Syntheses and Conformational Analyses of Glutamate Analogs: 2-(2-Carboxy-3-substituted-cyclopropyl)glycines as Useful Probes for Excitatory Amino Acid Receptors

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Received April 18, 1995[⊗]

An hypothesis that each subtype of glutamate receptors requires a specific conformation of L-glutamate for its selective activation was examined using the conformationally constrained analogs of L-glutamate, L-2-(2-carboxycyclopropyl)glycines (CCGs), and L-2-[2-carboxy-3-(methoxymethyl)cyclopropyl]glycines (MCGs). All MCG isomers were newly synthesized in a stereoselective manner via the common synthetic intermediate **5a** starting with the oxazolidine aldehyde **1**. The synthesis of the four MCG isomers was characterized by a stereoselective inversion of α-cyclopropyl acyl anion (e.g., from **10** to **11**). The spectroscopic studies, in particular, pH vs *J* correlation experiments of CCGs and MCGs using ^{1}H NMR and their molecular mechanics calculations, revealed that these analogs possessed an antiperiplanar conformation regarding the H−C2−C1′−H bond as a majority among the other possible rotamers in aqueous solution. The fact that each CCG and MCG exhibited potent and selective activities to the distinct types of glutamate receptors allowed us to extract an active conformation of L-glutamate. Thus, the conformational requirement of metabotropic glutamate receptors was speculated to be the anti-anti conformation (aa-A) because the conformations of CCG-I and *cis* and *trans*-MCG-I, selective agonists of the receptors, closely mimicked the rotamer A of L-glutamate. On the other hand, *N*-methyl-D-aspartate and kainate receptors, representative ionotropic glutamate receptors, would require glutamate's g^+g^+ rotamer E which was deduced from the conformation−activity relationship studies of the selective agonists CCG-IV, *cis*-MCG-IV, and *trans*-MCG-IV and the related analogs.

Introduction

L-Glutamic acid functions at many synapses in mammalian central nervous systems (CNS) as an excitatory neurotransmitter[1] and is implicated in the construction of memory and early learning[2] as well as in the pathogenesis of neuron damage to cause various neuronal diseases.[3] As with other neurotransmitter's receptors, glutamate receptors have been classified into two types: the ionotropic (iGluRs) and metabotropic (mGluRs) types. The former are further subdivided into *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainic acid (KA) receptors according to their selective actions as agonists.[1] The mGluRs are coupled to intracellular second-messenger systems.[4,5] It is suggested that glutamate neurotransmission in different synapses is mediated through distinct receptors and combinations of different receptors.[6] Therefore, development of selective and powerful agonists and antagonists seems to be essential for the investigation of molecular mechanisms of glutamate receptors and their physiological functions.

Our studies started from the hypothesis that each receptor subtype would require a particular conformation of glutamate for its selective activation, i.e., conformational requirements for activating receptors.[7] Thus, we synthesized four diastereomers of L-2-(carboxycyclopropyl)glycine (CCGs), which restricted the conformation of glutamate to an extended or folded form,[8] and examined their electrophysiological as well as biochemical characteristics in various biological preparations (Figure 1).[9,10] Among the four diastereomers of CCG, one of the extended types (CCG-I) was identified as a selective and powerful agonist of mGluRs.[10] On the other hand, CCG-IV, one of the folded types, exhibited potent affinity to NMDA receptors.[9a−d] These results strongly suggested that the conformational requirement of mGluRs was an extended conformation of glutamate, while that of NMDA receptors was a folded conformation. The other isomers, CCG-II and -III, were not potent agonists but were inhibitors of glutamate transport systems at the excitatory synapses.[9b,d−f] Thus, CCGs are not only used as a useful pharmacological tool in the neuroscience field but also provide proof that a specific conformation of glutamate is one of the most important factors for activation to distinct types of receptors.[8−11]

We wish to describe in this paper the syntheses and the conformational analyses of 3′-substituted CCG analogs, which would provide further experimental support to the above hypothesis.

Results and Discussion

1. Syntheses of MCGs. On the basis of the studies of CCGs, we designed 3′-substituted analogs of CCGs in which the substituent on the cyclopropyl ring would provide further information regarding the structural requirements for activating glutamate receptors. Furthermore, these studies would extend the scope for the synthesis of polymer-bounded CCG analogs, effective antagonists, and photoaffinity-labeled analogs. As a substituent at C3′, an hydroxymethyl group or its ester or ether analogs should be suitable for the above-mentioned purpose. Our first choice was the 3′-methoxymethyl-substituted CCGs, L-2-[2-carboxy-3-(meth-

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1995.

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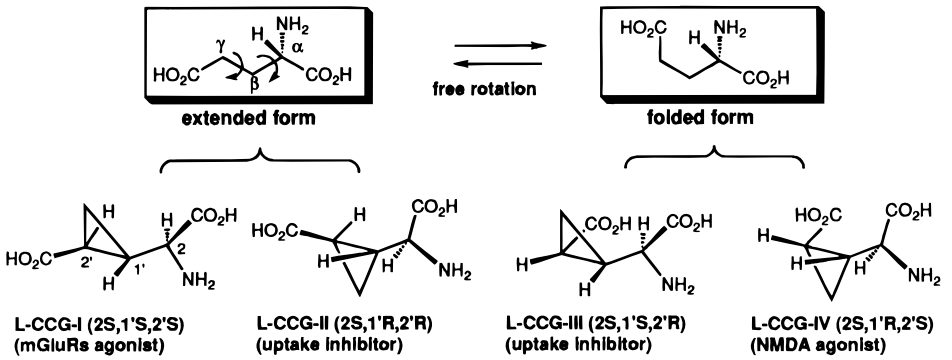


Figure 1. Structures of L-2-(carboxycyclopropyl)glycines CCG-I–IV.

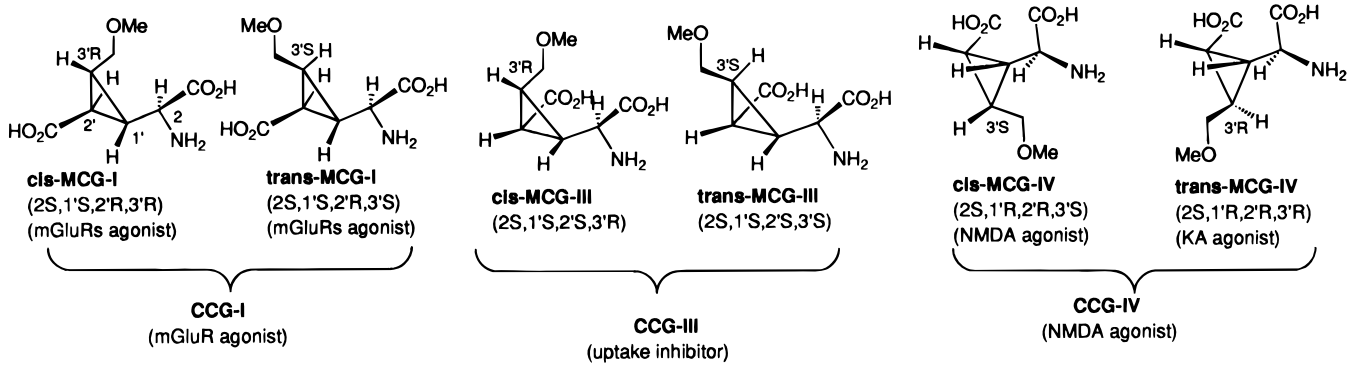
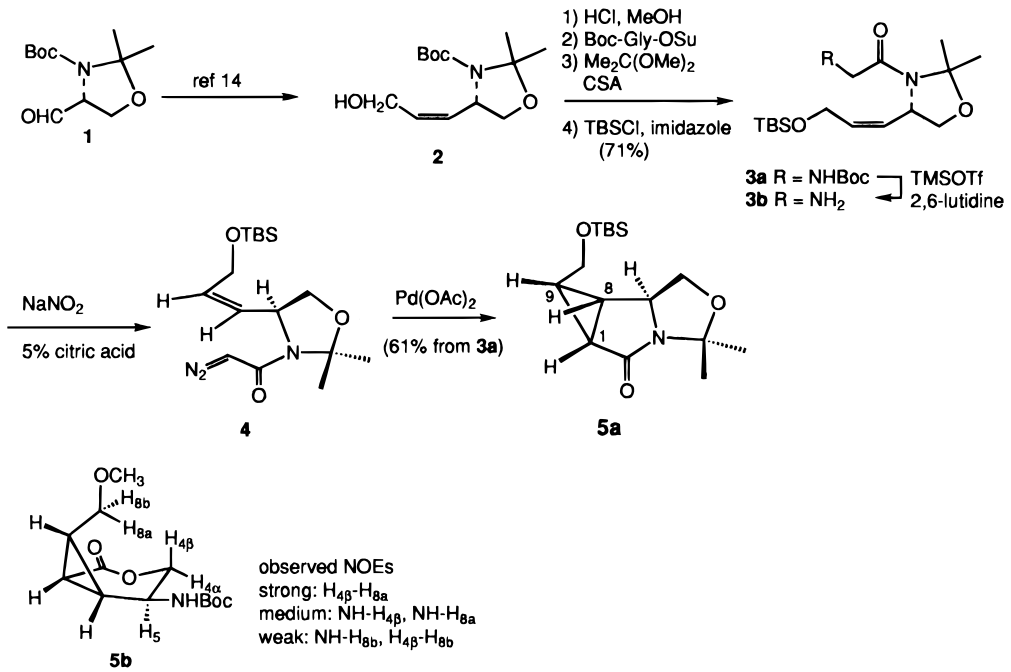


Figure 2. Structures of L-2-[2-carboxy-3-(methoxymethyl)cyclopropyl]glycines *cis*- and *trans*-MCGs.

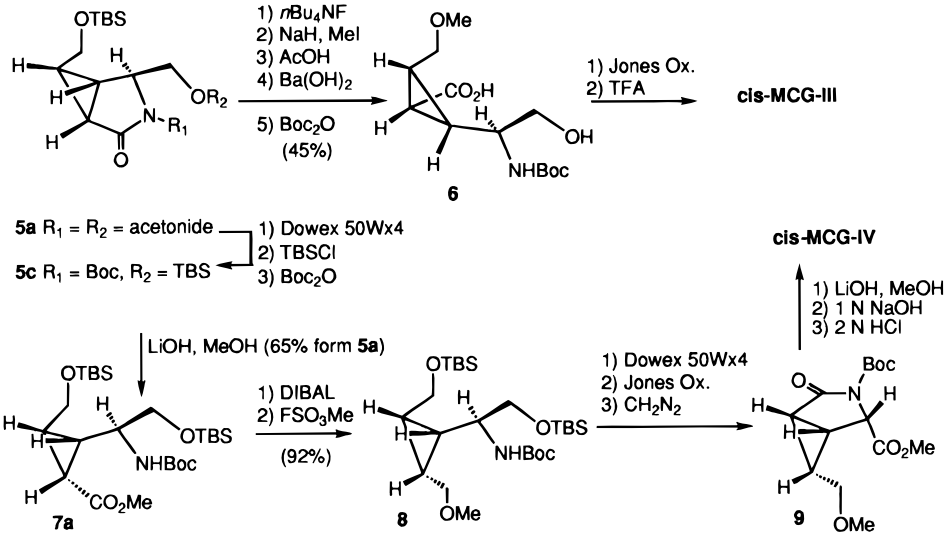
Scheme 1



oxymethyl)cyclopropyl]glycines (MCGs).[12] The methoxymethyl group would be chemically stable under physiological conditions. The synthesis involved (hydroxymethyl)cyclopropyl intermediates which would be convertible to a variety of 3′-alkoxy- or -(aryloxy)methyl-substituted CCG analogs. Thus, we synthesized both the cis and trans diastereomers at C3′ of CCG-I, -III, and -IV, a total of six MCG isomers (Figure 2).

Synthesis of *cis*-MCG-III and -IV via the Intramolecular Cyclopropanation of (*Z*)-Allyl Ether 3. We planned to employ the cycloadduct **5a** as the common synthetic intermediate for all MCGs (Scheme 1). Modification of the amide carbonyl and [(*tert*-butyldimethylsilyl)oxy]methyl groups of **5a** and/or inversion(s) of their configurations would open stereoselective routes to all MCGs. The synthesis began with the Horner–Emmons reaction of the aldehyde **1**,[13] prepared from D-serine. Reduction of the resulting (*Z*)-α,β-unsaturated ester with diisobutylammonium hydride (DIBAL-H) in toluene furnished (*Z*)-allyl alcohol **2**.[14] This was converted to the Boc-glycyl allyl ether **3a** by the following sequence of reactions: (1) removal of all protecting groups with HCl in MeOH, (2) condensation of the resulting amino group with (*tert*-butoxycarbonyl(Boc))glycine, and (3) introduction of an acetonide followed by protection with a *tert*-butyldimethylsilyl

Scheme 2



(TBS) group. The requisite amine **3b** was prepared from **3a** by the chemoselective removal of its Boc group in the presence of the TBS and acetonide groups using trimethylsilyl trifluoromethanesulfonate (TMSOTf)/2,6-lutidine.[15] Diazotization of **3b** with $NaNO_2$ in 5% citric acid gave the diazoacetoamide **4**. Intramolecular cyclopropanation of **4** was effected by a catalytic amount of $Pd(OAc)_2$ to give in 61% yield the exo-type cycloadduct **5a** as the sole stereoisomer.[16,17] The stereochemistry was unambiguously assigned to **5a** by converting it into the corresponding δ-lactone **5b**. The strong NOE observed between $H_{4\beta}$ and H_{8a} clearly indicated the lactone **5b** to have the 1*S*,5*S*,6*S*,7*R* stereochemistry.

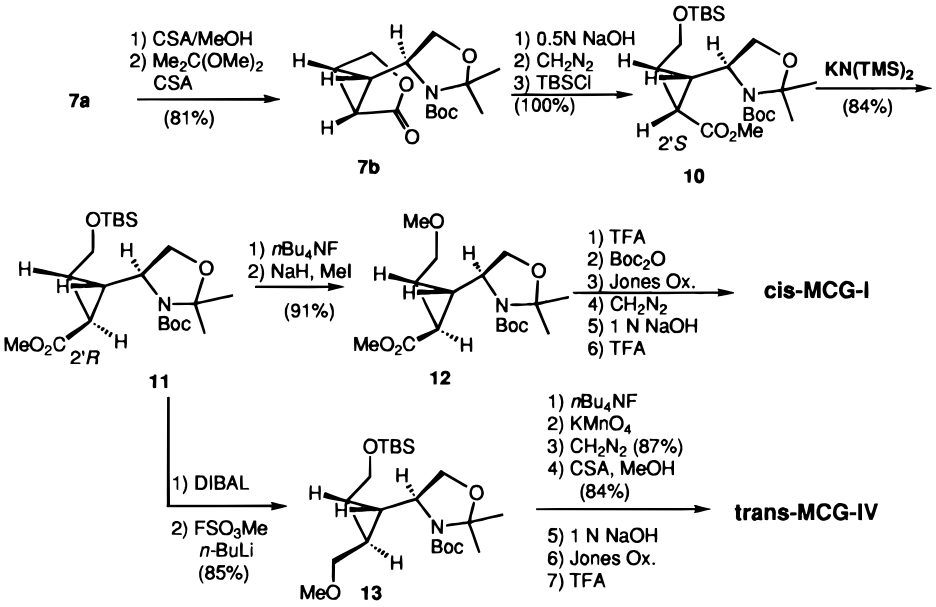
Treatment of the cycloadduct **5a** with tetra-*n*-butylammonium fluoride followed by MeI/NaH gave the corresponding methyl ether, which, upon hydrolysis of the lactam ring followed by protection of the resulting amine with a Boc group, furnished desired glycinol **6**. Jones oxidation of the primary alcohol and subsequent removal of the Boc group with trifluoroacetic acid (TFA) yielded *cis*-MCG-III.

The synthesis of *cis*-MCG-IV required selective transformations of the amide carbonyl group and the TBSO methyl group to the methoxymethyl group and the carboxyl group, respectively. Removal of all protecting groups of the cycloadduct **5a** under acidic conditions followed by reprotection of the resulting amide and hydroxyl groups gave *N*-Boc-γ-lactam **5c**. The lactam ring of **5c** was cleaved by a catalytic amount of LiOH in MeOH to give **7a** (65% overall yield from **5a**). Reduction of the ester group with DIBAL-H and subsequent methylation with *n*-butyllithium/methyl fluorosulfate gave desired 3′-methoxymethyl ether **8**.[18] Then, sequential treatment of **8** with (i) Dowex 50W × 4 (H^+)/MeOH, (ii) Jones reagent, and (iii) diazomethane gave γ-lactam **9**, which, upon treatment with LiOH/MeOH, afforded protected *cis*-MCG-IV. Deprotection with NaOH followed by 2 N HCl furnished *cis*-MCG-IV. Thus, the syntheses of the *cis*-3′-methoxymethyl CCG-III and -IV, each of which has the folded substructure of glutamate, were accomplished (Scheme 2).

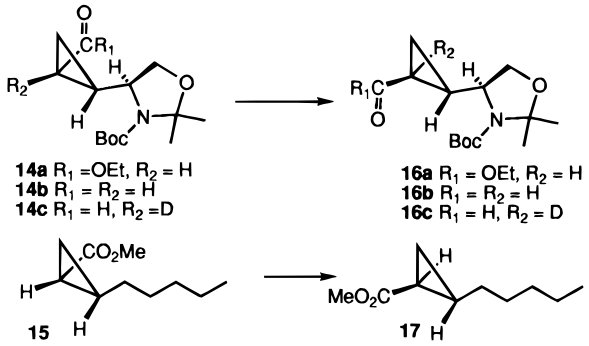
Inversion of α-Cyclopropyl Acyl Anion. Synthesis of *cis*-MCG-I and *trans*-MCG-I, -III, and -IV. Since all the substituents of **7a** were located cis on the cyclopropane ring, the isomerization of the C2′ ester group to the thermodynamically more stable 2′*R* (trans) isomer appeared feasible. The inversion product could be readily transformed into both *cis*-MCG-I and *trans*-MCG-IV (Scheme 3). Initial attempts at the inversion using (2′*S*)-**7a** with several bases such as lithium diisopropylamide (LDA), potassium bis(trimethylsilyl)amide ($KN(TMS)_2$), or *t*-BuOK were not succesful but rather gave, exclusively, γ-lactam due to anion formation at the amide nitrogen. Therefore, **7a** was converted to the acetonide **10** by the following sequence of reactions: (i) removal of the TBS group with *dl*-camphorsulfonic acid (CSA)/MeOH, (ii) simultaneous acetonide and γ-lactone formation with 2,2-dimethoxypropane/CSA, (iii) hydrolysis of the resulting lactone **7b** with 0.5 N NaOH, (iv) esterification with diazomethane, and (v) protection of the resulting alcohol with TBSCl/imidazole (81% overall yield from **7a**). Compound **10**, upon treatment with $KN(TMS)_2$ in THF followed by acetic acid quench, gave in 84% yield the desired trans isomer **11**, exclusively.[19] The inversion product **11** was efficiently converted to *cis*-MCG-I via **12** according to the same manner described as above. *trans*-MCG-IV was prepared from **11** via **13** simply by converting its ester group into the methoxymethyl group and the TBSO methyl group to the carboxyl group, respectively (Scheme 3).

The successful inversion of **10** to **11** led us to extensively examine work concerning (i) inversion mechanism and (ii) applicability to the other substrates such as cis-disubstituted cyclopropyl esters **14a**[20] and **15**.[21] Thus, the following experiments were carried out: (i) the treatment of **14a** and **15** with $KN(TMS)_2$ gave in excellent yields the trans isomers **16a** and **17**, respectively, (ii) the use of other bases such as KH, NaH, *t*-BuOK, and NaOMe, resulted in complete recovery of the starting materials, (iii) the treatment of each **10**, **14a**, and **15** with $KN(TMS)_2$ followed by CD_3CO_2D quench gave the corresponding inversion products in which no D atom was incorporated into C2′, (iv) the treatment of **10**, **14a**, and **15** with $NaOCD_3/CD_3OD$ gave neither inversion nor incorporation of D into their C2′ position (vide infra), and (v) the reaction of **10** was found to proceed even with the use of 0.3 equiv of $KN(TMS)_2$ to give **11** (81%). These results suggest that the trans esters are the thermodynamically more favored products than the cis isomers due to steric reasons. An initially formed α-cyclopropyl ester anion might rapidly invert to either a tetrahedral or a planar configuration, which

Scheme 3



Scheme 4



is inter- or intramolecularly reprotonated by the resulting $HN(TMS)_2$ to give thermodynamically more favored trans esters (Scheme 4). In these cases, the acidity of the α-proton might be decreased due to a difficulty in the formation of a planar enolate in a cyclopropane.[22,23]

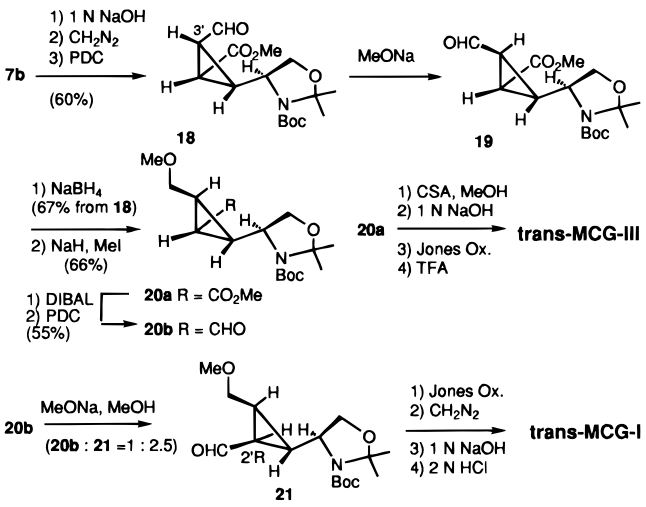
On the other hand, it has been reported that the α-proton in cyclopropanes with an electron-withdrawing substituent (CN, ketones, sulfones, etc.) is more acidic than that of the open chain analog owing to the greater *s*-character of the C−H bond in cyclopropane where the formation of the tetrahedron anion is suggested, i.e., the anion generated from an α-cyclopropyl nitrile was quenched by the solvent (NaOMe/MeOD) to give an α-deuterio product with retention of its configuration.[24] Thus, we turned our attention to examine the inversion studies using *cis*-α-cyclopropyl aldehyde **14b** of which C2′−H might be more acidic than the corresponding ester **14a**.[25] The treatment of **14b** in $CD_3OD/NaOCD_3$ at reflux gave a mixture of the cis and trans aldehydes **14c** and **16c** within 1 h (cis/trans = 1:2) in which a D atom was completely incorporated into the C2′ position. The ratio after 48 h changed to **14c**/**16c** = 1:>99. On the other hand, starting from the trans aldehyde **16b** (reflux, 5 h), none of the cis isomer **14b** or **14c** was detected and a mixture of starting **16b** and deuterated **16c** was recovered (**16b**/**16c** = 4:1). These results indicated that (i) the incorporation rate of a D atom into the trans isomer was much slower than that of cis isomer **14b** and (ii) protonation of an α-cyclopropyl formyl anion might be under an equilibrium to both cis and trans products to give the thermodynamically more favored trans isomer, exclusively.

This method was well applied to the aldehyde **18**, prepared from **7b** in three steps, which underwent desired inversion at C2′ to give the trans aldehyde **19**, exclusively. Thus, the present method would be useful for the syntheses of various MCGs. Reduction of **19** and subsequent methylation gave **20a**, which, upon standard synthetic procedure described as above, gave *trans*-MCG-III. We, next, examined the synthesis of *trans*-MCG-I which required an inversion of the C2′ ester group of **20a**. However, this transformation was not successful, and **20a** was recovered. On the other hand, the corresponding aldehyde **20b** with NaOMe/MeOH underwent inversion to give a mixture of desired 2′*R* isomer **21** and the starting material **20b** (**21**/**20b** = 2.5:1). The major isomer **21** was converted into *trans*-MCG-I according to the same procedures as above (Scheme 5). Thus, all six isomers of MCG were prepared from the cycloadduct **5a** in a highly stereoselective manner.

2. Neurobiological Activities of MCGs. The neurobiological actions of the synthetic MCGs in the mammalian CNS were examined mainly using electrophysiological methods (Table 1).[12,26−28] Both *cis*- and *trans*-MCG-I did not cause any depolarizing activities even at high concentrations (>1 mM) in the new born rat isolated spinal cord[12b,27,28] However, they potentially inhibited presynaptic neurotransmission.[27,28] Such inhibition has been known as one of the characteristic physiological actions of mGluRs.[29] Accordingly, they were classified as potent agonists of mGluRs. The potency of *trans*-MCG-I was 2 times of that of the cis isomer and almost the same as that of CCG-I.[10c,d] Neither *cis*- nor *trans*-MCG-III showed any activities (>1 mM) to the glutamate receptors and the transport system,[12a] although their parent compound, CCG-III, was a potent inhibitor of glutamate transport systems.[9] *cis*-MCG-IV retained the agonistic activity to NMDA receptors, while its activity was less than that of CCG-IV. To our surprise, *trans*-MCG-IV, which is a configurational isomer at C3′ of *cis*-MCG-IV, was found to activate KA receptors (vide infra).[12a,26]

3. Conformational Analyses of CCGs and MCGs.

Scheme 5



It would be possible to assume that the conformer of CCG or MCG which binds to glutamate receptors resembles one of the stable (minimum energy) conformers in aqueous solution. Thus, we next carried out the conformational analysis of CCGs and MCGs using ^{1}H NMR: (i) pH change and (ii) addition of inorganic metal cation. Initially, pK_a values of all CCG derivatives and the representative glutamate agonists[30−32] were measured (Table 2). These data indicated that all polar groups of CCGs and MCGs were ionized under the physiological conditions. The ^{1}H NMR in the presence of inorganic metal cations (Na^+, NH_4^+, Ca^{2+}, and Mg^{2+}), which are widely distributed in the CNS, showed no significant changes to either the signal pattern of all protons or the *J* value, suggesting that CCGs do not change their conformations by chelating with these metals.

In pH vs *J* correlation studies, each CCG isomer showed large $J_{2H\text{-}1'H}$ values (Figure 3). Among all CCGs, the *J* values of CCG-I and -III were rather constant in all pH ranges probably due to their H−C2−C1′−H antiperiplanar conformations which are favored in view of both steric hindrance as well as electrostatic effect. On the other hand, the *J* values of CCG-II and -IV were slightly affected by the pH change (pD 4, J = 11.0 Hz, and pD 8, J = 8.5 Hz for CCG-IV).[33] In these cases, their C2−C1′ bonds might fluctuate to some degree due to a balance between an electrostatic contribution (zwitterion formation) from the γ-carboxyl group to the α-amino group and a steric hindrance effect. The splitting *J* values of glutamate at C2 would be a result of a certain bias of several conformations (see Figure 5).

The *J* values of both *cis*-MCG-I and -IV were rather constant in all pH ranges compared to those of CCG-I and -IV.[34] On the other hand, *trans*-MCGs showed almost the same pattern as CCGs. Moreover, NOE experiments of *cis*-MCGs clearly indicated that the dihedral angle of H−C2−C1′−H is antiperiplanar, since a strong NOE between C2−H and one of the C3′ methylene protons was observed (Figure 4). These results suggest that *cis*-MCGs possess preferentially an antiperiplanar conformation owing to the increased steric hindrance effect of the substituent.

Examined next was molecular mechanics calculation of CCGs using the QUANTA/CHARMm system. The molecules were treated as their fully charged ionic states where the distance-dependent dielectric term (ϵ = 80) was employed.[35] The energy was calculated for each conformation which was obtained by rotating the C2−C1′ bond by 10°.[36] The global minimum conformation of all CCGs was their antiperiplanar one at H−C2−C1′−H (Table 3). The other energy minimum conformations had their dihedral angles to be ±60°. The C3′ methyl-substituted analogs of CCG-I and -IV instead of MCGs were treated as the model compounds to simplify the calculation.[37] *cis*-3′-Methyl-substituted CCG-IV indicated remarkable substituent effect for the increased energy difference between the antiperiplanar conformation and other conformations. *trans*-3′-Methyl CCG-I and -IV showed almost the same energy profiles as CCG-I and -IV, respectively. These results were in good agreement with those of the spectroscopic studies of CCGs and MCGs where the antiperiplanar rotamer existed as the major one among the other rotamers in aqueous solution. X-ray crystallographic analyses of three of the four CCG isomers indicated that each isomer has an antiperiplanar conformation in the solid state (see supporting information).[38]

4. Conformational Requirements of Glutamate Receptors. Glutamate has nine energetically stable rotamers consisting of the three anti (A−C) and six gauche (D−I) forms in aqueous solution (Figure 5).[39,40] On the basis of the conformation−activity studies of CCGs, MCGs, glutamate, and the other known agonists, it should be possible to extract a binding conformation of glutamate to a subtype receptor.[41]

Metabotropic Glutamate Receptors (mGluRs). We previously proposed that the conformational requirement of mGluRs was the extended form of L-glutamate based on the structure−activity relationship of CCG-I and the known mGluRs agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylate ((1*S*,3*R*)-ACPD).[42,43] The present results that *cis*- and *trans*-MCG-I were selective agonists of mGluRs similar to CCG-I support our proposed hypothesis, while the extended conformation of glutamate only concerns the anti relationship between the C2−C3 and C4−C5 bonds.[8b,11] Among the three anti forms of glutamate, aa-A and g^+a-B were well fitted for both (1*S*,3*R*)-ACPD and *cis*- and *trans*-MCG-I (Figure 6). We extracted the anti-anti rotamer A as a probable binding conformer of glutamate for mGluRs because the antiperiplanar conformer of *cis*-MCG-I, almost the exclusive conformer in aqueous solution, well superimposed on the rotamer A.[44] However, the energy barriers between the conformers of *cis*-MCG-I were too small. Therefore, we could not rule out the possibility that the rotamer B is also a possible binding conformer. The g^-a rotamer C would be excluded for the following reasons: (i) (1*S*,3*R*)-ACPD can not take such a conformation because of its 5-membered ring structure and (ii) the structure of the highly constrained derivative (2*S*,3*S*,4*R*,6*S*)-3,4-(6-carboxymethano)proline (CMP-II),[45,46] which well mimicked the rotamer C, showed no activities to glutamate receptors.[45,47]

NMDA Receptors. Watkins et al. first proposed the conformational requirements of NMDA receptors to be a folded form of L-glutamate.[7] This hypothesis was mainly based on the conformational as well as pharmacological studies of *cis*- and *trans*-2,3- and 2,4-piperidinedicarboxylates (PDAs).[7b] The compounds used were *dl*-form. Since D-isomers of acidic amino acids were found to preferentially activate NMDA receptors,[48]

Table 1. Neuropharmacological Profile of MCGs

	depolarization (RP)[a]		inhibition			
	spinal cord[b]	C-fiber[c]	uptake[d]	presynaptic[e]	cAMP formation[f]	agonist type
cis-MCG-I	−	−	−	+	++	mGluRs
trans-MCG-I	−	−	−	++	+++	mGluRs
cis-MCG-III	0.1	−	−	−	−	
trans-MCG-III	0.1	−	−	−	−	
cis-MCG-IV	25	0.1	−	−	−	NMDA
trans-MCG-IV	50	2.5	−	−	−	KA

[a] Relative potency values (RP) of the depolarizing action were calculated as follows: RP = concentration of glutamate to produce effect X/concentration of test compound to produce the same effect X. [b] Depolarizing action in the new born rat spinal cord; RPs were Glu (1), CCG-I (6), CCG-IV (100), NMDA (40), and KA (100), refs 9a and 26. [c] C-Fiber: KA sensitive neurons in the immature rat dosal roots; RPs were Glu (1), KA (9), CCG-IV (0.1), NMDA (−), and QA (−), ref 26. [d] Reference 9b. [e] Inhibition of spinal reflex evoked by electrical stimulation of the dosal root of the new born rat; DCG-IV (+++), CCG-I (++), (1*S*,3*R*)-ACPD (+), and GABA (+). IC_{50} values for DCG-IV, CCG-I, and (1*S*,3*R*)-ACPD were 0.07, 0.7, and 3 μM, refs 10d, 27, and 28. [f] Inhibition of forskolin-stimulated cAMP formation in the cultured spinal neurons; CCG-I (+++), DCG-IV (+++), and (1*S*,3*R*)-ACPD (+), IC_{50} values for CCG-I, DCG-IV, and (1*S*,3*R*)-ACPD were 0.1, 0.1, and 7 μM, refs 27 and 28.

Table 2. pK_a Values of the Representative Glutamate Agonists, CCGs, and MCGs[a]

	pK_a		
amino acid	pK_1	pK_2	pK_3
L-glutamic acid[b]	2.2	4.3	9.7
kainic acid[c]	2.1	4.3	10.1
quisqualic acid	1.8	3.6	9.0
AMPA	2.1	5.2	10.1
CCG-I	1.9	4.2	9.7
CCG-II	2.2	4.2	9.5
CCG-III	2.1	4.3	10.0
CCG-IV	2.5	4.6	10.0
cis-MCG-I	2.1	3.8	9.3
trans-MCG-I	2.0	4.4	9.5
cis-MCG-III	2.3	4.2	10.2
trans-MCG-III	2.1	4.0	9.7
cis-MCG-IV	2.0	5.1	10.1
trans-MCG-IV	2.2	4.7	9.6

[a] pK_a values are obtained by ^{1}H NMR titration experiments. pD of the solution was adjusted by adding DCl and NaOD. [b] Reference 30a. [c] Reference 30b.

the above hypothesis required proof using some other conformational probes with an L-configuration. Our previous CCG studies have provided a clear evidence to this point that a folded conformation of glutamate regarding the carbon chain is one of the crucial factors for the NMDA receptors because the structure of CCG-IV corresponds to the gauche form of glutamate's C2−C3 and C3−C5 bonds.[8b,9a,b,11] Several groups have reached a similar conclusion that the folded form is an active conformation of NMDA receptors by using molecular modeling techniques including the biological results of CCG-IV.[48b,49,50] The template molecule employed in their studies was 1-aminocyclobutane-1,3-dicarboxylate (*trans*-ACBD)[51] which is a meso compound.[49] Therefore, the required stereochemistry at the α-amino acid moiety of glutamate could not be determined by their studies because both L- and D-glutamate should equally fit with *trans*-ACBD in spite of the fact that L-glutamate is a much more potent agonist than D-glutamate. Alternatively, Ortwein's pharmacophore model obtained using MULTIFIT program could fit glutamate itself only with a relatively high energy cost.[50] Thus, these pharmacophore models remain to be refined.

The present conformational studies of CCG-IV and *cis*-MCG-IV indicated a bias toward their antiperiplanar conformations in aqueous solution. These results led us, further, to speculate which rotamer would be an active form of glutamate by using CCG-IV and *cis*-MCG-IV as template molecules. It was found that the three ionic functions of CCG-IV or *cis*-MCG-IV having the antiperiplanar conformation were well superimposed with those of the g^+g^+ rotamer E among six gauche rotamers (Figure 7).[52] On the other hand, the rotamers g^-g^+-F and g^-g^--I would be unfavorable because their three ionic functions matched well with those of the energetically much less stable (ΔE > 6 kcal/mol) conformer of *cis*-MCG-IV.[37] Furthermore, (2*S*,3*R*,4*S*,6*S*)-3,4-(6-carboxymethano)proline (CMP-III)[45,53] which was inactive to any glutamate receptors[45,47] fixed the conformation of glutamate to ag^+-D and ag^--G. Therefore, these rotamers could be ruled out from the probable candidates. In addition, conformational studies of NMDA and *trans*-ACBD performed by CHARMm indicated that each one of their energy-minimized conformers was superimposed with those of the antiperiplanar conformation of CCG-IV and g^+g^+-E (Figure 8). Therefore, we concluded that the rotamer E would be the most plausible conformation of glutamate required for binding to NMDA receptors.

KA and AMPA Receptors. Only a few kainate agonists which do not possess the kainoid skeleton[54] are known in the literature.[55] In the present studies, *trans*-MCG-IV was found to be a potent agonist of KA receptors which was ascertained by its electrophysiological assay using KA sensitive neurons in the new born rat C-fiber.[56] In addition, we recently demonstrated that the 5-membered ring analog of L-glutamate, L-2-(2-carboxy-3-methylenecyclopentyl)glycine (CPG-IV), possessing a folded substructure of glutamate and an exo methylene group at C3′, was a potent agonist of KA receptor.[57] Inspection of their conformations using molecular models as well as calculations using CHARMm revealed that the structures of CPG-IV and KA[58] with *trans*-MCG-IV (indicated as its antiperiplanar conformation) were well overlapped in view of their folded glutamate substructure which corresponds to the g^+g^+ rotamer of glutamate E (Figure 9). The most significant observation of these studies was that the 3′*R* substituent of *trans*-MCG-IV and the exo methylene group of CPG-IV could occupy the same space as the C−C double bond of KA.[59,60] These results suggest that the presence of the substituent of these agonists at the appropriate space is crucial to activate KA receptors. However, we have not reached a conclusion whether these groups act as a steric factor or a stereoelectronic factor. In spite of the absence of such a substituent in glutamate, glutamate can activate KA receptors. Further studies will be required to explain this substituent effect.

Structure−activity relationship studies are still insuf-

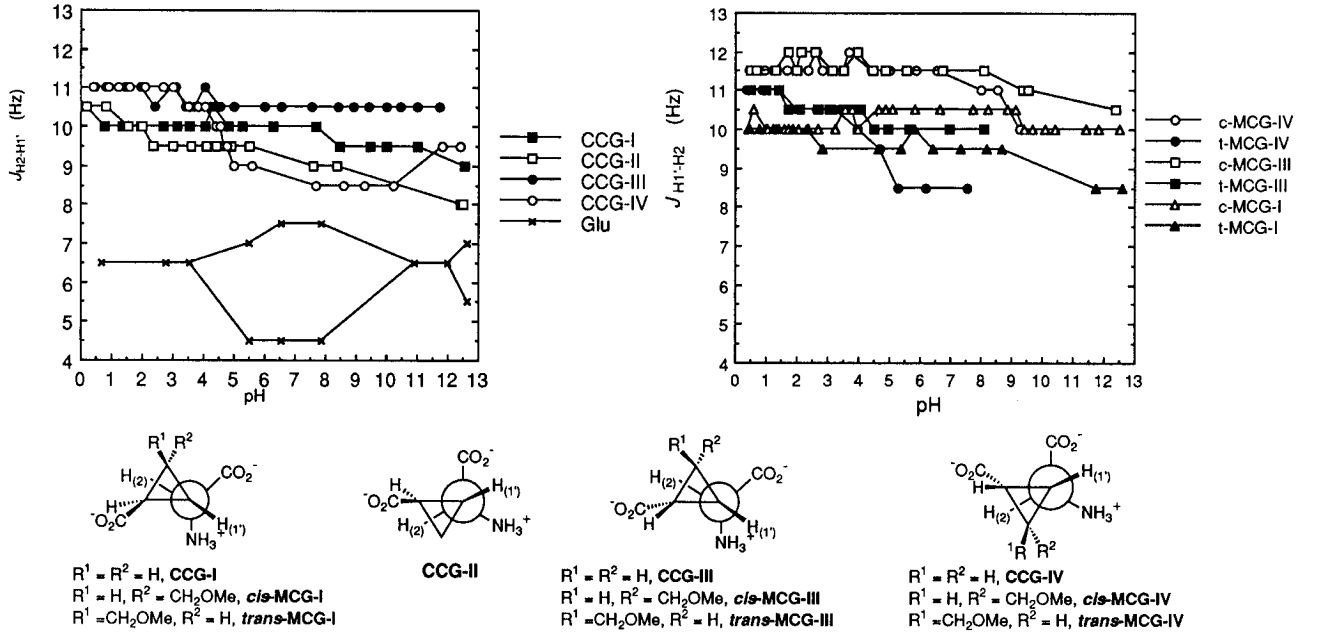


Figure 3. Correlation diagram between variable pH and $J_{2H\text{-}1'H}$ of CCGs and L-glutamate and MCGs.

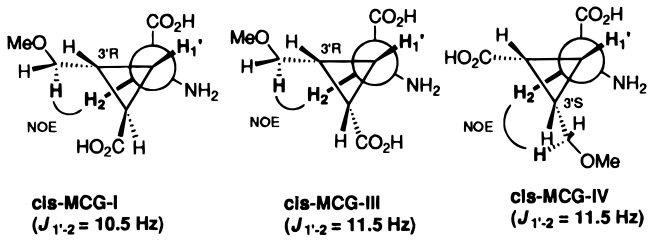


Figure 4. Probable conformations of *cis*-MCGs. An arc line indicates strong NOEs observed.

ficient for AMPA receptors, although potent agonists and antagonists have been developed recently.[55b,61,62] We could not obtain any information about AMPA receptors from the present studies. However, some of the 4,4-disubstituted glutamate analogs, synthesized as open chain analogs which might mimic a folded form of glutamate, did not activate mGluRs but activated the ionotropic glutamate receptors including the AMPA subtype.[63] A folded form of glutamate might be required for activating AMPA receptors.

Conclusions

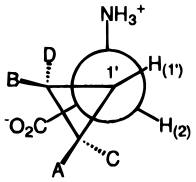
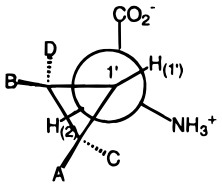
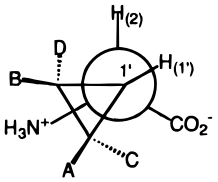
In spite of the recent progress concerning the structural analyses and the resultant subdivisions of glutamate receptors at the protein level, only a limited number of selective agonists and antagonists have been developed to date.[1,2,64] Therefore, the synthesis of effective drugs, which are necessary for investigating the physiological functions of glutamate receptors, is strongly hoped for by neuroscientists. From the point of view of chemists, it is of great interest how the distinct types of glutamate receptors recognize glutamate which is a conformationally flexible molecule and is capable of forming various conformations under physiological conditions. Our work on this problem started from the conformation−activity relationship studies of L-glutamate as described in this paper. Thus, we synthesized more than 10 species of conformationally constrained analogs of glutamate, CCGs, MCGs, and the other related amino acids. Some of them were found not only to be selective and powerful agonists for the receptors but also to provide useful information with regard to the conformational requirements of glutamate receptors. These amino acids are useful as leading compounds for further conformational studies of glutamate receptors as well as for developing effective drugs for various brain diseases.

Experimental Section

Melting point are uncorrected. ^{1}H NMR spectra were recorded on one of the following instruments: JEOL FX 100, EX-270, EX-400, GE GN-300, GN-500, and Nicolet NT-360. Chemical shifts are reported as δ values in ppm relative to $CHCl_3$ (7.26) in $CDCl_3$ or sodium 3-(trimethylsilyl)propionate-d_4 (TSP) (0.00) as an internal standard in D_2O. IR spectra were measured either on a Hitachi 270-30 or on a Perkin Elmer FT-IR 1640 spectrophotometer. Mass spectra (MS) and high-resolution mass spectra (HRMS) were obtained on a Hitachi M-80B spectrometer for secondary ionization mass spectrometry (SIMS) and electron-impact ionization (EI) or on a JEOL JMS-HX 110 spectrometer for fast atom bombardment ionization (FAB). Optical rotations were taken on a Perkin Elmer 241 polarimeter. All reactions were monitored by thin-layer chromatography (TLC), carried out on a 2 × 5 cm precoated TLC plates (silica gel 60F-254; layer thickness, 0.25 mm) manufactured by Merck, with UV light (254 nm), ninhydrin, or $KMnO_4$ solution (0.5 g dissolved in 100 mL of water). Silica gel (Merck 60, 70−230 mesh) was used for column chromatography. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials unless otherwise stated.

(4*S*)-3-*N*-(*tert*-Butoxycarbonyl)-4-[3-hydroxy-(1*Z*)-propenyl]-2,2-dimethyl-1,3-oxazolidine (2). To a suspension of NaH (60% oily suspension; 708 mg, 29.5 mmol) in THF (30 mL) at 0 °C under nitrogen was added, dropwise, a solution of bis(2,2,2-trifluoroethyl)[(methoxycarbonyl)methyl]phosphonate (9.38 g, 29.5 mL) in THF (40 mL). The solution was stirred at 0 °C for 30 min and then cooled to −78 °C. To this solution was added 18-crown-O-6 (39.6 g, 150 mmol) in THF (80 mL) and then (4*S*)-3-*N*-(*tert*-butoxycarbonyl)-4-formyl-2,2-dimethyl-1,3-oxazolidine (**1**)[13] (5.2 g, 22.7 mmol) in THF (20 mL). The reaction mixture was stirred at the same temperature for 2 h and the reaction quenched with aqueous saturated NH_4Cl solution (ca. 100 mL). The reaction mixture was extracted with Et_2O, dried over $MgSO_4$, and concentrated *in vacuo* to give an oily residue which was subjected to column chromatography on silica gel (Et_2O/hexane, 1:4) to give an α,β-unsaturated ester (5.3 g, 82%) as colorless crystals: mp 47.0−49.0 °C; $[\alpha]^{23}_D$ −32.6° (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 9 H), 1.46 (s), 1.52 (s), 1.57 (s), 1.64 (s) (total

Table 3. Minimized Conformations of CCGs, *cis*-Methyl CCGs, and *trans*-Methyl CCGs[a]

			
CCG-I	H2−H1′ = 55.9°	H2−H1′ = 179.1°	H2−H1′ = 58.6°
A = CO_2^-	*E* = 7.55	*E* = 5.28	*E* = 6.04
B, C, D = H	(Δ*E* = 2.27)	(Δ*E* = 0)	(Δ*E* = 0.76)
CCG-II	H2−H1′ = 52.8°	H2−H1′ = 176.6°	H2−H1′ = −61.3°
B = CO_2^-	*E* = 7.43	*E* = 5.23	*E* = 6.27
A, C, D = H	(Δ*E* = 2.20)	(Δ*E* = 0)	(Δ*E* = 1.04)
CCG-III	H2−H1′ = 82.9°	H2−H1′ = −178.5°	H2−H1′ = −55.4°
C = CO_2^-	*E* = 8.50	*E* = 5.37	*E* = 6.95
A, B, D = H	(Δ*E* = 3.13)	(Δ*E* = 0)	(Δ*E* = 1.58)
CCG-IV	H2−H1′ = 41.3°	H2−H1′ = 176.6°	H2−H1′ = −71.7°
D = CO_2^-	*E* = 8.34	*E* = 5.52	*E* = 6.87
A, B, C = H	(Δ*E* = 2.82)	(Δ*E* = 0)	(Δ*E* = 1.35)
cis-Me-CCG-I	H2−H1′ = 23.9°	H2−H1′ = 171.5°	H2−H1′ = −70.9°, −92.7°
A = CO_2^-	*E* = 9.61	*E* = 5.84	*E* = 8.24, 8.29
B, C = H	(Δ*E* = 3.77)	(Δ*E* = 0)	(Δ*E* = 2.40, 2.45)
D = CH_3			
trans-Me-CCG-I	H2−H1′ = 55.3°	H2−H1′ = 178.3°	H2−H1′ = −59.1°
A = CO_2^-	*E* = 8.01	*E* = 5.65	*E* = 6.53
B = CH_3	(Δ*E* = 2.36)	(Δ*E* = 0)	(Δ*E* = 0.88)
C, D = H			
cis-Me-CCG-IV	H2−H1′ = 44.0°	H2−H1′ = −173.7°	H2−H1′ = −63.7°
D = CO_2^-	*E* = 13.54	*E* = 7.14	*E* = 10.17
A, B = H	(Δ*E* = 6.40)	(Δ*E* = 0)	(Δ*E* = 3.03)
C = CH_3			
trans-Me-CCG-IV	H2−H1′ = 41.8°	H2−H1′ = 177.3°	H2−H1′ = −71.7°
D = CO_2^-	*E* = 8.48	*E* = 5.65	*E* = 6.92
A = CH_3	(Δ*E* = 2.84)	(Δ*E* = 0)	(Δ*E* = 1.27)
B, C = H			

[a] Dihedral angle of H−C2−C1′−H was represented as H2−H1′. *E* = kcal/mol (Δ*E* = difference from the global minimum energy).

6 H), 3.71 (s, 3H), 3.77 (dd, 1 H, *J* = 3.5, 9.5 Hz), 4.26 (m, 1 H), 5.38 (m, 1 H), 5.83 (m, 1 H), 6.21−6.39 (m, 1 H).

To a stirred solution of the ester (13.0 g, 45.6 mmol) in toluene (200 mL) at −78 °C under nitrogen was added a 1 M hexane solution of diisobutylaluminum hydride (DIBAL-H) (137 mL, 137 mmol). The reaction mixture was stirred at −78 °C for 1.5 h and the reaction quenched with MeOH (30 mL). The mixture was warmed to room temperature; then a small tip of ice (ca. 20 g) was added. The reaction mixture was stirred for 30 min. To the resulting white suspension was added $MgSO_4$ (ca. 50 g). After stirring for 30 min, the mixture was filtered through a pad of $MgSO_4$. The filtrate was concentrated *in vacuo* to give an oily residue which was subjected to column chromatography on silica gel (Et_2O/hexane, 1:1 and then 3:1) to give **2** (10.3, 88%) as an oil: $[\alpha]^{23}_D$ −28.7° (*c* 0.83, $CHCl_3$); IR (neat) 3436, 2984, 2940, 2884, 1700, 1684, 1674 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9 H), 1.49 (s, 3 H), 1.57 (s, 3 H), 3.70 (dd, 1 H, *J* = 1.5, 9.0 Hz), 3.90 (m, 1 H), 4.05 (dd, 1 H, *J* = 6.0, 9.0 Hz), 4.12 (dd, 1 H, *J* = 3.0, 8.5 Hz), 4.43 (m, 1 H), 4.92 (ddd, 1 H, *J* = 1.5, 6.0, 10.0 Hz), 5.53 (dd, 1 H, *J* = 10.0, 10.0 Hz), 5.85 (m, 1 H); MS (SIMS) *m*/*z* 258 $(M + H)^+$, 202, 184, 158, 144, 126, 57; HRMS (FAB) *m*/*z* calcd for $C_{13}H_{24}NO_4$ $(M + H)^+$ 258.1705, found 258.1976.

(4*S*)-3-*N*-[(*tert*-Butoxycarbonyl)glycyl]-4-[3-[(*tert*-butyldimethylsilyl)oxy]-(1*Z*)-propenyl]-2,2-dimethyl-1,3-oxazolidine (3a). To a solution of **2** (10.1 g, 39.3 mmol) in MeOH (30 mL) was added 1 N HCl/MeOH (150 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 16 h. The solvent was removed *in vacuo* to give an oily residue. A solution of the resulting mixture in water (50 mL) was adjusted to pH 7 by addition of 1 N NaOH and then to pH 9 by Et_3N. After removal of the solvent *in vacuo*, the mixture was dissolved in MeOH (100 mL) and THF (150 mL). If the solution was still acidic or neutral, additional Et_3N was added until the solution became pH 9. To this solution at 0 °C was added *N*-hydroxysuccinimide *N*-(*tert*-butoxycarbonyl)glycinate (Boc-Gly-OSu; 11.9 g, 44 mmol). The reaction mixture was warmed to room temperature and stirred for 30 min. The solvent was evaporated *in vacuo* to give an oily residue. The residue was dissolved in EtOAc, and the insoluble material was filtered off. The filtrate was concentrated *in vacuo* to give an oily residue which was subjected to column chromatography on silica gel (MeOH/$CHCl_3$, 1:20 and then 1:4) to give a Boc-glycyl compound. A solution of the oily product and CSA (300 mg) in acetone (100 mL) and 2,2-dimethoxypropane (50 mL) was heated at reflux for 2 h. After cooling down to room temperature, sodium bicarbonate powder (2 g) was added to the solution. The solvent was removed *in vacuo* to give a crude residue which was subjected to short pass silica gel column chromatography (100 g of silica gel, elution with MeOH/$CHCl_3$, 1:4) to give an oily residue. A solution of the oily residue (13 g) and *p*-toluenesulfonic acid (200 mg) in acetone (100 mL) and 2,2-dimethoxypropane (50 mL) was stirred at reflux for 1 h. The solvent was removed *in vacuo*. The resulting residue was again dissolved in benzene (150 mL) and 2,2-dimethoxypropane (50 mL) and heated at reflux for 3 h. The solution was concentrated *in vacuo* to a total volume of 100 mL. To this solution was added MeOH (20 mL). The solution was stirred at room temperature for 30 min and concentrated *in vacuo* to give a crude residue, which, upon column chromatography on silica gel (Et_2O/hexane, 1:1 and then 4:1), gave an ally alcohol (9.5 g, 78% from **2**) as an oil. To a solution of the allyl alcohol (9.4 g, 30 mmol) and imidazole (3.06 g, 45 mmol) in DMF (50 mL) at 0 °C under N_2 was added *tert*-butyldimethylsilyl chloride (5.4 g, 36 mmol) in DMF (20 mL). The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 1 h. The reaction mixture was poured into ice water (500 mL) and extracted with Et_2O three times. The combined organic phase was washed with water, dried over $MgSO_4$, and concentrated *in vacuo* to give an oily residue. The residue was purified by column chromatography on silica gel (Et_2O/hexane, 1:1) to give **3a** (10.03 g, 78%) as an oil: $[\alpha]^{23}_D$ −53.1° (*c* 1.76, $CHCl_3$); IR (neat) 3432, 2964, 2940, 2864, 1720, 1660 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (s, 3 H), 0.09 (s, 3 H), 0.90 (s, 9 H), 1.44 (s, 9 H), 1.56 (s, 3 H), 1.67 (s, 3 H), 3.77−3.93 (m, 3 H), 4.13 (dd, 1 H, *J* =

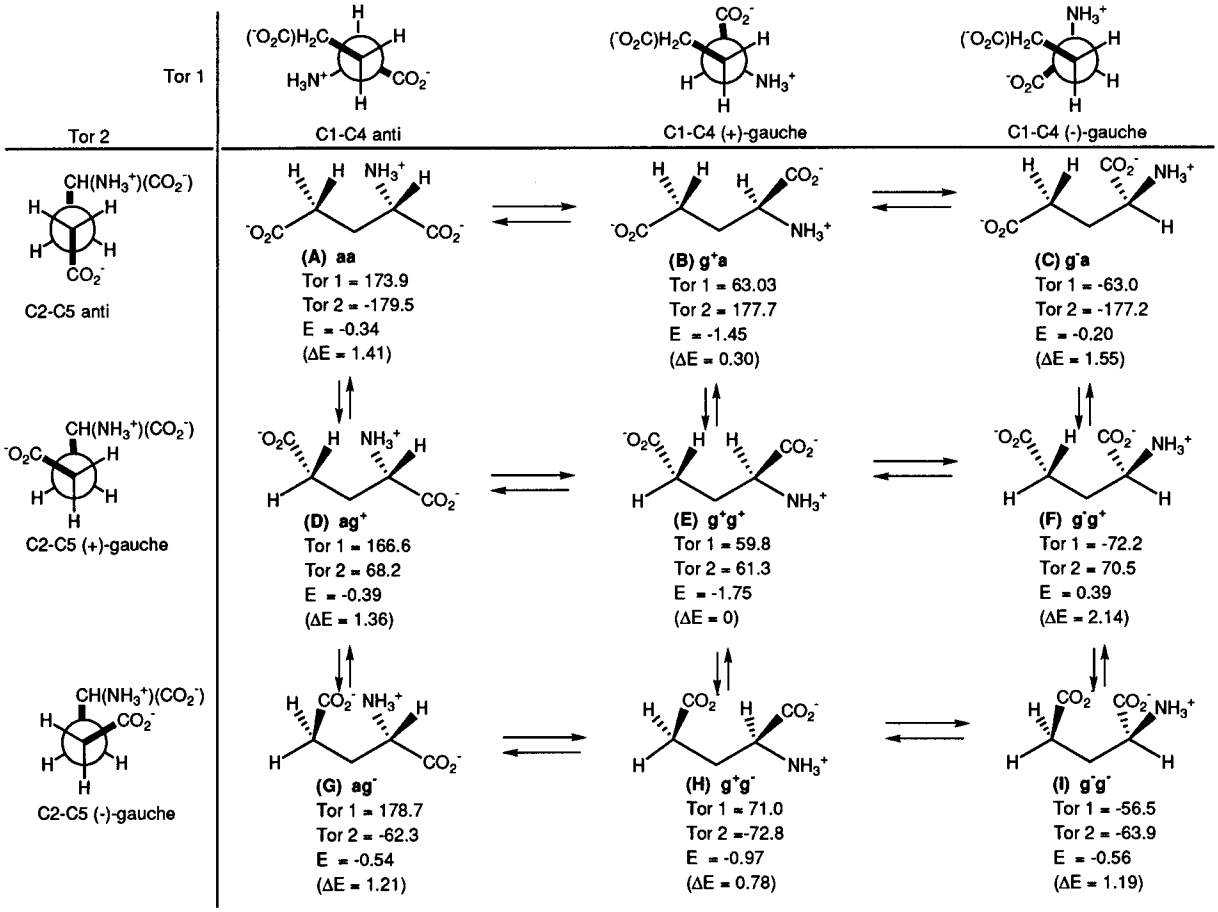


Figure 5. Newman projection and sawhorse model of the rotational isomers of L-glutamate. Tor 1 and Tor 2 indicate the torsions between the C1−C2 and C3−C4 bonds and between the C2−C3 and C4−C5 bonds, respectively (a = anti and g = gauche). E = kcal/mol (ΔE = difference from the minimum energy).

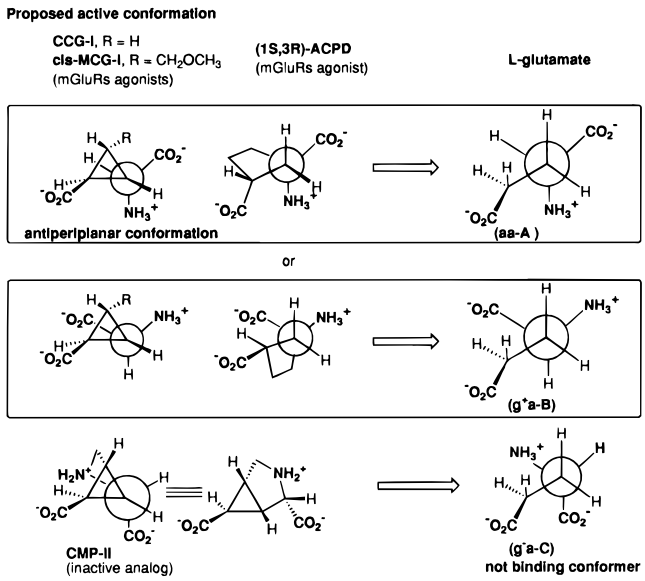


Figure 6. Proposed conformational requirements of mGluRs.

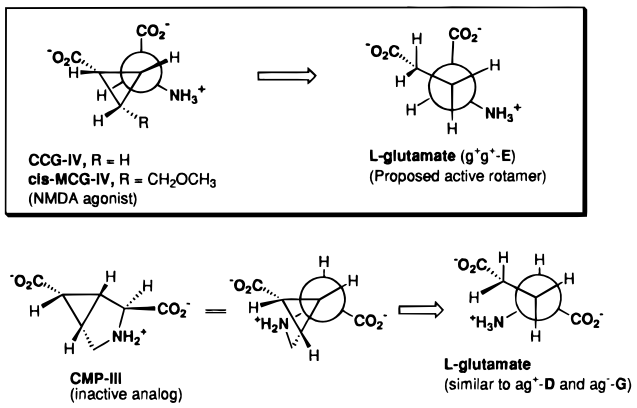


Figure 7. Proposed conformational requirements of NMDA receptors.

6.0, 9.0 Hz), 4.31 (m, 2 H), 4.81 (ddd, 1 H, J = 1.0, 7.0, 7.0 Hz), 5.32 (br s, 1 H), 5.53 (dddd, 1 H, J = 1.0, 1.0, 9.0, 11.5 Hz), 5.70 (ddd, 1 H, J = 6.0, 6.0, 11.5 Hz); MS (SIMS) m/z 429 $(M + H)^+$, 413, 315, 271, 257. Anal. ($C_{21}H_{40}N_2O_5Si$) C, H, N.

(1*S*,7*S*,8*S*,9*R*)-3-Aza-9-[[(*tert*-butyldimethylsilyl)oxy]-methyl]-4,4-dimethyl-5-oxa-tricyclo[6.1.0.0^{3,7}]nonan-2-one (5a). To a solution of **3a** (7.0 g, 16.3 mmol) and 2,6-lutidine (5.7 mL, 49 mmol) in CH_2Cl_2 (50 mL) at room temperature under N_2 was added TMSOTf (6.3 mL, 32.6 mmol). The reaction mixture was stirred for 15 min at room temperature. After cooling to 0 °C, the reaction was quenched with saturated aqueous NH_4Cl solution (10 mL). The mixture was extracted with Et_2O, washed with water, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give crude **3b** which was used for the next step without further purification. To a solution of **3b** in Et_2O (100 mL) was added $NaNO_2$ (5.65 g, 81.9 mmol) in water (50 mL) with intensive stirring at room temperature. To this suspension was added 5% citric acid, and the pH of the solution was adjusted to ~3. After vigorous stirring for 30 min at room temperature, the reaction mixture was extracted with Et_2O three times. The combined organic phase was washed with saturated aqueous $NaHCO_3$, water, and brine and dried over $MgSO_4$. The solvent was removed *in vacuo* to give **4** as an oily residue. The diazo ketone **4** without further purification was dissolved in benzene (300 mL). To this solution was added $Pd(OAc)_2$ (184 mg, 0.82 mmol), and the solution under N_2 was heated at 70 °C for 1 h. The solution was concentrated *in vacuo* to give an oily residue, which, upon column chromatography on silica gel (Et_2O/hexane, 1:1), gave **5a** (3.1 g, 61%) as a colorless oil: $[\alpha]^{23}_D$

Figure 8. Superimposition of the representative NMDA agonists and L-glutamate and CCG-IV. Green: CCG-IV (antiperiplanar rotamer), $\Delta E = 0$. Pink: L-glutamate (g^+g^+ rotamer E), $\Delta E = 0$, rms = 0.078. Yellow: NMDA, $\Delta E = 0.49$, rms = 0.265. Blue: *trans*-ACBD, $\Delta E = 0.59$, rms = 0.169. Only the best fit conformer of each agonist is shown. ΔE = difference from the global minimum energy (kcal/mol). rms = deviation between atoms employed in the fitting process (Å).

+62.1° (*c* 0.71, $CHCl_3$); IR (neat) 2940, 2864, 1706 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 0.07 (s, 3 H), 0.08 (s, 3 H), 0.91 (s, 9 H), 1.38 (s, 3 H), 1.68 (m, 1 H), 1.73 (s, 3 H), 2.06 (dd, 1 H, J = 6, 8 Hz), 2.16 (ddd, 1 H, J = 2, 6, 9 Hz), 3.48 (dd, 1 H, J = 7, 10 Hz), 3.62 (dd, 1 H, J = 9, 11 Hz), 3.94 (ddd, 1 H, J = 2, 6, 10 Hz), 4.00 (dd, 1 H, J = 6, 10 Hz), 4.03 (dd, 1 H, J = 6, 7 Hz); MS (SIMS) *m/z* 312 $(M + H)^+$, 254, 196, 180. Anal. ($C_{16}H_{29}NO_3Si$) C, H, N.

$Pd(OAc)_2$ used in this reaction was purified by the following procedures: Commercially available $Pd(OAc)_2$ (1.0 g) was dissolved in dry benzene (50 mL). The insoluble material was filtered off, and the filtrate was concentrated *in vacuo* to give a fine powder of $Pd(OAc)_2$ (~800 mg).[17]

(2*S*,1′*S*,2′*S*,3′*R*)-*N*-(*tert*-Butoxycarbonyl)-2-[2-carboxy-3-(methoxymethyl)cyclopropyl]glycinol (6). To a solution of **5a** (712 mg, 2.28 mmol) in THF (5 mL) was added *n*-Bu_4NF (1 M THF solution; 3.42 mL, 3.42 mmol) at 0 °C, and the reaction mixture was stirred for 10 min. The solvent was removed *in vacuo* to give a crude residue which was purified by column chromatography on silica gel (Et_2O and then MeOH/Et_2O, 1:19) to give an hydroxymethyl compound (423 mg, 94%) as an amorphous solid: $[\alpha]^{23}_D$ +156° (*c* 1.01, $CHCl_3$); IR (neat) 3420, 2992, 2944, 2888, 1678 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.39 (s, 3 H), 1.72 (s, 3 H), 1.76 (m, 1 H), 2.03 (br s, 1 H), 2.10 (dd, 1 H, J = 6.0, 8.0 Hz), 2.22 (ddd, 1 H, J = 2.0, 6.0, 9.0 Hz), 3.49 (dd, 1 H, J = 8.0, 10.0 Hz), 3.74 (br t, 1 H, J = 10.0 Hz), 3.92 (m, 2 H), 4.03 (dd, 1 H, J = 6.0, 8.0 Hz); MS (SIMS) *m/z* 198 $(M + H)^+$; HRMS (FAB) *m/z* calcd for $C_{10}H_{16}NO_3$ $(M + H)^+$ 198.1130, found 198.1128.

To a solution of the hydroxymethyl compound (400 mg, 2.0 mmol) in DMF (15 mL) was added NaH (60% oily suspension; 122 mg, 3.05 mmol) at 0 °C, and the reaction mixture was stirred for 20 min. To this suspension were added DMF (4 mL), *n*-Bu_4NI (37 mg, 0.1 mmol), and CH_3I (380 μL, 6.1 mmol) at room temperature, and the reaction mixture was stirred for 5 h. This was quenched with aqueous NH_4Cl and extracted with EtOAc. The organic layer was washed with water and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue which was purified by column chromatography on silica gel (Et_2O and then MeOH/Et_2O, 3:97) to give a methoxymethyl compound (353 mg, 82%) as an oil: $[\alpha]^{23}_D$ +117° (*c* 0.93, $CHCl_3$); IR (neat) 3592, 2988, 2940, 2900, 1708 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.40 (s, 3 H), 1.73 (s, 3 H), 1.74 (dddd, 1 H, J = 6.0, 8.0, 8.5, 9.0 Hz), 2.08 (dd, 1 H, J = 6.0, 8.0 Hz), 2.20 (ddd, 1 H, J = 2.0, 6.0, 8.5 Hz), 3.39 (s, 3 H), 3.46 (dd, 1 H, J = 9.0, 10.5 Hz), 3.48 (dd, 1 H, J = 7.5, 10.0 Hz), 3.71 (dd, 1 H, J = 6.0, 10.5 Hz), 3.93 (ddd, 1 H, J = 2.0, 6.0, 10.0 Hz), 4.03 (dd, 1 H, J = 6.0, 7.5 Hz); MS (SIMS) *m/z* 212 $(M + H)^+$, 180, 154, 31.

A solution of the methoxymethyl compound (353 mg, 1.67 mmol) in 60% aqueous acetic acid (10 mL) was stirred at room temperature for 12 h. The solvent was removed under reduced pressure. To a solution of the residue in EtOH (20 mL) were added $Ba(OH)_2 \cdot 8H_2O$ (1.58 g, 5.0 mmol) and water (2 mL), and the suspension was heated at 80 °C for 48 h. The reaction mixture was acidified to pH 2 with 10% H_2SO_4, and the pH of the solution was adjusted to 9 with aqueous $NaHCO_3$. The insoluble material was removed by filtration, and the filtrate was concentrated to 10 mL *in vacuo*. To the solution was added a solution of di-*tert*-butyl dicarbonate (Boc_2O; 576 μL, 2.5 mmol) in 1,4-dioxane (10 mL). The reaction mixture was stirred at room temperature for 16 h and washed with EtOAc. The pH of the aqueous layer was adjusted to 1 with 1 *N* HCl and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give **6** (281 mg, 58%) as colorless crystals: mp 53.5−55.0 °C; $[\alpha]^{23}_d$ −61.2° (*c* 0.73, $CHCl_3$); IR (neat) 3336, 2984, 1712 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.46 (s, 9 H), 1.86 (m, 3 H), 3.37 (s, 3 H), 3.80 (m, 5 H), 5.0−5.5 (br s, 2 H); MS (SIMS) *m/z* 290 $(M + H)^+$, 234, 216. Anal. ($C_{13}H_{23}NO_6$) C, H, N.

(1*S*,5*S*,6*S*,7*R*)-5-[*N*-(*tert*-Butoxycarbonyl)amino]-7-(methoxymethyl)-3-oxabicyclo[4.1.0]heptan-2-one (5b). A solution of **6** (56 mg, 0.19 mmol), 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide (WSCD) hydrochloride salt (45 mg, 0.23 mmol), 1-hydroxybenzotriazole (HOBt; 31 mg, 0.23 mmol), and Et_3N (32 mL, 0.23 mmol) in THF (4 mL) was stirred at 0 °C for 1 h and then at room temperature for 3 h. The reaction mixture was extracted with EtOAc and washed successively with 5% citric acid solution, water, and aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, and the solvent was evaporated *in vacuo* to give an oily residue. The residue was purified by column chromatography on silica gel (EtOAc) to give **5b** (24 mg, 46%) as colorless crystals: mp 140.0−141.0 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9 H), 1.86 (m, 1 H), 1.92 (m, 1 H), 2.16 (dd, 1 H, J = 9.0, 9.0 Hz), 3.28 (dd, 1 H, J = 11.0, 11.0 Hz), 3.41 (s, 3 H), 3.74 (dd, 1 H, J = 10.0, 12.0 Hz), 3.96 (dd, 1 H, J = 7.0, 11.0 Hz), 4.36 (dd, 1 H, J = 7.0, 12.0 Hz), 4.58 (m, 1 H), 5.52 (d, 1 H, J = 8.0 Hz); MS (SIMS) *m/z* 294 $(M + Na)^+$, 272 $(M + H)^+$, 216, 172; HRMS (FAB) *m/z* calcd for $C_{13}H_{22}NO_5$ $(M + H)^+$ 272.1498, found 272.1503.

(2*S*,1′*S*,2′*S*,3′*R*)-2-[2-Carboxy-3-(methoxymethyl)cyclopropyl]glycine (*cis*-MCG-III). To a solution of **6** (250 mg, 0.86 mmol) in acetone (8 mL) was added Jones reagent at 0 °C, and the reaction mixture was stirred at 0 °C for 3 h and then at room temperature for 2 h. The reaction was quenched with 2-propanol and the mixture extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue. To a solution of the residue in CH_2Cl_2 (1 mL) at 0 °C was added TFA (1 mL). The mixture was stirred for 30 min at room temperature and concentrated *in vacuo*. The residue was passed through a column of Dowex 50W × 4 (100−200 mesh) ion exchange resin (H_2O and then 1 N aqueous NH_3) to give a solution of the ammonium salt of *cis*-MCG-III. The solution was concentrated *in vacuo* and dissolved in water (5 mL). The pH of the solution was adjusted to 3 with 1 N HCl. The crystals that precipitated from the solution were collected by filtration. They were recrystallized from water to give *cis*-MCG-III (59 mg, 37%) as colorless crystals: mp 155.5−156.5 °C; $[\alpha]^{25}_D$ +85.9° (*c* 0.51, H_2O); IR (KBr) 3124, 2346, 1706, 1402 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 1.76 (ddd, 1 H, J = 8.0, 9.0, 12.0 Hz), 2.00 (m, 1 H), 2.05 (dd, 1 H, J = 7.0, 9.0 Hz), 3.32 (s, 3 H), 3.62 (m, 1 H), 3.96 (m, 1 H), 4.20 (d, 1 H, J = 11.5 Hz); MS (SIMS) *m/z* 204 $(M + H)^+$, 45, 31, 18. Anal. ($C_8H_{13}NO_5$) C, H, N.

**(1*S*,4*S*,5*S*,6*R*)-3-Aza-3-*N*-(*tert*-butoxycarbonyl)-4,6-bis-[[(*tert*-butyldimethylsilyl)oxy]methyl]bicyclo[3.1.0]-

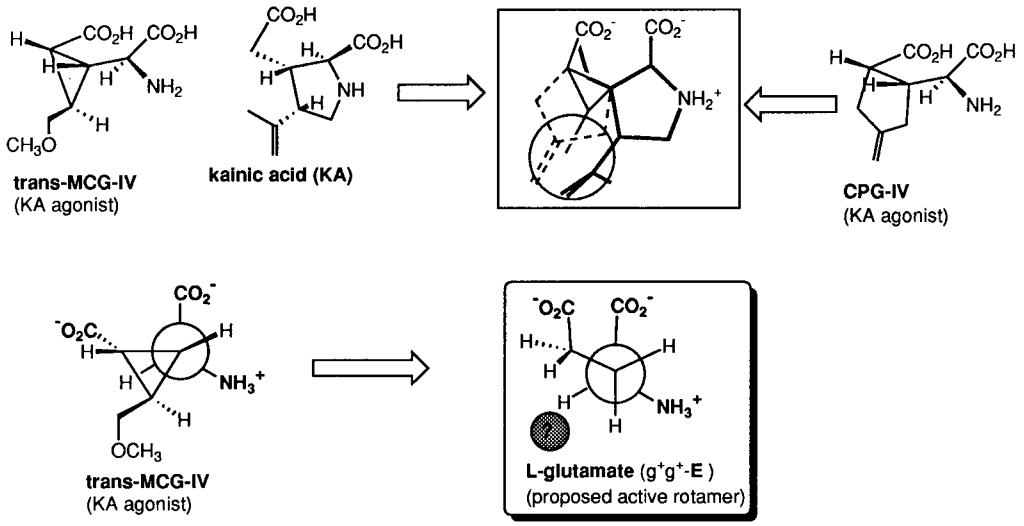


Figure 9. Proposed conformational requirements of KA receptors.

hexane-2-one (5c). A mixture of **5a** (830 mg, 2.66 mmol) and Dowex 50W × 4 (H^+ form, 100 mg) in MeOH (5 mL) was stirred at room temperature for 14 h. The resin was filtered off, and the filtrate was concentrated *in vacuo* to give an oily residue. To a solution of the residue and imidazole (724 mg, 10.6 mmol) in DMF (10 mL) was added *tert*-butyldimethylsilyl chloride (1.2 g, 7.99 mmol) in DMF (5 mL). The reaction mixture was stirred at room temperature for 16 h. The mixture was poured into cold water and extracted with Et_2O three times. The combined organic layer was dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue, which, upon column chromatography on silica gel (Et_2O/hexane, 1:2 and then 3:1), gave bis-TBS-imide (879 mg, 86%). A solution of the bis-TBS-imide (879 mg, 2.28 mmol), Et_3N (636 μL, 4.56 mmol), Boc_2O (780 μL, 3.42 mmol), and 4-(dimethylamino)pyridine (DMAP) (56 mg, 0.456 mmol) in THF (15 mL) was stirred at room temperature for 16 h. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed successively with 5% aqueous citric acid and water and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue. The residue was subjected to column chromatography on silica gel (Et_2O/hexane, 1:3) to give **5c** (1.28 g, 100%) as colorless crystals: mp 46.0−47.5 °C; $[\alpha]^{23}_D$ −45.3° (*c* 0.98, $CHCl_3$); IR (neat) 2936, 2864, 1790, 1758, 1714 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 0.04 (s, 12 H), 0.92 (s, 18 H), 1.45 (s, 9 H), 1.40−1.55 (m, 1 H), 1.95−2.15 (m, 2 H), 3.45 (dd, 1 H, *J* = 8, 11 Hz), 3.70−3.90 (m, 3 H), 4.05 (m, 1 H).

(1*S*,2*R*,3*R*,1′*S*)-2-[1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[(*tert*-butyldimethylsilyl)oxy]ethyl]-3-[[(*tert*-butyldimethylsilyl)oxy]methyl]cyclopropane-1-carboxylic Acid Methyl Ester (7a). A solution of **5c** (162 mg, 0.33 mmol) and LiOH (8 mg, 0.33 mmol) in anhydrous MeOH (3 mL) was stirred at room temperature for 16 h. The reaction was quenched with aqueous NH_4Cl solution (2 mL) and the mixture extracted with EtOAc. The organic layer was dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue. This was purified by column chromatography on silica gel (Et_2O/hexane, 1:1) to give **7a** (112 mg, 65%) as an oil: $[\alpha]^{23}_D$ −35.6° (*c* 0.88, $CHCl_3$); IR (neat) 3388, 2960, 2864, 1732, 1170 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 0.06 (s, 12 H), 0.91 (s, 18 H), 1.43 (s, 9 H), 1.25−1.4 (m, 1 H), 1.65−1.90 (m, 2 H), 3.64 (s, 3 H), 3.76 (m, 2 H), 3.92 (m, 1 H), 4.03 (m, 2 H), 4.74 (br d, 1 H, *J* = 8 Hz); MS (SIMS) *m*/*z* 518 $(M + H)^+$, 417, 414. Anal. ($C_{25}H_{51}NO_6Si_2$) C, H, N.

(1*R*,2*R*,3*S*,1′*S*)-1-[1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[(*tert*-butyldimethylsilyl)oxy]ethyl]-2-[[(*tert*-butyldimethylsilyl)oxy]methyl]-3-(methoxymethyl)cyclopropane (8). To a solution of **7a** (620 mg, 1.20 mmol) in CH_2Cl_2 (12 mL) was added DIBAL-H (1 M hexane solution; 3.6 mL, 3.6 mmol) at −78 °C, and the solution was stirred at −78 °C for 30 min. The solution was diluted with Et_2O and the reaction quenched with ice tips. To this mixture was added $MgSO_4$, and the insoluble material was filtered off. The filtrate was washed successively with 1 N HCl and brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue. This was subjected to column chromatography on silica gel (Et_2O/hexane, 1:1) to give an alcohol (584 mg, 99%). To a solution of the alcohol in THF/Et_2O (1:1, 5 mL) were added *n*-BuLi (1.6 M hexane solution; 352 μL, 0.56 mmol) and methyl fluorosulfonate (48 μL, 0.61 mmol) at −78 °C, and the reaction mixture was stirred at −78 °C for 2 h. To this solution were added additional *n*-BuLi (1.6 M hexane solution; 60 μL, 0.094 mmol) and methyl fluorosulfonate (11 μL, 0.14 mmol). After the mixture stirred for 2 h at −78 °C, the reaction was quenched with a solution of LiOH (54 mg, 2.26 mmol) in MeOH (2 mL), and the mixture was extracted with Et_2O. The organic layer was washed with water and dried over $MgSO_4$. The solvent was removed under reduced pressure to give an oily residue. This was purified by column chromatography on silica gel to give **8** (221 mg, 93%) as an amorphous solid: $[\alpha]^{23}_D$ −45.7° (*c* 0.53, $CHCl_3$); IR (neat) 3360, 2936, 2864, 1718 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 0.04 (s, 12 H), 0.89 (s, 18 H), 1.28 (m, 3 H), 1.44 (s, 9 H), 3.31 (s, 3 H), 3.46 (m, 1 H), 3.64 (m, 1 H), 3.72 (m, 2 H), 3.80 (m, 1 H), 4.90 (br d, 1 H, *J* = 10 Hz). Anal. ($C_{25}H_{53}NO_5Si_2$) C, H, N.

(2*S*,1′*R*,2′*R*,3′*S*)-*N*-(*tert*-Butoxycarbonyl)-2-[2-(methoxycarbonyl)-3-(methoxymethyl)cyclopropyl]-glycine Methyl Ester (Boc-*cis*-MCG-IV Dimethyl Ester). To a solution of **8** (150 mg, 0.30 mmol) in MeOH (5 mL) was added Dowex 50W × 4 (30 mg), and the reaction mixture was stirred at room temperature for 18 h. The resin was filtered, and the filtrate was concentrated *in vacuo* to give an oily residue. The residue was dissolved in acetone, and to this solution was added Jones reagent at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 1 h. The reaction was quenched with 2-propanol and the mixture extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue, which, upon esterification with CH_2N_2 in Et_2O, gave lactam **9** as an oily compound. This was immediately treated with LiOH (5 mg) in MeOH (2 mL). The reaction mixture was stirred at room temperature for 10 min and then extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give an oily residue, which, upon puarification by column chromatography on silica gel (Et_2O), gave Boc-*cis*-MCG-IV dimethyl ester (40 mg, 40%) as an oil: $[\alpha]^{23}_D$ +23.7° (*c* 0.78, $CHCl_3$); IR (neat) 3384, 2936, 1728, 1170 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.46 (s, 9 H), 1.69 (dd, 1 H, *J* = 6.0, 9.0 Hz), 2.05 (dd, 1 H, *J* = 9.0, 9.0 Hz), 3.36 (s, 3 H), 3.70 (s, 3 H), 3.72 (s, 3 H), 3.79 (dd, 1 H, *J* = 7.0, 9.0 Hz), 3.79 (m, 1 H), 4.73 (m, 1 H), 5.32 (br s, 1 H). Anal. ($C_{15}H_{25}NO_7$) C, H, N.

(2*S*,1′*R*,2′*R*,3′*S*)-2-[2-Carboxy-3-(methoxymethyl)cyclopropyl]glycine (*cis*-MCG-IV). To a solution of Boc-*cis*-MCG-IV dimethyl ester (40 mg, 0.127 mmol) in THF (1 mL) was added 0.5 N NaOH (870 μL, 0.435 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 14 h and at room

temperature for 1 h. The pH of the reaction mixture was adjusted to 1 with 2 N HCl, and the solution was stirred at room temperature for 4 h. The solvent was removed *in vacuo*. The residue was passed through a column of Dowex 50W × 4 (100−200 mesh) ion exchange resin (H_2O and then 1 N aqueous NH_3) to give a solution of the ammonium salt of *cis*-MCG-IV. The eluate was concentrated *in vacuo* and then dissolved in water (2 mL). The pH of the solution was adjusted to 3 with 1 N HCl. Crude crystals that precipitated from the solution were collected by filtration. The crystals were recrystallized from water to give *cis*-MCG-IV (14 mg, 55%) as colorless crystals: mp 147.0−151.0 °C dec; $[\alpha]^{25}_D$ +83.3° (*c* 0.52, H_2O); IR (KBr) 3512, 1636, 1412 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 1.72 (ddd, 1 H, J = 9.0, 9.0, 11.5 Hz), 1.86 (dddd, 1 H, J = 7.5, 9.0, 9.0, 9.0 Hz), 2.16 (dd, 1 H, J = 9.0, 9.0 Hz), 3.35 (s, 3 H), 3.82 (dd, 1 H, J = 7.5, 11.0 Hz), 3.84 (dd, 1 H, J = 9.0, 11.0 Hz), 4.36 (d, 1 H, J = 11.5 Hz); MS (SIMS) *m/z* 204 $(M + H)^+$, 172, 45, 31, 18; HRMS (FAB) *m/z* calcd for $C_8H_{14}NO_5$ $(M + H)^+$ 204.0872, found 204.0872.

(1*S*,5*R*,6*S*,4′*S*)-6-[*N*-(*tert*-Butoxycarbonyl)-2,2-dimethyl-1,3-oxazolidin-4-yl]-3-oxabicyclo[3.1.0]hexan-2-one (7b). To a solution of **7a** (1.2 g, 2.32 mmol) in MeOH (20 mL) was added CSA (20 mg). The solution was stirred under N_2 at room temperature for 5 h. After removal of the solvent *in vacuo*, the residue was dissolved in CH_2Cl_2 (30 mL) and heated at reflux for 1 h; then to this solution was added 2,2-dimethoxypropane (15 mL). The reaction mixture was heated at reflux for 1.5 h. The solvent was removed *in vacuo* to give a crude crystalline residue, which, upon chromatography on silica gel (MeOH/$CHCl_3$, 1:20), gave **7b** (560 mg, 81%) as colorless needles: mp 135.5−136.0 °C; $[\alpha]^{23}_D$ −14.4° (*c* 0.5, $CHCl_3$); IR (neat) 2988, 1772, 1696, 1390, 1368 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 9 H), 1.52 (s, 3 H), 1.59 (s, 3 H), 1.74 (m, 1 H), 2.24−2.34 (m, 2 H), 3.77 (m, 1 H), 3.89 (dd, 1 H, J = 1.4, 8.8 Hz), 4.06 (d, 1 H, J = 10.4 Hz), 4.07 (dd, 1 H, J = 6.0, 8.8 Hz), 4.38 (dd, 1 H, J = 5.3, 10.4 Hz); HRMS (FAB) *m/z* calcd for $C_{15}H_{24}NO_5$ $(M + H)^+$ 298.1655, found 298.1654.

(4*S*,1′*S*,2′*S*,3′*R*)-3-*N*-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-[3-[[(*tert*-butyldimethylsilyl)oxy]methyl]-2-(methoxycarbonyl)cyclopropyl]-1,3-oxazolidine (10). A solution of **7b** (560 mg, 1.89 mmol) in THF (10 mL) and 0.5 N NaOH (4.9 mL, 2.45 mmol) was stirred at 0 °C for 16 h. The pH of the solution was adjusted to 2 with 1 N HCl (or 5% citric acid) and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. After removal of the solvent *in vacuo*, the residue was dissolved in EtOAc (5 mL). To this solution was added a solution of CH_2N_2 in Et_2O. The resulting solution was concentrated *in vacuo* to give an amorphous solid which was then passed through a short column of silica gel (EtOAc) to give the corresponding methyl ester as an oil. This was immediately submitted to the following reaction to avoid a lactonization which results in the recovery of the starting **7b**. To a solution of the methyl ester and imidazole (256 mg, 3.78 mmol) in DMF (7 mL) at 0 °C under N_2 was added *tert*-butyldimethylsilyl chloride (428 mg, 2.84 mmol) in DMF (5 mL). The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 2 h. The mixture was poured into ice-cooled water (50 mL) and extracted with Et_2O three times. The combined organic layer was washed with water and brine and dried over $MgSO_4$. After removal of the solvent *in vacuo*, the residue was purified by column chromatography on silica gel (Et_2O/hexane, 1:20 and then 1:9) to give **10** (865 mg, 100%) as an oil: $[\alpha]^{23}_D$ −56.0° (*c* 0.5, $CHCl_3$); IR (neat) 2964, 2868, 1734, 1704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.20 (s, 6 H), 0.88 (s, 9 H), 1.46 (s, 9 H), 1.40−1.62 (m, 2 H), 1.58 (s, 3 H), 1.60 (s, 3 H), 1.92 (dd, 1 H, J = 8.8, 8.8 Hz), 3.72 (s, 3 H), 3.93 (br s, 1 H), 4.03 (dd, 1 H, J = 5.8, 8.8 Hz), 4.10 (br s, 2 H), 4.34 (br s, 1 H); HRMS (FAB) *m/z* calcd for $C_{22}H_{42}NO_6Si$ $(M + H)^+$ 444.2781, found 444.2764.

(4*S*,1′*S*,2′*R*,3′*R*)-3-*N*-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-[3-[[(*tert*-butyldimethylsilyl)oxy]methyl]-2-(methoxycarbonyl)cyclopropyl]-1,3-oxazolidine (11). To a solution of **10** (840 mg, 1.9 mmol) in THF (20 mL) at −78 °C under N_2 was added, dropwise, a 0.5 M solution of $KN(TMS)_2$ (4.16 mL, 2.08 mmol). The reaction mixture was stirred at −78 °C for 30 min, at −15 °C for 1.5 h, and then at −78 °C for 10 min. To this solution at −78 °C was added a solution of acetic acid (148 mg, 2.48 mmol) in THF (2 mL). The reaction mixture was diluted with Et_2O (50 mL), washed with brine, and dried over $MgSO_4$. After removal of the solvent *in vacuo*, the residue was purified by column chromatography on silica gel (Et_2O/hexane, 1:9) to give **11** (719 mg, 84%) as colorless crystals: mp 92.0−92.5 °C; $[\alpha]^{23}_D$ +7.5° (*c* 0.8, $CHCl_3$); IR (neat) 2936, 1726, 1698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, as a mixture of the rotamers) δ 0.04 (s, 3 H), 0.06 (s, 3 H), 0.88 (s, 9 H), 1.48 (s, 12 H), 1.54 (s, 3 H), 1.71 (m, 1 H), 1.95 (m, 0.7 H), 1.95 (m, 0.7 H), 2.17 (m, 0.3 H), 3.46 (dd, 1 H, J = 8.0, 10.5 Hz), 3.66 (s, 3 H), 3.70 (m, 0.7 H), 3.84 (m, 0.3 H), 3.96 (dd, 1 H, J = 5.2, 8.5 Hz), 3.98 (m, 1 H), 4.02 (dd, 1 H, J = 8.5, 8.5 Hz); HRMS (FAB) *m/z* calcd for $C_{22}H_{42}NO_6Si$ $(M + H)^+$ 444.2781, found 444.2777.

(4*S*,1′*S*,2′*R*,3′*R*)-3-*N*-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-[2-(methoxycarbonyl)-3-(methoxymethyl)cyclopropyl]-1,3-oxazolidine (12). To a solution of **11** (350 mg, 0.79 mmol) was added *n*-Bu_4NF (1 M THF solution; 0.8 mL, 0.8 mmol) at 0 °C under N_2, and the solution was stirred at room temperature for 15 min. The solvent was evaporated *in vacuo* to give an oily residue, which was subjected to column chromatography on silica gel (Et_2O/hexane = 1:3 and then 1:1) to give an hydroxymethyl compound (258 mg, 99%) as an oil: $[\alpha]^{23}_D$ +34.6° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz, as a mixture of the rotamers) δ 1.50 (s, 12 H), 1.62 (br s, 3 H), 1.70−1.92 (m, 2 H), 2.05 (m, 0.7 H), 2.20 (m, 0.3 H), 3.60 (m, 2 H), 3.70 (s, 3 H), 3.84−4.06 (m, 4 H).

To a suspension of NaH (60% oily suspension; 40 mg, 1.0 mmol) in DMF (5 mL) was added a solution of the hydroxymethyl compound (191 mg, 0.58 mmol) in DMF (7 mL) at 0 °C under N_2, and the solution was stirred for 30 min. To the reaction mixture were added successively MeI (284 mg, 2.0 mmol) and *n*-Bu_4NI (30 mg, 0.08 mmol). The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 2 h, and the reaction was quenched with ice tips. This was extracted with Et_2O, washed with brine, and dried over $MgSO_4$. The solvent was concentrated *in vacuo* to give an oily residue, which was purified by column chromatography on silica gel (Et_2O/hexane = 1:1) to give **12** (183 mg, 92%) as colorless crystals: mp 109.0−109.5 °C dec; $[\alpha]^{23}_D$ +34.2° (*c* 0.12, $CHCl_3$); IR (neat) 2940, 1770, 1730, 1696 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, as a mixture of the rotamers) δ 1.48 (s, 12 H), 1.62 (br s, 3 H), 1.80 (m, 2 H), 2.0 (m, 0.7 H), 2.2 (m, 0.3 H), 3.32 (s, 3 H), 3.32 (m, 1 H), 3.55−3.90 (m, 4 H), 3.92−4.01 (m, 2 H); HRMS (FAB) *m/z* calcd for $C_{17}H_{30}NO_6$ $(M + H)^+$ 344.2073, found 344.2070.

(2*S*,1′*S*,2′*R*,3′*R*)-*N*-(*tert*-Butoxycarbonyl)-2-[2-(methoxycarbonyl)-3-(methoxymethyl)cyclopropyl]-glycine Methyl Ester (Boc-*cis*-MCG-I Dimethyl Ester). To a solution of **12** (253 mg, 0.685 mmol) in CH_2Cl_2 (4 mL) was added TFA (4 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min and at room temperature for 10 min. This was concentrated *in vacuo*, and the residue was dissolved in dioxiane (2 mL) and water (2 mL). The pH of the solution was adjusted to 9 with Et_3N, and to this solution was added Boc_2O (500 μL). The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo*, and the residue was extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue, which, upon column chromatography on silica gel (Et_2O/hexane = 1:1), gave a glycinol (206 mg, 99%) as an oil: $[\alpha]^{23}_D$ +5.4° (*c* 0.65, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9 H), 1.67 (ddd, 1 H, J = 4.9, 9.3, 9.3 Hz), 1.74 (s, 1 H), 1.78 (dd, 1 H, J = 4.9, 4.9 Hz), 1.92 (dddd, 1 H, J = 4.9, 5.7, 9.3, 9.3 Hz), 2.95 (m, 1 H), 3.34 (s, 3 H), 3.34 (m, 1 H), 3.48 (m, 1 H), 3.60−3.75 (m, 2 H), 3.66 (s, 3 H), 4.88 (d, 1 H, J = 6.5 Hz).

To a solution of the glycinol (167 mg, 0.55 mmol) in acetone (5 mL) was added Jones reagent at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 1 h and the reaction quenched with 2-propanol at 0 °C. This was extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue, to which was added CH_2N_2 in Et_2O. The solvent was evaporated *in vacuo*, and the residue was purified by column chromatography on silica gel (Et_2O/hexane = 1:9) to give Boc-*cis*-MCG-I dimethyl ester (132 mg, 73%) as colorless crystals: mp 105.0−107.0 °C; $[\alpha]^{23}_D$ +50.0°

(*c* 0.2, $CHCl_3$); IR (neat) 3384, 2992, 1750, 1722, 1684 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.45 (s, 9 H), 1.72−1.88 (m, 2 H), 1.92 (dd, 1 H, J = 4.9, 5.2 Hz), 3.36 (s, 3 H), 3.49 (dd, 1 H, J = 5.4, 10.5 Hz), 3.67 (s, 3 H), 3.68 (dd, 1 H, J = 5.2, 10.5 Hz), 3.78 (s, 3 H), 4.14 (dd, 1 H, J = 8.5, 10.5 Hz), 5.22 (d, 1 H, J = 8.5 Hz); HRMS (FAB) *m/z* calcd for $C_{15}H_{26}NO_7$ $(M + H)^+$ 332.1709, found 332.1705.

(2*S*,1′*S*,2′*R*,3′*R*)-2-[2-Carboxy-3-(methoxymethyl)cyclopropyl]glycine (*cis*-MCG-I). To a solution of Boc-*cis*-MCG-I dimethyl ester (129 mg, 0.39 mmol) in THF (1.5 mL) was added 1 N NaOH (0.93 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h and at room temperature for 40 h. The pH of the solution was adjusted to 3 with 1 N HCl. The solution was concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2 (2 mL) and TFA (2 mL). The solution was stirred at room temperature for 30 min. The solvent was evaporated *in vacuo* to give an oily residue. This was subjected to column chromatography on Dowex 50W × 4 (H_2O and then 1 N aqueous NH_3) to give a solution of the ammonium salt of *cis*-MCG-I. The solution was concentrated *in vacuo*, and the residue was dissolved in water. The pH of the solution was adjusted to 2 with 1 N HCl. The crude crystals precipitated from the solution were recrystallized from water to give *cis*-MCG-I (53 mg, 67%) as colorless crystals: mp 208−212 °C dec; $[\alpha]^{23}_D$ +35.0° (*c* 0.5, H_2O); ^1H NMR (D_2O, 270 MHz) δ 1.75−1.93 (m, 3 H), 3.28 (s, 3 H), 3.33 (dd, 1 H, J = 8.6, 11.0 Hz), 3.44 (d, 1 H, J = 10.0 Hz), 3.81 (dd, 1 H, J = 4.6, 11.0 Hz); HRMS (FAB) *m/z* calcd for $C_8H_{14}NO_5$ $(M + H)^+$ 204.0872, found 204.0884.

(4*S*,1′*R*,2′*R*,3′*R*)-*N*-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-[2-[[(*tert*-butyldimethylsilyl)oxy]methyl]-3-(methoxymethyl)cyclopropyl]-1,3-oxazolidine (13). In a manner similar to the preparation of **8** from **7a**, **13** (114 mg, 85%) was obtained from **11** (140 mg, 0.315 mmol) as an amorphous solid: $[\alpha]^{23}_D$ −20.8° (*c* 0.52, $CHCl_3$); IR (neat) 2964, 2940, 2868, 1698, 1464 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.03 (s, 3 H), 0.06 (s, 3 H), 0.88 (s, 9 H), 1.05 (m, 2 H), 1.50 (s, 12 H), 1.60 (m, 4 H), 3.05−3.22 (m, 1 H), 3.31 (s, 3 H), 3.30−3.52 (m, 2 H), 3.62−3.84 (m, 1 H), 3.95 (dd, 1 H, J = 5.5, 8.5 Hz), 4.04 (dd, 1 H, J = 5.0, 12.0 Hz), 4.05 (m, 1 H); HRMS (FAB) *m/z* calcd for $C_{22}H_{44}NO_5Si$ $(M + H)^+$ 430.2989, found 430.2993.

(2*S*,1′*R*,2′*R*,3′*R*)-*N*-(*tert*-Butoxycarbonyl)-2-[2-carboxy-3-(methoxymethyl)cyclopropyl]glycinol. To a solution of **13** (49 mg, 0.114 mmol) was added *n*-Bu_4NF (1 M THF solution; 125 μL, 0.125 mmol) at 0 °C under N_2, and the solution was stirred at room temperature for 2 h. The solvent was evaporated *in vacuo* to give an oily residue, which, upon column chromatography on silica gel (Et_2O/hexane = 1:1), gave an oily residue. To a solution of the resulting primary alcohol in *tert*-butyl alcohol (2 mL) were added successively 1 N NaOH (0.46 mL, 0.46 mmol) and a solution of $KMnO_4$ (72 mg, 0.46 mmol) in H_2O (2 mL) at room temperature. The solution was stirred at room temperature for 2 h and the reaction quenched with 5% $Na_2S_2O_3$ aqueous solution (1 mL). The mixture was acidified with 1 N HCl and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give a crude oil, which was esterified with CH_2N_2 in Et_2O. The solvent was removed *in vacuo*, and the resulting residue was subjected to column chromatography on silica gel (Et_2O/hexane = 1:1) to give a methyl ester (34 mg, 87%): ^1H NMR ($CDCl_3$, 400 MHz, as a mixture of the rotamers) δ 1.45 (s, 9 H), 1.4−1.8 (m, 8 H), 2.12 (m, 0.7 H), 2.27 (m, 0.3 H), 3.22 (m, 1H), 3.32 (s, 3 H), 3.50 (m, 1 H), 3.66 (s, 3 H), 3.68 (m, 1 H), 3.90 (dd, 1 H, J = 5.5, 9.0 Hz), 4.12 (m, 0.7 H), 4.22 (m, 0.3 H).

A solution of the ester (22 mg, 0.064 mmol) and CSA (2 mg) in MeOH (1 mL) was stirred at room temperature for 16 h. The reaction mixture was extracted with EtOAc several times. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give an oily residue, which, upon column chromatography on silica gel (EtOAc), gave the methyl ester of the title compound (16 mg, 84%): ^1H NMR ($CDCl_3$, 400 MHz) δ 1.45 (s, 9 H), 1.50 (m, 1 H), 1.77 (dd, 1 H, J = 5.0, 9.0 Hz), 1.96 (m, 1 H), 2.82 (br s, 1 H), 3.33 (m, 1 H), 3.35 (s, 3 H), 3.39 (m, 1 H), 3.56 (m, 1 H), 3.63 (m, 1H), 3.68 (s, 3 H), 3.78 (m, 1 H), 5.00 (br s, 1 H).

To a solution of the methyl ester (16 mg, 0.052 mmol) in MeOH (0.4 mL) was added 1 N NaOH (200 μL, 0.2 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 5 h. The pH of the solution was adjusted to 2 with 1 N HCl. The solution was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give the title compound (13 mg, 85%) as colorless crystals: mp 113.0−114.0 °C; $[\alpha]^{23}_D$ −54.1° (*c* 0.54, $CHCl_3$); IR (neat) 3456, 2984, 1712 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 (m, 1 H), 1.44 (s, 9 H), 1.77 (dd, 1 H, J = 5.0, 9.0 Hz), 1.98 (m, 1 H), 3.32 (m, 1 H), 3.33 (s, 3 H), 3.38 (m, 1 H), 3.62 (m, 2 H), 3.78 (m, 1 H), 4.80 (br s). Anal. ($C_{13}H_{23}NO_6$) C, H, N.

(2*S*,1′*R*,2′*R*,3′*R*)-2-[2-Carboxyl-3-(methoxymethyl)cyclopropyl]glycine (*trans*-MCG-IV). To a solution of the glycinol (70 mg, 0.24 mmol) in acetone (2 mL) was added Jones reagent at 0 °C, and the reaction mixture was stirred at 0 °C for 3 h and then at room temperature for 2 h. The reaction was quenched with 2-propanol and the mixture extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue. To a solution of the residue in CH_2Cl_2 (1 mL) at 0 °C was added TFA (1 mL). The mixture was stirred for 30 min at room temperature and then concentrated *in vacuo*. The residue was passed through a column of Dowex 50W × 4 (100−200 mesh) ion exchange resin (H_2O and then 1 N aqueous NH_3) to give a solution of the ammonium salt of *trans*-MCG-IV. The solution was concentrated *in vacuo* and dissolved in water (2 mL). The pH of the solution was adjusted to 3 with 1 N HCl. The crude crystals that precipitated from the solution were collected by filtration. They were recrystallized from water to give *trans*-MCG-IV (14 mg, 28%) as colorless crystals: mp 185.5−187.0 °C dec; $[\alpha]^{25}_D$ +31.5° (*c* 0.47, H_2O); IR (KBr) 2981, 1710, 1624, 1578, 1400 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 1.68 (ddd, 1 H, J = 6.3, 9.1, 10.4 Hz), 1.90 (dddd, 1 H, J = 5.1, 6.1, 6.3, 7.7 Hz), 2.03 (dd, 1 H, J = 5.1, 9.1 Hz), 3.32 (dd, 1 H, J = 7.7, 10.8 Hz), 3.37 (s, 3 H), 3.58 (dd, 1 H, J = 6.1, 10.8 Hz), 3.90 (d, 1 H, J = 10.8 Hz); MS (SIMS) *m/z* 204 $(M + H)^+$, 45, 31, 18. Anal. ($C_8H_{13}NO_5$) C, H, N.

(4*S*,1′*S*,2′*S*)-*N*-(*tert*-Butoxycarbonyl)-4-[2-(ethoxycarbonyl)cyclopropyl]-2,2-dimethyl-1,3-oxazolidine (16a). To a solution of (4*S*,1′*S*,2′*R*)-*N*-(*tert*-butoxycarbonyl)-4-[2-(ethoxycarbonyl)cyclopropyl]-2,2-dimethyl-1,3-oxazolidine (**14a**)[8b] (75 mg, 0.24 mmol) was added a solution of $KN(TMS)_2$ in THF (0.5 M solution; 536 μL, 0.26 mmol) at −78 °C, and the reaction mixture was stirred at −78 °C for 30 min and at −15 °C for 1 h; then the reaction was quenched with AcOH (18 μL) in THF (1 mL) at −78 °C. The reaction mixture was extracted with AcOEt, and the organic layer was dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give **16a** (75 mg, 100%) as an oil: $[\alpha]^{23}_D$ +66.4° (*c* 1.25, $CHCl_3$); IR (neat) 2988, 2944, 1734, 1700, 1388 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, as a mixture of the rotamers) δ 0.78 (m, 1 H), 1.12 (ddd, 1 H, J = 5, 5, 9 Hz), 1.23 (t, 3 H, J = 7 Hz), 1.49 (s, 9 H), 1.44−1.65 (m, 6 H), 1.70 (dddd, 1 H, J = 4, 6.5, 8.5, 10.5 Hz), 1.89 (m, 0.5 H), 2.00 (m, 0.5 H), 3.36 (m, 0.5 H), 3.65−3.86 (m, 1.5 H), 3.94 (m, 1 H), 4.14 (m, 2 H).

(4*S*,1′*S*,2′*R*)-*N*-(*tert*-Butoxycarbonyl)-4-(2-formylcyclopropyl)-2,2-dimethyl-1,3-oxazolidine (14b). To a solution of (4*S*,1′*S*,2′*R*)-*N*-(*tert*-butoxycarbonyl)-4-[2-(ethoxycarbonyl)-cyclopropyl]-2,2-dimethyl-1,3-oxazolidine (**14a**)[8b] (576 mg, 1.83 mmol) was added DIBAL-H (1.5 M toluene solution; 4.2 mL, 6.3 mmol) at −78 °C. The reaction mixture was stirred for 30 min at 0 °C. The solution was diluted with Et_2O and the reaction quenched with ice tips. The solution was washed successively with 1 N HCl and brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue, which was subjected to column chromatography on silica gel (Et_2O) to give a primary alcohol as an oil. The alcohol was dissolved in DMSO (10 mL), and to this solution were added successively a solution of SO_3−pyridine complex (917 mg, 5.76 mmol) in DMSO (10 mL) and Et_3N (801 μL, 5.76 mmol). The solution was stirred at room temperature for 15 min and poured into ice water. The solution was extracted with AcOEt, washed successively with 5% aqueous citric acid and brine, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue, which was subjected to column chromatography on silica gel (Et_2O) to give **14b** (306 mg, 62%) as an amorphous solid: $[\alpha]^{23}_D$ −49.5° (*c* 0.95, $CHCl_3$); IR (neat) 2992, 2944, 2892, 1696, 1386 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz)

δ 1.10 (m, 2 H), 1.45 (s, 9 H), 1.58 (s, 6 H), 1.70 (m, 1 H), 2.11 (m, 1 H), 3.72 (br s, 1 H), 3.92 (d, 1 H, J = 8.5 Hz), 4.03 (dd, 1 H, J = 5.5, 8.5 Hz), 9.57 (d, 1 H, J = 4.0 Hz); HRMS (FAB) m/z calcd for $C_{14}H_{24}NO_4$ (M + H)$^+$ 270.1706, found 270.1687.

(4*S*,1′*S*,2′*S*)-*N*-(*tert*-Butoxycarbonyl)-4-(2-deuterio-2-formylcyclopropyl)-2,2-dimethyl-1,3-oxazolidine (16c). A solution of **14b** (20 mg, 0.074 mmol) in 0.1 M CD_3ONa/CD_3OD (1 mL) was heated at 65 °C for 2 days. The reaction was quenched with AcOH. The mixture was extracted with AcOEt and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue, which was subjected to column chromatography on silica gel (Et_2O) to give **16c** (17 mg, 85%) as colorless crystals: mp 74.5−76.0 °C; $[\alpha]^{23}_D$ +74.9° (*c* 0.67, $CHCl_3$); IR (neat) 2988, 2948, 2876, 1700, 1384 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz, as a mixture of the rotamers) δ 0.98 (dd, 1 H, J = 5.5, 5.5 Hz), 1.24 (dd, 1 H, J = 5.5, 9.0 Hz), 1.44 (s, 9 H), 1.60 (m, 6 H), 1.80 (dd, 1 H, J = 5.5, 9.0 Hz), 3.50 (m, 0.5 H), 3.78 (m, 1.5 H), 3.98 (m, 1 H), 9.02 (br s, 0.5 H), 9.25 (br s, 0.5 H); MS (FAB) m/z 271 (M + H)$^+$; HRMS (FAB) m/z calcd for $C_{14}H_{23}DNO_4$ (M + H)$^+$ 271.1768, found 271.1757.

(4*S*,1′*S*,2′*S*,3′*R*)-*N*-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-[3-formyl-2-(methoxycarbonyl)cyclopropyl]-1,3-oxazolidine (18). To a solution of **7b** (160 mg, 0.54 mmol) in MeOH (2 mL) was added 1 N NaOH (1 mL) at 0 °C, and the reaction mixture was stirred at the same temperature for 3 h. The pH of the solution was adjusted to 3 with 5% aqueous citric acid. This was extracted with AcOEt, and the organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue, which was treated with CH_2N_2 in Et_2O. The crude product was purified by silica gel column chromatography on silica gel (Et_2O) to give a methyl ester (163 mg, 92%). To a solution of the methyl ester (163 mg, 0.49 mmol) in CH_2Cl_2 (2 mL) was added pyridiunium dichromate (PDC) (558 mg, 1.48 mmol). The resulting suspension was stirred at room temperature for 20 h and diluted with Et_2O. The suspension was filtered, and the filtrate was concentrated *in vacuo* to give an oily residue, which, upon column chromatography on silica gel (Et_2O/hexane = 1:3 and then 1:1), gave **18** (119 mg, 74%) as colorless crystals: mp 84.0−86.6 °C; $[\alpha]^{23}_D$ −62.5° (*c* 0.60, $CHCl_3$); IR (neat) 3488, 2988, 2884, 1732, 1694 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9 H), 1.58 (s, 3 H), 1.61 (s, 3 H), 2.00 (ddd, 1 H, J = 9.0, 9.0, 9.0 Hz), 2.06 (m, 1 H), 2.48 (dd, 1 H, J = 9.0, 9.0 Hz), 3.79 (s, 3 H), 3.86 (d, 1 H, J = 9.0 Hz), 4.08 (dd, 1 H, J = 6.0, 9.0 Hz), 4.68 (m, 1 H), 9.89 (br s, 1 H); HRMS (FAB) m/z calcd for $C_{16}H_{26}NO_6$ (M + H)$^+$ 328.1760, found 328.1759.

(4*S*,1′*S*,2′*S*,3′*S*)-*N*-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-[3-(methoxymethyl)-2-(methoxycarbonyl)cycyopropyl]-1,3-oxazolidine (20a). A solution of **18** (112 mg, 0.34 mmol) in 0.1 M CH_3ONa/CH_3OH (10 mL) was stirred at 70 °C for 6 h. The reaction was quenched with acetic acid (100 μL) at 0 °C. The reaction mixture was concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel (Et_2O) to give **19** (111 mg, 100%) as an oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9 H), 1.58 (s, 6 H), 2.08 (ddd, 1 H, J = 5.0, 9.0, 9.0 Hz), 2.46 (m, 1 H), 2.55 (dd, 1 H, J = 4.5, 9.5 Hz), 3.78 (s, 3 H), 3.84 (d, 1 H, J = 9.0 Hz), 4.04 (dd, 1 H, J = 6.0, 9.0 Hz), 4.11 (m, 1 H), 9.36 (d, 1 H, J = 3.0 Hz).

To a solution of **19** (111 mg, 0.34 mmol) in EtOH was added $NaBH_4$ (60 mg, 1.6 mmol) at 0 °C. The solution was concentrated *in vacuo*, and the oily residue was extracted with AcOEt. The organic layer was dried over $MgSO_4$, and the solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (Et_2O) to give the 3′-hydroxymethyl compound (75 mg, 67%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9 H), 1.54−1.64 (m, 7 H), 1.66 (m, 1 H), 1.85 (dd, 1 H, J = 5.0, 9.0 Hz), 3.58 (m, 2 H), 3.74 (s, 3 H), 3.91 (d, 1 H, J = 7.5 Hz), 4.02 (m, 2 H).

To a solution of the hydroxymethyl compound (75 mg, 0.23 mmol) in DMF (2 mL) were added NaH (60% oily suspension; 18 mg, 0.46 mmol) and *n*-Bu_4NI (8 mg, 0.02 mmol) at 0 °C under N_2. The reaction mixture was stirred at 0 °C for 20 min. To the mixture was added MeI (42 mg, 0.69 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h. The reactino was quenched with 5% aqueous citric acid at 0 °C. The reaction mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to given an oily residue, which, upon column chromatography on silica gel (Et_2O/hexane = 1:3), gave **20a** (52 mg, 66%) as an oil: $[\alpha]^{23}_D$ −24.8° (*c* 1.12, $CHCl_3$); IR (neat) 2988, 2948, 1732, 1702, cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 12 H), 1.58−1.68 (m, 5 H), 1.83 (dd, 1 H, J = 5.0, 9.0 Hz), 3.25−3.36 (m, 2 H), 3.31 (s, 3 H), 3.73 (s, 3 H), 3.92 (d, 1 H, J = 7.0 Hz), 4.01 (m, 2 H); HRMS (FAB) m/z calcd for $C_{17}H_{30}NO_6$ (M + H)$^+$ 344.2073, found 344.2070.

(2*S*,1′*S*,2′*S*,3′*S*)-2-[2-Carboxy-3-(methoxymethyl)cyclopropyl]glycine (*trans*-MCG-III). In a manner similar to the preparation of *trans*-MCG-IV from (4*S*,1′*R*,2′*R*,3′*R*)-*N*-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-[2-(methoxycarbonyl)-3-(methoxymethyl)cyclopropyl]-1,3-oxazoline, *trans*-MCG-III was prepared from **20a** as colorless crystals: mp 195.0−198.0 °C; $[\alpha]^{25}_D$ +59.6° (*c* 0.54, H_2O); IR (KBr) 3152, 2871, 1702, 1617, 1560, 1485 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 1.67 (ddd, 1 H, J = 6.3, 8.1, 10.6 Hz), 1.90 (dd, 1 H, J = 5.3, 8.1 Hz), 2.05 (dddd, 1 H, J = 5.3, 5.4, 6.3, 7.8 Hz), 3.29 (dd, 1 H, J = 7.8, 8.1 Hz), 3.37 (s, 3 H), 3.68 (dd, 1 H, J = 5.4, 11.2 Hz), 4.01 (d, 1 H, J = 10.6 Hz). Anal. ($C_8H_{13}NO_5$) C, H, N.

(4*S*,1′*S*,2′*S*,3′*S*)-*N*-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-[2-formyl-3-(methoxymethyl)cyclopropyl]-1,3-oxazolidine (20b). To a solution of **20a** (69 mg, 0.20 mmol) was added DIBAL-H (1.5 M toluene solution; 402 μL, 0.60 mmol) at −78 °C. The reaction mixture was stirred for 30 min at 0 °C. The solution was diluted with Et_2O and the reaction quenched with ice tips. The solution was washed successively with 1 N HCl and brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue, which was subjected to column chromatography on silica gel (Et_2O) to give a primary alcohol (59 mg, 94%) as an oil. To a solution of the alcohol (34 mg, 0.12 mmol) in CH_2Cl_2 (2 mL) was added PDC (170 mg, 0.45 mmol). The suspension was stirred at room temperature for 20 h and diluted with Et_2O. The resulting mixture was filtered, and the filtrate was concentrated *in vacuo* to give an oily residue. This was purified by column chromatography on silica gel (Et_2O) to give **20b** (26 mg, 76%) as an amorphous solid: $[\alpha]^{23}_D$ +6.9° (*c* 0.65, $CHCl_3$); IR (neat) 2992, 2944, 1708, 1696 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 12 H), 1.60 (s, 3 H), 1.78 (ddd, 1 H, J = 5.0, 5.5, 11.5 Hz), 2.07 (ddd, 1 H, J = 4.0, 5.0, 9.0 Hz), 3.28 (m, 1 H), 3.31 (s, 3 H), 3.37 (dd, 1 H, J = 6.0, 10.0 Hz), 3.77 (br s, 1 H), 3.92 (dd, 1 H, J = 1.5, 9.0 Hz), 4.03 (dd, 1 H, J = 5.5, 9.0 Hz), 9.59 (d, 1 H, J = 4.0 Hz); HRMS (FAB) m/z calcd for $C_{16}H_{28}NO_5$ (M + H)$^+$ 314.1968, found 314.1952.

(2*S*,1′*S*,2′*R*,3′*S*)-*N*-(*tert*-Butoxycarbonyl)-2-[2-(methoxycarbonyl)-3-(methoxymethyl)cyclopropyl]-glycine Methyl Ester (Boc-*trans*-MCG-I Dimethyl Ester). A solution of **20b** (41 mg, 0.13 mmol) in 0.1 M CH_3ONa/CH_3OH (5 mL) was stirred at 70 °C for 22 h. The reaction was quenched with acetic acid (50 μL) at 0 °C, and the mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (Et_2O) to give a ∼2.5:1 mixture of (2′*R*)-**21** and the starting material **20b** (41 mg). (2′*R*)-**21**: ^{1}H NMR ($CDCl_3$, 400 MHz, 50 °C) δ 1.45 (s, 9 H), 1.59 (s, 3 H), 1.61 (s, 3 H), 1.69 (m, 1 H), 2.00 (dddd, 1 H, J = 5.5, 6.5, 7.5, 11.5 Hz), 2.35 (br s, 1 H), 3.28 (s, 3 H), 3.38 (dd, 1 H, J = 7.0, 10.0 Hz), 3.63 (dd, 1 H, J = 6.0, 10.0 Hz), 3.70 (m, 1 H), 3.76 (dd, 1 H, J = 1.5, 9.0 Hz), 3.96 (dd, 1 H, J = 6.0, 9.0 Hz), 9.56 (br s, 1 H).

To a solution of the mixture of the isomers (41 mg, 0.13 mmol) in acetone (2 mL) was added Jones reagent at 0 °C. The reaction mixture was stirred at room temperature for 5 h and the reaction quenched with 2-propanol at 0 °C. The reaction mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue, to which was added CH_2N_2 in Et_2O. The solvent was evaporated *in vacuo*, and the residue was purified by column chromatography on silica gel (Et_2O/hexane = 1:1) to give Boc-*trans*-MCG-I dimethyl ester (15 mg, 35%) as an oil: $[\alpha]^{23}_D$ +42.6° (*c* 1.00, $CHCl_3$); IR (neat) 3384, 2984, 1730, 1724 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (s, 9 H), 1.75 (m, 2 H), 1.93 (dd, 1 H, J = 5.5, 9.0 Hz), 3.28 (s, 3 H), 3.42 (dd, 1 H, J = 8.0, 10.5 Hz), 3.66 (dd, 1 H, J = 5.0, 10.5 Hz), 3.67 (s, 3 H), 3.76 (s, 3 H);

HRMS (FAB) *m/z* calcd for $C_{15}H_{26}NO_7$ $(M + H)^+$ 332.1709, found 332.1696.

(2*S*,1′*S*,2′*R*,3′*S*)-2-[2-Carboxy-3-(methoxymethyl)cyclopropyl]glycine (*trans*-MCG-I). To a solution of the dimethyl ester (20 mg, 0.06 mmol) in THF (0.5 mL) was added 1 N NaOH (0.2 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h and then at room temperature for 5 h. The pH of the solution was adjusted to 1 with 1 N HCl, and the solution was stirred at room temperature for 24 h. The solvent was evaporated *in vacuo* to give an oily residue. This was subjected to a column of Dowex 50W × 4 (H_2O and then 1 N aqueous NH_3) to give a solution of the ammonium salt of *trans*-MCG-I. The solution was concentrated *in vacuo* and dissolved in water. The pH of the solution was adjusted to 2 with 1 N HCl. The crude crystals precipitated from the solution were recrystallized from water to give *trans*-MCG-I (9.5 mg, 79%) as colorless crystals: mp 164−168 °C dec; $[\alpha]^{23}_D$ +19.9° (*c* 0.95, H_2O); ^1H NMR (D_2O, 400 MHz) δ 1.68 (ddd, 1 H, J = 4.5, 5.0, 10.0 Hz), 1.83 (dddd, 1 H, J = 5.0, 5.5, 8.5, 9.5 Hz), 1.92 (dd, 1 H, J = 4.5, 9.5 Hz), 3.28 (d, 1 H, J = 10.0 Hz), 3.35 (s, 3 H), 3.53 (dd, 1 H, J = 8.5, 11.0 Hz), 3.79 (dd, 1 H, J = 5.5, 11.0 Hz); HRMS (FAB) *m/z* calcd for $C_8H_{14}NO_5$ $(M + H)^+$ 204.0872, found 204.0869.

NMR Titrations. About 5 mg of the sample was dissolved in 1 mL of D_2O. To this solution was added a solution of TSP in D_2O as an internal standard. The pD value of the solution was adjusted by adding a small amount of DCl (20% solution in D_2O) and/or NaOD (40% solution in D_2O) through a capillary tube. After the pD value of the solution was recorded by glass electrode pH/ion meter (Iwaki Glass, M-225), ^1H NMR of the solution was measured. The pD dependence of the chemical shifts of each proton was recorded at appropriate 20−30 points through the range between pD 0.5 and 12.5.

Calculations. All molecular modelings were performed on an IBM workstation RS/6000 model 320 using QUANTA (version 3.3)/CHARMm system (Molecular Simulations Inc.). Construction of molecular structures and assignment of atom properties were excuted by using the ChemNote application. The CHARMm energy minimization (Newton−Raphson algorithm) with the distance-dependent dielectric term (ϵ = 80) was applied to all molecules.[17] The Conformation Search (Grid Scan) application was employed for CCGs, 3′-Me-CCGs, and glutamate as the following sequences: (1) the rotation barrier was obtained by the fixed grid torsion minimization and (2) the minimized structures and their energies were calculated by the torsion free minimization for each structure obtained by step 1. The defined tortions were as follows: for CCGs and 3′-Me-CCGs, ϕ_1 = C1−C2−C1′−C2′, 0−350° in 10° increments (36 structures); for glutamate, ϕ_1 = C1−C2−C3−C4, ϕ_2 = C2−C3−C4−C5, 0−330° in 30° increments (12 × 12 structures). The minimized conformations of other compounds were obtained from their plural initial structures which were constructed by Grid Scan application or Molecular Dynamics. The Molecular Similarity application was used for superimposing the refined structures. The corresponding nitrogen, α-carboxyl carbon, and the distal carboxyl carbon of each molecule were overlaid using a rigid body fit to the target CCG conformer.

Acknowledgment. We thank Drs. Haruhiko Shinozaki and Michiko Ishida, Tokyo Metropolitan Institute of Medical Sciences, for fruitful discussions. We are grateful to Dr. Susumu Yoshikawa, Osaka Industrial Technology Research Institute, Ministry of International Trade and Industries, Japan, for his kind permission to use the QUANTA/CHARMm system for molecular mechanics calculations of the glutamate analogs. We thank Dr. Glen Spears, Fujisawa Pharmaceutical Co. Ltd., for help in preparing the manuscript. This work was supported by a grant-in-aid from the Ministry of Education, Culture, and Science, Japan.

Supporting Information Available: X-ray crystallographic data of CCG-II−IV and drawings of the superimposed structures of CCG-I, (1*S*,3*R*)-ACPD, and aa-A, CMP-II and g⁻a-C, and CMP-III, ag⁺-D, and ag⁻-G (15 pages). Ordering information is given on any current masthead page.

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(37) Calculations of *cis*-MCG-I and -IV were performed by rotating the C2−C1′ bond and the two bonds of the C3′ methoxymethyl group (in 60° increments) and provided 32 and 23 minimized structures, respectively. Among them, the existence ratio of the H−C2−Cl′−H antiperiplanar type conformers were 96.6% for *cis*-MCG-I and 99.3% for *cis*-MCG-IV. These results were in good agreement with those of Me-substituted CCGs.

(38) We thank Dr. N. Hamanaka, Director, Minase Research Institute of Ono Pharmaceutical Co. Ltd., for X-ray crystallographic analysis of CCGs. We could not prepare crystals of CCG-I suitable for X-ray crystallographic analysis because this compound formed fine powders under numerous recrystallization conditions.

(39) The conformation of glutamate in the crystalline state was examined by X-ray crystallographic analysis and neutron diffraction analysis and found to be the gauche forms g^+g^+ (Tor 1 = 60.0°, Tor 2 = 68.3°) and ag^- (Tor 1 = −173.0°, Tor 2 = −73.1°), respectively. (a) Lehmann, M. S.; Nunes, A. C. A short hydrogen bond between near identical carboxyl group in the α modification of L-glutamic acid. *Acta Crystallogr.* **1980**, *B36*, 1621−1625. (b) Lehmann, M. S.; Koetzle, T. F.; Hamilton, W. C. Precision neutron diffraction structure determination of protein and nucleic acid components. VIII: the crystal and molecular structure of the β-form of the amino acid L-glutamic acid. *J. Crystallogr. Mol. Struct.* **1972**, *2*, 225−233.

(40) For previous conformational studies of glutamate, see: Ham, N. S. NMR studies of solution conformation of physiologically active amino-acids. In *Molecular and Quantum Pharmacology*; Bergmann, E., Pullman, B., Eds.; D. Reidel Publishing: Dordrecht, 1974; pp 261−268.

(41) For a review describing proposed conformational requirements of glutamate receptors based on the known glutamate agonists, see: Chamberlin, R.; Bridges, R. Conformationally constrained acidic amino acids as probes of glutamate receptors and transporters. In *Drug Design for Neuroscience*; Kozikowski, A. P., Ed.; Raven Press: New York, 1993; pp 231−259.

(42) Quisqualic acid and ibotenic acid have been known as potent agonists of not only metabotropic glutamate receptors but also ionotropic glutamate receptors.[1] Recently, we synthesized and investigaed the pharmacological actions of (2*S*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) which can be viewed as a hybrid form of CCG-I and -IV. DCG-IV was found to be a

potent and selective agonist of mGluRs, in particular, negatively coupled to adenyl cyclase activity among the other known mGluR agonists,[2c,5b,10d,19] although at high concentrations this compound binds to NMDA receptors. We believe that the extended partial structure of DCG-IV also contributes to its potent activity to mGluRs.

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(44) The rms value of E and CCG-I (antiperiplanar conformer) was 0.117 Å.

(45) CMP-II and -III have already been synthesized and characterized as L-*syn*-*exo*-MPDC and L-*anti*-*endo*-MPDC, respectively. Bridges, R. J.; Lovering, F. E.; Koch, H.; Cotman, C. W.; Chamberlin, A. R. A conformationally constrained competitive inhibitor of the sodium-dependent glutamate transporter in forebrain synaptosomes: L-*anti*-*endo*-3,4-methanopyrrolidine dicarboxylate. *Neuroscience Lett.* **1994**, *174*, 193−197.

(46) CMP-II was prepared from the 1*R*,2*S*,5*S*,6*R*-isomer[12a] of **5a** by the following sequence of reactions: (1) reduction with BH_3−Me_2S (81%), (2) removal of the TBS group (H^+/MeOH), (3) Jones oxidation (two steps, 72%), and (4) deprotection (NaOH and then TFA). Mp: >250 °C. $[\alpha]^{25}_D$ −45.7° (*c* 0.49, H_2O).

(47) Unpublished observations using electrophysiological method in the new born rat isolated spinal cord. There would be many reasons why CMPs are inactive to the receptors (e.g., steric hindrance, transport difficulties, and no flexibility to change its conformation). These factors could not be ruled out by the present studies.

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(53) The synthesis of CMP-III was accomplished from **5b** in a manner similar to that of CMP-II.[46] Mp: >250 °C. $[\alpha]^{25}_D$ −77.8° (*c* 0.51, H_2O).

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JM9502908