**. However, the irreversible inhibitors were comparable to vigabatrin as inactivators of GABA-AT. It is interesting that although **6** was designed to be a reversible inhibitor of GABA-AT because a simple elimination of HF was not initially apparent, it was found to be an irreversible inhibitor. Compounds **5** and **7**, which differ from **6** only by the length of the fluoroalkyl chain, are reversible inhibitors of GABA-AT. Although **8** and **9** are irreversible inhibitors of GABA-AT, as expected, introduction of a second trifluoromethyl group makes **10** a weak reversible inhibitor.

Scheme 2[a]
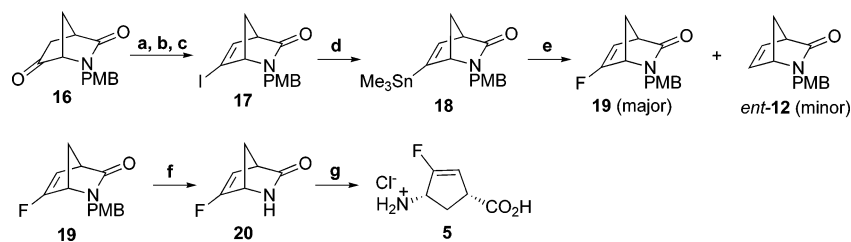
[a] Reagents and conditions: (a) $NH_2NH_2 \cdot H_2O$, Et_3N, EtOH, reflux, 1 h; (b) I_2, Et_3N, benzene, room temp, 2 h; (c) [t]BuOK, ether, room temp, 20 h, 60% in three steps; (d) $Me_3SnSnMe_3$, $Pd(PPh_3)_4$, PhMe, reflux, 30 min, 49%; (e) XeF_2, AgOTf, 2,6-bis([tert]-butyl)-4-methylpyridine, DCM, room temp, 8 min, 30%; (f) CAN, CH_3CN, H_2O, room temp, 2 h, 55%; (g) 4 N HCl(aq), 70 °C, 0.5−1 h, 64%.

Scheme 3[a]

a, Rf = CF_3; **b**, Rf = CF_3CF_2.
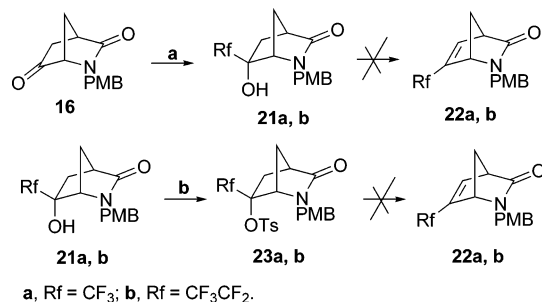
[a] Reagents and conditions: (a) TMSRf, TBAF(cat.), THF, room temp, 1 h, 95%; (b) TsCl, NaH, ether, 0 °C, 16 h, 79%.

Scheme 4[a]
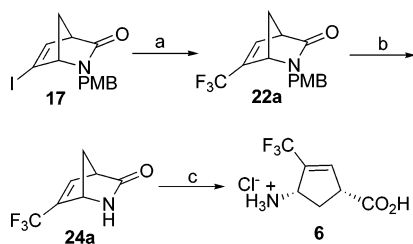
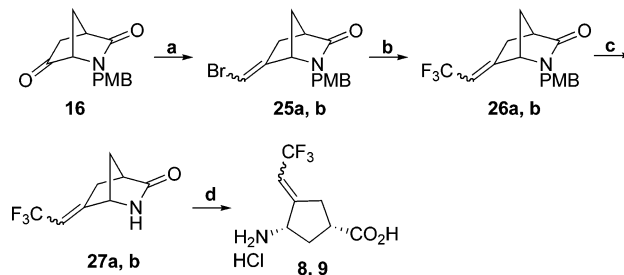[a] Reagents and conditions: (a) MFSDA, CuI, DMF, HMPA, 20 h, 75%; (b) CNA, $MeCN/H_2O$, room temp, 3 h, 61%; (c) 4 N HCl(aq), 70 °C, 0.5−1 h, 85%.

Scheme 5[a]
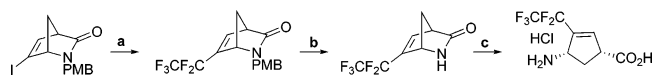
[a] Reagents and conditions: (a) $CF_3CF_2SiMe_3/KF/CuI$, NMO/DMF (1/1), 75 °C, 24 h, 57%; (b) CAN, CH_3CN, H_2O, room temp, 2 h, 76%; (c) 4 N HCl(aq), 70 °C, 0.5−1 h, 82%.

Exhaustive dialysis of GABA-AT that was inactivated by **6**, **8**, and **9** against potassium pyrophosphate buffer (pH 8.5) resulted in different extents of irreversibility of inhibition. Compared to the control, 48% and 13% of enzyme activity was recovered for **6** and **8**, respectively, after 22 h of dialysis, while the percentage of recovered enzyme activity for **9** continued to increase with extended dialysis. These results suggest that at least part of the adducts formed between the enzyme and **6** and **8** are stable, while at least one with **9** can be slowly hydrolyzed upon dialysis. Incubation of **6**, **8**, and **9** with GABA-AT in the presence of GABA resulted in a significant increase in their $t_{1/2}$ of inactivation (slower rate of inactivation), indicating active-site binding to GABA-AT by these compounds.[32]

A possible mechanism for inactivation of GABA-AT by **6** is shown in Scheme 8. Initial Schiff base formation with the PLP followed by tautomerization gives **31**. Michael addition to this trifluoromethyl-activated Michael acceptor[33] by Lys329 (X =

Scheme 6[a]

[a] Reagents and conditions: (a) $BrCH_2PPh_3 \cdot Br$, [t]BuOK, THF, −78 °C, 20 h, 72% E/Z; (b) MFSDA, CuI, DMF, HMPA, 75 °C, 20 h, 95%; (c) CAN, CH_3CN, H_2O, room temp, 2 h, 76%; (d) 4 N HCl(aq), 70 °C, 10−12 h, 79%.

Scheme 7[a]
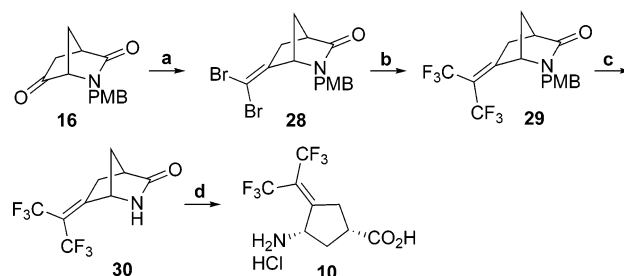
[a] Reagents and conditions: (a) CBr_4, PPh_3, toluene, reflux 22 h, 86%; (b) MFSDA, CuI, DMF, HMPA, 75 °C, 50 h, 82%; (c) CAN, CH_3CN, H_2O, room temp, 1 h, 56%; (d) 4 N HCl(aq), 70 °C, 10−12 h, 77%.

**Table 1.** Kinetic Constants for **5−10**

compd	k_{inact} (min^{-1})	K_I (mM)	k_{inact}/K_I (min^{-1} mM^{-1})	K_i (mM)
5				1.3
6	0.68	4.11	0.17	
7				2.8
8	6.96	39.23	0.18	
9	2.89	7.74	0.37	
10				4.2
vigabatrin[a]	0.24	0.85	0.28	

[a] From ref 25.

Lys329) or hydroxide (X = HO$^-$) results in **32**, which can eliminate fluoride ion to give a highly reactive exocyclic difluoromethylene group[32] conjugated to the PMP iminium (**33**). This should lead to rapid nucleophilic attack,[32] either by the enzyme (pathway a) or by water (pathway b) to give **34** or **36**, respectively. Fluoride ion elimination and hydrolysis would give **35** (inactivation) or **38** (turnover). Inactivation by **8** and **9** may be related to the mechanism of inactivation proposed for **4**.[25]

To test this mechanism, fluoride ion release was monitored during inactivation. GABA-AT was incubated with all six compounds for 2 h, and the fluoride ion concentration was monitored. With **6**, 270 fluoride ions were released per enzyme

Scheme 8
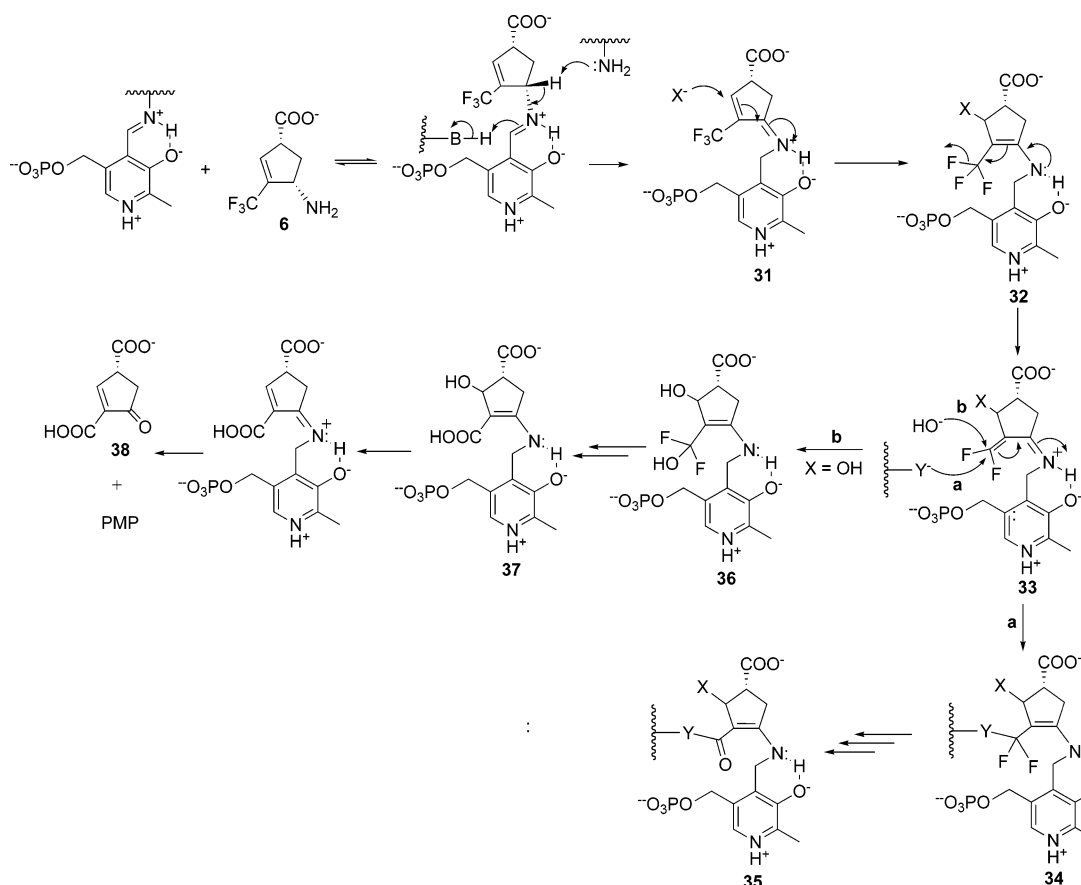
dimer inactivated. This suggests that possibly 90 molecules of **6** are turned over for each inactivation event (partition ratio of 89). Possibly, the activated intermediate **33** undergoes reaction with water (pathway b) 89 times for each attack by an active site residue (pathway a). Depending on what enzyme residue reacts with **33**, the carboxylic acid derivative product formed may or may not be stable to conditions of dialysis, leading to partial reactivation of the enzyme during dialysis. For **7**, 109 fluoride ions were released, and for the other four compounds, less than 6 fluoride ions were released during inactivation.

Enzyme activity was monitored concomitantly with fluoride ion release during inactivation of GABA-AT by **6**. A good correlation was observed (Figure 3), which suggests that inactivation occurs as a consequence of fluoride ion release. When GABA-AT was substituted by human albumin, no fluoride ion was released, indicating that the presence of a protein is not responsible for the fluoride ion release.
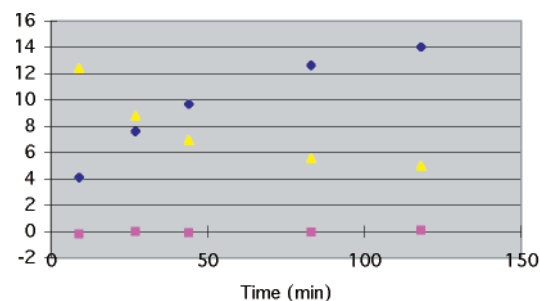

Figure 3. Enzyme activity and fluoride ion release during inactivation of GABA-AT with **6** (2 mM) as a function of time: fluoride ion concentration when GABA-AT was incubated with **6** (diamonds); fluoride ion concentration when human albumin was incubated with **6** (squares); remaining relative enzyme activity compared to control when GABA-AT was incubated with **6** (triangles).

Figure 4 shows the time-dependent increase in fluoride ion concentration when GABA-AT was incubated with **7**, a weak reversible inhibitor. The fluoride ion concentration reached it highest level in 2−3 h. No fluoride ion was released when **7** was incubated with albumin, indicating a GABA-AT-dependent reaction. This suggests that the corresponding reactive intermediate (related to **33** in Scheme 8) only undergoes hydrolysis (possibly because of steric hindrance by the trifluoromethyl group of **7** that replaces the fluoride in **6**).
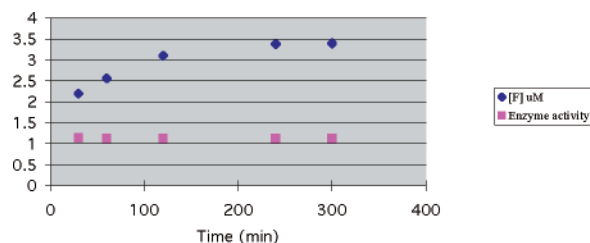
Figure 4. Enzyme activity and fluoride ion release during inactivation of GABA-AT with **7** (2 mM) as a function of time: fluoride ion concentration when GABA-AT was incubated with **7** (diamonds); remaining relative enzyme activity compared to control when GABA-AT was incubated with **7** (squares).

Conclusion

Two series of fluorinated compounds were designed as potential inhibitors and inactivators of GABA-AT based on the high potency of **4**. The k_{inact}/K_I value for **8** (0.37 mM^{-1} min^{-1}) is greater than that for the epilepsy drug vigabatrin (0.28 mM^{-1} min^{-1}); that for **6** (0.16 mM^{-1} min^{-1}) and for **9** (0.18 mM^{-1} min^{-1}) are slightly lower. None of these, however, is as efficient an inactivator as **4** (52 mM^{-1} min^{-1}). Compounds **5−7** were designed as reversible inhibitors, so it was unexpected that

6 was a potent time-dependent inactivator. It appears that there is more to the effective binding of compounds to GABA-AT than just the fluorine atoms. Other structures that more closely resemble **4** are being constructed.

Experimental Section

General Methods and Reagents. Optical spectra and GABA-AT assays were recorded on a Perkin-Elmer Lambda 10 UV/vis spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz NMR spectrometer. Chemical shifts are reported as δ values in parts per million downfield from TMS as the internal standard in CDCl$_3$. For samples run in D$_2$O, the HOD resonance was arbitrarily set at 4.60 ppm. CClF$_3$ was selected as an external standard with δ 0.0 ppm for ^{19}F NMR. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Combustion analyses were performed by Oneida Research Laboratories, NY. High-resolution mass spectra and accurate mass spectra were obtained on a VG-70-250SE high-resolution spectrometer and a Micromass Quattro II LC/MS spectrometer. An Orion Research model 702A pH meter with a general combination electrode was used for pH measurements. Fluoride ion concentration measurements were obtained using an Orion Research model 702A pH meter with an Orion Research model 96-09 combination fluoride electrode. Flash column chromatography was carried out with Merck silica gel 60 (230−400 mesh ASTM). TLC was run with EM Science silica gel 60 F254 precoated glass plates.

All reagents were purchased from Aldrich Chemical Co. and were used without further purification except anhydrous ether and tetrahydrofuran (THF), which were distilled from sodium metal under nitrogen and anhydrous dichloromethane, which was distilled from calcium hydride.

(1S,4R)-6-Iodo-2-(4-methoxybenzyl)-2-azabicyclo[2.2.1]hept-5-en-3-one (17). Compound **16** (0.246 g, 1.0 mmol)[29] was dissolved in anhydrous ethanol (5 mL) and was added via syringe to a solution of hydrazine hydrate (51% of hydrazine, 1.02 mL, 21.0 mmol) and Et$_3$N (2.18 mL, 15.7 mmol) in anhydrous ethanol (5 mL) while stirring. The resulting colorless solution was heated to reflux and stirred under argon for 1 h. The reaction mixture was then evaporated under vacuum to give the crude hydrazone product as a colorless oil, which was used directly in the next step without further purification. I$_2$ (0.508 g, 2.0 mmol) was dissolved in anhydrous benzene (6 mL) and was added dropwise into a solution of the above hydrazone and Et$_3$N (1.1 mL, 8.0 mmol) in anhydrous benzene (5 mL) with stirring at room temperature. The resulting brown suspension was stirred at room temperature under argon for 1.5 h. Water (20 mL) was added, and the resulting two layers were separated; the aqueous layer was further extracted with ether (20 mL × 3). The combined organic layers were washed with aqueous HCl (0.5 N, 10 mL), water (10 mL), saturated aqueous NaHCO$_3$ (20 mL), and brine (20 mL × 2) and dried over MgSO$_4$. The solvent was removed under vacuum, and the resulting crude product was purified via column chromatography on silica gel (hexanes/EtOAc, 9:1) to give the diiodo intermediate as a light-brown oil, which was dissolved in anhydrous ether and treated by dropwise addition of two equiv of ^{t}BuOK at room temperature; the resulting brown suspension was stirred overnight. Water was added, and the two layers were separated. The aqueous layer was further extracted with ether, and the combined ether solutions were washed with brine and dried over anhydrous MgSO$_4$. Ether was removed under vacuum, and the resulting crude product was purified via column chromatography on silica gel (hexanes/EtOAc, 9:1) to give **17** as a light-green oil (213 mg, 60%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.19 (2H, d, J = 8.5 Hz), 6.96 (1H, s), 6.89 (2H, d, J = 8.5 Hz), 4.53 (1H, d, J = 14.5 Hz), 4.06 (1H, s), 4.01 (1H, d, J = 14.5 Hz), 3.82 (3H, s), 3.36 (1H, s), 2.31 (1H, d, J = 7.5 Hz), 2.22 (1H, d, J = 7.5 Hz). ^{13}C NMR (100 MHz, CDCl$_3$) δ 178.50, 159.36, 144.04, 129.84, 128.73, 114.32, 98.91, 71.65, 58.13, 56.33, 55.38, 46.83. MS m/z (ESI, MeOH): 378 (M + Na)$^+$. HRMS calcd for C$_{14}$H$_{14}$O$_2$NI: 355.0064. Found: 355.0057.

(1S,4R)-2-(4-Methoxybenzyl)-6-trimethylstannanyl-2-azabicyclo-[2.2.1]hept-5-en-3-one (18). Compound **17** (41 mg, 0.115 mmol) was dissolved in anhydrous toluene (4 mL) under argon, followed by addition of Pd(PPh$_3$)$_4$ (20 mg, 0.017 mmol) and Me$_3$SnSnMe$_3$ (45 mg, 0.139 mmol). A light-yellow solution formed, which was heated to reflux under argon and stirred for 1 h. Toluene was evaporated under vacuum, and the residue was purified via column chromatography on silica gel (hexanes/EtOAc, 3:1) to give **18** (25 mg, 56%) as a colorless oil. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.14 (2H, d, J = 8.5 Hz), 6.88 (2H, d, J = 8.5 Hz), 6.85 (1H, m), 4.67 (1H, d, J = 15.0 Hz), 4.13 (1H, s), 3.81 (3H, s), 3.47 (1H, s), 3.41 (1H, d, J = 15.0 Hz), 2.24 (1H, d, J = 7.5 Hz), 2.00 (1H, d, J = 8.0 Hz), 0.16 (9H, s). ^{13}C NMR (CDCl$_3$, 125.6 MHz) δ 179.83, 159.03, 153.07, 148.19, 129.26, 129.12, 114.12, 66.20, 58.27, 55.68, 55.34, 47.56, −9.28. MS m/z (EI, DCM): 389.0 (M)$^+$, 374.0 (M − CH$_3$)$^+$, 211.0 (M − PMB − CONH − CH$_3$)$^+$, 121.0 (PMB)$^+$. HRMS calcd for C$_{17}$H$_{23}$O$_2$N^{116}Sn (M)$^+$: 389.0741. Found: 389.0749. HRMS calcd for C$_{16}$H$_{20}$O$_2$N^{116}Sn (M − CH$_3$)$^+$: 374.0506. Found: 374.0504.

(1S,4R)-6-Fluoro-2-(4-methoxybenzyl)-2-azabicyclo[2.2.1]hept-5-en-3-one (19). AgOTf (48 mg, 0.187 mmol) was dissolved in dry DCM under argon. Compound **18** (73.4 mg, 0.187 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (19 mg, 0.094 mmol) in dry DCM (1 mL) were added via syringe, followed by immediate addition of XeF$_2$ (38 mg, 0.225 mmol) in DCM. The resulting reaction mixture was shielded from light and stirred at room temperature for 15 min. The reaction mixture was then partitioned between saturated aqueous NaHCO$_3$ and CHCl$_3$, and the aqueous layer was further extracted with CHCl$_3$ (20 mL). The combined CHCl$_3$ solutions were washed with brine (20 mL) and dried over anhydrous MgSO$_4$. The CHCl$_3$ was removed under vacuum, and the residue was purified via column chromatography on silica gel (hexanes/EtOAc, 3:1) to give **19** (20 mg, 30%) as a light-yellow oil. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.15 (2H, d, J = 8.8 Hz), 6.86 (2H, d, J = 8.4 Hz), 5.57 (1H, s), 4.48 (1H, d, J = 14.8 Hz), 4.08 (1H, d, J = 14.8 Hz), 3.89 (1H, s), 3.80 (1H, s), 3.24 (1H, s), 2.33 − 2.38 (2H, m). ^{19}F NMR (376 MHz, CDCl$_3$) δ −119.72 (1F, s). ^{13}C NMR (CDCl$_3$, 100.63 MHz) δ 177.01, 173.97, 159.20, 129.68, 128.27, 114.11, 105.37 (d, J = 7.3 Hz), 62.64, 57.16, 55.33, 49.62, 47.12. MS m/z (EI, DCM): 247.1 (M)$^+$, 163.1, 121.1 (PMB)$^+$. HRMS calcd for C$_{14}$H$_{14}$O$_2$NF (M)$^+$: 247.1003. Found: 247.1003.

(1S,4R)-6-Fluoro-2-azabicyclo[2.2.1]hept-5-en-3-one (20). This compound was synthesized from **19** using general procedure II (see below) in a 55% yield. ^{1}H NMR (400.168 MHz, CDCl$_3$) δ 6.41 (1H, s), 5.59 (1H, s), 4.12 (1H, s), 3.10 (1H, s), 2.44 (2H, s). ^{19}F NMR (376.492 MHz, CDCl$_3$) δ −120.59 (1F, s). ^{13}C NMR (CDCl$_3$, 125.614 MHz) δ 184.95, 175.63 (d, J_{FC} = 304.9 Hz), 105.88, 59.52, 58.49, 49.45. MS m/z (CI, DCM): 128.1 (M + 1)$^+$, 85.3 (M − CON)$^+$. HRMS calcd for C$_6$H$_7$ONF: 128.0506. Found: 128.0511.

(1R,4S)-4-Amino-3-fluorocyclopent-2-enecarboxylic Acid (5). This compound was synthesized from **20** using general procedure III in a yield of 64% as a light-yellow solid, mp 201.0−202.5 °C. ^{1}H NMR (D$_2$O, 499.511 MHz) δ 5.68 (1H, s), 4.45 (1H, d, J = 2.9 Hz), 3.66 (1H, d, J = 4.0 Hz), 2.83 (1H, dt, J = 14.5 Hz, 9.0 Hz), 2.20 (1H, dt, J = 14.5 Hz, 4.5 Hz). ^{19}F NMR (D$_2$O, 376.493 MHz) δ −128.97 (1F, d, J = 4.5 Hz). ^{13}C NMR (D$_2$O, 125.614 MHz) δ 177.18, 157.61 (d, J_{FC} = 280.2 Hz), 109.21 (d, J_{FC} = 12.1 Hz), 51.37 (d, J_{FC} = 22.2 Hz), 42.57 (d, J_{FC} = 8.2 Hz), 29.32 (d, J_{FC} = 4.0 Hz). MS m/z (EI, MeOH): 145.1 (M)$^+$, 126.1, 100.1 (M − CO$_2$H)$^+$. HRMS calcd for C$_6$H$_8$O$_2$NF: 145.0534. Found: 145.0530. Anal. (C$_6$H$_8$O$_2$NF·0.5H$_2$O) C, H, N.

(1S,4S,6SR)-6-Hydroxy-6-trifluoromethyl-2-(4′-methoxybenzyl)-azabicyclo[2.2.1]heptan-3-one (21a). To a solution of **16** (0.123 g, 0.5 mmol) in dry THF (4 mL) was added TMSCF$_3$ (0.107 mg, 0.75 mmol). Tetrabutylammonium fluoride (TBAF) (1.0 M solution in THF, 50 μL, 0.05 mmol) was added dropwise. The resulting mixture was stirred under nitrogen for 2 h and was concentrated under vacuum. The residue was purified by column chromatography (hexanes/EtOAc, 2:1 to 1:1), giving **21a** (0.149 g, 94%) as a white solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.19 (2H, d, J = 8.4 Hz), 6.87 (2H, d, J = 8.4 Hz), 5.00 (1H, d, J = 14.8 Hz), 4.56 (1H, br.

s), 4.04 (1H, d, *J* = 14.4 Hz), 3.81 (3H, s), 3.74 (1H, s), 2.87 (1H, s), 2.33 (1H, dd, *J* = 14.0 Hz, 3.6 Hz), 1.80−1.91 (3H, m). ^{19}F NMR (376 MHz, CDCl$_3$) δ −78.19 (3F, s). MS *m/z* (EI, DCM): 316.1 (M + 1)$^+$, 315.1 (M)$^+$, 121.1 (CH$_3$OC$_6$H$_4$CH$_2$)$^+$. HRMS calcd for C$_{15}$H$_{16}$O$_3$NF$_3$: 315.1070. Found: 315.1077.

(1*S*,4*S*,6*SR*)-6-Hydroxyl-6-pentafluoroethyl-2-(4′-methoxy-benzyl)azabicyclo[2.2.1]heptan-3-one (21b). This compound was synthesized by the same method as for **21a**. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.17 (2H, d, *J* = 8.8 Hz), 6.87 (2H, d, *J* = 7.6 Hz), 4.98 (1H, d, *J* = 15.2 Hz), 4.97 (1H, s), 4.01 (1H, d, *J* = 14.8 Hz), 3.86 (1H, s), 3.80 (3H, s), 2.84 (1H, s), 2.42 (1H, d, *J* = 13.6 Hz), 1.78−1.89 (3H, m). ^{19}F NMR (376 MHz, CDCl$_3$) δ −79.02 (3F, s), −120.08 (2F, ab, *J* = 277.5 Hz). MS *m/z* (EI, DCM): 366.1 (M + 1)$^+$, 365.1 (M)$^+$, 121.1 (CH$_3$OC$_6$H$_4$CH$_2$)$^+$. HRMS calcd for C$_{16}$H$_{16}$O$_3$NF$_5$: 365.1050. Found: 365.1045.

(1*S*,4*R*,6*S*)-Toluene-4-sulfonic Acid 2-(4-Methoxybenzyl)-3-oxo-6-trifluoromethyl-2-azabicyclo[2.2.1]hept-6-yl Ester (23a). NaH in mineral oil (14 mg, 60%, 0.3 mmol) was washed with anhydrous pentane, suspended in dry ether (3 mL), and cooled to 0 °C in an ice bath. Compound **21a** (52 mg, 0.16 mmol) in dry ether (1 mL) was then added dropwise via syringe. The resulting white suspension was stirred at 0 °C for 15 min under argon followed by addition of TsCl (64 mg, 0.3 mmol) in dry ether (1 mL). The resulting white suspension was allowed to slowly warm up to room temperature and was stirred under argon for 16 h. Water was added, and the two layers were separated. The aqueous layer was further extracted with ether (10 mL × 3), and the combined ether solutions were washed with brine (10 mL × 2) and concentrated under vacuum. The residue was separated by flash column chromatography on silica gel (hexanes/EtOAc, 1:1) to give **23a** (58 mg, 79%) as a white solid. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.82 (2H, d, *J* = 8.0 Hz), 7.40 (2H, d, *J* = 6.0 Hz), 7.04 (2H, d, *J* = 6.4 Hz), 6.82 (2H, d, *J* = 6.4 Hz), 4.75 (1H, d, *J* = 12.0 Hz), 3.84 (1H, s), 3.78 (3H, s), 3.43 (1H, d, *J* = 12.0 Hz), 3.09 (1H, d, *J* = 12.0 Hz), 2.96 (1H, s), 2.47 (3H, s), 2.40 (1H, dd, *J* = 12.4 Hz, 2.8 Hz), 1.81−1.84 (2H, m). ^{19}F NMR (376 MHz, CDCl$_3$) δ −74.41 (3F, s). ^{13}C NMR (CDCl$_3$, 125.6 MHz) δ 175.17, 159.24, 145.72, 134.22, 130.13, 129.54, 128.30, 127.76, 123.16 (q), 114.21, 63.32, 55.31, 45.83, 45.03, 39.13, 31.11, 21.80.

(1*S*,4*R*,6*S*)-Toluene-4-sulfonic Acid 2-(4-Methoxybenzyl)-3-oxo-6-pentafluoroethyl-2-azabicyclo[2.2.1]hept-6-yl Ester (23b). This compound was synthesized from **21b** similar to the synthesis of **23a**. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.84 (2H, d, *J* = 8.0 Hz), 7.40 (2H, d, *J* = 8.8 Hz), 7.04 (2H, d, *J* = 8.4 Hz), 6.83 (2H, d, *J* = 8.0 Hz), 4.95 (1H, d, *J* = 15.6 Hz), 3.90 (1H, s), 3.78 (3H, s), 3.54 (1H, d, *J* = 14.8 Hz), 3.29 (1H, d, *J* = 15.6 Hz), 2.96 (1H, s), 2.48−2.51 (4H, m), 1.85 (1H, d, *J* = 11.2 Hz), 1.79 (1H, d, *J* = 10.4 Hz). ^{19}F NMR (376 MHz, CDCl$_3$) δ −78.99 (3F, s), −116.41 (2F, ab). ^{13}C NMR (CDCl$_3$, 125.6 MHz) δ 174.98, 159.22, 145.86, 133.85, 130.04, 129.62, 127.98, 114.21, 63.21, 55.33, 46.13, 44.59, 30.77, 21.87. MS *m/z* (EI, MeOH): 519.0 (M)$^+$, 520.0 (M + 1)$^+$, 365 (M − CH$_3$C$_6$H$_4$SO$_2$)$^+$, 121.0. HRMS calcd for C$_{23}$H$_{22}$O$_5$NF$_5$S: 519.183. Found: 519.1135.

(1*S*,4*R*)-6-Trifluoromethyl-2-(4-methoxybenzyl)-2-azabicyclo-[2.2.1]hept-5-en-3-one (22a). General Procedure I. Methyl fluorosulfonyldifluoroacetate (MFSDA; 48 mg, 0.25 mmol) in anhydrous DMF (1 mL) was added dropwise via syringe to a suspension of **17** (35.5 mg, 0.1 mmol) and CuI (23 mg, 0.12 mmol) in anhydrous DMF (2 mL) and HMPA (1 mL) at 75 °C under argon over a period of 1 h. The resulting suspension was stirred at 75 °C under argon for 72 h and evaporated under high vacuum. The residue was purified via column chromatography on silica gel (hexanes/EtOAc, 3:1) to give **22a** (22.1 mg, 75%) as a light-brown oil. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.18 (2H, d, *J* = 8.5 Hz), 7.11 (1H, s), 6.89 (2H, d, *J* = 8.5 Hz), 4.77 (1H, d, *J* = 15.0 Hz), 4.23 (1H, s), 3.82 (3H, s), 3.56 (1H, s), 3.54 (1H, d, *J* = 15.0 Hz), 2.40 (1H, d, *J* = 8.0 Hz), 2.32 (1H, d, *J* = 8.0 Hz). ^{19}F NMR (376.49 MHz, CDCl$_3$) δ −64.69 (3F, s). ^{13}C NMR (CDCl$_3$, 125.6 MHz) δ 178.04, 159.30, 143.97 (q, *J*$_{CF}$ = 36.6 Hz), 141.52 (q, *J*$_{CF}$ = 5.4 Hz), 129.54, 128.26, 122.44 (q, *J*$_{CF}$ = 267.8 Hz), 114.25, 61.35, 59.31, 55.35, 54.06, 46.81. MS *m/z* (EI, MeOH): 297.1 (M)$^+$, 298.1

(M + 1)$^+$, 163.1 (M − PMBN)$^+$, 121.0 (PMB)$^+$. HRMS calcd for C$_{15}$H$_{14}$O$_2$NF$_3$: 297.0971. Found: 297.0970.

(1*S*,4*R*)-6-Trifluoromethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (24a). General Procedure II. Ceric ammonium nitrate (CAN; 1.38 g, 2.525 mmol) in water (3 mL) was added to a solution of **22a** (150 mg, 0.505 mmol) in acetonitrile (6 mL). The resulting red solution was stirred at room temperature for 1.5 h. The reaction mixture was diluted with ethyl acetate, washed with saturated aqueous NaHCO$_3$ and then brine, and dried over anhydrous MgSO$_4$. The solution was concentrated under vacuum, and the residue was purified via column chromatography on silica gel (hexanes/EtOAc, 1:1) to give **24a** (54 mg, 61%) as a white solid. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.07 (1H, s), 6.15 (1H, br. s), 4.51 (1H, s), 3.42 (1H, s), 2.54 (1H, d, *J* = 8.0 Hz), 2.43 (1H, d, *J* = 8.0 Hz). ^{19}F NMR (376 MHz, CDCl$_3$) δ −66.42 (3F, s). ^{13}C NMR (CDCl$_3$, 125.6 MHz) δ 182.3, 144.3 (m), 141.0 (q, *J*$_{FC}$ = 5.5 Hz), 122.4 (q, *J*$_{FC}$ = 268.2 Hz), 60.1, 58.6, 53.7. MS *m/z* (CI, MeOH): 178.3 (M + 1)$^+$, 115.2. HRMS calcd for C$_7$H$_7$ONF$_3$ (M + H): 178.0474. Found: 178.0481.

(1*R*,4*S*)-4-Amino-3-trifluoromethylcyclopent-2-enecarboxylic Acid (6). General Procedure III. Compound **24a** (44 mg, 0.249 mmol) was dissolved in 4 N aqueous HCl (5 mL), and the resulting clear solution was heated to 70−75 °C and stirred for 40−45 min. The resulting colorless clear solution was extracted with EtOAc (3 mL × 2) and evaporated to dryness under high vacuum to give the crude product, which was further purified via ion exchange chromatography (0.15 N aqueous HCl as eluant) to give **6** as a white solid (49 mg, 85%), mp 195.0−196.0 °C. ^{1}H NMR (D$_2$O, 400.169 MHz) δ 6.96 (1H, s), 4.69 (1H, s), 3.92 (1H, s), 2.87−2.95 (1H, m), 2.29−2.35 (1H, m). ^{19}F NMR (D$_2$O, 376.493 MHz) δ −64.35 (3F, s). ^{13}C NMR (D$_2$O, 125.614 MHz) δ 175.12, 142.54 (q, *J*$_{FC}$ = 4.6 Hz), 130.82 (q, *J*$_{FC}$ = 33.7 Hz), 121.62 (q, *J*$_{FC}$ = 269.7 Hz), 53.64, 48.80, 32.11. MS *m/z* (EI, MeOH): 196.1 (M)$^+$, 179.1 (M − OH)$^+$, 159.1. HRMS calcd for C$_7$H$_9$O$_2$NF$_3$: 196.0580. Found: 196.0576. Anal. (C$_7$H$_9$O$_2$NF$_3$) C, H, N.

(1*S*,4*R*)-6-Pentafluoroethyl-2-(4-methoxybenzyl)-2-azabicyclo-[2.2.1]hept-5-en-3-one (22b). A 5 mL screw-capped Pyrex tube was charged with CuI (19 mg, 0.10 mmol), KF (14.5 mg, 0.25 mmol), and a stirring bar. The tube was dried with a heat gun under vacuum for 20 min and filled with argon. Compound **17** (18 mg, 0.05 mmol) in anhydrous NMP (1 mL) was added via syringe to the above reaction tube followed by TMSC$_2$F$_5$ (48 mg, 0.25 mmol) in anhydrous DMF (1 mL). The resulting suspension was heated to 80 °C and stirred vigorously for 48 h. The reaction mixture was then evaporated under high vacuum, and the residue was purified via column chromatography on silica gel (hexanes/EtOAc, 3:1) to give **22b** (10 mg, 57%) as a light-yellow oil. ^{1}H NMR (400.168 MHz, CDCl$_3$) δ 7.17−7.19 (3H, m), 6.89 (2H, d, *J* = 8.8 Hz), 4.80 (1H, d, *J* = 15.2 Hz), 4.26 (1H, s), 3.82 (3H, s), 3.60 (1H, s), 3.52 (1H, d, *J* = 14.4 Hz), 2.40 (1H, d, *J* = 6.4 Hz), 2.32 (1H, d, *J* = 8.0 Hz). ^{19}F NMR (376.49 MHz, CDCl$_3$) δ −84.12 (3F, s), −113.67 (2F, ab, *J*$_{ab}$ = 279.4 Hz). ^{13}C NMR (CDCl$_3$, 125.6 MHz) δ 177.59, 159.30, 144.14 (t, *J*$_{CF}$ = 8.0 Hz), 143.03 (t, *J*$_{CF}$ = 26.0 Hz), 129.51, 128.21, 118.81 (dt, *J*$_{CF}$ = 285.4 Hz, 37.8 Hz), 114.24, 61.90, 59.79, 55.32, 54.54, 46.64. MS *m/z* (EI, DCM): 347.1 (M)$^+$, 121.0 (PMB)$^+$. HRMS calcd for C$_{16}$H$_{14}$O$_2$NF$_5$: 347.0939. Found: 347.0933.

(1*S*,4*R*)-6-Pentafluoroethyl-2-azabicyclo[2.2.1]hept-5-en-3-one (24b) was synthesized from **22b** in a yield of 74% using general procedure II. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.14 (1H, s), 6.64 (1H, br. s), 4.52 (1H, s), 3.43 (1H, s), 2.52 (1H, d, *J* = 7.0 Hz), 2.41 (1H, d, *J* = 7.0 Hz). ^{19}F NMR (376 MHz, CDCl$_3$) δ −84.45 (3F, s), −116.41 (2F, ab, *J*$_{ab}$ = 276.3 Hz, Δ*ν* = 268.h Hz). ^{13}C NMR (CDCl$_3$, 125.6 MHz) δ 182.28, 143.40−143.78 (m, 2C), 111.80−122.15 (m, 2C, C$_2$F$_5$), 60.72, 59.23, 54.13. MS *m/z* (CI, DCM): 228.0 (M + 1)$^+$, 185.3 (M − CON)$^+$, 165.3 (M − CONH − F)$^+$. HRMS calcd for C$_{16}$H$_{14}$O$_2$NF$_5$: 347.0939. Found: 347.0933.

(1*R*,4*S*)-4-Amino-3-pentafluoroethylcyclopent-2-enecarboxylic acid (7) was synthesized using general procedure III from **24b** as a white solid in a yield of 82%, mp 187.0−188.5 °C. ^{1}H NMR (D$_2$O, 499.511 MHz) δ 7.00 (1H, s), 4.71 (1H, d, *J* = 3.5 Hz),

3.92 (1H, s), 2.85−2.91 (1H, m), 2.29−2.33 (1H, m). ^{19}F NMR (D$_2$O, 376.493 MHz) δ −84.60 (3F, t, J = 18.8 Hz), −113.07 (2F, ab, J = 283.9 Hz, Δ = 62.5 Hz). ^{13}C NMR (D$_2$O, 125.614 MHz) δ 174.89, 145.37 (t, J_{FC} = 7.2 Hz), 129.63 (t, J_{FC} = 25.0 Hz), 109.70−119.59 (2C, m), 54.61, 49.27, 32.10. MS m/z (EI, MeOH): 246.1 (M + 1)$^+$, 245.1 (M)$^+$, 200.1 (M − CO$_2$H)$^+$, 176.1. HRMS calcd for C$_8$H$_8$O$_2$NF$_5$: 245.0470. Found: 245.0475. Anal. (C$_8$H$_8$O$_2$NF$_5$·1.5H$_2$O) C, H, N.

(1S,4R)-6-Bromomethylene-2-(4-methoxybenzyl)-2-azabicyclo-[2.2.1]heptan-3-one (25). (Bromomethyl)triphenylphosphonium bromide (0.567 g, 1.3 mmol) was suspended in dry THF (4 mL), cooled to −78 °C, and then treated by addition of ^{t}BuOK (1.0 M in THF, 1.2 mL, 1.2 mmol). The resulting brown suspension was stirred at −78 °C for 1.5 h and then treated with **16** (0.246 g, 1.0 mmol) in dry THF (1 mL). The resulting light-brown mixture was allowed to gradually warm to room temperature and was stirred overnight. The reaction was quenched via addition of water, and the mixture was extracted with dichloromethane (10 mL × 3). The combined organic layers were washed with brine (20 mL × 2) and dried over anhydrous MgSO$_4$. The solvent was removed under vacuum, and the residue was purified via column chromatography on silica gel (hexanes/EtOAc, 1:1) to give **25a** (Z-isomer, 100 mg, 31%) as a light-yellow oil and **25b** (E-isomer, 131 mg, 41%) as a white solid.

25a: ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.26 (2H, d, J = 8.0 Hz), 6.88 (2H, d, J = 8.0 Hz), 6.01 (1H, s), 4.76 (1H, d, J = 15.0 Hz), 4.35 (1H, s), 3.81 (3H, s), 3.70 (1H, d, J = 15.0 Hz), 3.02 (1H, s), 2.47 (1H, d, J = 16.0 Hz), 2.31 (1H, d, J = 15.5 Hz), 1.97 (1H, d, J = 9.5 Hz), 1.54 (1H, d, J = 10.0 Hz). ^{13}C NMR (126 MHz, CDCl$_3$) δ 177.51, 159.15, 143.88, 129.64, 128.94, 114.01, 97.82, 61.88, 55.31, 45.81, 44.36, 39.78, 32.77. MS m/z (ESI, MeOH): 344.6 (M + Na)$^+$, 346.5 (M + 2 + Na)$^+$. HRMS calcd for C$_{15}$H$_{16}$O$_2$NBr: 321.0359. Found: 321.0359.

25b: ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.14 (2H, d, J = 8.5 Hz), 6.86 (2H, d, J = 8.5 Hz), 6.10 (1H, s), 4.62 (1H, d, J = 15.0 Hz), 3.86 (1H, s), 3.80 (3H, s), 3.79 (1H, d, J = 15.0 Hz), 2.97 (1H, s), 2.43 (1H, dt, J = 16.5 Hz, 3.0 Hz), 2.26 (1H, d, J = 16.5 Hz), 2.06 (1H, d, J = 9.5 Hz), 1.60 (1H, d, J = 9.5 Hz). ^{13}C NMR (126 MHz, CDCl$_3$) δ 177.79, 159.19, 144.28, 129.42, 128.53, 114.15, 100.00, 63.46, 55.33, 44.89, 43.95, 41.16, 33.49. MS m/z (ESI, MeOH): 344.6 (M + Na)$^+$, 346.2 (M + 2 + Na)$^+$. HRMS calcd for C$_{15}$H$_{16}$O$_2$NBr: 321.0359. Found: 321.0359.

(Z)-(1S,4R)-2-(4-Methoxybenzyl)-6-(2,2,2-trifluoroethylidene)-2-azabicyclo[2.2.1]heptan-3-one (26a) was synthesized in a 95% yield from **25a** using general procedure I (reaction time, 20 h). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.18 (2H, d, J = 8.8 Hz), 6.88 (2H, d, J = 8.4 Hz), 5.68 (1H, q, J = 8.0 Hz), 4.81 (1H, d, J = 14.8 Hz), 4.44 (1H, s), 3.82 (1H, s), 3.58 (1H, d, J = 15.2 Hz), 2.95 (1H, s), 2.57 (1H, d, J = 17.2 Hz), 2.41 (1H, d, J = 17.2 Hz), 2.06 (1H, d, J = 8.8 Hz), 1.58 (1H, d, J = 10.4 Hz). ^{19}F NMR (376.49 MHz, CDCl$_3$) δ −58.02 (3F, m). MS m/z (EI, DCM): 311.1 (M)$^+$, 312.1 (M + 1)$^+$, 121.0 (PMB)$^+$. HRMS calcd for C$_{16}$H$_{16}$O$_2$NF$_3$: 311.1128. Found: 311.1133.

(E)-(1S,4R)-2-(4-Methoxybenzyl)-6-(2,2,2-trifluoroethylidene)-2-azabicyclo[2.2.1]heptan-3-one (26b) was synthesized in 95% yield from **25b** using general procedure I (reaction time, 20 h). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.15 (2H, d, J = 8.8 Hz), 6.87 (2H, d, J = 8.4 Hz), 5.51 (1H, q, J = 7.2 Hz), 4.57 (1H, d, J = 14.4 Hz), 3.90 (1H, d, J = 15.2 Hz), 3.86 (1H, s), 3.80 (3H, s), 2.98 (1H, s), 2.62 (1H, dq, J = 17.2 Hz, 2.4 Hz), 2.48 (1H, dq, J = 17.2 Hz, 2.4 Hz), 2.05 (1H, d, J = 9.2 Hz), 1.56 (1H, d, J = 10.0 Hz). ^{19}F NMR (376.49 MHz, CDCl$_3$) δ −60.98 (3F, m). MS m/z (EI, DCM): 311.1 (M)$^+$, 312.1 (M + 1)$^+$, 121.0 (PMB)$^+$. HRMS calcd for C$_{16}$H$_{16}$O$_2$NF$_3$: 311.1128. Found: 311.1133.

(Z)-(1S,4R)-6-(2,2,2-Trifluoroethylidene)-2-azabicyclo[2.2.1]-heptan-3-one (27a) was synthesized in 74% yield from **26a** using general procedure II. ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.30 (1H, s), 5.58 (1H, q, J = 8.0 Hz), 4.61 (1H, s), 2.83 (1H, s), 2.55 (1H, d, J = 17.2 Hz), 2.36 (1H, d, J = 16.4 Hz), 2.18 (1H, dd, J = 10.4 Hz, 1.2 Hz), 1.63 (1H, d, J = 9.6 Hz). ^{19}F NMR (376 MHz, CDCl$_3$) δ −58.28 (3F, m). ^{13}C NMR (CDCl$_3$, 125.6 MHz) δ 179.92, 152.44

(q, J_{FC} = 5.5 Hz), 122.93 (q, J_{FC} = 270.1 Hz), 110.02 (q, J_{FC} = 34.8 Hz), 56.67, 43.58, 41.49, 32.23. MS m/z (EI, DCM): 192 (M + 1)$^+$, 191 (M)$^+$, 79.1. HRMS calcd for C$_8$H$_8$ONF$_3$: 191.0553. Found: 191.0559.

(E)-(1S,4R)-6-(2,2,2-Trifluoroethylidene)-2-azabicyclo[2.2.1]-heptan-3-one (27b) was synthesized in a 74% yield from **26b** using general procedure II. ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.57 (1H, s), 5.77 (1H, d, J = 7.6 Hz), 4.13 (1H, s), 2.87 (1H, s), 2.65 (1H, d, J = 16.0 Hz), 2.48 (1H, d, J = 16.8 Hz), 2.14 (1H, d, J = 9.6 Hz), 1.62 (1H, d, J = 9.6 Hz). ^{19}F NMR (376 MHz, CDCl$_3$) δ −61.08 (3F, m). ^{13}C NMR (CDCl$_3$, 125.6 MHz) δ 180.27, 151.69 (q, J_{FC} = 4.6 Hz), 123.20 (q, J_{FC} = 270.1 Hz), 110.82 (q, J_{FC} = 35.7 Hz), 60.10, 44.60, 41.52, 30.53. MS m/z (EI, DCM): 192 (M + 1)$^+$, 191 (M)$^+$, 163 (M − CO)$^+$, 79. HRMS calcd for C$_8$H$_8$ONF$_3$: 191.0553. Found: 191.0553.

(1S,3S)-(Z)-3-Amino-4-(2,2,2-trifluoroethylidene)cyclopen-tanecarboxylic acid (8) was synthesized in a 66% yield as a white solid from **27a** using general procedure III; mp 212.0−214.0 °C. ^{1}H NMR (D$_2$O, 499.511 MHz) δ 5.97 (1H, q, J = 7.5 Hz), 4.37 (1H, s), 3.09−3.16 (2H, m), 2.92−2.95 (1H, m), 2.56−2.62 (1H, m), 1.98 (1H, q, J = 12.5 Hz). ^{19}F NMR (D$_2$O, 376.493 MHz) δ −60.78 (3F, d, J_{HF} = 6.0 Hz). ^{13}C NMR (D$_2$O, 125.614 MHz) δ 177.93, 150.92 (q, J_{FC} = 5.0 Hz), 123.11 (q, J_{FC} = 270.2 Hz), 114.77 (q, J_{FC} = 35.3 Hz), 53.76, 40.69, 32.95, 32.43. MS m/z (EI, MeOH): 209.1 (M)$^+$, 208.1 (M − 1)$^+$, 189.1 (M − H$_2$O − 2)$^+$, 164.1 (M − CO$_2$H)$^+$. HRMS calcd for C$_8$H$_{10}$O$_2$NF$_3$: 209.0658. Found: 277.0656. Anal. (C$_8$H$_{10}$O$_2$NF$_3$·0.25H$_2$O) C, H, N.

(1S,3S)-(Z)-3-Amino-4-(2,2,2-trifluoroethylidene)cyclopen-tanecarboxylic acid (9) was synthesized from **27b** in a 79% yield as a white solid using general procedure III, mp 206.0−207.5 °C. ^{1}H NMR (D$_2$O, 499.749 MHz) δ 6.09 (1H, qd, J = 9.0 Hz, 1.5 Hz), 4.65 (1H, s), 3.02−3.09 (1H, m), 2.91−2.92 (2H, m), 2.57−2.63 (1H, m), 2.13−2.18 (1H, m). ^{19}F NMR (D$_2$O, 376.493 MHz) δ −60.37 (3F, d, J_{HF} = 7.9 Hz). ^{13}C NMR (D$_2$O, 125.673 MHz) δ 178.64, 149.42 (q, J_{FC} = 5.4 Hz), 120.43 (q, J_{FC} = 269.3 Hz), 116.73 (q, J_{FC} = 34.3 Hz), 50.15, 40.74, 37.00, 34.44. MS m/z (EI, MeOH): 210.1 (M + 1)$^+$, 209.1 (M)$^+$, 191.1 (M − H$_2$O)$^+$, 164.1 (M − CO$_2$H)$^+$. HRMS calcd for C$_8$H$_{10}$O$_2$NF$_3$: 209.0664. Found: 277.0675. Anal. (C$_8$H$_{10}$O$_2$NF$_3$·0.25H$_2$O) C, H, N.

(1S,4R)-6-Dibromomethylene-2-(4-methoxybenzyl)-2-azabi-cyclo[2.2.1]heptan-3-one (28). Compound **16** (0.246 g, 1.0 mmol), carbon tetrabromide (0.498 g, 1.5 mmol), and triphenylphosphine (0.787 g, 3.0 mmol) were dissolved in anhydrous toluene (10 mL), and the resulting light-yellow suspension was stirred while refluxing under argon for 22 h. The reaction mixture was then filtered, washed with benzene, and evaporated to give the crude product, which was purified via column chromatography on silica gel (hexanes/EtOAc, 1:1) to give **28** (377 mg, 94%) as a light-yellow oil. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.21 (2H, m), 6.85 (2H, m), 4.72 (1H, d, J = 12.5 Hz), 4.20 (1H, s), 3.73−3.78 (4H, m), 3.03 (1H, s), 2.45 (1H, d, J = 16.5 Hz), 2.29 (1H, d, J = 16.5 Hz), 2.02 (1H, s), 1.63 (1H, s). ^{13}C NMR (126 MHz, CDCl$_3$) δ 177.41, 159.15, 145.83, 129.45, 128.51, 114.04, 81.58, 64.27, 55.28, 45.66, 44.49, 40.84, 36.61. MS m/z (ESI, MeOH): 399.9 (M)$^+$, 401.9 (M + 2)$^+$, 404 (M + 4)$^+$, 322.0 (M − Br)$^+$, 293.9 (M − CH$_3$OC$_6$H$_4$)$^+$. HRMS calcd for C$_{15}$H$_{15}$O$_2$NBr$_2$: 398.9464. Found: 398.9460.

(1S,4R)-2-(4-Methoxybenzyl)-6-(2,2,2-trifluoro-1-trifluoro-methylethylidene)-2-azabicyclo[2.2.1]heptan-3-one (29) was synthesized from **28** in an 82% yield using general procedure I (50 h). ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.15 (2H, d, J = 8.8 Hz), 6.88 (2H, d, J = 8.8 Hz), 4.82 (1H, d, J = 15.2 Hz), 4.62 (1H, s), 3.81 (3H, s), 3.65 (1H, d, J = 15.2 Hz), 3.03 (1H, s), 2.82 (1H, d, J = 18.4 Hz), 2.73 (1H, d, J = 18.4 Hz), 2.12 (1H, d, J = 10.4 Hz), 1.63 (1H, d, J = 10.4 Hz). ^{19}F NMR (376.49 MHz, CDCl$_3$) δ −57.12 to −57.03 (3F, m), −59.97 to −59.91 (3F, m). MS m/z (EI, DCM): 379.1 (M)$^+$, 380.1 (M + 1)$^+$, 136.1 (PMB + NH)$^+$, 121.0 (PMB)$^+$. HRMS calcd for C$_{16}$H$_{16}$O$_2$NF$_3$: 379.1001. Found: 379.1003.

(1S,4R)-6-(2,2,2-Trifluoro-1-trifluoromethylethylidene)-2-aza-bicyclo[2.2.1]heptan-3-one (30) was synthesized from **29** in a 56% yield using general procedure II. ^{1}H NMR (400 MHz, CDCl$_3$) δ

5.62 (1H, s), 4.78 (1H, s), 2.96 (1H, s), 2.83 (1H, s), 2.74 (1H, s), 2.29 (1H, s), 1.71 (1H, s). ^{19}F NMR (376 MHz, CDCl$_3$) δ −57.32 (3F, s), −60.25 (3F, s). MS m/z (EI, DCM): 260.1 (M + 1)$^+$, 259.1 (M)$^+$, 189.1 (M − CF$_3$)$^+$. HRMS calcd for C$_9$H$_7$ONF$_6$: 259.0426. Found: 259.0434.

(1S,3S)-3-Amino-4-(2,2,2-trifluoro-1-trifluoromethylethylidene)-cyclopentanecarboxylic acid (10) was synthesized from **30** in a 77% yield as a white solid using general procedure III; mp 198.0− 199.5 °C. ^{1}H NMR (D$_2$O, 400.169 MHz) δ 4.92 (1H, s), 3.29− 3.34 (1H, m), 3.16−3.20 (2H, m), 2.57−2.65 (1H, m), 2.20−2.27 (1H, m). ^{19}F NMR (D$_2$O, 376.493 MHz) δ −59.97 (3F, q, J = 9.0 Hz), −60.47 (3F, q, J = 9.0 Hz). ^{13}C NMR (D$_2$O, 100.749 MHz) δ 178.08, 158.14, 122.40, 119.16−119.67 (m), 52.56 (d, J_{FC} = 2.3 Hz), 40.73, 36.31 (q, J_{FC} = 3.8 Hz), 33.68. MS m/z (EI, MeOH): 278.1 (M + 1)$^+$, 277.1 (M)$^+$, 259.1 (M − H$_2$O)$^+$, 232.0 (M − CO$_2$H)$^+$. HRMS calcd for C$_9$H$_9$O$_2$NF$_6$: 277.0538. Found: 277.0545. Anal. (C$_9$H$_9$O$_2$NF$_6$·H$_2$O) C, H, N.

Enzyme and Assays. GABA aminotransferase was isolated from pig brains by a known procedure.[34] Succinic semialdehyde dehydrogenase (SSDH) was isolated from GABAse, a commercially available mixture of SSDH and GABA aminotransferase, by inactivation of the GABA aminotransferase with gabaculine as described previously.[35] GABA activity assays were carried out using a modification of the coupled assay developed by Scott and Jakoby.[36] The assay solution contains 11 mM GABA, 5.3 mM α-ketoglutarate, 1.1 mM NADP$^+$, and 5 mM β-mercaptoethanol in 100 mM potassium pyrophosphate, pH 8.5, and excess SSDH. By use of this assay, the change in absorbance at 340 nm indicates production of NADPH, which is directly proportional to the activity of GABA aminotransferase.

Time-Dependent Inactivation of GABA Aminotransferase. Incubation solutions (200 μL) contained enzyme (30 μL), potassium pyrophosphate buffer (volume dependent on volume of inhibitor, 50 mM, pH 8.5), α-ketoglutarate (20 μL, 16 mM in 50 mM potassium pyrophosphate buffer, pH 8.5), 2-mercaptoethanol (2.0 mM), and various volumes of the inhibitor solutions (**6**, **8**, and **9**, 0.2 M in doubly distilled H$_2$O). The concentration of inhibitors was calculated on the basis of a total volume of 200 μL. At time intervals, aliquots (27 μL) from the incubation solution were added to the assay solution (568 μL) with excess succinic semialdehyde dehydrogenase (SSDH). Rates were measured spectrophotometrically at 340 nm, and the logarithm of the remaining activity (percentage) was plotted against time for each concentration to determine the half-time. Then a secondary plot of half-time versus the reciprocal of the inhibitor concentration was obtained to determine the k_{inact} and K_I values.

Exhaustive Dialysis of 6, 8, and 9 Inactivated GABA-AT with Potassium Pyrophosphate Buffer. Incubation solutions (200 μL) contained enzyme (30 μL), potassium pyrophosphate buffer (147 μL, 50 mM, pH 8.5), α-ketoglutarate (20 μL, 16 mM in 50 mM potassium pyrophosphate buffer, pH 8.5), 2-mercaptoethanol (2.0 mM), and the inhibitor solutions (**6**, **8**, and **9**, 3.0 μL, 0.2 M in doubly distilled H$_2$O). A control solution (200 μL) containing enzyme (30 μL), potassium pyrophosphate buffer (150 μL, 50 mM, pH 8.5), α-ketoglutarate (20 μL, 16 mM in 50 mM potassium pyrophosphate buffer, pH 8.5), 2-mercaptoethanol (2.0 mM), and no inhibitor solution was also prepared. The incubation solution was allowed to stand at room temperature for about 1 h to ensure that most of the enzyme was inactivated. The incubation solutions and the control solution were injected into a Slide-A-Lyzer dialysis cassette (extra strength, 10000 MWCO, 0.5−3.0 mL capacity), respectively, and stirred in a 1 L beaker containing a stirring bar and 800 mL of 50 mM potassium pyrophosphate buffer (pH 8.5). The buffer was changed every 4 h, at which point an aliquot was taken out to assay.

Substrate Protection against Inhibition of GABA-AT by Compounds 6, 8, and 9. Incubation solutions (200 μL) contained enzyme (30 μL), potassium pyrophosphate buffer (volumes dependent on the volume of substrate, 50 mM, pH 8.5), α-ketoglutarate (20 μL, 16 mM in 50 mM potassium pyrophosphate buffer, pH 8.5), 2-mercaptoethanol (2.0 mM), inhibitor solutions (2 μL or

0 μL in control, **6**, **8**, or **9** in doubly distilled H$_2$O, 0.2 M), and GABA solution (0 μL (control), 17.6 and 35.2 μL). The concentrations of GABA in the incubation solutions were 0 (control), 3, and 6 mM, respectively. At time intervals, aliquots (27 μL) from the incubation solution were added to the assay solution (568 μL) with excess succinic semialdehyde dehydrogenase (SSDH). Rates were measured spectrophotometrically at 340 nm, and the remaining activity (percentage) was determined against the control and plotted versus time to determine the $t_{1/2}$.

Fluoride Ion Released During Inhibition of GABA-AT by 5−10. GABA-AT (25 μL) was incubated for 3 h with each of the six compounds **5**−**10** (0.8 mM) in 50 mM potassium pyrophosphate buffer, pH 8.5, containing 2.0 mM β-mercaptoethanol and 0.64 mM α-ketoglutarate in a total volume of 250 μL at 25 °C. A control containing no enzyme (25 μL of buffer was added instead) was also run. The fluoride ion concentration (200 μL) of each sample mixed with 1.00 mL of total ionic strength adjusting buffer (TISAB low level), 790 μL of potassium pyrophosphate buffer, and 10 μL of diluted standard fluoride solution (2.38 × 10^{-4} M), was measured with a fluoride ion electrode.

Fluoride Ion Release during Inhibition of GABA-AT by 6 and 7 with Time. GABA-AT (120 μL) was incubated with **6** or **7** (12 μL, 0.2 M in doubly distilled water) in 50 mM potassium pyrophosphate buffer, pH 8.5, containing 2.0 mM β-mercaptoethanol and 0.64 mM α-ketoglutarate in a total volume of 1.2 mL at 25 °C. Another two samples containing no GABA-AT but serum albumin or just potassium pyrophosphate buffer (control) were also run. At time intervals, 200 μL of each sample was removed and mixed with 1.00 mL of total ionic strength adjusting buffer (TISAB low level), 790 μL of potassium pyrophosphate buffer, and 10 μL of diluted standard fluoride solution (2.38 × 10^{-4} M). The fluoride ion concentration was measured with a fluoride ion electrode.

Supporting Information Available: Results from combustion analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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