Synthesis, Pharmacological Characterization, and Molecular Modeling of Heterobicyclic Amino Acids Related to (+)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid (LY354740): Identification of Two New Potent, Selective, and Systemically Active Agonists for Group II Metabotropic Glutamate Receptors

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As part of our ongoing research program aimed at the identification of highly potent, selective, and systemically active agonists for group II metabotropic glutamate (mGlu) receptors, we have prepared novel heterobicyclic amino acids (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268, **(−)-9**) and (−)-2-thia-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY389795, **(−)-10**). Compounds **(−)-9** and **(−)-10** are structurally related to our previously described nanomolar potency group II mGlu receptor agonist, (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate (LY354740 monohydrate, **5**), with the C4-methylene unit of **5** being replaced with either an oxygen atom (as in **(−)-9**) or a sulfur atom (as in **(−)-10**). Compounds **(−)-9** and **(−)-10** potently and stereospecifically displaced specific binding of the mGlu2/3 receptor antagonist ([^{3}H]LY341495) in rat cerebral cortical homogenates, displaying IC_{50} values of 15 ± 4 and 8.4 ± 0.8 nM, respectively, while having no effect up to 100 000 nM on radioligand binding to the glutamate recognition site on NMDA, AMPA, or kainate receptors. Compounds **(−)-9** and **(−)-10** also potently displaced [^{3}H]LY341495 binding from membranes expressing recombinant human group II mGlu receptor subtypes: **(−)-9**, K_i = 14.1 ± 1.4 nM at mGlu2 and 5.8 ± 0.64 nM at mGlu3; **(−)-10**, K_i = 40.6 ± 3.7 nM at mGlu2 and 4.7 ± 1.2 nM at mGlu3. Evaluation of the functional effects of **(−)-9** and **(−)-10** on second-messenger responses in nonneuronal cells expressing human mGlu receptor subtypes demonstrated each to be a highly potent agonist for group II mGlu receptors: **(−)-9**, EC_{50} = 2.69 ± 0.26 nM at mGlu2 and 4.58 ± 0.04 nM at mGlu3; **(−)-10**, EC_{50} = 3.91 ± 0.81 nM at mGlu2 and 7.63 ± 2.08 nM at mGlu3. In contrast, neither compound (up to 10 000 nM) displayed either agonist or antagonist activity in cells expressing recombinant human mGlu1a, mGlu5a, mGlu4a, or mGlu7a receptors. The agonist effects of **(−)-9** and **(−)-10** at group II mGlu receptors were not totally specific, however, as mGlu6 agonist activity was observed at high nanomolar concentrations for **(−)-9** (EC_{50} = 401 ± 46 nM) and at micromolar concentrations (EC_{50} = 2 430 ± 600 nM) for **(−)-10**; furthermore, each activated mGlu8 receptors at micromolar concentrations (EC_{50} = 1 690 ± 130 and 7 340 ± 2 720 nM, respectively). Intraperitoneal administration of either **(−)-9** or **(−)-10** in the mouse resulted in a dose-related blockade of limbic seizure activity produced by the nonselective group I/group II mGluR agonist (1*S*,3*R*)-ACPD (**(−)-9** ED_{50} = 19 mg/kg, **(−)-10** ED_{50} = 14 mg/kg), indicating that these molecules effectively cross the blood-brain barrier following systemic administration and suppress group I mGluR-mediated limbic excitation. Thus, heterobicyclic amino acids **(−)-9** and **(−)-10** are novel pharmacological tools useful for exploring the functions of mGlu receptors in vitro and in vivo.

Introduction

(*S*)-Glutamic acid ((*S*)-Glu) is the primary excitatory neurotransmitter in the mammalian central nervous system (CNS), participating in an expansive list of physiological and pathophysiological processes.[1,2] The neuronal effects of (*S*)-Glu are mediated by two heterogeneous families of cell membrane-associated receptors, the ion-channel-linked or ionotropic glutamate (iGlu) receptors (iGluRs) and the G-protein-coupled or metabotropic glutamate (mGlu) receptors (mGluRs).[3,4]

The mGlu receptors are highly heterogeneous with respect to their structure, function, and localization within the CNS. There are currently eight known mGlu

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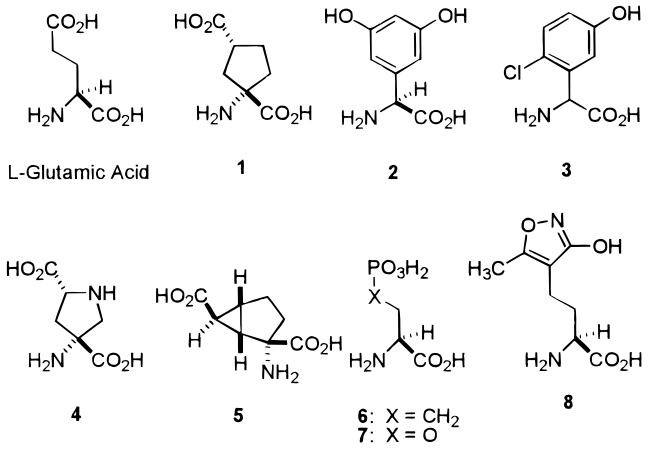
receptor subtypes (mGlu1−8) that have been classified into three subfamilies, or groups, based on their amino acid sequence homology, signal transduction mechanisms, and responses to prototypic agonists.[3−8] Group I mGluRs (mGlu1, mGlu5, and splice variants) are positively coupled through G_s to phospholipase C (PLC). When activated, these receptors induce phosphoinositide hydrolysis and subsequent calcium mobilization within the target cell. Group I mGluRs are nonselectively activated by micromolar concentrations of (1*S*,3*R*)-ACPD (**1**)[8] and selectively activated by 3,5-dihydroxyphenylglycine (**2**).[8−11] More recently, *rac*-2-chloro-5-hydroxyphenylglycine (**3**) has been reported as an mGluR5 subtype-selective agonist.[12] Group II mGluRs (mGlu2 and mGlu3) are negatively coupled through G_i to adenylyl cyclase (AC). Agonist activation of group II mGluRs results in the inhibition of 3′,5′-cyclic adenosine monophosphate (c-AMP) formation. Compound **1** activates group II mGluRs but only at concentrations that also produce agonist effects at the group I receptors.[8,13] Selective agonists for the group II mGlu receptors include two molecules previously described by our laboratory, (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate ((2*R*,4*R*)-APDC, **4**), a compound that activates mGlu2 and mGlu3 receptors at high nanomolar to low micromolar concentrations,[14,15] and (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate (LY354740, **5**), a compound possessing low nanomolar potency at these target proteins.[16,17] Group III mGluRs (mGlu4, mGlu6, mGlu7, and mGlu8) are also negatively coupled to AC and are activated by (*S*)-2-amino-4-phosphonobutyrate ((*S*)-AP4, **6**) and (*S*)-serine *O*-phosphate ((*S*)-SOP, **7**).[8,18−22] More recently, mGluR6 subtype-selective agonist activity has been reported for (*S*)-homo-AMPA (**8**).[23]



As part of our overall research program directed toward the preparation of highly potent and selective ligands for mGlu receptor subtypes, we have continued to focus our efforts on the identification of group II mGluR agonists. In this account, we report the preparation of two novel heterobicyclic amino acids related to **5**, (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (**(−)-9**, LY379268) and (−)-2-thia-4-aminobicyclo-[3.1.0]hexane-4,6-dicarboxylate (**(−)-10**, LY389795), and their pharmacological characterization as potent group II mGlu receptor agonists.

Chemistry

The preparation of racemic and nonracemic **9** is depicted in Scheme 1. Carboxycyclopropanation of furan was accomplished by way of rhodium-catalyzed condensation with ethyl diazoacetate and afforded bicyclic adduct **11** in consistent, though low (20−40%), overall yields.[24] The relative stereochemistry about the cyclopropane ring of **11** was confirmed by ^{1}H NMR analysis which revealed a 5.7-Hz couple between the ring fusion protons H1 and H5 and a 2.5-Hz couple between each of the ring fusion protons and H6. Regiospecific incorporation of a hydroxyl functionality at the C4-position was achieved in 87% yield by hydroboration of **11** followed by in situ conversion of the intermediate organoborane to the corresponding carbinol **12** by treatment with buffered hydrogen peroxide or sodium perborate.[25] Further oxidation of **12** under Swern conditions afforded heterobicyclic ketone **13** in 84% yield. Conversion of the ketone functionality of **13** to diastereomerically pure 5′-spirohydantoin **14** was effected by treatment of this ketone with ammonium carbonate and potassium cyanide in methanol. Compound **14** was obtained in 79% yield by direct crystallization from this reaction mixture.[26] Hydrolysis of **14** then afforded **(±)-9** in 62% yield. Selective saponification of the C6-ester functionality of **14** afforded *rac*-carboxylic acid **15** which was conveniently resolved by selective crystallization of either the (*R*)- or (*S*)-2-phenylglycinol salts **(−)-16** or **(+)-16**, respectively. The so resolved hydantoin carboxylates **(−)-15** and **(+)-15** were isolated from aqueous HCl, and the enantiomeric excess of each isomer was then established by chiral chromatography to be >98%.[27] Exhaustive alkaline hydrolysis of each of the hydantoin enantiomers **(−)-15** or **(+)-15** then afforded the nonracemic amino acid products **(−)-9** and **(+)-9**, respectively. Single-crystal X-ray analysis of **(−)-9** (Figure 1) firmly established the relative stereochemistry of this molecule.[28]

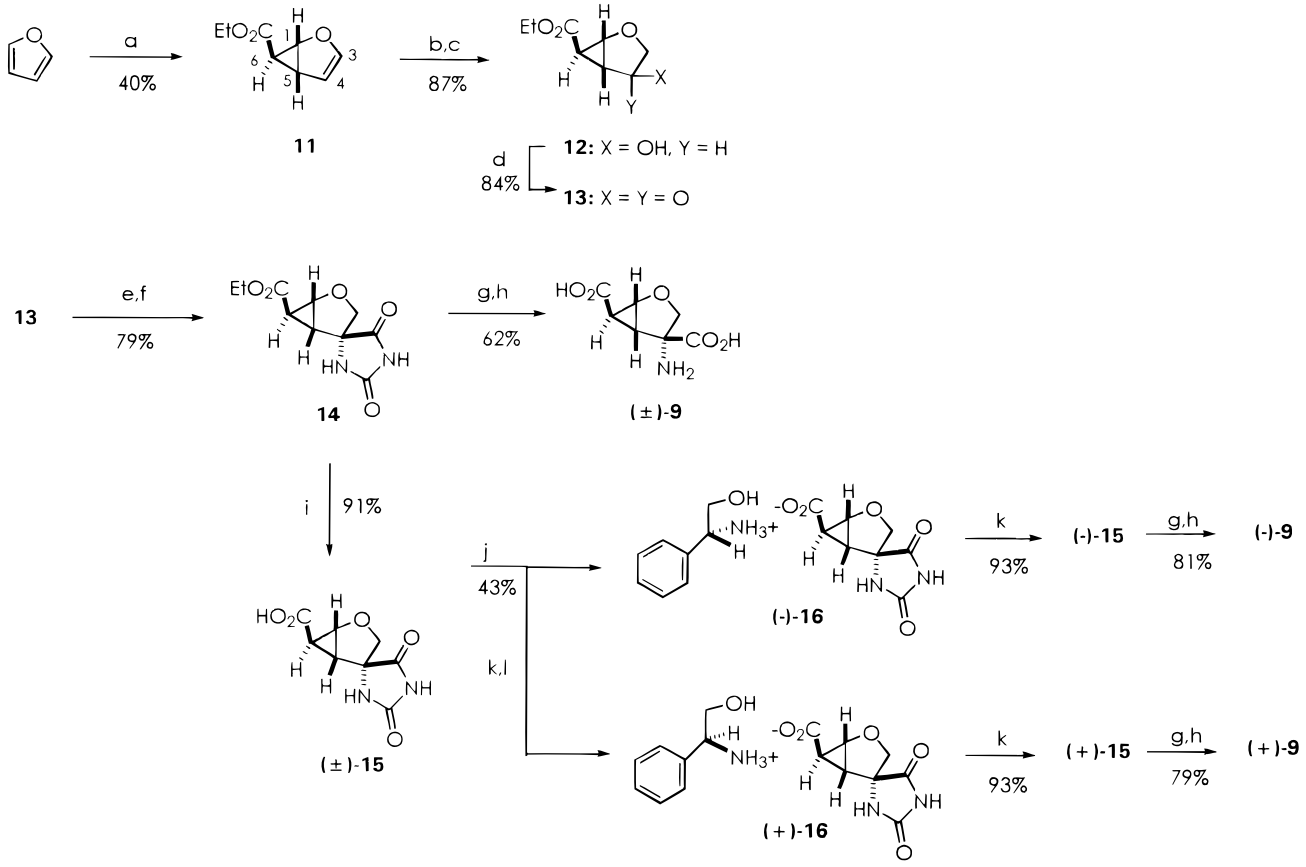
In a strictly analogous manner, the preparation of racemic and nonracemic **10** was achieved in similar overall chemical yield and enantiomeric purity by employing thiophene as the heteroaromatic partner in the initial carboxycyclopropanation reaction (Scheme 2).[24−27]

Pharmacological Methods

Rat Brain Glutamate Receptor Affinity. Glutamate receptor affinity was determined in rat forebrain homogenates by examining the ability of test ligands to displace [^{3}H]CGP39653, [^{3}H]AMPA, [^{3}H]kainic acid, and [^{3}H]LY341495 from NMDA, AMPA, kainate, and group II mGlu receptors, respectively, as previously described.[29−32] In those cases where an IC_{50} value of less than 100 μM could be determined, experiments were performed in triplicate. Data are expressed as the mean IC_{50} ± standard error.

Affinity For Recombinant Human mGlu Receptor Subtypes. Test compounds were evaluated for their ability to displace [^{3}H]LY341495 from recombinant human mGlu2 and mGlu3 receptor subtypes individually expressed in RGT cells.[33,34] The K_i values (± standard error) were calculated from the IC_{50} values

Scheme 1[a]



[a] Reagents and conditions: (a) ethyl diazoacetate, $Rh_2(OAc)_4$, 10 °C, 2 h; (b) thexylborane, THF, 0 °C, 2 h; (c) 30% H_2O_2, phosphate buffer (pH 7), room temperature, overnight; (d) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −65 °C; (e) $(NH_4)_2CO_3$, KCN, MeOH; (f) crystallization at pH 7; (g) 2 N NaOH, reflux, overnight; (h) anion-exchange chromatography; (i) 2 N NaOH, room temperature, 0.5 h; (j) (*R*)-(−)-2-phenylglycinol, EtOH/H_2O (5:1); (k) 1 N HCl, EtOAc extraction; (l) (*S*)-2-phenylglycinol, EtOH.

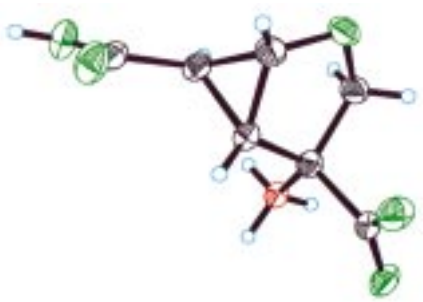


Figure 1. Thermal elipsoid representation of **(−)-9**.[28] The non-H atom elipsoids enclose the 50% probability limits.

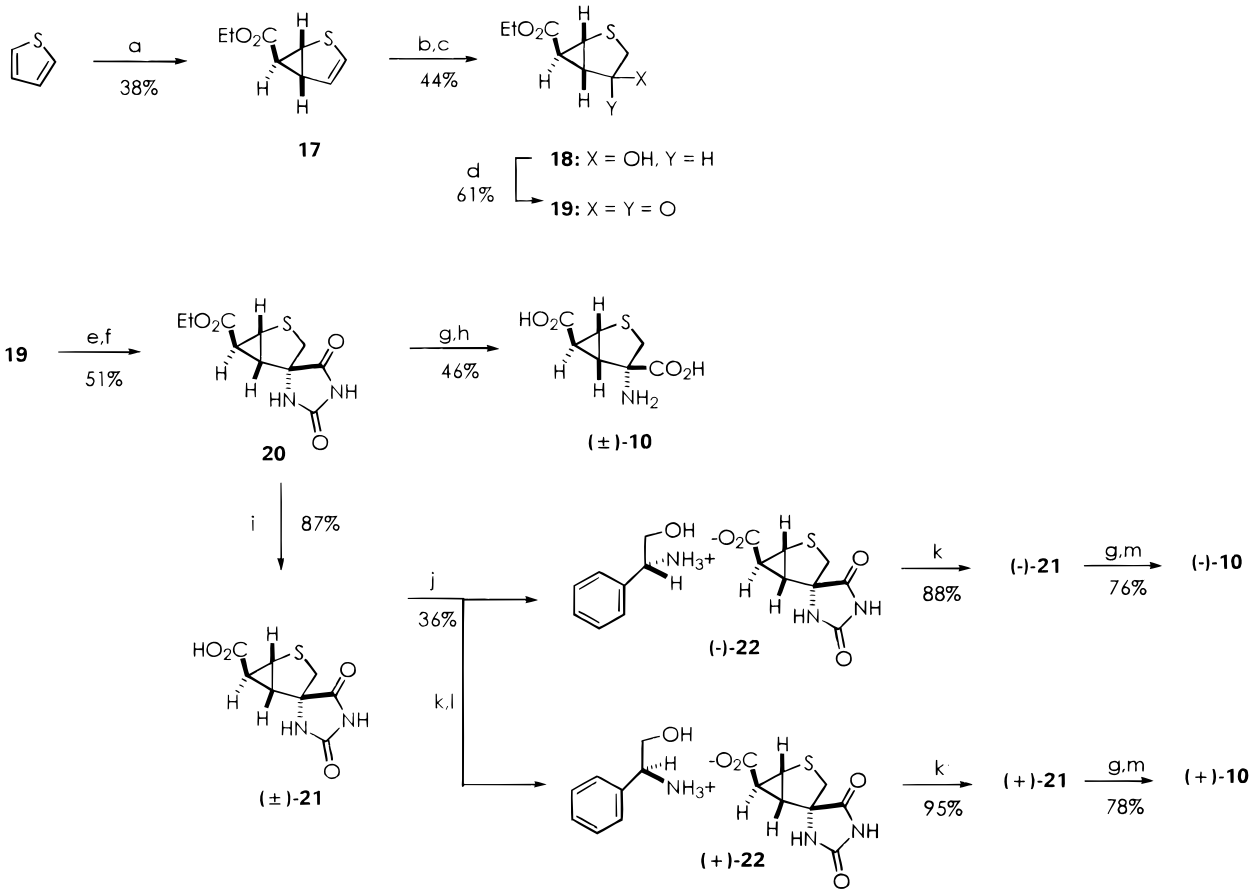
employing the Cheng−Prusoff equation[35] and represent the mean of at least three separate experiments.

Second-Messenger Responses in Cells Expressing Recombinant mGlu Receptors. Test compounds were evaluated for their ability to influence the production of second messengers in "RGT" cells. RGT cells are nonneuronal AV12-664 cells coexpressing both GLAST (a recombinant glutamate transporter to minimize constituent glutamate activity) and a recombinant human group I (mGlu1a or mGlu5a), group II (mGlu2 or mGlu3), or group III (mGlu4a, mGlu6, mGlu7a, or mGlu8) receptor employing methods previously described.[16,36]

Mouse Limbic Seizure Assay. Evaluation of the effects of test compounds on limbic seizure activity produced by intracerebral administration of **1** was performed as previously described.[37] Briefly, male NIH swiss mice were physically restrained to allow unilateral intracerebral (ic) injections of **1**, which were made using a 10-μL Hamilton microsyringe. Animals were pretreated at the time indicated with either sterile water (5 μL, ic) or test compound at the dose indicated. Animals were observed for 30 min following administration of **1**. Limbic seizures in treated mice were characterized by the presence of at least one episode of clonic forelimb contractions followed by hind limb 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.

Molecular Modeling. All initial models were derived from the X-ray structure of **5**.[17] The acid functions were modeled in their deprotonated states and the amines as either the neutral or protonated form, to represent the major forms present in aqueous solution at physiological pH 7.4. The suffix $-NH_2$ indicates the neutral amine form and the suffix $-NH_3$ the protonated amine form of a compound. Structures were optimized by a direct Hartree−Fock approach, as implemented in Spartan v5.0. The basis set used was 6-31G**. Charges were derived by fitting to the electrostatic potential. Molecular similarity calculations were performed using the carbo index, a 3-component Gaussian function, exhaustive rigid search at 15° intervals followed by a simplex optimization, as implemented in ASP v3.21.

Scheme 2[a]



[a] Reagents and conditions: (a) ethyl diazoacetate, $Rh_2(OAc)_4$, 70 °C, 3 h; (b) thexylborane, THF, room temperature, 2 h; (c) $NaBO_3 \cdot 4H_2O$, phosphate buffer (pH 7), room temperature, 1 h; (d) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −40 °C; (e) $(NH_4)_2CO_3$, KCN, EtOH/H_2O (2.5:1), 35 °C; (f) crystallization from 2-propanol; (g) 2 N NaOH, reflux; (h) anion-exchange chromatography; (i) 2 N NaOH, room temperature, 0.5 h; (j) (*R*)-(−)-2-phenylglycinol, EtOH/H_2O (5:1); (k) aqueous HCl, 0 °C; (l) (*S*)-2-phenylglycinol, EtOH/H_2O (5:1); (m) pH 3, 0 °C.

Results

Glutamate Receptor Radioligand Binding in Vitro. Racemic heterobicyclic amino acids **(±)-9** and **(±)-10** each potently displaced [^{3}H]LY341495 binding from rat forebrain homogenates (IC_{50} = 29 ± 4 nM for **(±)-9** and 26.1 ± 1.5 nM for **(±)-10**) (Figure 2, Table 1). This effect was determined to be highly stereoselective, with the mGlu receptor-active enantiomers **(−)-9** (IC_{50} = 15 ± 4 nM) and **(−)-10** (IC_{50} = 8.4 ± 0.8 nM) displaying approximately 300- and 90-fold higher affinity for [^{3}H]LY341495-labeled sites than the corresponding inactive enantiomers **(+)-9** and **(+)-10**. Furthermore, the potencies of **(−)-9** and **(−)-10** in this assay compare favorably to that observed for the related carbobicyclic amino acid **5** (IC_{50} = 254 ± 86 nM). In contrast, at concentrations up to 100 000 nM, neither racemic nor nonracemic **9** or **10** displaced radioligand binding to NMDA, AMPA, or kainate receptors expressed in rat forebrain homogenates.

As expected based on the foregoing results and the known mGlu2/3 receptor selectivity for [^{3}H]-LY341495,[32−34] heterobicyclic amino acids **9** and **10** potently displaced this radioligand from recombinant human mGlu2 (**(±)-9**, K_i = 36.5 ± 1.3 nM; **(±)-10**, K_i = 67.2 ± 4.8 nM) and mGlu3 (**(±)-9**, K_i = 14.3 ± 0.4 nM; **(±)-10**, K_i = 9.4 ± 0.3 nM) receptors expressed in membranes prepared from RGT cells (Figure 2, Table 1). As was observed in the rat brain homogenate binding experiments, a high degree of stereoselectivity was also evident for binding of the individual enantiomers to the glutamate site on cloned group II mGlu receptors, with the (−)-isomers displaying high-affinity binding to mGlu2 (**(−)-9**, K_i = 14.1 ± 1.4 nM; **(−)-10**, K_i = 40.6 ± 3.7 nM) and mGlu3 (**(−)-9**, K_i = 5.8 ± 0.6 nM; **(−)-10**, K_i = 4.7 ± 1.2 nM) receptors relative to the (+)-isomers, which displaced [^{3}H]LY341495 binding only at high ligand concentrations (**(+)-9**, K_i = 1 995 ± 34.3 nM at mGlu2 and 1 037 ± 89 nM at mGlu3; **(+)-10**, K_i = 818.8 ± 81.3 nM at mGlu2 and 169.7 ± 20.2 nM at mGlu3). In comparison, compound **5** displayed significantly lower affinity for both mGlu2 (K_i = 74.9 ± 9.1 nM) and mGlu3 (K_i = 93.3 ± 2.6) receptors than that observed for either **(−)-9** or **(−)-10**.

Second-Messenger Responses. Heterobicyclic amino acids **(−)-9** and **(−)-10** potently inhibited forskolin-stimulated c-AMP production in RGT cells expressing recombinant human mGluR2 (**(−)-9**, EC_{50} = 2.69 ± 0.26 nM; **(−)-10**, EC_{50} = 3.91 ± 0.81 nM) or mGluR3 (**(−)-9**, EC_{50} = 4.58 ± 0.04 nM; **(−)-10**, EC_{50} = 7.63 ± 2.08 nM). The data are shown in Figure 3A,B and summarized in Table 2. Agonist activity was also evinced by these molecules in the mGlu6 receptor-expressing cells (**(−)-9**, EC_{50} = 401 ± 46 nM; **(−)-10**, EC_{50} = 2 430 ± 600 nM) and in the mGlu8 receptor-expressing cells (**(−)-9**,

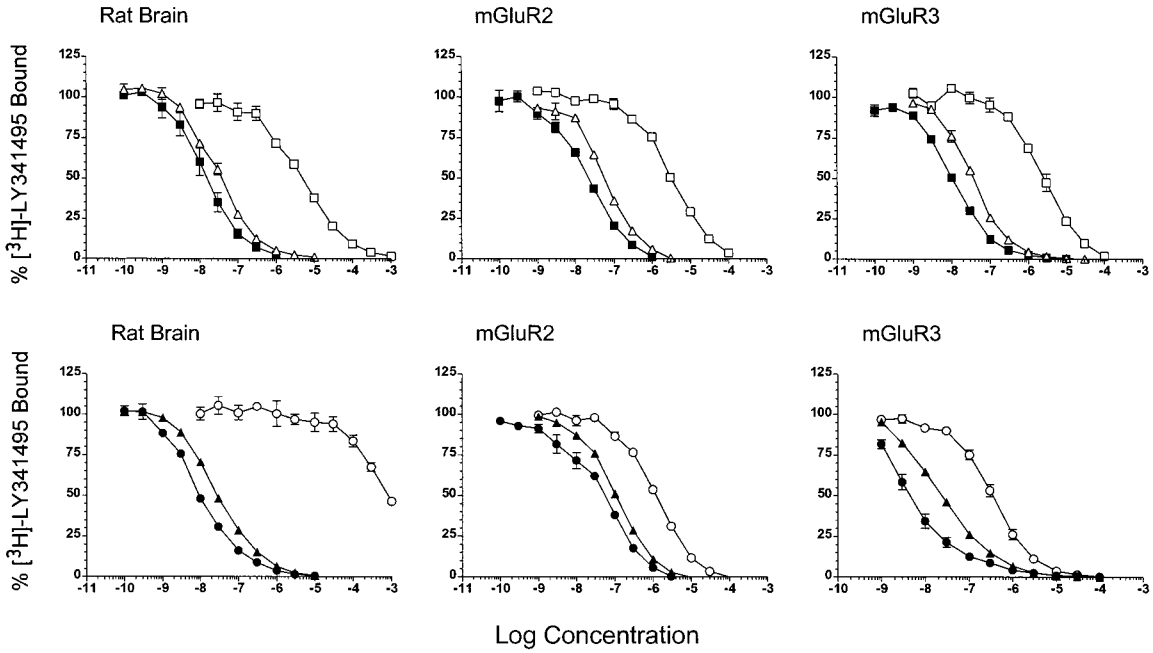


Figure 2. Displacement of [^{3}H]LY341495 binding to rat forebrain homogenates,[32] human mGluR2,[33,34] and human mGluR3[33,34] by racemic and nonracemic **9** (top three panels) and **10** (bottom three panels). Legend: **(±)-9** (△), **(−)-9** (■), **(+)-9** (□), **(±)-10** (▲), **(−)-10** (●), **(+)-10** (○).

Table 1. Effects of **5**, **9**, and **10** on Radioligand Binding to Glutamate Receptors in Rat Brain and to Recombinant Human mGlu2 and mGlu3 Receptors Expressed in RGT Cells

	IC_{50} (nM) ± SEM				K_i (nM) ± SEM	
compd	[^{3}H]LY341495[a] rat brain	[^{3}H]CGP39653[b] rat brain	[^{3}H]AMPA[c] rat brain	[^{3}H]KA[d] rat brain	[^{3}H]LY341495 mGlu2[e]	[^{3}H]LY341495 mGlu3[e]
5	254 ± 86	>100 000	>100 000	>100 000	74.9 ± 9.1	93.3 ± 2.6
(±)-**9**	29 ± 4	>100 000	>100 000	>100 000	36.5 ± 1.3	14.3 ± 0.4
(−)-**9**	15 ± 4	>100 000	>100 000	>100 000	14.1 ± 1.4	5.8 ± 0.6
(+)-**9**	5 040 ± 260	>100 000	>100 000	>100 000	2 000 ± 34	1 040 ± 89
(±)-**10**	26.1 ± 1.5	>100 000	>100 000	>100 000	67.2 ± 4.8	9.4 ± 0.3
(−)-**10**	8.4 ± 0.8	>100 000	>100 000	>100 000	40.6 ± 3.7	4.7 ± 1.2
(+)-**10**	792 ± 27.6	>100 000	>100 000	>100 000	819 ± 81	170 ± 20

[a] See ref 32. [b] See ref 29. [c] See ref 30. [d] See ref 31. [e] See refs 33 and 34.

EC_{50} = 1 690 ± 130 nM; **(−)-10**, EC_{50} = 7 340 ± 2 720 nM). In contrast, at concentrations up to 10 000 nM (in most instances up to 100 000 nM) neither **(−)-9** nor **(−)-10** produced either agonist or antagonist effects in cells expressing recombinant group I (mGlu1a, mGlu5a) or other recombinant group III (mGlu4a, mGlu7a) receptors (Table 2).

Mouse Limbic Seizure Model. Intracerebral administration of **1** (400 nmol) produced a characteristic limbic seizure behavior in NIH Swiss mice which persisted throughout the ensuing 20-min observation period.[37] Intraperitoneal administration of **(−)-9** at either 1, 2, or 4 h prior to **1** resulted in a dose-related decrease in the number of mice exhibiting seizures (Table 3). The calculated ED_{50} values for 1-, 2-, and 4-h preadministration times of **(−)-9** were 19, 6, and 26 mg/kg, respectively. In a similar fashion, **(−)-10** blocked seizure behaviors with ED_{50} values of 14, 15, and 14 mg/kg when administered by the ip route at 1, 2, or 4 h prior to **1**.

Molecular Modeling. The optimization calculations were carried out in the gas phase, with no solvent or dielectric corrections. This can lead to structures which are considerably different from those in solution, especially for charged species. The optimized structure of **5**-NH_2 (nonprotonated amine form) was superimposed onto its X-ray structure,[17] and the rms deviation over all atoms was determined to be 0.4 Å. In fact, all the deviations occurred in the α-amino acid group, with the carboxylate groups being oriented at an angle of 103° with respect to each other and the amine being more pyramidal in the optimized structure. In all other respects, the structures were identical so that the effect of omitting solvent on the calculated molecular geometry of **5**-NH_2 is small. Similarity calculations were used to establish whether the heteroatom for methylene substitutions has a measurable effect on the overall shape and/or electrostatic profile of the compounds. Thus, similarity calculations relating the protonated ($-NH_3$) and nonprotonated ($-NH_2$) forms of **5**, **(−)-9**, and **(−)-10** (Table 4) were performed using a 1:1 weighting of the shape and electrostatic components of similarity. These data indicate a high degree of similarity between protonated and nonprotonated forms of each of the individual molecules ($r^2 \geq 0.93$ in each case), as well as between different analogues of similar ($r^2 \geq 0.90$) or dissimilar ($r^2 \geq 0.87$) protonation states. An overlay of the nonprotonated forms of **5**, **(−)-9**, and **(−)-10** is shown in Figure 4 (left).

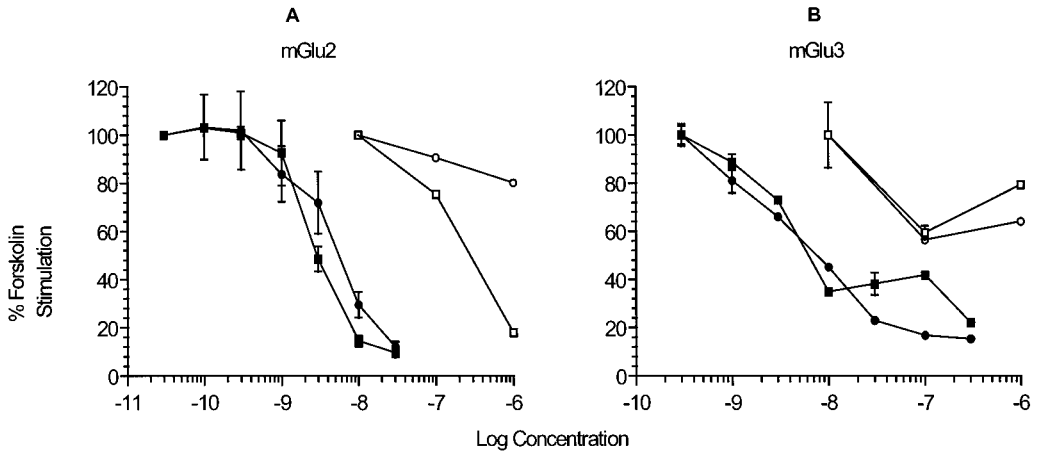


Figure 3. Forskolin-stimulated c-AMP responses to **(−)-9** (■), **(−)-10** (●), and their inactive enantiomers **(+)-9** (□) and **(+)-10** (○) in RGT cells expressing recombinant human mGlu2 (left) or mGlu3 (right) receptors. Methods are described in detail in ref 16.

Table 2. Effects of **5**, (−)-**9**, and (−)-**10** on Second-Messenger Responses in RGT Cells Expressing Recombinant Human mGlu Receptor Subtypes

	EC_{50} (nM) ± SEM							
compd	mGlu1a[a]	mGlu2[a,b]	mGlu3[a,c]	mGlu4a[a,d]	mGlu5a[a]	mGlu6[e]	mGlu7a[a,d]	mGlu8[d]
5	>100 000	11.1 ± 2.5	38.0 ± 3.07	>100 000	>100 000	2 990 ± 120	>100 000	11 500 ± 200[f]
(−)-**9**	>100 000	2.69 ± 0.26	4.58 ± 0.04	21 100 ± 8 600[g]	>100 000	401 ± 46	>100 000	1 690 ± 130[h]
(−)-**10**	>100 000	3.91 ± 0.81	7.63 ± 2.08	>100 000	>100 000	2 430 ± 600	>100 000	7 340 ± 2 720[i]

[a] See ref 16. [b] B_{max} = 29.2 pmol/mg of protein; E_{max} for all compounds tested ≥ 90%. [c] B_{max} = 35 ± 1.2 pmol/mg of protein; E_{max} for all compounds tested ≥ 90%. [d] See ref 36. [e] B_{max} = 1.58 pmol/mg of protein; E_{max} for all compounds tested > 95%. [f] E_{max} = 50%. [g] E_{max} = 46%. [h] E_{max} = 91%. [i] E_{max} = 80%.

Table 3. Effect of Systemically Administered (−)-**9** or (−)-**10** on Limbic Seizures Induced by **1** (400 nmol, ic) in NIH Swiss Mice[a]

	(−)-**9**			(−)-**10**		
dose (mg/kg, ip)	1 h	2 h	4 h	1 h	2 h	4 h
control[b]	0/10	0/10	0/10	0/10	0/10	0/10
control[c]	9/10	9/10	9/10	9/10	9/10	9/10
3		6/10		7/10		
10	7/10	4/10	7/10	5/10	7/10	6/10
30	3/10	3/10	5/10	4/10	2/10	3/10
100	2/10	2/10	2/10	3/10	0/10	2/10
ED_{50} (mg/kg)	19	6	26	14	15	14

[a] Compound (−)-**9** or (−)-**10** was administered by the ip route at the indicated time point prior to ic administration of **1**. Data are expressed as the number of mice observed to have exhibited limbic seizures/number treated at any time during the 15-min observation period following administration of **1**.[37] [b] Mice were treated with H_2O (ip) at the indicated time point prior to ic administration of H_2O. [c] Mice were treated with H_2O (ip) at the indicated time point prior to ic administration of **1**.

Quantum mechanical calculations were used to probe more subtle effects, in particular the charge and potential distributions about the ionizable functional groups in these molecules. Figure 4 (right) shows the electrostatic potential map projected onto an isoelectron surface. As can be seen, there is very little difference between the maps generated for **5**-NH_2, **(−)-9**-NH_2, and **(−)-10**-NH_2. The charges on the oxygen atoms of the two carboxylate groups are essentially identical for all three molecules. The properties that provide the greatest discrimination between the NH_2 form of these compounds are the HOMO and LUMO orbitals (Figures 5). As shown in Figure 5, the HOMO coefficients (left panel) are larger at the ring heteroatom position for **(−)-10**-NH_2 and, to a lesser extent, **(−)-9**-NH_2 when compared with **5**-NH_2. The coefficients on the LUMO orbital for the NH_2 species (Figure 5, right panel) show a marked difference between **(−)-10**-NH_2 when compared with either **(−)-9**-NH_2 or **5**-NH_2 and no significant difference when comparing **(−)-9**-NH_2 to **5**-NH_2. The LUMO in **(−)-10**-NH_2 seems to be strongly localized around the soft sulfur atom, whereas the LUMO in both **5** and **(−)-9** is primarily localized on the amino face of these compounds.

Table 4. Matrix of Combined Shape and Electrostatic Potential Energy Similarity Coefficients (1:1 weighting) for Protonated ($-NH_3$) and Nonprotonated ($-NH_2$) Forms of **5**, (−)-**9**, and (−)-**10**

compd	**5**-NH_2	**5**-NH_3	(−)-**9**-NH_2	(−)-**9**-NH_3	(−)-**10**-NH_2	(−)-**10**-NH_3
5-NH_2	1					
5-NH_3	0.93	1				
(−)-**9**-NH_2	0.99	0.93	1			
(−)-**9**-NH_3	0.93	0.90	0.92	1		
(−)-**10**-NH_2	0.90	0.91	0.90	0.87	1	
(−)-**10**-NH_3	0.93	0.97	0.93	0.93	0.94	1

Comparison of $-NH_3$ Species. The protonated ($-NH_3$) species show a greater variation between the three compounds, with **(−)-10** being clearly differentiated from **(−)-9** and **5**. The potential maps for the acid and the amine faces of these molecules (Figure 6 top) show clear differences at both of the acid positions and on the amine. This is mirrored in the HOMO and LUMO orbitals (Figure 6, bottom) in which it is evident that the sulfur atom is having a major effect on both HOMO and LUMO coefficients in compound **(−)-10**-NH_3. In **5**-NH_3 and **(−)-9**-NH_3, the HOMO is focused on the C6-carboxylic acid and the LUMO around the amine; in **(−)-10**-NH_3, both of these effects are diluted by the sulfur atom.

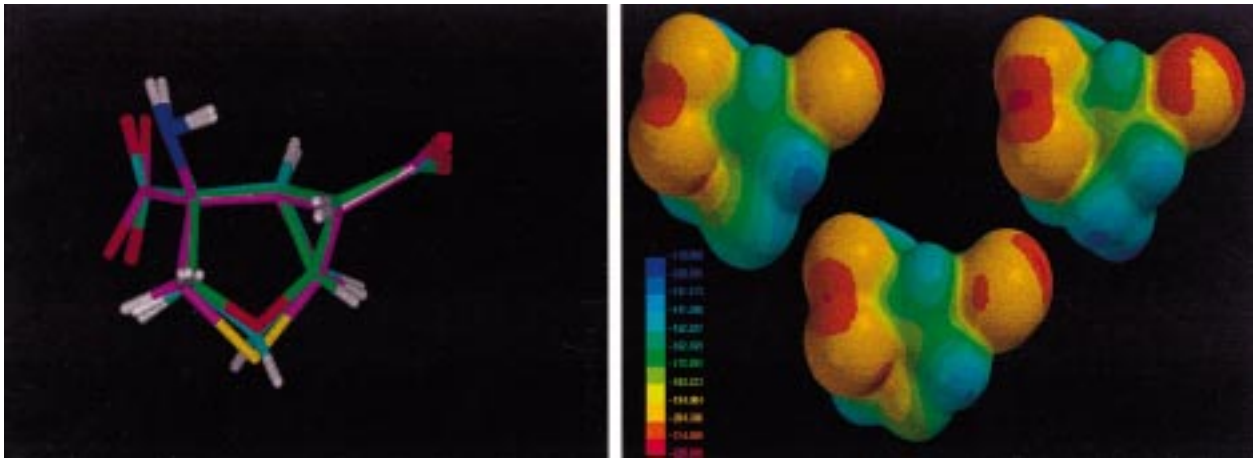


Figure 4. Left: Overlay of energy-minimized structures of **5**-NH_2 (cyan), **(−)-9**-NH_2 (green), and **(−)-10**-NH_2 (purple) showing the close match of the acids and bases and the slight differences in ring geometry at the 2-position. Right: Map of the electrostatic potential projected on the surface defined by a constant electron density of 0.002 electron/au^3 and contoured between −110 and −225 au. The molecules are from left to right: **(−)-10**-NH_2, **(−)-9**-NH_2, and **5**-NH_2.

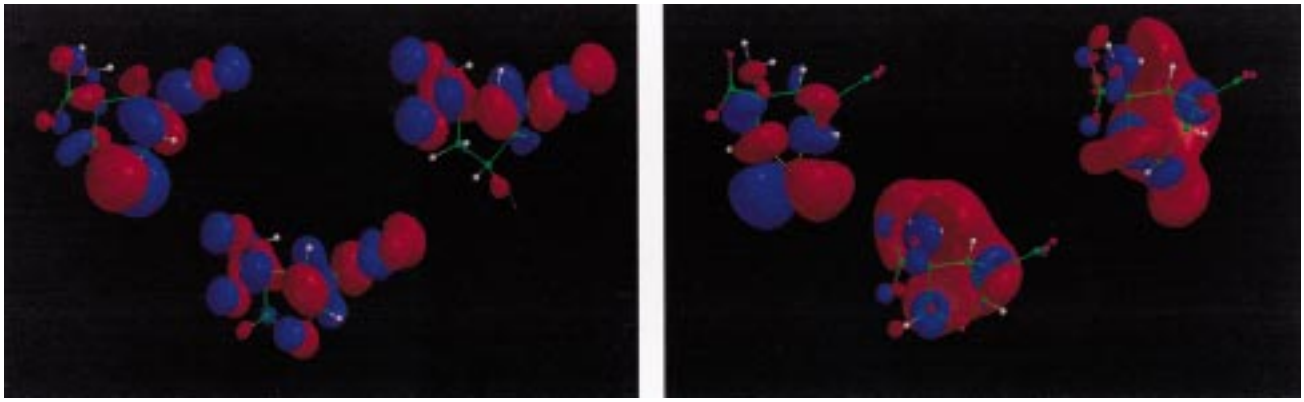


Figure 5. HOMO (left) and LUMO (right) orbital coefficients of the $-NH_2$ species. The molecules are from left to right: **(−)-10**-NH_2, **(−)-9**-NH_2, and **5**-NH_2.

Discussion

Metabotropic glutamate research has been greatly facilitated with the appearance of highly potent agonists and antagonists capable of discriminating between the individual receptor subtypes. Our laboratory has focused on the development of ligands directed toward the group II (mGlu2/3) receptors, with particular attention to the development of novel agonists. The characterization of **5** as a highly potent and selective agonist for group II mGluRs has provided important information regarding the conformation that the naturally occurring agonist, glutamate, likely assumes when binding to and activating these receptors. With this in mind, we set out to prepare molecules that maintain the conformational features of **5** while simultaneously avoiding the incorporation of additional steric bulk about the core bicyclic ring system that might interfere with high-affinity receptor recognition and/or group II selectivity.[38]

Both **(−)-9** and **(−)-10** bind with high ($IC_{50} \sim 10$ nM) affinity to group II mGlu receptors expressed in native rat brain homogenates and to cell membranes expressing recombinant human mGlu2 and mGlu3 receptor subtypes as measured by specific displacement of the group II-selective radioligand [^{3}H]LY341495. Affinity of these compounds for the group II mGluRs was found to be highly stereoselective, with the corresponding (+)-enantiomers displacing [^{3}H]LY341495 binding only at substantially higher concentrations, where isomeric contamination by the active enantiomer cannot be entirely ruled out. As has been shown for **5**, neither **(−)-9** nor **(−)-10** have any measurable affinity at native rat brain NMDA, AMPA, or kainate receptors up to 100 000 nM.

The affinity of **(−)-9** and **(−)-10** for native and recombinant group II mGluRs compares favorably to that observed for the parent carbocyclic amino acid **5**, with each being slightly greater than 1 order of magnitude more potent than this compound in binding to both native group II and recombinant mGlu3 receptors. While enhanced affinity was also observed for these analogues relative to **5** at recombinant mGlu2 receptors, it was of a more modest (approximately 2−5-fold) nature. As a consequence, **(−)-9** and **(−)-10** possess significantly different mGlu2/mGlu3 receptor selectivity ratios (K_i ratio for mGlu2/mGlu3 = 2.4 for **(−)-9** and 8.6 for **(−)-10**) compared to the parent amino acid (K_i ratio for mGlu2/mGlu3 = 0.8). Thus, while **5** possesses approximately equal affinity for both mGlu2 and mGlu3 receptors, the heterocyclic analogues are considerably more mGlu3 receptor-selective.

Functionally, both **(−)-9** and **(−)-10** potently suppressed forskolin-stimulated c-AMP formation in cells expressing recombinant human mGlu2 and mGlu3 receptors, indicative of group II mGluR agonist activity.

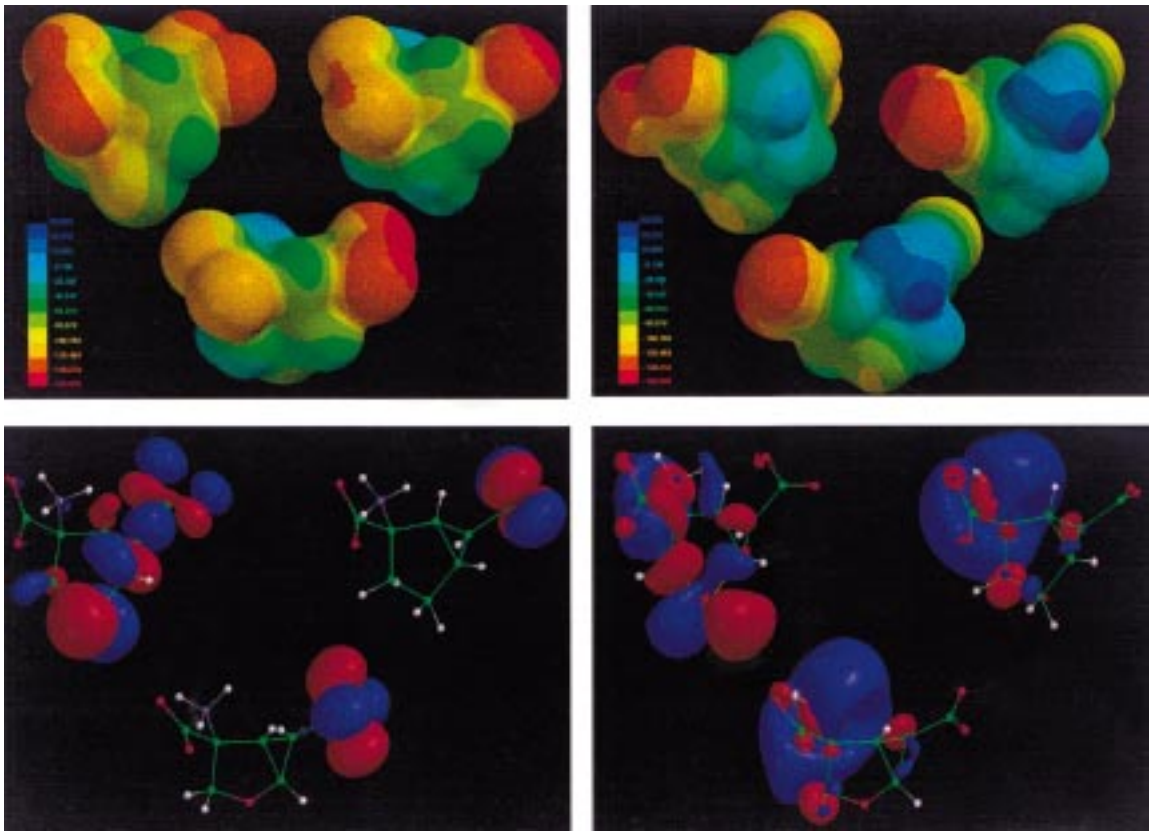


Figure 6. Top panels: Maps of the electrostatic potential contoured between −160 and 60 au with views from the acid face (left) and amine face (right) of the molecules. Bottom: HOMO (left) and LUMO (right) orbital coefficients. The molecules are from left to right: **(−)-10**-NH_3, **(−)-9**-NH_3, and **5**-NH_3.

However, despite the higher affinity of these molecules for mGlu3 receptors, each was equipotent, if not slightly more potent, as an agonist in the mGlu2 receptor-expressing cell line (EC_{50} values of 2.69 and 3.91 nM for **(−)-9** and **(−)-10**, respectively) in comparison to their effects in cells expressing mGlu3 receptors (EC_{50} values of 4.58 and 7.63 nM, respectively). This observation cannot be explained simply on the basis of differential levels of receptor expression in the two cell lines, as the B_{max} values for each are similar (29.2 and 35 pmol/mg of protein for mGlu2 and mGlu3, respectively). It is possible that the lack of direct correlation between binding affinity and agonist potency may relate to differences in the relationships that exist between expression level and functional coupling of these receptors to G_i protein subunits within the RGT cell line.

In addition to their high degree of potency at mGlu2 and mGlu3 receptors, compounds **(−)-9** and **(−)-10** also demonstrated a high degree of group II mGluR agonist selectivity when compared to their effects at other mGluR subtypes. Neither compound produced agonist or antagonist activity at mGlu1a, mGlu5a, or mGlu7 receptors up to 100 000 nM (10 000−100 000 times the EC_{50} values for mGlu2/3 receptor activation). In cells expressing recombinant mGlu4a receptors, high concentrations of **(−)-9** produced a partial agonist effect (EC_{50} = 21 000 nM, E_{max} approximately 50%), while **(−)-10** and **5** were inactive as either an agonist or antagonist up to 100 000 nM at this group III receptor. Each compound also suppressed forskolin-stimulated c-AMP formation in the mGlu8 receptor-expressing cell line with similar (micromolar) potencies. However, the heterocyclic amino acids **(−)-9** and **(−)-10** could be distinguished from **5** at this target in that they produced a nearly full agonist response (E_{max} = 80−90%) compared with the partial agonist effects (E_{max} = 50%) observed for **5**. Finally, compound **(−)-9** could be clearly differentiated from **5** and **(−)-10** in its agonist potency in cells expressing mGlu6, an mGlu receptor localized primarily in the retina.[39] Thus, while both **5** and **(−)-10** produced agonist responses in this cell line at micromolar concentrations (EC_{50} values = 2 990 and 2 430 nM, respectively), **(−)-9** more potently suppressed the stimulated c-AMP response with an EC_{50} value of 401 nM, approximately 2 orders of magnitude higher than that required for group II activation. This level of activity is noteworthy as **(−)-9** represents one of the more potent compounds yet described for this mGlu receptor subtype.[40] Hence, while **(−)-9** and **(−)-10** in general share a common pharmacological profile with each other and with **5**, and are highly selective for the group II receptor class, the oxabicyclic analogue **(−)-9** appears to possess modestly greater potency at mGlu4a and mGlu8 receptors and significantly greater potency at mGlu6 receptors than either the thiabicyclic or carbobicyclic amino acid **(−)-10** or **5**.

We have previously demonstrated that selective group II mGluR agonists are capable of blocking limbic seizures produced by intracerebral administration of **1** (acting through group I mGlu receptor activation) and have used this assay as a means to determine functional group II mGluR agonist activity following systemic administration of test ligands in mice.[15,17] In the current studies, compounds **(−)-9** and **(−)-10**, when given by the intraperitoneal route 1, 2, or 4 h prior to 400 nmol of **1**, blocked the limbic seizure activity produced by this

molecule in a dose-related manner (Table 3). The dose−response in this assay was similar (10−100 mg/kg) for both molecules, as was the influence of preadministration time, with both compounds producing a similar degree of efficacy when administered either 1 h (ED_{50} = 19 and 14 mg/kg for **(−)-9** and **(−)-10**, respectively) or 4 h (ED_{50} = 26 and 14 mg/kg for **(−)-9** and **(−)-10**, respectively) prior to **1**.

In an attempt to understand the underlying physical properties of **(−)-9** and **(−)-10** that give rise to the enhanced affinity at group II (especially mGlu3) receptors compared to **5**, we have examined the influence of the ring heteroatoms in **(−)-9** and **(−)-10** on molecular conformation, electrostatic potential energies, and HOMO/LUMO localization and compared these results to those derived from **5**. As shown in Table 4, heterobicyclic amino acids **(−)-9** and **(−)-10** are highly similar with each other and with **5** in terms of both electronic and conformational properties. Furthermore, the similarity calculations do not shed light on whether the charge on the amino group is important to binding, as the similarity coefficients between any nonprotonated ($-NH_2$) and protonated ($-NH_3$) pair are high. Ab initio calculations were subsequently performed on **(−)-9**, **(−)-10**, and **5** in order to evaluate more closely the effects of the ring heteroatoms in **(−)-9** and **(−)-10** on the distribution of the highest occupied and lowest unoccupied molecular orbitals relative to those of the parent carbocycle **5** in similarly charged species. While clear distinctions can be observed in comparing either the HOMO or LUMO of thiabicyclic analogue **(−)-10** to carbocycle **5** in either the NH_2 (Figure 5) or NH_3 (Figure 6) forms, no such differences could be observed between **5** and oxabicyclic amino acid **(−)-9**. Hence, the enhanced group II mGluR potency of **(−)-9** and **(−)-10** relative to **5** cannot be explained by changes in the distribution of these molecular orbitals in similarly charged species.

Conclusion

LY379268 (**(−)-9**) and LY389795 (**(−)-10**) are the most potent agonists yet described for group II metabotropic glutamate receptors. The high mGlu2/3 potency and selectivity evinced by these heterobicyclic amino acids lend further experimental evidence supporting the hypothesis that glutamate interacts with mGlu2/3 receptors in a fully extended conformation. Like **5**, heterocycles **(−)-9** and **(−)-10** effectively penetrate into the CNS following systemic administration and block seizures induced by group I mGlu receptor activation. Thus, like **5**, these agents may be useful for studying the functions of mGlu2/3 receptors both in vitro and in vivo.

Experimental Section

Melting points were obtained using a Thomas-Hoover capilliary melting point apparatus and are uncorrected. ^1H and ^{13}C NMR data 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 AG1-X8 anion-exchange resin (hydroxide form). Chiral HPLC analyses were performed using a Chirobiotic V 4.6- × 250-mm column utilizing 20% THF/0.1% trifluoroacetic acid/water as the eluent system at a solvent flow rate of 0.6 mL/min. Detection was performed at λ = 230 nm.

(±)-Ethyl 2-Oxabicyclo[3.1.0]hex-3-ene-6-carboxylate (11).[24] A solution of ethyl diazoacetate (100 g, 877 mmol) in furan (250 mL) was added dropwise to a solution of $Rh_2(OAc)_4$ (0.5 g, 1.1 mmol) in furan (250 mL) with stirring at 10 °C over a period of about 2−2.5 h. A further 0.1 g (0.2 mmol) of $Rh_2(OAc)_4$ was added about two-thirds of the way into the addition. After HPLC analysis showed complete consumption of ethyl diazoacetate, a solution of $NaHSO_3$ (200 g) in water (400 mL) was added, and the resultant two-phase mixture was allowed to warm to ambient temperature with stirring for 1−2 h. The reaction mixture was then extracted with methyl *tert*-butyl ether (500 mL) and the organic phase washed with water (400 mL) and saturated NaCl (300 mL) and then dried over Na_2SO_4. The solvent was then removed by evaporation, and the resultant oil was vacuum-distilled (45 °C at 0.2 mmHg) to afford **11** (54 g, 351 mmol) in 40% yield: FDMS M^+ = 154; ^1H NMR ($CDCl_3$) δ 0.95 (d, 1H, J = 2.5 Hz), 1.26 (t, 3H, J = 7 Hz), 2.78 (dd, 1H, J = 2.5 and 5.7 Hz), 4.13 (q, 2H, J = 7 Hz), 4.85 (d, 1H, J = 5.7 Hz), 5.48 (t, 1H, J = 2.6 Hz), 6.39 (d, 1H, J = 2.5 Hz); ^{13}C NMR ($CDCl_3$) δ 14.24, 22.10, 31.54, 60.59, 67.01, 106.31, 147.21, 172.82.

(±)-Ethyl 4-Hydroxy-2-oxabicyclo[3.1.0]hexane-6-carboxylate (12). A solution of thexylborane was prepared by adding a solution of 2,3-dimethyl-2-butene (4 M, 53.0 mL) in THF via a syringe to borane−dimethyl sulfide complex (10 M, 21.2 mL) in a dry flask under nitrogen at approximately 0 °C. The solution was stirred for 2 h at this temperature before use. Compound **11** (32.73 g, 212.30 mmol) was dissolved in 150 mL of THF under N_2. The resultant solution was cooled with stirring to −0.5 °C. While the stirring solution was cooling, the system was evacuated and purged twice with N_2. The entire thexylborane solution prepared above was added via cannula over 40 min, maintaining the temperature between 0 and 5 °C. After the mixture stirred for 2 h at this temperature, 87 mL of 30% H_2O_2 was added slowly, over 70 min, to maintain the temperature at <30 °C. Following the peroxide addition, 15 mL of pH 7 phosphate buffer (1 M KH_2PO_4 and 1 M in K_2HPO_4) was added, and the mixture was allowed to stir for 14 h while warming to ambient temperature. The mixture was cooled to <5 °C, and 25 mL of saturated aqueous $Na_2S_2O_3$ was added slowly; 75 mL of EtOAc was then added, followed slowly by 75 mL of saturated aqueous $Na_2S_2O_3$. The mixture was stirred for 15 min and then partitioned between 75 mL of EtOAc and 30 mL of saturated aqueous $Na_2S_2O_3$. The aqueous layer was back-extracted three times with 50 mL of EtOAc. The combined organic layers were washed with 30 mL of brine and dried over Na_2SO_4. The solvent was removed to afford 54.44 g of an oil. The oil was purified by a flash chromatography (370 g of silica gel, wet packed with 3:2 hexanes:EtOAc) eluting with 3:2 hexanes:EtOAc, to afford **12** (31.72 g, 16% EtOAc by ^1H NMR, corrected yield = 26.6 g, 154.5 mmol) in 73% yield. An analytical sample was prepared by further evaporation of solvent (with some product loss): FDMS M^+ = 172; ^1H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.1 Hz), 1.82 (dd, 1H, J = 1.0 and 3.7 Hz), 2.25 (t, 1H, J = 4.2 Hz), 2.25 (br s, 1H), 3.55 (dd, 1H, J = 4.4 and 11.1 Hz), 3.94 (d, 1H, J = 11.1 Hz), 4.10 (q, 2H, J = 7.1 Hz), 4.36 (dd, 1H, J = 1.0 and 4.9 Hz), 4.45 (d, 1H, J = 4.4 Hz); ^{13}C NMR (DMSO-d_6) δ 14.09, 21.25, 30.94, 60.74, 64.41, 72.35, 72.39, 74.49, 170.77. Anal. ($C_8H_{12}O_4$) C, H.

(±)-Ethyl 4-Oxo-2-oxabicyclo[3.1.0]hexane-6-carboxylate (13). Oxalyl chloride (25.70 g, 202.44 mmol) in CH_2Cl_2 (300 mL) under N_2 was added dropwise over 35 min to a solution of DMSO (28.74 g, 367.8 mmol) in CH_2Cl_2 (75 mL) while keeping the temperature below −65 °C. The solution was stirred for 10 min and cooled back to −70 °C. A solution of 31.68 g of **12** (26.29 g, 152.71 mmol, corrected for residual EtOAc) dissolved in 100 mL of CH_2Cl_2 was added dropwise over 40 min while maintaining the temperature at −67 °C. The mixture was stirred for 5 min; then 62 mL (45.01 g, 444.83 mmol) of triethylamine was added dropwise over 15 min, keeping the temperature below −50 °C. After stirring for 15 min, TLC indicated complete reaction, and the mixture was allowed to warm to about −40 °C. The mixture was filtered and washed through with 300 mL of CH_2Cl_2. The filtrate was extracted two times with 150 mL of 1 N HCl. The aqueous layer was back-extracted with 50 mL of CH_2Cl_2. The combined organic layers were washed with 75 mL of brine and dried over Na_2SO_4. Most of the solvent was removed by rotary evaporation to leave 44.36 g of liquid. A few seed crystals were added, and the flask was blanketed with N_2 and stirred at ambient temperature for 30 min while a thin slurry formed. To the room temperature slurry was slowly added 20 mL of hexanes. The slurry was stirred for 90 min at ambient temperature and then for 3 h in an ice/NaCl/water bath. The solids were filtered, washed with 25 mL of 5:1 hexanes:EtOAc, and dried under vacuum to afford **13** (19.48 g, 114.6 mmol) as white crystals. A second crop of crystals (2.28 g, 13.4 mmol) was obtained. The combined yield was 84%: mp 62−65 °C; FDMS $M^+ = 170$; ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 3H, $J = 7$ Hz), 2.35 (dd, 1H, $J = 0.8$ and 3.5 Hz), 2.47 (t, 1H, $J = 4.1$ Hz), 3.83 (d, 1H, $J = 17.6$ Hz), 4.0 (d, 1H, $J = 17.8$ Hz), 4.08 (q, 2H, $J = 7$ Hz), 4.75 (d, 1H, $J = 4.4$ Hz); ^{13}C NMR ($CDCl_3$) δ 14.04, 27.25, 31.85, 61.48, 67.94, 69.97. Anal. ($C_8H_{10}O_4 \cdot 0.25H_2O$) C, H.

(±)-Ethyl 4-(Spiro-5′-hydantoin)-2-oxabicyclo[3.1.0]-hexane-6-carboxylate (14). To a slurry of ammonium carbonate (5.65 g, 58.8 mmol) and potassium cyanide (2.01 g, 30.9 mmol) in 25 mL of methanol at ambient temperature was added a solution of **13** (5.0 g, 29.4 mmol) in 25 mL of methanol. The mixture was stirred at ambient temperature and monitored by HPLC. After 23 h the reaction was judged complete by TLC. The mixture was diluted with 100 mL of water, cooled, and seeded. The pH was adjusted from 9.6 to 7.0 with 6 N HCl giving a white solid. The slurry was stirred at 0−5 °C for 1.5 h, filtered, and washed with 75 mL of cold water−methanol (2:1). The white solid was dried under vacuum at 40 °C affording **14** (5.55 g, 23.1 mmol) in 79% yield: mp 161−163 °C; FDMS $M^+ = 240$; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, 3H, $J =$ 7 Hz), 2.38 (dd, 1H, $J = 5.4$ and 3.9 Hz), 2.42 (dd, 1H, $J = 3.9$ and 1.5 Hz), 3.37 (d, 1H, $J = 10.3$ Hz), 4.05 (q, 2H, $J = 7$ Hz), 4.07 (d, 1H, $J = 10.3$ Hz), 4.39 (dd, 1H, $J = 5.4$ and 1.5 Hz), 8.14 (s, 1H), 10.80 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 14.05, 21.95, 30.90, 60.40, 65.79, 68.21, 70.57, 156.18, 169.40, 175.41. Anal. ($C_{10}H_{12}N_2O_5 \cdot 0.6H_2O$) C, H, N.

(±)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic Acid ((±)-9). A solution of **14** (0.33 g, 1.37 mmol) in 2 N NaOH (10 mL) was warmed under reflux for 24 h in a glass flask. The pH was adjusted to approximately 11 with 1 N HCl, and the resulting solids (presumably silicic acid from the reaction vessel) were removed by filtration. After the volume was reduced to approximately 5 mL under reduced pressure, the solution was applied to AG1-X8 anion-exchange resin. The product was eluted with 3 N AcOH and concentrated to dryness. The amorphous solid was crystallized from 15 mL of H_2O (saturated solution pH ∼ 3) to afford **(±)-9** (0.158 g, 0.84 mmol) in 62**%** yield: mp > 200 °C dec; FDMS M^+ (+H) = 188; ^{1}H NMR (D_2O + KOD) δ 2.15 (br d, 1H, $J = 4$ Hz), 2.31 (dd, 1H, $J = 4.1$ and 4.6 Hz), 3.45 (d, 1H, $J = 9.8$ Hz), 4.20 (d, 1H, $J = 9.8$ Hz), 4.39 (br d, 1H, $J = 5$ Hz); ^{13}C NMR (D_2O + KOD, 63 MHz) δ 26.35, 33.61, 66.70, 75.12, 179.05 (one carbonyl not observed). Anal. ($C_7H_9NO_5$) C, H, N.

(±)-4-(Spiro-5′-hydantoin)-2-oxabicyclo[3.1.0]hexane-carboxylic Acid ((±)-15). A solution of **14** (7.59 g, 31.6 mmol) in 2 N NaOH (63.2 mL) was stirred for 30 min at ambient temperature. The hydrolysis was then quenched by the addition of 12 N HCl (5.27 mL, 63.2 mmol). The reaction mixture was stirred for 3 h at 0 °C and then vacuum-filtered. The solid collected was dried under vacuum at 50 °C overnight, affording **(±)-15** (6.12 g, 28.9 mmol) in 91% yield: mp 258−261 °C; FDMS $M^+ = 212$; ^{1}H NMR (DMSO-d_6) δ 2.24 (s, 1H), 2.26 (s, 1H), 3.35 (d, 1H, $J = 11$ Hz), 4.05 (d, 1H, $J = 11$ Hz), 4.39 (d, 1H, $J = 5$ Hz); ^{13}C NMR (DMSO-d_6) δ 22.14, 30.75, 65.74, 68.32, 70.61, 156.32, 171.11, 175.63. Anal. ($C_8H_8N_2O_5$) C, H, N.

4-(Spiro-5′-hydantoin)-2-oxabicyclo[3.1.0]hexane-6-carboxylic Acid, (*R*)-2-Phenylglycinol Salt ((−)-16). To a solution of (*R*)-2-phenylglycinol (0.52 g, 3.8 mmol) in ethanol (20 mL) and water (4 mL) was added **(±)-15** (0.80 g, 3.8 mmol). The mixture was heated to reflux, and an additional 1 mL of water was added, producing a homogeneous solution. After approximately 30 min at reflux, the mixture was allowed to cool to ambient temperature. After stirring overnight, the reaction mixture was filtered, washed with 1 mL of a cold 25:5 mixture of ethanol and water, and dried under vacuum at 50 °C overnight, to afford **(−)-16** (0.57 g, 1.63 mmol) in 43% yield as a white solid: mp 198−201 °C; optical rotation $\alpha_D = -111°$ ($c = 1$, H_2O); ^{1}H NMR (DMSO-d_6) δ 2.05 (t, 1H, $J = 3.3$ Hz), 2.20 (d, 1H, $J = 3$ Hz), 3.30 (d, 1H, $J = 11$ Hz), 3.50 (m, 1H), 3.55 (m, 1H), 4.0 (d, 1H, $J = 11$ Hz), 4.1 (m, 1H), 4.18 (d, 1H, $J = 6$ Hz), 7.25 (m, 1H), 7.30 (m, 2H), 7.35 (m, 2H). Anal. ($C_{16}H_{19}N_3O_6$) C, H, N. The enantiomeric excess was determined to be 98.8% by chiral HPLC.[27]

4-(Spiro-5′-hydantoin)-2-oxabicyclo[3.1.0]hexane-6-carboxylic Acid, (*S*)-2-Phenylglycinol Salt ((+)-16). Combined filtrates from various crystallizations of the (*R*)-2-phenylglycinol salt **(−)-16** were concentrated to dryness (4.61 g, 13.2 mmol), then treated with 1 N HCl, and extracted with hot EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated to afford the optically enriched hydantoin 6-carboxylate **(+)-15** (2.8 g, 13.2 mmol, ee ∼ 70%). This was stirred in hot EtOH (100 mL) and treated in one portion with a solution of (*S*)-2-phenylglycinol in hot EtOH (50 mL). The resulting mixture was warmed to boiling as the volume was reduced to approximately 200 mL. The solids were filtered, and the filtrate was allowed to slowly cool to room temperature overnight. Three crops of crystals of **(+)-16** were obtained (2.35 g, 6.7 mmol) in 51% yield from enriched **(+)-15**. Each crop was found to possess an ee > 98% and were thus combined: mp 217−220 °C; optical rotation $\alpha_D = 103°$ ($c = 1$, H_2O). Anal. ($C_{16}H_{19}N_3O_6 \cdot 0.5H_2O$) C, H, N.

(+)-4-(Spiro-5′-hydantoin)-2-oxabicyclo[3.1.0]hexane-6-carboxylic Acid ((+)-15). Resolved salt **(+)-16** (2.30 g, 6.6 mmol) was partitioned between hot EtOAc and 1 N HCl. The layers were separated, and the aqueous one was extracted with hot EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated to afford **(+)-15** (1.3 g, 6.1 mmol) in 93% yield: mp 267−269 °C; FDMS $M^+ = 212$; optical rotation $\alpha_D = 128°$ ($c = 1$, methanol); ee = 99%. Anal. ($C_8H_8N_2O_5 \cdot 0.4$AcOH) C, H, N.

(−)-4-(Spiro-5′-hydantoin)-2-oxabicyclo[3.1.0]hexane-6-carboxylic Acid ((−)-15). Resolved salt **(−)-16** (3.8 g, 10.9 mmol) was partitioned between hot EtOAc and 1 N HCl. The layers were separated, and the aqueous one was extracted with hot EtOAc. The organic phases were combined, dried over $MgSO_4$, and concentrated to afford **(−)-15** (2.15 g, 10.1 mmol) in 93% yield: mp 260−262 °C; FDMS M^+ (+H) = 213; optical rotation $\alpha_D = -134°$ ($c = 1$, methanol); ee = 99.6%. Anal. ($C_8H_8N_2O_5$) C, H, N.

(+)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic Acid ((+)-9). A solution of **(+)-15** (1.2 g, 5.6 mmol) in 2 N NaOH (15 mL) was warmed under reflux overnight. The reaction mixture was cooled to room temperature, treated with 6 N HCl to pH 1, and concentrated to dryness. The solids were reconstituted in H_2O, and 1 N NaOH was added to adjust the pH to 12. The solution was subjected to ion-exchange chromatography (Bio-Rad AG 1-X8, elute with 3 N AcOH) to afford

(+)-9 (0.83 g, 4.4 mmol) in 79% yield as a white solid after evaporation: mp > 275 °C dec; FDMS $M^+(+H) = 188$; optical rotation $\alpha_D = 62°$ ($c = 1$, H_2O). Anal. ($C_7H_9NO_5 \cdot 0.3H_2O$) C, H, N.

(−)-4-Amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic Acid ((−)-9). A solution of **(−)-15** (2.1 g, 10 mmol) in 2 N NaOH (35 mL) was warmed under reflux overnight. The reaction mixture was cooled to room temperature, treated with 6 N HCl to pH 1, and concentrated to dryness. The solids were reconstituted in H_2O, and 1 N NaOH was added to adjust the pH to 12. The solution was subjected to ion-exchange chromatography (Bio-Rad AG 1-X8, elute with 3 N AcOH) to afford **(−)-9** (1.51 g, 8.07 mmol) in 81% yield as a white solid after evaporation: mp > 275 °C dec; FDMS $M^+(+H) = 188$; ^{1}H NMR (D_2O + KOD) δ 1.94 (br d, 1H, $J = 4$ Hz), 2.08 (dd, 1H, $J =$ 4.1 and 4.6 Hz), 3.15 (d, 1H, $J = 9.8$ Hz), 3.95 (d, 1H, $J = 9.8$ Hz), 4.15 (br d, 1H, $J = 5$ Hz); ^{13}C NMR (D_2O + KOD, 63 MHz) δ 26.79, 31.40, 66.50, 73.46, 174.64, 177.57; optical rotation $\alpha_D = -63°$ ($c = 1$, H_2O). Anal. ($C_7H_9NO_5$) C, H, N.

(±)-Ethyl 2-Thiabicyclo[3.1.0]hex-3-ene-6-carboxylate (17). A solution of ethyl diazoacetate (11.4 g, 100 mmol) in thiophene (20 mL) was added dropwise to a 70 °C solution of $Rh_2(OAc)_4$ in thiophene (100 mL). Upon complete addition, the reaction mixture was warmed under reflux for 3 h, concentrated to an orange-colored oil, and purified by preparative HPLC (10% EtOAc/hexanes) to afford **17** (6.51 g, 38.2 mmol) in 38% yield: FDMS $M^+ = 170$; ^{1}H NMR ($CDCl_3$) δ 1.09 (dd, 1H, $J = 3$ and 3 Hz), 1.26 (t, 3H, $J = 7$ Hz), 3.04 (ddd, 1H, J = 2.6, 3.3, and 7.5 Hz), 3.49 (ddd, 1H, $J = 1.8$, 3.0, and 7.5 Hz), 4.15 (q, 2H, $J = 7$ Hz), 5.88 (dd, 1H, $J = 5.6$ and 2.6 Hz), 6.15 (dd, 1H, $J = 1.8$ and 5.6 Hz); ^{13}C NMR ($CDCl_3$) δ 14.29, 23.92, 34.06, 38.99, 60.89, 122.94, 127.66, 174.05. Anal. ($C_8H_{10}O_2S$) C, H.

(±)-Ethyl 4-Hydroxy-2-thiabicyclo[3.1.0]hexane-6-carboxylate (18). A solution of thexylborane was prepared by dropwise addition of a solution of borane−dimethyl sulfide complex (10 M, 5.46 mL, 54.6 mmol mL) to a solution of 2,3-dimethyl-2-butene (6.49 mL, 54.6 mmol) in THF (10 mL) at 0 °C. The solution was stirred for 2 h at this temperature and then was added via cannula to a solution of **17** (9.30 g, 54.6 mmol) in THF (50 mL) at 0 °C. Upon complete addition, the cooling bath was removed and the reaction was allowed to warm to ambient temperature and remain there for 2 h. The reaction mixture was then cooled to 0 °C, and 71 mL of pH 7 phosphate buffer (1 M KH_2PO_4 and 1 M in K_2HPO_4) was added dropwise, followed immediately by $NaBO_3 \cdot 4H_2O$ (10.1 g, 65.5 mmol). The resulting mixture was allowed to stir at ambient temperature for 1 h. The mixture was then partitioned between H_2O and EtOAc, and the product was extracted with EtOAc. The organic layers were combined, dried (Na_2SO_4), and concentrated. The carbinol was purified by preparative HPLC (10% EtOAc/hexanes to 50% EtOAc/hexanes eluent) affording **18** (4.50 g, 23.9 mmol) in 44% yield: FDMS $M^+ = 188$; ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 3H, $J = 7$ Hz), 1.59 (dd, 1H, $J = 3$ Hz and 3 Hz), 2.29−2.32 (m, 1H), 2.64 (dd, 1H, $J = 13$ and 4 Hz), 2.81−2.88 (m, 3H), 4.07 (q, 2H, $J = 7$ Hz), 4.54 (d, 1H, $J = 4$ Hz); ^{13}C NMR ($CDCl_3$) δ 14.09, 25.10, 29.29, 35.86, 37.63, 60.90, 74.42, ester carbonyl not observed. Anal. ($C_8H_{12}O_3S$) C, H.

(±)-Ethyl 4-Oxo-2-thiabicyclo[3.1.0]hexane-6-carboxylate (19). A solution of **18** (5.5 g, 29.2 mmol) in CH_2Cl_2 (25 mL) was added slowly to a solution of oxalyl chloride (3.8 mL, 43.8 mmol) and DMSO (4.1 mL, 58.4 mmol) in CH_2Cl_2 (325 mL) at −78 °C. The rate of addition of **18** was monitored so to maintain an internal temperature below −60 °C. Upon complete addition, the reaction was allowed to slowly warm to −40 °C, then was chilled to −78 °C, and treated dropwise with triethylamine (15.18 g, 150 mmol). The reaction mixture was allowed to warm to room temperature and then was partitioned between 1 N HCl and Et_2O. The product was extracted with Et_2O; the organic phases were combined, washed with brine, dried ($MgSO_4$), and concentrated. The product was purified by preparative HPLC (5% EtOAc/hexanes to 75% EtOAc/hexanes) to afford **19** (3.34 g, 18 mmol) in 61% yield: mp 56−57 °C; FDMS $M^+ = 186$; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, $J = 7$ Hz), 2.34 (dd, 1H, $J = 3$ Hz and 3 Hz), 2.58−2.60 (m, 1H), 3.19−3.23 (m, 1H), 3.30 (d, 2H, $J = 2$ Hz), 4.16 (q, 2H, $J = 7$ Hz); ^{13}C NMR ($CDCl_3$) δ 14.15, 29.26, 30.80, 34.08, 34.93, 61.71, 168.83, 206.40. Anal. ($C_8H_{10}O_3S$) C, H.

(±)-Ethyl 4-(Spiro-5′-hydantoin)-2-thiabicyclo[3.1.0]-hexane-6-carboxylate (20). A solution of **19** (3.22 g, 17.3 mmol) in EtOH (25 mL) and H_2O (10 mL) at ambient temperature was treated consecutively with $(NH_4)_2CO_3$ (3.37 g, 43.3 mmol) and KCN (1.41 g, 21.6 mmol) and warmed at 35 °C until the reaction was judged complete by TLC. The reaction mixture was acidified with 6 N HCl, aqueous NaCl was added, and the product was extracted with EtOAc. All of the organic phases were combined, dried ($MgSO_4$), and concentrated. Crystallization from 2-propanol afforded **20** (2.25 g, 8.8 mmol) in 51% yield: mp 197−200 °C; FDMS $M^+ = 256$; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, 3H, $J = 7$ Hz), 2.16 (dd, 1H, J = 3.4 and 3.9 Hz), 2.39 (dd, 1H, $J = 3.9$ and 6.9 Hz), 2.72 (dd, 1H, $J = 1$ and 12.8 Hz), 2.96 (dd, 1H, $J = 3.4$ and 6.9 Hz), 3.17 (dd, 1H, $J = 1$ and 12.8 Hz), 4.08 (q, 2H, $J = 7$ Hz), 8.07 (d, 1H, $J = 1$ Hz), 10.79 (s, 1H) (also observed protons for 2-propanol, integrated ratio = 0.75 eq); ^{13}C NMR (DMSO-d_6) δ 13.99, 24.33, 25.44, 29.99, 33.99, 34.90, 60.55, 61.98, 70.55, 155.87, 169.93, 174.85. Anal. ($C_{10}H_{12}N_2O_4S \cdot 0.75$ 2-propanol) C, H, N.

(±)-4-Amino-2-thiabicyco[3.1.0]hexane-4,6-dicarboxylate ((±)-10). A solution of **20** (0.85 g, 3.30 mmol) in 2 N NaOH (20 mL) was warmed under reflux for 4 days. The reaction mixture was then acidified with 6 N HCl and concentrated to dryness. The solid was reconstituted in H_2O at pH 11, applied to AG1-X8 anion-exchange resin, eluted with 3 N AcOH, and concentrated to dryness. The product was triturated in a mixture of hot H_2O/2-propanol mixture and filtered to afford **(±)-10** (0.31 g, 1.5 mmol) in 46% yield: mp > 250 °C; FDMS $M^+ = 203$; ^{1}H NMR (D_2O + KOD) δ 1.55 (dd, 1H, $J = 3.1$ and 3.8 Hz), 2.02 (dd, 1H, $J = 4.1$ and 6.9 Hz), 2.25 (d, 1H, $J = 12.4$ Hz), 2.41 (dd, 1H, $J = 3.1$ and 6.9 Hz), 2.79 (d, 1H, $J = 12.4$ Hz); ^{13}C NMR (D_2O + KOD) δ 24.89, 26.60, 35.16, 36.11, 67.32, 177.31, 178.10. Anal. ($C_7H_9NO_4S \cdot 0.5H_2O$) C, H, N.

(±)-4-(Spiro-5′-hydantoin)-2-thiabicyclo[3.1.0]hexane-6-carboxylate ((±)-21). To a 1000-mL recovery flask were added **20** (93.6 g, 365 mmol) and 365 mL (730 mmol) of 2 N NaOH. The resulting red homogeneous solution was stirred at ambient temperature for approximately 30 min. HPLC analysis showed consumption of starting material and formation of **21**. The reaction was quenched by the addition of 12 N HCl (60.9 mL, 730 mmol). The resulting mixture was cooled to 0 °C in an ice−water bath and stirred for 3 h before being vacuum-filtered through a glass frit. The precipitated product was dried under vacuum at 50 °C overnight, affording **(±)-21** (72.3 g, 317 mmol) in 87% yield as an off-white solid: mp > 280 °C dec; $M^+ = 228$; ^{1}H NMR (DMSO-d_6) δ 2.02 (t, 1H, $J =$ 2 Hz), 2.30 (dd, 1H, $J = 3$ and 3 Hz), 2.69 (d, 1H, $J = 11$ Hz), 2.89 (dd, 1H, $J = 4$ and 4 Hz), 3.15 (d, 1H, $J = 11$ Hz); ^{13}C NMR (DMSO-d_6) δ 24.62, 29.73, 34.19, 34.87, 70.70, 156.01, 171.62, 175.08. Anal. ($C_8H_8N_2O_4S$) C, H, N.

(±)-4-(Spiro-5′-hydantoin)-2-thiabicyclo[3.1.0]hexane-6-carboxylate (*R*)-2-Phenylglycinol Salt ((−)-22). To a 5-L three-neck round-bottom flask were added **(±)-21** (70.1 g, 307 mmol) and (*R*)-2-phenylglycinol (42.1 g, 307 mmol) in 3500 mL of 95% ethanol (toluene-denatured) and 700 mL of water. The resulting brown heterogeneous mixture was heated to reflux. At reflux all the solid material dissolved, producing a homogeneous dark amber solution. After refluxing for approximately 15 min, the reaction mixture was allowed to slowly cool to room temperature. After stirring at room temperature overnight (approximately 16 h) the mixture was vacuum-filtered through a glass frit. The solid collected on the frit was rinsed with 2 × 5-mL portions of a cold mixture of 95% ethanol (toluene-denatured) and water (50:10) before being dried under vacuum at 60 °C overnight. The product **(−)-22** (40.16 g, 110 mmol) was obtained in 36% yield as a white solid: mp 228−230 °C; ^{1}H NMR (DMSO-d_6) δ 1.95 (t, 1H, $J = 3$ Hz), 2.13 (m, 1H), 2.68 (d, 1H, $J = 11$ Hz), 2.71 (m, 1H), 3.11 (d, 1H, $J = 11$ Hz),

3.59 (m, 2 H), 4.1 (m, 1H), 7.25 (m, 1H), 7.31 (m, 2H), 7.38 (m, 2H); 99.9% ee (by HPLC); optical rotation $[\alpha]_D = -149.56$ (c = 1.0, methanol). Anal. ($C_{16}H_{19}N_3O_5S \cdot 0.75H_2O$) C, H; N: calcd, 11.10; found, 10.28.

(±)-4-(Spiro-5′-hydantoin)-2-thiabicyclo[3.1.0]hexane-6-carboxylate (*S*)-2-Phenylglycinol Salt ((+)-22). The filtrate from resolution of 5.5 g of **(±)-21** using (*R*)-2-phenylglycinol was concentrated under reduced pressure, and the residue was dissolved in 50 mL of H_2O. As 7 mL of 6 M HCl was added, a precipitate formed. The precipitate was collected by filtration and washed with H_2O. The solid was dried in a vacuum oven at 80 °C for 6 h to afford 2.26 g of optically enriched **(+)-21**. The acid was combined with 1.35 g of (*S*)-2-phenylglycinol, 113 mL of ethanol, and 23 mL of H_2O. The mixture was heated to reflux to afford a solution and was then allowed to cool to room temperature overnight. The resulting solid was collected by filtration and dried in a vacuum oven at 50 °C for 6 h to afford 2.42 g of **(+)-22** (2.15 g corrected for residual ethanol, 60% yield from enriched **(+)-21**) as a white solid: 98.4% ee by chiral HPLC; mp 230−232 °C; ^{1}H NMR (DMSO-d_6) δ 1.93 (t, 1, J = 5 Hz), 2.11 (dd, 1H, J = 5 and 7 Hz), 2.68 (m, 2H), 3.11 (d, 1H, J = 12 Hz), 3.55 (m, 2H), 4.10 (dd, 1H, J = 5 and 7 Hz), 7.25−7.45 (m, 5H), 8.12 (br s, 1H); optical rotation $[\alpha]_D$ = 147.87 (c = 1.0, methanol). Anal. ($C_{16}H_{19}N_3O_5S \cdot 0.90H_2O$) C, H; N: calcd, 11.01; found, 10.49.

(−)-4-(Spiro-5′-hydantoin)-2-thiabicyclo[3.1.0]hexane-6-carboxylate ((−)-21). To a 2-L three-neck round-bottom flask were added **(−)-22** (113 g, 308 mmol) and 675 mL of water. The resulting heterogeneous brown mixture was stirred at ambient temperature for approximately 15 min during which time all the solid material was observed to dissolve producing a homogeneous brown solution. HCl (12 N) (28.4 mL, 339 mmol) was then added dropwise over 5 min. An off-white precipitate formed. An ice−water bath was placed underneath the flask and its contents stirred at 0 °C for 60 min before being vacuum-filtered through a glass frit. The off-white solid collected on the frit was rinsed with 2 × 5-mL portions of cold water before being dried under vacuum at 60 °C overnight to afford **(−)-21** (61.72 g, 270.5 mmol) in 88% yield as an off-white solid: mp > 280 °C dec; M^+ = 228; ^{1}H NMR (DMSO-d_6) δ 2.01 (t, 1H, J = 2 Hz), 2.29 (dd, 1H, J = 3 and 8 Hz), 2.69 (d, 1H, J = 12 Hz), 2.89 (dd, 1H, J = 3 and 8 Hz), 3.15 (d, 1H, J = 12 Hz), 8.10 (br s, 1H), 10.75 (br s, 1H), 12.52 (br s, 1H); ^{13}C NMR (DMSO) δ 24.61, 29.70, 34.22, 34.86, 70.67, 155.98, 171.56, 175.02; optical rotation $[\alpha]_D = -202.3$ (c = 1.0, methanol). Anal. ($C_8H_8N_2O_4S$) C, H, N.

(+)-4-(Spiro-5′-hydantoin)-2-thiabicyclo[3.1.0]hexane-6-carboxylate ((+)-21). To a slurry of 2.32 g (2.07 g corrected for ethanol content, 98.4% ee) of **(+)-22** in 18 mL of H_2O was added 2.1 mL of 3 N HCl. The resulting slurry was stirred for 5 min at room temperature and for 40 min at 0 °C. The solid was collected by filtration and rinsed with H_2O. The solid was dried in a vacuum oven at 70 °C for 1.5 h to afford 1.23 g (95%) of **(+)-21** as a tan solid: 98.6% ee by chiral HPLC; mp >280 °C dec; FDMS M^+ = 228; ^{1}H NMR (DMSO-d_6) δ 2.01 (t, 1H, J = 4 Hz), 2.32 (dd, 1H, J = 3 and 8 Hz), 2.67 (d, 1H, J = 12 Hz), 2.90 (dd, 1H, J = 3 and 8 Hz), 3.15 (d, 1H, J = 12 Hz), 8.12 (br s, 1H), 10.75 (br s, 1H), 12.51 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 24.61, 29.70, 34.23, 34.86, 70.67, 155.98, 171.56, 175.02; optical rotation $[\alpha]_D = 203.9$ (c = 1.0, methanol). Anal. ($C_8H_8N_2O_4S$) C, H, N.

(−)-4-Amino-2-thiabicyco[3.1.0]hexane-4,6-dicarboxylate ((−)-10). To 30.6 g (134 mmol) of (−)-**21** in an autoclave was added 450 mL (900 mmol) of 2 M aqueous sodium hydroxide. The solution was heated at 100 °C for 23 h. The reaction mixture was filtered though Celite, and the resulting orange/brown solution was evaporated to approximately one-half the original volume using a rotary evaporator (bath 50 °C). The solution was cooled in an ice/water bath, and 57 mL of 12 M HCl was added over 5 min. The bath was removed, and additional 12 N HCl was added to adjust the pH to approximately 3. The slurry was stirred for 30 min at room temperature and for 1 h in an ice/water bath. The solid was collected by filtration and rinsed with cold water. The collected solid was dried in a vacuum oven at 70 °C for 18 h to afford crude **(−)-10** (22.0 g, 108.2 mmol) in 81% yield. This material was combined with a separate batch of similarly prepared material (24.3 g, 120 mmol) in 231 mL of water, and the thick slurry was heated at reflux for 2 h. The mixture was allowed to stir at room temperature overnight and subsequently for 1.5 h in an ice/water bath. The solid was collected by filtration, washed with 25 mL of cold water, and dried at 75 °C, affording **(−)-10** (41.69 g 205.0 mmol) in 76% overall yield: mp 273−274 °C dec; FDMS M^+ = 203.4; ^{1}H NMR (D_2O + KOD, 300 MHz) δ 1.58 (t, 1, J = 3.5 Hz), 2.07 (dd, 1, J = 7.0 and 4.2 Hz), 2.29 (d, 1, J = 12.5 Hz), 2.44 (dd, 1, J = 7.0 and 3.0 Hz), 2.84 (d, 1, J = 12.5 Hz); ^{13}C NMR (D_2O + KOD, 63 MHz) δ 28.71, 30.64, 36.43, 37.20, 70.04, 176.55, 179.25; optical rotation $[\alpha]_D = -159.6$ (c = 1, H_2O). Anal. ($C_7H_9NO_4S$) C, H, N.

(+)-4-Amino-2-thiabicyco[3.1.0]hexane-4,6-dicarboxylate ((+)-10). A solution of 1.12 g (4.9 mmol) of **(+)-21** in 15 mL of 2 M NaOH (29 mmol, 6 equiv) was placed in a Teflon sleeve inside a 50-mL Parr bomb. The mixture was heated at 97 °C for 16 h. After cooling to room temperature, the solution was transferred to a flask and treated with 12 N HCl to lower the pH to about 3 (2.5 mL). The resulting slurry was stirred at room temperature for 30 min and at 0 °C for 1 h. The solid was collected by filtration and rinsed with cold H_2O. After drying with vacuum on the filter for 30 min, the soapy solid was dried in a vacuum oven at 70 °C for 1 h to afford 0.78 g (78%) of **(+)-10** as a white solid: mp >280 °C dec; FDMS M^+ = 203; ^{1}H NMR (D_2O + KOD) δ 1.68 (t, 1H, J = 3.7 Hz), 2.15 (dd, 1H, J = 4.0 and 7.1 Hz), 2.39 (d, 1H, J = 12.4 Hz), 2.55 (dd, 1H, J = 3.3 and 7.1 Hz), 2.94 (d, 1H, J = 12.4 Hz); ^{13}C NMR (D_2O + KOD) δ 27.82, 29.92, 38.08, 38.98, 70.43, 180.77, 181.20; optical rotation $[\alpha]_D = 168.2$ (c = 1.0, H_2O). Anal. ($C_7H_9NO_4S$) C, H, N.

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Supporting Information Available: X-ray crystallographic data pertaining to **(−)-9** and tablulated combustion analyses on new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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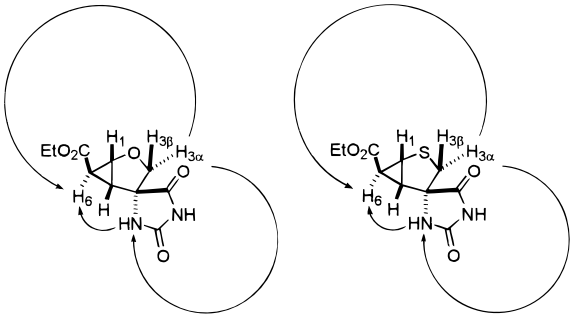

(27) The enantiomeric purities of (−)-**15**, (+)-**15**, (−)-**21**, and (+)-**21** were determined by chiral HPLC (see General Methods, Experimental Section, for details). Under these conditions, (−)-**15** and (+)-**15** exhibited elution times of 9.5 and 8.3 min, respectively. (−)-**21** and (+)-**21** exhibited elution times of 10.5 and 9.4 min, respectively.
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