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Fluorinated Conformationally-Restricted γ -Aminobutyric Acid Aminotransferase Inhibitors

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Abstract

Based on the structures of several potent inhibitor molecules for γ -aminobutyric 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), 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 inactivators¹⁴ of GABA-AT¹⁵ 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 drugs¹⁸ and currently is prescribed in over 60 countries worldwide, but it was not approved

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Supporting Information

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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 addiction²⁰ 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-methylenecyclopentane carboxylic 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 substituents 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. Based on that hypothesis, we designed six new fluorine-containing conformationally-restricted vigabatrin analogues (**5-10**; Figure 2). Compounds **5**, **6** and **7** are C-3 fluorine or fluoroalkyl substituted analogues of **2**, while **8**, **9** and **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 previously²⁹ (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 (PhSO₂)₂NF/nBuLi resulted in the *ent*-**12** reduction product as the major product. Conversion of **17** to organotin intermediate **18**, followed by fluorine-metal exchange

with XeF₂ 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 = CF₃ or CF₃CF₂) 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 CF₃Cu from FSO₂CF₂CO₂Me (methyl fluorosulfonyldifluoroacetate; MFSDA) and CuI³¹ 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 CF₃CF₂CO₂Na/CuI at 140 °C only resulted in decomposition of the substrate. Treatment of **17** with CF₃CF₂SiMe₃/KF/CuI, however, afforded **22b** in good yields.

A Wittig reaction of **16** with CHBr=PPh₃, generated *in situ* from bromomethyltriphenylphosphonium bromide and *tert*-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 CF₃Cu 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 CBr₄ and PPh₃ in toluene afforded **28**, which was double trifluoromethylated with *in situ* generated CF₃Cu 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 one hour when the compounds are treated with 4 *N* aq. 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.

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 acceptor³³ by Lys329 (X = Lys329) or hydroxide (X = HO⁻) results in **32**, which can eliminate fluoride ion to give a highly reactive exocyclic difluoromethylene group³² 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 two hours, and the fluoride ion concentration was monitored. With **6**, 270 fluoride ions were released per enzyme 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 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 its highest level in 2-3 hours. 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**).

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** ($0.37 \text{ mM}^{-1}\text{min}^{-1}$) is greater than that for the epilepsy drug vigabatrin ($0.28 \text{ mM}^{-1}\text{min}^{-1}$); that for **6** ($0.16 \text{ mM}^{-1}\text{min}^{-1}$) and **9** ($0.18 \text{ mM}^{-1}\text{min}^{-1}$) are slightly lower. None of these, however, is as

efficient an inactivator as **4** ($52 \text{ mM}^{-1}\text{min}^{-1}$). 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.

4. Experimental

General Methods and Reagents

Optical spectra and GABA-AT assays were recorded on a Perkin-Elmer Lambda 10 UV/Vis spectrophotometer. ^1H 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_2O , 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 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_3N (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_3N (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 $\times$ 3). The combined organic layers were washed with aqueous HCl (0.5 N, 10 mL), water (10 mL), saturated aqueous NaHCO_3 (20 mL), brine (20 mL $\times$ 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 *tert*-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%). ^1H NMR (400MHz, 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 (100MHz, 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. m/z (ESI, MeOH): 378 (M + Na)⁺. HRMS calcd. For C₁₄H₁₄O₂Ni: 355.0064. Found 355.0057.

(1*S*,4*R*)-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(PPh₃)₄ (20 mg, 0.017 mmol) and Me₃SnSnMe₃ (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. ¹H NMR (500MHz, CDCl₃) 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). ¹³C NMR (CDCl₃, 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. m/z (EI, DCM): 389.0 (M)⁺, 374.0 (M – CH₃)⁺, 211.0 (M – PMB – CONH – CH₃)⁺, 121.0 (PMB)⁺. HRMS calcd. for C₁₇H₂₃O₂N¹¹⁶Sn (M)⁺: 389.0741. Found 389.0749; calcd for C₁₆H₂₀O₂N¹¹⁶Sn (M – CH₃)⁺: 374.0506. Found 374.0504.

(1*S*,4*R*)-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) was added *via* syringe, followed by immediate addition of XeF₂ (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 aq. NaHCO₃ and CHCl₃, and the aqueous layer was further extracted with CHCl₃ (20 mL). The combined CHCl₃ solutions were washed with brine (20 mL) and dried over anhydrous MgSO₄. The CHCl₃ 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. ¹H NMR (400MHz, CDCl₃) 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). ¹⁹F NMR (376MHz, CDCl₃) δ -119.72 (1F, s). ¹³C NMR (CDCl₃, 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. m/z (EI, DCM): 247.1 (M)⁺, 163.1, 121.1 (PMB)⁺. HRMS calcd. for C₁₄H₁₄O₂NF (M)⁺: 247.1003. Found 247.1003.

(1*S*,4*R*)-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. ¹H NMR (400.168 MHz, CDCl₃) δ 6.41 (1H, s), 5.59 (1H, s), 4.12 (1H, s), 3.10 (1H, s), 2.44 (2H, s). ¹⁹F NMR (376.492 MHz, CDCl₃) δ -120.59 (1F, s). ¹³C NMR (CDCl₃, 125.614 MHz) δ 184.95, 175.63 (d, *J*_{FC} = 304.9 Hz), 105.88, 59.52, 58.49, 49.45. m/z (CI, DCM): 128.1 (M+1)⁺, 85.3 (M-CON)⁺. HRMS calcd. For C₆H₇ONF: 128.0506. Found 128.0511.

(1*R*,4*S*)-4-Amino-3-fluoro-cyclopent-2-ene carboxylic 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: ¹H NMR (D₂O, 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). ¹⁹F NMR (D₂O, 376.493 MHz) δ -128.97 (1F, d, *J* = 4.5 Hz). ¹³C NMR (D₂O, 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). m/z (EI, MeOH): 145.1 (M)⁺, 126.1, 100.1 (M-CO₂H)⁺. HRMS calcd. For C₆H₈O₂FCNF: 145.0534. Found 145.0530. Anal. (C₆H₈O₂NF.0.5H₂O) 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 TMSCF₃ (0.107 mg, 0.75 mmol). Tetrabutylammonium fluoride (TBAF) (1.0 M solution in THF, 50 μ L, 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. ¹H NMR (400MHz, CDCl₃) 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). ¹⁹F NMR (376MHz, CDCl₃) δ -78.19 (3F, s). *m/z* (EI, DCM): 316.1 (*M* + 1)⁺, 315.1 (*M*)⁺, 121.1 (CH₃OC₆H₄CH₂)⁺. HRMS calcd. For C₁₅H₁₆O₃NF₃: 315.1070. Found 315.1077.

(1S,4S,6SR)-6-hydroxyl-6-pentafluoroethyl-2-(4'-methoxybenzyl)-azabicyclo [2.2.1]heptan-3-one (21b)—This compound was synthesized by the same method as for **21a**. ¹H NMR (400MHz, CDCl₃) 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). ¹⁹F NMR (376MHz, CDCl₃) δ -79.02 (3F, s), -120.08 (2F, *ab*, *J* = 277.5 Hz). *m/z* (EI, DCM): 366.1 (*M* + 1)⁺, 365.1 (*M*)⁺, 121.1 (CH₃OC₆H₄CH₂)⁺ HRMS calcd. For C₁₆H₁₆O₃NF₅: 365.1050. Found 365.1045.

(1S,4R,6S)-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 $\times$ 3), and the combined ether solutions were washed with brine (10 mL $\times$ 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. ¹H NMR (500MHz, CDCl₃) 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). ¹⁹F NMR (376MHz, CDCl₃) δ -74.41 (3F, s). ¹³C NMR (CDCl₃, 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.

(1S,4R,6S)-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**. ¹H NMR (400MHz, CDCl₃) 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). ¹⁹F NMR (376MHz, CDCl₃) δ -78.99 (3F, s), -116.41 (2F, *ab*). ¹³C NMR (CDCl₃, 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. *m/z* (EI, MeOH): 519.0 (*M*)⁺, 520.0 (*M* + 1)⁺, 365 (*M* - CH₃C₆H₄SO₂)⁺, 121.0. HRMS calcd. For C₂₃H₂₂O₅NF₅S: 519.183. Found 519.1135.

(1S,4R)-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. ¹H NMR (500MHz, CDCl₃) 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). ¹⁹F NMR (376.49 MHz, CDCl₃) δ -64.69 (3F, s). ¹³C NMR (CDCl₃, 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. *m/z* (EI, MeOH): 297.1 (M)⁺, 298.1 (M + 1)⁺, 163.1 (M – PMBN)⁺, 121.0 (PMB)⁺. HRMS calcd. For C₁₅H₁₄O₂NF₃: 297.0971. Found 297.0970.

(1S,4R)-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 aq. NaHCO₃, then brine, and dried over anhydrous MgSO₄. 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. ¹H NMR (500MHz, CDCl₃) 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). ¹⁹F NMR (376MHz, CDCl₃) δ -66.42 (3F, s). ¹³C NMR (CDCl₃, 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. *m/z* (CI, MeOH): 178.3 (M + 1)⁺, 115.2. HRMS calcd. for C₇H₇ONF₃ (M + H): 178.0474. Found 178.0481.

(1R,4S)-4-Amino-3-trifluoromethyl-cyclopent-2-ene carboxylic acid (6). General procedure III—Compound **24a** (44 mg, 0.249 mmol) was dissolved in 4 *N* aq. HCl (5 mL), and the resulting clear solution was heated to 70 to 75 °C and stirred for 40 to 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* aq. HCl as eluant) to give **6** as a white solid (49 mg, 85%), mp 195.0-196.0 °C; ¹H NMR (D₂O, 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). ¹⁹F NMR (D₂O, 376.493 MHz) δ -64.35 (3F, s). ¹³C NMR (D₂O, 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. *m/z* (EI, MeOH): 196.1 (M)⁺, 179.1 (M – OH)⁺, 159.1. HRMS calcd. For C₇H₉O₂NF₃: 196.0580. Found 196.0576. Anal. (C₇H₉O₂NF₃) C, H, N.

(1S,4R)-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 TMSC₂F₅ (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. ¹H NMR (400.168 MHz, CDCl₃) 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). ¹⁹F NMR (376.49 MHz, CDCl₃) δ -84.12 (3F, s), -113.67 (2F, ab, *J*_{ab} = 279.4 Hz). ¹³C NMR (CDCl₃, 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. *m/z*

(EI, DCM): 347.1 (M)⁺, 121.0 (PMB)⁺. HRMS calcd. For C₁₆H₁₄O₂NF₅: 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. ¹H NMR (500MHz, CDCl₃) 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). ¹⁹F NMR (376MHz, CDCl₃) δ -84.45 (3F, s), -116.41 (2F, ab, *J*_{ab} = 276.3 Hz, Δ*ν* = 268.6 Hz). ¹³C NMR (CDCl₃, 125.6 MHz) δ 182.28, 143.40–143.78 (m, 2C), 111.80–122.15 (m, 2C, C₂F₅), 60.72, 59.23, 54.13. *m/z* (CI, DCM): 228.0 (M+1)⁺, 185.3 (M-CON)⁺, 165.3 (M-CONH-F)⁺. HRMS calcd. For C₁₆H₁₄O₂NF₅: 347.0939. Found 347.0933.

(1*R*,4*S*)-4-Amino-3-pentafluoroethyl-cyclopent-2-ene carboxylic 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: ¹H NMR (D₂O, 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). ¹⁹F NMR (D₂O, 376.493 MHz) δ -84.60 (3F, t, *J* = 18.8 Hz), -113.07 (2F, ab, *J* = 283.9 Hz, Δ = 62.5 Hz). ¹³C NMR (D₂O, 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. *m/z* (EI, MeOH): 246.1 (M+1)⁺, 245.1 (M)⁺, 200.1 (M-CO₂H)⁺, 176.1. HRMS calcd. For C₈H₈O₂NF₅: 245.0470. Found 245.0475. Anal. (C₈H₈O₂NF₅·1.5H₂O) C, H, N.

(1*S*,4*R*)-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 *tert*-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 up 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 MgSO₄. 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: ¹H NMR (500MHz, CDCl₃) 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). ¹³C NMR (126MHz, CDCl₃) 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. *m/z* (ESI, MeOH): 344.6 (M + Na)⁺, 346.5 (M + 2 + Na)⁺. HRMS calcd. For C₁₅H₁₆O₂NBr: 321.0359. Found 321.0359.

25b: ¹H NMR (500MHz, CDCl₃) 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). ¹³C NMR (126MHz, CDCl₃) 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. *m/z* (ESI, MeOH): 344.6 (M + Na)⁺, 346.2 (M + 2 + Na)⁺. HRMS calcd. For C₁₅H₁₆O₂NBr: 321.0359. Found 321.0359.

(*Z*)-(1*S*,4*R*)-2-(4-Methoxybenzyl)-6-(2,2,2-trifluoro-ethylidene)-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). ¹H NMR (400MHz, CDCl₃) 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). m/z (EI, DCM): 311.1 (M^+), 312.1 ($\text{M} + 1$) $^+$, 121.0 (PMB) $^+$. HRMS calcd. For $\text{C}_{16}\text{H}_{16}\text{O}_2\text{NF}_3$: 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 a 95% yield from **25b** using general procedure I (reaction time 20 h). ^1H 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). m/z (EI, DCM): 311.1 (M^+), 312.1 ($\text{M} + 1$) $^+$, 121.0 (PMB) $^+$. HRMS calcd. For $\text{C}_{16}\text{H}_{16}\text{O}_2\text{NF}_3$: 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 a 74% yield from **26a** using general procedure II. ^1H NMR (400MHz, 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 (376MHz, CDCl_3) δ -58.28 (3F, m). ^{13}C NMR (CDCl_3 , 125.6 MHz) δ 179.92, 152.44 (q, $J_{\text{FC}} = 5.5$ Hz), 122.93 (q, $J_{\text{FC}} = 270.1$ Hz), 110.02 (q, $J_{\text{FC}} = 34.8$ Hz), 56.67, 43.58, 41.49, 32.23. m/z (EI, DCM): 192 ($\text{M} + 1$) $^+$, 191 (M) $^+$, 79.1. HRMS calcd. for $\text{C}_8\text{H}_8\text{ONF}_3$: 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 **26a** using general procedure II. ^1H NMR (400MHz, 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 (376MHz, CDCl_3) δ -61.08 (3F, m). ^{13}C NMR (CDCl_3 , 125.6 MHz) δ 180.27, 151.69 (q, $J_{\text{FC}} = 4.6$ Hz), 123.20 (q, $J_{\text{FC}} = 270.1$ Hz), 110.82 (q, $J_{\text{FC}} = 35.7$ Hz), 60.10, 44.60, 41.52, 30.53. m/z (EI, DCM): 192 ($\text{M} + 1$) $^+$, 191 (M) $^+$, 163 (M-CO) $^+$, 79. HRMS calcd. for $\text{C}_8\text{H}_8\text{ONF}_3$: 191.0553. Found 191.0553.

(1S,3S)-(Z)-3-Amino-4-(2,2,2-trifluoroethylidene)cyclopentane carboxylic acid

(8)—was synthesized in a 66% yield as a white solid from **27a** using general procedure III; mp 212.0-214.0 $^\circ\text{C}$; ^1H NMR (D_2O , 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_2O , 376.493 MHz) δ -60.78 (3F, d, $J_{\text{HF}} = 6.0$ Hz). ^{13}C NMR (D_2O , 125.614 MHz) δ 177.93, 150.92 (q, $J_{\text{FC}} = 5.0$ Hz), 123.11 (q, $J_{\text{FC}} = 270.2$ Hz), 114.77 (q, $J_{\text{FC}} = 35.3$ Hz), 53.76, 40.69, 32.95, 32.43. m/z (EI, MeOH): 209.1 (M^+), 208.1 ($\text{M}-1$) $^+$, 189.1 ($\text{M}-\text{H}_2\text{O}-2$) $^+$, 164.1 ($\text{M}-\text{CO}_2\text{H}$) $^+$. HRMS calcd. For $\text{C}_8\text{H}_{10}\text{O}_2\text{NF}_3$: 209.0658. Found 209.0656. Anal. ($\text{C}_8\text{H}_{10}\text{O}_2\text{NF}_3 \cdot 0.25\text{H}_2\text{O}$) C, H, N.

(1S,3S)-(Z)-3-Amino-4-(2,2,2-trifluoroethylidene)cyclopentane carboxylic acid

(9)—was synthesized from **27b** in a 79% yield as a white solid using general procedure III; mp 206.0-207.5 $^\circ\text{C}$; ^1H NMR (D_2O , 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_2O , 376.493 MHz) δ -60.37 (3F, d, $J_{\text{HF}} = 7.9$ Hz). ^{13}C NMR (D_2O , 125.673 MHz) δ 178.64, 149.42 (q, $J_{\text{FC}} = 5.4$ Hz), 120.43 (q, $J_{\text{FC}} = 269.3$ Hz), 116.73 (q, $J_{\text{FC}} = 34.3$ Hz), 50.15, 40.74, 37.00, 34.44. m/z (EI, MeOH): 210.1 ($\text{M}+1$) $^+$, 209.1 (M) $^+$, 191.1 ($\text{M}-\text{H}_2\text{O}$) $^+$, 164.1 ($\text{M}-\text{CO}_2\text{H}$) $^+$. HRMS calcd. For $\text{C}_8\text{H}_{10}\text{O}_2\text{NF}_3$: 209.0664. Found 209.0675. Anal. ($\text{C}_8\text{H}_{10}\text{O}_2\text{NF}_3 \cdot 0.25\text{H}_2\text{O}$) C, H, N.

(1*S*,4*R*)-6-Dibromomethylene-2-(4-methoxybenzyl)-2-azabicyclo[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. ¹H NMR (500MHz, CDCl₃) 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). ¹³C NMR (126MHz, CDCl₃) 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. *m/z* (ESI, MeOH): 399.9 (M)⁺, 401.9 (M + 2)⁺, 404 (M + 4)⁺, 322.0 (M - Br)⁺, 293.9 (M - CH₃OC₆H₄)⁺. HRMS calcd. For C₁₅H₁₅O₂NBr₂: 398.9464. Found 398.9460.

(1*S*,4*R*)-2-(4-Methoxybenzyl)-6-(2,2,2-trifluoro-1-trifluoromethylethylidene)-2-azabicyclo[2.2.1]heptan-3-one (29)

—was synthesized from **28** in an 82% yield using general procedure I (50 h). ¹H NMR (400MHz, CDCl₃) 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). ¹⁹F NMR (376.49 MHz, CDCl₃) δ -57.12 - -57.03 (3F, m), -59.97 - -59.91 (3F, m). *m/z* (EI, DCM): 379.1 (M)⁺, 380.1 (M + 1)⁺, 136.1 (PMB + NH)⁺, 121.0 (PMB)⁺. HRMS calcd. For C₁₆H₁₆O₂NF₃: 379.1001. Found 379.1003.

(1*S*,4*R*)-6-(2,2,2-Trifluoro-1-trifluoromethylethylidene)-2-azabicyclo[2.2.1]

heptan-3-one (30)—was synthesized from **29** in a 56% yield using general procedure II. ¹H NMR (400MHz, CDCl₃) 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). ¹⁹F NMR (376MHz, CDCl₃) δ -57.32 (3F, s), -60.25 (3F, s). *m/z* (EI, DCM): 260.1 (M + 1)⁺, 259.1 (M)⁺, 189.1 (M - CF₃)⁺. HRMS calcd. for C₉H₇ONF₆: 259.0426. Found 259.0434.

(1*S*,3*S*)-3-Amino-4-(2,2,2-trifluoro-1-trifluoromethylethylidene)cyclopentane

carboxylic acid (10)—was synthesized from **30** in a 77% yield as a white solid using general procedure III; mp 198.0-199.5 °C; ¹H NMR (D₂O, 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). ¹⁹F NMR (D₂O, 376.493 MHz) δ -59.97 (3F, q, *J* = 9.0 Hz), -60.47 (3F, q, *J* = 9.0 Hz). ¹³C NMR (D₂O, 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. *m/z* (EI, MeOH): 278.1 (M+1)⁺, 277.1 (M)⁺, 259.1 (M-H₂O)⁺, 232.0 (M-CO₂H)⁺. HRMS calcd. For C₉H₉O₂NF₆: 277.0538. Found 277.0545. Anal. (C₉H₉O₂NF₆·H₂O) 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. Using 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 μ L) contained enzyme (30 μ L), potassium pyrophosphate buffer (volume dependent on volume of inhibitor, 50 mM, pH 8.5), α -ketoglutarate (20 μ L, 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**, 0.2M in doubly distilled H₂O). The concentration of inhibitors was calculated based on a total volume of 200 μ L. At time intervals, aliquots (27 μ L) from the incubation solution were added to the assay solution (568 μ L) 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 μ L) contained enzyme (30 μ L), potassium pyrophosphate buffer (147 μ L, 50 mM, pH 8.5), α -ketoglutarate (20 μ L, 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 μ L, 0.2 M in doubly distilled H₂O). A control solution (200 μ L) containing enzyme (30 μ L), potassium pyrophosphate buffer (150 μ L, 50 mM, pH 8.5), α -ketoglutarate (20 μ L, 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 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 μ L) contained enzyme (30 μ L), potassium pyrophosphate buffer (volumes dependent on the volume of substrate, 50 mM, pH 8.5), α -ketoglutarate (20 μ L, 16 mM in 50 mM potassium pyrophosphate buffer, pH 8.5), 2-mercaptoethanol (2.0 mM), inhibitor solutions (2 μ L or 0 μ L in control, **6**, **8** or **9**, respectively in doubly distilled H₂O, 0.2 M) and GABA solution [0 μ L (control), 17.6 μ L and 35.2 μ L]. The concentrations of GABA in the incubation solutions were 0 mM (control), 3 mM, and 6 mM, respectively. At time intervals, aliquots (27 μ L) from the incubation solution were added to the assay solution (568 μ L) 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 μ L) 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 μ L at 25 °C. A control containing no enzyme (25 μ L of buffer was added instead) was also run. The fluoride ion concentration (200 μ L) of each sample mixed with 1.00 mL of total ionic strength adjusting buffer (TISAB low level), 790 μ L of potassium pyrophosphate buffer, and 10 μ L 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 μ L) was incubated with **6** or **7** (12 μ L, 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 ($2.38 \times 10^{-4} \text{ M}$). The fluoride ion concentration was measured with a fluoride ion electrode.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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

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Figure 1.
Vigabatrin (**1**) and conformationally-restricted analogues

Figure 2.
New fluorinated conformationally-restricted potential inhibitors and inactivators of GABA-AT

Scheme 1.

Reagents and conditions: **a**, PMBCl, NaH, Bu₄NI, DMF, 0 °C - r.t., 2h, 83%; **b**, DBDMH, HOAc, r.t., 20h, 97%; **c**, Bu₃SnH, AIBN, PhH, reflux, 12h, 95%; **d**, K₂CO₃, MeOH, H₂O, r.t., 2h, 87%; **e**, NMO, TPAP, DCM, r.t., 24h, 70%.

Scheme 2.

Reagents and conditions: **a**, $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, Et_3N , EtOH , reflux, 1 h; **b**, I_2 , Et_3N , benzene, r.t., 2 h; **c**, $t\text{-BuOK}$, ether, r.t., 20 h, 60% in three steps; **d**, $\text{Me}_3\text{SnSnMe}_3$, $\text{Pd}(\text{PPh}_3)_4$, PhMe , reflux, 30 min, 49%; **e**, XeF_2 , AgOTf , 2,6-bis(*tert*-butyl)-4-methylpyridine, DCM , r.t., 8 min, 30%; **f**, CAN , CH_3CN , H_2O , r.t., 2 h, 55%; **g**, 4N HCl (aq.), 70 °C, 0.5-1 h, 64%

Scheme 3.

Reagents and conditions: **a**, TMSRf, TBAF (cat.), THF, r.t., 1 h, 95%; **b**, TsCl, NaH, ether, 0 °C, 16 h, 79%.

Scheme 4.

Reagents and conditions: **a**, MFSDA, CuI, DMF, HMPA, 20 h, 75%; **b**, CAN, MeCN/H₂O, r.t., 3 h, 61%; **c**, 4N HCl (aq.), 70 °C, 0.5-1 h, 85%.

Scheme 5.

Reagents and conditions: **a**, $\text{CF}_3\text{CF}_2\text{SiMe}_3/\text{KF}/\text{CuI}$, $\text{NMO}/\text{DMF}(1/1)$, 75°C , 24 h, 57%; **b**, CAN , CH_3CN , H_2O , r.t., 2 h, 76%; **c**, 4N HCl (aq.), 70°C , 0.5-1 h, 82%.

Scheme 6.

Reagents and conditions: **a**, $\text{BrCH}_2\text{PPh}_3 \cdot \text{Br}$, *tert*-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 , r.t., 2 h, 76%; **d**, 4N HCl (aq.), 70 °C, 10-12 h, 79%.

Scheme 7.

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 , r.t., 1 h, 56%; d, 4N HCl (aq.), 70°C , 10-12 h, 77%.

Scheme 8.

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 as compared to control when GABA-AT was incubated with **6** (triangles).

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 as compared to control when GABA-AT was incubated with **7** (squares).

Table 1Kinetic constants for **5-10**

Compound	k_{inact} (min^{-1})	K_{I} (mM)	$k_{\text{inact}}/K_{\text{I}}$ ($\text{min}^{-1}\text{mM}^{-1}$)	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 reference ²⁵