A New Class of Conformationally Rigid Analogues of 4-Amino-5-halopentanoic Acids, Potent Inactivators of γ-Aminobutyric Acid Aminotransferase

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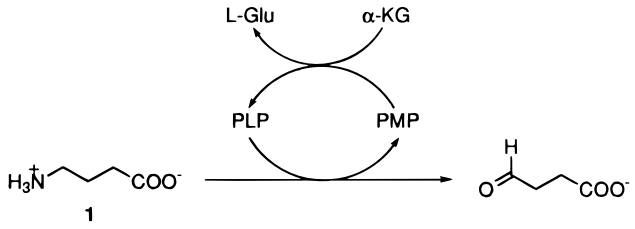
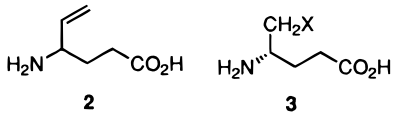
Recently, we found (Qiu, J.; Pingsterhaus, J. M.; Silverman, R. B. *J. Med. Chem.* **1999**, *42*, 4725−4728) that conformationally rigid analogues of the GABA aminotransferase (GABA-AT) inactivator vigabatrin were not inactivators of GABA-AT. To determine if this is a general phenomenon of GABA-AT inactivators, several mono- and di-halogen-substituted conformationally rigid analogues (**7**−**15**) of other GABA-AT inactivators, 4-amino-5-halopentanoic acids, were synthesized as potential inactivators of GABA-AT. Four of them, (+)-**7**, (−)-**9**, (+)-**10**, and (+)-**15**, were inactivators, although not as potent as the corresponding open-chain analogues. The maximal inactivation rate constants, k_{inact}, for the fluoro- and bromo-substituted analogues were comparable, indicating that cleavage of the C−X bond is not rate determining. Consistent with that observation is the finding that [3-^{2}H]-**10** exhibits a deuterium isotope effect on inactivation of 3.3, suggesting that C−H bond cleavage is the rate-determining step. The rate of inactivation of GABA-AT by the fluorinated analogue **7** is 1/15 that of inactivation by the corresponding open-chain analogue, 4-amino-5-fluoropentanoic acid (**3a**). Whereas inactivation by **3a** releases only one fluoride ion, inactivation by **7** releases 148 fluoride ions, accounting for the less efficient inactivation rate. Inactivation leads to covalent attachment of 2 equiv of inactivator after gel filtration; upon urea denaturation, 1 equiv of radioactivity remains bound to the enzyme. This suggests that, unlike the open-chain anlogue, the conformationally rigid analogue becomes, at least partially, attached to an active-site residue. It appears that the conformational constraint has a larger effect on inactivators that inactivate by a Michael addition mechanism than by an enamine mechanism.

γ-Aminobutyric acid aminotransferase (GABA-AT) is a pyridoxal 5′-phosphate (PLP)-dependent enzyme that catalyzes the transformation of the inhibitory neurotransmitter γ-aminobutyric acid (**1**, GABA) to succinic semialdehyde and to the excitatory neurotransmitter L-glutamic acid (Scheme 1).[1] Inhibitors of GABA-AT exhibit anticonvulsant activity by increasing the concentration of **1** in the brain.[2] Vigabatrin (**2**) is a time-dependent, mechanism-based, inactivator of GABA-AT currently in medical use for the treatment of epilepsy[3] and is under investigation as a treatment for drug addiction.[4] Mechanism-based inactivators are unreactive prior to their turnover by the target enzyme, so they tend to cause fewer side reactions than affinity labeling agents.[5] One of the general strategies for the design of mechanism-based inactivators of PLP-dependent enzymes is to incorporate a good leaving group in the β-position of an amino acid that acts as a substrate for the targeted enzyme.[5] For example, β-haloalanines are valuable mechanism-based inactivators of aminotransferases,[6] racemases,[7] and decarboxylases.[8] 4-Amino-5-halopentanic acids (**3**) were rationally designed as mechanism-based inactivators of GABA-AT by introduction of a good leaving group β to the amino group.[9] The fluoro analogue of this series (**3a**) was shown to be a potent inactivator of GABA-AT with a highly efficient partition ratio (release of product per inactivation event) of 0.[10] Its inactivation mechanism was shown to proceed via

Scheme 1





a, X = F
b, X = Cl
c, X = Br
d, X = OH

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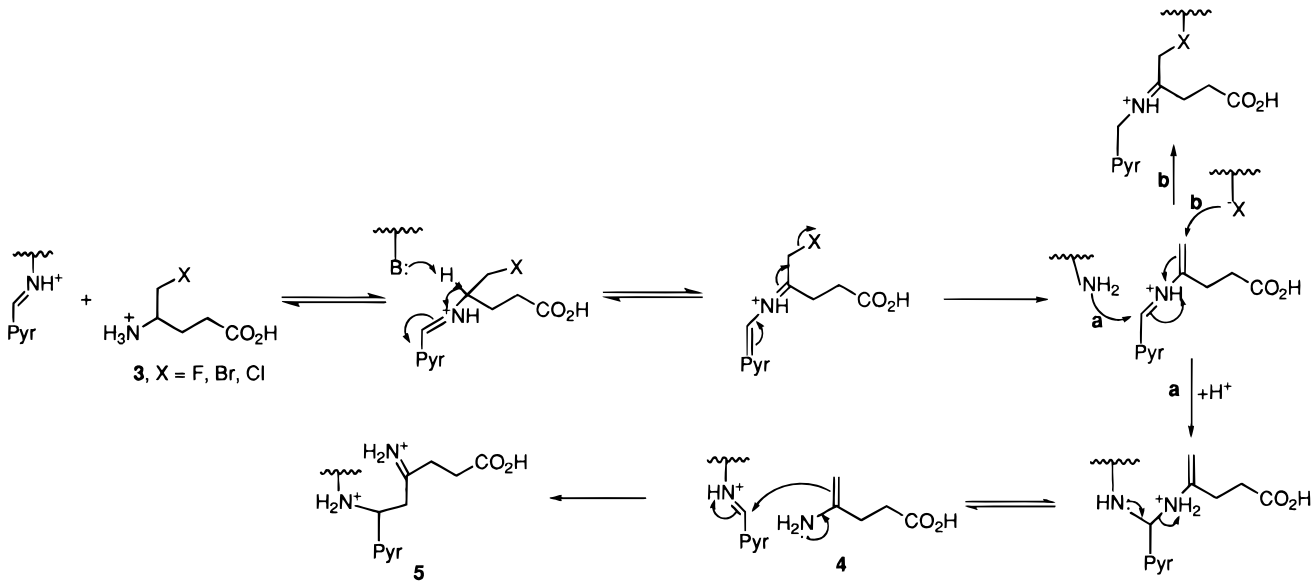
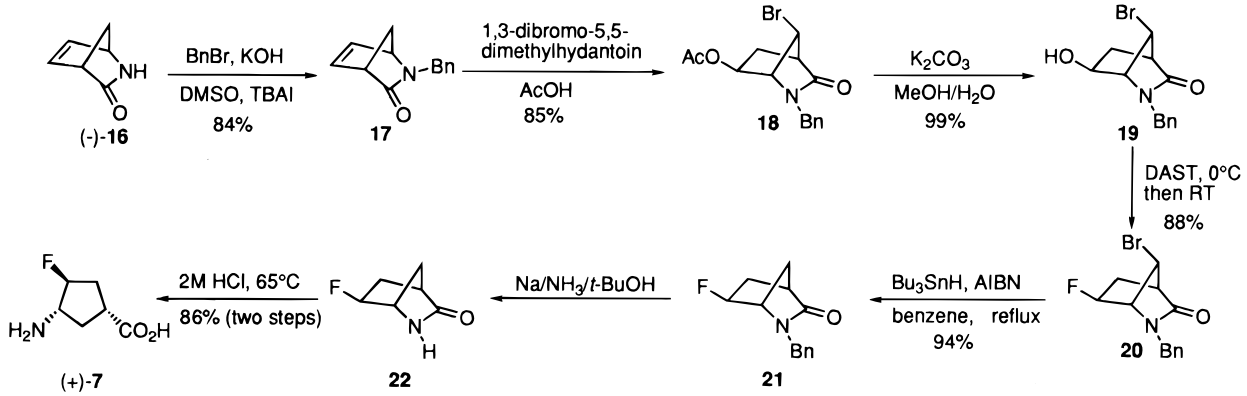
Scheme 2

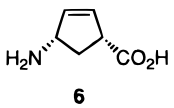


Scheme 3



an enamine pathway in which an active enamine intermediate (**4**), generated by GABA-AT, modifies the enzyme-bound PLP (**5**, Scheme 2).

The anticonvulsant drug vigabatrin (**2**) inactivates GABA-AT by two mechanisms, mostly by a Michael addition pathway, but a small amount by an enamine pathway.[11] Recently, (1*R*,4*S*)-4-aminocyclopent-2-ene-1-carboxylic acid (**6**), a conformationally rigid analogue of vigabatrin, was shown to be a potent inhibitor and substrate of GABA-AT, but it was not a time-dependent inactivator.[12] It was surprising that the corresponding conformationally rigid analogue (**6**) exhibited no time-



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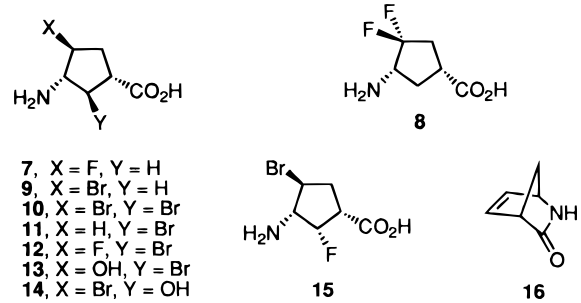
dependent inhibition. To determine if conversion of acyclic inactivators into cyclic ones generally interferes with mechanism-based inactivation of GABA-AT, we constructed a variety of conformationally rigid analogues of 4-amino-5-halopentanoic acids (**3**), compounds known to inactivate GABA-AT exclusively by an enamine pathway (pathway a, Scheme 2). Several of the analogues described here are time-dependent, mechanism-based inactivators, as in the case of the corresponding open-chain (GABA) analogues.

Chemistry

All analogues (**7**−**15**) were synthesized from a common starting material, 2-azabicyclo[2.2.1]hept-5-en-3-one (**16**), which is commercially available in both enantiomeric forms from Aldrich. It is necessary to protect the lactam of **16** to prevent formation of complex mixtures during the syntheses. Enantiopure (+)-**7** was synthesized from enantiopure (−)-**16** as shown in Scheme 3. Treatment of (−)-**16** with benzylbromide in the presence of powdered potassium hydroxide gave the



7, X = F, Y = H
9, X = Br, Y = H
10, X = Br, Y = Br
11, X = H, Y = Br
12, X = F, Y = Br
13, X = OH, Y = Br
14, X = Br, Y = OH

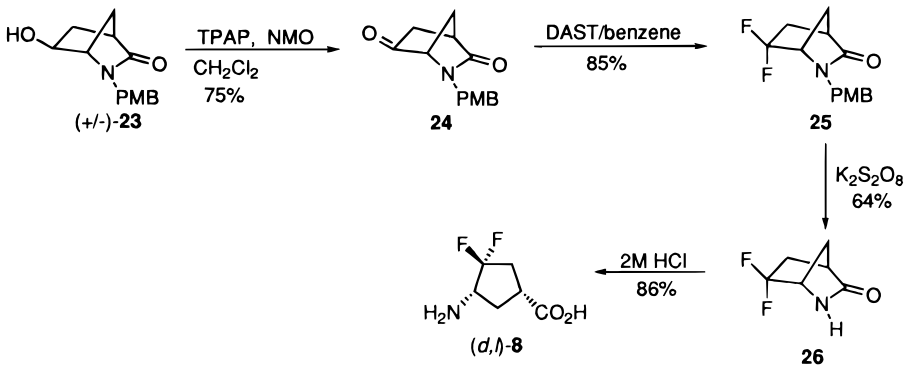
8

15

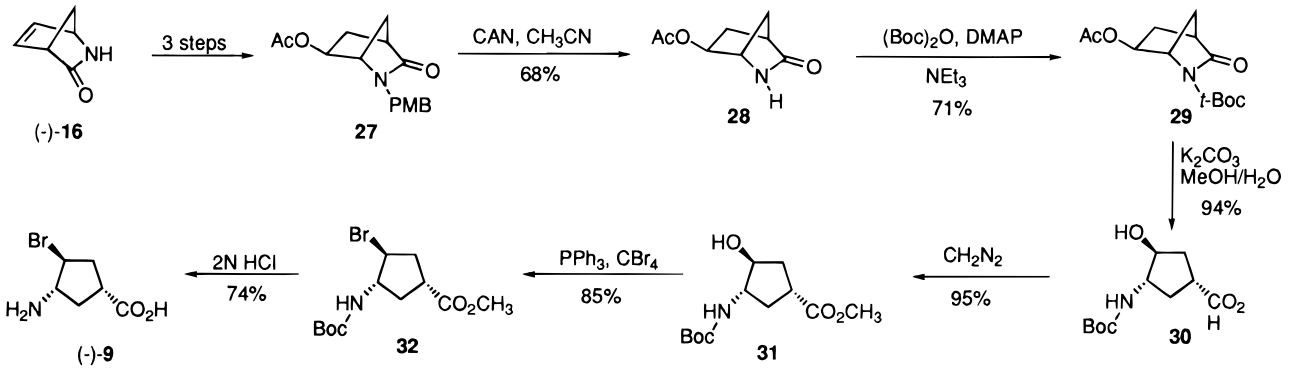
16

protected lactam **17**,[13] which was treated with a solution of 1,3-dibromo-5,5-dimethylhydantoin in glacial acetic acid at room temperature to afford **18**. The absolute stereochemistry of **17** is switched in going to **18**, as

Scheme 4



Scheme 5



previously reported,[14] but this is the required stereochemistry for the final product (+)-**7**. Basic hydrolysis of **18** with potassium carbonate and fluorination of **19** with (diethylamino)sulfur trifluoride (DAST)[15] gave desired product **20**; dehydrobromination of **20** with tributyltin hydride in the presence of a catalytic ammount of azoisobutyronitrile (AIBN) in dry benzene furnished the monofluoro lactam **21** in an excellent yield.[14] Birch reduction was applied to deprotect the benzyl group in **21** and afford lactam **22**. Without any purification, the resultant lactam **22** was hydrolyzed in 2 N HCl/acetic acid at 65 °C to give the final product (+)-**7**.

The difluoro analogue (±)-**8** was synthesized from (±)-**23** (Scheme 4), which was prepared in four steps.[14] The oxidation of **23** with a catalytic amount of tetrapropylammonium perruthenate (TPAP) in the presence of the co-oxidant, 4-methylmorpholine *N*-oxide (NMO), afforded ketone **24**.[16] Difluorination of **24** with DAST, in benzene at room temperature, gave the difluorolactam **25**, which was deprotected with potassium persulfate[17] in the presence of potassium hydrogen phosphate at 80 °C to afford the difluorolactam **26**. Unsuccessful attempts at deprotection included oxidation of the 4-methoxybenzyl group in **25** with ceric ammonium nitrate (CAN), which predominantly afforded the oxidation product instead of the required deprotected product **26** (less than 10% yield), and neat, anhydrous trifluoroacetic acid[18] and trifluoroacetic acid-anisole in boiling 1,2-dichloroethane.[19] Acidic hydrolysis of **26** gave the final racemic product (**8**).

Compound (−)-**9** was synthesized starting from (−)-**16** (Scheme 5). The enantiopure compound **28** was obtained in four steps (Scheme 5).[14] Protection of **28**[14] gave the *t*-Boc protected lactam **29**, which was hydrolyzed in a suspension of potassium carbonate, methanol, and water to **30**. Treatment of **30** with diazomethane afforded **31** in high yield. Bromination of **31** with carbon tetrabromide and triphenyl phosphine gave **32** with retention of the stereochemistry at C-4, as evidenced by the observation of a NOE between H-1 and H-3 and between H-4 and one of the protons on C-2. The lack of a NOE between H-4 and H-1 strongly supports a trans relationship for protons H-4 and H-1. Retention of the stereochemistry in going from **31** to **32** was probably facilitated by participation of the neighbor group (*t*-Boc)[20] in the bromination step (Figure 1). Acid hydrolysis gave (−)-**9**.

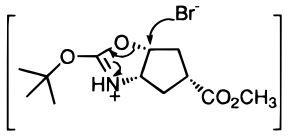
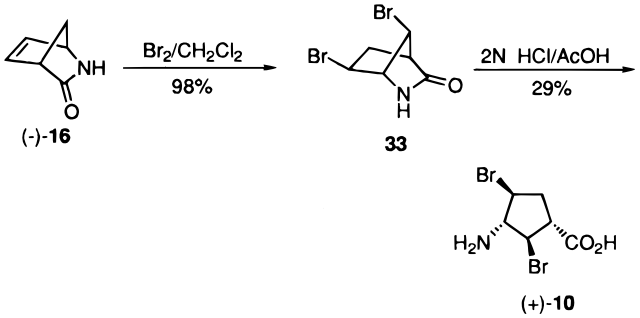


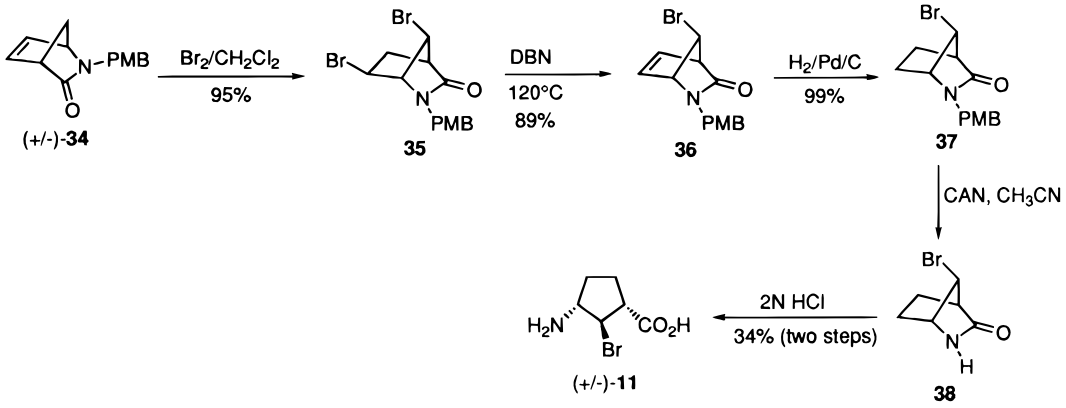
Figure 1. Achimeric assistance by the carbamate group of **31** to give retention of configuration in **32**.

Scheme 6

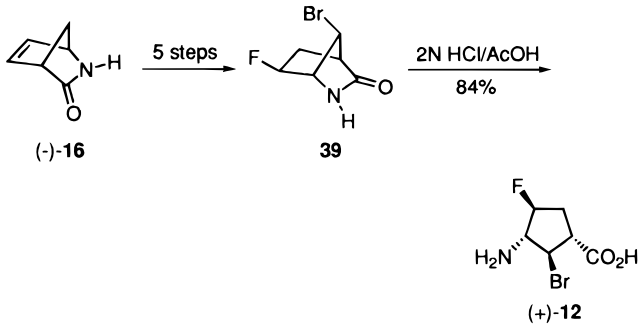


The synthesis of dibromo analogue **10** was straightforward starting from (−)-**16** (Scheme 6). Bromination

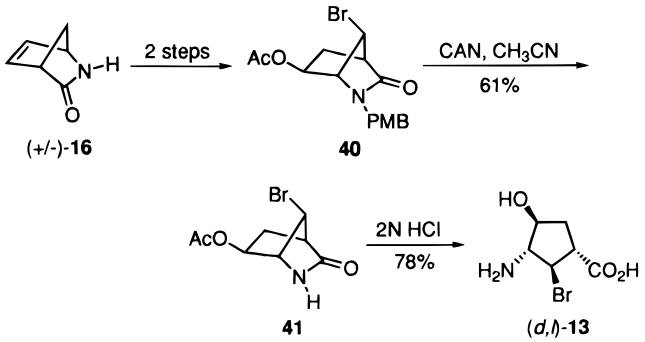
Scheme 7



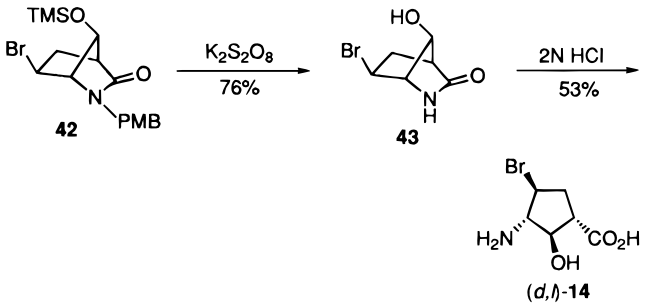
Scheme 8



Scheme 9



Scheme 10

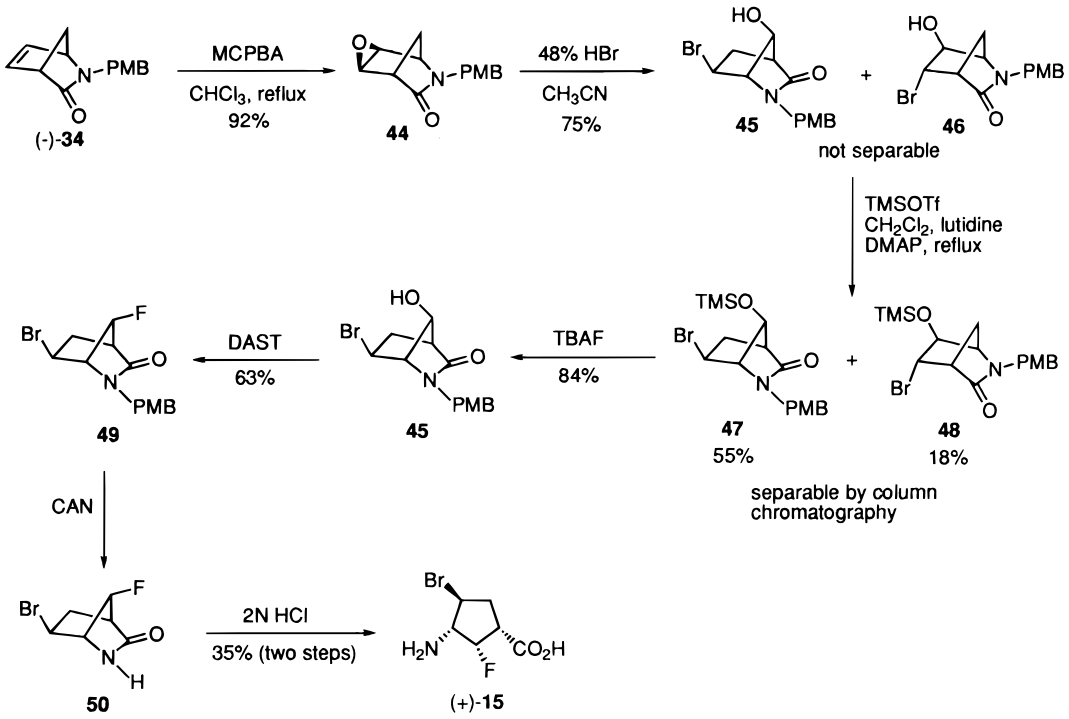


of (−)-**16** had been shown to give dibromo compound **33** by way of an elegant "molecular somersault", which was hydrolyzed to (+)-**10** (Scheme 6).[21] The synthesis of the 2-bromo analogue **11** was achieved from racemic 4-methoxybenzyl protected lactam **34**[14] (Scheme 7). Treatment of (±)-**34** with bromine at room temperature gave dibromolactam **35** in high yield. Elimination of hydrobromide in **35**, by treatment with 1,5-diazobicyclo[4.3.0]-non-5-ene (DBN) in refluxing toluene and xylene, did not give satisfactory results. In refluxing toluene the reaction did not go to completion and gave rise to a complex mixture of **35** and **36**. In refluxing xylene the reaction temperature was too high, thereby decomposing the starting material. However, the elimination of hydrobromide from **35** to **36** was successful using neat DBN at 120 °C. Compound **36** was converted by hydrogenation, deprotection (CAN), and acidic hydrolysis to (±)-**11**, which was purified by ion-exchange chromatography.

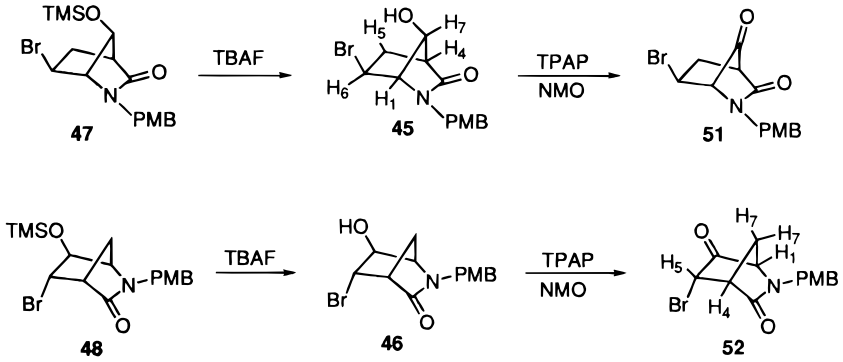
Enantiomerically pure analogue **12** was made by hydrolysis of enantiopure lactam **39** (Scheme 8), which was prepared in five steps following a known procedure.[14] Analogue **13** was obtained as a racemic mixture starting from racemic **16** (Scheme 9). Deprotection of the 4-methoxybenzyl group of **40**[14] gave lactam **41**; acid hydrolysis of **41** gave (±)-**13**. The synthesis of analogue **14** was straightforward (Scheme 10). Deprotection of (±)-**42** with $K_2S_2O_8$ gave **43**, which was hydrolyzed to racemic **14**.

The synthesis of (+)-**15** is shown in Scheme 11. Epoxidation of (−)-**34** with *m*-chloroperbenzoic acid in boiling $CHCl_3$ gave exclusively the *exo*-epoxide[21] **44**. Opening of the epoxide was attempted under different conditions, e.g., HBr(g) in dry dichloromethane at 0 °C, HBr(g) in CH_3CN, and aqueous HBr (48%) in CH_3CN at 0 °C. None of these conditions gave one exclusive product. It was expected that there would be only one rearranged product, as was the case after acidic opening of norbornene oxide.[22] The mixture of **45** and **46**, resulting from the opening of **44** with 48% HBr, could not be separated by either flash column chromatography on silica gel or recrystallization from ethyl acetate/hexane. However, after the hydroxyl groups of **45** and **46** were masked by trimethylsilylation, the resulting products (**47** and **48**) could be easily separated by flash column chromatography on silica gel. The structures of **45** and **46** were identified by a chemical derivatization method (Scheme 12) and by NOE experiments. After separation by column chromatography, **47** and **48** were each treated with tetrabutylammonium fluoride (TBAF) to afford pure **45** and **46**, respectively. Oxidation of **45** and **46** with TPAP afforded ketones **51** and **52**. NMR spectral analyses of these products readily identified the protons attached to the carbons bearing the hydroxyl groups in **45** and **46**. Furthermore, NOE experiments with **45** showed that there were no interactions between H-7 and H-5, and between H-6 and H-4, but there are

Scheme 11



Scheme 12



interactions between H-6 and H-5 and between H-6 and H-1. These NOE experiments support the proposed structure for the rearranged product **45** depicted in Scheme 11. The structure of **46** was identified indirectly by determining the structure of **52** as depicted in Scheme 12. NOE experiments performed on **52** illustrated interactions between H-4 and H-5, H-5 and H-7, and no interactions between H-5 and H-1. The opening of the epoxide **44** with HBr gave two products: the required rearranged product **45**, and the directly opened product **46**. The optimized conditions for ring opening were found to be aqueous 48% HBr in CH_3CN at 0 °C, giving the highest ratio of **45** to **46** (3:1) in a 75% overall yield. Fluorination of **45** with DAST gave a single product (**49**), via an S_N2 mechanism (Scheme 11). The final product ((+)-**15**) was obtained after deprotection of **49** and acid hydrolysis of **50**.

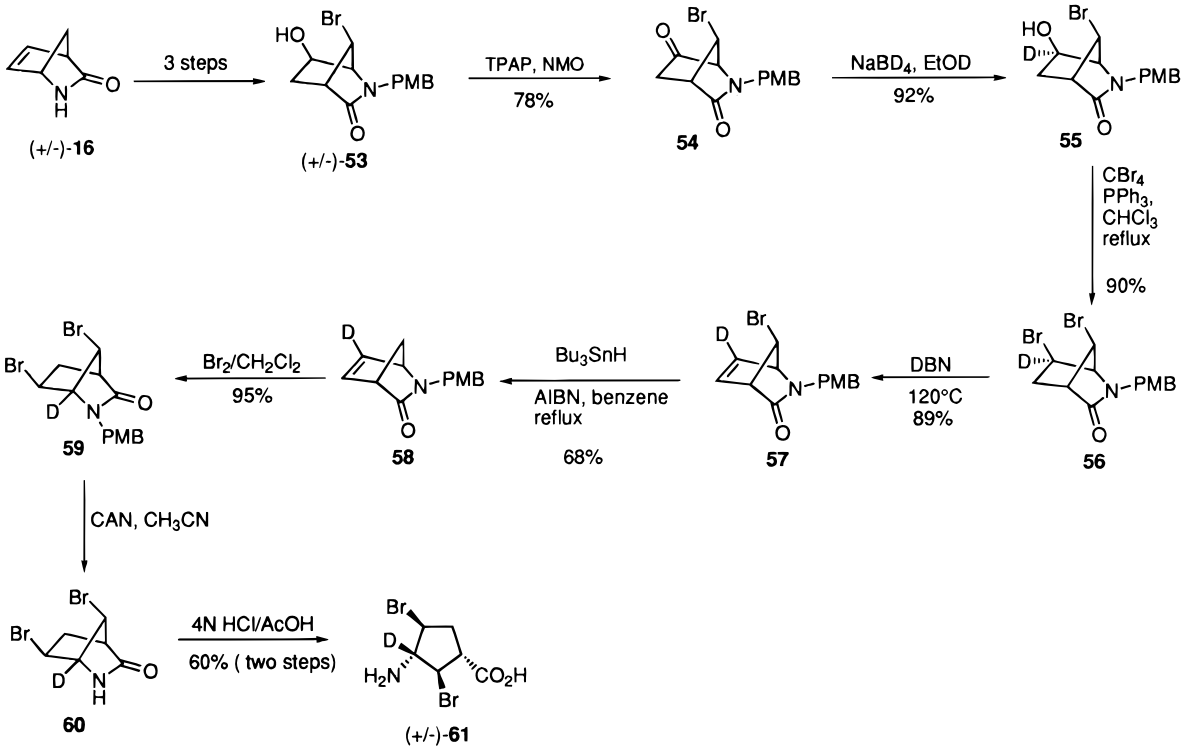
Two isotopically labelled analogues also were synthesized for mechanistic studies. The deuterated dibromo analogue ((±)-**61**; Scheme 13) was synthesized from (±)-**53**. TPAP oxidation of the alcohol gave ketone **54**. Introduction of the deuterium from the favored endo side with $NaBD_4$ gave **55**, which was converted to the dibromide **56**. Elimination of HBr followed by tributyltin hydride-induced debromination gave **58**. Bromination, deprotection, and hydrolysis produced the deuterated analogue **61**.

To incorporate a radiolabel into **10** (Scheme 14), enantiopure **62** was treated with tributyltin chloride and tritiated sodium borohydride (all of the reactions in this scheme were first performed in deuterated forms, then with tritium). Tritiated **63** (**63**-T) was dibrominated and deprotected and the lactam hydrolyzed to give [5-^{3}H]-(+)-**66** (i.e., tritiated (+)-**10**).

Results and Discussion

Kinetic constants for the inhibition and/or inactivation of GABA-AT by each of the conformationally rigid analogues are given in Table 1. Analogues (+)-**7**, (−)-**9**, (+)-**10**, and (+)-**15** are time-dependent inactivators of GABA-AT. After incubation of GABA-AT with each of these compounds until less than 10% of the enzyme activity remained, gel filtration to remove excess and noncovalently bound inactivators did not restore the enzyme activity. This shows that all four of these analogues irreversibly inactivate GABA-AT. Inactivation by (+)-**10** was prevented by addition of GABA to the incubation solution, indicating that inactivation is

Scheme 13



Scheme 14

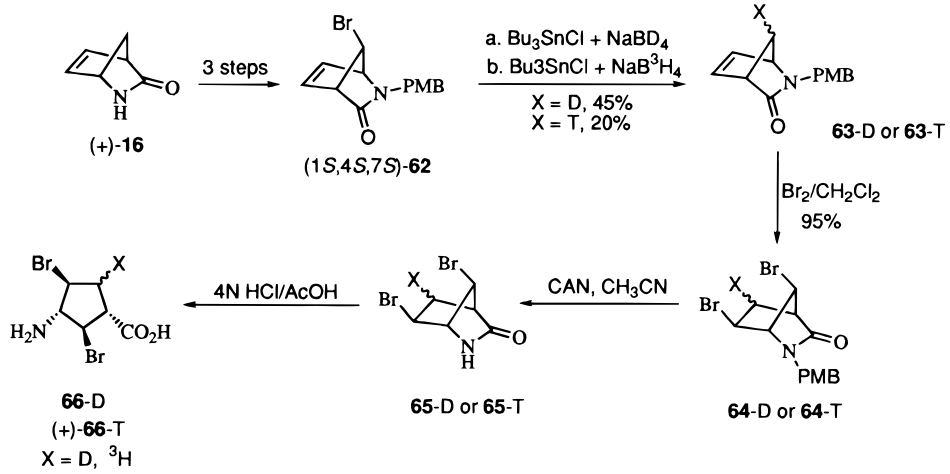


Table 1. Kinetic Constants for Inhibition and Inactivation of GABA-AT by Conformationally Rigid Analogues

compd	K_I (mM)	k_{inact} (min^{-1})	k_{inact}/K_I (min^{-1} mM^{-1})	K_i (mM)
(+)-**7**	0.43	0.03	0.070	0.012 ± 0.002
(D,L)-**8**	no inactivation			0.19 ± 0.03
(−)-**9**	16	0.02	0.0037	3.6 ± 0.4
(+)-**10**	49	0.18	0.0013	4.2 ± 0.3
(D,L)-**11**	no inactivation			12 ± 2
(+)-**12**	no inactivation			4.7 ± 0.6
(D,L)-**13**	no inactivation			13 ± 2
(D,L)-**14**	no inactivation			5.9 ± 0.6
(+)-**15**	3.14	0.023	0.0073	1.6 ± 0.2

an active-site phenomenon. If the PLP is converted to PMP with GABA in the absence of α-ketoglutarate to convert it back to PLP, then (+)-**10** does not inactivate the enzyme. Therefore, PLP is required for inactivation.

Of the four inactivators, (+)-**7** is the most potent based on the values of k_{inact}/K_I. The K_I value for the open-chain derivative, (±)-4-amino-5-fluoropentanoic acid (**3a**; K_I = 0.40 mM),[9b] is comparable to that for (+)-**7**, but the k_{inact} value of **3a** (0.50 min^{-1}) is 17 times greater. The smaller k_{inact} value for **7** could be a function of its constrained structure, which could either misorient the proton abstracted so that it does not have maximal orbital overlap with the PLP p-orbitals, thereby slowing down the reaction rate, or misorient the reaction of the activated species with the enzyme. However, it was found that for (+)-**7** 148 ± 8 fluoride ions are released per enzyme dimer inactivated (i.e., the partition ratio is 147 ± 8), whereas for **3a** the partition ratio is 0 (only one fluoride ion is released during inactivation).[10] The lower inactivation rate, therefore, is, more likely, a function of the lack of efficiency of inactivation with **7** relative to **3a** as a result of the constraint of the molecule. With both **3a** and **7**, no transamination occurs during inactivation, only fluoride ion release. The fact that **7** is an inactivator at all, however, is quite surpris-

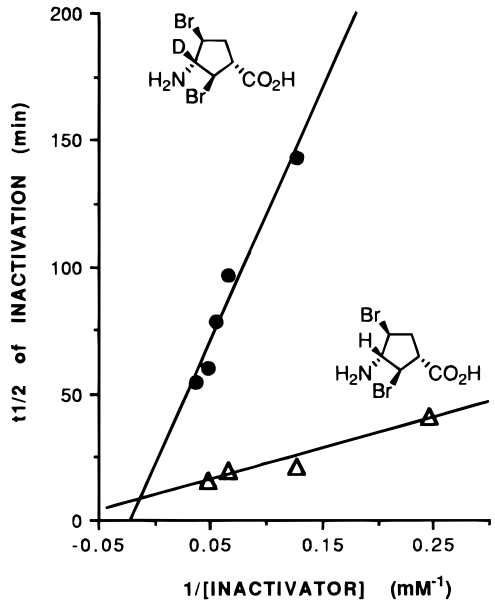


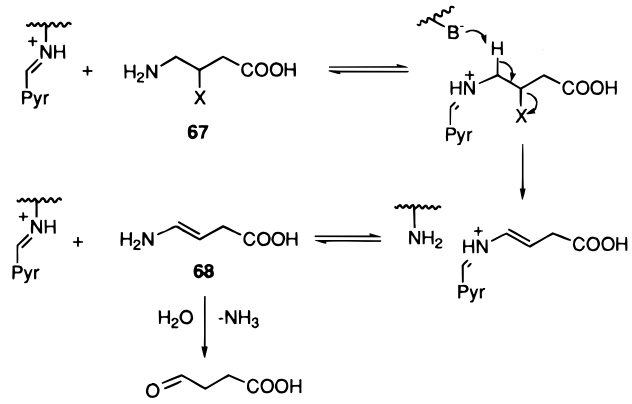
Figure 2. Deuterium isotope effects on the rate of inactivation of GABA-AT by (±)-**10** and (±)-**61**. From a plot of the log of the remaining activity vs the time of incubation, values for the $t_{1/2}$ of inactivation at varying concentrations of (±)-**10** (△) and (±)-**61** (●) were obtained.

ing because the conformationally rigid analogue of 4-amino-5-hexenoic acid (vigabatrin, **2**), namely **6**, is not an inactivator. This may be a function of the difference in inactivation mechanisms between vigabatrin (a Michael addition mechanism; see Scheme 2, pathway b for a Michael addition mechanism with **3a**) and **3a** (an enamine mechanism; Scheme 2, pathway a) and suggests that the leaving group orientation (such as the fluorine in **7**) is not as important as the double bond orientation (in **6**) for inactivation.

The similarity of the maximal inactivation rate constants (k_{inact}) for (−)-**9** and (+)-**7**, which differ only by the halogen atom, suggests that the cleavage of the carbon−halogen bond is not the rate-determining step; if it were, then the k_{inact} for **9** (X = Br) would have been greater than that for **7** (X = F). Further support for this conclusion comes from a comparison of the kinetic constants for (±)-**10** with (±)-**61** (i.e., [3-^{2}H]-(±)-**10**; see Scheme 13). As calculated from the results in Figure 2, $^{H}k_{inact}/^{D}k_{inact}$ = 3.3 and $(^{H}k_{inact}/^{H}K_I)/(^{D}k_{inact}/^{D}K_I)$ = 8.1. Therefore, abstraction of the proton adjacent to the amino group is the rate-determining step, as is the case with **3a**.[9c] The isotope effect result also confirms that **10** is a mechanism-based inactivator; inactivation is coupled to an enzyme-catalyzed process, namely, proton removal.

Because of the effectiveness of (+)-**7** as an inactivator, and the fact that 4-amino-5,5-difluoropentanoic acid (the difluoro analogue of **3a**) also is an inactivator of GABA-AT,[23] the corresponding conformationally rigid analogue of 4-amino-5,5-difluoropentanoic acid, (±)-**8**, was synthesized. Surprisingly, **8** exhibits no time-dependent inhibition, although it is a competitive inhibitor with a K_i value of 190 μM (Table 1). Incubation of GABA-AT with **8** in the presence of [^{14}C]-α-ketoglutarate, even for 16 h, produces no [^{14}C]-L-glutamate, indicating that **8** does not act as a substrate, reducing PLP to PMP. However, a fluoride ion electrode was used to show that fluoride ion is slowly released from **8** by GABA-AT;

Scheme 15



about 22% of the total of one fluoride ion (2.4 ± 0.1 mM fluoride ion) was released in a period of 16 h. Elimination of HF from **8**, apparently, does not lead to inactivation. Presumably, the product of elimination is not susceptible to Michael addition by an active-site nucleophile (Scheme 2, pathway b) nor can it undergo the enamine mechanism (Scheme 2, pathway a).

Above, it was demonstrated that **10** inactivates GABA-AT, and the rate-determining step is C−H bond cleavage. In the case of **10**, abstraction of the C-3 proton gives a stabilized carbanion that can lead to elimination of one or both of the two bromines in the molecule. It was previously shown that 4-amino-3-halobutanoic acids (**67**, Scheme 15) are good substrates for GABA-AT but do not cause inactivation; GABA-AT-dependent elimination of the halide ion and transfer of the PLP back onto the active-site lysine residue gives enamine **68**, which presumably is not oriented properly for addition to the enzyme-bound PLP (Scheme 15).[24] This suggests that, in the case of **10**, elimination of the C-2 bromide should not be responsible for inactivation but elimination of the C-4 bromide should, because the C-4 bromide mimics the structure of the 4-amino-5-halopentanoic acid inactivators. Analogues (−)-**9** and (±)-**11** support that hypothesis. Only (−)-**9**, the 4-amino-5-halopentanoic acid mimic, is an inactivator, not **11**, the 4-amino-3-halobutanoic acid mimic (Table 1). Unfortunately, **11** was too unstable to allow for isolation of the expected elimination product.

Because elimination of both the C-2 and C-4 bromines can occur, we wondered if competition between these pathways could be influenced by a change in the leaving group ability of the substituent. Consequently, (+)-**12** was synthesized. Because the better leaving group is at C-2, preferential HBr elimination should occur, leading to the enamine pathway, such as in Scheme 15, which does not cause inactivation. In fact (Table 1), (+)-**12** is not an inactivator of GABA-AT. However, when these two halogens are reversed (the compound in which the fluorine has *R* stereochemistry is much more difficult to synthesize, so the fluorine was incorporated with *S* stereochemistry), as in the case of (+)-**15**, the compound is an inactivator; elimination of HBr from enzyme-bound **15** gives an intermediate equivalent to that from the inactivator (+)-**10**. Similarly, **13** does not inactivate GABA-AT. When the bromine and hydroxyl groups were reversed (**14**), which should give a good inactivator, only slow inactivation (10% loss of enzyme activity in 4 h at 19.4 mM concentration) was observed.

Possibly, there is hydrogen bonding to the hydroxyl group which interferes with the compound from assuming the appropriate orientation for inactivation.

Inactivation by, at least, (+)-**10** is a covalent process. Following inactivation of GABA-AT by **66-T** (i.e., [5-^{3}H]-**10**), dialysis, and gel filtration, 2.2 ± 0.4 equiv of tritium was found to remain associated with the protein. After urea denaturation of the inactivated enzyme, 1.06 ± 0.08 equiv remained bound per dimer. This suggests that, at least, part of the inactivation occurs by protein modification, which is different from what was observed with **3a**. Possibly, the structural constraints now permit the Michael addition pathway to be a viable option as well as the enamine pathway, which is the case with vigabatrin (**2**).

All of the compounds are competitive reversible inhibitors of GABA-AT (Table 1), ranging from a K_i value of 12 μM for (+)-**7** to 13 mM for (±)-**13**. It appears that bulkiness at C-2 is not very well tolerated.

In conclusion, unlike the five-membered conformationally rigid analogue of vigabatrin (**6**), which does not inactivate GABA-AT, five-membered conformationally rigid analogues of 4-amino-5-halopentanoic acids exhibit inactivation properties similar to, although not as potent as, the corresponding open-chain analogues. Because the inactivation mechanisms require strict orbital overlap requirements, constraint of these orbitals can have a detrimental effect on inactivation rate constants; in some cases, the constraint prohibits inactivation. From the studies with conformationally rigid analogues of vigabatrin and 4-amino-5-halopentanoic acids, it appears that conformational constraint affects inactivation by a Michael addition mechanism more so than inactivation by an enamine pathway.

Experimental Section

General Methods. 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 Me_4-Si (δ 0.0) 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. Radioactivity was measured by liquid scintillation counting using a Packard Tri-Carb 2100TR counter and Packard Ultima Gold scintillation cocktail. HPLC analyses and preparations were performed using a Beckman System Gold system with a 125 solvent delivery module and a 166 UV detector. High-resolution mass spectra and accurate mass spectra were obtained on a VG70-250SE high-resolution spectrometer and a Micromass Quatro 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.

Reagents. 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. Sodium borohydride [^{3}H] (100 mCi, 15 Ci/mmol) was purchased from the American Radiolabeled Chemicals, Inc.

General Procedure for the Conversion of a Hydroxyl Group into Fluorine. (Diethylamino)sulfur trifluoride (DAST) (1.2 equiv) was added to a stirred solution of an alcohol (1.0 equiv) in anhydrous CH_2Cl_2 (15 mL) at −80 °C. After the resulting solution was stirred for 1.5 h at −80 °C, it was allowed to stir for 9 h at room temperature, then it was diluted with dichloromethane (100 mL). The resulting organic layer was washed with saturated $NaHCO_3$ (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate/hexane, to afford the fluorobromo product.

General Procedure for the 4-Methoxybenzyl Deprotection by Cerium(IV) Ammonium Nitrate (CAN). A solution of cerium(IV) ammonium nitrate (CAN) (3.0 equiv) in water (5 mL) was added to a stirred solution of the 4-methoxybenzyl protected lactam (1.0 equiv) in acetonitrile (20 mL). After being stirred for 5 h, the reaction mixture was diluted with ethyl acetate (200 mL). The combined organic solvents were washed with water (4 × 5 mL) and brine (25 mL) and dried over anhydrous Na_2SO_4. The solvent was concentrated under reduced pressure to afford crude deprotected lactam. The resultant lactam was normally not purified and used directly for the next step.

General Procedure for the Acidic Hydrolysis of the Lactam to the Corresponding Amino Acid. A lactam was added to a stirred solution of acetic acid (10 mL) and 4 N HCl (10 mL). The resulting solution was stirred at 70 °C for 5 h and concentrated in vacuo to afford a solid mixture. The solid was purified by ion-exchange chromatography (AG 50W-X8), eluting with either 1 M aqueous pyridine or a gradient from 0.4 N to 2.0 N HCl, giving the final amino acid hydrochloride product.

(1*R*,4*R*,6*S*,7*R*)-6-Acetoxy-7-bromo-2-benzyl-2-azabicyclo-[2.2.1]heptan-3-one (18). 1,3-Dibromo-5,5-dimethylhydantoin (1.10 g, 3.78 mmol) was added to a stirred solution of (1*R*,4*S*) 2-benzyl-2-azabicyclo[2.2.0]heptan-3-one[14] (**17**; 1.37 g, 6.9 mmol) in acetic acid (15 mL). After being stirred overnight, the resulting solution was diluted with dichloromethane (200 mL). The organic layer was washed with water (3 × 20 mL), 10% $NaHSO_3$ (30 mL), saturated $NaHCO_3$ (3 × 20 mL), and brine (30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate/hexane (1:3) to afford the desired product (**18**; 1.91 g, 85%) as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28−7.41 (5H, m, C_6H_5), 4.79 (1H, m, H_6), 4.77 (1H, d, *J* 14.7 Hz, CH_2Ph), 4.20 (1H, m, H_7), 4.01 (1H, d, *J* 14.7 Hz, CH_2Ph), 3.87 (1H, m, H_1), 2.96 (1H, m, H_4), 2.37 (2H, m, H_5), 2.07 (3H, s, CH_3CO) ppm; ^{13}C NMR (300 MHz, $CDCl_3$) δ 173.5, 171.2, 136.1, 129.5, 128.9, 128.7, 73.1, 64.5, 51.0, 48.8, 45.1, 30.5, 21.5; *m*/*z* (EI) 339, 337, 216, 186, 91. HRMS (EI) $C_{15}H_{16}{}^{79}BrO_3N$ calcd M 337.0314, found M 337.0310; $C_{15}H_{16}{}^{81}BrO_3N$ calcd M 339.0294, found M 339.0305.

(1*R*,4*R*,6*S*,7*R*)-7-Bromo-6-hydroxy-2-benzyl-2-azabicyclo[2.2.1]heptan-3-one (19). K_2CO_3 (0.46 g, 3.4 mmol) was added to a stirred solution of (1*R*,4*R*,6*S*,7*R*)-**18** (0.27 g, 0.84 mmol) in methanol (5 mL) and water (1 mL). The resulting mixture was stirred for 2 h. After methanol was removed under reduced pressure, the resulting aqueous layer was extracted with $CHCl_3$ (60 mL). The organic layer was washed with brine (15 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to afford **19** (0.22 g, 99%) as a solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (5H, m, C_6H_5), 4.68 (1H, d, *J* 14.7 Hz, CH_2Ph), 4.27 (1H, m, H_6), 4.20 (1H, m, H_7), 4,01 (1H, d, *J* 14.7 Hz, CH_2Ph), 3.96 (1H, m, H_7), 3.87 (1H, m, H_1), 2.96 (1H, m, H_4), 2.37 (2H, m, H_5) ppm; ^{13}C NMR (300 MHz, $CDCl_3$) 172.8, 136.1, 129.3, 128.4, 73.1, 65.9, 50.8, 50.4, 44.7, 33.8 ppm; *m*/*z* (EI) 295, 297, 216, 186, 173, 91. HRMS (EI) $C_{13}H_{14}{}^{79}BrO_2N$ calcd M 295.0208, found M 295.0209; $C_{13}H_{14}{}^{81}BrO_2N$ calcd M 297.0189, found M 297.0231.

(1*S*,4*R*,6*S*,7*R*)-2-Benzyl-7-bromo-6-fluoro-2-azabicyclo-[2.2.1]heptan-3-one (20). Following the general fluorination procedure described above, (1*R*,4*R*,6*S*,7*R*)-**19** (0.15 g, 0.51 mmol) was treated with DAST (0.11 g, 0.66 mmol) to afford the fluorobromo product **20** (0.15 g, 88%) as a solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (5H, m, C_6H_5), 4.79 (1/2H, m, H_6), 4.61 (1/2H, m, H_6), 4.63 (1H, d, *J* 14.8 Hz, CH_2Ph), 4.27 (1H, m, H_7), 4.10 (1H, d, *J* 14.8 Hz, CH_2Ph), 3.94 (1H, m, H_1), 3.87 (1H, m, H_1), 2.99 (1H, m, H_4), 2.30−2.60 (2H, m, H_5) ppm; ^{19}F NMR (300 MHz, $CDCl_3$) −173 (1F, ddd, *J* 57, 29, 12 Hz) ppm; *m*/*z* (EI) 296, 298, 218, 172, 91. HRMS (EI) $C_{13}H_{13}{}^{79}BrONF$ calcd M 297.0164, found M 297.0172; $C_{13}H_{14}{}^{81}BrONF$ M calcd 299.0145, found M 299.0142.

(1*S*,4*R*,6*S*)-2-Benzyl-6-fluoro-2-azabicyclo[2.2.1]heptan-3-one (21). Tributyltin hydride (1.35 g, 4.6 mmol) and 2,2′-azobisisobutyronitrile (AIBN) (20 mg) were added to a solution of (1*S*,4*R*,6*S*,7*R*)-**20** (1.06 g, 3.87 mmol) in dry benzene (25 mL). The resultant solution was heated at reflux and stirred for 12 h. After the reaction was concentrated under reduced pressure, the residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate/hexane (1:3), to afford the desired debrominated product **21** (0.71 g, 94%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (5H, m, C_6H_5), 4.74 (1/2H, m, H_6), 4.57 (1/2H, m, H_6), 4.52 (1H, d, *J* 15.1 Hz, CH_2Ph), 4.14 (1H, d, *J* 15.1 Hz, CH_2Ph), 3.80 (1H, m, H_1), 2.83 (1H, m, H_4), 2.17−1.70 (4H, m, H_7 and H_5) ppm; ^{19}F NMR (300 MHz, $CDCl_3$) −173 (1F, ddd, *J* 50, 32, 18 Hz) ppm; *m*/*z* (EI) 235, 219, 173, 91, 65. HRMS (EI) $C_{13}H_{14}ONF$ calcd M 235.1008, found M 235.1008.

(1*R*,3*S*,4*S*)-3-Amino-4-fluorocyclopentane-1-carboxylic Acid ((+)-7). Freshly cut pieces of sodium metal (0.45 g) were added to a stirred solution of liquid NH_3 (8 mL) and *tert*-butyl alcohol (2 mL) at −78 °C to afford a deep blue solution. Then a solution of (1*S*,4*R*,6*S*)-**21** (0.71 g, 3.0 mmol) in THF (8 mL) was added portionwise to the stirred sodium−liquid ammonium solution at −78 °C. After the resulting solution was stirred for 10 min at −78 °C, the temperature was allowed to rise to −30 °C and stirring continued for 4 min. The solution was cooled back to −78 °C, and acetic acid (2 mL) was added slowly. After the solution was allowed to warm to room temperature, the resultant slurry was filtered and washed with ethyl acetate (100 mL). The organic solution was concentrated under reduced pressure to afford a crude solid residue (**22**, 0.80 g). Without any purification, the residue **22** was hydrolyzed following the general acidic hydrolysis procedure, with a basic elution, to give the product (**7**, 0.46 g, 86%) as a colorless solid: mp 225 °C (decomp.); $[\alpha]^{23.5} = +8.85°$ (*c*, 1.92, H_2O); ^{1}H NMR (300 MHz, D_2O) 5.25 (0.5H, m, H_4), 5.08 (0.5H, m, H_4), 3.76 (1H, dtd, *J* 21.7, 7.8, 3.6 Hz, H_3), 2.94 (1H, quintet, J, 8.0 Hz, H_1), 1.69−2.45 (4H, m, H_2 and H_5) ppm; ^{19}F NMR (300 MHz, D_2O) −173.5 (sextet, *J* 54, 27 Hz) ppm; *m*/*z* (EI) 148, 127, 102, 83, 56. HRMS (EI) calcd for $C_6H_{11}O_2NF$ M 148.0774, found M 148.0775.

6-Oxo-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (24). 4-Methylmorpholine *N*-oxide (0.77 g, 6.6 mmol), tetrapropylammonium perruthenate (TPAP) (5 mg), and 4 Å sieves were added to a stirred solution of (±)-**23**[14] (0.67 g, 2.7 mmol) in anhydrous CH_2Cl_2 (10 mL). After being stirred for 14 h, the mixture was concentrated under reduced pressure. The resultant slurry was loaded onto a flash silica gel column directly, eluting with ethyl acetate/hexane (2:3), to afford **24** (0.5 g, 75%) as a colorless solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (2H, m, ArH), 6.85 (2H, m, ArH), 4.68 (1H, d, *J* 14.7 Hz, $ArCH_2$), 3.86 (1H, d, *J* 14.7 Hz, $ArCH_2$), 3.78 (3H, s, OCH_3), 3.50 (1H, m, H_1), 3.01 (1H, m, H_4), 2.33−2.15 (4H, m, H_5 and H_7) ppm; *m*/*z* (EI) 246, 230, 184, 104, 91. HRMS (EI) calcd for $C_{14}H_{15}NO_3$ M 245.1052, found M 245.1058.

2-(4′-Methoxybenzyl)-6,6-difluoro-2-azabicyclo[2.2.1]-heptan-3-one (25). DAST (0.72 g, 4.4 mmol) was added to a stirred solution of **24** (0.32 g, 1.30 mmol) in anhydrous benzene under N_2. The solution was stirred for 4 days until no starting material was observed by TLC. The resultant solution was poured into a saturated $NaHCO_3$ (30 mL) solution, and the aqueous solution was extracted with ethyl acetate (3 × 30 mL). The organic solution was washed with brine (25 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford **25** (0.3 g, 85%) as a solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (2H, m, ArH), 6.86 (2H, m, ArH), 4.97 (1H, d, *J* 14.7 Hz, $ArCH_2$), 3.87 (1H, d, *J* 15.3 Hz, $ArCH_2$), 3.78 (3H, s, OCH_3), 3.59 (1H, m, H_1), 2.83 (1H, m, H_4), 2.40−1.80 (4H, m, H_5 and H_7) ppm; ^{13}C NMR (300 MHz, $CDCl_3$) δ 171.1, 154.5, 128.8, 125.3, 124.8, 123.5, 121.9, 100.5, 56.9 (quartet), 50.5, 40.6 (d), 38.9 (t), 34.1 (t), 32.0 (t) ppm; ^{19}F NMR −92.2 (1/2F, m), −93.0 (1/2F, m), −111.5 (1/2F, m), −112.3 (1/2F, m) ppm; *m*/*z* (EI) 229, 187, 135, 91, 77, 45. HRMS (EI) calcd for $C_{14}H_{15}F_2NO_3$ M 267.1071, found M 267.1076.

6,6-Difluoro-2-azabicyclo[2.2.1]heptan-3-one (26). Potassium persulfate (3.5 g, 12.9 mmol) and potassium hydrogen phosphate trihydrate (1.7 g, 7.4 mmol) were added to a stirred solution of **25** (0.6 g, 2.2 mmol) in CH_3CN/H_2O (40 mL, 1:1). The resultant suspended solution was heated and stirred at 82 °C for 1.5 h. After removal of CH_3CN under reduced pressure, the aqueous residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford **26** (0.21 g, 64%) as a solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.54 (1H, m, H_1), 2.83 (1H, m, H_4), 2.40−1.80 (4H, m, H_5 and H_7) ppm; ^{19}F NMR −95.3 (1/2F, m), −96.1 (1/2F, m), −111.8 (1/2F, m), −112.6 (1/2F, m) ppm; *m*/*z* (EI) 147, 128, 84. HRMS (EI) calcd for $C_6H_7F_2NO$ M 147.1256, found M 147.1259.

3*β*-Amino-4,4-difluorocyclopentane-1*β*-carboxylic Acid (8). Following the general acid hydrolysis procedure, compound (±)-**26** (0.2 g, 1.35 mmol) was treated with HCl/acetic acid, followed by ion-exchange chromatography with basic elution, to afford **8** (0.20 g, 86%) as a colorless solid: mp 207−209 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.99 (1H, m, H_3), 3.20 (1H, m, H_1), 2.40−2.70 (3H, m, H_2 and H_5), 2.06 (1H, m, H_2) ppm; ^{19}F NMR −97.0 (1/2F, m), −97.8 (1/2F, m), −102.15 (1/2F, m), −103.0 (1/2F, m) ppm; *m*/*z* (EI) 145, 120, 100, 83, 56, 36. HRMS (EI) calcd for ($C_6H_{10}O_2NF_2 + H^+$) (M + H) 166.0691, found M + H 166.0687.

(1*S*,4*R*,6*S*)-6-Acetoxy-2-(*tert*-butoxycarbonyl)-2-azabicyclo[2.2.1]heptan-3-one (29). Di-*tert*-butyl dicarbonate (1.79 g, 8.0 mmol), DMAP (200 mg), and triethylamine (4 mL) were added to a stirred suspended solution of (1*S*,4*R*,6*S*)-6-acetoxy-2-azabicyclo[2.2.1]heptan-3-one **28**[14] (0.62 g, 4.0 mmol) in dichloromethane (15 mL). After being stirred for 5 h, the resultant mixture was diluted with ethyl acetate (100 mL) and washed with 5% HCl (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate/hexane (1/3), to yield **29** (0.74 g, 71%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 4.92 (1H, m, H_6), 4.43 (1H, m, H_1), 2.77 (1H, m, H_4), 2.30−1.60 (4H, m, H_4 and H_7), 2.02 (3H, s, CH_3CO_2), 1.47 (9H, s, -O-*t*-$C(CH_3)_3$) ppm; *m*/*z* (EI) 269, 227, 196, 171, 109, 83, 58. HRMS (EI) calcd for $C_{13}H_{19}NO_5$ M 269.1263, found M 269.1261.

Methyl (1*R*,3*S*,4*S*)-3-*tert*-butoxycarbonylamido-4-hydroxycyclopentane-1-carboxylate (31). K_2CO_3 (1.18 g, 8.4 mmol) was added to a stirred solution of *t*-Boc protected lactam **29** (0.72 g, 2.8 mmol) in methanol (15 mL) and water (5 mL). After being stirred for 4 h, the reaction mixture was concentrated under reduced pressure to remove the methanol. The resultant aqueous solution was carefully acidified with 1 N HCl to a pH of 2.0, monitoring with a pH meter. Then, the acidified aqueous solution was extracted with ethyl acetate (4 × 50 mL). The organic solution was washed with brine (25 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resultant residue **30** (0.64 g, 94%) was dissolved in methanol (10 mL) and ether (10 mL). Excess of freshly made diazomethane in ether was added portionwise to the stirred methanol−ether solution until the yellow color did not disappear. After being stirred for another 30 min, the solution was concentrated under reduced pressure to afford **31** (0.64 g, 95%) as a colorless solid.

For (1*R*,3*S*,4*S*)-**30**: ^{1}H NMR (300 MHz, DMSO) δ 6.72 (1H, br, N−H), 4.78 (d, *J* 4.2 Hz, OH), 3.77 (1H, m, H_4), 3.51 (1H, m, H_3), 2.78 (1H, m, H_1), 1.4−2.2 (4H, m, H_2 and H_5), 1.35 (9H, s, O-*t*-Bu) ppm; *m*/z (EI) 171 (M−OC_4H_9−H), 154, 126, 102, 83, 58. HRMS (ES), calcd for $C_{11}H_{19}NO_5$H M + 1 246.1341, found 246.1330.

For methyl (1*R*,3*S*,4*S*)-**31**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.12 (1H, br, N−H), 4.09 (1H, m, H_4), 3.75 (1H, m, H_3), 3.66 (3H, s, OCH_3), 3.02 (1H, m, H_1), 1.6−2.4 (4H, m, H_2 and H_5), 1.44 (9H, s, O-*t*-Bu) ppm; *m*/z (EI) 185 (M−OC_4H_9−H), 140, 116, 83, 58. HRMS (ES) calcd for $C_{12}H_{21}NO_5$H M + H 260.1497, found M + H 260.1479.

Methyl (1*R*,3*S*,4*S*)-4-Bromo-3-*tert*-butoxycarbonylamidocyclopentane-1-carboxylate (32). Carbon tetrabromide (0.67 g, 2.0 mmol), triphenylphosphine (0.53 g, 2.0 mmol), and tetra-*n*-butylammonium bromide (20 mg) were added to a stirred solution of methyl (1*R*,3*S*,4*S*)-**31** (0.27 g, 1.0 mmol) in anhydrous THF (15 mL). After being stirred for 16 h, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford **32** (0.28 g, 85%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.93 (1H, d, br, *J* 8.0 Hz, N−H), 4.51 (1H, m, H_4), 3.97 (1H, m, H_3), 3.71 (3H, s, OCH_3), 2.88 (1H, m, H_1), 2.62 (2H, m, H_2), 2.05−2.30 (2H, m, H_5), 1.44 (9H, s, O-*t*-Bu) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) 175.99, 156.20, 1.20.43, 80.93, 58.14, 56.01, 53.30, 39.88, 38.10, 32.97, 29.39 ppm; *m*/z (EI) 321, 248, 216, 190, 185, 142, 126, 82, 58. HRMS (EI) calcd for $C_{12}H_{20}{}^{79}BrNO_4$ M 321.0576, found M 321.0571, calcd for $C_{12}H_{20}{}^{81}BrNO_4$ M 323.0555, found M 323.0559.

(1*R*,3*S*,4*S*)-3-Amino-4-bromocyclopentane-1-carboxylic Acid ((−)-9). Methyl (1*R*,3*S*,4*S*)-**32** (0.24 g, 0.72 mmol) was hydrolyzed according to the general acid hydrolysis procedure, and the product was purified by recrystallization from ethanol/ether, to give (−)-**9** (0.15 g, 74%) as a colorless solid: mp 172−174 °C; $[\alpha]^{23.5} = -12.4°$ (*c*, 0.88, H_2O); ^{1}H NMR (300 MHz, DMSO) δ 4.62 (1H, m, H_4), 3.55 (1H, m, H_3), 2.96 (1H, m, H_3), 2.68 (1H, m, H_1), 2.43 (2H, m, H_2), 2.20−2.30 (2H, m, H_5) ppm; *m*/z (EI) 164, 162, 121, 101, 82, 56. HRMS (ES) calcd for $C_6H_9{}^{79}BrNO_2$H M + H 207.9974, found M + H 208.0005; calcd for $C_6H_9{}^{81}BrNO_2$H M + H 209.9948, found M + H 209.9939.

(1*R*,2*S*,3*R*,4*S*)-3-Amino-2,4-dibromocyclopentane-1-carboxylic Acid ((+)-10). Bromine (0.80 g, 5.0 mmol) in CH_2Cl_2 (2 mL) was added to a stirred solution of (1*R*)-(−)-azabicyclo-[2.2.1]hept-5-en-3-one (**16**, 0.50 g, 4.58 mmol) in CH_2Cl_2 (10 mL) at 0 °C. After being stirred for 2 h, the reaction mixture was concentrated under reduced pressure to (1*R*,4*R*,6*S*,7*R*)-6,7-dibromo-2-azabicyclo[2.2.1]heptan-3-one (**33**; 1.20 g, 98%). Without any purification, the lactam **33** was hydrolyzed following the general acid hydrolysis procedure, followed by ion-exchange chromatography with a basic elution, giving a light yellow solid. The solid was dissolved in warm water and briefly treated with charcoal. After filtration of the charcoal and lyophilization, the product was obtained as a colorless solid ((+)-**10**, 0.38 g, 29%).

For the dibromolactam **33**: ^{1}H NMR (300 MHz, D_2O) δ 8.48 (1H, s, NH), 4.57 (1H, d, *J* 1.38 Hz, H_6), 4.08 (2H, m, H_1 and H_7), 2.76 (1H, m, H_4), 2.41 (2H, m, H_5) ppm.

For (+)-**10**: mp 134 °C (decomp.); $[\alpha]^{23.5} = +48.6°$ (*c*, 1.36, H_2O); ^{1}H NMR (300 Hz, D_2O) δ 4.27 (2H, m, H_2 and H_4), 4.10 (1H, m, H_3), 3.27 (1H, quart. *J* 9.0 Hz, H_1), 2.55 (2H, m, H_5) ppm; *m*/z (ES) M^+ 286.0 (50%), 288.0 (100%), 290 (50%). HRMS calcd for $C_6H_9NO_2{}^{79}Br_2H$ M + H 285.9079, found M + H 285.9048; calcd for $C_6H_9NO_2{}^{79}Br^{81}BrH$ M + H 287.9054, found M + H 287.9109; calcd for $C_6H_9NO_2{}^{81}Br_2H$ M + H 289.9038, found M + H 289.9097.

6-*exo*-7-*anti*-Dibromo-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (35). Bromine (0.45 g, 2.8 mmol) was added to a stirred solution of 2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]hept-5-en-3-one ((±)-**34**)[14] (0.62 g, 2.5 mmol) in dichloromethane (10 mL) at 0 °C. After being stirred for 1 h, the reaction mixture was diluted with dichloromethane (50 mL) and washed with saturated Na_2SO_3 solution (20 mL) and brine (20 mL). The organic solution was dried over $MgSO_4$ and concentrated under reduced pressure to give **35** (0.95 g, 95%) as a colorless solid, which was used directly in the next step: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (2H, m, ArH), 6.91 (2H, m, ArH), 4.60 (1H, d, *J* 14.6 Hz, $ArCH_2$), 4.24 (1H, m, H_7), 4.00 (1H, d, *J* 14.6 Hz, $ArCH_2$), 3.91 (1H, m, H_6), 3.84 (3H, s, $-OCH_3$), 3.79 (1H, m, H_1), 2.98 (1H, m, H_4), 2.52−2.71 (2H, m, H_5) ppm; *m*/z (EI) 388 (M + H), 308, 198, 162, 121, 77, 65. HRMS (EI) calcd for $C_{14}H_{15}NO_2{}^{79}Br_2$ calcd M 386.9470, found M 386.9468; $C_{14}H_{15}NO_2{}^{79}Br^{81}Br$ calcd M 388.9450, found M 388.9443; $C_{14}H_{15}NO_2{}^{81}Br_2$ calcd M 390.9420, found M 390.9418.

7-*anti*-Bromo-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]-hept-5-en-3-one (36). Dibromolactam **35** (0.80 g, 2.1 mmol) was mixed with neat 1,5-diazobicyclo[4.3.0]non-5-ene (DBN) (2 g), stirred, and heated to 120 °C for 5 h. The resultant dark oil was diluted with dichloromethane (100 mL), then the organic layer was washed with 1 N HCl (2 × 25 mL) and brine (25 mL) and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford **36** (0.48 g, 89%) as a colorless solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (2H, m, ArH), 6.86 (2H, m, ArH), 6.43 (2H, m, H_5 and H_6), 4.63 (1H, m, H_7), 4.29 (1H, d, *J* 14.4 Hz, $ArCH_2$), 4.12 (1H, d, *J* 14.6 Hz, $ArCH_2$), 4.08 (1H, m, H_1), 3.80 (3H, s, $-OCH_3$); 3.49 (1H, m, H_4) ppm; *m*/z (EI) 307, 228, 163, 121, 76, 65. HRMS (EI) calcd for $C_{14}H_{14}{}^{79}BrNO_2$ M 307.0208, found M 307.0204; calcd for $C_{14}H_{14}{}^{81}BrNO_2$ M 390.0201, found M 390.0186.

7-*anti*-Bromo-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]-heptan-3-one (37). Palladium on charcoal (10%, 20 mg) was added to a solution of **36** (0.20 g) in ethyl acetate (8 mL). The resultant mixture was stirred under a hydrogen atmosphere for 2 h. After careful filtration of the catalyst through a bed of Celite 450 and evaporation of the solvent under reduced pressure, **37** (0.20 g, 98%) was obtained as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (2H, m, ArH), 6.86 (2H, m, ArH), 6.43 (2H, m, H_5 and H_6), 4.75 (1H, d, *J* 14.4 Hz, $ArCH_2$), 4.63 (1H, m, H_7), 4.01 (1H, d, *J* 14.6 Hz, $ArCH_2$), 3.80 (3H, s, $-OCH_3$), 3.65 (1H, m, H_1), 2.89 (1H, m, H_4), 2.20−2.10 (2H, m, H_5 or H_6), 1.4−1.75 (2H, m, H_5 or H_6) ppm; *m*/z (EI) 309, 229, 164, 122, 78, 65. HRMS (EI) calcd for $C_{14}H_{16}{}^{79}BrNO_2$ M 309.0365, found M 309.0370, calcd for $C_{14}H_{16}{}^{81}BrNO_2$ M 311.0344, found M 311.0350.

3-α-Amino-2β-bromocyclopentane-1α-carboxylic Acid (11). Following the general deprotection procedure with CAN, compound (±)-**37** (0.14 g, 0.45 mmol) was treated with CAN (0.74 g, 1.35 mmol) to afford crude **38** (0.14 g). Without further purification, **38** was hydrolyzed following the general acid hydrolysis procedure and purified by ion-exchange chromatography with acid elution, to yield (±)-**11** (0.034 g, 34%) as a light-yellow solid.

For **38**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.24 (1H, m, H_7), 3.80 (3H, s, $-OCH_3$), 3.93 (1H, m, H_1), 2.87 (1H, m, H_4), 2.23−2.40 (2H, m, H_5 or H_6), 1.20−1.60 (2H, m, H_5 or H_6) ppm; *m*/z (EI) 189, 135, 110, 82, 69, 45. HRMS (EI) calcd for $C_6H_8{}^{79}BrNO$ M 188.9790, found M 188.9798; calcd for $C_6H_8{}^{81}BrNO$ M 190.9769, found M 190.9768.

For (±)-**11**: mp 130 °C (decomp); ^{1}H NMR (300 MHz, D_2O) δ 4.19 (1H, t, *J* 9.0 Hz, H_2), 3.73 (1H, dd, *J* 9.0, 8.7, H_3), 3.02 (1H, dd, *J* 9.3, 8.7 Hz, H_1), 2.02−2.30 (2H, m, H_4 or H_5), 1.60−1.90 (2H, m, H_4 or H_5) ppm. HRMS calcd for $C_6H_{10}{}^{79}BrNO_2H$ M + H 207.9938, found M + H 207.9974; calcd for $C_6H_{10}{}^{81}BrNO_2H$ M + H 209.9953, found M + H 210.0036.

(1*S*,4*R*,6*S*,7*R*)-7-Bromo-6-fluoro-2-azabicyclo[2.2.1]-heptan-3-one (39). Compound **39-PMB**[14] (0.68 g, 2.08 mmol) was treated with CAN (3.4 g, 6.24 mmol), following the general CAN deprotection procedure, to afford crude **39**. Further purification by flash column chromatography on silica gel, eluting with ethyl acetate/hexane (1:1), gave **39** (0.36 g, 84%) as a colorless solid: mp 160 °C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.21 (1H, s, NH), 4.97 (1/2H, d, *J* 6 Hz, H_6), 4.79 (1/2H, d, *J* 6 Hz, H_6), 4.12 (1H, s, H_1), 2.86 (1H, s, H_4), 2.24−2.55 (2H, m, H_5 and H_6) ppm; ^{19}F NMR (300 MHz, $CDCl_3$) −171.4 (ddd, *J* 56.7, 29.4, 12.0 Hz) ppm; *m*/z (EI) 207, 127, 108, 82, 59. HRMS (EI) calcd for $C_6H_7{}^{79}BrFNO$ M 206.9696,

found M 206. 9701; calcd for $C_6H_7{}^{81}BrFNO$ M 208.9675, found M 208. 9681.

(1*S*,2*R*,3*R*,4*S*)-3-Amino-2-bromo-4-fluorocyclopentane-1-carboxylic Acid ((+)12). Lactam **39** (0.36 g, 1.4 mmol) was hydrolyzed to afford crude **12** by the general acid hydrolysis procedure. Further recrystallization from ethanol/ether gave pure (+)-**12** (0.22 g, 48%) as the HCl salt: mp 160 °C (decomp.); $[\alpha]^{23.5} = +36.5°$ (*c*, 1.70, H_2O); ^{1}H NMR(300 MHz, D_2O) δ 5.30 (1/2H, m, H_4), 5.17 (1/2H, m, H_4), 4.32 (1H, t, *J* 10 Hz, H_2), 4.13 (1H, ddd, *J* 23.1, 10, 4.4 Hz, H_3), 3.54 (1H, q, *J* 10.4 Hz, H_1), 2.30−2.55 (2H, m, H_5) ppm; ^{13}C NMR (D_2O) 175.83, 94.53, 92.70, 65.31, 65.03, 50.38, 45.78, 45.73, 34.36 ppm; ^{19}F NMR −175. 5 (1F, m) ppm; *m*/*z* (EI) 182 (M−HCO_2), 180 (M−HCO_2), 146, 126, 100, 74. HRMS (ES) calcd for $C_6H_{10}{}^{79}BrFNO_2H$ M + H 225.9879, found M + H 225.9894; calcd for $C_6H_{10}{}^{81}BrFNO_2H$ M + H 227.9859, found M + H 227.9865.

6-*exo*-Acetoxy-7-*anti*-bromo-2-azabicyclo[2.2.1]heptan-3-one (41). 6-*exo*-Acetoxy-7-*anti*-bromo-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (**40**)[14] (1.33 g, 3.6 mmol) was treated with CAN (5.9 g, 10.8 mmol) to afford crude **41** (general deprotection by CAN). Purification by flash column chromatography on silica gel, eluting with ethyl acetate/hexane (1:1), afforded **41** (0.54 g, 61%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.30 (1H, s, NH), 4.81 (1H, m, H_6), 4.30 (1H, m, H_7), 4.06 (1H, m, H_1), 2.86 (1H, m, H_4), 2.32 (2H, m, H_5), 2.08 (3H, s, CH_3CO_2) ppm. HRMS (ES) calcd for $C_8H_9{}^{79}BrNO_3$ M 245.9766, found M 245.9781; calcd for $C_8H_9{}^{81}BrNO3$ M 247.9657, found M 247.9691.

3α-Amino-2*β*-bromo-4*β*-hydroxycyclopentane-1α-carboxylic Acid ((±)-13). The deprotected lactam (±)-**41** (0.46 g, 1.86 mmol) was hydrolyzed by the general acid hydrolysis procedure, then purified by ion-exchange chromatography with acid elution, to give (±)-**13** (0.32 g, 78%) as the HCl salt: mp 39−42 °C; ^{1}H NMR (300 MHz, D_2O) δ 4.07−4.21 (2H, H_2 and H_4), 3.55 (1H, dd, *J* 7.2, 9.0 Hz, H_3), 3.30 (1H, q, *J* 9.4 Hz, H_1), 2.20−1.96 (2H, m, H_5) ppm; ^{13}C NMR (D_2O) 175.7, 71.2, 65.4, 49.1, 45.1, 34.6 ppm. HRMS (ES) calcd for $C_6H_{11}O_3N^{79}Br$ (M + H) 223.9917, found (M + H) 223.9938; calcd for $C_6H_{11}O_3N^{81}$-Br (M + H) 225.9897, found (M + H) 225.9957.

6-*exo*-Bromo-7-*anti*-hydroxy-2-azabicyclo[2.2.1]heptan-3-one (43). Following the same procedure for the preparation of **26** from **25**, 6-*exo*-bromo-2-(4′-methoxybenzyl)-7-*anti*-trimethylsiloxy-2-azabicyclo[2.2.1]heptan-3-one (**42**, 0.76 g, 1.15 mmol) was treated with $K_2S_2O_8$ (1.87 g, 6.93 mmol) and potassium hydrogen phosphate trihydrate (0.89 g, 3.91 mmol) to afford **43** (0.21 g, 53%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.83 (1H, s, NH), 4.32 (1H, m, H_7), 4.00 (1H, m, H_6), 3.92 (1H, m, H_1), 2.80 (1H, m, H_4), 2.50−2.52 (2H, m, H_5) ppm. HRMS (ES) calcd for $C_6H_9{}^{79}BrNO_2$ (M + H) 205.9811, found (M + H) 205.9801; calcd for $C_6H_9{}^{81}BrNO_2$ (M + H) 207.9791, found (M + H) 207.9818.

3α-Amino-4*β*-bromo-2*β*-hydroxycyclopentane-1α-carboxylic Acid ((±)-14). Lactam (±)-**43** (0.2 g, 0.97 mmol) was hydrolyzed by the general acid hydrolysis procedure and purified by ion-exchange with acid elution to afford (±)-**14** (0.16 g, 76%) in the HCl form: mp 48−50 °C; ^{1}H NMR (300 MHz, D_2O) δ 4.19−4.08 (2H, H_2 and H_4), 3.59 (1H, t, *J* 9.0 Hz, H_3), 2.97 (1H, m, H_1), 2.53 (1H, m, H_{5a} or H_{5b}), 2.35 (1H, m, H_{5a} or H_{5b}) ppm; ^{13}C NMR (D_2O) 175.7, 71.2, 65.4, 49.1, 45.1, 34.6 ppm. HRMS (ES) calcd for $C_6H_{11}O_3N^{79}Br$ (M + H) 223.9917, found (M + H) 223.9938; calcd for $C_6H_{11}O_3N^{81}Br$ (M + H) 225.9897, found (M + H) 225.9957.

(1*R*,4*S*,5*R*,6*S*)-5,6-Epoxy-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (44). An excess of *m*CPBA (1.9 g, 57%) was added to a stirred solution of (1*R*,4*S*)-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]hept-5-en-3-one (−)-**34** (1.30 g, 5.7 mmol) in chloroform (20 mL) and was heated at reflux for 8 h. After being cooled, the resultant mixture was diluted with chloroform (80 mL) and washed with saturated Na_2SO_3 solution (30 mL), saturated Na_2CO_3 solution (2 × 30 mL), and brine (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resultant residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate/hexane (1/2), to afford **44** (1.28 g, 92%) as a light brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (2H, m, ArH), 6.84 (2H, m, ArH), 4.42 (1H, d, *J* 14.6 Hz, $ArCH_2$), 4.20 (1H, d, *J* 14.6 Hz, $ArCH_2$), 3.77 (3H, s, OCH_3), 3.74 (1H, m, H_1), 3.50 (1H, m, H_6), 3.20 (1H, m, H_5), 2.96 (1H, m, H_4), 1.53−1.77 (2H, m, H_7) ppm; *m*/*z* (EI) 245, 217, 163, 121, 77, 66. HRMS (EI) calcd for $C_{14}H_{15}NO_3$ M 245.1052, found M 245.1052.

Mixture of (1*S*,4*S*,6*S*,7*R*)-6-Bromo-7-hydroxy-2-(4′ethoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (45) and (1*R*,-4*R*,5*S*,6*S*)-5-Bromo-6-hydroxy-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (46). HBr (48%, 1.5 mL) was added to a stirred solution of **44** (1.2 g, 3.67 mmol) in acetonitrile (30 mL) at 0 °C. After being stirred for 6 h, the resultant reaction mixture was diluted with ethyl acetate (120 mL) and washed with saturated sodium bicarbonate (30 mL) and brine (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resultant residue was recrystallized from hexane and ethyl acetate to afford a mixture (1.2 g, 75%) of **45** and **46** as a colorless solid.

(1*S*,4*S*,6*S*,7*R*)-6-Bromo-2-(4′-methoxybenzyl)-7-trimethylsiloxy-2-azabicyclo[2.2.1]heptan-3-one (47) and (1*R*,4*R*,5*S*,6*S*)-5-Bromo-2-(4′-methoxybenzyl)-6-trimethylsiloxy-2-azabicyclo[2.2.1]heptan-3-one (48). 2,6-Lutidine (1.10 mL, 14.6 mmol), trimethylsilyl triflate (2.0 mL, 14.6 mmol) and DMAP (0.2 g) were added to a stirred solution of the mixture of **45** and **46** (1.8 g, 5.5 mmol) in anhydrous CH_2Cl_2 (20 mL). After being stirred overnight, the resultant reaction mixture was diluted with CH_2Cl_2 (100 mL) and briefly washed with a 5% HCl solution (2 × 20 mL) and brine (25 mL). The resultant mixture was stirred and heated at reflux overnight. After being cooled, the dark mixture was concentrated under reduced pressure. The resultant residue was purified by flash column chromatography on silica gel, eluting with ethyl acetate/hexane (1/4), giving **47** (1.2 g, 55%) and **48** (0.4 g, 18%), both as colorless solids.

For **47**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (2H, m, ArH), 6.90 (2H, m, ArH), 4.52 (1H, d, *J* 14.6 Hz, $ArCH_2$), 4.10 (1H, d, *J* 14.6 Hz, $ArCH_2$), 4.07 (1H, m, H_7), 3.83 (3H, s, OCH_3), 3.71 (1H, m, H_6), 3.58 (1H, m, H_1), 2.71 (1H, m, H_4), 2.39−2.46 (2H, m, H_5), 0.141 (9H, s, $Si(CH_3)_3$) ppm; *m*/*z* (EI) 397, 320, 227, 162, 121, 73. HRMS (EI) calcd for $C_{17}H_{24}{}^{79}BrNO_3Si$ M 397.0708, found M 397.0710; calcd for $C_{17}H_{24}{}^{81}BrNO_3Si$ M 399.0689, found M 399.0688.

For **48**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (2H, m, ArH), 6.91 (2H, m, ArH), 4.53 (1H, d, *J* 14.6 Hz, $ArCH_2$), 4.25 (1H, d, *J* 14.6 Hz, $ArCH_2$), 3.89 (1H, m, H_5), 3.82 (3H, s, OCH_3), 3.82 (1H, m, H_6), 3.36 (1H, m, H_1), 2.98 (1H, m, H_4), 2.04 (2H, m, H_7), 0.141 (9H, s, $Si(CH_3)_3$) ppm; *m*/*z* (EI) 397, 320, 246, 180, 121, 78, 66. HRMS (EI) calcd for $C_{17}H_{24}{}^{79}BrNO_3Si$ M 397.0708, found M 397.0712; calcd for $C_{17}H_{24}{}^{81}BrNO_3Si$ M 399.0689, found M 399.0694.

(1*S*,4*S*,6*S*,7*R*)-6-Bromo-7-hydroxy-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (45). Tetra-*n*-butylammonium fluoride (1 M) in THF (1.5 mL) was added to a stirred solution of **47** (0.39 g, 0.98 mmol) in THF (10 mL). After being stirred for 30 min, the solvent was concentrated under reduced pressure. The resultant residue was diluted with ethyl acetate (100 mL), and the solution was washed with 0.5 N HCl (25 mL), saturated $KHCO_3$ (25 mL), and brine (25 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resultant residue was purified by recrystallization from hexane/ethyl acetate to give **45** (0.27 g, 84%) as colorless flakes: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (2H, m, ArH), 6.90 (2H, m, ArH), 4.54 (1H, d, *J* 14.6 Hz, $ArCH_2$), 4.19 (1H, m, H_7), 4.06 (1H, d, *J* 14.6 Hz, $ArCH_2$), 3.84 (3H, s, OCH_3), 3.76 (1H, m, H_6), 3.72 (1H, m, H_1), 2.85 (1H, m, H_4), 2.48−2.51 (2H, m, H_5) ppm; *m*/*z* (EI) 325, 327, 246, 162, 121, 83. HRMS (EI) calcd for $C_{14}H_{16}{}^{79}BrNO_3$ M 325.0314, found M 325.0318; calcd for $C_{14}H_{16}{}^{81}BrNO_3$ M 327.0294, found M 327.0288.

(1*S*,4*S*,6*S*,7*S*)-6-Bromo-7-fluoro-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (49). Following the general fluorination procedure, **45** (0.6 g, 1.8 mmol) was treated with

DAST (0.35 g, 2.2 mmol) affording **49** (0.37, 63%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (2H, m, ArH), 6.88 (2H, m, ArH), 4.92 (1/2H, m, H_7), 4.74 (1/2H, m, H_7), 4.49 (1H, dd, *J* 2.4, 14.7 Hz, $ArCH_2$), 4.10 (1H, d, *J* 14.7 Hz, $ArCH_2$), 3.86 (1H, m, H_6), 3.81 (3H, s, OCH_3), 3.79 (1H, m, H_1), 2.99 (1H, m, H_4), 2.37−2.55 (2H, m, H_5) ppm; *m/z* (EI) 327, 248, 162, 136, 121, 78. HRMS (EI) calcd for $C_{14}H_{15}{}^{79}BrFNO_2$ M 327.02706, found M 327.02702; calcd for $C_{14}H_{15}{}^{81}BrFNO_2$ M 329.0250, found M 329.0227.

(1*R*,2*S*,3*S*,4*S*)-3-Amino-4-bromo-2-fluorocyclopentane-1-carboxylic Acid ((+)-15). In the general deprotection procedure by CAN, the protected lactam **49** (0.37 g, 1.1 mmol) was treated with CAN (1.8 g, 3.4 mmol), giving **50** as a solid. Without further purification, the solid was hydrolyzed by the general acid hydrolysis procedure with a basic elution from an ion-exchange column, giving (+)-**15** (0.08 g, 35%) as a colorless solid. The product was further purified by recrystallization from methanol/ether.

For **50**: ^{1}H NMR (300 MHz, D_2O) δ 5.17 (1/2H, m, H_7), 4.97 (1/2H, m, H_7), 4.21 (1H, m, H_6), 4.14 (1H, m, H_1), 2.85 (1H, m, H_4), 2.30−2.60 (2H, m, H_5); ^{19}F NMR (300 MHz, D_2O) −182.38 (dd, *J* 59.5, 37.5 Hz) ppm; *m/z* (EI) 207, 127, 108, 82, 59. HRMS (EI) calcd for $C_6H_7{}^{79}BrFNO$ M 206.9695, found M 206.9696; calcd for $C_6H_7{}^{81}BrFNO$ M 208.9675, found M 208.9679.

For (+)-**15**: mp 126 °C (decomp.); $[\alpha]^{23.5} = +45.7°$ (*c*, 1.36, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 5.16 (1/2H, t, *J* 6.0 Hz, H_2), 4.99 (1/2H, t, *J* 6.0 Hz, H_2), 4.12 (1H, quart., *J* 8.4 Hz, H_4), 3.90 (1H, m, H_3), 3.06 (1H, m, H_1), 2.38−2.53 (2H, m, H_5); ^{19}F NMR (300 MHz, D_2O) −179.85 (dt, *J* 55.8, 21.6 Hz) ppm; *m/z* (EI) 199, 201, 162, 121, 74, 56. HRMS (ES) calcd for $C_6H_9{}^{79}BrFNO_2H$ M + H 225.9879, found M + H 225.9889; calcd for $C_6H_9{}^{81}BrFNO_2H$ M + H 227.9859, found M + H 227.9845.

6-*exo*-Bromo-2-(4′-methoxybenzyl)-7-oxo-2-azabicyclo-[2.2.1]heptan-3-one (51). Following the same oxidation procedure from **23** to **24**, compound **45** (20 mg) was treated with excess of NMO in the presence of TPAP to afford crude **51.** Purification by flash column chromatography yielded pure (±)-**51** (14 mg, 70%) as a solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (2H, m, ArH), 6.92 (2H, m, ArH), 4.64 (1H, d, *J* 14.7 Hz, $ArCH_2$), 4.40 (1H, d, *J* 14.7 Hz, $ArCH_2$), 3.84 (3H, s, OCH_3), 3.74 (1H, m, H_6), 3.72 (1H, m, H_1), 3.04 (1H, dd, *J* 4.1, 1.2 Hz, H_4), 2.62 (1H, dd, *J* 14.3, 9.1 Hz, H_{5-endo} or H_{5-exo}), 2.49 (1H, m, H_{5-endo} or H_{5-exo}) ppm.

5-*endo*-Bromo-2-(4′-methoxybenzyl)-6-oxo-2-azabicyclo-[2.2.1]heptan-3-one (52). Following the same procedure as for the preparation of **45** (20 mg), compound **48** was treated with excess TBAF to afford crude **46**. Without any purification, compound **46** was oxidized to form **52** (10 mg, 50%) by the same method as for the preparation of **51**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (2H, m, ArH), 6.86 (2H, m, ArH), 4.70 (1H, d, *J* 14.8 Hz, $ArCH_2$), 4.29 (1H, d, *J* 4.0 Hz, H_5), 3.89 (1H, d, *J* 14.8 Hz, $ArCH_2$), 3.78 (3H, s, OCH_3), 3.68 (1H, s, H_1), 3.25 (1H, m, H_4), 2.50 (1H, m, H_{7-syn}), 2.02 (1H, m, H_{7-anti}) ppm.

7-*anti*-Bromo-2-(4′-methoxybenzyl)-6-oxo-2-azabicyclo-[2.2.1]heptan-3-one (54). Following the same oxidation procedure used to convert **23** to **24**, 7-*anti*-bromo-6-*exo*-hydroxyl-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (**53**)[11] (2.3 g, 7.0 mmol) was treated with NMO (1.64 g, 14 mmol) in the presence of a catalytic amount of TPAP (5 mg) to afford **54** (1.8 g, 78%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (2H, m, ArH), 6.88 (2H, m, ArH), 4.72 (1H, d, *J* 14.6 Hz, $ArCH_2$), 4.37 (1H, m, H_7), 3.90 (1H, d, *J* 14.6 Hz, $ArCH_2$), 3.81 (3H, s, OCH_3), 3.68 (1H, m, H_1), 3.17 (1H, m, H_4), 2.72 (2H, d, *J* 17.8, 4.1 Hz, H_5), 2.23 (2H, d, *J* 17.8, 2.5 Hz, H_5) ppm; *m/z* (EI) 323, 325, 244, 216, 121. HRMS (EI) calcd for $C_{14}H_{14}{}^{79}BrNO_3$ M 323.0157, found M 323.0153; calcd for $C_{14}H_{14}{}^{81}BrNO_3$ M 325.0138, found M 323.0134.

7-*anti*-Bromo-6-deuterium-6-hydroxy-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (55). $NaBD_4$ (0.13 g, 3.1 mmol) was added to a stirred solution of **54** (1.02 g, 3.1 mmol) in EtOD (10 mL). After being stirred for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was treated with 0.5 N HCl (10 mL), and the aqueous portion was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate/hexane (2:3), giving **55** (0.94 g, 92%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (2H, m, ArH), 6.91 (2H, m, ArH), 4.60 (1H, d, *J* 14.8 Hz, $ArCH_2$), 4.27 (1H, d, *J* 1.3 Hz, H_7), 3.93 (3H, s, OCH_3), 3.91 (1H, d, *J* 14.6 Hz, $ArCH_2$), 3.73 (1H, m, H_1), 2.99 (1H, m, H_4), 2.45 (2H, m, H_5), 2.23 (2H, m, H_5) ppm; *m/z* (EI) 327, 325, 201, 199, 121, 77. HRMS (EI) calcd for $C_{14}H_{15}{}^{79}BrNO_3D$ M 326.0376, found M 326.0368; calcd for $C_{14}H_{15}{}^{81}BrNO_3$ M 328.0357, found M 328.0390.

6-*exo*-7-*anti*-Dibromo-6-deuterium-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (56). Triphenylphosphine (1.41 g, 5.4 mmol), carbon tetrabromide (1.80 g, 5.4 mmol), and terta-*n*-butylammonium bromide (100 mg) were added to a stirred solution of **55** (0.90 g, 2.7 mmol) in anhydrous THF (20 mL). The resultant solution was heated at reflux for 9 h. The organic solvents were concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel, eluting with ethyl acetate/hexane (1:4), yielding **56** (0.95 g, 90%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (2H, m, ArH), 6.91 (2H, m, ArH), 4.62 (1H, d, *J* 14.8 Hz, $ArCH_2$), 4.25 (1H, d, *J* 1.3 Hz, H_7), 4.00 (1H, d, *J* 14.8 Hz, $ArCH_2$), 3.89 (1H, m, H_1), 3.84 (3H, s, $-OCH_3$), 2.99 (1H, m, H_4), 2.52−2.71 (2H, m, H_5) ppm; *m/z* (EI) 390, 311, 309, 121. HRMS (EI) calcd for $C_{14}H_{14}NO_2{}^{79}Br_2D$ M 387.9532, found M 387.9549; calcd for $C_{14}H_{14}NO_2{}^{79}Br{}^{81}BrD$ M 389.9512, found M 389.9515; calcd for $C_{14}H_{15}NO_2{}^{81}Br_2D$ M 391.9493, found M 391.9466.

7-*anti*-Bromo-6-deuterium-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]hept-5-en-3-one (57). Following the preparation of **36** from **35**, dibromolactam **56** (0.80 g, 2.1 mmol) was treated with neat DBN (2 g) to afford **57** (0.48 g, 89%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (2H, m, ArH), 6.87 (2H, m, ArH), 6.47 (1H, m, H_6), 4.66 (1H, m, H_7), 4.32 (1H, d, *J* 14.5 Hz, $ArCH_2$), 4.16 (1H, d, *J* 14.5 Hz, $ArCH_2$), 4.12 (1H, m, H_1), 3.82 (3H, s, $-OCH_3$), 3.55 (1H, m, H_4) ppm; *m/z* (EI) 311, 309, 241, 243, 229, 199, 163, 121. HRMS (EI) calcd for $C_{14}H_{13}{}^{79}BrNO_2D$ M 308.0271, found M 308.0279; calcd for $C_{14}H_{13}{}^{81}BrNO_2D$ M 310.0252, found M 310.0299.

6-Deuterium-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]-hept-5-en-3-one (58). As described in the preparation of **21** from **20**, compound **57** (0.46 g, 1.5 mmol) was allowed to react with tributyltin hydride (0.65 g, 2.23 mmol) in the presence of AIBN (20 mg), giving **58** (0.22 g, 68%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (2H, m, ArH), 6.87 (2H, m, ArH), 6.47 (1H, dd, *J* 1.38, 3.2 Hz, H_6), 4.3 (1H, d, *J* 14.8 Hz, $ArCH_2$), 4.00 (1H, d, *J* 14.8 Hz, $ArCH_2$), 4.05 (1H, m, H_1), 3.82 (3H, s, $-OCH_3$); 3.40 (1H, m, H_4), 2.30 (1H, m, H_7), 2.09 (1H, m, H_7) ppm; *m/z* (EI) 230, 163, 121, 69. HRMS (EI) calcd for $C_{14}H_{14}NO_2D$ M 230.1166, found M 230.1166.

6-*exo*-7-*anti*-Dibromo-1-deuterium-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]heptan-3-one (59). Bromine (0.15 g, 0.94 mmol) was added to a stirred solution of **58** (0.18 g, 0.78 mmol) in dichloromethane (10 mL) at 0 °C. After being stirred for 1 h, the reaction mixture was diluted with dichloromethane (50 mL) and washed with a saturated Na_2SO_3 solution (20 mL) and brine (20 mL). The organic solution was dried over $MgSO_4$ and concentrated under reduced pressure to afford **59** (0.29 g, 95%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (2H, m, ArH), 6.91 (2H, m, ArH), 4.60 (1H, d, *J* 14.8 Hz, $ArCH_2$), 4.24 (1H, t, J 1.5 Hz, H_7), 4.00 (1H, d, *J* 14.8 Hz, $ArCH_2$), 3.84 (3H, s, $-OCH_3$), 3.78 (1H, m, H_6), 2.99 (1H, m, H_4), 2.68 (1H, m, H_5), 2.52 (1H, m, H_5) ppm. HRMS (ES) calcd for $C_{14}H_{14}NO_2{}^{79}Br_2D$ + H 388.9600, found M + H 388.9648; calcd for $C_{14}H_{14}NO_2{}^{79}Br{}^{81}BrD$ + H 390.9590, found M + H 390.9620; calcd for $C_{14}H_{14}NO_2{}^{81}Br_2D$ + H 392.9570, found M + H 392.9598.

3α-Amino-2*β*,4*β*-dibromo-3*β*-deuteriumcyclopentane-1α-carboxylic Acid (61). Following the general deprotection procedure by CAN, compound **59** (0.22 g, 0.45 mmol) was treated with CAN (0.92 g, 1.7 mmol) to afford crude **60**.

Without further purification, **60** was hydrolyzed following the general acid hydrolysis procedure, then purified by ion-exchange chromatography with 1 N aqueous pyridine, giving **61** (0.09 g, 60%) as a light-yellow solid: mp 134 °C (decomp.); ^{1}H NMR (300 MHz, D_2O) δ 4.14−4.22 (2H, H_4 and H_2), 3.16 (1H, quart., J 9.0, H_1), 2.41−2.48 (2H, m, H_5) ppm. HRMS calcd for $C_6H_8{}^{79}Br_2NO_2D$ + H M + H 286.9520, found M + H 286.9518; calcd for $C_6H_8{}^{81}Br^{79}BrNO_2D$ + H M + H 288.9946, found M + H 288.9956; calcd for $C_6H_8{}^{81}Br_2NO_2D$ + H M + H 290.9848, found M + H 290.9865.

7-Deuterium-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]-hept-5-en-3-one (63-D). Tributyltin chloride (0.058 g, 0.18 mmol) in anhydrous ethanol (1 mL) was added to a stirred suspension of $NaBD_4$ (0.026 g, 0.60 mmol) in ethanol (2 mL) at 0 °C. A precipitate formed. Then 7-*anti*-bromo-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]hept-5-en-3-one (**62**, 0.28 g, 0.90 mmol), which was prepared following the same procedure as the preparation of **36**, in anhydrous ethanol (3 mL) was added to the above solution. The resultant mixture was stirred and heated at reflux for 15 h and then was diluted with ethyl acetate (100 mL). The organic solution was washed with saturated $NaHCO_3$ and brine and dried over anhydrous Na_2SO_4. After the organic solvents were concentrated under reduced pressure, the residue was purified by column chromatography on silica gel, eluting with ethyl acetate/hexane (1:2), to afford **63-D** (48 mg, 40%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (2H, m, ArH), 6.84 (2H, m, ArH), 6.52 (2H, m, H_5 and H_6), 4.34 (1H, d, J 14.7 Hz, CH_2Ar), 4.02 (1H, m, H_1), 4.01 (1H, d, J 14.7 Hz, CH_2Ar), 3.80 (3H, s, OCH_3), 3.37 (1H, m, H_4), 2.26 (1/2 H, s, H_{7-syn} or H_{7-anti}), 2.04 (1/2H, s, H_{7-syn} or H_{7-anti})) ppm.

6-*exo*-7-*anti*-Dibromo-5-deuterium-2-(4′-methoxybenzyl)-2-azabicyclo[2.2.1]hept-5-en-3-one (64-D). Following the same procedure as for the preparation of **59**, compound **63-D** (48 mg, 0.2 mmol) was treated with excess bromine to yield **64-D** (75 mg, 95%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (2H, m, ArH), 6.91 (2H, m, ArH), 4.60 (1H, d, J 14.8 Hz, $ArCH_2$), 4.24 (1H, m, H_7), 4.00 (1H, d, J 14.8 Hz, $ArCH_2$), 3.96 (1H, m, H_1), 3.84 (3H, s, $-OCH_3$), 3.78 (1H, m, H_6), 2.99 (1H, m, H_4), 2.68 (1/2H, m, H_{5-exo} or H_{5-endo}), 2.52 (1/2H, m, H_{5-exo} or H_{5-endo}) ppm.

3α-Amino-2*β*,4*β*-dibromo-5α/*β*-deuteriumcyclopentane-1α-carboxylic Acid (66-D). Following the same procedure as for **61**, **64-D** (75 mg, 0.19 mmol) was deprotected with cerium(IV) ammonium nitrate (0.32 g, 0.60 mmol), giving **65-D** (65 mg). Acid hydrolysis of **65-D** and ion-exchange chromatography, as with **10**, gave **66-D** (29 mg, 53%): ^{1}H NMR (300 Hz, D_2O) δ 4.27 (2H, m, H_2 and H_4), 4.10 (1H, m, H_3), 3.27 (1H, m, H_1), 2.55 (1H, m, H_5) ppm.

(1*R*,2*S*,3*R*,4*S*)-3-Amino-2,4-dibromo-5α/*β*-tritiumcyclopentane-1-carboxylic Acid ((+)-66-T). Following the same procedure used for the preparation of **63-D**, 100 mCi of [^{3}H]-$NaBH_4$ (15 Ci/mmol) in anhydrous ethanol (1 mL) was added to a stirred solution of tributyltin chloride (150 mg) in anhydrous ethanol (1 mL). After being stirred for 25 min, $NaBH_4$ (25 mg) in anhydrous ethanol (1 mL) was added to the above solution, then (1*S*,4*R*)-**62** (198 mg) in anhydrous ethanol (2 mL) was added. The resultant solution was stirred and heated at reflux for 20 h. Workup and column purification afforded the tritium-labeled product **63-T** (0.36 mCi) with a radioactivity yield of 1.4%.

Following the same procedure described for the preparation of **64-D**, compound **63-T** was treated with excess bromine (60 mg) to afford the crude dibromo product **64-T** (40 mg).

The crude **64-T** was treated with cerium(IV) ammonium nitrate (310 mg), which gave **65-T** (0.32 mCi). Hydrolysis of **65-T** with 4 N HCl (5 mL) and acetic acid (5 mL) at 75 °C afforded **66-T**, which was purified with AG 50W-X8 and washing with water and then with 1 M aqueous pyridine solution to give **66-T** (0.22 mM; specific radioactivity 40 mCi/mmol). The total activity yield was 0.9%. Compound **66-T** was further purified on a Hypresil C-18 Elite analytical column (5.4 × 250 mm), initially eluting with water for 10 min, then eluting with methanol solution in a gradient from 0% to 100% in 30 min at a flow rate of 1.0 mL/min. The column effluent was monitored at 214 nm. The collected fraction was 1 mL/min. All fractions were also checked by liquid scintillation counting. Compound **66-T** has a rention time of 22 min. Finally, **66-T** was mixed with (+)-**10** to make a 100 mM solution with a specific radioactivity of 4.2 mCi/mmol for the enzyme inactivation studies.

Enzyme and Assays. GABA aminotransferase was isolated from pig brains by a modified procedure.[25] 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.[26] GABA activity assays were carried out using a modification of the coupled assay developed by Scott and Jakoby.[27] 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 by 7−15 and 61. GABA aminotransferase (15 μL, 1.80 mg/mL) was added to solutions of **7**−**15** (110 μL in final, with varied concentrations of compounds), in 100 mM potassium pyrophosphate buffer, pH 8.5, containing 5 mM α-ketoglutarate and 1 mM β-mercaptoethanol at 25 °C. For **8**, **11**, **12**, **13**, and **14**, concentrations of 15 mM and incubation times of more than 1 h were used with no time-dependent inhibition observed. Concentrations of (+)-**7**, (−)-**9**, (+)-**10**, and (+)-**15** used were as follows: (+)-**7**, 22, 51, 64, 88, and 130 μM; (−)-**9**, 4.6, 9.2, 14, and 19 mM; (+)-**10**, 2.8, 5.5, 8.3, 11, 17, and 25 mM; (+)-**15**, 3.0, 6.1, 9.1, 14, and 18 mM. For the measurement of the deuterium isotope effect, the concentrations of (±)-**10** and (±)-**61** were as follows: 4.2, 8.4, 17, 25, and 34 mM for (±)-**10** and 8.4, 17, 21, 25, and 34 mM for (±)-**61**. At timed intervals, aliquots (15 μL) were withdrawn and added to the assay solution (585 μL) with excess SSDH. Rates were measured spectrophotometrically at 340 nm, and the logarithm of the remaining activity was plotted against time for each concentration of inhibitor. A secondary plot of $t_{1/2}$ obtained from the first plot versus 1/[inactivator] was constructed to determine K_I and k_{inact} values[28] for those four inactivators.

Substrate Protection and Requirement for PLP for Inactivation. Substrate protection was carried out as described for inactivation of GABA-AT except **10** (12 mM) was incubated with GABA aminotransferase in the presence of GABA (0.6 mM and 5.1 mM, respectively) and in the absence of GABA.

To determine the importance of PLP for inactivation, GABA aminotransferase (30 μL, 1.80 mg/mL) was preincubated with 1.2 mM GABA in a 100 mM potassium pyrophosphate buffer (160 μL, pH 8.5) containing 1 mM β-mercaptoethanol at 25 °C, but no α-ketoglutarate, for 1 h. The incubation solution was divided equally into two parts. One part was treated with (+)-**10** (20 mM) in the absence of α-ketoglutarate, and the other part was treated with (+)-**10** (20 mM) in the presence of α-ketoglutarate (5 mM). The remaining enzyme activities were monitored following the procedure described in the time-dependent inactivation experiment.

Competitive Inhibition of GABA Aminotransferase by 7−15. The activity of GABA aminotransferase (20 μL, 0.15 mg/mL) at 25 °C in 600 μL of 120 mM potassium pyrophosphate buffer (pH 8.5) containing excess succinic semialdehyde dehydrogenase, 5.3 mM α-ketoglutarate, and 1 mM $NADP^+$ was determined upon the introduction of varying concentrations of **7**−**15** (two different concentrations for each GABA concentration) and at different GABA concentrations. The percentage of inhibition was obtained by comparison to an untreated enzyme control. The K_i values were calculated based on the following equation: % Inhibition = $100[I]/\{[I] + K_i(1 + [GABA]/K_m)\}$.[29] The range of GABA concentrations is from 2.7 to 6.0 mM. The concentrations for these analogues are as follows: 0.04, 0.053, and 0.080 mM for (+)-**4**; 0.68 and 1.4 mM for **5**;

4.1, 6.8, 11, and 14 mM for (+)-**6**; 2.5, 5.0, 6.2, and 12 mM for (−)-**7**; 6.2, 9.3, and 15 mM for **8**; 3.8, 4.9, 5.4, and 11.4 mM for (+)-**9**; 4.0, 12, 24, and 28 mM for **10**; 1.78, 5.35, 8.9, and 11 mM for **11**; 0.56, 1.12, and 1.7 mM for (+)-**12**.

Release of Fluoride Ions during Inactivation GABA-AT by (+)-7. Five different amounts of GABA-AT (28, 38, 47, 56, and 75 μg) were incubated for 16 h with (+)-**7** (16 mM) in 100 mM potassium pyrophosphate buffer, pH 8.5, containing 5.0 mM β-mercaptoethanol and 3.0 mM α-ketoglutarate in a total volume of 200 μL at 23 °C. No enzyme activity was observed. A control containing no enzyme also was run. After each sample (200 μL) was mixed with 5.00 mL of distilled water and 5.00 mL of total ionic strength adjusting buffer (TISAB II, Orion), the concentration of fluoride ions in each sample (200 μL) was measured with a fluoride ion electrode.

Release of Fluoride Ions during Inactivation GABA-AT by (±)-8. Analogue (±)-**8** (11.6 mM) was incubated for 16 h with GABA-AT (40 μL, 0.94 mg/mL) in 100 mM potassium pyrophosphate buffer, pH 8.5, containing 5.0 mM β-mercaptoethanol and 3.0 mM α-ketoglutarate in a total volume of 200 μL. The resultant incubation solution was mixed with 5.00 mL of distilled water and 5.00 mL of total ionic strength adjusting buffer (TISAB II, Orion) for the fluoride ion concentration measurement.

Transamination Events during Incubation with (+)-7 and (±)-8. Analogue (+)-**7** or (±)-**8** (16 mM) was incubated for 16 h with GABA-AT (30 μL, 0.94 mg/mL) in 100 mM potassium pyrophosphate buffer, pH 8.5, containing 5.0 mM β-mercaptoethanol in a total volume of 200 μL in the presence of 5.0 mM [^{14}C]-α-ketoglutarate at 23 °C. No [^{14}C]-L-glutamate was detected.

Determination of the Number of Equivalents of 66-T Bound to GABA Aminotransferase after Inactivation. In a typical experiment **66-T** (16 mM, 4.2 mCi/mmol) was incubated with GABA-AT (50 μL, 1.80 mg/mL) in the presence of 2 mM α-ketoglutarate and 5 mM β-mercaptoethanol in 100 mM potassium pyrophosphate buffer (pH 8.5) for 16 h. More than 60% of the enzyme was inactivated. The incubation solution was loaded into a Centricon concentrator (10 000 MW cutoff 3000) and was diluted to 1.5 mL with 50 mM potassium pyrophosphate buffer (pH 8.5). Then the resultant solution was concentrated to about 0.1 mL. The concentrated solution was diluted to 1 mL with the same buffer and then was concentrated to 0.1 mL. After the removal of excess inactivator by the method of Penefsky[30] (Sephadex G-50, presoaked with 50 mM potassium pyrophosphate buffer, pH 8.5), a 30 μL aliquot of the inactivated enzyme sample was counted by liquid scintillation. The protein concentrations of the inactivated enzyme sample (20 μL) and BSA standard (20 μL) were determined in 50 mM potassium pyrophosphate buffer, pH 8.5. A control experiment also was carried out with the PMP form of GABA-AT, which was obtained by pretreatment of GABA-AT with GABA (5 mM) in the absence of α-ketoglutarate. The number of equivalents of inactivator bound to the enzyme was calculated as the ratio of millimoles of inactivator in the sample (determined by scintillation counting and the specific activity of the inactivator) to the millimoles of enzyme in the sample (determined by protein assay). This calculation also was used for the control experiment. Finally, the number of equivalents of inactivator bound to the enzyme was obtained after subtraction of the control experiment from the experiment.

Equivalents of 66-T Bound to GABA Aminotransferase after Inactivation and Denaturation with Urea. Tritium-labeled dibromo analogue **66-T** (10 mM, 4.2 mCi/mmol) was incubated for 16 h with GABA aminotransferase (80 μL, 0.94 mg/mL) in 100 mM potassium pyrophosphate buffer, pH 8.5, containing 5.0 mM β-mercaptoethanol and 3.0 mM α-ketoglutarate in a total volume of 200 μL; >50% of the enzyme was inactivated. The resultant incubation solution was loaded into a Slide-A-Lyzer mini dialysis unit (Pierce, 10 000 molecular weight cutoff) and dialyzed against 8 M urea (200 mL, changed each 8 h) for 4 days. The resultant protein sample was further purified by the Penefsky spin method[30] (Sephadex G-50, presoaked with 8 M urea). A 40 μL aliquot of the inactivated enzyme sample was counted by liquid scintillation. The protein concentrations of enzyme sample (20 μL in 8 M urea) and BSA standard (20 μL in 8 M urea) with 700 μL of Coomassie Plus Protein Assay Reagent (Pierce) were measured by a UV spectrometer at 595 nm.

Supporting Information Available: Elemental analyses, time-dependent inactivation plots for (+)-**7**, (−)-**9**, (+)-**10**, and (+)-**15**, substrate protection of (+)-**10**, and effect of α-ketoglutarate on inactivation of (+)-**10**. This information is available free of charge via the Internet at http://pubs.acs.org.

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