Design, Synthesis, and Pharmacological Characterization of (+)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid (LY354740): A Potent, Selective, and Orally Active Group 2 Metabotropic Glutamate Receptor Agonist Possessing Anticonvulsant and Anxiolytic Properties

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2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (**9**) was designed as a conformationally constrained analog of glutamic acid. For **9**, the key torsion angles (τ_1 and τ_2) which determine the relative positions of the α-amino acid and distal carboxyl functionalities are constrained where $\tau_1 = 166.9°$ or 202° and $\tau_2 = 156°$, respectively. We hypothesized that **9** would closely approximate the proposed bioactive conformation of glutamate when acting at group 2 metabotropic glutamate receptors (mGluRs). The racemic target molecule (±)-**9**, its C2-diastereomer (±)-**16**, and its enantiomers (+)-**9** (LY354740) and (−)-**9** (LY366563) were prepared by an efficient, stereocontrolled, and high-yielding synthesis from 2-cyclopentenone. Our hypothesis that **9** could interact with high affinity and specificity at group 2 mGluRs has been supported by the observation that (±)-**9** ($EC_{50} = 0.086 \pm 0.025$ μM) and its enantiomer (+)-**9** ($EC_{50} = 0.055 \pm 0.017$ μM) are highly potent agonists for group 2 mGluRs in the rat cerebral cortical slice preparation (suppression of forskolin-stimulated cAMP formation) possessing no activity at other glutamate receptor sites (iGluR or group 1 mGluR) at concentrations up to 100 μM. Importantly, the mGluR agonist effects of (+)-**9** are evident following oral administration in mice in both the elevated plus maze model of anxiety ($ED_{50} = 0.5$ mg/kg) and in the ACPD-induced limbic seizure model ($ED_{50} = 45.6$ mg/kg). Thus, (+)-**9** is the first orally active group 2 mGluR agonist described thus far and is an important tool for studying the effects of compounds of this class in humans.

Introduction

L-Glutamic acid is the principal excitatory amino acid (EAA) neurotransmitter in the mammalian central nervous system.[1] Glutamate exerts its effects via activation of two types of receptors: those which form ligand-gated cation channels (ionotropic glutamate receptors, iGluRs) and those which are coupled via G-proteins to intracellular enzyme systems which influence the production of second messengers (metabotropic glutamate receptors, mGluRs). There are currently eight distinct mGluR proteins (mGluR1−8) which have been pharmacologically distinguished into three groups based on amino acid sequence homology, signal transduction mechanisms, and agonist pharmacology.[2−6]

Group 1 mGluRs (mGluR1 and mGluR5) are positively coupled to phospholipase C (PLC). When activated, these mGluRs induce the breakdown of cellular phosphoinositides (PI), producing inositol triphosphate (IP3) and diacylglycerol (DAG) within the target neuron. Group 1 mGluRs are activated by (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (**1**), although this compound produces group 2 mGluR agonist effects at similar concentrations.[7] More recently, 3,5-dihydroxyphenylglycine (**2**) has been demonstrated to be a selective agonist for group 1 mGluRs possessing no effects at group 2 mGluRs, group 3 mGluRs, or iGluRs.[8−10] Group 2 mGluRs (mGluR2 and mGluR3) are negatively coupled to adenylyl cyclase (AC). Agonist activation of this receptor class effects the inhibition of 3′,5′-cyclic adenosine monophosphate (c-AMP) formation. (2*S*,3*S*,4*S*)-2-(carboxycyclopropyl)glycine (L−CCG-1, **3**)[11,12] and (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)-glycine (DCG-IV, **4**)[13,14] are highly potent agonists for group 2 mGluRs but possess agonist effects at other glutamate receptor sites at somewhat higher concentrations (**3** at group 1 mGluRs, **4** at NMDA receptors). More

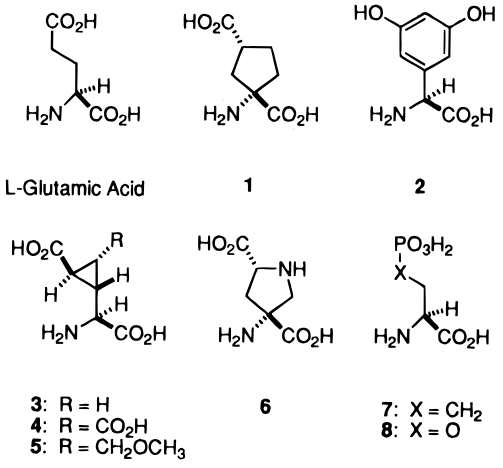

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Table 1. Torsion Angle Measurements and CHARMm Energy Calculations for Various Conformations of Compounds **3** and **9**

	conformer of **3**[a]						conformer of **9**[b]	
measurement	a	b	c	d	e	f	A (flap up)	B (flap down)
τ_1 angle (deg)[c]	35	85	153	217	274	332	166.9	200.2
τ_2 angle (deg)[c]	140.9	136.6	150.0	146.3	141.3	143.9	155.9	157.0
absolute CHARMm (energy (kcal/mol^{-1})[d]	6.64	7.47	3.35	6.97	5.09	7.99	13.02	15.60
relative CHARMm energy (kcal/mol^{-1})[d]	2.30	3.12	0	2.63	0.74	3.64	0	2.59
4−31G nuclear repulsion energy (E_h)[e]	651.328		642.168		651.192		862.407	862.410
4−31G dipole (debye)[f]	12.82		8.70		11.44		8.77	8.77

[a] See Figure 1 for graphical representation. [b] See Figure 2 for Newman projection representations. [c] See ref 24 for definition. [d] CHARMm force field, refs 25 and 26.

selective (although less potent) group 2 mGluR agonists include (2*S*,1′*S*,2′*R*,3′*R*)-2-(2-carboxy-3-(methoxymethyl)cyclopropyl)glycine (*cis*-MCG-I, **5**)[15] and (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate (2*R*,4*R*-APDC, **6**).[16,17] Group 3 mGluRs (mGluR4, mGluR7, and mGluR8) are also negatively coupled to AC, but can be distiguished from the group 2 class both by protein sequence homology and agonist pharmacology. Group 3 mGluRs are selectively activated by 2-amino-4-phosphonobutyrate (AP4, **7**) and L-serine *O*-phosphate (L-SOP, **8**).[18−22] As part of our research program directed toward the preparation of highly potent and selective ligands for mGluRs, we have focused on the identification of highly potent, selective, and systemically active group 2 mGluR agonists and antagonists.

Molecular Modeling

Because of the high degree of potency and good selectivity evinced by **3** toward group 2 mGluRs, this partially constrained glutamic acid analog has been the subject of inquiry regarding the preferred conformation of glutamate when interacting at these receptor sites.[23] In this study, a computer-assisted comparison of the low-energy conformers of **1** and **3** was made, from which the likely bioactive conformation of glutamate for group 2 mGluR protein−agonist interaction was inferred. Specifically, the group 2 mGluR-active conformation of glutamate was hypothesized to be one in which the τ_1 and τ_2 angles[24] approximate 177° and −174°, respectively (e.g. a nearly fully extended glutamate backbone conformation). This hypothesis is further supported by the observation that **6**, a highly selective group 2 mGluR agonist, preferentially adopts a fully extended glutamate conformation in the vacuum phase.[17] We have ourselves examined the effect of rotation about the C2−C3 bond of **3** on the CHARMm energy[25,26] for this molecule (Figure 1, Table 1) and have arrived at a similar conclusion—the lowest energy conformer of **3** exhibits values for τ_1 and τ_2 of 153° and 150°, respectively (conformer "c", Figure 1, Table 1). However, two other low-energy conformations of **3** (conformers "a" and "e", Figure 1, Table 1) cannot be ruled out as possible group 2 mGluR-active forms of **3** as these would be expected to be energetically accessible at physiologically relevant temperatures.

To more fully test the hypothesis that a fully extended glutamate backbone is required for optimal group 2 mGluR protein−ligand interaction, we designed bicyclic amino acid **9** in which the glutamic acid skeleton is incorporated into a fused bicyclo[3.1.0]hexane nucleus (Figure 2). For compound **9**, two low-energy conformations (envelope flap down and up, Figure 2) were observed which differed by only 2.59 kcal/mol (Table 1).

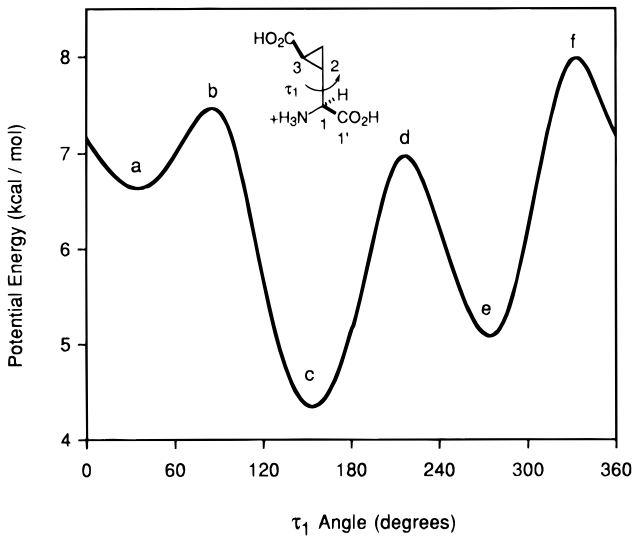
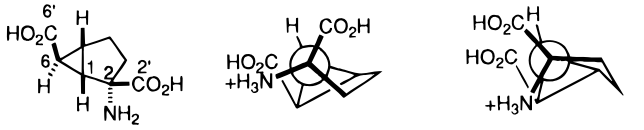


Figure 1. Effect of rotation about τ_1[24] of **3** on the calculated CHARMm potential energy. See molecular modeling section for details.



9 **9** (Conformation A) (envelope flap down) **9** (Conformation B) (envelope flap up)

Figure 2. Numbering system for **9** and Newman projections of calculated low-energy conformations. Reader is viewing **9** down the axis of the C2−C1 carbon−carbon bond.

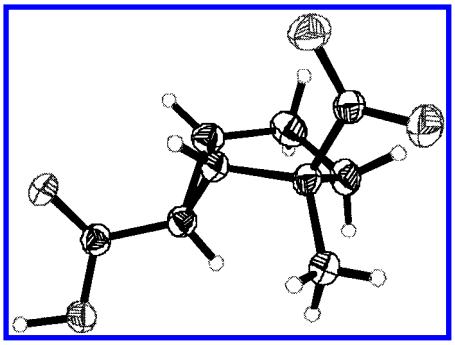
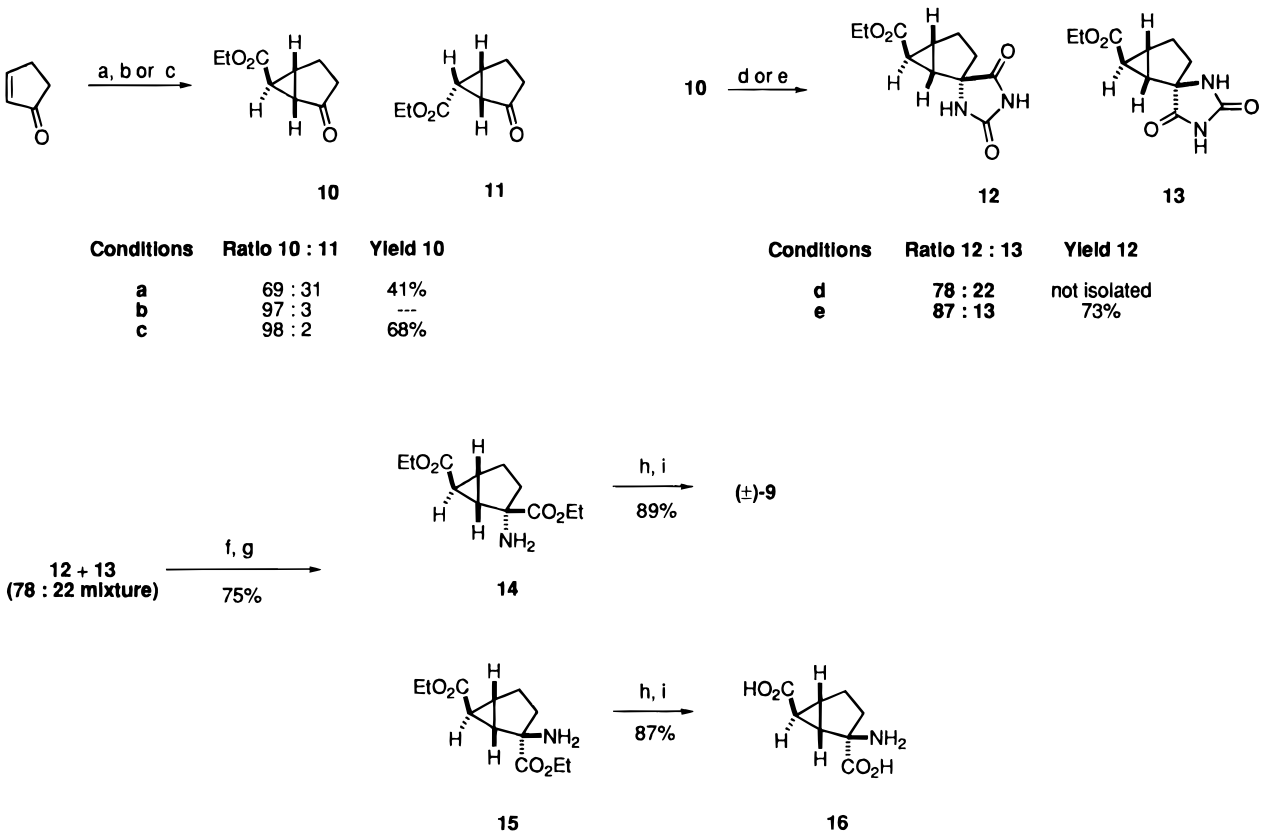


Figure 3. Thermal elipsoid representation of (+)-**9**. The non-H atom elipsoids enclose the 50% probability limits.

For each of these conformers, the τ_1 angle[24] is close to 180° (166.9° for conformer A, 202° for conformer B) and the τ_2 angle[24] is constrained at 156° in conformer A and at 157° in conformer B. In close agreement with these calculated values, the τ_1 and τ_2 angles observed in the X-ray crystal structure of (+)-**9** (Figure 3) were determined to be 171.38° and 150.60°, respectively.

Scheme 1



[a] (a) Ethyl (dimethylsufuranylidene)acetate, benzene, 50 °C; (b) ethyl (dimethylsufuranylidene)acetate, toluene, room temperature; (c) ethyl (dimethylsulfonium)acetate bromide, DBU, toluene, room temperature; (d) $(NH_4)_2CO_3$, KCN, EtOH, H_2O, 50 °C; (e) $(NH_4)_2CO_3$, KCN, EtOH, H_2O, 35 °C; (f) NaOH, H_2O, reflux; (g) EtOH, $SOCl_2$, reflux; (h) NaOH, H_2O, room temperature; (i) ion-exchange chromatography.

Chemistry

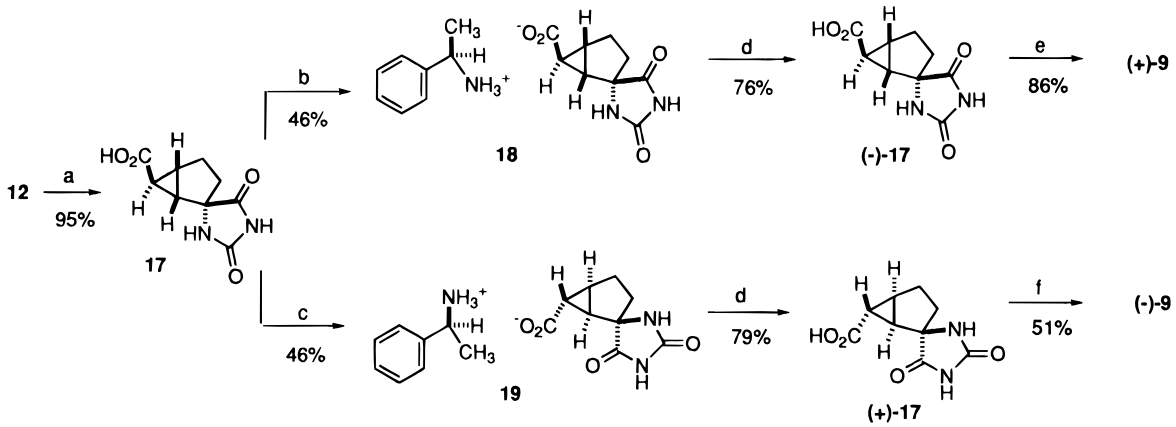
The preparation of (±)-**9** and its racemic C2-diastereoisomer (±)-**16** is depicted in Scheme 1. Carboxycyclopropanation of 2-cyclopentenone utilizing ethyl (dimethylsulfuranylidene)acetate (EDSA)[27] under standard conditions (80 °C, benzene) afforded a 69:31 ratio of cycloadducts **10** and **11** in a combined yield of 64% (41% isolated yield of **10**). We found that the diastereoselectivity of this reaction could be dramatically enhanced (**10**:**11** = 97:3) by simply performing the cycloaddition at room temperature either with isolated EDSA as above or with EDSA generated in situ with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Thus, treatment of ethyl dimethylsulfonium bromide with DBU in toluene at room temperature followed by addition of 2-cyclopentenone afforded the highly enriched mixture of diastereomers from which **10** was isolated in 68% yield after chromatography. The structure of **10** was confirmed by single-crystal X-ray analysis.[28] Ketone **10** was then subjected to hydantoin formation under Bucherer−Bergs conditions ($(H_4N)_2CO_3$, KCN, EtOH, H_2O, 50−60 °C), yielding an inseparable mixture of **12** and **13** (78:22 ratio based on analytical reverse phase HPLC analysis). Lowering the reaction temperature to 30−35 °C resulted in a significant enhancement in the observed diastereoselectivity, affording a mixture of **12** and **13** (87:13 ratio). From this mixture **12** could be isolated by selective crystallization in 73% yield.[29] Alternatively, exhaustive hydrolysis of the 78:22 mixture of **12** and **13** followed by esterification and chromatographic separation afforded the diastereomerically related amino diethyl esters **14** and **15** (75:25 isolated ratio), with **14** being isolated in 66% overall yield from **10**. Alkaline hydrolysis of **14** followed by ion exchange chromatography then yielded the target racemic amino acid (±)-**9**, while saponification of **15** afforded (±)-**16**.

Optical resolution of the **12** was achieved as depicted in Scheme 2. Alkaline hydrolysis of the C6-ester functionality of **12** afforded carboxylic acid **17** in 95% yield. Treatment of **17** with either (*R*)- or (*S*)-1-phenylethylamine in acetone:H_2O (1.6:1) at room temperature followed by filtration afforded the diastereomerically pure salts **18** (from (*R*)-1-phenylethylamine) and **19** (from (*S*)-1-phenylethylamine), which were subsequently converted to the free carboxylic acids (−)-**17** and (+)-**17**, respectively.[30] Alkaline hydrolysis of (−)-**17** afforded (+)-**9**, while acid hydrolysis of (+)-**17** provided (−)-**9**. The structure of (+)-**9** was confirmed by single-crystal X-ray analysis (Figure 3).[28]

Molecular Modeling

All molecular mechanics calculations and conformational searches were carried out under CHARMm 23.1 with a continuum dielectric constant of 80 and in the fully protonated form. Charges were assigned using CHARMm templates, smoothed over all atoms, and a unit positive charge of +1 applied. Both **3** and **9** were initially minimized and subjected to conformational searches in order to determine the optimal orientations of the polar functional groups. Protonated NH_3^+ groups were used to avoid complicating symmetry problems which would arise from using an unprotonated NH_2

Scheme 2



[a] (a) NaOH, H_2O, room temperature; (b) (*R*)-(+)-1-phenylethylamine, acetone, H_2O; (c) (*S*)-(−)-1-phenylethylamine, acetone, H_2O; (d) HCl, H_2O; (e) NaOH, H_2O, reflux, then precipitation at pH = 3; (f) 48% HBr, reflux, then precipitation at pH = 3.

group. The conformational searches on **9** were 10° incremental grid scans about the C2−C2′ and C6−C6′ axes (see Figure 2). The torsions were fixed at each increment, and 250 steps of ABNR minimization were performed. The open chain molecule **3** presented a more complex conformational searching problem since the range of conformations is increased by the freely rotating τ_1 angle. In order to be as thorough as possible on these searches, combinations of grid scans were executed around the C1−C1′, C3−C3′ and C1−C2 bonds (see Figure 1) using the same protocol as outlined for **9**. From the best minima determined at this stage, further one-bond conformational searches were carried out around τ_1 at 1° intervals with 250 steps ABNR performed at each increment. These searches were carried out in two ways, the first in which the the τ_1 angle was held fixed at each 1° increment and the second in which τ_1 was allowed to fully relax from each 1° increment.

Pharmacological Methods

Biochemistry. Radioligand binding to NMDA, AMPA, kainate, and metabotropic glutamate receptors was performed utilizing [^{3}H]CGS19755, [^{3}H]AMPA, [^{3}H]kainate, and [^{3}H]glutamate as the radioligands, respectively.[31−34] Measurement of cyclic adenosine monophosphate (cAMP) and tritiated inositol monophosphates (3H-IP) levels in rat cerebral cortical slices was performed as previously described.[16,35]

Mouse Limbic Seizure Assay. Evaluation of the effects of compound **(+)-9** in NIH Swiss mice in the presence and absence of **1** was performed as previously described.[36] Briefly, male NIH Swiss mice (20−25 g, Charles River Labs., Inc., Portage, MI) were physically restrained to allow unilateral intracerebral (ic) injections, which were made using a 10 μL Hamilton microsyringe. The entry site was 2 mm lateral from bregma, with the syringe placed parallel to the midline angled 45° posterior and the injection was made to a depth of 3.7 mm. Animals were pretreated (20 min) with either of sterile water (5 μL, ic), (+)-**9**, or (−)-**9** at the doses indicated. Animals were observed for 30 min following administration of 1*S*,3*R*-ACPD. Limbic seizures in treated mice were characterized by the presence of at least one episode of clonic forelimb contractions followed by hindlimb rearing to a praying stance, then loss of balance, and falling. The onset of these behaviors was within 1 min following injection; behaviors lasted approximately 20 min.

Automated Elevated Plus-Maze Assay. Male NIH Swiss mice (20−35 g) were obtained from Harlan Sprague−Dawley (Cumberland, IN) and acclimated at least 3 days before study onset. Mice were housed at 23 ± 2 °C (relative humidity 30−70%) and given food and water ad libitum. The photoperiod was 12 h of light and 12 h of dark, with dark onset at approximately 1800 h. All experiments were carried out in accordance with USDA Animal Care and Use Guidelines. Construction of the elevated plus-maze was based on a design validated for mice.[37] The entire maze was made of clear Plexiglas. The maze possesses two open arms (30 × 5 × 0.25 cm) and two enclosed arms (30 × 5 × 15 cm). The floor of each maze arm was corrugated to provide texture. The arms extended from a central platform and were angled at 90° from each other. The maze was elevated to a height of 45 cm above the floor and illuminated by red light. Individual infrared photocells were mounted along each arm of the maze to monitor closed arm, open arm, or nosepoke activity. Mice were individually placed on the closed platform of the maze, and the number of closed arm, open arm, and nosepoke counts were recorded and used as a measure of arm entries and time spent on various sections of the maze over a 5-min test period. All compounds were administered to the mice orally 30 min before evaluation in the maze. Elevated plus maze data were evaluated by an analysis of variance followed by a Tukey's standardized range test for post hoc comparisons of group means. Statistical analyses were performed using Statistical Analysis Systems (SAS Institute, Inc., 1985).

Results

In Vitro EAA Radioligand Binding. Compounds **3** (Tocris Neuramin, Bristol, U.K.), (±)-**9**, (+)-**9**, (−)-**9**, and (±)-**16** were initially evaluated for their ability to displace [^{3}H]CGS19755 (10 nM),[31] [^{3}H]AMPA (5 nM),[32] [^{3}H]kainate (5 nM),[33] and ACPD-sensitive [^{3}H]glutamate binding (10 nM)[34] in rat forebrain membranes (Table 2). Compounds **3** and **9** (racemate and enantiomers) were ineffective in displacing [^{3}H]CGS19755, [^{3}H]-AMPA, or [^{3}H]kainate binding at concentrations up to 100 μM. Compound **16** evinced some displacement of [^{3}H]CGS19755 binding, but only at high concentrations (IC_{50} = 62.4 μM). In contrast, **3**, (±)-**9**, and (+)-**9**

Table 2. Glutamate Receptor Radioligand Displacement and Second Messenger Responses Produced by Conformationally Constrained Glutamic Acid Analogs

	IC_{50} (μM)				EC_{50} (μM)	
compd	ACPD-sensitive ^{3}H]glutamate[a]	[^{3}H]CGS19755[b]	[^{3}H]AMPA[c]	[^{3}H]KA[d]	inhibition of forskolin-stimulated cAMP[e]	stimulation of ^{3}H]IP formation[f]
3	0.454 ± 0.038	>100	>100	>100	1.1 ± 0.29	25.4 ± 0.2
(±)-**9**	0.318 ± 0.091	>100	>100	>100	0.086 ± 0.025	NT[h]
(±)-**16**	>100	62.4[g]	>100	>100	NT[h]	NT[h]
(+)-**9**	0.180 ± 0.052	>100	>100	>100	0.055 ± 0.017	>300
(−)-**9**	>100	>100	>100	>100	>300	NT[h]

[a] See ref 34. [b] See ref 31. [c] See ref 32. [d] See ref 33. [e] See ref 16. [f] See ref 35. [g] Single analysis (triplicate analysis at 1, 10, and 100 μM). [h] Not tested.

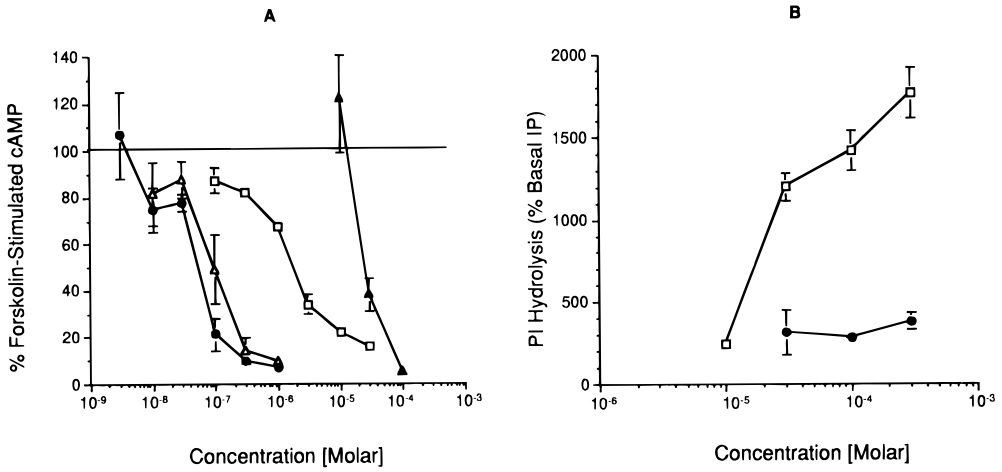


Figure 4. Comparison of **3** (open squares), (±)-**9** (open triangles), (+)-**9** (closed circles), and (−)-**9** (closed triangles) on forskolin (30 μM)-stimulated cAMP formation in adult rat cerebral cortical slices (A) and on [^{3}H]phosphoinositide hydrolysis in neonatal rat cerebral cortical slices (B). *p < 0.05 when compared to the basal value.

potently displaced ACPD-sensitive [^{3}H]glutamate binding, wtih IC_{50} values of 0.454 ± 0.038, 0.318 ± 0.091, and 0.180 ± 0.052 μM, respectively (N = 3). Compounds **16** and (−)-**9** were each ineffective in displacing ACPD-sensitive [^{3}H]glutamate binding at concentrations up to 100 μM.

In Vitro mGluR Functional Responses. Compounds **3**, (±)-**9**, (+)-**9** and (−)-**9** were then evaluated in adult rat cerebral cortical slices for their ability to influence forskolin-stimulated cAMP formation (Figure 4A)[16] or basal [^{3}H]IP formation (compounds **3** and (+)-**9** only, Figure 4B)[35]. As shown in Figure 4A, **3**, (±)-**9**, and (+)-**9** are effective in decreasing forskolin-stimulated cAMP formation in the adult rat cerebral cortex, with EC_{50} values of 1.1 ± 0.29, 0.086 ± 0.025,and 0.055 ± 0.017 μM for **3**, (±)-**9** and (+)-**9**, respectively. The agonist effect of (+)-**9** is highly stereospecific, as its enantiomer, (−)-**9**, evinced an agonist effect only at high concentrations (EC_{50} = 22 ± 3.4 μM) which may be attributed to the presence of a small amount (ca. 0.25%) of (+)-**9** enantiomeric contamination. Moreover, while **3** is effective in stimulating the formation of [^{3}H]IP formation in the adult rat cerebral cortex (EC_{50} = 25.4 ± 0.2 μM), (+)-**9** (up to 300 μM) does not stimulate PI hydrolysis above basal levels in this tissue (Figure 4B). These results are summarized in Table 2.

Mouse Limbic Seizure Model. When compound **1** is administered (400 nmol, ic) to NIH Swiss mice, a characteristic limbic seizure is subsequently observed which persists for the entire 20 min observation period (Figure 5).[36] Direct ic administration of (+)-**9** (12.5−200 nmol, ic) did not result in limbic seizure activity on its own. Intraperatoneal (ip) administration of (+)-**9** (30 min prior to direct injection of **1**) produced a dose-related decrease in the number of animals exhibiting limbic seizures over the 15 min observation period (ED_{50} = 17 mg/kg, ip, Figure 5, Table 3). The anticonvulsant effect of (+)-**9** in this assay was also observed following oral administration (30 min pretreatment time, ED_{50} = 45.6 mg/kg, po, Figure 5, Table 3). This effect was stereospecific, as (−)-**9** (up to 100 mg/kg, ip) was ineffective in blocking ACPD-induced limbic seizures (Table 3).

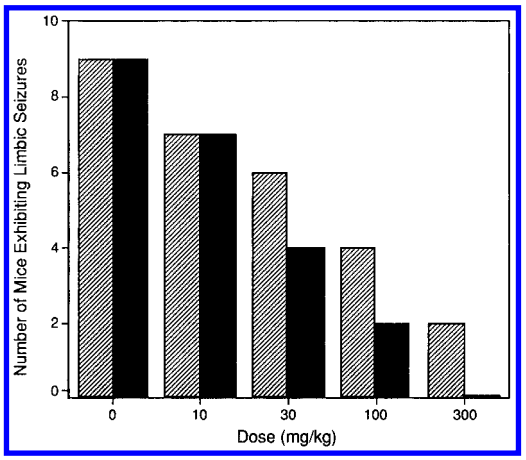


Figure 5. Anticonvulsant effect of (+)-**9** on limbic seizures produced by **1** (400 nmol, ic) in NIH Swiss mice.[36] Compound (+)-**9** was administered by either intraperatoneal (solid bar) or oral (hashed bar) route to NIH Swiss mice 30 min prior to the administration of **1**.

Elevated Plus Maze. Oral administration of (+)-**9** (0.3−10 mg/kg) resulted in dose-related increases in open arm activity with significance at 1, 3, and 10 mg/kg and an oral ED_{50} of 0.5 mg/kg (Figure 6, Table 3). Closed arm activity counts were not significantly altered

Table 3. Effect of (+)-**9** or (−)-**9** in the Mouse Limbic Seizure Model[a] and in the Automated Elevated plus Maze Assay[b]

compd	assay	route	ED_{50} (mg/kg)
(+)-**9**	limbic seizure	ip	17
	limbic seizure	po	45.6
	elevated plus maze	po	0.5
(−)-**9**	limbic seizure	ip	>100
	elevated plus maze	ip	>100

[a] See ref 36. [b] See ref 37.

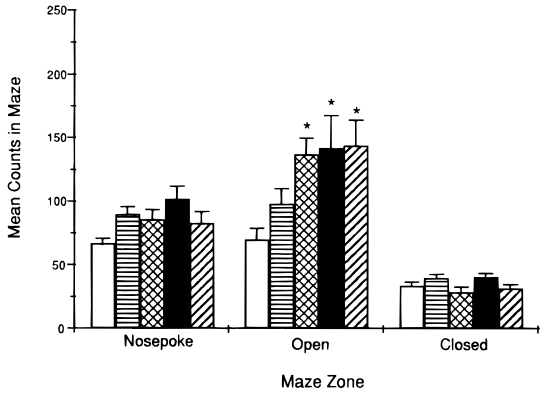


Figure 6. The effect of (+)-**9** on automated elevated plus maze following oral administration in NIH Swiss mice. Nosepoke counts represent incomplete movements (head only) into the open arm from the closed arm. Open arm counts represent activity in the open (sideless) arms of the maze. Closed arm counts represent activity in the enclosed arms of the maze. (+)-**9** was administered 30 min before testing on the maze. Values represent mean ± SE of activity counts in each zone during a 5 min test period. *Significantly different from control, $p < 0.05$.

at any dose of (+)-**9**. The anxiolytic effect of (+)-**9** was stereospecific, as (−)-**9** (10 mg/kg) had no effect on open or closed arm activity (Table 3).

Discussion

As part of our ongoing program aimed at delineating the structural and conformational aspects of agonist and antagonist interactions with excitatory amino acid receptors, we designed and prepared the conformationally constrained glutamic acid analog (±)-**9** and its constituent enantiomers (+)-**9** and (−)-**9** in which the two torsion angles (τ_1 and τ_2) which define the geometric relationship between the α-amino acid and distal carboxylic acid functionalities are constrained in an extended arrangement. Evaluation of the ability of these analogs to displace radioligand binding from iGluRs and mGluRs demonstrated that (±)-**9** potently displaces ACPD-sensitive [^{3}H]glutamate binding (an indication of group 2 mGluR affinity)[38,39] from rat forebrain homogenates and that this activity is evinced exclusively by the (+)-enantiomer. Furthermore, (+)-**9** is devoid of binding affinity for the glutamate recognition site on the NMDA, AMPA, and kainate receptors. In rat cerebral cortical slices, both (±)-**9** and (+)-**9** potently activate mGluRs negatively coupled to AC, while demonstrating no agonist activity for stimulation of PI hydrolysis in this tissue. Thus, as a result of restricting the conformation of glutamic acid by its incorporation into the bicyclo[3.1.0]hexane framework, we have simultaneously achieved a high degree of potency and selectivity for (+)-**9** at mGluRs negatively coupled to AC, presumably group 2 mGluRs.[39] We have therefore investigated (+)-**9** in vivo so as to study the physiological and behavioral consequences of activation of this class of mGluRs.

A substantial body of evidence now exists which suggests that a number of mGluRs, in particular mGluR2, mGluR4, and mGluR7, are presynaptically localized and function as inhibitory autoreceptors which regulate neurotransmitter release in the CNS.[5] In support of the hypothesis that mGluR2 may serve in this capacity, the mixed group 1 mGluR/group 2 mGluR agonist **1** depresses excitatory neuronal transmission in a number of brain regions by a presynaptic mechanism.[5] Previous studies have shown that direct intrathalamic administration of **1** to mice produces (by a group 1 mGluR mechanism) limbic seizures that are blocked by either direct or systemic administration of selective group 2 mGluR agonists, such as **6**.[17] However, while **6** is an extremely useful tool for studying group 2 mGluR function in vitro, its utility is limited by its relatively low (μM) agonist potency. We thus examined the more potent group 2 mGluR agonist (+)-**9** for its effects on ACPD-induced seizures in mice. Like **6**, systemic administration of (+)-**9** produced a dose dependent decrease in the number of mice exhibiting limbic seizures in this model (ED_{50} = 17 mg/kg, ip). Importantly, the anticonvulsant activity of (+)-**9** was maintained following oral administration (ED_{50} = 45.6 mg/kg, po). These data provide further evidence for the lack of group 1 mGluR agonist (seizure-producing) activity in (+)-**9**, and the anticonvulsant activity in (+)-**9** is consistent with its selective effects on decreasing cAMP formation (group 2 mGluR activity).

The elevated plus maze is a widely used test of anxiety which has been validated for both rats and mice and is sensitive to both anxiolytic and anxiogenic drugs.[37,40,41] The test procedure is based upon the rodent's natural aversion to heights and open spaces. Clinically proven anxiolytics such as diazepam (Valium) and buspirone (Buspar) are effective at increasing open arm activity (reducing fear) in the elevated plus maze.[41,42] However, all clinically available anxiolytics are restricted in use by their adverse side effect profile, dependence producing properties[43,44] or limited efficacy.[45,46] The benzodiazepines are postulated to produce their anxiolytic effects by suppression of excitatory pathways within the limbic system.[47] Agonists acting at group 2 or group 3 mGluRs have been shown to attenuate excitatory synaptic transmission within certain limbic pathways including the amygdala,[48] as well as the perforant[49] and mossey fiber[50] pathways of the hippocampus. The ability of (+)-**9** to suppress ACPD-induced limbic seizures is consistent with such a mechanism. With this in mind, we profiled the effects of (+)-**9** on the murine automated evaluated plus maze model of anxiety. When examined for its effects in this assay, (+)-**9** (0.3−10 mg/kg, po) was highly efficacious in increasing open arm activity while not affecting closed arm activity in mice (ED_{50} = 0.5 mg/kg, po), indicative of anxiolytic effects. This degree of oral efficacy compares favorably with that observed for diazepam.[41] Furthermore, the lack of effect of (+)-**9** at any dose on closed arm activity suggests that this compound is neither enhancing nor diminishing locomotor activity at these doses. The effect of (+)-**9** was stereospecific, as the mGluR inactive enantiomer (−)-**9**

was ineffective in producing an effect on either open or closed arm activity at doses up to 10 mg/kg (data not shown). These data support the hypothesis that suppression of excitatory neurotransmission in limbic structures in the brain, possibly through the inhibition of excitatory neurotransmitter release, can produce anxiolytic effects in rodents. Further evaluation of this important pharmacological tool is currently underway and will be reported in due course.

Summary

Compound **9** was designed as a constrained glutamic acid analog which closely mimics the proposed bioactive conformation of this neurotransmitter when acting at group 2 mGluRs. This hypothesis has been supported by the observation that (±)-**9** and (+)-**9** are exceptionally potent agonists at group 2 mGluRs, possessing no activity at other glutamate receptors at concentrations several orders of magnitude greater than that required to suppress forskolin-stimulated cAMP formation in rat cerebral cortical slices. Importantly, the mGluR agonist effects of (+)-**9** are stereospecific and apparent following oral administration in mice in the elevated plus maze model of anxiety and in the ACPD-induced limbic seizure assay. Thus, (+)-**9** is the first orally active group 2 mGluR agonist described thus far and may be an important tool for studying the effects of compounds of this class in humans.

Experimental Section

General Procedures. Melting points were obtained using a Thomas-Hoover capilliary melting point apparatus and are uncorrected. Analytical gas chromatography (GC) was performed on a Hewlett-Packard 5890 series II gas chromatograph utillizing an Ultra 2 (cross-linked 5% Ph Me silicone) capilliary column (25 m × 0.32 mm × 0.52 μm film thickness). ^{1}H and ^{13}C NMR spectra were obtained at 300.15 and 75.48 MHz, respectively, with TMS as an internal standard. Field desorption mass spectroscopy (FDMS) was performed using either a VG 70SE or Varian MAT 731 instrument. Optical rotations were obtained using the Perkin-Elmer 241 polarimeter and are reported at the sodium D line (589 nm), unless otherwise noted. Preparative HPLC was performed with the Waters Prep LC2000 apparatus using dual silica gel PrepPAK-500 cartridges. Solvent systems employed are given in parentheses for each example. Preparative centrifugal thin layer chromatography (PC-TLC) was performed on a Harrison Model 7924A chromatotron using Analtech silica gel GF rotors. The solvent system employed is indicated in the particular example. Cation-exchange chromatography was performed with Dowex 50 × 8−100 ion-exchange resin and anion-exchange chromatography with Bio-Rad AG 1-X8 anion-exchange resin (acetate form converted to hydroxide form). Chiral HPLC was performed on a Chriobotic 4.6 × 250 mm column utilizing 20% THF/0.1% TFA/water (0.6 mL/min) as the eluent system at a solvent flow rate of 0.6 mL/min. Detection was performed at λ = 230 nm.

(1*SR*,5*RS*,6*SR*)-Ethyl 2-Oxobicyclo[3.1.0]hexane-6-carboxylate (10) and (1*SR*,5*RS*,6*RS*)-Ethyl 2-Oxobicyclo-[3.1.0]hexane-6-carboxylate (11). The procedure of Payne[27] was followed. A solution of 2-cyclopentenone (19.0 g, 231.9 mmol) and ethyl (dimethylsufuranylidene)acetate (17.2 g, 115.9 mmol) in benzene (150 mL) was heated at 50 °C for 6 h and then allowed to stir at room temperature overnight. The reaction mixture (GC: 69:31 ratio of **10**:**11**) was concentrated to dryness and then subjected to preparative HPLC (10% EtOAc in hexane eluent), affording **10** (7.95 g, 47.3 mmol) in 41% yield and **11** (1.76 g, 10.5 mmol) in 9.0% yield. **10:** crystallized on standing, mp 42−44 °C; FDMS M$^+$ = 168; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7 Hz, 3H), 2.0−2.4 (m, 6H), 2.48−2.55 (m, 1H), 4.15 (q, J = 7 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 14.19 (CH_3), 22.49 (CH_2), 26.53 (CH), 29.24 (CH), 31.93 (CH_2), 35.81 (CH), 61.28 (CH_2), 170.47 (CO_2Et), 211.80 (C=O). Anal. Calcd for $C_9H_{12}O_3$: C, H. **11:** FDMS M$^+$ = 168; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7 Hz, 3H), 2.05−2.15 (m, 1H), 2.20−2.50 (m, 6H), 4.15 (q, J = 7 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 14.50 (CH_3), 20.35 (CH_2), 28.99 (CH), 30.24 (CH), 34.56 (CH), 38.30 (CH_2), 61.52 (CH_2), 170.18 (CO_2Et), 213.78 (C=O). Anal. ($C_9H_{12}O_3$) C, H.

(1*SR*,5*RS*,6*SR*)-Ethyl 2-Oxobicyclo[3.1.0]hexane-6-carboxylate (10) by in Situ Generation of EDSA. A suspension of ethyl (dimethylsulfonio)acetate bromide (45.5 g, 198.6 mmol)[27] in toluene (350 mL) was stirred at room temperature as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was added in one portion. The resulting mixture was stirred at room temperature for 1 h. 2-Cyclopenten-1-one (19.57 g, 238 mmol) was then added, and the reaction mixture was allowed to stir at room temperature overnight. The mixture was poured into a suspension of 1 N HCl in brine, extracted with Et_2O, dried over $MgSO_4$, and concentrated under reduced pressure. Capilliary GC analysis revealed the presence of **10** and **11** in a ratio of 98:2. Chromatography (HPLC: 10% EtOAc/hexanes to 50% EtOAc/hexanes) afforded pure cycloadduct **10** which was identical in every respect to that which was isolated in the preceding example.

(1*SR*,2*SR*,5*RS*,6*SR*)-Diethyl 2-Aminobicyclo[3.1.0]-hexane-2,6-dicarboxylate (14) and (1*SR*,2*RS*,5*RS*,6*SR*)-Diethyl 2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylate (15). To an ethanolic solution (200 mL) of **10** (22.81 g, 135.6 mmol) was added an aqueous solution (200 mL) of KCN (9.71 g, 149.2 mmol) and $(H_4N)_2CO_3$ (21.2 g, 271.2 mmol) at room temperature. The reaction mixture was then stirred at 50 °C overnight to produce a mixture of **12** and **13**. The reaction mixture was cooled to room temperature, NaOH (16.2 g, 407 mmol) was added, and the resulting solution was refluxed for 48 h. The reaction mixture was then cooled to 0 °C, acidified to pH 1 with concentrated HCl, and concentrated to dryness. The residue was reconstituted in EtOH and treated at 0 °C with thionyl chloride (80.6 g, 678 mmol), and the resulting reaction mixture was refluxed for 48 h. After the reaction mixture was concentrated to dryness under reduced pressure, the products were partitioned between 1 N NaOH and Et_2O. The organic phase was separated, and the aqueous one was extracted with Et_2O (3 × 100 mL). The combined organic phases were dried over K_2CO_3 and concentrated under reduced pressure to afford the mixture of **14** and **15** (24.65 g, 102 mmol, 75:25 GC ratio) in 75% combined yield from **10**.

(1*SR*,2*SR*,5*RS*,6*SR*)-Diethyl 2-Aminobicyclo[3.1.0]-hexane-2,6-dicarboxylate (14). To a stirred solution of the preceding mixture (20.71 g, 85.8 mmol) in EtOAc (200 mL) was added a solution of oxalic acid (15.46 g, 171.7 mmol) in EtOH (50 mL). The resulting reaction mixture was stirred at room temperature for 1 h. Additional EtOH (50 mL) was then added, and the mixture was allowed to stir at room temperature overnight. The resulting salt (5.8 g, 17.5 mmol) was removed by filtration. The filtrate, which was now enriched to a 10:1 ratio (GC) favoring diastereomer **14**, was concentrated to dryness under reduced pressure, partitioned with 1 N NaOH, extracted with Et_2O, washed with brine, dried over K_2CO_3, and concentrated. Chromatography (HPLC: CH_2Cl_2/5% NH_4OH:MeOH, 97:3) afforded **14** (13.48 g, 55.9 mmol) in 66% isolated yield: FDMS M$^+$ (+H) = 242; ^{1}H NMR ($CDCl_3$) δ 1.10−1.21 (m, 1H), 1.26 (t, J = 7 Hz, 3H), 1.34 (t, J = 7 Hz, 3H), 1.75 (t, J = 2 Hz, 1H), 1.80−2.20 (m, 7H), 4.12 (q, J = 7 Hz, 2H), 4.21 (q, J = 7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.17 (CH_3), 14.23 (CH_3), 21.17 (CH), 27.23 (CH_2), 29.23 (CH), 34.07 (CH_2), 37.24 (CH), 60.59 (CH_2), 61.38 (CH_2), 65.64 (C), 173.04 (C=O), 175.61 (C=O). Anal. ($C_{12}H_{19}NO_4$) C, H, N.

(1*SR*,2*RS*,5*RS*,6*SR*)-Diethyl 2-Aminobicyclo[3.1.0]-hexane-2,6-dicarboxylate (15). The oxalic acid salt from above (GC: 63.4:36.6 ratio of **15**:**14**) was converted to the corresponding free base and subjected to preparative chromatography (HPLC: CH_2Cl_2/5% NH_4OH:MeOH, 97:3) to yield an additional 1.93 g (8.0 mmol) of **14** (total yield of **14** including that obtained above = 15.41 g, 63.9 mmol, 74.4%) and 3.65 g (15.1 mmol) of **15** (17.6%): FDMS M$^+$ (+H) = 242; ^{1}H NMR ($CDCl_3$) δ 1.06 (t, J = 7 Hz, 3H), 1.08 (t, J = 7 H, 3H), 1.31 (dd, J = 13.7, 8.2 Hz, 1H),1.53−1.65 (m 3H), 1.71−1.78 (m,

3H), 1.83−1.93 (m, 2H), 3.88 (q, J = 7 Hz, 2H), 3.93−4.04 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.01 (CH_3), 14.08 (CH_3), 22.27 (CH), 25.70 (CH_2), 27.13 (CH), 32.96 (CH_2), 37.43 (CH), 60.38 (CH_2), 61.18 (CH_2), 64.84 (C), 172.64 (C=O), 174.96 (C=O). Anal. ($C_{12}H_{19}NO_4$) C, H, N.

(1*SR*,2*SR*,5*RS*,6*SR*)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylate [(±)-9]. The diethyl ester **14** (3.1 g, 12.8 mmol) was stirred at room temperature in a 1:1 mixture of 2 N NaOH/THF (50 mL total volume) overnight. The THF was removed under reduced pressure, and the subsequent aqueous solution was adjusted to pH 11. Ion exchange chromatography (Bio-Rad AG1-X8 anion-exchange: elute with 50% HOAc−H_2O) afforded (±)-**9** (2.12 g, 11.4 mmol) in 89% yield: mp >250 °C dec; FDMS M^+ (+H) = 186; ^{1}H NMR (D_2O + KOD) δ 1.1−1.35 (m, 1H), 1.55 (m, 1H), 1.8−2.15 (m, 5H); ^{13}C NMR (D_2O + KOD) δ 24.71 (CH), 27.39 (CH_2), 28.73 (CH), 32.90 (CH_2), 34.70 (CH), 67.61 (C), 180.00 (C=O), 181.99 (C=O). Anal. ($C_8H_{11}NO_4$) C, H, N.

(1*SR*,2*RS*,5*RS*,6*SR*)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylate (16). The diethyl ester **15** (1.0 g, 4.1 mmol) was stirred at room temperature in a 1:1 mixture of 1 N NaOH/THF (30 mL total volume) overnight. The THF was removed under reduced pressure, and the subsequent aqueous solution was adjusted to pH 11. Ion exchange chromatography (Bio-Rad AG1-X8 anion-exchange: elute with 3 N AcOH) afforded (±)-**16** (0.66 g, 3.6 mmol) in 87% yield, mp >260 °C dec; FDMS M^+(+H) = 186; ^{1}H NMR (D_2O + KOD) δ 1.35−1.45 (m, 2H), 1.60−1.75 (m, 3H), 1.80−2.00 (m, 2H); ^{13}C NMR (D_2O + KOD) δ 24.61 (CH), 25.02 (CH_2), 25.52 (CH), 31.73 (CH_2), 36.53 (CH), 65.99 (C), 176.52 (C=O), 180.80 (C=O). Anal. ($C_8H_{11}NO_4$) C, H, N.

(1*SR*,2*SR*,5*RS*,6*SR*)-Ethyl Bicyclo[3.1.0]hexane-6-carboxylate-2-spiro-5′-hydantoin (12). A mixture of **10** (5.05 g, 30.0 mmol), KCN (2.15 g, 33.0 mmol), and $(NH_4)_2CO_3$ (5.77 g, 73.9 mmol) in a mixture of EtOH (30 mL) and H_2O (12 mL) was stirred at 35 °C for 15 h. The reaction mixture was cooled to 0 °C, and H_2O (33 mL) was added to the mixture. After 2 h at 0 °C, the precipitate was isolated by filtration and dried to give **12** (5.23 g, 22.0 mmol) in 73% yield: mp 219−221 °C; FDMS M^+ = 238; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, J = 7 Hz, 3H), 1.30 (m, 1H), 1.82 (m, 5H), 1.97 (m, 1H), 4.02 (q, J = 7 Hz, 2H), 7.89 (s, 1H, NH) 10.57 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 14.01 (CH_3), 20.02 (CH), 25.58 (CH_2), 27.37 (CH), 29.45 (CH_2), 32.95 (CH), 60.15 (CH_2), 68.61 (C), 156.14 (C=O), 171.75 (C=O), 177.43 (C=O). Anal. ($C_{11}H_{14}N_2O_4$) C, H, N.

(1*SR*,2*SR*,5*RS*,6*SR*)-Bicyclo[3.1.0]hexane-6-carboxylate-2-spiro-5′-hydantoin (17). A mixture of **12** (16.32 g, 68.6 mmol) and 2 N NaOH (137 mL) was stirred at room temperature for 1 h. Concentrated HCl was added to adjust the pH to 1.0. The resulting precipitate was isolated by filtration and dried to give **17** (13.70 g, 65.2 mmol) in 95% yield: mp 278−280 °C; FDMS M^+ = 210; ^{1}H NMR (DMSO-d_6) δ 1.28 (m, 1H), 1.68 (m, 2H), 1.85 (m, 3H), 7.91 (s, 1H, NH), 10.54 (s, 1H, NH), 12.16 (s, 1H, CO_2H); ^{13}C NMR (DMSO-d_6) δ 20.10 (CH), 25.59 (CH_2), 27.01 (CH), 29.55 (CH_2), 32.71 (CH), 68.62 (C), 156.15 (C=O), 173.35 (C=O), 177.50 (C=O). Anal. ($C_9H_{10}N_2O_4$) C, H, N.

***(R)*-(+)-1-Phenylethylamine Salt (18).** Compound **17** (10.51 g, 50.0 mmol), acetone (102 mL), and water (64 mL) were combined and heated to 55 °C. (*R*)-(+)-1-phenylethylamine (6.06 g, 50.0 mmol) was added quickly over several minutes. The heating mantle was removed, and the solution was allowed to cool slowly to room temperature. The product crystallized almost immediately. The reaction was stirred at room temperature for several hours and filtered. The cake was washed with acetone (20 mL) and dried to give **18** (7.62 g, 23.0 mmol) in 46% yield: mp 211−217 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (m, 1H) 1.27 (d, 3H), 1.72 (m, 5H), 1.93 (m, 1H), 4.05 (q, 1H), 7.26 (m, 5H), 7.60 (s, 2H, NH_2), 7.93 (s, 1H, NH); $[\alpha]_D$ = −27.00° (c = 1.01, H_2O). Anal. ($C_{17}H_{21}N_3O_4 \cdot {}^1/_2H_2O$) C, H, N.

***(S)*-(−)-1-Phenylethylamine Salt (19).** Compound **17** (10.51 g, 0.05 mol), acetone (102 mL), and water (64 mL) were combined and heated to 55 °C. (*S*)-(−)-1-Phenylethylamine (6.06 g, 50.0 mmol) was added quickly over several minutes. The heating mantle was removed, and the solution was allowed to cool slowly to room temperature. The product crystallized almost immediately. The reaction mixture was stirred at room temperature for several hours and filtered. The cake was washed with acetone (20 mL) and dried to afford **19** (7.64 g, 23.1 mmol) in 46% yield: mp 218−224 °C; ^{1}H NMR (DMSO-d_6) δ 1.30 (m, 4H), 1.69 (m, 5H), 1.93 (m, 1H), 4.09 (q, 1H), 6.70 (s, 2H, NH_2), 7.28 (m, 5H), 7.94 (s, 1H, NH); $[\alpha]_D$ = 32.73° (c = 1.02, H_2O) Anal. ($C_{17}H_{21}N_3O_4 \cdot {}^1/_2H_2O$) C, H, N.

(−)-Bicyclo[3.1.0]hexane-6-carboxylate-2-spiro-5′-hydantoin [(−)-17]. A mixture of **18** (0.74 g, 2.2 mmol) and water (10 mL) was stirred at 25 °C while the pH was adjusted to 1.0 using 1 N HCl. The reaction mixture was stirred for 1 h, and the product was collected by filtration and dried to give (−)-**17** (0.35 g, 1.7 mmol) in 76% yield: mp 310 °C dec; FDMS M^+ = 210; ^{1}H NMR (DMSO-d_6) δ 1.28 (m, 1H), 1.68 (m, 2H), 1.85 (m, 3H), 7.91 (s, 1H, NH), 10.54 (s, 1H, NH), 12.16 (s, 1H, CO_2H); $[\alpha]_D$ = −24.22° (c = 1.0, MeOH). Anal. ($C_9H_{10}N_2O_4$) C, H, N. Chiral HPLC (see general methods section) indicated that the product was 99.9% ee (retention time = 9.61 min)

(+)-Bicyclo[3.1.0]hexane-6-carboxylate-2-spiro-5′-hydantoin [(+)-17]. A mixture of **19** (5.10 g, 15.4 mmol) and water (51 mL) was stirred at room temperature while the pH was adjusted to 1.0 using 1 N HCl. The reaction mixture was stirred for 30 min. The product was collected by filtration and dried to give (+)-**17** (2.55 g, 12.1 mmol) in 79% yield: mp 310 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.28 (m, 1H), 1.68 (m, 2H), 1.85 (m, 3H), 7.91 (s, 1H, NH), 10.54 (s, 1H, NH), 12.16 (s, 1H, CO_2H); $[\alpha]_D$ = 25.25° (c = 1.01, MeOH). Anal. ($C_9H_{10}N_2O_4$) C, H, N. Chiral HPLC (see general methods section) indicated that the product was 99.9% ee (retention time = 8.95 min).

(+)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid [(+)-9]. A mixture consisting of (−)-**17** (184 g, 876.2 mmol) and 3 N NaOH (1750 mL) was heated at reflux until the reaction was judged complete by HPLC. After 28 h, the solution was cooled to room temperature and filtered through glass paper to remove trace amounts of insoluble material. The pH of the solution was adjusted to 3.0 using concentrated HCl. The reaction mixture was stirred 1 h at room temperature and 2 h at 0 °C. The precipitated product was collected by filtration, washed with cold water (170 mL), and dried to afford (+)-**9** (152.5 g, 750.5 mmol) in 86% yield: mp 271 °C; FDMS M^+ (+H) = 186; ^{1}H NMR (trifluoroacetic acid-*d*) δ 1.75 (m, 1H), 2.13 (m, 2H), 2.40 (m, 3H), 2.57 (m, 1H); ^{13}C NMR (D_2O + KOD) δ 24.82 (CH), 27.57 (CH_2), 29.29 (CH), 31.66 (CH_2), 33.38 (CH), 67.40 (C), 177.13 (C=O), 180.96 (C=O); $[\alpha]_D$ = 23.18° (c = 1.0, 1 N HCl). Anal. ($C_8H_{11}NO_4 \cdot H_2O$) C, H, N.

(−)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid [(−)-9]. A mixture of (+)-**17** (1.05 g, 5.0 mmol) and 48% HBr (25 mL) was heated for 48 h at reflux. The reaction was filtered while hot through diatomaceous earth. Upon the filtrate being cooled to room temperature a crystalline product formed and was collected by filtration. The solid was dissolved in water (7 mL) and the pH adjusted to 3.0 using 5 N NaOH. The product which precipitated was stirred for 1 h at a pH of 3.0 and then collected by filtration and dried to give (−)-**9** (0.52 g, 2.6 mmol) in 51% yield: mp 277 °C; FDMS M^+ (+H) = 186; ^{1}H NMR (trifluoroacetic acid-*d*) δ 1.75 (m, 1H), 2.13 (m, 2H), 2.40 (m, 3H), 2.57 (m, 1H); ^{13}C NMR (D_2O + KOD) δ 25.11 (CH), 27.60 (CH_2), 29.13 (CH), 32.02 (CH_2), 33.62 (CH), 67.40 (C), 177.85 (C=O), 181.56 (C=O); $[\alpha]_D$ = −22.08° (c = 1.01, 1 N HCl). Anal. ($C_8H_{11}NO_4 \cdot H_2O$) C, H, N.

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Supporting Information Available: Combustion analyses for all numbered compounds and X-ray crystallographic data pertaining to compound **(+)-9** (6 pages). Ordering information is given on any current masthead page.

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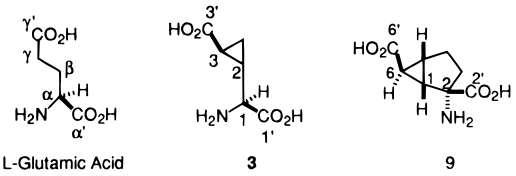
(24) Torsion angles τ_1 and τ_2 are defined in the following manner:

glutamic acid: τ_1, $C_{\alpha'}-C_\alpha-C_\beta-C_\gamma$; τ_2, $C_\alpha-C_\beta-C_\gamma-C_{\gamma'}$

3: τ_1, $C_1'-C_1-C_2-C_3$; τ_2, $C_1-C_2-C_3-C_{3'}$

9: τ_1, $C_{2'}-C_2-C_1-C_6$; τ_2, $C_2-C_1-C_6-C_6'$



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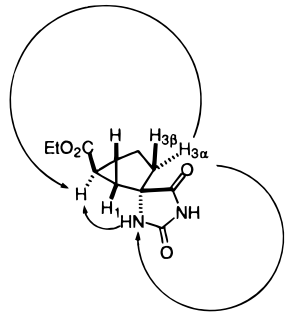
(28) The X-ray structure and coordinates for (+)-**9** and **10** have been filed with the Cambridge crystallagraphic database. Crystallographic data obtained for (+)-**9** is available as Supporting Information.

(29) The relative stereochemistry of **12** was determined by NOE analysis. Thus,as depicted below, NOEs were observed between the "amide" NH of the hydantoin ring, $H_{3\alpha}$ and H_6, whereas no NOEs were observed between the "amide" NH and either H_1 or $H_{3\beta}$. This assignment was subsequently confirmed with the determination of the X-ray crystal structure of (+)-**9**.



(30) The enantiomeric purity of (+)-**17** and (−)-**17** were determined by chiral HPLC (see general methods, Experimental Section for details). Under these conditions, (+)-**17** and (−)-**17** exhibited elution times of 8.95 and 9.61 min, respectively.

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