Synthesis and Neuroexcitatory Action of Conformationally Constrained Glutamate Analogues

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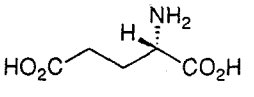
Contents

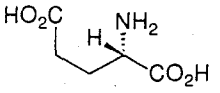
Introduction

Recently L-glutamic acid (L-Glu) in mammalian central nervous systems (CNS) has attracted much attention from the fields of life science because of its neurotransmitter actions which links to a variety of physiological functions such as memory and early learning as well as movement and reflex.[1] Moreover, L-Glu exhibits a potent excitotoxic action[2] which causes serious cell damage and induces various acute and chronic brain diseases, for example, Huntington's chorea, Parkinsonism, and epilepsy.

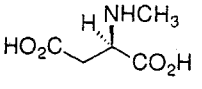
At the present time, the excitatory amino acid receptors are classified into at least three subtypes[3~5] which have a particular affinity for: (1) *N*-methyl-D-aspartic acid (NMDA)[6,7] (2) kainic acid (KA)[8], and (3) quisqualic acid (QA)[9] (Figure 1 and 2). These three types of receptor are known as the ionotropic receptors. They are believed to be directly coupled with ion channels, through which positive ions enter inside the neuron



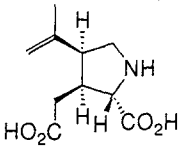
L-Glutamic acid (L-Glu)
extended form



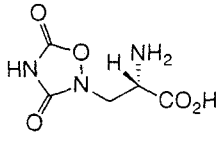
L-Glutamic acid (L-Glu)
folded form



NMDA
(*N*-methyl-D-aspartic acid)



KA (kainic acid)



QA (quisqualic acid)

Figure 1

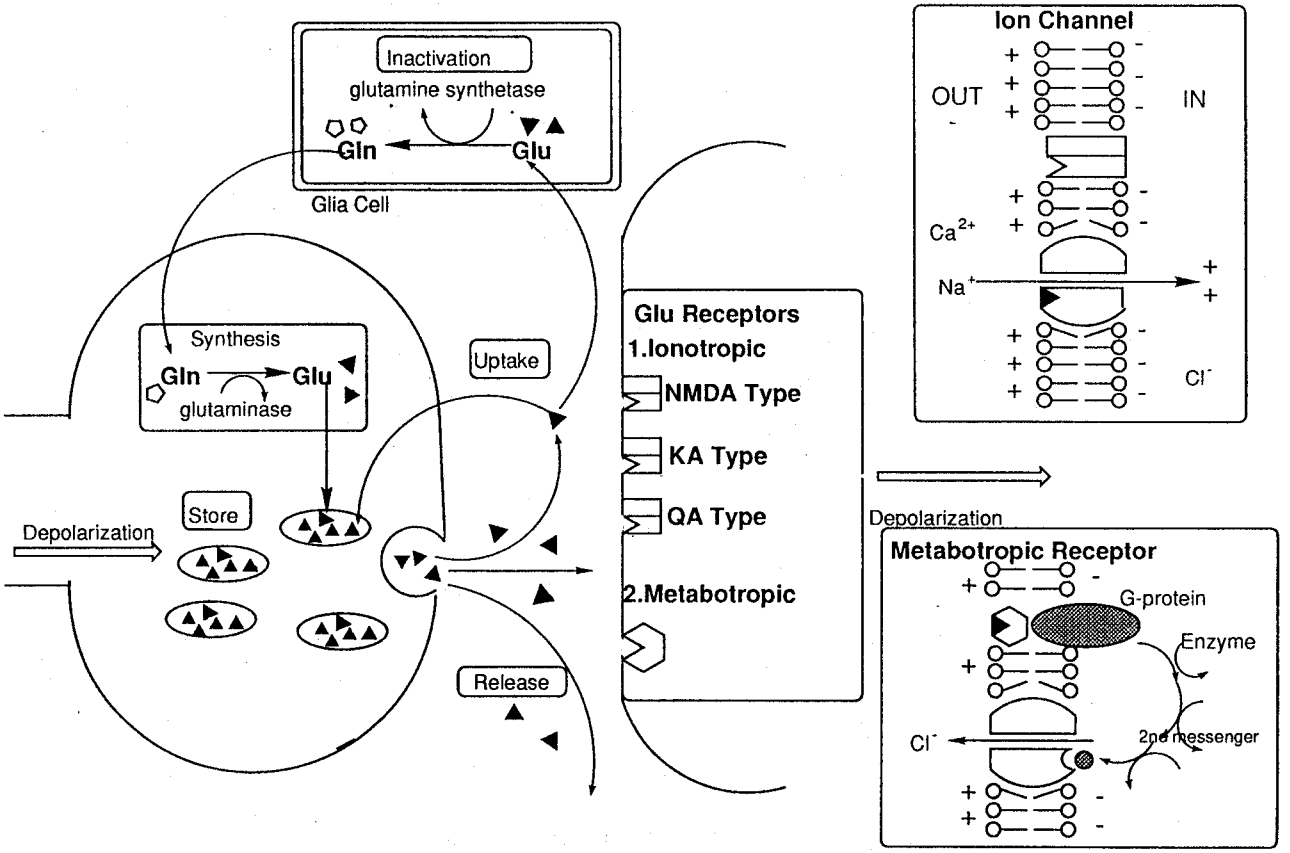
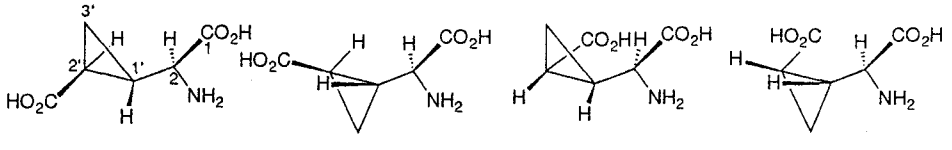


Figure 2 Schematic view of the glutamate receptors.

from the synaptic cleft to cause excitatory response.[10] In addition to these ionotropic receptors, recent studies have demonstrated the existence of a metabotropic receptor which is coupled with the intracellular metabolic pathway[11,12] (Figure 2, see also Figure 4-1 and Table 4-1).

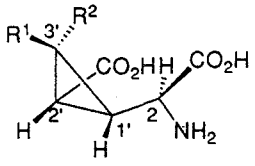
However, neither the physiological roles of the receptors nor the structural requirements of L-Glu in activating these receptors have been clarified yet. Why are there so many types of receptor? How does L-Glu activate the different receptors? Recent structure-activity studies revealed that all the functional groups of L-Glu are essential to activate its receptors since any modification of these groups resulted in a significant decrease or complete loss of activity. I was thus interested in the conformations of L-Glu molecule that activate specifically its receptors, assuming that each L-Glu receptor can recognize an optimal conformation of L-Glu as either the extended or the folded form, since L-Glu is a conformationally flexible molecule.[13]

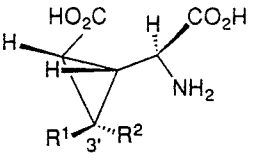
It is interesting to study conformationally restricted analogues of L-Glu in order to clarify the active conformation of L-Glu that activates each receptor subtype. Thus, four stereoisomers of L-2-(carboxycyclopropyl)glycines[14] (CCG-I~IV: **1~4**),



CCG-I (1) (2 *S*,1' *S*,2' *S*) **CCG-II (2)** (2 *S*,1' *R*,2' *R*) **CCG-III (3)** (2 *S*,1' *S*,2' *R*) **CCG-IV (4)** (2 *S*,1' *R*,2' *S*)

Figure 3

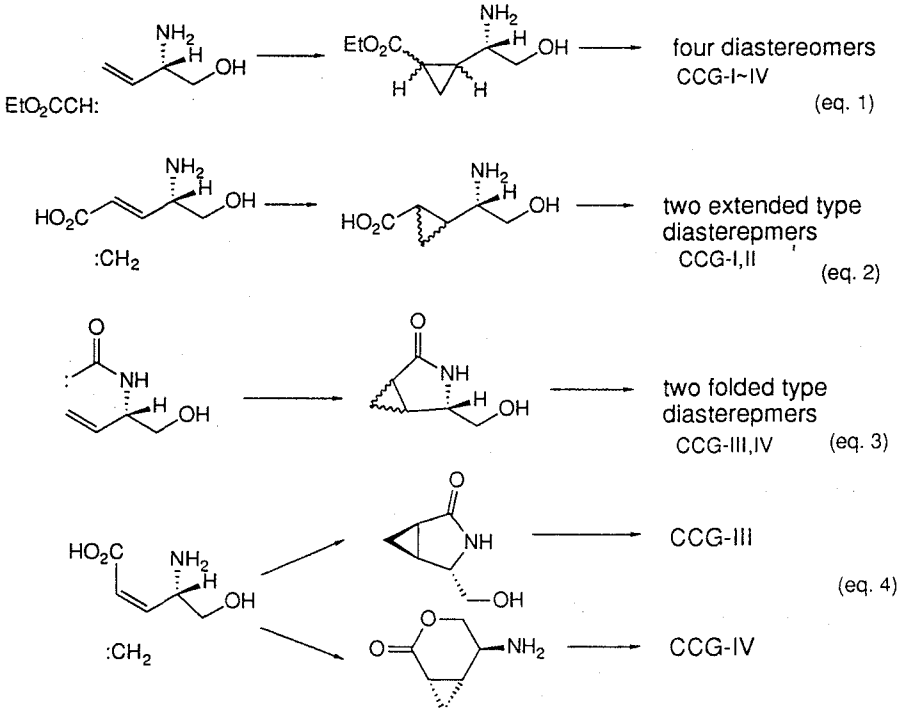




***t*-MCG-III (24)** $R^1 = CH_2OCH_3$, $R^2 = H$ ***t*-MCG-IV (26)** $R^1 = CH_2OCH_3$, $R^2 = H$
***c*-MCG-III (25)** $R^1 = H$, $R^2 = CH_2OCH_3$ ***c*-MCG-IV (27)** $R^1 = H$, $R^2 = CH_2OCH_3$

Figure 4

where the cyclopropyl group fixes the glutamate substructure in an extended or a folded form, were synthesized. In addition, in order to elucidate the steric role of the cyclopropane ring, C-3' substituted CCG analogues [2-(2-carboxy-3-methoxymethyl-cyclopropyl)glycines, MCGs; **24**~**27**) were designed. Described in

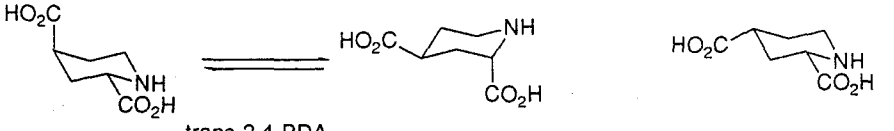


Chapter 1 is the efficient synthesis of all the diastereomeric CCGs via an intermolecular cyclopropanation of a vinylglycine derivative with ethyl diazoacetate (eq. 1).[15] In Chapter 2, the stereoselective syntheses of CCGs (in particular folded isomers which were found to be important in view of their interesting neurobiological activities) are described (eq. 2~4).[15,16] Described in Chapter 3 are the syntheses of C-3' substituted CCG analogues (MCGs; **24~27**).[17] In Chapter 4, neurobiological activities of CCGs and MCGs, and their conformation-activity relationship will be discussed.[18,19] These studies supported a speculation that the conformational requirement of L-Glu plays a crucial role for activating its receptor.

Chapter 1

Efficient Synthesis of All Diastereomers of 2-(Carboxycyclopropyl)glycine

A hypothesis that either the extended or the folded conformer of L-glutamic acid (L-Glu) is responsible for activating the excitatory amino acid receptors has been proposed based on structure-activity relationship studies between L-Glu and its exogenous agonists. For example, *trans*- and *cis*-piperidinedicarboxylic acids (PDAs) were synthesized to explore the conformational requirements of L-Glu.[20,21] Since these compounds are capable of existing as a mixture of different conformers, they are not satisfactory enough to determine the conformation of L-Glu when it interacts with the receptor.



trans-2,4-PDA
(weak NMDA agonist)

cis-2,4-PDA
(weak NMDA antagonist)

Therefore, I planned to restrict the conformation of L-Glu by the introduction of a cyclopropane ring into its carbon chain. Thus, L-2-(carboxycyclopropyl)glycines (CCG-I~IV: **1~4**) are synthesized. Among these compounds, CCG-I (**1**) and III (**3**) were isolated from the immature fruits of *Aesculus parviflora* and *Blighia sapida* by Fowden.[14] These fruits are known to induce the symptoms of hypoglycemia in the animal. However, the biological activity of these amino acids themselves had not been examined. The cyclopropyl group in the CCGs fixes the glutamate substructure in either an extended or a folded form. Of the four possible diastereomers, CCG-I (**1**) and II (**2**) restrict

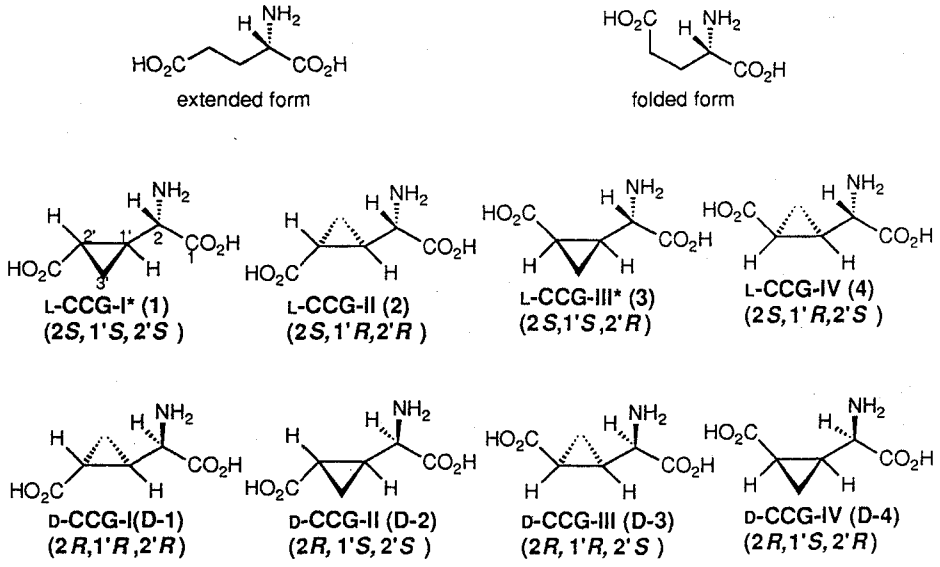
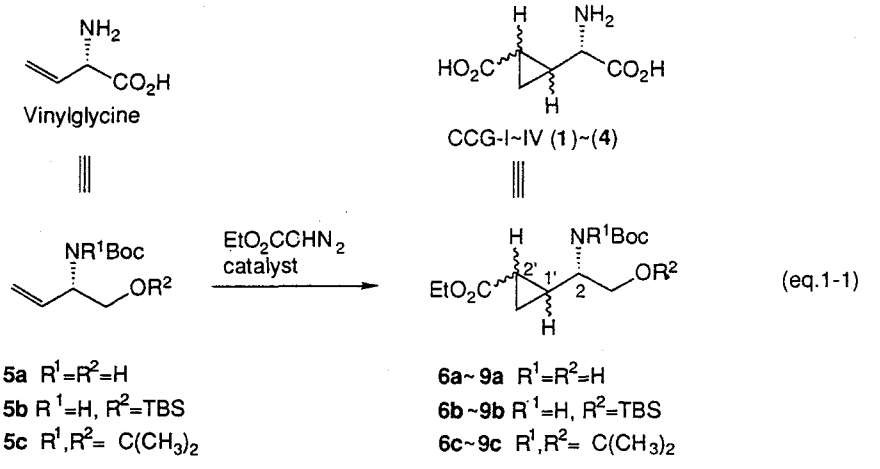


Figure 1-1

the conformation of "L-Glu" to the extended form, and CCG-III (**3**) and IV (**4**) to the folded form. In addition, each set of CCG isomers (**1** and **2**, and **3** and **4**) possesses the same extended or the folded conformation but has an opposite configuration of the cyclopropane ring, respectively. The neurobiological effect induced by each set of CCG was expected to provide information about the three dimensional structure of the receptor surface. Moreover, the D-isomers of CCG were intriguing owing to the fact that some of the D-amino acids such as NMDA interact with L-Glu receptors. Thus, I planned to synthesize all the diastereomers of CCG in order to elucidate the conformational requirements of L-Glu receptors at the molecular level.

[1] Syntheses of CCG-I~IV (1~4). In order to synthesize the four diastereomers of L-CCG, all of which were required for



the neurobiological assay in the mammalian central nervous system, cyclopropanation of an unsaturated amino acid with diazoacetate was examined first.[15] This method was expected to provide in a single reaction all the possible diastereomers of the cyclopropyl amino acid. The elucidation of the configuration was expected to be rather easy by comparisons of all the stereoisomers in hand with the natural CCG-I and III. L-Vinylglycine or its equivalent compounds were envisaged as the starting substrates for this reaction. In order to avoid racemization of the chiral center, the corresponding amino alcohol was chosen as a starting material for the cyclopropanation. Oxidation of the hydroxyl group of the resulting cyclopropyl amino alcohol should give the desired amino acid without racemization. Therefore, (2*S*)-2-*N*-*tert*-butoxycarbonylamino-3-butenol (**5a**), readily available from L-methionine,[22] was chosen as the starting material.

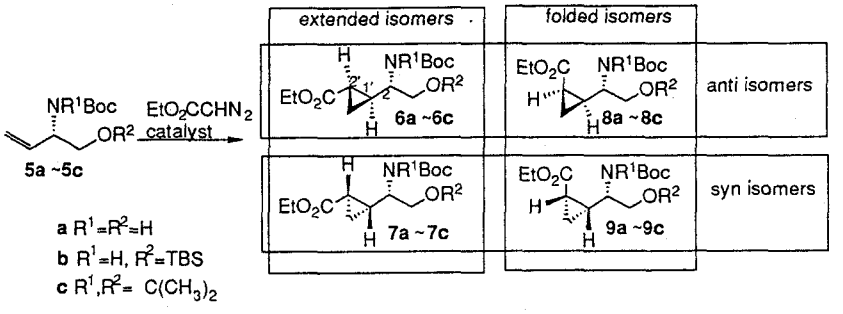
Initially, the free hydroxyl group of **5a** was protected, because the reaction using unprotected **5a** was sluggish and gave

an inseparable mixture of desired products contaminated with the starting material and unidentified polymers produced from diazoacetate. Thus, the hydroxyl group of **5a** was protected as the *tert*-butyldimethylsilyl (TBS) ether (TBSCl, imidazole, DMF, 92%) since TBS group is known to be stable under a variety of chemical transformations including organometallic species. The cyclopropanation of the TBS ether **5b** with ethyl diazoacetate in the presence of a catalytic amount of $PdCl_2 \cdot (CH_3CN)_2$ yielded a mixture of the desired cycloadducts (**6b~9b**) in moderate yield and the resulting cycloadducts could be separated from polymers. To improve the yield and to optimize the reaction conditions, other palladium (II) catalysts such as $Pd(OAc)_2$[23], $PdCl_2$, and $Pd(NO_3)_2$ were examined. Among these Pd catalysts, $Pd(OAc)_2$ was found to be most convenient and efficient to give the best yield (88%). Copper catalyst such as $CuCl_2$, $CuCl \cdot P(i\text{-}PrO)_3$ produced the desired cycloadducts only in poor yield. $Rh_2(OAc)_4$, which is frequently used as a catalyst for cyclopropanation,[24,25] was not effective, and led to the complete recovery of the starting material. Therefore, it was concluded that the use of $Pd(OAc)_2$ as the catalyst is the most efficient method for this cyclopropanation (*vide infra*).

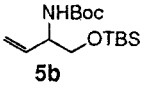
The cycloadducts prepared from **5b** were chromatographically inseparable, at this stage. However, the corresponding hydroxyl derivatives **6a~9a**, which were prepared by simple desilylation of the cycloadducts **6b~9b** [(±)-10-camphorsulfonic acid (CSA), EtOH, room temperature, 16 h], were separable by HPLC.[*1] The HPLC analysis of the mixture indicated that the product ratio was **6a/7a/8a/9a** = 1.2/3.5/1/1 (Table 1-1, entry 1). Although the details of the determination of the stereochemistry will be

described later, among the cycloadducts obtained, **6a** and **7a** were found to be the extended isomers and **8a** and **9a** to be the folded isomers. Regarding the anti/syn relationship between the amino group at C-2 and the cyclopropane methylene at C-1', **6a** and **8a** were found to be the anti compounds with (2*S*,1'*S*) con-

Table 1-1. Cyclopropanation of the 2-amino-3-butenol derivatives

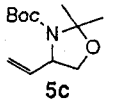


entry	catalyst	substrate	yield (%)	product ratio 6	7	8	9	extended (6+7) / folded (8+9)	anti (6+8) / syn (7+9)
1	$Pd(OAc)_2$	(S)-**5b**	88	1.2	3.5	1	1	4.7 / 1	1 / 2
2	$Pd(OAc)_2$	(S)-**5c**	14	3.3	1.8	2.2	1	1.6 / 1	2 / 1
3	(R)-7644 [a]	(S)-**5b**	23	5	5	2	1	3.3 / 1	1.2 / 1
4	(R)-7644	(R)-**5b**	26	5	5	2	1	3.3 / 1	1.2 / 1
5	(R)-7644	(S)-**5c**	7	5	5	1	1	5 / 1	1 / 1
6	(R)-7644	(R)-**5c**	6	5	6	1	1	5.5 / 1	1 / 1.2

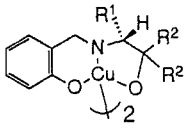


5b

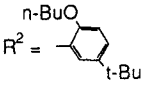
[a]Structure of (R)-7644.



5c



$R^1 = CH_2Ph$, R^2 =



Footnote*1 Column: Develosil ODS-5 (Nomura Chemical, Nagoya, Japan); flow rate: 2 ml/min; eluent: MeOH/H_2O, 1/1; retention times: **6a**, 32.2 min; **7a**, 34.4 min; **8a**, 26.6 min; **9a**, 29.0 min.

figuration while **7a** and **9a** to be the syn compounds with (2*S*,1'*R*) stereochemistry*2. The ratio of anti isomers (**6a**+**8a**) to syn isomers (**7a**+**9a**) was about 2:1. Moreover, among the syn isomers, the syn-extended compound **7b** was more predominant in the cyclopropanation reaction of **5b**. The conformation of olefin **5b** appears to be flexible (Figure 1-2, **A** and **B**) because its C-2 and C-3 bond can freely rotate. In addition, the olefin's mode of coordination with palladium should be considered. Thus, the reason why **7b** was the major product is not clearly understood at this stage.

In order to elucidate its stereoselectivity, I examined the cyclopropanation of the acetonide derivative **5c**. The acetonide group of **5c** somehow constrains the free rotation of the C2-C3 bond (Figure 1-2, **C** and **D**). The reaction gave cycloadducts **6c**~**9c** in 14% yield (85% recovery of the starting material). Since the cycloadducts were not separable, the mixture was converted into the separable derivatives, **6a**~**9a**, by successive treatment with (1) trifluoroacetic acid (TFA), H_2O and (2) di-*tert*-butyl dicarbonate (Boc_2O), $NaHCO_3$, dioxane. Although the yield of this reaction was low, the product ratio was **6a**/**7a**/**8a**/**9a** = 3.3/1.8/2.2/1, which was different from that observed using the TBS ether **5b** (Table 1-1, entry 2). Especially, the formation of the anti type products, **6** and **8**, was favored over the

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Footnote*2 In this paper, the anti/syn relationship is defined as follows.

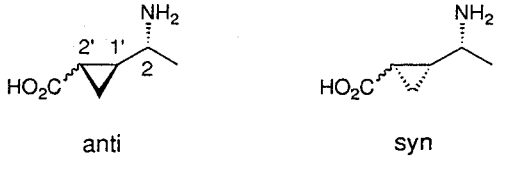
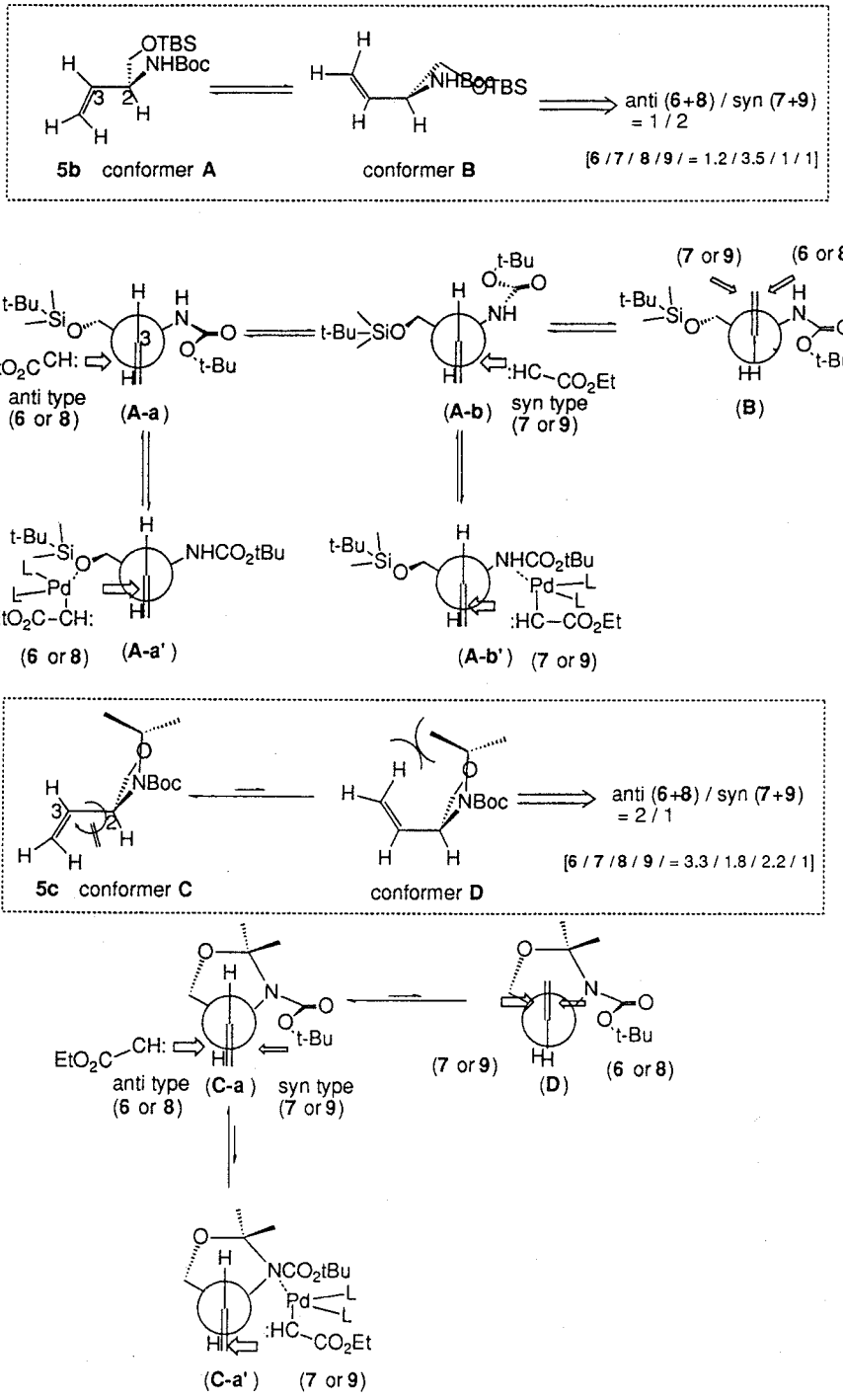


Figure 1-2


OTBS
NHBoc
anti (6+8) / syn (7+9)
= 1 / 2
[6 / 7 / 8 / 9 / = 1.2 / 3.5 / 1 / 1]
5b conformer A
conformer B
anti type
(6 or 8)
(A-a)
(A-b)
syn type
(7 or 9)
(7 or 9)
(6 or 8)
(B)
(6 or 8)
(A-a')
(A-b')
(7 or 9)
anti (6+8) / syn (7+9)
= 2 / 1
[6 / 7 / 8 / 9 / = 3.3 / 1.8 / 2.2 / 1]
5c conformer C
conformer D
anti type
(6 or 8)
(C-a)
syn type
(7 or 9)
(7 or 9)
(D)
(6 or 8)
(C-a')
(7 or 9)

syn type [anti (**6**+**8**)/syn (**7**+**9**) = ~2/1]. This result is completely opposite to that observed in the cyclization of **5b**. As shown in Figure 1-2, the conformer **C** would be thermodynamically more favored than the conformer **D** in view of its steric interactions. If the carbene attacks **C** from its less hindered face, the formation of anti type products, **6** and **8**, will be predominant than the syn isomers, **7** and **9**. If the amino group chelates the Pd-carbene complex, the reaction would proceed through the transition state conformer **C-a'** to produce the syn isomers **7** and **9** as the major products. The reaction using acetonide **5c** produced the anti isomers, **6** and **8**, predominantly over syn isomers, **7** and **9**. This suggested that in the cyclopropanation reaction of **5c**, steric factor are more important than chelation effects.

These results allow the following description of the mechanism of the palladium catalyzed cyclopropanation of **5c** with ethyl diazoacetate; (1) the carbene attacks from the less hindered face of the more favored conformer **C**, in which the steric interaction between H_3 and the protecting group of the hydroxyl group is minimum, (2) the chelation effect of the Pd-carbene complex with the nitrogen atom and/or Boc group is weak. In the case of TBS ether **5b**, it is difficult to conclud whether conformer **A-b**, in which TBS group occupies a proximal position to olefin and blocks the attack from the side of TBS group, is predominant over the conformer **A-a**, in which the side of Boc group is hindered, or the chelation of Pd-carbene complex with the amide group of conformer **A-b'** contributes to the stereoselectivity (Figure 1-2).

Next examined was the use of chiral copper catalyst (en-

tries 3~6), because the transition state of the cyclopropanation using chiral catalysts has been well examined. The catalyst, (R)-7644, has been employed for the syntheses of optically active pyrethroids.[26] It has been reported that the excellent enantio- and/or diastereoselectivity in the syntheses of pyrethroids is due to the steric bulkiness of the catalyst-ligand complex in the four-membered metallacycle intermediate. The reaction of (2*S*)-**5b** with (R)-7644 was expected to give the anti-extended (2*S*,1'*S*,2'*S*) isomer **6a** as the major product (Figure 1-3).[26b] Prior to cyclopropanation, the solution of (R)-7644 was heated at 90 °C with a small amount of ethyl diazoacetate. The reaction of olefin (**5b** or **5c**) with the Cu(I)-catalyst and large excess of ethyl diazoacetate gave cycloadducts, **6b~9b** or **6c~9c**, even though in poor yield (45 °C, 18 h). The cycloadducts were converted into alcohols **6a~9a** in order to analyze the products ratio.

The reaction of TBS ether **5b** provided the extended isomers as the major product [extended (**6**+**7**) /folded (**8**+**9**) = 3.3/1]; however, the anti/syn selectivity was reduced [anti (**6**+**8**) /syn (**7**+**9**) = 1.2/1, especially, the extended isomers anti (**6**) /syn (**7**) = 1/1] (entry 3). The reaction of (2*S*)-**5c** also provided the extended isomers predominantly [extended (**6**+**7**) /folded (**8**+**9**) = 5/1, anti (**6**+**8**) /syn (**7**+**9**) = 1/1] (entry 5). Masamune proposed that matched or mismatched combination of the chiral catalyst with a chiral substrate affects diastereoselectivity (double asymmetric synthesis).[27] It was expected that the (2*R*)-isomers match with the catalyst better than the (2*S*)-isomers because in *Cu-(2*S*)-**5c** complex a large steric interaction between the Boc group and R^2 group of the catalyst was expected (Figure 1-4).

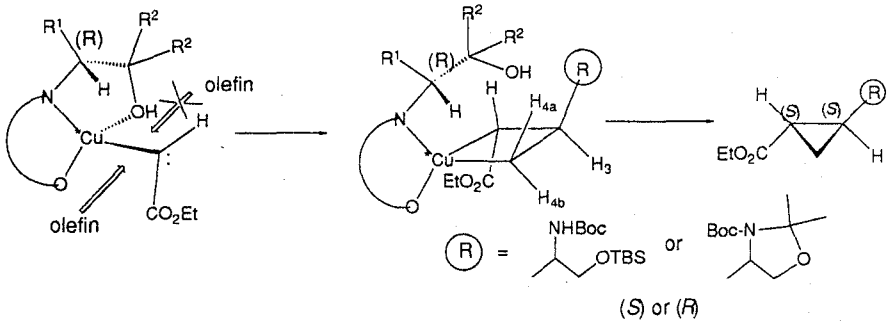
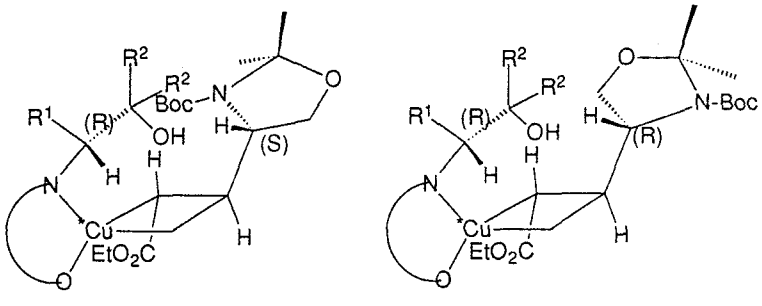


Figure 1-3

However, the use of (2*R*)-**5b** and (2*R*)-**5c** with (R)-7644 gave a mixture of the cycloadducts in almost the same yields as (2*S*)-**5b** and (2*S*)-**5c**, respectively.

Since the double asymmetric effect was not observed and the yield was quite poor, the four membered metallacycle intermediate probably decomposed or was not formed under the reaction conditions. Also probably the catalyst activity was reduced because the copper catalyst was poisoned by the chelation with the amide group of the substrate. The poor yields were also attributed to the lower reactivity of the C-C double bond



*Cu-(2 *S*)-**5c** *Cu-(2 *R*)-**5c**

Figure 1-4

of **5b** and **5c** as compared with the substrates, possessing aryl or alkenyl substituents on the C-C double bond, used for the pyrethroid syntheses.

Best yields were obtained by using $Pd(OAc)_2$ as catalyst in the cyclopropanation reaction of **5b** employing the following procedure; both Et_2O solutions of ethyl diazoacetate (10 equiv) and the $Pd(OAc)_2$ (0.05 equiv) were added simultaneously, drop by drop, to an ethereal solution of **5b** at room temperature. Dropwise addition of both the solutions of ethyl diazoacetate and the catalyst was essential; otherwise the reaction did not go to completion. A mixture of cycloadducts **6b~9b** was produced in 88% yield. This procedure can be performed in a ~10 g scale. Desilylation of a mixture of **6b~9b** (CSA, EtOH, room temperature, 16 h) gave alcohols **6a~9a** which were separated and converted into the desired amino acids through a short sequence of reactions (*vide infra*).

As mentioned above, the cycloadducts **6b~9b** were inseparable. However, the desilylated alcohols **6a~9a** were found to be separable. Analysis by TLC or medium pressure column chromatography on SiO_2 (ether/hexane, 3:1) showed the mixture of **6a~9a** to consist of at least three components, *Rf* = 0.46, 0.38, and 0.30, respectively. HPLC analysis and ^1H NMR data indicated that the least polar component with *Rf* = 0.46 and the most polar component with *Rf* = 0.30 are single diastereomer and that the component with *Rf* = 0.38 was a mixture of two diastereomers.

The structure of these compounds, to be extended or folded, were preliminarily assigned by the inspection of their *J* values. The least polar alcohol showed $J_{1',2'}$ = 4.0 Hz and the

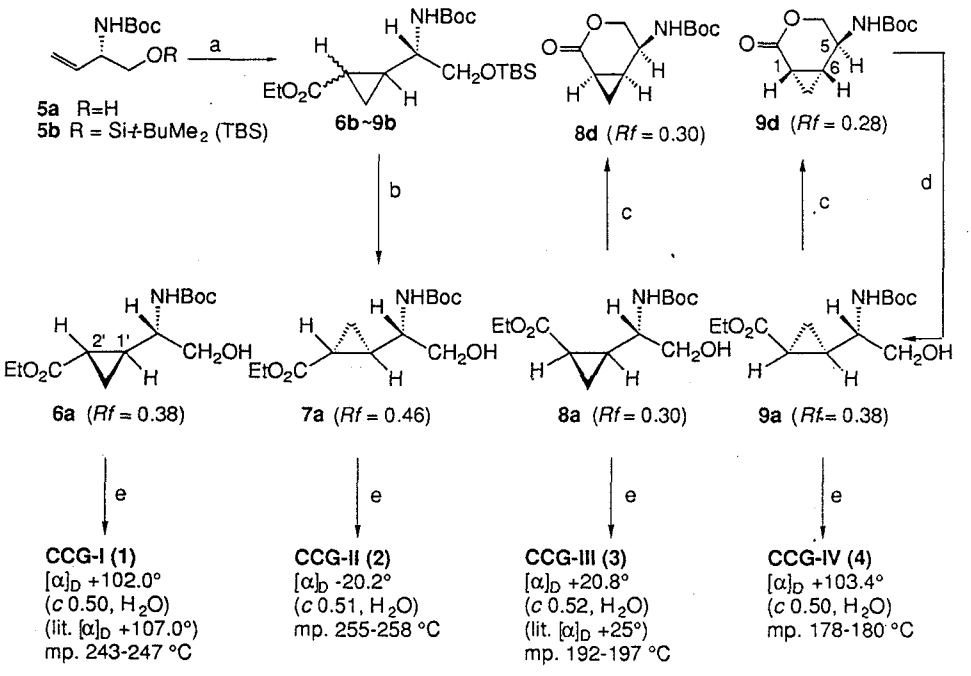
most polar alcohol showed $J_{1',2'}$ = 8.5 Hz. These results suggested that the least polar isomer was the extended isomer and the most polar isomer would be the folded one.

It was expected that only the folded isomers would form the corresponding δ-lactones under acidic conditions. Thus, each alcohol was treated with a catalytic amount of CSA in CH_2Cl_2. The most polar one (*Rf* = 0.30) gave a δ-lactone (**8d** or **9d**), which showed the same *Rf* value as that of the starting material (*Rf* = 0.30) but was a different product. On the other hand, the least polar alcohol (*Rf* = 0.46) remained unchanged. Therefore, it was concluded that the most polar alcohol is one of the folded isomers (**8a** or **9a**) and the least polar alcohol is one of the extended isomers (**6a** or **7a**).

Therefore, the other component with *Rf* = 0.38 should be a mixture of an extended isomer and a folded isomer. The treatment of this mixture with CSA in CH_2Cl_2 gave a separable mixture of two compounds with *Rf* = 0.38 and 0.28. This result suggested that the less polar compound (recovered unchanged) is an extended alcohol (**6a** or **7a**) and the more polar compound, the δ-lactone (**8d** or **9d**). This δ-lactone was re-converted, in excellent yield, into its corresponding alcohol ethyl ester (**8a** or **9a**) by ethanolysis (catalytic amount of K_2CO_3, EtOH, 87%). ^1H NMR of the product showed a large *J* value ($J_{1',2'}$ = 9.0 Hz) which is consistent with a folded configuration of the alcohol. Thus, all the diastereomers of alcohol **6a**~**9a** were separated by using chromatographic and/or chemical method and the relative stereochemistry (either the extended or the folded) of each compound was determined.

Next, the structures of the δ-lactone whether it was **8d** or

Scheme 1-1



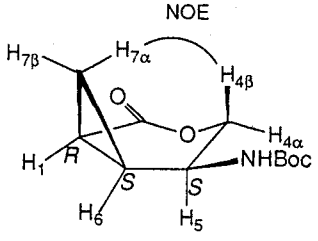
(a) EtO_2CCHN_2, 0.05 equiv $Pd(OAc)_2$ (88%); (b) (1) CSA, EtOH; (2) SiO_2 column chromatography; (c) CSA, CH_2Cl_2 (**8d** 69%, **9d** 98%); (d) K_2CO_3, EtOH (87%); (e) (1) Jones reagent; (2) CH_2N_2; (3) 1 *M* NaOH; (4) TFA; (5) Dowex 50Wx4 (**1** 39%, **2** 65%, **3** 27%, **4** 61%).

9d was determined by the ^1H NMR study and NOESY experiments. As depicted in Figure 1-5, the data clearly indicate that the less polar δ-lactone (*Rf* = 0.30) is the (1*R*,5*S*,6*S*)-isomer, **8d**; a strong NOE is observed between $H_{4\beta}$ and $H_{7\alpha}$. And the more polar isomer (*Rf* = 0.28) is **9d**; a strong NOE is observed between $H_{4\alpha}$ and $H_{7\alpha}$. The amino group adopts an axial orientation in the six membered ring with a half-chair like conformation, where the steric repulsion between H_5 and the cyclopropyl ring is reduced. These data helped me to unambiguously assign structure **8a** to the most polar alcohol with *Rf* = 0.30 and structure **9a** to the isomer with *Rf* = 0.38. The stereochemistry of the extended isomers was determined by converting them into the amino acids,

1 and **2**, respectively (*vide infra*).

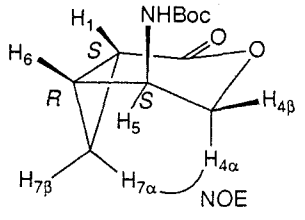
The conversion of **6a~9a** into CCG-I~IV (**1~4**) was effected in four steps, respectively: (1) oxidation of the primary alcohol with Jones reagent, (2) esterification of the carboxyl group with CH_2N_2 for purification, (3) hydrolysis of the ester groups with 1 *M* aqueous NaOH, and (4) removal of Boc group with TFA. The resulting acidic solution was then passed through a column filled with Dowex 50Wx4 ion exchange resin (elution with 1 *M* aqueous NH_3). The pH of the eluent containing the amino acid as its the ammonium salt was adjusted to 3 with 1 *M* aqueous HCl to precipitate the free amino acid as colorless crystals.

The structures of the synthetic CCG-I~IV were established by comparing their physical properties, especially spectral data, with authentic natural CCG-I and III.*[3,14] The amino acid



8d *Rf* = 0.30
$H_{4\alpha}$: δ 4.27 $J_{H4\alpha\text{-}H5}$ = 3.5 Hz
$H_{4\beta}$: δ 3.64 $J_{H4\beta\text{-}H5}$ = 11.5 Hz

[a]NOEs (s) $H_{4\alpha}$-$H_{4\beta}$, $H_{4\beta}$-$H_{7\alpha}$, $H_{7\alpha}$-$H_{7\beta}$;
(m) $H_{4\beta}$-NH;
(w) H_1-H_6, H_1-$H_{7\beta}$, H_6-$H_{7\beta}$.



9d Rf = 0.28
$H_{4\alpha}$: δ 4.12 $J_{H4\alpha\text{-}H5}$ = 2.5 Hz
$H_{4\beta}$: δ 4.28 $J_{H4\beta\text{-}H5}$ = 1.5 Hz

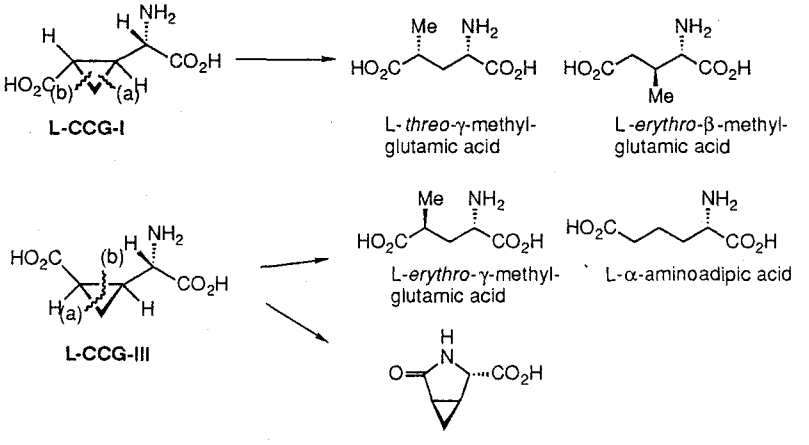
[a]NOEs (s) $H_{4\alpha}$-$H_{4\beta}$, $H_{4\alpha}$-$H_{7\alpha}$;
(m) H_1-$H_{7\beta}$, H_6-$H_{7\beta}$;
(w) $H_{7\alpha}$-$H_{7\beta}$.

[a]Observed NOEs (NOESY, 300 MHz, $CDCl_3$) of the compounds **8d** and **9d** were as follows. The strength of NOEs in parentheses is designated strong (s), medium (m), and weak (w).

Figure 1-5

($[\alpha]_D$ +102°) derived from the extended alcohol with *Rf* = 0.38 was identical to natural **1** (lit.$[\alpha]_D$ +109°). The configuration of the amino acid and the alcohol was assigned as (2*S*,1'*S*,2'*S*). Therefore the other extended alcohol (*Rf* = 0.46) was unambiguously assigned **7a** with the (2*S*,1'*R*,2'*R*) configuration. The structure of amino acid ($[\alpha]_D$ -20°) obtained from **7a** was further confirmed to be (2*S*,1'*R*,2'*R*)-isomer **2** by comparison with authentic (±)-**2** reported by Ohfune.[28] On the other hand, synthetic **3** ($[\alpha]_D$ +21°) derived from **8a** was completely identical to natural CCG-III (**3**) (lit.$[\alpha]_D$ +25°). Therefore, the structure of amino acid ($[\alpha]_D$ +103°) derived from the other folded alcohol **9a** was unambiguously assigned to be (2*S*,1'*R*,2'*S*)-isomer **4** as depicted.

Footnote*3 The structure of natural CCG-I and III were determined by Fowden et al., as evidensed by converting them to the known compounds as follows.[14] Hydrogenation of CCG-I under weakly acidic conditions produced a 1:1 mixture of *threo*-γ-methylglutamic acid (route a) and *erythro*-β-methylglutamic acid (route b). On the other hand, CCG-III produced *erythro*-γ-methyl-glutamic acid (route a) as the major product accompanied by a small amount of α-aminoadipic acid (route b). The cis relationship of CCG-III was confirmed by the formation of the γ-lactam ring.



Thus, all the diastereomers of L-CCG could be synthesized via the cyclopropanation of a common starting material (2*S*)-**5b** with ethyl diazoacetate. The availability of all the diastereomers in hand permitted the facile and reliable structure determination. In addition, D-isomers of CCG (**D-1**~**D-4**) were prepared from (2*R*)-**5b** in the same manner as L-series.[29]

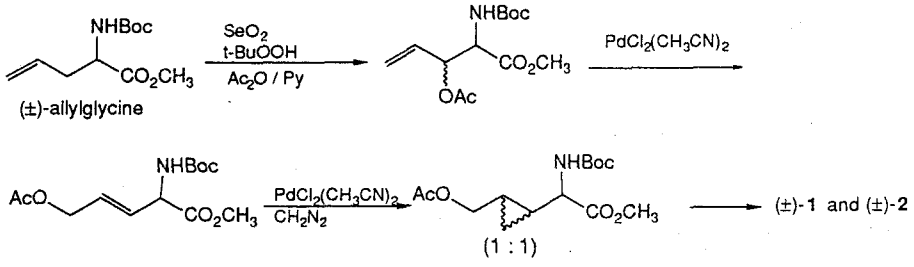
Optical purity of all the synthetic CCGs were analyzed by HPLC using an optically active column, CROWNPAK CR(+) (Daicel Chem. Ind. Ltd.: column size; 0.4 x 15 cm: flow rate; 0.4 mL/min: elution with 1% aqueous $HClO_4$, pH 2.0). Retention time of each of the compounds is as follows; **1**, 5.3 min; **2**, 4.6 min; **3**, 9.5 min; **4**, 9.4 min; **D-1**, 4.0 min; **D-2**, 3.8 min; **D-3**, 4.3 min; **D-4**, 4.2 min. Each isomer gave a single peak and was ascertained to be ~99% enantiomerically pure. Thus, the eight possible diastereomers of CCG could be obtained in an optically pure form. The high optical purity enables the quantitatively trustworthy evaluation of their neurobiological activity.

Chapter 2
Stereoselective Approaches to the Synthesis of CCGs

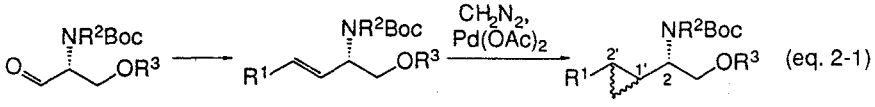
The eight diastereomers of CCG (**1~4**, and **D-1~D-4**), the conformational and configurational variants of L-Glu, were synthesized in a convenient manner as described in Chapter 1. The neurobiological activity of these CCGs were examined by the electrophysiological experiments as well as receptor binding assay using rat central nervous system. All the isomers exhibited characteristic neurobiological actions and were found to be useful as tools for the neuropharmacological studies (details of the activities will be discussed in Chapter 4). Examined next was approaches to the stereoselective synthesis of each diastereomer of CCG.

[1] Syntheses of CCG-I (1) and II (2). Palladium Catalyzed Cycloaddition of Diazomethane to (*E*)-Olefins. From the neurobiological studies, CCG-I (**1**) and II (**2**) were found to activate the metabotropic receptor. Especially, CCG-I (**1**) was found to be a potent and selective agonist and expected to afford information about the metabotropic receptor; the information is of great importance for the elucidation of the not well documented physiological role of this receptor. Previously, Ohfune and Kurokawa reported the syntheses of the extended isomers, (±)-**1** and (±)-**2**, by carbene addition to the allyl acetate prepared from (±)-allylglycine (Scheme 2-1).[28] However, the intermediate allyl acetate was unstable under the ambient conditions and its C-C double bond gradually isomerized to afford the α,β-

Scheme 2-1 [28]



unsaturated ester. It was concluded that this route was not suitable for the synthesis of optically active **1** and **2**. Therefore, I examined the synthesis of **1** and **2** by the use of other intermediates employing the carbene cycloaddition strategy according to eq. 2-1.



R^1 = CO_2Me, CO_2Et, CH_2OH, CH_2OAc, CH_2OTBS

Initially, the cyclopropanation of (*E*)-olefins using diazomethane was examined (eq. 2-1). For the study of carbene addition, a variety of olefins with an *E* double bond (eq. 2-1, R^1 = CO_2Me, CO_2Et, CH_2OAc, etc.) were prepared from D-serinal derivatives via Wittig reaction. Although $PdCl_2 \cdot (CH_3CN)_2$ was used as a catalyst in the syntheses of (±)-**1** and (±)-**2**, I chose $Pd(OAc)_2$ [24,30] (see Chapter 1). The cycloaddition reaction of diazomethane in the presence of $Pd(OAc)_2$ gave a mixture of anti and syn products as summarized in Table 2-1. The reaction proceeded smoothly to give a mixture of cycloadducts both with the α,β-unsaturated esters and the allyl alcohols. In the case of allyl alcohols, about 50% of the starting material was recov-

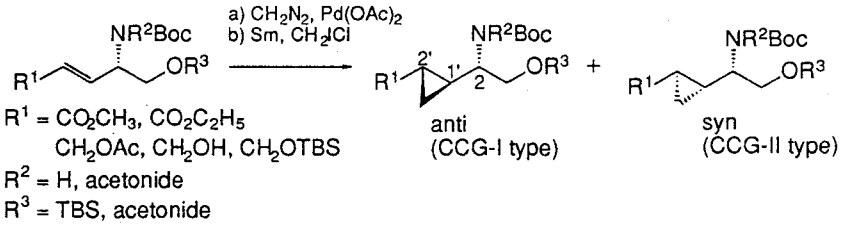
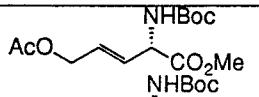
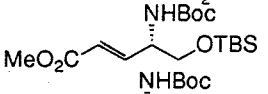
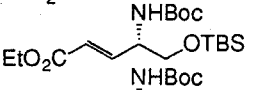
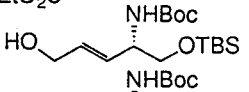
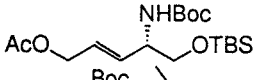
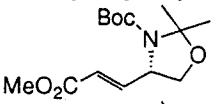
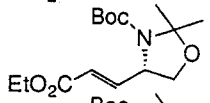
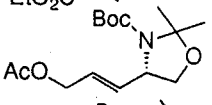
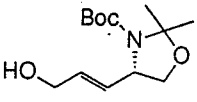


Table 2-1. Cyclopropanation of *E* olefins. [a]

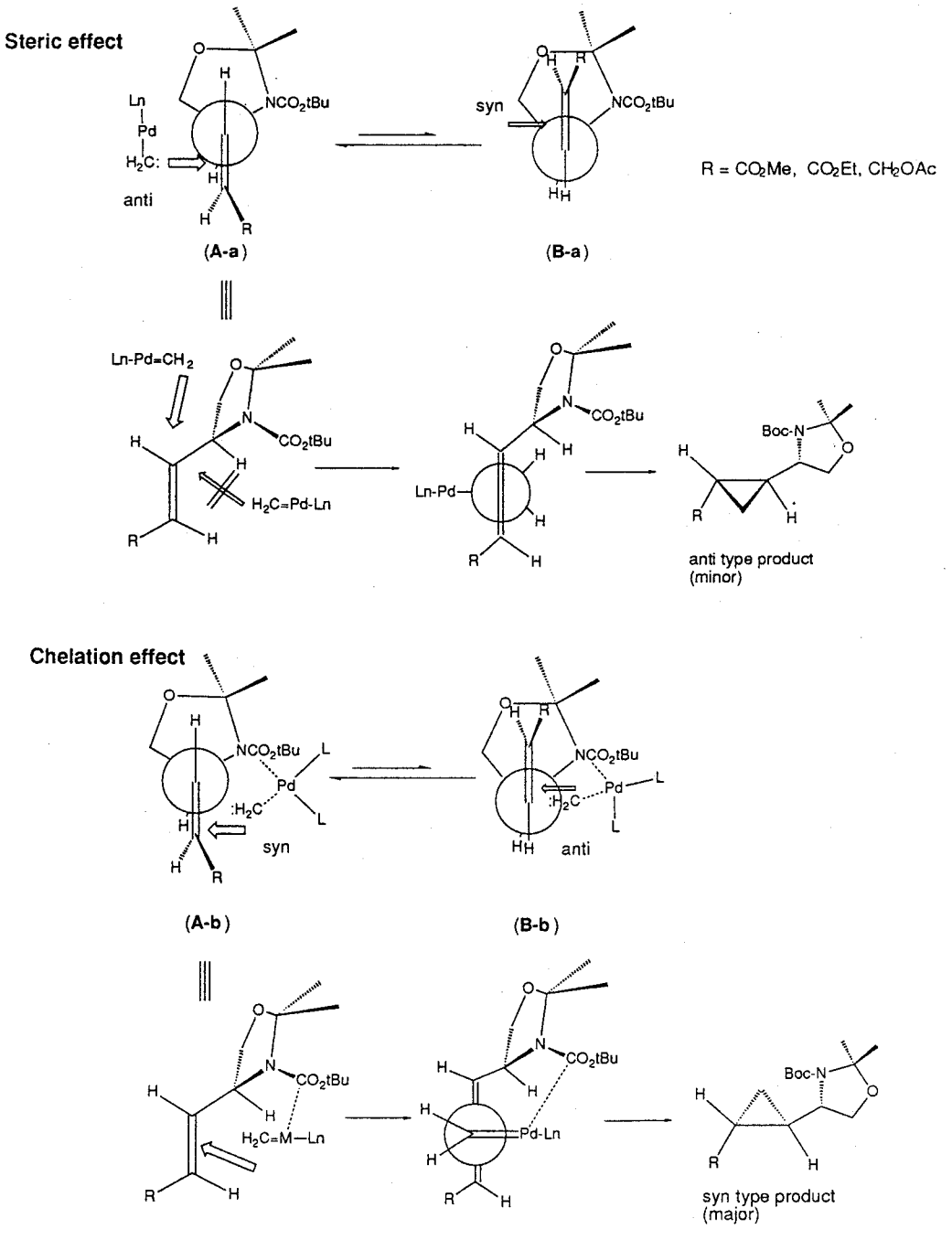
entry	substrate	reaction conditions	yield (%)	products ratio[d] anti : syn
1[b]		a	68[c]	1 : 1
2		a	48[e]	1 : 1
3		a	73[c]	1 : 3
4		a b	40[e] 39[c]	1 : 2 3 : 4
5		a	50[e]	4 : 3
6		a	87[c]	1 : 2.8
7		a	90[c]	1 : 4.6
8		a	53[e]	1 : 6
9		b	39[c]	2 : 1

[a]Reaction conditions: (a) excess CH_2N_2, 0.05 equiv of $Pd(OAc)_2$, room temperature; (b) 4 equiv of Sm powder, CH_2ICl, room temperature. [b]Kurokawa and Ohfune, ref 28. [c]Isolated yield. [d]Stereochemistry of the cycloadducts was determined by converting them into the known diastereomers **6b** and **7b** or their corresponding dimethyl esters. Products ratio was obtained by the chromatographic isolation or ^1H NMR analysis of the resulting mixture. [e]Yield of the cycloadducts was determined by the ^1H NMR analysis.

ered unchanged. Generally, a dropwise addition of diazomethane to the suspension of Pd catalyst and olefin in Et_2O was crucial for the reaction. Otherwise large excess of diazomethane caused an insertion of carbene onto the acidic N-H or O-H group to give large quantities of *N*- or *O*-methylated compounds (entries 1~5). The stereoselectivity of the cycloaddition reaction was only slightly dependent on the substrate structures. The reaction provided the syn isomer as the major product except for entry 5. To avoid *N*- or *O*-methylation and to constrain the conformation of the substrate, olefins with a protected amino alcohol moety as an acetonide were examined next (entries 6~8). The acetonide derivatives gave superior yields in comparison to the olefins possessing N-H group and the ratio of syn products slightly increased. Contrary to the case of ethyl diazoacetate discussed in Chapter 1, the carbene attacked from the more hindered face of conformer **A** (Figure 2-1), which was assumed to be much more stable than conformer **B** in terms of steric hindrance. It is speculated that the *N*-Boc group of the reaction intermediate coordinates with the palladium-carbene complex and that the carbene is delivered to C-C double bond internally (intramolecular carbene transfer to C-C double bond). Thus the reaction gave rise to the preferred formation of the syn products (CCG-II type) as indicated. The behavior of the diazomethane-Pd complex seemed to be much different from that of ethyl diazoacetate-Pd complex, which did not chelate the substrate (see Chapter 1, Fig 1-2).

On the other hand, it is well known that the stereoselectivity of cyclopropanation of allylalcohols, using Simmons-Smith ($Zn\text{-}CH_2I_2$)[31] reaction conditions or $Sm\text{-}CH_2X_2$ reaction con-

Figure 2-1


Steric effect
Ln
Pd
H2C:
anti
NCO2tBu
syn
R = CO2Me, CO2Et, CH2OAc
(A-a)
(B-a)
Ln-Pd=CH2
H2C=Pd-Ln
Ln-Pd
Boc-N
anti type product
(minor)
Chelation effect
L
:H2C
syn
anti
(A-b)
(B-b)
H2C=M–Ln
Pd-Ln
syn type product
(major)

ditions[32], depends on the configuration of the hydroxyl group: the carbene-metal complex chelates with the hydroxyl group and attacks the double bond from the same side of the hydroxyl group to give cycloadducts with excellent diastereoselectivity.

In the case of the substrates I had to use, Simmons-Smith reaction (Zn-Cu, CH_2I_2) was not effective due to the cleavage of the Boc group probably by ZnI_2 attack and the desired cycloadducts were not obtained. In order to synthesize CCG-I type anti isomer, examined next was the reaction of allylalcohol using Sm/CH_2ICl[32] (entries 4b, 9). The reaction using Sm provided a mixture of the anti and syn isomers in a 2:1 ratio in 39% yield. The observed stereoselectivity suggested an initial chelation of Sm carbene complex with the hydroxyl group, followed by delivery of carbene to the less hindered side of intermediate (Figure 2-2) to afford the anti isomer as the major product. The approach of the carbene from the other side of the double bond is obviously hindered by the bulky Boc group. For the synthesis of CCG-I (**1**), the stereoselectivity of the reaction using the acetonide derivative with Sm was superior to that with diazomethane. But the yield was not satisfactory and needed to be improved.

Finally, the resulting cycloadducts were converted into CCG-I (**1**) and II (**2**) in a similar manner to that described in Chapter 1.

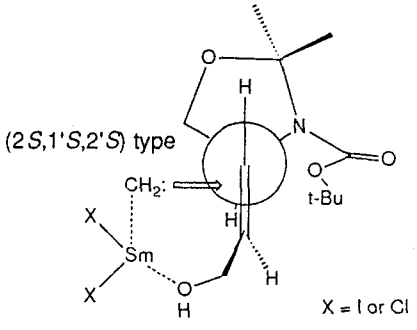
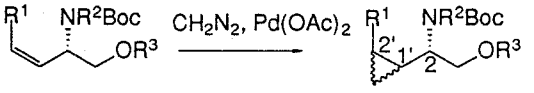


Figure 2-2

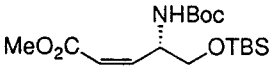
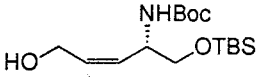
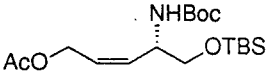
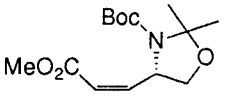
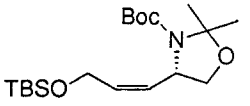
On the other hand, attempts to obtain cycloadducts from the

corresponding (*Z*)-olefin and diazomethane in the same manner as (*E*)-olefin (eq 2-2) were unsuccessful. These reactions didn't proceed at all or gave only poor yield (Table 2-2). The poor yield could be result of the following: (1) accompanying the *N*- and/or *O*-methylation of starting material (Table 2-2,. entry 1~3), (2) the rate of the carbene addition was so slow that the catalyst-carbenoid complex decomposed. The poor reactivity of (*Z*)-olefins was probably due to a steric repulsion between the (*Z*)-substituent and the palladium ligand in the hypothetical transition state, in which palladium coordinated with the nitrogen of the amide group or the carbonyl group of the Boc group (Figure 2-3). The reason why the carbene did not attack, without chelation to nitrogen or Boc group, from the opposite



(eq. 2-2)

Table 2-2. Cyclopropanation of *z* olefins.

entry	substrate	cycloadducts	
1		not detected	A small amount of 1,3-dipolar cycloaddition product was detected.
2		< 10%	*O*- or/and *N*-methylation was observed.
3		< 5%	*N*-Methylation was observed. (> 65%)
4		not detected	no reaction
5		< 5%	Starting material was recovered.

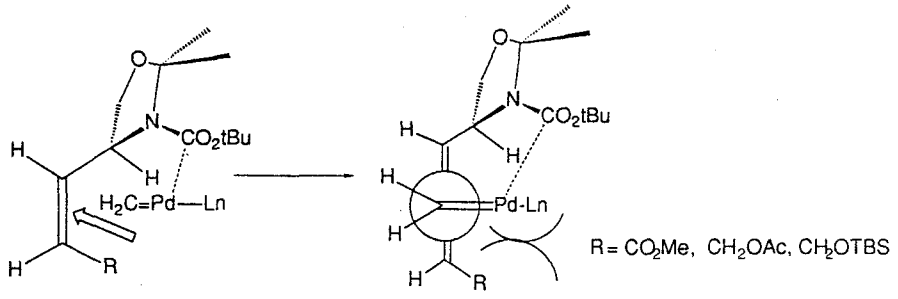
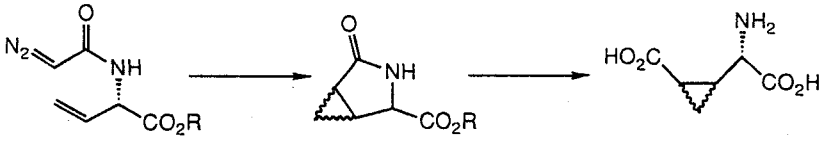


Figure 2-3

side of the *N*-Boc group (less hindered side) is not understood. It is interesting to note that only (*E*)-olefins underwent cyclopropanation, especially, (*E*)-α,β-unsaturated esters gave good yields. Some other steric and/or stereoelectronic factors in the transition state remains to be solved. Alternative routes to the synthesis of the folded isomers (**3**) and (**4**) were hence examined.

[2] Syntheses of CCG-III (3) and IV (4) via Intramolecular Cyclopropanation. The folded isomers of CCG (**3** and **4**) showed important activity, such as potentiation of the response of L-Glu by L-CCG-III (**3**) or potent excitatory action by L-CCG-IV (**4**). As described in the previous section, approaches to the synthesis of the folded isomers using (*Z*)-olefins with diazomethane were unsuccessful. I next examined the intramolecular cycloaddition for the synthesis of the folded isomers.[15] Intramolecular reaction would contribute to entropic activation resulting in an increase in the reactivity of the internal olefin with the carbene to give cycloadducts in much improved yield. Also the stereoselectivity of the intramolecular reaction attracted much interest. As shown in eq.2-3 intramolecular



(eq.2-3)

cyclopropanation may provide the cis fused bicyclo[3.1.0]hexane skeleton which has appropriate configuration to be convertible to **3** and/or **4**.

(2*S*)-2-*N*-*tert*-butoxycarbonylamino-3-butenol (**5a**), which was used for the synthesis of all the diastereomers in Chapter 1, was used as the starting material. After removal of the Boc group of **5a** with TFA, the product was converted into *N*-glycyl-2-amino-3-butenol (**10**) by treatment with *N*-*tert*-butoxycarbonyl-glycine *N*-hydroxysuccinimide ester (Boc-Gly-OSu) and Et_3N (-20 °C, 3 h). To avoid carbene insertion to the amide or hydroxyl group in the cycloaddition step, these groups were protected as *N,O*-acetonide (2,2-dimethoxypropane, acetone, catalytic amount of CSA, 80 °C, 16 h) to give **11** (3 steps from **5a**; 62%).*1

To generate diazoacetamide, it was necessary to remove the Boc group chemoselectively in the presence of the acetonide group, both of which are labile under acidic conditions. Recently, Sakaitani and Ohfune have reported the removal of the Boc group under neutral conditions using *tert*-butyldimethyl-

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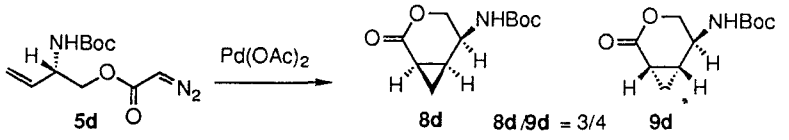
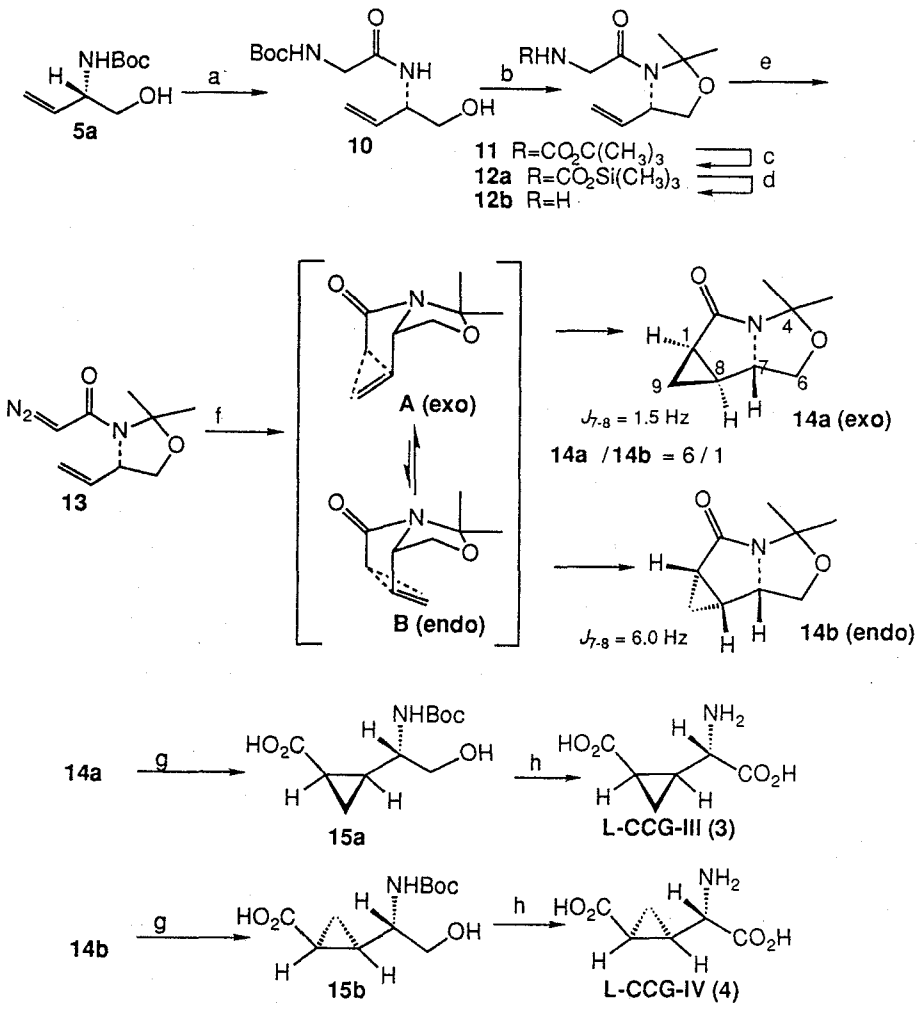
Footnote*1 Carbene insersion took place between N-H of the amide to give *N*-alkylated products. Actually attempted intramolecular cyclopropanation of diazoacetoxy compound **5d** gave a sluggish mixture of unidentified products and the yield of δ-lactones (**8d**/**9d** = 3/4) was only 8%.

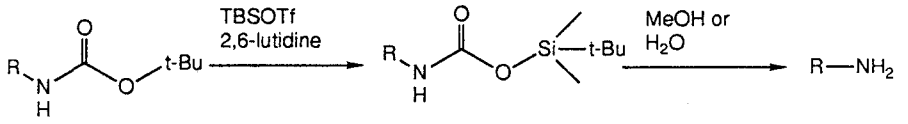


Scheme 2-2



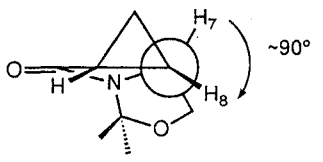
(a) (1) TFA; (2) Boc-Gly-OSu, Et_3N, THF; (b) $(CH_3)_2C(OCH_3)_2$, CSA, acetone (3 steps, 62%); (c) 1.5 equiv of TMSOTf, 3 equiv of 2,6-lutidine, CH_2Cl_2; (d) MeOH; (e) $NaNO_2$, citric acid buffer (pH 3) (71%); (f) 0.05 equiv of $Pd(OAc)_2$, toluene, 80 °C (43%); (g) (1) 60% AcOH; (2) 0.5*M*NaOH; (3) Boc_2O, $NaHCO_3$; (h) (1) Jones reagent, acetone; (2) TFA (**3** 59 % 5 steps from **14a** ; **4** 39% 5 steps from **14b**)

Conversion of Boc group to silylcarbamate [33]



Boc group Silyl carbamate

silyl trifluoromethanesulfonate (TBSOTf).[33] This method involves an initial conversion of the *tert*-butyl group of Boc group into the TBS group and subsequent removal of the TBS group to give the amine. I have modified this method by using trimethylsilyl trifluoromethanesulfonate (TMSOTf) instead of TBSOTf, since TMS carbamate could be removed much easily compared to TBS carbamate. Thus, treatment of the Boc-acetonide **11** with 1.5 equiv of TMSOTf and 3.0 equiv of 2,6-lutidine gave the TMS carbamate **12a**. The TMS carbamate was readily hydrolyzed by addition of methanol to give the desired amine **12b**. The amine **12b** was converted into diazoacetamide **13** with $NaNO_2$/citric acid (pH 3). This, without further purification, was treated with a catalytic amount of $Pd(OAc)_2$ in toluene at 90 °C to give a mixture of cis fused 3-aza-5-oxatricyclo[6.1.0.$0^{3,7}$]nonan-2-one (**14a** and **14b**) in a ratio of 6/1 in 43% yield. The stereochemistry of the products was ascertained by the combination of ^1H NMR (**14a**; J_{7-8} = 1.5 Hz: **14b**; J_{7-8} = 6.0 Hz, see Figure 2-4)



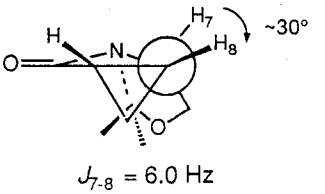
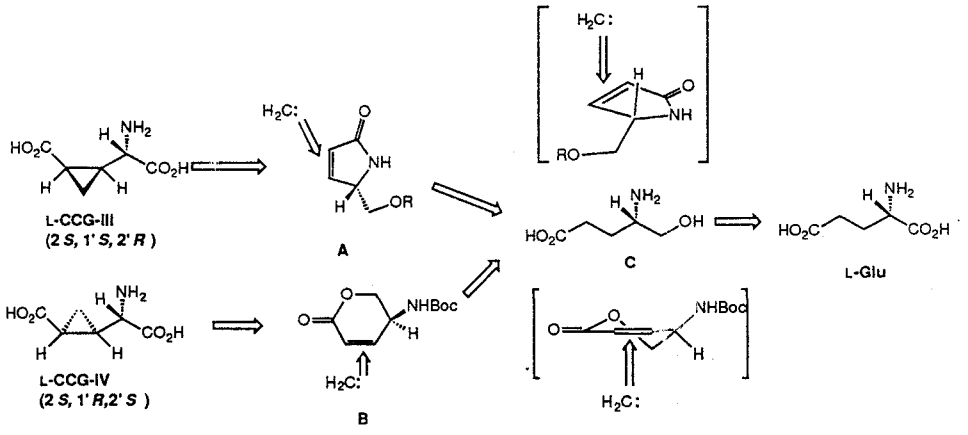


Figure 2-4

and their chemical conversion into **3** and **4**, respectively. The product ratio (exo/endo) may be influenced by the thermodynamic stability of the conformers (**A** and **B**) in the transition state, in which **A** would be sterically less hindered than **B**. The conversion of each isomer into cis-CCG (**3** or **4**) was carried out using the following sequence of reactions: (1) removal of the acetonide with 60% AcOH (room temperature, 8 h), (2) hydrolysis of the amide bond with 0.5 *M* aqueous NaOH (70 °C, 4 h), (3) protection of the amino group with the Boc group (Boc_2O, room temperature, 16 h), (4) oxidation of the hydroxyl group using Jones reagent (0 °C, 14 h), and (5) removal of the Boc group with TFA. The yield of **3** from the exo-adduct **14a** was 59% and that of **4** from the endo-adduct **14b** was 39%.

[3] Stereoselective Synthesis of CCG-III (3) and IV (4). In order to develop a more efficient method for the synthesis of both CCG-III and IV, stereoselective routes for the synthesis of these isomers were examined.[16] In particular, synthesis of CCG-IV (**4**), which was the minor product in the previous strategy, was intensely required. Although the cycloaddition of diazomethane with acyclic (*Z*)-olefins shown in Table 2-2 was not satisfactory, I attempted this reaction again but this time using cyclic α,β-unsaturated compounds. Some of the reason for this choice are (1) these compounds were expected to be more reactive owing to the ring strain, and (2) the cycloaddition of a carbene to **A** should occur from the side opposite to the bulky allylic substituent to give CCG-III type adduct, also CCG-IV type of cycloadduct should be available from **B** because the bulky Boc group would act as a directing factor. Thus, cyclo-

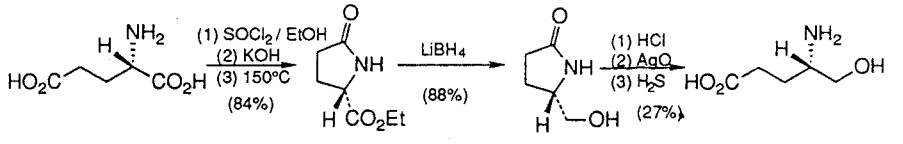
Scheme 2-3



propanation of the cyclic α,β-unsaturated intermediates **A** and **B** with diazomethane is the key step in this strategy (Scheme 2-3).

[3-1] Regioselective Reduction of the α-Carboxyl Group of L-Glu. 4-Amino-5-hydroxypentanoic acid **C** was envisaged as a common intermediate for the synthesis of both **A** and **B**. L-Glu provides both a source of chirality and the required carbon framework for these intermediates (Scheme 2-3). Recently, Silverman et al. reported the synthesis of 4-amino-5-hydroxypentanoic acid via pyroglutamic acid starting from L-Glu.[35] However, this method suffers from several disadvantages: (1) considerable racemization occurred during the reduction of pyroglutamic acid, (2) it is difficult to handle water soluble intermediates, and (3) hydrolysis of the lactam ring was a low

Synthesis of 4-amino-5-hydroxypentanoic acid (Silverman's route) [35]

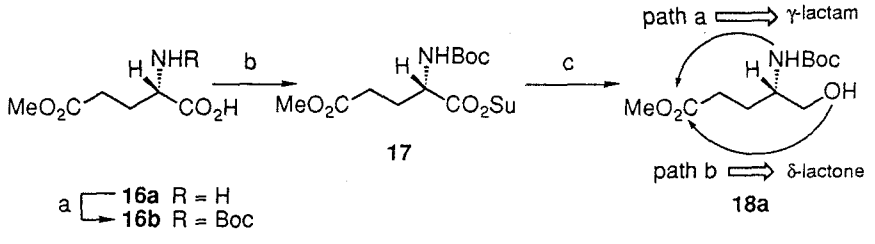


yielding process.

Therefore I focussed my attention onto the regioselective reduction of the α-carboxyl group of L-Glu to avoid the difficulties in the reported procedure. The regioselective reduction of the α-carboxylic acid of L-Glu γ-methyl ester **16a**, which is commercially available, was successfully performed by the reduction of the active ester **17** with $NaBH_4$.[36] After protection of the amine in **16a** with Boc group, treatment of the resulting carboxylic acid with dicyclohexylcarbodiimide (DCC) / *N*-hydroxy-succinimide (HOSu) provided Boc-Glu(OMe)-OSu. This, upon reduction with $NaBH_4$ in THF-EtOH, gave the desired primary alcohol **18a** (mp 40.5-41.5 °C, $[\alpha]_D$ -13.2°) in 83% yield from the half ester **16a**. Thus, 4-amino-5-hydroxypentanoic acid could be obtained in a protected form.

Usually, borane reduction of carboxylic acid is much faster than that of ester. However, the direct reduction of **16a** with THF-B_2H_6 or $Me_2S{\cdot}BH_3$ complex gave a mixture of **18a**, α,γ-diol, and the starting material. In this case, the borane may chelate to the amino group, and delivers the hy-

Scheme 2-4



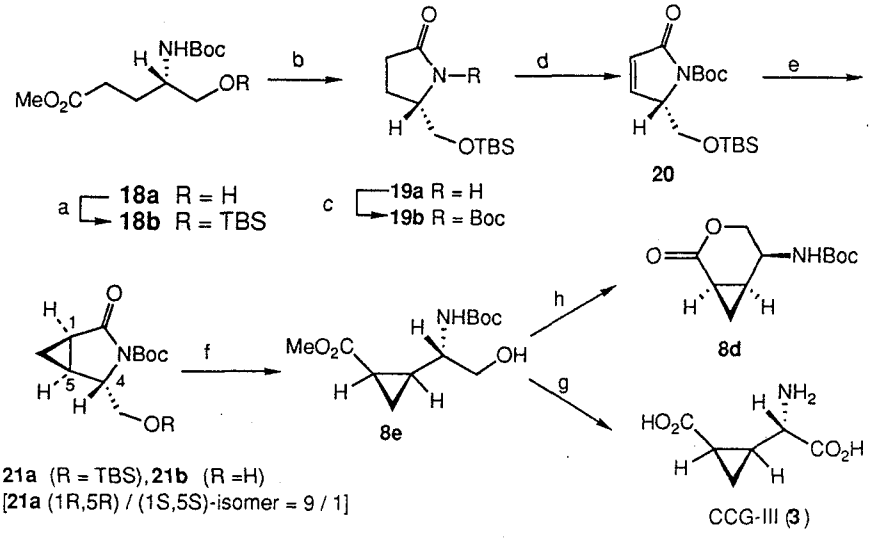
(a) Boc_2O, $NaHCO_3$, dioxane-H_2O (1/1); (b) HOSu, DCC, AcOEt (83% from **16a**); (c) $NaBH_4$, THF-EtOH (3/1) (92%).

dride intramolecularly to the ester group, which accelerates even more the reduction.

The 4-amino-5-hydroxypentanoic acid derivative **18a** is a key intermediate both for γ-lactam **20** (path a) and for δ-lactone **22a** (path b). No racemization accompanied this procedure as was ascertained by converting the alcohol **18a** into the known γ-lactam **19b**.[37]

[3-2] Synthesis of CCG-III (3). With compound **18a** in hand, and in order to synthesize CCG-III, α,β-unsaturated γ-lactam **20** was prepared in a straight forward way. Protection of the primary hydroxyl group as a TBS ether and treatment of **18b** with NaH in Et_2O gave γ-lactam **19a**. Even though during this reaction the Boc group was cleaved, this was re-introduced by upon expo-

Scheme 2-5

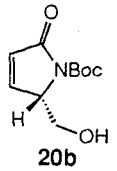


(a) TBSCl, imidazole, DMF (92%). (b) 1 equiv of NaH, Et_2O; (c) Boc_2O, Et_3N, DMAP, THF (87%); (d) (1) LDA, PhSeCl, THF (85%); (2) O_3, NaOAc, CH_2Cl_2 (87%); (e) CH_2N_2, 0.05 equiv of $Pd(OAc)_2$, Et_2O (100%; **21a**/(1S,5S)-isomer = 9/1); (f) (1) CSA, MeOH; (2) LiOH, MeOH (83%, from **21a**); (g) (1) Jones reagent, acetone; (2) 1*M* NaOH; (3) TFA, CH_2Cl_2 (36%15 steps from **16a**) (h) CSA, CH_2Cl_2.

sure of γ-lactam **19a** to Boc_2O/Et_3N/0.2 equiv of dimethylaminopyridine (DMAP) to afford the protected γ-lactam **19b** (87% from **18b**). Its ^1H NMR data and optical rotation value ($[\alpha]_D$ -62°) were identical to those previously reported by Ohfune (lit. $[\alpha]_D$ -61°).[37] Thus no racemization occurred in the reduction step (**16a** to **18a**). The conversion of **19b** to α,β-unsaturated γ-lactam **20** was carried out using the reported method[37]: (1) Phenylselenylation of **19b** with PhSeCl/lithium diisopropylamide (LDA), (2) oxidative removal of the selenide of **19c** with O_3.

Cycloaddition of **20** with diazomethane proceeded smoothly in the presence of a catalytic amount of $Pd(OAc)_2$ (0.05 equiv) to give the desired (1*R*,5*R*)-**21a** as the major product (100% yield as a 9/1 mixture of diastereomers: the ratio was determined by ^1H NMR).*2 The structure of (1*R*,5*R*)-**21a** was ascertained by converting it to the known δ-lactone **8d** (vide infra). Although (1*R*,5*R*)-**21a** was inseparable from its (1*S*,5*S*)-isomer, the desired alcohol (1*R*,5*R*)-**21b**, fortunately crystallized after desilylation of the mixture (CSA, MeOH, room temperature, 14 h). Recrystallization from Et_2O gave pure (1*R*,5*R*)-**21b**. Cleavage of the lactam ring was effected by methanolysis (LiOH, MeOH, room temperature, 16 h, 83% from **21a**) to give the methyl ester **8e**, the structure of which was confirmed by converting it into the δ-lactone **8d** (CSA, CH_2Cl_2). The conversion of **8e** into CCG-III

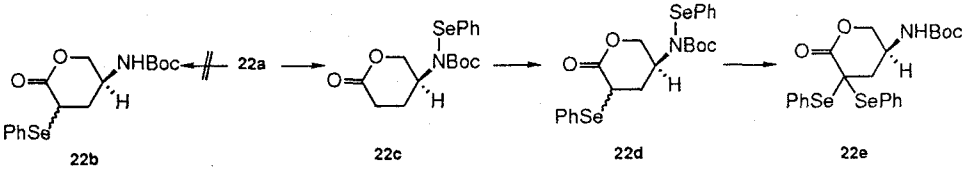
Footnote*2 Attempts to perform Simmons-Smith reaction (Zn-Cu, CH_2I_2) on the hydroxylactam **20b** were not successful. They resulted in a cleavage of the Boc group owing probably to ZnI_2 catalysis under the reaction conditions. Reaction using Sm/CH_2ClI provided no cycloadducts.



20b

(**3**) was carried out by repeating the same procedure as described in Chapter 1. The overall yield of CCG-III (**3**) starting from L-Glu half ester **16a** was 36% (15 steps).

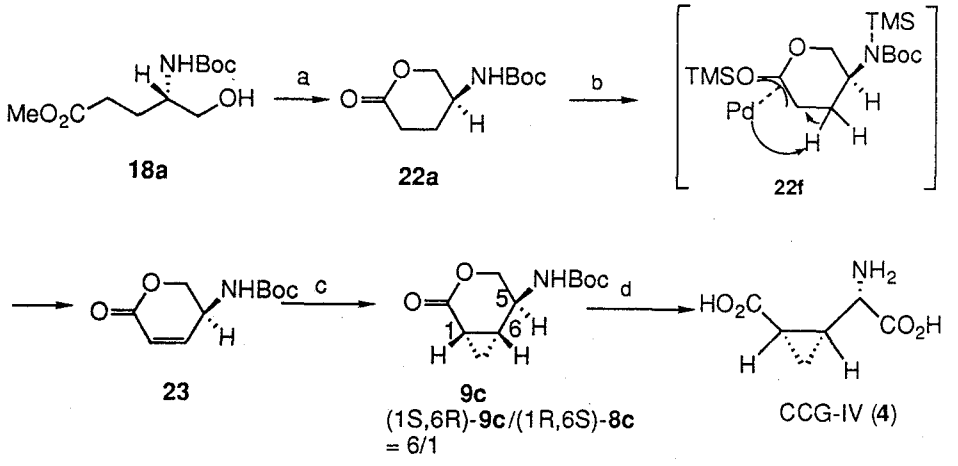
[3-3] Synthesis of CCG-IV (4). For the synthesis of CCG-IV (**4**) I chose to apply the diazomethane cycloaddition to the α,β-unsaturated δ-lactone intermediate **23**. Thus, the hydroxy-ester **18a** was converted into the δ-lactone **22a** with CSA/benzene in 92% yield*3,4. The next step involved the conversion of the δ-lactone **22a** into the α,β-unsaturated δ-lactone **23**. The selenylation-deselenylation procedure for the conversion of **22a** to **23** proved unsuccessful in my hand. The selenylation of **22a** did not give the desiderd monoselenylated compound **22b** but a mixture of 2,2-diselenylated compound **22e** and recovered starting material **22a**. It was assumed that the presence of the excess anion caused the disproportionation of the monoselenide **22b** to give **22a** and **22e**. In order to reduce the anion concentration, reversed addition of the enolate into a cooled solution of PhSeCl in THF was performed. However, it provided only *N*-selenylated product **22c**. I assumed that initial selenylation occurred at



Footnote*3 It was found that hydroxypentanoic acid **18a** partially dimerized and/or polymerized when it was stored at room temperature for a week. To avoid this, lactonization of **18a** was carried out under high dilution conditions (8 mM benzene solution).

Footnote*4 On the other hand, *t*-butyl ester of **18a**, which was obtained from L-Glu γ-*tert*-butyl ester in excellent yield, was stable enough to be stored for a long time without dimerization, while lactonization reaction did not go tom completion (~50% yield).

Scheme 2-6



(a) CSA, benzene (92%); (b) (1) 2.2 equiv of TMSCl, 2.2 equiv of LiHMDS, THF; (2) 1.2 equiv of $Pd(OAc)_2$, CH_3CN (70%); (c) CH_2N_2, 0.2 equiv of $Pd(OAc)_2$, Et_2O (46%). (d) (1) 1 *M* NaOH; (2) Jones reagent, acetone; (3) TFA, CH_2Cl_2 (14%, 12 steps from **16a**).

the amide group to give **22c** and subsequent selenylation at C-6 gave **22d**. Subsequent intra- or intermolecular migration of *N*-selenyl group to C-6 might have afforded the diselenide **22e**. Therefore I turned my attention to examine the Saegusa's method[38a] for the introduction of the requisite C-C double bond into **22a**. Treatment of **22a** with 2 equiv of lithium hexamethyldisilazide (LiHMDS) and 2 equiv of trimethylsilyl chloride (TMSCl) gave the *N*-trimethylsilylated *O*-trimetylsilyl enol lactone **22f**. This, upon formation of the oxo-π-allyl complex using $Pd(OAc)_2$ followed by oxidative dehydrogenation of β-position, furnished the desired α,β-unsaturated δ-lactone **23** in 70% yield.*5

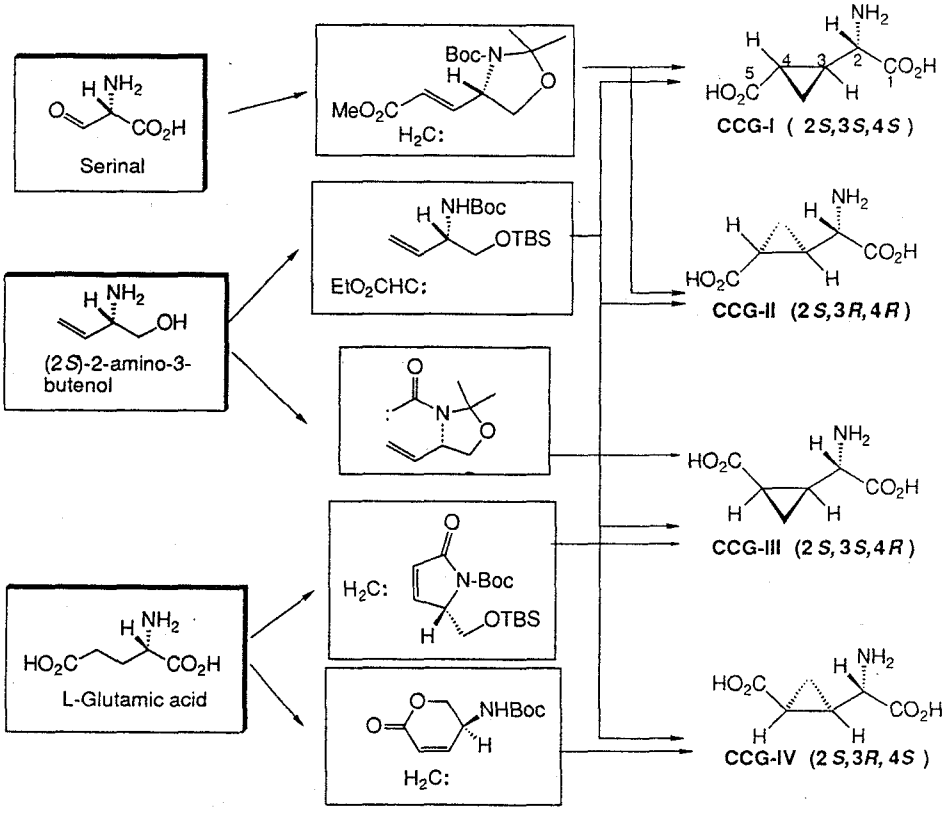
In contrast to the carbene addition to α,β-unsaturated γ-lactam (**20**), cycloaddition of diazomethane to **23** proceeded very

Footnote*5 Attempts to improve this process by the use of diallycarbonate and catalytic amount of $Pd(OAc)_2$ [38b] were unsuccessful, and gave an *N*-allyl compound or an allyl ester resulted from the δ-lactone opening.

slowly and required large excess of diazomethane and 0.2 equiv of $Pd(OAc)_2$ catalyst. The reaction was not reproducible in a large scale (>10 mmol) and gave a mixture of ~20% the cycloadduct and 70% of the starting material **23**. Also the reaction did not proceed to completion even in the presence of more than 0.2 equiv of $Pd(OAc)_2$, this is probably due to the inactivation of the catalyst (formation of orange or brown precipitate). When 1 mmol of **23** was employed, the yield was approximately 50%. The cyclopropanation gave a 6:1 mixture of cycloadducts (**8d** and **9d**). The major product was the desired (1*R*,5*S*)-isomer **9d**. These isomers were separable by column chromatography on silica gel. The conversion of **9d** to **4** has already been described in Chapter 1. The overall yield of **4** from **16a** was 14% (12 steps). Thus, the folded isomers of CCG (**3** and **4**) were prepared in an efficient manner from L-Glu. In addition, these unsaturated γ-lactam and δ-lactone should be useful as chiral synthons for the synthesis of related compounds. Synthesis of β-substituted glutamate analogues using these compounds as the key intermediates has been reported[35] and the syntheses of other glutamate analogues are now under investigation in our laboratory.

In summary, the synthesis of CCGs described in Chapter 1 and 2 are featured by the following points (Scheme 2-7): (i) all the diastereomers of CCG were obtained by the cycloaddition of ethyl diazoacetate to (2*S*)-2-amino-3-butenol derivative prepared from L-methionine, (ii) the cycloaddition of diazomethane to the (*E*)-olefins gave the extended isomers CCG-I (**1**) and II (**2**), (iii) the intramolecular cyclopropanation of the diazoacetamide derived from (2*S*)-2-amino-3-butenol provided the folded isomers CCG-III (**3**) and CCG-IV (**4**), (iv) the cycloaddi-

Scheme 2-7



tion of diazomethane to the α,β-unsaturated γ-lactam derived from L-Glu afforded CCG-III (**3**), stereoselectively, (v) the cycloaddition of diazomethane to the α,β-unsaturated δ-lactone derived from L-Glu produced CCG-IV (**4**). Thus, all isomers or each isomer of CCG can be synthesized by the methods described above. It is worthy to note that in each approach the unsaturated amino acid or alcohol readily prepared from the commercially available amino acids (L-methionine, D-serine, or L-glutamic acid) was subjected to the cyclopropanation reaction.

Chapter 3

Syntheses of 3'-Substituted Analogues of L-CCG-III and IV

The syntheses of the eight diastereomers of CCGs has been described in the preceding chapters. The results of the neurobiological assays using synthetic CCGs demonstrated that both the folded isomers (**3** and **4**) activated one of L-Glu receptor subtypes; the activity of L-CCG-IV (**4**) was much more than that of L-CCG-III (**3**). On the other hand, the latter isomer **3** was a potent inhibitor of L-Glu uptake (details of these results will be described in the next Chapter).

Because both CCG-III (**3**) and IV (**4**) exhibited marked neurobiological action, I was interested in synthesizing other CCG analogues possessing a substituent on the C-3' position of the cyclopropane ring of both **3** and **4**. Introduction of a substituent on the cyclopropane ring would provide further information

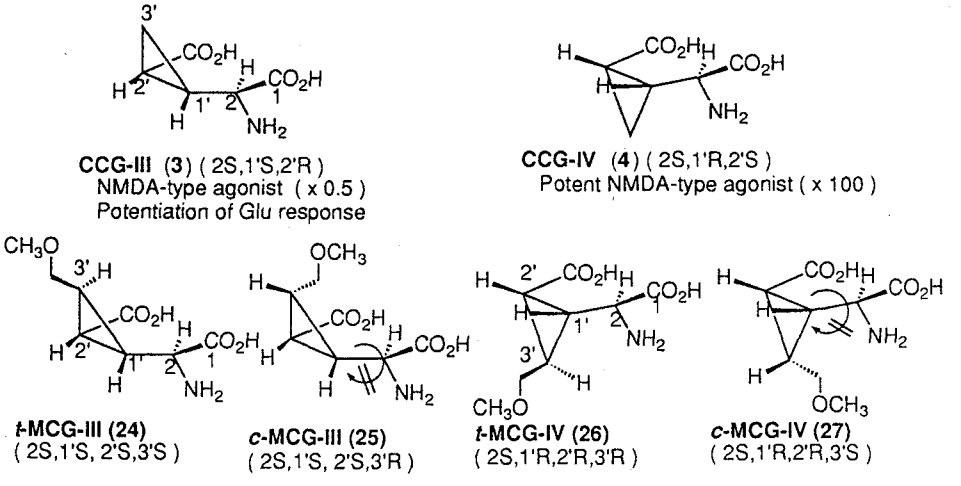
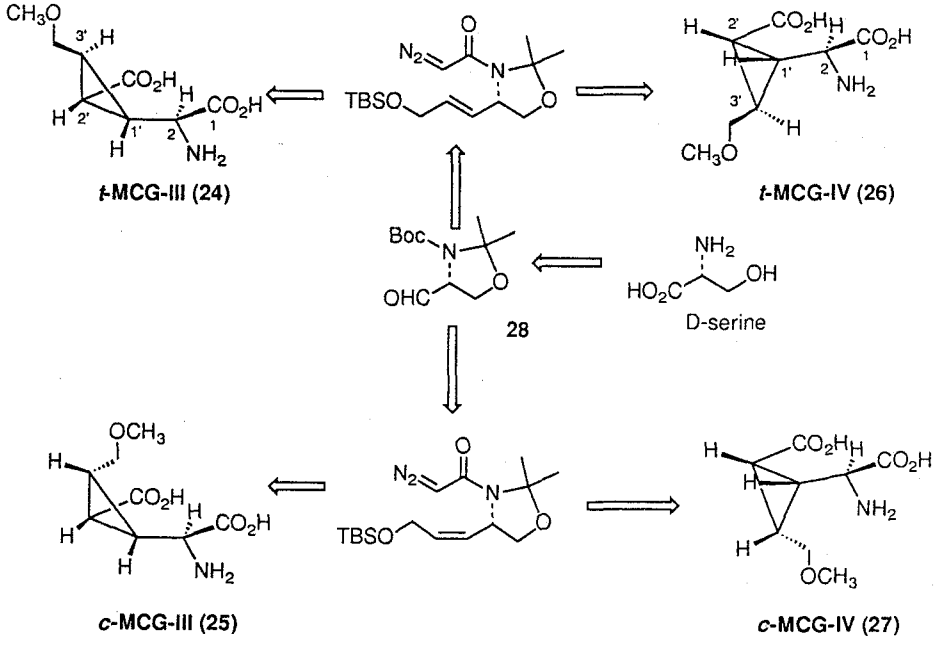


Figure 3-1

regarding the structural requirements for activating the L-Glu receptors as well as extending the scope of these studies, i.e., synthesis of polymer bounded CCG analogues, synthesis of effective antagonists, synthesis of photoaffinity labelled analogues, etc.

As a substituent at C-3', hydroxymethyl group or its ether or ester analogues should be suitable for the purpose mentioned above. The methoxymethyl group was my first choice because this group is chemically and physiologically stable. Described in this Chapter are the stereoselective syntheses of the four possible diastereomers which are substituted at C-3' in CCG-III and IV; *trans* and *cis*-2-(3'-methoxymethyl-2'-carboxycyclopropyl)glycines [*t*-MCG-III (**24**), *c*-MCG-III (**25**), *t*-MCG-IV (**26**), *c*-MCG-IV (**27**)].

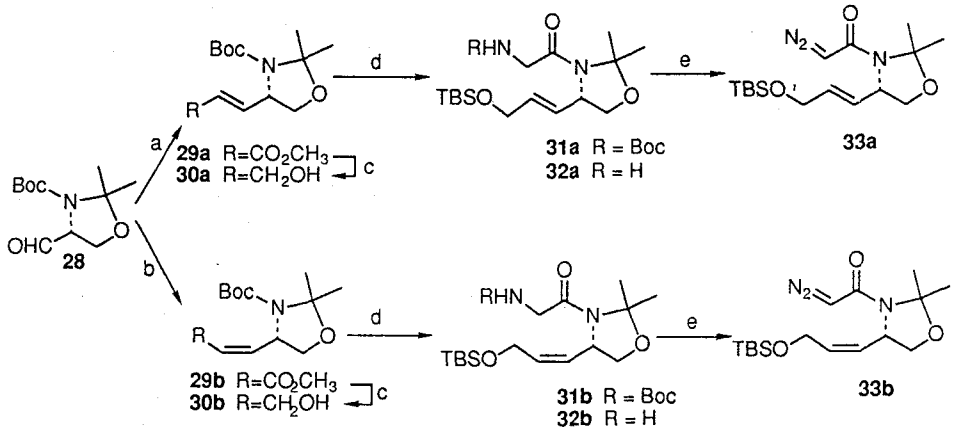
Scheme 3-1



[1] Syntheses of Diazoacetamide with (*E*)- or (*Z*)-Allylalcohol. The synthetic plan to obtain the methoxymethyl substituted CCG analogues (MCGs), **24~27**, was based on the synthesis of CCG-III and IV described in Chapter 2. The key step involves an intramolecular cyclopropanation of the (*E*)- or (*Z*)-allyl alcohol intermediates which can be prepared from D-serinal derivative, (4*R*)-*N*-Boc-2,2-dimethyl-4-formyl-1,3-oxazolidine (**28**)[39] (Scheme 3-1).

Wittig reaction of **28** with methyl triphenylphosphoranylideneacetate ($Ph_3P{=}CHCO_2CH_3$) in benzene gave (*E*)-α,β-unsaturated ester **29a**, exclusively. While Horner-Emmons reaction of **28** with methyl bis(2,2,2-trifluoroethyl)phosphonoacetate [$(CF_3CH_2O)_2P(O)CH_2CO_2CH_3$], NaH, 18-crown-6 in CH_2Cl_2 gave (*Z*)-α,β-unsaturated ester **29b** as a major product (*Z*/*E* = 9/1).*[1,34a] Attempted reduction of the ester group of the (*E*)-isomer **29a** using several reducing agents such as $LiAlH_4$, *i*-Bu_2AlH (DIBAL),

Scheme 3-2



(a) $Ph_3PCHCO_2CH_3$, benzene (95%); (b) $(CF_3CH_2O)_2P(O)CH_2CO_2CH_3$, NaH, 18-crown-6, THF, -78 °C, 2 h (85%); (c) *n*-Bu(*i*-Bu)$_2$LiAlH, toluene; **30a** (87%), **30b** (86%); (d) (1) HCl, MeOH; (2) Boc-Gly-OSu, Et_3N, THF, MeOH; (3) $(CH_3)_2C(OCH_3)_2$, CSA, acetone, then MeOH; (4) TBSCl, imidazole, DMF; **31a** (63%, 4 steps from **30a**), **31b** (71%, 4 steps from **30b**); (5) TMSOTf, 2,6-lutidine, CH_2Cl_2; (e) $NaNO_2$, citric acid buffer (pH 3).

affected predominantly the saturated alcohol and aldehyde. The desired allyl alcohol **30a** was obtained only in a low yield. The reduction with $LiAlH_4$, DIBAL and DIBAL/BF_3·OEt_2[40] yielded **30a** in 12%, 31% and 43%, respectively. These results suggested that aluminum reagents initially chelate with the amide nitrogen, and subsequent internal hydride attack from this complex to the C-C double bond in a 1,4-addition mode leads to the formation of saturated compounds. After numerous unsuccessful attempts, fortunately I found that the use of bulky *ate* complex, LiAl(*n*-Bu)(*i*-Bu)$_2$H prepared from DIBAL and *n*-BuLi,[41] provided the desired allyl alcohol **30a** in an excellent yield. In this case, the reaction was accompanied with less than 10% of the saturated alcohol.

After removal of the protecting groups in **30a**, the glycyl unit was introduced to the amino group. Then, its functional groups were reprotected using the following four step sequence of which led to acetonide **31a**; (1) HCl, MeOH, 0 °C, 16 h, (2) Boc-Gly-OSu, Et_3N, 0 °C, 1 h; (3) $(CH_3)_2C(OCH_3)_2$, CSA, 60 °C, 2 h, then MeOH, room temperature, 30 min, (4) TBSCl, imidazole, room temperature, 3 h, (4 steps, 63%). Attempts to remove Boc group of **30a** via silylcarbamate, in which **30a** was expected to be converted into the glycyl-acetonide derivative **31a** directly, were unsuccessful because the carboxyl group of the silylcarbamate attacked the double bond intramolecularly to give the cyclic carbamate.

Next the Boc group in **31a** was removed chemoselectively

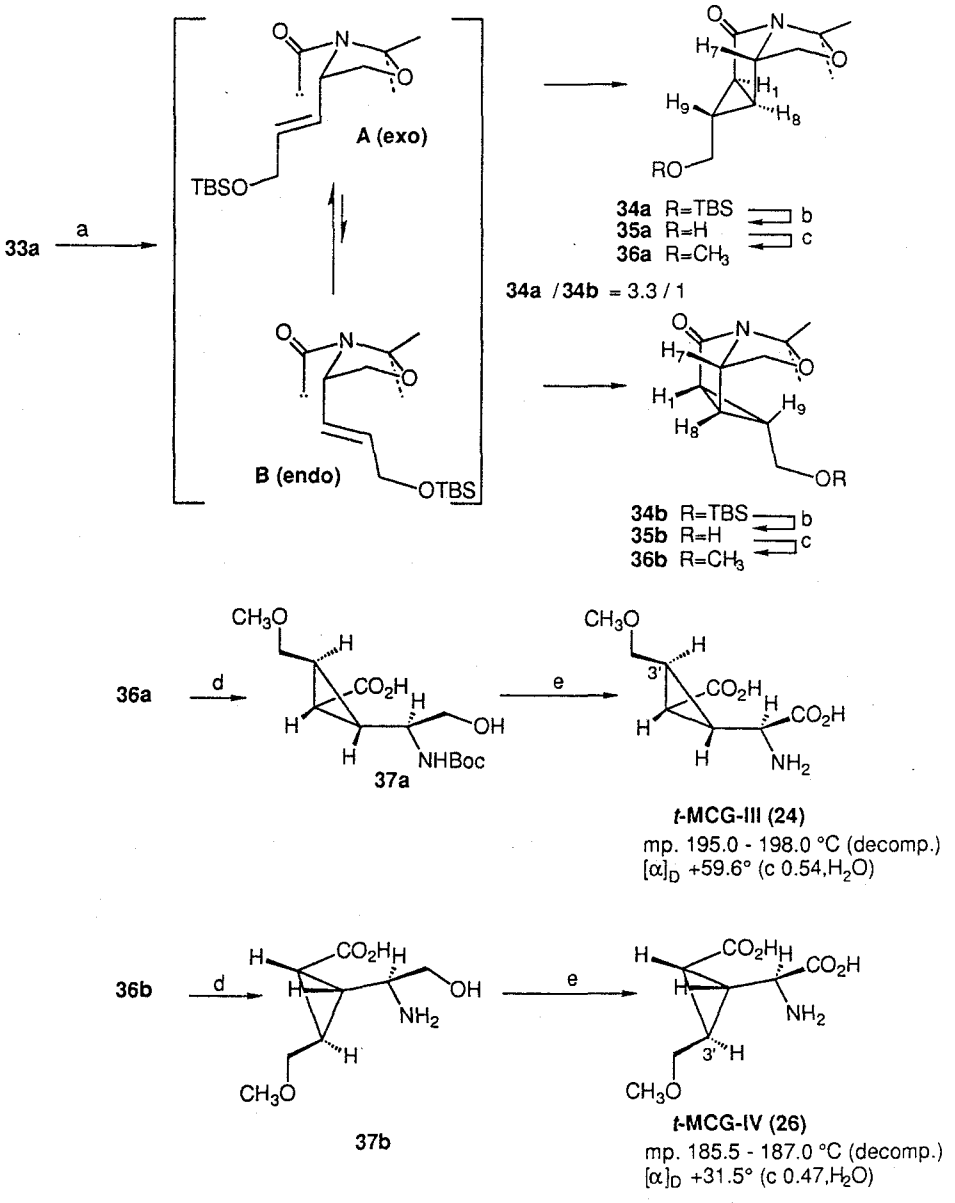
Footnote*1 In the case of acyclic serinal derivative, in which the hydroxyl group was protected as a TBS ether, Wittig reaction was accompanied by ~30% racemization, however, when acetonide **28** was used, the configuration was retained and the product was obtained in >95% ee.

with TMSOTf and 2,6-lutidine to give the amine **32a** in the same manner as described in Chapter 2. The resulting amine **32a**, upon diazotization by treatment with $NaNO_2$ in citric acid buffer, provided diazoacetamide **33a**. The diazoacetamide **33a** was subjected to cyclopropanation without further purification. The diazoacetamide **33b** was obtained from (*Z*)-allyl alcohol **30b** by the same procedure as (*E*)-isomer **33a**.

[2] Syntheses of the Trans Substituted Isomer, *t*-MCG-III (24) and *t*-MCG-IV (26). Cyclopropanation of **33a** in the presence of $Pd(OAc)_2$ gave a mixture of the cycloadducts in the ratio of 3.3/1 (**exo-34a/endo-34b**). The structure of the isomers was determined by comparisons of the coupling constants of their ^1H-NMR (**34a**; J_{7-8} = 1.4 Hz: **34b**; J_{7-8} = 6.0 Hz) with those of the unsubstituted compounds, **14a** and **14b** (see Figure 2-2), and NOESY experiments on their corresponding δ-lactones, **40a** and **40b**, (*vide infra*).

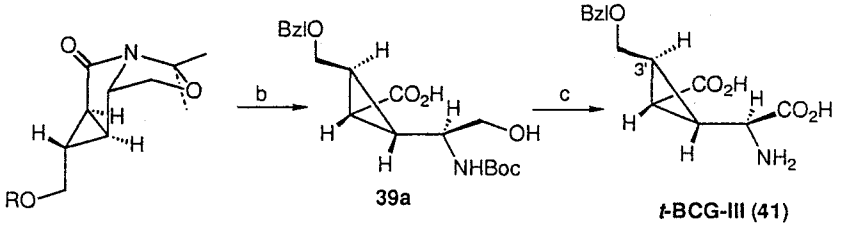
The silyl group of the exo adduct **34a** was converted into the methyl ether in two steps (1) *n*-Bu_4NF (65%) and (2) NaH/MeI (63%) to give **36a**. Successive hydrolysis of the acetonide of **36a** with 60% AcOH followed by lactam cleavage with $Ba(OH)_2$ gave the free amine which was re-protected with Boc_2O to provide the primary alcohol **37a** (3 steps, 73%). Finally, this was converted into *t*-MCG-III (**24**) (mp. 195-198 °C, $[\alpha]_D$ +59.6°) by the following sequence of reactions; (1) oxidation of the hydroxyl group of **37a** with Jones reagent and (2) deprotection of the Boc group with TFA. The endo-isomer **34b** was transformed into *t*-MCG-IV (**26**) (mp. 185.5-187 °C, $[\alpha]_D$ +31.5°) in the same manner as above (yields are shown in Scheme 3-3).

Scheme 3-3



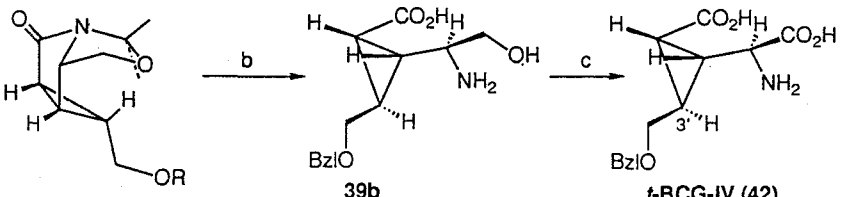
(a) 0.05 equiv $Pd(OAc)_2$, toluene, 90 °C;**34a** (33%, 3 steps from **32a**), **34b** (10%, 3 steps from **32a**); (b) *n*-Bu_4NF; **35a** (86%), **35b** (65%); (c) NaH, CH_3I, *n*-Bu_4NI, DMF; **36a** (87%), **36b** (63%); (d) (1) 60% AcOH; (2) $Ba(OH)_2$, EtOH/H_2O; (3) Boc_2O; **37a** (3 steps 73%), **37b** (3 steps, 79%); (e) (1) Jones reagent, acetone; (2) TFA; (3) Dowex 50Wx4; ***t*-MCG-III** (3 steps 34%), ***t*-MCG-IV** (3 steps, 28%).

[a]**Scheme 3-4**



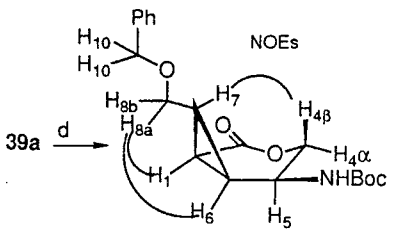
35a R=H
38a R=Bzl
a

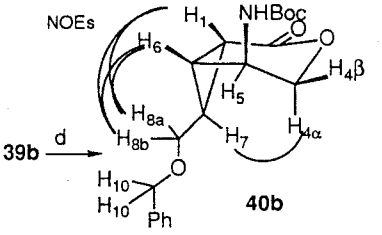
mp. 195.0 - 196.0 °C (decomp.)
$[\alpha]_D$ +63.5° (c 0.55,2*M* HCl)



35b R=H
38b R=Bzl
a

mp. 188.0-189.0 °C (decomp.)
$[\alpha]_D$ +40.2° (c 0.45,2*M* HCl)





$H_{4\alpha}$;δ 4.26
$H_{4\beta}$;δ 3.60, $J_{H4\beta\text{-}H5}$ = 11.0 Hz

$H_{4\alpha}$;δ 4.08 $J_{H4\alpha\text{-}H5}$ = 2.5 Hz
$H_{4\beta}$;δ 4.26 $J_{H4\beta\text{-}H5}$ = 1.5 Hz

[b]NOEs
(s) $H_{4\beta}$-H_7, H_{8a}-H_{8b}, $H_{4\alpha}$-$H_{4\beta}$;
(m) H_1-H_{8a}, H_5-H_6, H_6-H_{8a}, H_{8a}-H_{10}, H_{8b}-H_{10};
(w) H_5-NH, H_7-H_{8a}, H_7-H_{10}.

[b]NOEs
(s) $H_{4\alpha}$-$H_{4\beta}$, $H_{4\alpha}$-H_7, H_{8a}-H_{8b}, H_{8a}-H_{10}, H_{8b}-H_{10};
(m) H_1-H_{8a}, H_1-H_{8b}, H_6-H_{8a}, H_6-H_{8b};
(w) H_5-H_6, H_{10}-Ph.

[a](a) NaH, BzlBr, *n*-Bu_4NI, THF-DMF(1:1); **38a** (69%), **38b** (72%); (b) (1) 60% AcOH; (2) $Ba(OH)_2$, EtOH-H_2O; (3) Boc_2O, dioxane; **39a** (3 steps, 71%), **39b** (3 steps, 65%); (c) (1) Jones reagent, acetone; (2) CH_2N_2; (3) 1 *M* NaOH, EtOH-H_2O; (4) TFA; (5) Dowex50Wx4, **41** (5 steps, 41%), **42** (5 steps, 24%); (d) CSA, CH_2Cl_2; **40a** (16%), **40b** (83%).

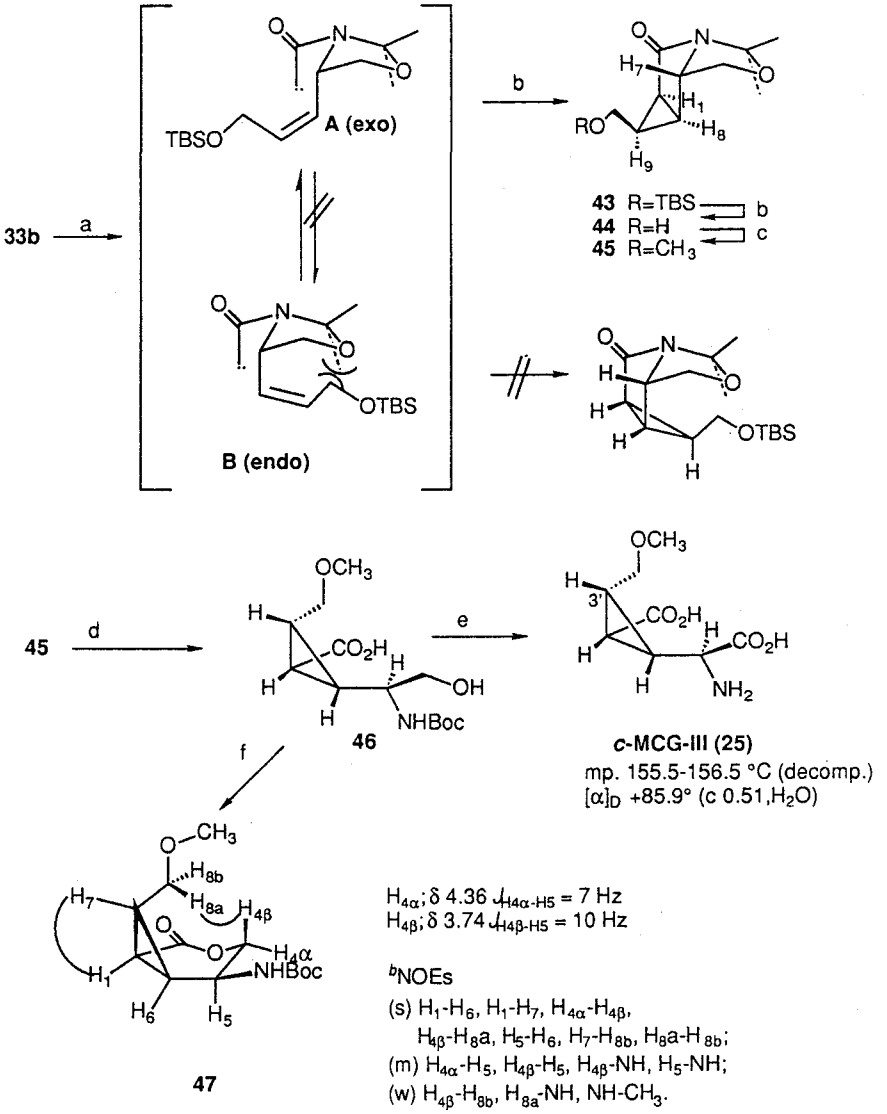
[b]Observed NOEs (NOESY, 300 MHz, $CDCl_3$). The strength of the NOE is designated strong (s), medium (m), and weak (w).

In addition, in order to prove the possibility of the binding to the spacer group of the resin for affinity column chromatography, benzyloxymethyl derivatives, *t*-BCG-III (**41**) and *t*-BCG-IV (**42**), were prepared. Benzylation of **35a** and **35b** with NaH/BzlBr/*n*-Bu_4NI gave **38a** and **38b**, respectively, which were converted into *t*-BCGs, **41** and **42**, in the same manner as *t*-MCGs, **24** and **26**.

The stereochemistry of the alcohols (**39a** and **39b**) was also ascertained after their conversion into δ-lactones **40a** and **40b** by the NOESY experiments. The data of **40a** indicated that the configuration of the cyclopropane ring was CCG-III type as depicted in Scheme 3-4, because a strong NOE was observed between $H_{4\beta}$ and H_7. The trans relationship between the benzyloxymethyl group and lactone ring was confirmed because NOEs were observed between H_1 and H_{8a}, and H_6 and H_{8a}. On the other hand, the configuration of **40b** was found to be CCG-IV type, as a consequence of the observation of a strong NOE between $H_{4\alpha}$ and H_7. The amino group of **40b** has an axial orientation similar to the unsubstituted δ-lactone **9d**. The relationship between the benzyloxymethyl group and lactone ring was also trans because NOEs were observed between H_1 and $H_{8a,b}$, and H_6 and $H_{8a,b}$. Thus, the stereochemistry of *t*-MCG-III (**24**) and IV (**26**), and *t*-BCG-III (**41**) and IV (**42**) were unambiguously confirmed as depicted.

[3] Syntheses of the Cis Substituted Isomers, *c*-MCG-III (25) and *c*-MCG-IV (27). In contrast to the cyclopropanation of the (*E*)-isomer (**33a** to **34a,b**), the cycloaddition of the diazoacetamide **33b**, obtained from the (*Z*)-allyl alcohol **30b**, provided the exo-adduct **43**, exclusively. The high stereoselectivity may

[a]**Scheme 3-5**



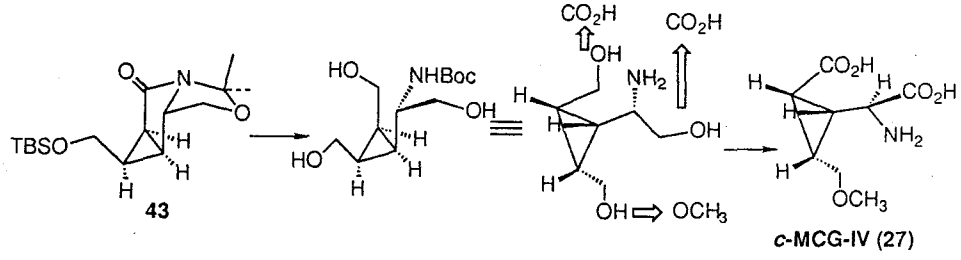
[a](a) 0.05 equiv of $Pd(OAc)_2$, benzene (61%); (b) *n*-Bu_4NF, THF; (94%);(c) NaH, CH_3I, *n*-Bu_4NI, DMF (82%); (d) (1) 60% AcOH; (2) $Ba(OH)_2$, EtOH/H_2O; (3) Boc_2O, dioxane (3 steps, 58%); (e) (1) Jones reagent, acetone; (2) TFA; (3) Dowex 50Wx4 (37%); (f) WSCD-HCl, HOBt, Et_3N, THF, 0 °C, 1 h, then room temperature, 3 h (46%).

[b]Observed NOEs (NOESY, 300 MHz, $CDCl_3$). The strength of the NOE is designated strong (s), medium (m), and weak (w).

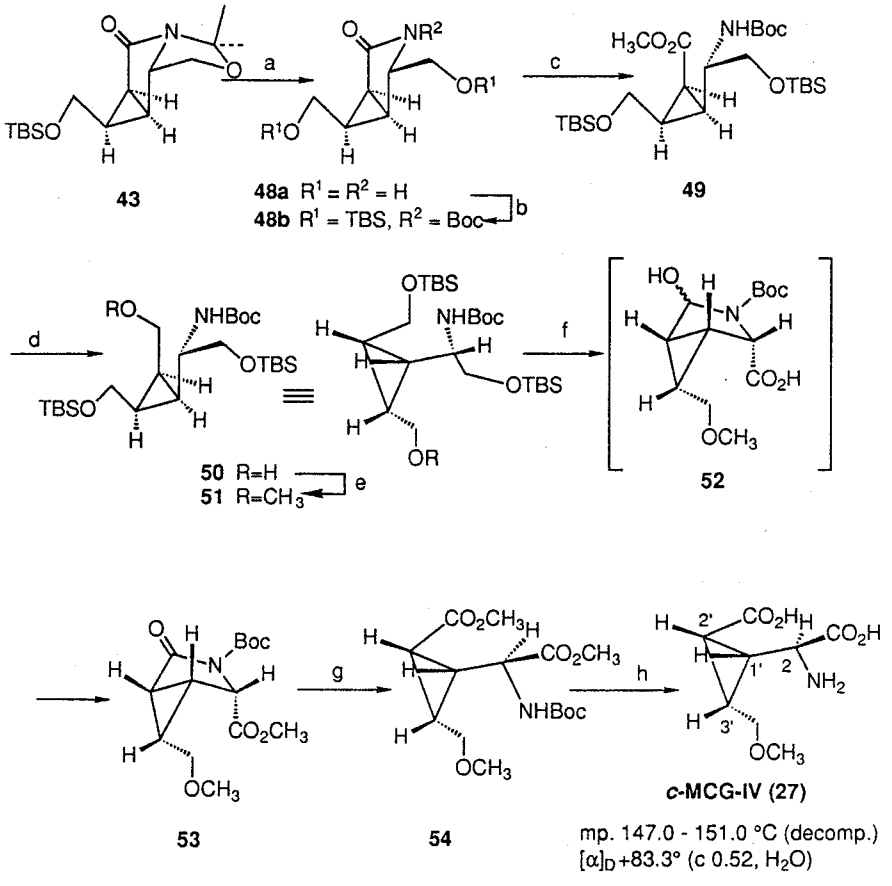
be attributed to severe steric interaction between the methyl group of the acetonide and the TBS group in the endo type transition state. The structure of **43** was temporarily determined by its ^{1}H NMR (J_{7-8} = 2.0 Hz) and was confirmed by converting it into the δ-lactone **47** via the primary alcohol **46**. These transformations were performed as summarized in Scheme 3-5. The configuration of the cyclopropane ring of **47** was found to be CCG-III type because a strong NOE was observed between $H_{4\beta}$ and H_{8a} as shown in the Scheme 3-5. A strong NOE observed between H_1 and H_7 clearly indicated that **47** is an all-cis-isomer. The *c*-MCG-III (**25**: mp. 155.5-156.5 °C; $[\alpha]_D$ +85.9°) was obtained by oxidation of the alcohol **46** in the same manner as above (yields are shown in Scheme 3-5).

Since no endo-type adduct was formed in the above cycloaddition reaction of the diazoacetamide with (*Z*)-allylalcohol **33b**, I planned to derive the other cis-type methoxymethyl analogue, *c*-MCG-IV (**27**), from the exo-adduct **43**. For this purpose it was necessary to convert the carboxyl group in the amide function of **43** to the methoxymethyl group and the hydroxyl group protected by TBS group to the carboxyl group as shown in Scheme 3-6. Thus, both the TBS and acetonide groups of **43** were removed by the treatment with Dowex 50Wx4 in MeOH to give diol

Scheme 3-6



Scheme 3-7



(a) Dowex50Wx4, MeOH; (b) (1) TBSCl, imidazole, DMF; (2) Boc_2O, Et_3N, DMAP, THF (3 steps, 69%); (c) LiOH, MeOH (65%); (d) DIBAL, CH_2Cl_2 (99%); (e) *n*-BuLi, FSO_3CH_3, THF-Et_2O (1:1) (93 %); (f) (1) Dowex50Wx4, MeOH; (2) Jones reagent, acetone; (3) CH_2N_2, Et_2O; (g) LiOH, MeOH (40%, 4 steps from **51**); (h) (1) 0.5 *M* NaOH, THF-H_2O; (2) 2 *M* HCl, THF; (3) Dowex50Wx4 (3 steps, 55%).

48a. This was re-protected with (1) TBSCl, imidazole, and (2) Boc_2O, Et_3N, DMAP (3 steps from **43**, 69%) to give di-TBS derivative **48b**. The lactam ring of **48b** was effectively cleaved with LiOH in MeOH (65%) to yield the methyl ester **49**. This was reduced with DIBAL to give the desired primary alcohol **50** in quantitative yield. Methylation of the hydroxyl group of **50** using the standard conditions (NaH/MeI) provided a mixture of the desired *O*-methylated product **51** (~50 %) and the *N*-methylated product of **50** (~50%). However, treatment of **50** with *n*-BuLi/ FSO_3Me[42] afforded, exclusively, the desired *O*-methylated product **51** in excellent yield. Thus, the required functional group was introduced into the C-3' position.

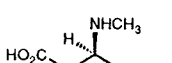
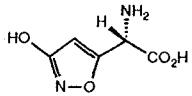
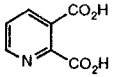
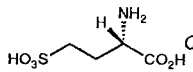
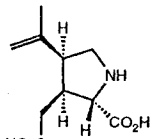
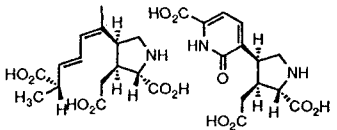
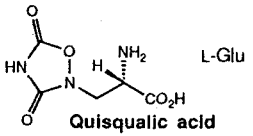
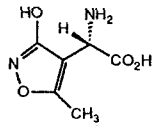
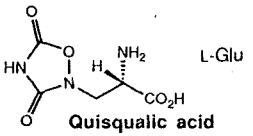
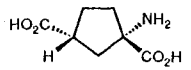
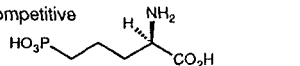
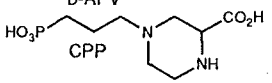
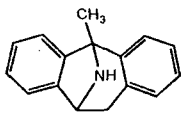
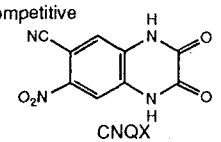
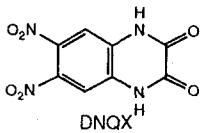
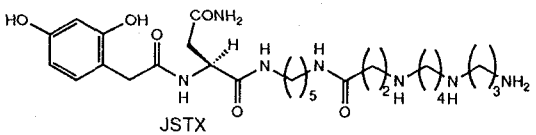
The conversion of **51** into the *c*-MCG-IV **27** was accomplished by the following sequence of reactions. After removal of the TBS ether of **51**, the resulting hydroxyl compound was oxidized with Jones reagent, followed by esterification of the resulting carboxyl group with diazomethane to give γ-lactam **53**. This γ-lactam was produced via aminal derivative **52**. The lactam **53** was subjected to methanolysis (LiOH/MeOH, room temperature, 15 min) to provide protected *c*-MCG-IV **54** (40%, 4 steps from **51**). Finally, removal of the protecting groups in **51** in 2 steps afforded *c*-MCG-IV (**27**) (mp. 147-151 °C, $[\alpha]_D$ +83.3 °): (1) 0.5 *M* aqueous NaOH, THF, H_2O, 0 °C, 14 h, (2) 2 *M* aqueous HCl, THF, H_2O, room temperature, 4 h.

Thus, the analogues of CCG-III and IV possessing 3'-substituent on its cyclopropane ring were stereoselectively synthesized from D-serinal derivative. These approaches would be practicable for the syntheses of polymer bounded CCGs and photoaffinity labelled compounds by replacing the substituent.

Chapter 4
Neurobiological Activities of CCGs and Their Derivatives. The Structure-Activity Relationships.

As described in Introduction, L-Glu receptors are classified into at least four types [*N*-methyl-D-aspartic acid (NMDA), kainic acid (KA), quisqualic acid (QA), and metabotropic receptors].[4] The ionotropic receptors (NMDA, KA, and QA type) are coupled with the ion channel directly. Because of the ready availability of the selective NMDA type antagonists, for example D-2-amino-5-phosphonovaleric acid (D-APV),[43] 3-(*dl*-carboxypiperazine-4-yl)propyl-1-phosphonic acid (CPP),[44] and Mg^{2+},[45] the physiological function of this receptor has been well studied.[44,46] Recent studies suggested that the NMDA receptor is closely linked to (1) construction of memory and early learning, (2) neuronal plasticity, (3) neuron damage which causes the brain diseases such as Huntington's chorea, Parkinsonism, Alzheimer symptom, and epilepsy. It is interesting that D-amino acids, such as NMDA, D-Glu, and D-APV, have selective affinity to the NMDA receptor. On the other hand, KA and QA receptors are believed to relate to movement and reflex. The common antagonists for these receptors, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)[47], facilitated their classification into non-NMDA type receptors. However, since no selective antagonist was available for each receptor type, it was not possible to distinguish KA type from QA type of receptors, electrophysiologically. It is intensely required to develop more selective and efficient antagonists for the above receptor

Table 4-1 Classification of Excitatory Amino Acid Receptors and Thier Agonists and Antagonists

Class	Ionotropic Receptors			Metabotropic Receptors
Subtype	**NMDA type** (N-Methyl-D-aspartic acid)	**KA type** (Kainic acid)	**QA type** (Quisqualic acid)	
Agonist	 NMDA L-Glu L-Asp D-Glu D-Asp  Ibotenic acid  Quinolic acid  Homocysteic acid	 Kainic acid L-Glu QA  Domoic acid Acromelic acid A	 Quisqualic acid L-Glu  AMPA	 Quisqualic acid L-Glu  t-ACPD
Antagonist	competitive  D-APV  CPP channel blocker  MK-801 Mg^{2+}	competitive  CNQX  DNQX channel blocker  JSTX		

subtypes.

In addition to these ionotropic receptor subtypes, recent studies demonstrated the presence of the metabotropic receptor which stimulates cell metabolism including the activation of G-protein, enzyme systems, second messengers, and mobilization of intracellular Ca^{2+} (Figure 4-1).[11,12] Since the metabotropic

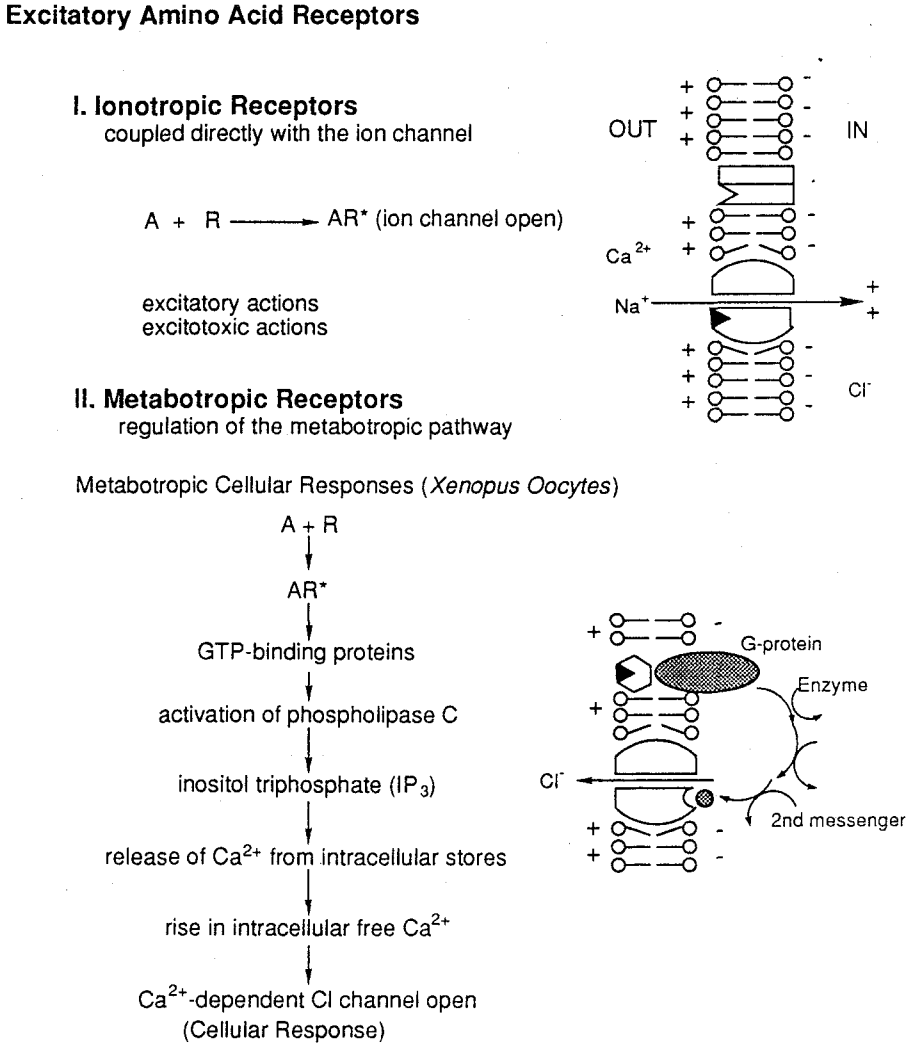
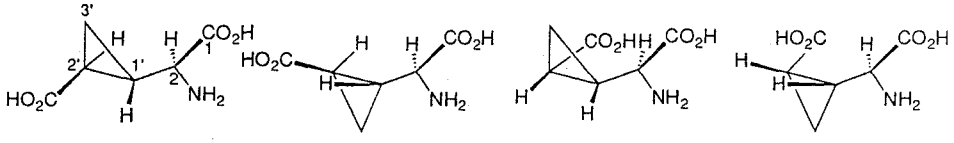


Figure 4-1

receptor has been investigated only over the last few years, its physiological role has not yet been well documented. Quisqualic acid is known to be a potent metabotropic receptor agonist, but it stimulates also the ionotropic QA receptor. Potent and selective agonists as well as antagonists are essential to investigate the metabotropic receptor.

[1] Neurobiological Activities of CCGs and The Conformation-Activity Relationships. It is assumed that each of the L-Glu receptors can recognize an optimal conformation of L-Glu. Thus, the four synthetic diastereomers of L-CCG (**1**~**4**) were subjected to neurobiological assays as the conformational variants of L-Glu. The cyclopropyl group of CCGs fixes the extended or the folded conformation of L-Glu, except for the rotamers around the α-amino acid moiety.

To elucidate the conformational requirements of L-Glu for activating its different types of receptor, depolarizing activity of CCGs was measured first, electrophysiologically, by the use of the new born rat spinal cord.[18] Each diastereomer showed various depolarizing responses (Figure 4-3). Relative potency ratios of L-isomers, **1**~**4**, and other representative excitatory amino acids were as follows: QA (300) > **4** (100) = KA (100) > NMDA (40) > **1** (6) > L-Glu (1) > **3** (0.5) > **2** (0.3). It is noted



CCG-I (1) (2 *S*,1' *S*,2' *S*) **CCG-II (2)** (2 *S*,1' *R*,2' *R*) **CCG-III (3)** (2 *S*,1' *S*,2' *R*) **CCG-IV (4)** (2 *S*,1' *R*,2' *S*)

Figure 4-2

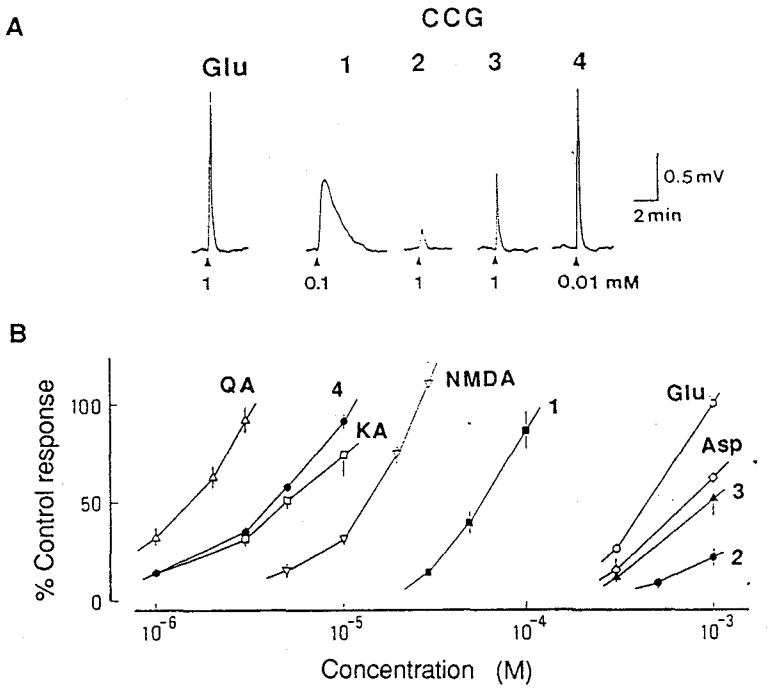


Figure 4-3

A: Sample records of responses to L-Glu and CCGs (1~4).

B: Concentration-activity relationship of excitatory amino acids and CCGs in the new born rat spinal cord. Responses from individual preparations have been normarized, so that results are expressed as a percentage of the control depolarization to 1 mM L-Glu. Vertical bars represent S.E.M. (*n*, at least 3). Responses were recorded from the vertral root extracelluraly in Mg^{2+}-free, tetrodotoxin (0.5 μM)-containing solution. All test samples were added to the bathing solution for 10 s.

that the activity of L-CCG-IV (4) was much more potent than L-Glu or NMDA and almost equal to KA but less than QA.

Examined next was the characterization of these CCGs into the receptor subtypes. The use of selective NMDA antagonists allowed the classification of the CCGs as NMDA agonists or non-NMDA agonists. D-APV and CPP are employed as competitive NMDA antagonists while Mg^{2+} is known to be an ion channel blocker (Table 4-1). They almost blocked the depolarizing actions of

the folded isomers, **3** and **4**. Accordingly, **3** and **4** were classified as NMDA agonists. On the other hand, the responses of the extended isomers (**1** and **2**) were hardly affected by these antagonists. These results suggested that NMDA receptor is activated by the folded confomer of L-Glu. In order to confirm this, the receptor binding studies in rat cerebral cortical membranes using [^{3}H]CPP[48] were undertaken. Among the CCG isomers, the folded isomer **4** showed the highest and selective affinity to the NMDA receptor. Relative potency ratios were as follows; **4** (17) > L-Glu (1) > NMDA (0.2) >> **1** (0.006) > **2**, **3** (~0). Among the known agonists and antagonists of NMDA receptor, **4** showed the highest activity. The results obtained by both electrophysiological experiments and receptor binding assay led to the conclusion that the conformational requirement of L-Glu for activating NMDA receptor is the folded form.

Moreover, L-CCG-IV (**4**) was found to be a superior NMDA agonist than NMDA itself. In fact, recent investigations demonstrated that **4**, by its interaction with NMDA receptor, induced intracellular Ca^{2+} mobilization and consequently neurotoxicity which caused cell death.*[1,50] Therefore L-CCG-IV (**4**) would provide important information not only about the conformational requirements of L-Glu for binding to the NMDA receptor but also about the physiological and pharmacological role of L-Glu such

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Footnote*1 CCG-IV (**4**) induced the increase of intracellular free Ca^{2+} concentration 20 times more potently than L-Glu and caused cell death, which was inhibited by NMDA antagonists. This study demonstrated the relation between the NMDA receptor and neurotoxicity. Since known NMDA agonists including NMDA induced only weak effects, the relationship has been examined only by the use of antagonists. This is the first example using NMDA agonist.

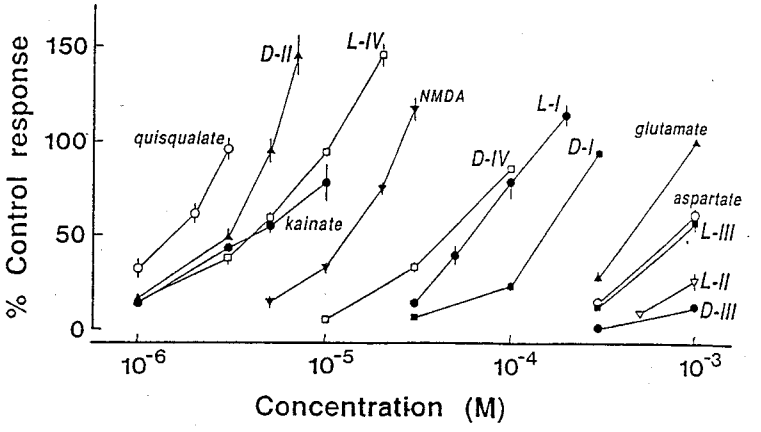

Figure 4-4

The concentration-response curves of eight CCG isomers and representative excitatory amino acids. Percentage of the control depolarization to 1 mM L-Glu are plotted against the concentrations (M).

as neurotoxicity and cell death.

However, the results of D-CCGs were contradictory to those of L-isomers. The relative potency ratio of **D-1**~**D-4** were as follows: **D-2** (200) > **D-4** (10) > **D-1** (3) > L-Glu (1) > **D-3** (0.1). All D-isomers were found to be NMDA agonists. Among them, the extended isomer **D-2** was the most potent NMDA agonist as understood from electrophysiological experiment. However by the binding assay to NMDA receptor, the potency of D-amino acids was much less than that by electrophysiological assay; **D-4** (1.5) > L-Glu (1) > **D-2** (0.8) > NMDA (0.2) > **D-1**, **D-3** (~0).*2,48 It is proposed that NMDA receptor has tolerance for the D-configuration of agonists[46], although the real transmitter has the L-configuration. The structural relationship between L-Glu

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Footnote*2 The binding study using [^{3}H]Glu was reported.[29a] Although the details were not discussed, this report discussed that **D-4** was about twice more potent than **4**.

and **D-2**, between L-Glu and NMDA, or between **D-2** and **D-4** cannot be found. It is difficult to explain why D-amino acids show such potent and selective NMDA type depolarizing action. The mechanism of activation by D-amino acids remains to be solved.

On the other hand, attempts to classify the activity of **1** and **2**, whose responses were hardly affected by the NMDA antagonists, were further examined using the non-NMDA (KA and/or QA) type antagonist, CNQX. Surprisingly, their responses were not blocked by CNQX and remained unchanged. These results suggested either the presence of a new type of ionotropic receptor or an activation of the metabotropic L-Glu receptor, because both **1** and **2** were classified as neither the NMDA type nor non-NMDA type.

Recently, Shinozaki has demonstrated that the extended isomer **1** is a selective agonist of the metabotropic receptor.[37] This was ascertained by the following experiments: (1) The depolarizing action of **1** was markedly reduced by lowering the temperature. Because the metabotropic pathway involves the enzyme reaction, the response to the activation of the metabotropic receptor would be expected to depend on the temperature. (2) The extended isomer **1** also induced oscillatory response in *Xenopus oocytes* injected with rat brain mRNA. This response is characteristic for the activation of the metabotropic receptor. It has been shown that the oscillatory responses are due to the increase of chloride conductance as a consequence of the stimulation of metabotropic pathway.[11]

In addition, Nakagawa has reported that the IP_3 metabolism was enhanced by **1**.[51] Thus, **1** being a metabotropic receptor agonist was further confirmed by a biochemical method. Only *trans-*

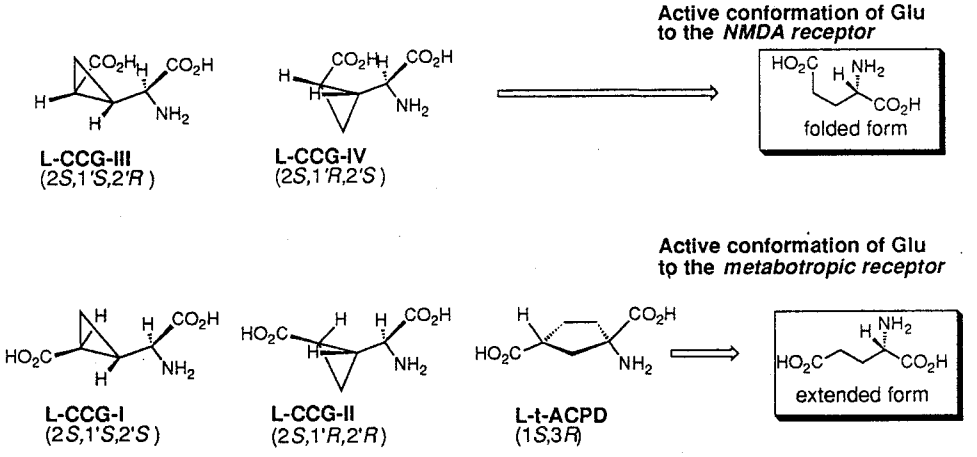


Figure 4-5

dl-1-amino-1,3-cyclopentanedicarboxylic acid (*t*-ACPD) has been introduced as a selective agonist for this receptor.[12] Although the potency of **1** was much less than QA [electrophysiological assay; QA (300) >> **1** (6) > *t*-ACPD (2) > L-Glu: enhancement of IP_3 metabolism; QA (350) >> **1** (5) > *t*-ACPD = L-Glu (1)], **1** showed the most potent effects as a selective agonist of the metabotropic receptor. Since QA is an agonist of both the ionotropic and metabotropic receptors, the extended isomer **1** is expected to be a useful tool to examine the physiological role of the metabotropic receptor. The fact that the extended isomer **1** showed selective activity in this receptor strongly suggested that the extended conformer of L-Glu is responsible for the activation of the metabotropic receptor.

Although a selective metabotropic receptor agonist *t*-ACPD has been used in a racemic form, (*1S,3S*)-ACPD has recently proven to be an active enantiomer.[52] This is a flexible molecule and can adopt different conformers. One of its conformers

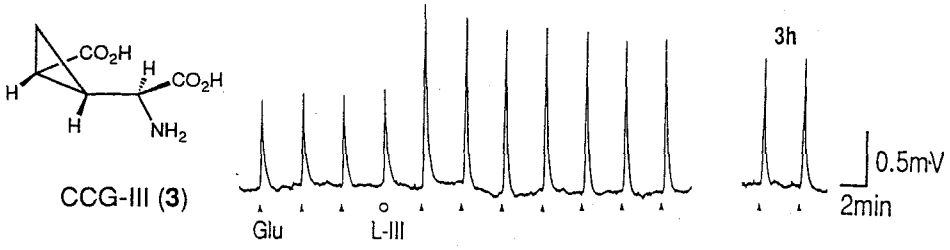


Figure 4-6

The potentiation of the L-Glu responses induced by CCG-III (**3**). L-Glu was added to the bathing solution for a period 10 s in a concentration of 1 mM. CCG-III (**3**) was added for 15 s in a concentration of 1 mM.

showed good agreement with the extended form of L-Glu (Figure 4-5).

Besides potent depolarizing activity induced by CCG-I (**1**) and IV (**4**), marked potentiation of L-Glu response was observed by one of the folded isomers, **3**, although its depolarizing activity was less than L-Glu.[18] This effect lasted for several hours. This observation was temporarily explained as the inhibition of L-Glu uptake in the synaptic environment. Binding assay of **3** inhibited D-Asp uptake, which supported the explanation that CCG-III (**3**) is a potent uptake inhibitor.[48,53] The inhibition of L-Glu uptake from the synaptic environment, inevitably, causes an increase of L-Glu concentration, which results in excess stimulation of its receptor. Therefore, this experimental observation may provide important information about neurotoxicity of L-Glu as a result of an increase of intracellular Ca^{2+} concentration, as well as long term potentiation (LTP)[1c,54] which is assumed to be a model of the molecular mechanism of memory and learning in mammalian brain.

As mentioned above, each isomer of CCG showed the characteristic activity summerized in Table 4-2. The crucial role of

Table 4-2. Neurobiological Activities of CCGs

CCG	Depolarizing Activity[a] (L-Glu = 1)	Binding Affinity[b] (L-Glu = 1)	Receptor Subtype	
extended				
CCG-I (1)	6	0.006	Metabotropic	L-Glu < t-ACPD < **1** <QA Selective agonist
CCG-II (2)	0.3	~0	Metabotropic	
folded				
CCG-III (3)	0.5	~0	Ionotropic (mainly NMDA type)	Potentiation of L-Glu response (Potent inhibitor of L-Glu uptake)
CCG-IV (4)	100	17	Ionotropic (NMDA type)	L-Glu < NMDA <**4** Potent agonist
extended				
D-CCG-I (D-1)	3	~0	Ionotropic (NMDA type)	
D-CCG-II (D-2)	200	0.8	Ionotropic (NMDA type)	L-Glu < NMDA <**4** < **D-2** Potent agonist (depolarizing action)
folded				
D-CCG-III (D-3)	0.1	~0	Ionotropic (NMDA type)	
D-CCG-IV (D-4)	10	1.5	Ionotropic (NMDA type)	

[a] In the rat spinal cord.
[b] Binding affinity to NMDA receptor in the rat cerebral cortex. ([^{3}H]CPP)

the optimal conformation of L-Glu for activating its receptors became obvious using CCG isomers as conformationally restricted analogues of L-Glu.

[2] Effect of the Cyclopropyl Ring. Next, the steric effect of the cyclopropane ring of CCGs was analyzed. The activities (depolarizing action, binding affinity, and potentiation) between the extended isomers [CCG-I (**1**) and II (**2**)], and between the folded isomers [CCG-III (**3**) and IV (**4**)] were much different from each other. Both **1** and **2** activate the same metabotropic receptor but the response of **1** was 10 times more potent than that of **2**. Similarly, both **3** and **4** activate the NMDA

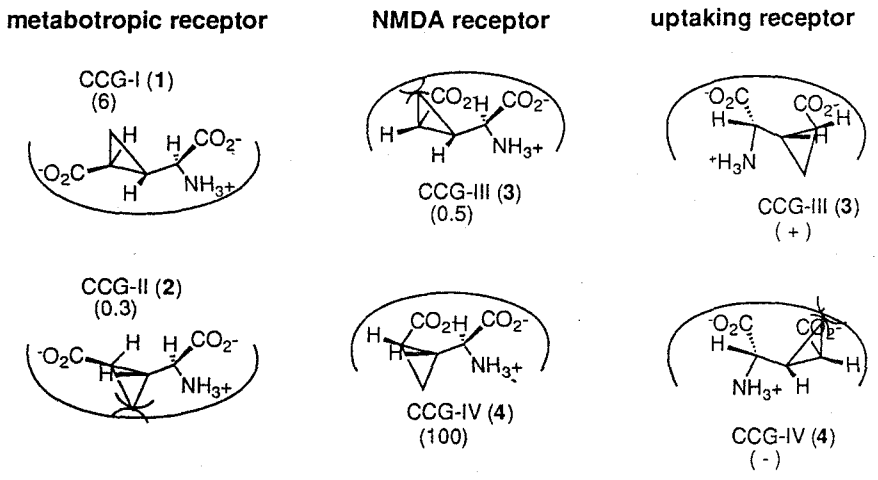
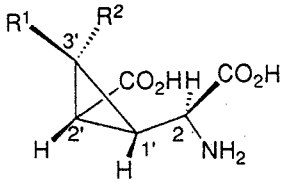
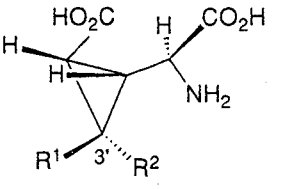


Figure 4-7

receptor but the response of **4** was 200 times more potent than that of **3**. These results suggest that the cyclopropane ring of **2** and **3** might act as a steric hindrance at the receptor binding site, while that of **1** and **4** might occupy an open space on the receptor surface. In contrast, the cyclopropane ring of **4** might be rejected from the uptake receptor, because **3** inhibited L-Glu uptake, while **4** did not show such effect.

[3] Biological Activities of the 3'-Substituted Derivatives of CCG-III and IV. The fact that CCG-III (**3**) and IV (**4**), both of which mimic the folded form of L-Glu, showed quite different and important activities initiated further interest regarding the conformational as well as configurational requirements of L-Glu for activating its receptors. Therefore, 3'-substituted analogues of CCG-III and IV (**24~27**) were designed. It was expected that these analogues would provide information to the following issues;





***t*-MCG-III (24)** $R^1 = CH_2OCH_3$, $R^2 = H$ ***t*-MCG-IV (26)** $R^1 = CH_2OCH_3$, $R^2 = H$
***c*-MCG-III (25)** $R^1 = H$, $R^2 = CH_2OCH_3$ ***c*-MCG-IV (27)** $R^1 = H$, $R^2 = CH_2OCH_3$

Figure 4-8

(1) Verification of the conformational demand upon L-Glu, especially, finding information about the optimal rotamer of α-amino acid moiety; the cis substituent on C-3' was expected to restrict even more the free rotation of the α-amino acid moiety.

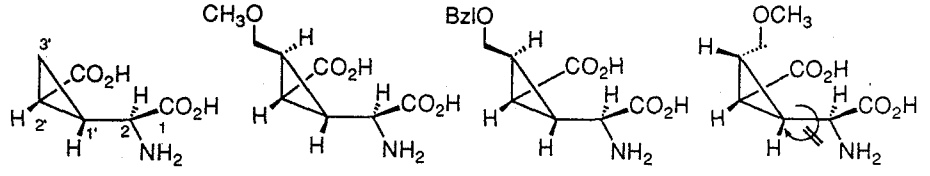
(2) Investigation of the conformational requirements for L-Glu to activate the non-NMDA receptors; although both the NMDA and non-NMDA receptors couple with ion channel, rational explanation for the non-NMDA receptor could not be obtained by the use of four CCG isomers.

(3) Elucidation of the steric role of the cyclopropane ring; either the trans or the cis substituent on C-3' was expected to add an extra bulkiness at the receptor surface. The cyclopropane ring of active species was postulated to occupy an open space of the receptor. The MCG analogues may confirm this speculation.

(4) Examination of the synthesis of polymer bounded CCG analogues (for affinity chromatography); if the 3'-substituted analogues retain the activity, the CCG analogues, bounded to a spacer group of resin, would make it possible to isolate the receptor protein.

(5) Examination of the possibility of the synthesis of CCG analogues with photoaffinity label; the labelled CCG analogues would contribute to the isolation of the receptor protein and determination of the binding site of L-Glu on the receptor protein.

As described in Chapter 3, C-3' methoxymethyl and benzyloxymethyl derivatives of CCG-III and IV, MCGs (**24**~**27**) and BCGs



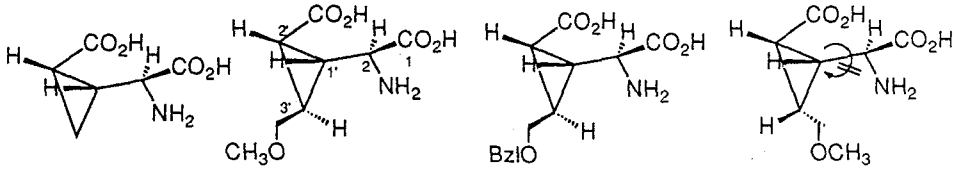
CCG-III (3) | ***t*-MCG-III (24)** | ***t*-BCG-III (41)** | ***c*-MCG-III (25)**

Depolarizing Activity*

(0.3; NMDA-type) | (0.1) | (0.1) | (-)

Potentoation of Glu Response

(+) | (-) | (-) | (-)



CCG-IV (4) | ***t*-MCG-IV (26)** | ***t*-BCG-IV (42)** | ***c*-MCG-IV (27)**

Depolarizing Activity*

(100) NMDA-type | (50) Non-NMDA-type (KA or QA) | (5) Non-NMDA-type (KA or QA) | (20) NMDA-type

* Depolarizing activity of Glu = (1)

Figure 4-9

(**41** and **42**) were synthesized. The depolarizing activities of these MCGs and BCGs were measured in the new born rat spinal cord[19]. Relative potency ratios of the deporlarizing action were as follows; L-CCG-IV **4** (100) > *t*-MCG-IV **26** (50) > *c*-MCG-IV **27** (25) > *t*-BCG-IV **42** (5) > L-Glu (1) > CCG-III **3** (0.3) > *t*-MCG-III **25**, *t*-BCG-III **41** (<0.1) > *c*-MCG-III (**25**) (0) (Figure 4-9).

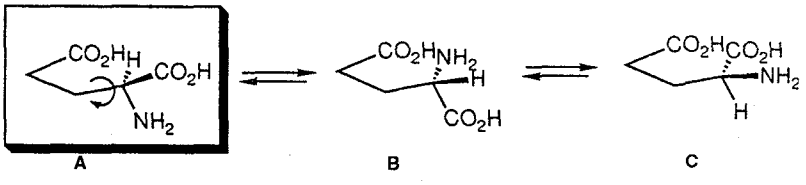
In the case of CCG-III type isomers, both the trans isomers (**24** and **41**) and cis isomer (**25**) showed only a weak depolarizing action. This may be due to the steric repulsion between the substituents on cyclopropane ring and receptor surface. The enhanced steric bulkiness of these compounds compared to that of CCG-III (**3**) reduced clearly their activities. This result supported the speculation that the cyclopropane ring of CCG-III (**3**) was rejected by the receptor for steric reasons.

Unlike CCG-III (**3**), *t*-MCG-III (**25**) and *t*-BCG-III (**41**) did not show any potentiation of the response of L-Glu. The complete loss of the potentiation activity may be the result of the steric repulsion between the 3'-substituent and the receptor. Only the cyclopropane ring without 3'-substituent may be responsible for the uptake of L-Glu in the synaptic environment.

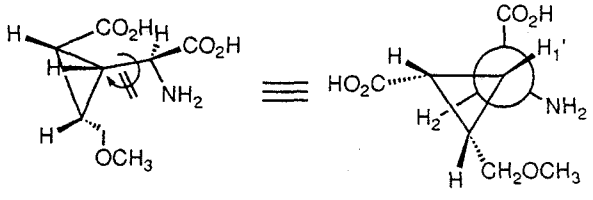
On the other hand, all 3'-substituted derivatives of CCG-IV (**26**, **27**, and **42**) showed potent depolarizing action. The activities of these analogues were about 5~50 times greater than that of L-Glu. Among them, only the response of the cis type compound, *c*-MCG-IV (**27**), was blocked by an NMDA antagonist. Thus **27** was found to be an NMDA type agonist similar to its

parent compound, CCG-IV (**4**). This diastereomer was designed as an analogue of L-Glu with restricted free rotation of the α-amino acid moiety as a consequence of the presence of the C-3'-cis-substituent (Figure 4-10). The large J value of H_2 ($J_{2-1'}$ = 11.5 Hz) of **27** suggested that the vicinal dihedral angle (H2-C2-C1'-H1') was almost 180° (antiperiplanar). The conformation of *c*-MCG-IV (**27**) at ambient temperature in an aqueous solution would be the conformer **A** as shown in Figure 4-10. Since **27** activated the NMDA receptor much more potently than L-Glu, it is concluded that the active conformer of L-Glu, when it interacts with the NMDA receptor, could be shown in Figure 4-10 including α-amino acid moiety.

Contrary to the effect induced by the cis isomer **27** and the parent compound CCG-IV (**4**), even though the depolarizing action of the trans isomers (**26** and **42**) could not be inhibited



The folded conformers of L-Glu



c-MCG-IV (27)

$J_{2-1'}$ = 11.5 Hz

Figure 4-10

by an NMDA antagonist, they were, surprisingly, completely blocked by a non-NMDA antagonist (CNQX). These results indicate that the action of the trans isomers are non-NMDA (KA or QA) type. Accordingly, the different types of receptor were recognized as a function of the configuration at C-3'-position. There is no antagonist which is capable of distinguishing either KA type or QA type substrate. Therefore, it could not be concluded as to which type of receptor was activated by *t*-MCG-IV (**26**) and *t*-BCG-IV (**42**). It is known, however, that the immature rat isolated dosal roots have selective affinity to KA and its analogous kainoid compounds, such as domoic acid and acromelic acid.*[3,55] In this system, QA and AMPA (a selective agonist of the ionotropic QA receptor) showed only weak depolarizing effect[56]. Thus, the activity of **26** and **42** were examined by the use of the above assay system. The action of these compounds were more potent than L-Glu; domoic acid (300) >> KA (9) >*t*-BCG-IV **42** (3.5) > *t*-MCG-IV **26** (2.5) > L-Glu (1) > CCG-IV **4** (0.4) > QA (0.3) > AMPA (0.1) > *c*-MCG-IV **27** > NMDA. These results strongly suggested that these compounds are KA-type agonists which do not have a kainoid skeleton. Only the kainoids were so far believed to activate the KA receptor. Thus, the trans substituted CCG derivatives (**26** and **42**) are the novel compounds classified as agonists of the KA receptor.

Due to the conformational flexibility of L-Glu substructure in KA, optimal conformation of L-Glu for activating KA receptor could not be deduced in spite of the precise ^{1}H-NMR ex-

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Footnote*3 "Kainoid compound" is the general term for the compound which has kainic acid substructure, such as domoic acid and acromelic acid (see Table 4-1).

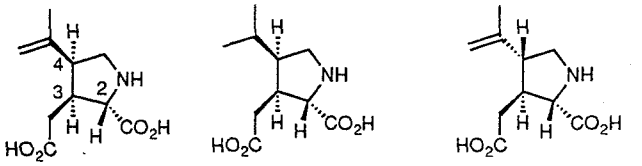


Figure 4-11

periments. On the other hand, effects of the C-4 substituents were well examined.[55] The presence of the C-C double bond of the isopropenyl group and C-4 configuration on the pyrrolidine ring are crucial, because the saturated analogue (dihydrokainic acid) and C-4 epimer (allokainic acid) were completely inacitve (Figure 4-11). The fact that *t*-MCG-IV **26** and *t*-BCG-IV **42** activate KA receptor initiated me to examine the structure-activity relationship between KA, **26**, and **42**.

Inspection of molecular models revealed that not only the L-Glu substructure of **26** and **42** were superimposable with that of one of the conformer of KA, but also that the 3'*R* substituent of **26** and **42** could occupy the same space as the isopropenyl group of KA as shown in Figure 4-12. These results led me to believe that both of the following factors are necessary for

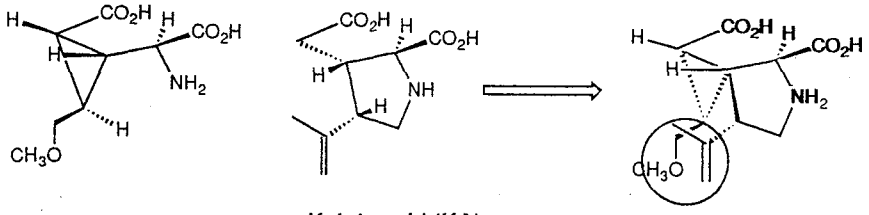


Figure 4-12

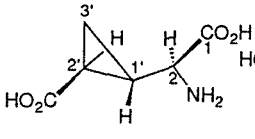
the activation of the KA receptor; (1) the folded confomer of L-Glu and (2) the existence of a three dimensional trigger such as methoxymethyl or isopropenyl group. It is interesting that both NMDA and KA type receptors were activated by the folded isomers of CCG and MCG. The conformational similarity of L-Glu (folded form) might be general feature for activating the ionotropic receptors.

Although the isopropenyl group of KA and methoxymethyl group of *t*-MCG-IV (**27**) were assumed as a trigger for the activation of KA receptor, it was not concluded whether these group act as a steric factor (occupation of the space) or stereoelectronic factor (affinity of the receptor with π-electron of isopropenyl group, or electronegative oxygen atom). Despite the fact that L-Glu has no substituent as a trigger, it can activate the KA receptor. Some other co-factor, *e.g.* another amino acid, would be needed together with L-Glu for the activation of the KA receptor. In order to determine the real function of the trigger, the effects of other CCG-IV derivatives possessing different C-3'substituents such as carboxyl, alkyl, and alkenyl groups should be examined. The hydroxyl compound **35b** can be used as the common intermediate for the syntheses of these analogues. Nevertheless the results obtained herein must give clues for the design of more effective agonists or antagonists for the KA receptor.

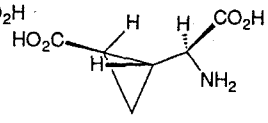
As mentioned in this chapter, eight isomers of CCG and four isomers of MCG have provided reasonable answers for elucidating the conformational requirements of L-Glu for activating its receptors.

Conclusion

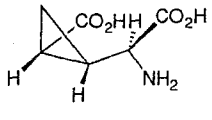
In order to elucidate the conformational requirements of L-Glu for activating its receptors, four diastereomeric L-2-(carboxycyclopropyl)glycines (**1**~**4**) were designed as conformationally restricted analogues of L-Glu. Four diastereomers of L-CCGs as well as four D-isomers were synthesized in an efficient manner using the following reactions assisted by a palladium catalyst; (i) cycloaddition of the (2*S*)-2-amino-3-butenol derivative (**5b**) with ethyl diazoacetate led to the successful synthesis of all isomers of CCG (**1**~**4**), (ii) cycloaddition of (*E*)-olefins with diazomethane gave CCG-I (**1**) and II (**2**), (iii) intramolecular cycloaddition of the (2*S*)-2-*N*-diazoacetylamino-3-butenol derivative **13** afforded CCG-III (**3**), (iv) cyclopropanation of α,β-unsaturated γ-lactam **20** and δ-lactone **23** with diazomethane provided efficient and stereoselective routes for the synthesis of CCG-III (**3**) and IV (**4**), respectively. Thus, it be-



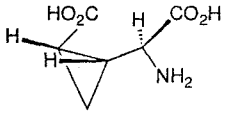
CCG-I (1)
(2 *S*,1' *S*,2' *S*)



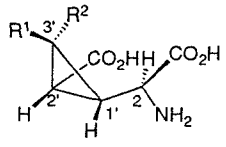
CCG-II (2)
(2 *S*,1' *R*,2' *R*)

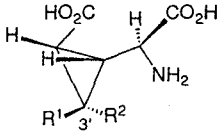


CCG-III (3)
(2 *S*,1' *S*,2' *R*)



CCG-IV (4)
(2 *S*,1' *R*,2' *S*)





***t*-MCG-III (24)** $R^1 = CH_2OCH_3$, $R^2 = H$ ***t*-MCG-IV (26)** $R^1 = CH_2OCH_3$, $R^2 = H$
***c*-MCG-III (25)** $R^1 = H$, $R^2 = CH_2OCH_3$ ***c*-MCG-IV (27)** $R^1 = H$, $R^2 = CH_2OCH_3$

came possible to synthesize all the isomers or each isomer of CCG by using the above synthetic methods. The syntheses of the 3'-substituted analogues of CCG-III and IV (MCGs; **24~27**) were also performed via an intramolecular cycloaddition reaction as the key step.

The neurobiological actions of these CCGs and MCGs were examined using the electrophysiological study and the receptor binding assay. These studies supported, in a precise manner, the hypothesis that the conformation of L-Glu plays a crucial role for activating its receptor subtypes. The extended conformer of L-Glu activates the metabotropic receptor because the extended diastereomer, CCG-I (**1**), was a potent and selective agonist of the metabotropic receptor. On the other hand, the folded confomer of L-Glu activates NMDA and KA receptors (ionotropic receptors) because the folded diastereomer, CCG-IV (**4**), and its derivative *c*-MCG-IV (**27**) were potent agonists of NMDA receptor while *t*-MCG-IV (**26**) was an agonist of KA receptor. Moreover, CCG-III (**3**) was found to be a potent inhibitor of L-Glu uptake.

The various physiological functions of L-Glu were investigated using these compounds as powerful pharmacological tools. These results would initiate the work to elucidate the molecular mechanism of the neurotransmission; for example, the binding site of the receptor protein, the mode of action of the receptor and the ion channel protein, the regulation mechanism of the release and the inactivation of L-Glu, etc. Utilizing affinity column chromatography, photolabelled compounds, and radioisotope labelled compounds would contribute to these issues.

Furthermore, the analogues synthesized in the present work are expected to play a role as a probe for designing effective agonists and antagonists, which are required in view of not only the tools for the investigation on the neurotransmission but also chemotherapy for brain diseases.

Experimental Section

Melting points are uncorrected. ^1H NMR spectra were recorded on one of the following instruments: JEOL FX 100, GE GN-300, 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 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 on 2 x 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. Medium-pressure liquid chromatography (MPLC) was carried out by LiChroprep Si 60 Lobar column sizes A, B, and C (Merck). Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials unless otherwise stated.

Chapter 1

(2*S*)-*N*-*tert*-Butoxycarbonyl-2-(ethoxycarbonylcyclopropyl)glycinol (6a-9a) and (1*S*, 5*S*, 6*R*)-5-*N*-(*tert*-butoxycarbonyl)amino-3-oxobicyclo[3.1.0]heptan-2-one (9d). To a solution of $Pd(OAc)_2$ (168 mg, 0.75 mmol) and **5b** (4.34 g, 15 mmol) in Et_2O (150 mL) at room temperature was simultaneously added, drop by drop, a solution of ethyl diazoacetate (17.1 g, 150 mmol) in Et_2O (300 mL) and a solution of $Pd(OAc)_2$ (168 mg, 0.75 mmol) in Et_2O (200 mL) over 3 h. The mixture was filtered. The filtrate was concentrated *in vacuo* to give a crude oil, which, upon column chromatography on silica gel (Et_2O/hexane, 1 : 4), gave a mixture of the four diastereomers **6b-9b** as a colorless oil (4.00 g, 88%). The mixture was dissolved in EtOH (100 mL) and was treated with *dl*-10-camphorsulfonic acid (CSA) (5 mg) at room temperature for 16 h. Then the solvent was evaporated under reduced pressure. The residue was purified by medium-pressure column chromatography on silica gel (Et_2O/hexane, 3 : 1) to give (2*S*,1'*R*,2'*R*)-**7a** (932 mg), (2*S*,1'*S*,2'*R*)-**8a** (175 mg), and a mixture of (2*S*,1'*S*,2'*S*)-**6a** and (2*S*,1'*R*,2'*S*)-**9a** (363 mg), along with a mixture of **6a-9a** (197 mg). The mixture of **6a** and **9a** was treated with CSA (5 mg) in CH_2Cl_2 (10 mL) at room temperature for 18 h. The mixture was then washed with aqueous $NaHCO_3$. The organic phase was dried and the solvent was evaporated *in vacuo*

to give an oily residue. This was purified by medium-pressure column chromatography on silica gel (Et_2O/hexane, 3 : 1) to give unchanged **6a** (190 mg) as a colorless oil and (1*S*,5*S*,6*R*)-δ-lactone **9d** (80 mg) as colorless crystals: **(2*S*,1'*S*,2'*S*)-6a**: Oil; $[\alpha]^{25}_D$ +72.9° (*c* 0.50, $CHCl_3$); IR (neat) 3372, 2984, 1712 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.89 (ddd, 1 H, *J* = 4.5, 6.5, 9.0 Hz), 1.20 (ddd, 1 H, *J* = 4.5, 4.5, 9.0 Hz), 1.25 (t, 3 H, *J* = 7.0 Hz), 1.45 (s, 9 H), 1.55 (dddd, 1 H, *J* = 4.5, 6.5, 9.0, 9.0 Hz), 1.76 (ddd, 1 H, *J* = 4.5, 4.5, 9.0 Hz), 2.40 (br s, 1 H), 3.15 (m, 1 H), 3.68 (m, 1 H), 3.76 (m, 1 H), 4.118 (dq, 1 H, *J* = 7.0, 11.0 Hz), 4.120 (dq, 1 H, *J* = 7.0, 11.0 Hz); MS (SIMS) *m/z* 274 $(M+H)^+$, 218, 174. Anal. Calcd for $C_{13}H_{23}O_5N$: C, 57.13; H, 8.48; N, 5.12. Found: C, 56.70; H, 8.71; N, 5.02. **(2*S*,1'*R*,2'*R*)-7a**: Mp 88.0-89.0 °C; $[\alpha]^{23}_D$ -47.2° (*c* 0.55, $CHCl_3$); IR (neat) 3460, 3028, 1712 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.02 (ddd, 1 H, *J* = 4.5, 6.5, 8.5 Hz), 1.18 (ddd, 1 H, *J* = 4.0, 4.5, 9.0 Hz), 1.26 (t, 3 H, *J* = 7.0 Hz), 1.44 (s, 9 H), 1.54 (dddd, 1 H, *J* = 4.0, 4.0, 6.5, 8.5 Hz), 1.59 (ddd, 1 H, *J* = 4.0, 6.5, 9.0 Hz), 2.35 (br s, 1 H), 3.22 (dddd, 1 H, *J* = 4.0, 5.0, 6.5, 8.5 Hz), 3.68 (ddd, 1 H, *J* = 5.0, 5.0, 10.5 Hz), 3.76 (ddd, 1 H, *J* = 4.0, 6.5, 10.5 Hz), 4.115 (dq, 1 H, *J* = 7.0, 11.0 Hz), 4.120 (dq, 1 H, *J* = 7.0, 11.0 Hz); MS (SIMS) *m/z* 274 $(M+H)^+$, 218, 174. Anal. Calcd for $C_{13}H_{23}O_5N$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.19; H, 8.46; N, 5.12. **(2*S*,1'*S*,2'*R*)-8a**: Mp 94.0-95.0 °C; $[\alpha]^{23}_D$ -56.0° (*c* 0.48, $CHCl_3$); IR (neat) 3392, 2984, 1716 cm^{-1}; ^1H NMR

($CDCl_3$, 360 MHz) δ 1.13 (ddd, 1 H, *J* = 5.0, 8.0, 8.5 Hz), 1.18 (ddd, 1 H, *J* = 5.0, 6.0, 7.0 Hz), 1.27 (t, 3 H, *J* = 7.0 Hz), 1.42 (s, 9 H), 1.53 (dddd, 1 H, *J* = 7.0, 7.0, 8.0, 8.5 Hz), 1.78 (ddd, 1 H, *J* = 6.0, 8.5, 8.5 Hz), 3.02 (br s, 1 H), 3.61 (dddd, 1 H, *J* = 3.5, 6.0, 7.0, 10.5 Hz), 3.70 (br m, 1 H), 3.84 (br m, 1 H), 4.13 (dq, 1 H, *J* = 7.0, 11.0 Hz), 4.17 (dq, 1 H, *J* = 7.0, 11.0 Hz); MS (SIMS) *m/z* 274 $(M+H)^+$, 218, 174. Anal. Calcd for $C_{13}H_{23}O_5N$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.03; H, 8.45; N, 5.09. **(1*S*, 5*S*, 6*R*)-δ-Lactone (9d)**: Mp 152.0-154.0 °C; $[\alpha]^{23}_D$ -59.4° (*c* 0.46, $CHCl_3$); IR (neat) 3328, 2984, 1734, 1710 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.29 (ddd, 1 H, *J* = 6.5, 8.0, 9.5 Hz), 1.38 (ddd, 1 H, *J* = 5.0, 5.0, 6.5 Hz), 1.45 (s, 9 H), 1.88 (dddd, 1 H, *J* = 3.0, 5.0, 7.5, 8.0 Hz), 1.96 (ddd, 1 H, *J* = 5.0, 7.5, 9.5 Hz), 4.12 (dd, 1 H, *J* = 2.5, 13.0 Hz), 4.21 (dddd, 1 H, *J* = 1.5, 2.5, 3.0, 7.0 Hz), 4.28 (dd, 1 H, *J* = 1.5, 13.0 Hz), 4.95 (br d, 1 H, *J* = 7.0 Hz); MS (SIMS) *m/z* 228 $(M+H)^+$, 172, 128. Anal. Calcd for $C_{11}H_{17}O_4N$: C, 58.14; H, 7.54; N, 6.16. Found: C, 58.08; H, 7.68; N, 6.05.

(2*S*,1'*R*,2'*S*)-*N*-*tert*-Butoxycarbonyl-2-(ethoxycarbonylcyclopropyl)glycinol (9a). A mixture of **9d** (514 mg, 2.26 mmol), K_2CO_3 (20 mg), and EtOH (30 mL) was stirred at room temperature for 24 h. The mixture was then extracted with EtOAc several times. The combined extracts were washed with water, dried ($MgSO_4$) and concentrated *in vacuo*. The oily residue was purified by column

chromatography on silica gel (Et_2O/hexane, 3 : 1) to give **9a** as colorless crystals (540 mg, 87%): mp 109.0-110.5 °C; $[\alpha]^{23}_D$ -49.3° (*c* 1.04, $CHCl_3$); IR (neat) 3380, 2984, 2940, 1726 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.10 (ddd, 1 H, *J* = 5.0, 8.0, 8.0 Hz), 1.24 (m, 1 H), 1.27 (t, 3 H, *J* = 7.0 Hz), 1.47 (s, 9 H), 1.50 (m, 1 H), 1.80 (ddd, 1 H, *J* = 5.5, 8.0, 9.0 Hz), 2.85 (br s, 1 H), 3.56 (m, 1 H), 3.67 (m, 2 H), 4.15 (q, 2 H, *J* = 7.0); MS (SIMS) *m/z* 274 $(M+H)^+$, 218, 174. Anal. Calcd for $C_{13}H_{23}O_5N$: C, 57.13; H, 8.48; N, 5.12. Found: C, 56.97; H, 8.51; N, 5.03.

***(1R, 5S, 6S)*-5-(*N*-*tert*-Butoxycarbonyl)amino-3-oxo-bicyclo[3.1.0]heptan-2-one (8d).** A solution of **8a** (100 mg, 0.37 mmol) and CSA (5 mg) in CH_2Cl_2 (5 mL) was stirred at room temperature for 24 h. The mixture was then washed with aqueous $NaHCO_3$. The organic phase was dried and the solvent was evaporated *in vacuo* to give an oily residue. This was purified by column chromatography on silica gel (Et_2O/hexane, 3 : 1) to give **8d** (58 mg, 69%) as colorless crystals: mp 123.0-125.0 °C; $[\alpha]^{23}_D$ +70.4° (*c* 0.68, $CHCl_3$); IR (neat) 3348, 2984, 2940, 1714 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.17 (ddd, 1 H, *J* = 6.0, 7.5, 9.5 Hz), 1.45 (s, 9 H), 1.50 (m, 1 H), 1.90 (m, 1 H), 2.02 (ddd, 1 H, *J* = 4.5, 8.0, 9.5 Hz), 3.64 (t, 1 H, *J* = 11.5 Hz), 4.27 (dd, 1 H, *J* = 3.5, 11.5 Hz), 4.43 (m, 2 H), 4.49 (m, 1 H); MS (SIMS) *m/z* 228 $(M+H)^+$, 172, 128. Anal. Calcd for $C_{11}H_{17}O_4N$: C, 58.14; H, 7.54; N, 6.16. Found: C, 57.75; H, 7.52; N, 6.09.

***(2S, 1'S, 2'S)*-2-(Carboxycyclopropyl)glycine**

(1: CCG-I). To a solution of **6a** (1.00 g, 3.66 mmol) in acetone (10 mL) at 0 °C was added a 1.5-fold excess of Jones reagent drop-by-drop over 5 min. The mixture was stirred at 0 °C for 3 h and then at room temperature for 1.5 h. The excess Jones reagent was decomposed with 2-propanol. The mixture was then extracted with EtOAc several times. The combined extracts were washed with water, dried ($MgSO_4$), and concentrated *in vacuo*. The residual oil was dissolved in Et_2O and was treated with an Et_2O solution of CH_2N_2. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (Et_2O/hexane, 3 : 1). A solution of the crude product and 1 *M* aqueous NaOH (9 mL, 9 mmol) in MeOH (5 mL) was stirred at 0 °C for 19 h. The mixture was then acidified to pH 2 with 1 *M* aqueous HCl, saturated with solid NaCl, and extracted with EtOAc several times. The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo* to give an oily residue. To a solution of the residue (crude *N*-*t*-Boc **1**) in CH_2Cl_2 (2 mL) at 0 °C was added TFA (2 mL). The mixture was stirred for 30 min at room temperature, then was concentrated *in vacuo*. The residue was passed through a column of Dowex 50Wx4 (100-200 mesh) ion exchange resin (H_2O, then 1 *M* aqueous NH_3) to give a solution of the ammonium salt of **1**. The eluate was concentrated *in vacuo* and then was dissolved in water (2 mL). The pH of the solution was adjusted to 3 with 1 *M* aqueous HCl. The crystals that precipitated from the solution were collected by filtration. They were recrystallized

from water to give **1** (228 mg, 39% from **6a**) as colorless crystals: mp 243-247 °C (decomp); $[\alpha]^{21}_{D}$ +102.0° (*c* 0.50, H_2O); IR (KBr) 2935, 1688, 1625, 1586 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.23 (ddd, 1 H, *J* = 5.1, 6.2, 8.7 Hz), 1.32 (ddd, 1 H, *J* = 5.0, 5.1, 9.1 Hz), 1.68 (dddd, 1 H, *J* = 4.1, 6.2, 9.1, 10.2 Hz), 1.76 (ddd, 1 H, *J* = 4.1, 5.0, 8.7 Hz), 3.23 (d, 1 H, *J* = 10.2 Hz); MS (SIMS) *m/z* 160 $(M+H)^+$, 142, 115. Anal. Calcd for $C_6H_9O_4N$: C, 45.28; H, 5.70; N, 8.80. Found: C, 45.10; H, 5.76; N, 8.65.

(2*S*,1'*R*,2'*R*)-2-(Carboxycyclopropyl)glycine (2: CCG-II). In a manner similar to that used to prepare **1**, **2** (75.2 mg, 65%) was prepared from **7a** (200 mg, 0.73 mmol). **2**: Colorless crystals; mp 255-258 °C (decomp); $[\alpha]^{25}_{D}$ -20.2° (*c* 0.51, H_2O); IR (KBr) 3162, 1695, 1628, 1575 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.10 (ddd, 1 H, *J* = 5.0, 6.5, 9.0 Hz), 1.25 (ddd, 1 H, *J* = 5.0, 5.0, 9.0 Hz), 1.72 (dddd, 1 H, *J* = 4.0, 6.5, 9.0, 9.0 Hz), 1.84 (ddd, 1 H, *J* = 4.0, 5.0, 9.0 Hz), 3.39 (d, 1 H, *J* = 9.0 Hz); MS (SIMS) *m/z* 160 $(M+H)^+$, 115. Anal. Calcd for $C_6H_9O_4N$: C, 45.28; H, 5.70; N, 8.80. Found: C, 45.21; H, 5.69; N, 8.71.

(2*S*,1'*S*,2'*R*)-2-(Carboxycyclopropyl)glycine (3: CCG-III). In a manner similar to that used to prepare **1**, **3** (16.1 mg, 27%) was prepared from **8a** (100 mg, 0.37 mmol). **3**: Colorless crystals; mp 192-197 °C (decomp); $[\alpha]^{22}_{D}$ +20.8° (*c* 0.52, H_2O); IR (KBr) 3141, 1700, 1615, 1578 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.26 (ddd, 1 H, *J* = 5.0, 6.0, 7.0 Hz), 1.39 (ddd, 1 H, *J* = 5.0, 9.0, 9.0 Hz), 1.61 (dddd, 1 H, *J* = 7.0, 8.0, 9.0, 11.0 Hz), 1.86 (ddd, 1 H,

J = 6.0, 8.0, 9.0 Hz), 3.89 (d, 1 H, J = 11.0 Hz); MS (SIMS) m/z 160 $(M+H)^+$, 115. Anal. Calcd for $C_6H_9O_4N$: C, 45.28; H, 5.70; N, 8.80. Found: C, 45.19; H, 5.58; N, 8.79.

(2*S*,1'*R*,2'*S*)-2-(Carboxycyclopropyl)glycine (4: CCG-IV). In a manner similar to that used to prepare **1**, **4** (159 mg, 61%) was prepared from **9a** (450 mg, 1.65 mmol). **4**: Colorless crystals; mp 178-180 °C; $[\alpha]^{26}_D$ +103.4° (*c* 0.50, H_2O); IR (KBr) 3372, 3170, 1712, 1630, 1560 cm^{-1}; ^1H NMR (D_2O, 360 MHz) δ 1.05 (ddd, 1 H, J = 5.0, 6.0, 7.0 Hz), 1.20 (ddd, 1 H, J = 5.0, 8.5, 8.5 Hz), 1.60 (dddd, 1 H, J = 7.0, 8.5, 9.0, 9.0 Hz), 1.93 (ddd, 1 H, J = 6.0, 8.5, 9.0 Hz), 3.89 (d, 1 H, J = 9.0 Hz); MS (SIMS) m/z 160 $(M+H)^+$, 110. Anal. Calcd for $C_6H_9O_4N$-H_2O: C, 40.68; H, 6.26; N, 7.91. Found: C, 40.53; H, 6.30; N, 7.88.

General procedure for the (R)-7644-catalyzed cycloaddition of ethyl diazoacetate. To a solution of the olefin (0.17 mmol) and (R)-7644 (7.5 mg, 0.01 mmol) in toluene (0.7 mL) was added a solution of ethyl diazoacetate (68 mg, 0.6 mmol) in toluene (2 mL). The mixture was heated at 80 °C until the solution became brown in color. Then a solution of ethyl diazoacetate (500 mg, 4.4 mmol) in toluene (15 mL) was added, drop by drop, at 45 °C over 6 h. The mixture was stirred at 45 °C for 18 h. Then it was concentrated *in vacuo* to give an oily residue. Purification of the residue by flash column chromatography on silica gel (Et_2O/hexane, 1 : 1) gave a mixture of cycloadducts. The mixture of diastereomers was

dissolved in EtOH (2 mL). The solution was treated with a catalytic amount of CSA at room temperature for 16 h. The solvent was then evaporated under reduced pressure to give a mixture of **6a**~**9a**. This was analyzed by HPLC to determine the product ratio.[14] The yields and product ratio are given in Table 1-1.

Chapter 2

General procedure for the cycloaddition of diazomethane. To a suspension of the olefin (0.7 mmol), $Pd(OAc)_2$ (8 mg, 0.035 mmol), and Et_2O (10 mL) was added, drop by drop, a solution of CH_2N_2 in Et_2O (200 mL) at room temperature over 30 min. The mixture was then filtered. The filtrate was concentrated *in vacuo* to give an oily residue. This, upon purification by column chromatography on silica gel (Et_2O/hexane, 1 : 3), gave a mixture of cycloadducts. The yields and product ratio are given in Table 2-1.

General procedure for cyclopropanation catalyzed by samarium. To a suspension of Sm metal (316 mg, 2.1 mmol) and THF (5 mL) was added a solution of $HgCl_2$ (54 mg, 0.21 mmol) in THF (5 mL). The mixture was stirred for 15 min, then a solution of the allylic alcohol (0.5 mmol) in THF (2 mL) was added. Freshly distilled CH_2ICl (146 μL, 2.0 mmol) was added drop by drop and the blue suspension that resulted was stirred for 2 h at room temperature. The mixture was then quenched with aqueous NH_4Cl and was extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to give an oily residue. This was purified by column chromatography on silica gel (Et_2O/hexane, 3 : 1) to give the cycloadducts. The yields and product ratio are given in Table 2-1.

(4*S*)-3-(*N*-*tert*-butoxycarbonylglycyl)-2,2-dimethyl-4-vinyl-1,3-oxazolidine (11). To a solu-

tion of **5a** (1,38 g, 7,4 mmol) in CH_2Cl_2 (5 mL) was added TFA at 0 °C and the solution was stirred for 1 h. The solvent was removed *in vacuo* and the residue was dissolved in THF (15 mL). The pH of a solution was adjusted to 9 with Et_3N at -20 °C. To this solution was added *N'*-hydroxysuccinimide *N*-tert-butoxycarbonylglycinate (Boc-Gly-OSu; 2.42 g, 8.9 mmol) and the solution was stirred at room temperature for 3 h. The solvent was evaporated *in vacuo*. The residue was dissolved in EtOAc and the solution was successively washed with 10% aqueous citric acid, water, aqueous $NaHCO_3$, and water. 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 ($MeOH/CHCl_3$, 1 : 4) to give **10** (1.48 g, 82%) as an oil. This was treated with CSA (10 mg) and 2,2-dimethoxypropane (10 mL) / acetone (10 mL) at 80 °C for 16 h. The reaction mixture was extracted with ether and the organic layer was washed with aqueous $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$. The solvent was removed *in vacuo* to give an oily residue. This was purificated by column chromatography on silica gel ($MeOH/CHCl_3$, 1 : 19) to give **11** (1.14 g, 75.4%) as an oil: $[\alpha]^{23}_D$ -12.4° (*c* 0.85, $CHCl_3$); IR (neat) 3432. 3100, 1714, 1662 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.44(s, 9 H), 1.55 (s, 3 H), 1.67 (s, 3 H), 3.87 (m, 3 H), 4.14 (m, 2 H), 5.25 (m, 2 H), 5.34 (m, 1 H) 5.84 (ddd, 1 H, J = 7, 9, 16 Hz); Anal. Calcd. for $C_{14}H_{24}N_2O_4$: C, 59.14;

H, 8.51; N, 9.85. Found: C, 58.63; H, 8.53; N, 9.57.

(1*S*,7*S*,8*S*)-3-Aza-4,4-dimethyl-5-oxatricyclo-[6.1.0.0^{3,7}]nonan-2-one (14a) and (1R,7*S*,8R)-3-Aza-4,4-dimethyl-5-oxatricyclo[6.1.0.0^{3,7}]nonan-2-one (14b). To a solution of **11** (992 g, 3.49 mmol) in CH_2Cl_2 (7 mL) were added 2,6-lutidine (812 μL, 6.98 mmol) and TMSOTf (1.01 mL, 5.23 mmol) at room temperature for 15 min. The reaction mixture was quenched with MeOH at 0 °C. The solution was subjected to column chromatography on silica gel ($CHCl_3$, then MeOH/$CHCl_3$, 1 : 9) to give **12b**. To a solution of **12b** in ether (10 mL) was added $NaNO_2$ (240 mg, 3.48 mmol) in water (5 mL) with intensively stirring. The pH of the solution was adjusted to 3 with citric acid. The organic layer was washed successive with aqueous $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$. The solution was evaporated *in vacuo* to give **13** (473 mg, 70%) as an yellow oil; IR (neat) 2936, 2108, 1616 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.60 (s, 3 H), 1.68 (s, 3 H), 3.86 (m, 1 H), 4.09 m, 2 H), 4.84 (s, 1 H), 5.26 (m, 2 H), 5.84 (m, 1 H). To the solution of $Pd(OAc)_2$ (13 mg) in toluene (30 mL) was added, drop by drop, the solution of **13** (23 mg, 1.17 mmol) in toluene (30 mL) at 80 °C for 2 h. The solvent was removed *in vacuo* to give an oily residue. This, upon purification by column chromatography on silica gel (Et_2O), gave (1*S*,7*S*,8*S*)-**14a** (73.3 g, 37%) and (1*R*,7*S*,8*R*)-**14b** (11.6 mg, 6%). **14a**: Amorphous solid; $[\alpha]^{23}_D$ +52.0° (*c* 0.58, $CHCl_3$); IR (neat) 3080, 2988, 1706 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.98 (m, 1 H), 1.22

(m, 1 H), 1.36 (s, 3 H), 1.77 (s, 3 H), 1.94 (m, 2 H), 3.39 (dd, 1 H, J = 9, 12 Hz), 3.98 (m, 2 H); HRMS (FAB) m/z Calcd for $C_9H_{14}NO_2$ $(M+H)^+$, 168.1024; Found: 168.1000. **14b**: Amorphous solid; $[\alpha]^{23}_D$ +22.0° (c 0.50, $CHCl_3$); IR (neat) 3780, 2988, 1682 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 0.76 (m, 1 H), 0.98 (m, 1 H), 1.42 (s, 3 H), 1.48 (s, 3 H), 1.70 (m, 1 H), 2.04 (m, 1 H), 3.39 (t, 1 H, J = 9 Hz), 3.96 (dd, 1 H, J = 6, 9 Hz), 4.49 (ddd, 1 H, J = 6, 6, 9 Hz); HRMS (EI) m/z Calcd for $C_9H_{13}NO_2$ M^+, 167.0945; Found: 167.0962.

(2*S*,1'*R*,2'*S*)-2-(carboxycyclopropyl)glycine (CCG-III; 3). A solution of **14a** (60 mg, 0.36 mmol) in 60% aqueous acetic acid (2 mL) was stirred at room temperature for 8 h. The solvent was removed under reduced pressure. To a solution of the residue in THF (1 mL) were added 0.5 M aqueous $Na(OH)_2$ (1 mL) and the solution was heated at 70 °C for 4 h. The reaction mixture was neutralized with 1 M aqueous HCl and the pH of the solution was adjusted to 9 with $NaHCO_3$. To the solution were added Boc_2O (125 mL, 0.54 mmol) and 1,4-dioxane (2 mL). The reaction mixture was stirred at room temperature for 16 h and was washed with Et_2O. The pH of the aqueous layer was adjusted to 2 with 1 M aqueous 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 **15a** (62 mg, 71%) as colorless crystalline. To a solution of **15a** (62 mg, 0.25 mmol) in acetone (2 mL) was added Jones reagent at 0 °C and the reaction mixture was

stirred at 0 °C for 14 h. This was quenched with 2-propanol and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give a residue. To a solution of the residue in CH_2Cl_2 (1 mL) at 0 °C was added TFA (0.5 mL). The mixture was stirred for 30 min at room temperature, then was concentrated *in vacuo*. The residue was passed through a column of Dowex 50Wx4 (100-200 mesh) ion exchange resin (H_2O, then 1 *M* aqueous NH_3) to give a solution of the ammonium salt of **3**. The eluate was concentrated *in vacuo* and then was dissolved in water (2 mL). The pH of the solution was adjusted to 3 with 1 *M* aqueous HCl. The crystals that precipitated from the solution were collected by filtration. They were recrystallized from water to give **3** (13 mg, 59%) as colorless crystals:

Methyl (4*S*)-4-*N*-(*tert*-butoxycarbonyl)amino-5-hydroxypentanoate (18a). To a solution of L-glutamic acid γ-methyl ester **16a** (18.4 g, 114 mmol) and $NaHCO_3$ (24 g, 285 mmol) in water (300 mL) was added a solution of Boc_2O (31 mL, 137 mmol) in 1,4-dioxane (300 mL). The mixture was stirred for 16 h at room temperature, then was washed with Et_2O. The aqueous layer was acidified with 1 *M* aqueous HCl to pH 2, and was extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. To a solution of the residue and *N*-hydroxysuccinimide (15.8 g, 137 mmol) in EtOAc (500 mL) was added DCC (28.2 g, 137 mmol) at 0 °C. The mixture was warmed to room temperature and was stirred for 3 h. The

mixture was then filtered. The filtrate was washed successively with aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated *in vacuo* to give crude **17** as colorless crystals (51 g). To a solution of crude **17** (20 g, 55 mmol) in THF (150 mL) was added $NaBH_4$ (2.1 g, 56 mmol) at 0 °C. Then EtOH (50 mL) was added. The resulting suspension was stirred for 15 min at 0 °C, and then was quenched with aqueous NH_4Cl. The mixture was extracted with EtOAc. The extract was dried ($MgSO_4$) and concentrated under reduced pressure to give an oily residue. This, upon column chromatography on silica gel (Et_2O), gave **18a** as colorless crystals. Recrystallization (Et_2O/hexane) gave pure **18a** (11.4 g, 83%): mp 40.5-41.5 °C; $[\alpha]^{23}_D$ -13.2° (*c* 1.00, $CHCl_3$); IR (neat) 3372, 2980, 1708 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.43 (s, 9 H), 1.78 (m, 1 H), 1.88 (ddt, 1 H, *J* = 5.0, 8.0, 8.0, 15.0 Hz), 2.418 (dt, 1 H, *J* = 8.0, 8.0, 16.0 Hz), 2.422 (dt, 1 H, *J* = 8.0, 8.0, 16.0 Hz), 2.51 (br s, 1 H), 3.60 (m, 1 H), 3.65 (m, 2 H), 3.67 (s, 3 H), 4.80 (d, 1 H, *J* = 7 Hz); MS (SIMS) *m/z* 248 $(M+H)^+$, 192, 148. Anal. Calcd for $C_{11}H_{21}O_5N$: C, 53.43; H, 8.56; N, 5.66. Found: C, 53.57; H, 8.76; N, 5.62.

Methyl (4*S*)-4-*N*-(*tert*-butoxycarbonyl)amino-5-*tert*-butyldimethylsilyloxypentanoate (18b). To a solution of **14a** (2.95 g, 11.9 mmol) in DMF (15 mL) was added imidazole (1.62 g, 23.9 mmol) and *t*-$BuMe_2SiCl$ (2.69 g, 17.9 mmol). The solution was stirred at room temperature for 3 h, then was quenched with MeOH. The mixture was extracted with EtOAc. The extract was washed with

water. The extract was then dried ($MgSO_4$) and was concentrated *in vacuo* to give an oily residue. This was purified by column chromatography on silica gel to give **18b** (3.97 g, 92%) as an oil: $[\alpha]^{23}_D$ -21.9° (*c* 2.00, $CHCl_3$); IR (neat) 3468, 2960, 1744, 1722 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 0.05 (*s*, 6 H), 0.91 (s, 9 H), 1.45 (s, 9 H), 1.84 (m, 2 H), 2.39 (t, 2 H, *J* = 8 Hz), 3.60 (m, 3 H), 3.67 (s, 3 H), 4.64 (br s, 1 H); MS (SIMS) *m/z* 362 $(M+H)^+$, 306, 262, 248. Anal. Calcd for $C_{17}H_{35}O_5N$: C, 56.47; H, 9.76; N, 3.87. Found: C, 56.67; H, 9.83; N, 3.83.

(5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldimethylsilyloxymethyl-2-pyrrolidone (19b). To a solution of **18b** (1.00 g, 2.76 mmol) in Et_2O (100 mL) was added NaH (83 mg, 2.76 mmol) at 0 °C. The resulting suspension was stirred for 18 h at room temperature, then was quenched with aqueous NH_4Cl solution and was washed with water. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to give **19a** as an oil. To a solution of **19a** in THF (50 mL) was added Boc_2O (760 μL, 3.31 mmol), Et_3N (460 μL, 3.31 mmol) and DMAP (67 mg, 0.55 mmol). The mixture was stirred for 4 h at room temperature, then was extracted with EtOAc. The extract was washed successively with 5% aqueous citric acid and brine and was dried ($MgSO_4$). The solvent was evaporated under reduced pressure to give an oily residue. This, upon purification by silica gel column chromatography (Et_2O/hexane, 1 : 1), gave **19b** (796 mg, 87%) as an oil: $[\alpha]^{23}_D$ -62.2° (*c* 1.23, $CHCl_3$); IR (neat) 2960, 1790, 1756, 1714 cm^{-1}; ^1H NMR

($CDCl_3$, 100 MHz) δ 0.03 (s, 3 H), 0.04 (s, 3 H), 0.88 (s, 9 H), 1.53 (s, 9 H), 1.90-2.30 (m, 2 H), 2.46 (m, 1 H), 2.66 (m, 1 H), 3.68 (dd, 1 H, *J* = 3, 11 Hz), 3.90 (dd, 1 H, *J* = 5, 11 Hz), 4.14 (m, 1 H); MS (SIMS) *m/z* 330 $(M+H)^+$, 274, 236, 230, 216, 172, 156. Anal. Calcd for $C_{16}H_{31}O_4NSi$: C, 58.32; H, 9.48; N, 4.25; Found. C, 58.07; H, 9.40; N, 4.27.

(5*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldimethylsilyloxymethyl-3-pyrrolin-2-one (20). To a solution of **19b** (3.20 g, 9.73 mmol) in THF (5 mL) was added, drop-by-drop, a solution of *i*-Pr_2NLi [prepared from diisopropylamine (1.58 mL, 11.3 mmol) and *n*-BuLi (1.6 *M* in hexane solution, 6.95 mL, 11.1 mmol)] in THF (25 mL) at -78 °C under N_2. The solution was stirred for 30 min at -78 °C. Then was added a solution of PhSeCl (2.05 g, 10.7 mmol) in THF (15 mL). After 5 min, aqueous NH_4Cl was added. The mixture was allowed to warm to room temperature, then was extracted with Et_2O. The extract was dried ($MgSO_4$) and concentrated *in vacuo* to give an oily residue. This was purified by column chromatography on silica gel (Et_2O/hexane; 1 : 9, then 1 : 3) to give the phenylselenyl compound as a mixture of diastereomers (4.00 g, 85%). Ozone was passed through a solution of (5*S*)-*N*-*tert*-butoxycarbonyl-5-*tert*-butyldimethylsilyloxymethyl-3-phenylseleno-pyrrolidine-2-one (3.2 g, 6.6 mmol) in anhydrous CH_2Cl_2 (80 mL) at -78 °C until the solution became slightly blue in color. Excess ozone was removed from the solution by passing a stream of O_2 through the solution.

To the solution was then added NaOAc (6.6 g, 80 mmol). The mixture was allowed to warm to 0 °C, then was stirred for 40 min. The mixture was washed with water, and the organic layer was dried (Na_2SO_4). The solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (Et_2O/hexane, 1 : 4) to give **20** (1.73 g, 87%) as colorless crystals: mp 64.0-65.0 °C; $[\alpha]^{25}_D$ -175.6° (*c* 0.9, $CHCl_3$); IR (neat) 2936, 1768, 1714 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 0.06 (s, 6 H), 0.88 (s, 9 H), 1.56 (s, 9 H), 3.70 (dd, 1 H, *J* = 7, 10 Hz), 4.15 (dd, 1 H, *J* = 4, 10 Hz), 4.60 (m, 1 H), 6.11 (dd, 1 H, *J* = 2, 6 Hz), 7.24 (dd, 1 H, *J* = 2, 6 Hz); MS (SIMS) *m/z* 328 $(M+H)^+$, 272, 228, 214, 170. Anal. Calcd for $C_{16}H_{29}O_4NSi$: C, 58.68; H, 8.93, N, 4.28. Found: C, 58.68; H, 8.72; N, 4.24.

(1*R*, 4*S*, 5*R*)-*N*-*tert*-Butoxycarbonyl-3-aza-4-*tert*-butyldimethylsilyloxymethylbicyclo[3.1.0]-hexan-2-one (21a). To a solution of $Pd(OAc)_2$ (14 mg, 0.06 mmol) and **20** (200 mg, 0.609 mmol) in Et_2O (5 mL) was added a solution of CH_2N_2 in Et_2O (30 mL) at room temperature. The resulting suspension was filtered. The filtrate was concentrated *in vacuo* to give an oily residue. This was purified by column chromatography on silica gel (Et_2O/hexane, 1 : 1) to give a 9 : 1 mixture of (1*R*,4*S*,5*R*)-**21a** and its (1*S*,4*S*,5*S*)-isomer (208 mg, 100%). **(1*R*, 4*S*, 5*R*)-21a**: Oil; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.03 (s, 3 H), 0.04 (s, 3 H), 0.72 (dt, 1 H, *J* = 3, 4.5, 4.5 Hz), 0.85 (s, 9 H), 1.13 (dt, 1 H, *J* = 4.5, 8.0, 8.0 Hz), 1.50 (s, 9 H), 1.91

(ddd, 1 H, J = 4.5, 6.5, 8.0 Hz), 1.94 (dddd, 1 H, J = 1.5, 3.0, 6.5, 8.0 Hz), 3.76 (dd, 1 H, J = 5.5, 10.0 Hz), 3.85 (dd, 1 H, J = 2.5, 10.0 Hz), 4.02 (ddd, 1 H, J = 1.5, 2.5, 5.5 Hz). **(1*S*, 4*S*, 5*S*)-isomer**: Oil; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.03 (s, 3 H), 0.04 (s, 3 H), 0.72 (m, 1 H), 0.85 (s, 9 H), 1.13 (m, 1 H), 1.50 (s, 9 H), 1.91 (m, 1 H), 1.94 (m, 1 H), 3.69 (dd, 1 H, J = 2.5, 10.0 Hz), 3.91 (dd, 1 H, J = 4.0, 10.0 Hz), 4.16 (ddd, 1 H, J = 2.5, 4.0, 7.0 Hz).

(2*S*,1'*S*,2'*S*)-*N*-*tert*-Butoxycarbonyl-2-(methoxycarbonylcyclopropyl)glycinol (8e). To solution of **21a** (177 mg, 0.517 mmol) in MeOH (5 mL) was added CSA (5 mg). The mixture was stirred at room temperature for 14 h. It was then concentrated *in vacuo* and was extracted with EtOAc. The extract was dried ($MgSO_4$) and concentrated *in vacuo* to give a crystalline residue. This was recrystallized (Et_2O) to give (1*R*,4*S*,5*R*)-**21b** as colorless crystals: mp 99.0-100.0 °C; $[\alpha]^{23}_D$ -39.9° (*c* 0.91, $CHCl_3$); IR (neat) 3436, 2984, 1770, 1718 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz), δ 0.77 (ddd, 1 H, J = 3.5, 4.0, 5.0 Hz), 1.19 (ddd, 1 H, J = 5.0, 8.0, 9.0 Hz), 1.50 (s, 9 H), 1.88 (ddd, 1 H, J = 4.0, 6.0, 8.0 Hz), 2.00 (dddd, 1 H, J = 1.5, 3.5, 6.0, 9.0 Hz), 2.31 (t, 1 H, J = 6.0 Hz), 3.82 (ddd, 1 H, J = 4.0, 6.0, 11.0 Hz), 3.86 (ddd, 1 H, J = 4.0, 6.0, 11.0 Hz), 4.11 (dt, 1 H, J = 1.5, 4.5, 4.5 Hz); MS(SIMS) *m/z* 228 $(M+H)^+$, 172, 128. Anal. Calcd for $C_{11}H_{17}O_4N$: C, 58.14; H, 7.54; N, 6.16. Found: C, 58.04; H, 7.67; N, 6.13.

To a solution of **21b** in MeOH (5 mL) was added LiOH

(14 mg, 0.57 mmol). The solution was stirred at room temperature for 16 h. Then it was extracted with EtOAc. The extract was dried ($MgSO_4$) and concentrated under reduced pressure to give an oily residue. This was purified by column chromatography on silica gel (Et_2O) to give **8e** (111 mg, 83% from **21a**) as a colorless amorphous solid: $[\alpha]^{23}_D$ -52.7° (*c* 1.09, $CHCl_3$); IR (neat) 3384, 2984, 1718 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.14 (ddd, 1 H, *J* = 5.0, 8.0, 9.5 Hz), 1.17 (ddd, 1 H, *J* = 5.0, 5.5, 7.0 Hz), 1.42 (s, 9 H), 1.55 (dddd, 1 H, *J* = 7.0, 7.5, 9.0, 9.5 Hz), 1.80 (ddd, 1 H, *J* = 5.5, 8.0, 9.0 Hz), 2.80 (br s, 1 H), 3.62 (dddd, 1 H, *J* = 3.5, 6.0, 7.5, 7.5 Hz), 3.70 (dd, 1 H, *J* = 6.0, 11.0 Hz), 3.70 (s, 3 H), 3.83 (dd, 1 H, *J* = 3.5, 11.0 Hz), 4.93 (br s, 1 H); MS (SIMS) *m/z* 260 $(M+H)^+$, 204, 160. Anal. Calcd for $C_{12}H_{21}O_5N$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.44; H, 8.29; N, 5.40.

(4*S*)-4-*N*-(*tert*-Butoxycarbonyl)amino-5-pentanolide (22a). A solution of **18a** (1.00 g, 4.04 mmol) and CSA (10 mg) in benzene (500 mL) was refluxed for 3 h. The mixture was washed with aqueous $NaHCO_3$ and water, and dried ($MgSO_4$). Concentration *in vacuo* gave a crystalline residue. This was recrystallized (Et_2O) to give **22a** (799 mg, 92%) as colorless crystals: mp 104.0-104.5 °C; $[\alpha]^{23}_D$ -37.4° (*c* 1.09, $CHCl_3$); IR (neat) 3356, 2984, 1756, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 1.44 (s, 9 H), 1.87 (m, 1 H), 2.22 (m, 1 H), 2.58 (dt, 1 H, *J* = 7.5, 7.5, 17.0 Hz), 2.66 (dt, 1 H, *J* = 7.0, 7.0, 17.0 Hz), 4.03 (m, 1 H), 4.19 (dd, 1 H, *J* = 5.0, 11.5 Hz), 4.40

(dd, 1 H, J = 0.5, 4.0, 11.5 Hz), 4.72 (br s, 1 H); MS (SIMS) m/z 216 (M+H)+, 160, 116. Anal. Calcd for $C_{10}H_{17}O_4N$: C, 55.80; H, 7.96; N, 6.51. Found: C, 55.81; H, 8.03; N, 6.36.

(5*S*)-5-*N*-(*tert*-Butoxycarbonyl)amino-5,6-dihydro-2-pyrone (23). To a solution of **22a** (3.00 g, 13.9 mmol) in THF (70 mL) was added, successively, Me_3SiCl (3.85 mL, 30.7 mmol) and lithium hexmethyldisilazane (30.7 mL of a 1 *M* solution in THF, 30.7 mmol) at -78 °C. The solution was stirred for 15 min, then was added to a solution of $Pd(OAc)_2$ (3.74 g, 16.7 mmol) in CH_3CN (80 mL). The mixture was stirred at room temperature for 45 min, and then was quenched with aqueous NH_4Cl. The resulting suspension was filtered. The filtrate was concentrated *in vacuo* to give an oily residue. This was dissolved in Et_2O (100 mL) and the solution was washed with brine. The Et_2O solution was dried and the solvent was evaporated *in vacuo* to give an oily residue. This, upon purification by column chromatography on silica gel (Et_2O/hexane, 3 : 1), gave **23** (2.08 g, 70%) as colorless crystals: mp 128.0-129.0 °C; $[\alpha]^{23}_D$ +113° (*c* 1.07, $CHCl_3$); IR (neat) 3340, 2988, 1724, 1686 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz), δ 1.47 (s, 9 H), 4.2-4.6 (m, 3 H), 4.80 (br s, 1 H), 6.70 (d, 1 H, J = 10 Hz), 6.86 (dd, 1 H, J = 5, 10 Hz); MS (SIMS) m/z 214 $(M+H)^+$, 158, 114. Anal. Calcd for $C_{10}H_{15}O_4N$: C, 56.33; H, 7.09; N, 6.57. Found: C, 56.15; H, 7.07; N, 6.43.

(1*S*,5*S*,6*R*)-5-*N*-(*tert*-Butoxycarbonyl)amino-3-oxa-bicyclo[3.1.0]heptan-2-one (9d). To a suspension

of $Pd(OAc)_2$ (21 mg, 0.094 mmol) and **23** (200 mg, 0.938 mmol) in Et_2O (30 mL) was added, drop-by-drop, a solution of CH_2N_2 in Et_2O at room temperature over 2 h. The mixture was then filtered. The filtrate was concentrated *in vacuo* to give an oily residue. This, upon purification by flash column chromatography on silica gel (Et_2O/hexane, 3 : 1), gave **9d** as colorless crystals (98 mg, 46%).

Chapter 3

Methyl 3-[(4*S*)-3-*N*-*tert*-butoxycarbonyl-2,2-dimethyl-1,3-oxazolidin-4-yl]-(2*E*)-porpionate (29a). To a solution of **28** (5.55 g, 24.2 mmol) in benzene (100 mL) was added methyl triphenylphosphoranilideneacetate ($Ph_3PCHCO_2CH_3$) (9.73 g, 29.1 mmol) and the reaction mixture was stirred at room temperature for 16 h. The solvent was removed *in vacuo* and the oily residue was subjected to a column chromatography on silica gel (Et_2O/hexane, 1 : 1) to give crude crystals. They were recrystallized (Et_2O) to give **29a** (6,58 g, 95%) as colorless crystals: mp 47.0-49.0 °C; $[\alpha]^{23}_D$ +61.7° (*c* 1.10, $CHCl_3$); IR (neat) 2984, 1732, 1704, 1666 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 1.48 (s, 9 H), 1.56 (s, 3H), 1.65 (s, 3 H), 3.79 (s, 3 H), 3.81 (dd, 1 H, *J* = 3, 10 Hz), 4.11 (dd, 1 H, *J* = 7, 10 Hz), 4.50 (br s, 1 H), 5.93 (d, 1 H, *J* = 16 Hz), 6.85 (dd, 1 H, *J* = 7, 16 Hz); MS (SIMS) *m/z* 286 $(M+H)^+$, 212, 199, 186. Anal. Calcd. for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 59.05; H, 8.14; N, 4.77.

(4*S*)-3-*N*-*tert*-butoxycarbonyl-4-[3-hydroxy-(1*E*)-propenyl]-2,2-dimethyl-1,3-oxazolidine (30a). To a solution of DIBAL (1 *M* hexane solution) (74 mL, 74 mmol) in toluene (120 mL) was added *n*-BuLi (1.6 *M* hexane solution) (46 mL, 74 mmol) at 0 °C and the mixture was stirred for 30 min. This solution was added to a solution of **29a** (7.00 g, 24.5 mmol) in toluene (360 mL) at -78 °C, drop by drop, over 20 min. The reaction mixture was stirred at -78 °C for 40 min and at 0 °C for 30 min. It was quenched with MeOH (5 mL) and extracted

with 1 *M* aqueous HCl and Et_2O. The organic layer was washed with water and 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 **30a** (5,5 g, 87%) as an oil: $[\alpha]^{23}_D$ +11.1° (*c* 1.13, $CHCl_3$); IR (neat) 3456, 2984, 2940, 2876, 1698 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 1.46 (s, 9 H), 1.50 (s, 3 H), 1.59 (s, 3 H), 2.04 (br s, 1 H), 3.73 (dd, 1 H, *J* = 2, 9 Hz), 4.04 (dd, 1 H, *J* = 7, 9 Hz), 4.13 (d, 2 H, *J* = 6 Hz), 4.32 (br s, 1 H), 5.71 (m, 2 H); MS (SIMS) *m/z* 258 $(M+H)^+$, 202, 184. Anal. Calcd. for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.45; H, 9.11; N, 5.34.

(4*S*)-3-*N*-*tert*-butoxycarbonyl-4-[3-hydroxy-(1*Z*)-propenyl]-2,2-dimethyl-1,3-oxazolidine (30b). In a manner similar to that used to prepare **30a**, **30b** (5.70 g, 86%) was prepared from **29b** (7.40 g, 25.9 mmol). **30b**: Oil: $[\alpha]^{23}_D$ -28.7° (*c* 0.83, $CHCl_3$); IR (neat) 3436, 2984, 2940, 2884, 1700, 1684, 1674 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.47 (s, 9 H), 1.49 (s, 3 H), 1.57 (s, 3 H), 3.72 (dd, 1 H, *J* = 3, 9 Hz), 3.7-4.2 (m, 2 H), 4.06 (dd, 1 H, *J* = 6, 9 Hz), 4.43 (br t, 1 H, *J* = 11 Hz), 4.91 (m, 1 H), 5.53 (t, 1 H, *J* = 10 Hz), 5.80 (m, 1 H); HRMS (FAB) *m/z* Calcd for $C_{13}H_{24}NO_4$ $(M+H)^+$, 258.1705; Found: 258.1976.

(4*S*)-3-*N*-*tert*-Butoxycarbonylglycyl-4-[3-*tert*-butyl-dimethylsilyloxy-(1*E*)-propenyl]-2,2,-dimethyl-1,3-oxazolidine (31a). To a solution of **30a** (4.7 g, 18.3 mmol) in MeOH (30 mL) was added 1 *M* HCl/MeOH (30 mL) at 0 °C and the reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated *in vacuo* to give a residue.

This was dissolved in MeOH again and concentrated *in vacuo* to remove HCl. The pH of the solution of this residue in THF (20 mL) / MeOH (5 mL) was adjusted to 9 with Et_3N at 0 °C. To this solution was added *N*-hydroxysuccinimide *N*-tert-butoxycarbonyl-glycinate (Boc-Gly-OSu; 5.97 g, 21.9 mmol) and the pH of the solution was again adjusted to 9 with Et_3N. The solvent was evaporated *in vacuo*. The residue was dissolved in EtOAc and the insoluble material was filterd. The filtrate was concentrated *in vacuo* to give an oily residue. This was subjected to column chromatography on silica gel ($MeOH/CHCl_3$, 4 : 1). A solution of the oily product and CSA (100 mg) in acetone (50 mL) and 2,2-dimethoxypropane (50 mL) was stirred at 60 °C for 2 h. The mixture was extracted with EtOAc and aqueous $NaHCO_3$. The organic layer was washed with water and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue. This was treated with CSA and 2,2-dimethoxypropane (75 mL) / acetone (25 mL) at 80 °C for 5 h. The reaction mixture was extracted in the same manner as above. A solution of the oily residue in 1,2-dichloroethane and CSA (50 mg) was heated at 90 °C for 2 h and then MeOH (20 mL) was added. The reaction mixture was stirred at room temperature for 30 min. This was extracted with EtOAc and the organic layer was dried over $MgSO_4$. The solvent was removed *in vacuo* to give an oily residue. To a solution of this residue in DMF (50 mL) were added *t*-$BuMe_2SiCl$ (3.32 g, 21.9 mmol) and imidazole (1.87 g, 27.5 mmol) and the reaction mixture was stirred at room temperature for 3 h. This was extracted with Et_2O and the organic layer was dried. The solvent was evaporated *in vacuo* to give an oily

residue. This was purificated by column chromatography on silica gel (Et_2O/hexane, 1 : 1) to give **31a** (4.92 g, 63%) as an oil: $[\alpha]^{23}_D$ +29.8° (*c* 0.85, $CHCl_3$); IR (neat) 3436. 3340, 2940, 2864, 1718, 1660 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 0.08 (s, 6 H), 0.92 (s, 9 H), 1.46(s, 9 H), 1.56 (s, 3 H), 1.68 (s, 3 H), 3.8-4.0 (m, 3 H), 4.0-4.3 (m, 3 H), 4.30 (m, 1 H), 5.38 (br s, 1 H), 5.76 (m, 2 H); MS (SIMS) *m/z* 429 $(M+H)^+$, 371, 315, 271. Anal. Calcd. for $C_{21}H_{40}N_2O_5Si$: C, 58.84; H, 9.41; N, 6.54. Found: C, 58.68; H, 9.50; N, 6.31.

(4*S*)-3-*N*-tert-Butoxycarbonylglycyl-4-[3-tret-butyldimethylsilyloxy-(1*Z*)-propenyl]-2,2,-dimethyl-1,3-oxazolidine (31b). In a manner similar to that used to prepare **31a**, **31b** (6.74 g, 71%) was prepared from **30b** (7.40 g, 25.9 mmol). **31b**: Oil: $[\alpha]^{23}_D$ -53.1° (*c* 1.76, $CHCl_3$); IR (neat) 3432, 2964, 2940, 2864, 1720, 1660 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$, δ 0.09 (s, 6 H), 0.90 (s, 9 H), 1.44 (s, 9 H), 1.56 (s, 3 H), 1.67 (s, 3 H), 3.76-3.94 (m, 3 H), 4.13 (dd, 1 H, *J* = 6, 9 Hz), 4.30 (d, 2 H, *J* = 6 Hz), 4.81 (ddd, 1 H, *J* = 1, 7, 7 Hz), 5.30 (br s, 1 H), 5.4-5.85 (m, 2 H). Anal. Calcd. for $C_{21}H_{40}N_2O_5Si$: C, 58.84; H, 9.41; N, 6.54. Found: C, 58.74; H, 9.41; N, 6.50.

(1*R*, 7*S*, 8*R*, 9*R*)-3-Aza-9-*tert*-butyldimethylsilyloxy-methyl-4,4-dimethyl-5-oxatricyclo[6.1.0.$0^{3,7}$]nonan-2-one (34b) and (1*S*, 7*S*, 8*S*, 9*S*)-3-Aza-9-*tert*-butyl-dimethyl-silyloxymethyl-4,4-dimethyl-5-oxatricyclo[6.1.0.$0^{3,7}$]-nonan-2-one (34a). To a solution of **31a** (6.38 g, 14.9 mmol) in CH_2Cl_2 (30 mL) were added 2,6-lutidine (3.5 mL, 29.8 mmol) and TMSOTf (4.3 mL, 22.4 mmol) at room temperature for 15 min.

The reaction mixture was quenched with aqueous NH_4Cl at 0 °C. This was extracted with EtOAc and washed with water. The solvent was evaporated *in vacuo* to give **32a**. To a solution of **32a** in toluene (100 mL) was added $NaNO_2$ (5.15 g, 74.5 mmol) in water (50 mL) with intensively stirring. The pH of the solution was adjusted to 3 with citric acid. The organic layer was washed successively with aqueous $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$. The desiccant was filtered and to the filtrate was added $Pd(OAc)_2$ (167 mg, 0.745 mmol). The reaction mixture was heated at 90 °C for 30 min. The solvent was removed *in vacuo* to give an oily residue. This, upon column chromatographic purification on silica gel (50% Et_2O/hexane, 1 : 1), gave (1*R*,7*S*,8*R*,9*R*)-**34b** (465 mg, 10%) and (1*S*,7*S*,8*S*,9*S*)-**34a** (1.51 g, 33%). **34b**: Oil; $[\alpha]^{23}_D$ +20.2° (*c* 0.58, $CHCl_3$); IR (neat) 2940, 2864, 1702 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 0.03 (s, 3 H), 0.04 (s, 3 H), 0.87 (s, 9 H), 1.30 (m, 1 H), 1.44 (s, 3 H), 1.51 (s, 3 H), 1.72 (ddd, 1 H, *J* = 3.5, 6.0, 6.0 Hz), 2.04 (dd, 1 H, *J* = 3.0, 6.0 Hz), 3.45 (dd, 1 H, *J* = 8.5, 9.0 Hz), 3.52 (dd, 1 H, *J* = 5.5, 10.5 Hz), 3.83 (dd, 1 H, *J* = 4.0, 10.5 Hz), 3.97 (dd, 1 H, *J* = 6.0, 8.5 Hz), 4.50 (ddd, 1 H, *J* = 6.0, 6.0, 9.0 Hz); MS (CI) *m/z* ; Anal. Calcd. for $C_{16}H_{29}NO_3Si$: C, 61.69; H, 9.38; N, 4.50. Found: C, 61.58; H, 9.49; N, 4.43. **34a**: Oil; $[\alpha]^{23}_D$ +103.7° (*c* 1.09, $CHCl_3$); IR (neat) 3596, 2940, 2896, 2864, 1710 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 0.04 (s, 6 H), 0.87 (s, 9 H), 1.33 (s, 3 H), 1.50 (dddd, 1 H, *J* = 2.9, 3.5, 4.0, 5.1 Hz), 1.71 (s, 3 H), 1.93 (dd, 1 H, *J* = 3.5, 6.1 Hz), 1.96 (ddd, 1 H, *J* = 1.4, 2.9, 6.1 Hz), 3.39 (dd, 1 H, *J* = 9.5, 11.8 Hz), 3.54 (dd, 1 H, *J* = 5.1, 10.7 Hz), 3.78 (dd, 1 H, *J* =

4.0, 10.7 Hz), 3.97 (dd, 1 H, *J* = 5.6, 9.5 Hz), 3.98 (ddd, *J* = 1.4, 5.6, 11.8 Hz); MS (EI) *m/z* 296 $(M-CH_3)^+$, 254, 196, 178, 166, 152; (CI) *m/z* 312 $(M+H)^+$, 254; HRMS (EI) *m/z* Calcd for $C_{16}H_{29}NO_3Si$ M^+, 312.1993; Found: 312.1970. Anal. Calcd. for $C_{16}H_{29}NO_3Si$: C, 61.69; H, 9.38; N, 4.50. Found: C, 61.41; H, 9.45; N, 4.47.

(1*S*, 7*S*, 8*S*, 9*R*)-3-Aza-9-*tert*-butyldimethylsilyloxymethyl-4,4-dimethyl-5-oxatricyclo[6.1.0.$0^{3,7}$]nonan-2-one (43). In a manner similar to that used to prepare **34a**, **43** (1.33 g, 61%) was prepared from **31b** (3.00 g, 7.0 mmol). **43**: Oil; $[\alpha]^{23}_D$ +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). Anal. Calcd. for $C_{16}H_{29}NO_3Si$: C, 61.69; H, 9.38; N, 4.50. Found: C, 61.42; H, 9.44; N, 4.49.

(1*R*, 7*S*, 8*R*, 9*R*)-3-Aza-9-hydroxymethyl-4,4-dimethyl-5-oxatricyclo[6.1.0.$0^{3,7}$]nonan-2-one (35b). To a solution of **34b** (300 mg, 0.96 mmol) in THF (2 mL) was added *n*-Bu_4NF (1 *M* THF solution; 1.4 mL, 1.4 mmol) at 0 °C and the reaction mixture was stirred for 10 min. The solvent was removed to give a residue. This was purificated by column chromatography on silica gel (Et_2O, then MeOH/Et_2O, 1 : 19) to give **35b** (123 mg, 65%) as an amorphous solid. $[\alpha]^{23}_D$ +29.5° (*c* 1.10, $CHCl_3$); IR (neat) 3432, 2996, 2916, 2892, 1668 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.38 (br s, 2 H), 1.41 (s, 3 H), 1.48 (s, 3 H), 1.71

(m, 1 H), 2.05 (dd, 2 H, J = 3, 6 Hz), 3.39 (t, 1 H, J = 9 Hz), 3.55 (d, 2 H, J = 5 Hz), 3.96 (dd, 1 H, J = 6, 9 Hz), 4.48 (ddd, 1 H, J = 6, 6, 9 Hz); HRMS (FAB) *m/z* Calcd for $C_{10}H_{16}NO_3$ $(M+H)^+$, 198.1130; Found: 198.1115.

(1*S*, 7*S*, 8*S*, 9*S*)-3-Aza-9-hydroxymethyl-4, 4-dimethyl-5-oxatricyclo[6.1.0.$0^{3,7}$]nonan-2-one (35a). In a manner similar to that used to prepare **35b**, **35a** (520 mg, 86%) was prepared from **34a** (950 mg, 3.05 mmol). **35a**: Amorphous solid; $[\alpha]^{23}_D$ +156° (*c* 1.04, $CHCl_3$); IR (neat) 3376, 2992, 2944, 2880, 1678 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.35 (s, 3 H), 1.60 (s, 1 H), 1.72 (s, 3 H), 1.94 (m, 2 H), 2.45 (br s, 1 H), 3.38 (dd, 1 H, J = 9, 12 Hz), 3.57 (d, 2 H, J = 6 Hz), 3.98 (dd, 1 H, J = 6, 9 Hz); MS (EI) *m/z* 197 (M^+), 182, 136, 126, 108, 96, 82; HRMS (EI) *m/z* Calcd for $C_{10}H_{15}NO_3$ M^+, 197.1050; Found: 197.1041. Anal. Calcd. for $C_{10}H_{15}NO_3$: C, 60.90; H, 7.67; N, 7.10. Found: C, 60.61; H, 7.53; N, 6.90.

(1*S*, 7*S*, 8*S*, 9*R*)-3-Aza-9-hydroxymethyl-4, 4-dimethyl-5-oxatricyclo[6.1.0.$0^{3,7}$]nonan-2-one (44). In a manner similar to that used to prepare **35b**, **44** (423 mg, 94%) was prepared from **43** (712 mg, 2.28 mmol). **44**: 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, 100.0 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); HRMS (FAB) *m/z* Calcd for $C_{10}H_{16}NO_3$ $(M+H)^+$, 198.1130; Found: 198.1128.

(1*R*, 7*S*, 8*R*, 9*R*)-3-Aza-9-methoxymethyl-4, 4-dimethyl-

5-oxatricyclo[6.1.0.0^{3,7}]nonan-2-one (36b). To a solution of **35b** (115 mg, 0.58 mmol) in THF (2 mL) was added NaH (40% oil suspension: 28 mg, 0.70 mmol) at 0 °C and the reaction mixture was stirred for 20 min. To this suspension were added DMF (1 mL), *n*-Bu_4NI (5 mg), CH_3I (55 mL, 0.64 mmol) at room temperature and the reaction mixture was stirred for 2 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. This was purificated by column chromatography on silica gel (Et_2O, then MeOH/Et_2O, 3 : 97) to give **36b** (77 mg, 63%) as an oil: $[\alpha]^{23}_D$ +16.5° (*c* 0.55, $CHCl_3$); IR (neat) 3512, 2988, 2940, 2884, 1702 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.40 (s, 3 H), 1.41 (m, 1 H), 1.48 (s, 3 H), 1.70 (m, 1 H), 2.01 (dd, 1 H, *J* = 3, 7 Hz), 3.13 (dd, 1 H, *J* = 7, 10 Hz), 3.32 (s, 3 H), 3.45 (t, 1 H, *J* = 9 Hz), 3.53 (dd, 1 H, *J* = 8, 10 Hz), 3.98 (dd, 1 H, *J* = 6, 9 Hz), 4.49 (ddd, 1 H, *J* = 6, 6, 9 Hz); HRMS (FAB) *m/z* Calcd for $C_{11}H_{16}NO_3$ $(M+H)^+$: 212.1287; Found: 212.1287.

(1*S*, 7*S*, 8*S*, 9*S*)-3-Aza-9-methoxymethyl-4,4-dimethyl-5-oxatricyclo[6.1.0.0^{3,7}]nonan-2-one (36a). In a manner similar to that used to prepare **36b**, **36a** (92 mg, 87%) was prepared from **35a** (100 mg, 0.50 mmol). **36a**: Oil; $[\alpha]^{23}_D$ +162° (*c* 1.03, $CHCl_3$); IR (neat) 3392, 2992, 2940, 2880, 1708 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.33 (s, 3 H), 1.60 (m, 1 H), 1.71 (s, 3 H), 1.93 (m, 2 H), 3.11 (dd, 1 H, J = 7, 10 Hz), 3.35 (s, 3 H), 3.37 (dd, 1 H, *J* = 8, 12 Hz), 3.50 (dd, 1 H, *J* = 5, 10 Hz), 3.82-4.10 (m, 2 H). MS (EI) *m/z* 196 $(M-CH_3)^+$, 140, 126, 112, 96, 82; (CI) *m/z* 212 $(M+H)^+$, 172; HRMS (EI) *m/z* Calcd

for $C_{11}H_{17}NO_3$ M^+, 211.1207; Found: 212.1205.

(1*S*, 7*S*, 8*S*, 9*R*)-3-Aza-9-methoxymethyl-4,4-dimethyl-5-oxatricyclo[6.1.0.0^{3,7}]nonan-2-one (45). In a manner similar to that used to prepare **36a**, **45** (353 mg, 82%) was prepared from **44** (400 mg, 2.00 mmol). **45**: 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); Anal. Calcd. for $C_{11}H_{17}NO_3$: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.54; H, 8.16; N, 6.56.

(2*S*, 1'*R*, 2'*R*, 3'*R*)-*N*-*tert*-butoxycarbonyl-2-(2-carboxy-3-methoxymethylcyclopropyl)glycinol (37b). A solution of **36b** (72 mg, 0.34 mmol) in 60% aqueous acetic acid (2 mL) was stirred at room temperature for 12 h. The solvent was removed under reduced pressure. To a solution of the residue in ethanol (2 mL) were added $Ba(OH)_2 \cdot 6H_2O$ (323 mg, 1.02 mmol) and water (2 mL) and the suspension was heated at 80 °C for 14 h. The reaction mixture was neutralized with aqueous H_2SO_4 and the pH of the solution was adjusted to 9 with $NaHCO_3$. The insoluble material was removed by filteration and the filtrate was concentrated to 2 mL *in vacuo*. To the solution were added Boc_2O (156 mL, 0.68 mmol) and 1,4-dioxane (2 mL). The reaction mixture was stirred at room temperature for 16 h and washed with EtOAc. The pH of the aqueous layer was ad-

justed to 1 with 1 *M* aqueous 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 **37b** (77 mg, 79%) as colorless crystalline: 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 (100 MHz, $CDCl_3$) δ 1.40 (m, 1 H), 1.46 (s, 9 H), 1.77 (m, 1 H), 1.98 (m, 1 H), 3.33 (s, 3 H), 3.2-3.4 (m, 2 H), 3.48 (m, 2 H), 3.64 (br s, 2 H), 3.70 (br s, 1 H), 5.06 (m, 1 H). Anal. Calcd. for $C_{13}H_{23}NO_6$: C, 53.97; H, 8.01; N, 4.84; Found: C, 54.03; H, 7.86; N, 4.69.

(2*S*,1'*S*,2'*S*,3'*S*)-*N*-*tert*-butoxycarbonyl-2-(2-carboxy-3-methoxymethylcyclopropyl)glycinol (37a). In a manner similar to that used to prepare **37b**, **37a** (119 mg, 73%) was prepared from **36a** (120 mg, 0.57 mmol). **37a**: Colorless crystals; mp 51.5-53.0 °C; $[\alpha]^{23}_D$ -4.9° (*c* 0.45, $CHCl_3$); IR (neat) 3396, 2984, 1710 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.43 (s, 9 H), 1.50 (m, 1 H), 1.65-1.98 (m, 2 H), 3.13 (dd, 1 H, J = 7, 10 Hz), 3.34 (s, 3 H), 3.54 (dd, 1 H, J = 6, 10 Hz), 3.60 (m, 1 H), 3,73 (m, 2 H), 4.41 (m, 2 H), 5.14 (br s, 1 H); MS (SIMS) *m/z* 290 (M+H)+, 246, 234, 216, 190. Anal. Calcd. for $C_{13}H_{23}NO_6$: C, 53.97; H, 8.01; N, 4.84. Found: C, 53.65; H, 8.19; N, 4.64.

(2*S*,1'*S*,2'*S*,3'*R*)-*N*-*tert*-butoxycarbonyl-2-(2-carboxy-3-methoxymethylcyclopropyl)glycinol (46). In a manner similar to that used to prepare **37a**, **46** (281 mg, 58%) was prepared from **45** (353 mg, 1.67 mmol). **46**: 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, 5b H). Anal. Calcd. for $C_{13}H_{23}NO_6$: C, 53.97; H, 8.01; N, 4.84; Found: C, 53.93; H, 8.14; N, 4.81.

(1*S*, 5*S*, 6*S*, 7*R*)-5-*N*-(*tert*-Butoxycarbonyl)amino-7-methoxymethyl-3-oxobicyclo[3.1.0]heptan-2-one (47). A solution of **46** (56 mg, 0.19 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSCD) HCl salt (45 mg, 0.23 mmol), 1-hydroxybenzotriazole (HOBt) (31 mg, 0.23 mmol), and Et_3N (32 μL, 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 MgSO4 and the solvent was evaporated *in vacuo* to give an oily residue. This was purified by column chromatography on silica gel (EtOAc) to give **47** (24 mg, 46%) as colorless crystals; mp 140.0-141.0 °C; 1H NMR (300 MHz, CDCl3) δ 1.45 (s, 9 H), 1.86 (m, 1 H), 1.92 (m, 1 H), 2.16 (t, 1 H, *J* = 9 Hz), 3.28 (t, 1 H, *J* = 11 Hz), 3.41 (s, 3 H), 3.74 (dd, 1 H, *J* = 10, 12 Hz), 3.96 (dd, 1 H, *J* = 7, 11 Hz), 4.36 (dd, 1 H, *J* = 7, 12 Hz), 4.58 (m, 1 H), 5.52 (d, 1 H, *J* = 8 Hz); HRMS (FAB) *m/z* Calcd for $C_{13}H_{22}NO_5$ (M+H)$^+$, 272.1498; Found: 272.1503.

(2*S*, 1'*R*, 2'*R*, 3'*R*)-2-(2-carboxy-3-methoxymethylcyclopropyl)glycine (26: t-MCG-IV). To a solution of **37b** (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, then at room temperature for 2 h. The mixture was quenched with 2-propanol and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was

evaporated *in vacuo* to give a 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, then was concentrated *in vacuo*. The residue was passed through a column of Dowex 50Wx4 (100-200 mesh) ion exchange resin (H_2O, then 1 *M* aqueous NH_3) to give a solution of the ammonium salt of **26**. The eluate was concentrated *in vacuo* and then was dissolved in water (2 mL). The pH of the solution was adjusted to 3 with 1 *M* aqueous HCl. The crystals that precipitated from solution were collected by filtration. They were recrystallized from water to give **26** (14 mg, 28%) as colorless crystals: mp 185.5-187.0 °C (decomp.); $[\alpha]^{25}_D$ +31.5° (*c* 0.47, H_2O); IR (KBr) 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). Anal. Calcd. for $C_8H_{13}NO_5$: C, 47.29; H, 6.45; N, 6.89; Found: C, 47.17; H, 6.38; N, 6.82.

(2*S*,1'*S*,2'*S*,3'*S*)-2-(2-carboxy-3-methoxymethylcyclopropyl)glycine (24: t-MCG-III). In a manner similar to that used to prepare **26**, **24** (26 mg, 34%) was prepared from **37a** (110 mg, 0.38 mmol). **24**: Colorless crystals; mp 195.0-198.0 °C; $[\alpha]^{25}_D$ +59.6° (*c* 0.54, H_2O); IR (KBr) cm^{-1}; ^1H 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. Calcd. for $C_8H_{13}NO_5$: C, 47.29; H, 6.45; N, 6.89; Found: C,

47.02; H, 6.43; N, 6.76.

(2*S*,1'*S*,2'*S*,3'*R*)-2-(2-carboxy-3-methoxymethylcyclopropyl)glycine (25: c-MCG-III). In a manner similar to that used to prepare **26**, **25** (59 mg, 37%) was prepared from **46** (250 mg, 0.86 mmol). **25**: Colorless crystals; mp 155.5-156.5 °C; $[\alpha]^{25}_{D}$ +85.9° (*c* 0.51, H_2O); IR (KBr) 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). Anal. Calcd. for $C_8H_{13}NO_5$: C, 47.29; H, 6.45; N, 6.89; Found: C, 47.01; H, 6.39; N, 6.67.

(1*R*,7*S*,8*R*,9*R*)-3-aza-9-benzyloxymethyl-4,4-dimethyl-5-oxatricyclo[6.1.0.$0^{3,7}$]nonan-2-one (38b). To a solution of **35b** (30 mg, 0.15 mmol) in THF/DMF (1:1, 2 mL) was added NaH (10 mg, 0.25 mmol) at 0 °C and the solution was stirred for 20 min. To this suspension was added *n*-Bu_4NI (5 mg, 0.015 mmol) and benzyl bromide (36 mL, 0.30 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. To this suspension were added NaH (10 mg, 0.25 mmol) and benzyl bromide (24 mL, 0.20 mmol) and the reaction mixture was stirred at room temperature for additional 2 h. This was quenched with aqueous NH_4Cl and was extracted with EtOAc. The organic layer was washed with water and dried over $MgSO_4$. The solvent was removed *in vacuo* to give an oily residue. This was purificated by column chromatography on silica gel (Et_2O/hexane, 1 : 1) to give **38b** (32 mg, 72%) as an oil; $[\alpha]^{23}_{D}$ +12.4° (c 1.33, $CHCl_3$); IR (neat) 3490, 2880, 2696 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) d 1.40 (m, 1 H), 1.42 (s, 3

H), 1.50 (s, 3 H), 1.70 (ddd, 1 H, J = 4.5, 6.0, 6.0 Hz), 2.02 (dd, 1 H, J = 3.0, 6.0 Hz), 3.25 (dd, 1 H, J = 7.0, 10.5 Hz), 3.44 (t, 1 H, J = 8.5 Hz), 3.61 (dd, 1 H, J = 5.0, 10.5 Hz), 3.97 (dd, 1 H, J = 6.0, 8.5 Hz), 4.48 (ddd, 1 H, J = 6.0, 6.0, 8.5 Hz), 4.49 (d, 1 H, J = 12.0 Hz), 4.51 (d, 1 H, J = 12.0 Hz), 7.25-7.40 (m, 5 H). HRMS (FAB) m/z Calcd for $C_{17}H_{23}NO_6$ $(M+H)^+$, 380.2073; Found: 380.2087.

(1*R*, 7*S*, 8*R*, 9*R*)-3-aza-9-benzyloxymethyl-4, 4-dimethyl-5-oxatricyclo[6.1.0.0^{3,7}]nonan-2-one (38a). In a manner similar to that used to prepare **38b**, **38a** (478 mg, 69%) was prepared from **35a** (480 mg, 2.40 mmol). **38a**: Oil; $[\alpha]^{23}_D$ +73.8° (c 1.00, $CHCl_3$); IR (neat) 3356, 2876, 1686 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 3 H), 1.62 (dddd, 1 H, J = 3.0, 3.5, 5.0, 7.0 Hz), 1.71 (s, 3 H), 1.92 (dd, 1 H, J = 3.5, 6.0 Hz), 1.96 (ddd, 1 H, J = 1.5, 3.0, 6.0 Hz), 3.23 (dd, 1 H, J = 7.0, 10.5 Hz), 3.37 (dd, 1 H, J = 10.0, 12.5 Hz), 3.65 (dd, 1 H, J = 5.0, 10.5 Hz), 3.96 (dd, 1 H, J = 6.0, 10.0 Hz), 3.98 (ddd, 1 H, J = 1.5, 6.0, 12.5 Hz), 4.49 (d, 1 H, J = 12.0 Hz), 4.54 (d, 1 H, J = 12.0 Hz), 7.25-7.40 (m, 5 H). Anal. Calcd for $C_{17}H_{21}NO_3$: C, 71.06; H, 7.37; N, 4.87. Found: C, 70.78; H, 7.22; N, 4.86.

(2*S*, 1'*R*, 2'*R*, 3'*R*)-*N*-*tert*-butoxycarbonyl-2-(3-benzyloxymethyl-2-carboxycyclopropyl)glycinol (39b). A solution of **38b** (126 mg, 0.44 mmol) in 60% aqueous acetic acid (2 mL) was stirred at room temperature for 5 h. The solvent was removed under reduced pressure. To a solution of the residue in ethanol (2 mL) were added $Ba(OH)_2 \cdot 6H_2O$ (277 mg, 0.88 mmol) and water (2 mL) and the suspension was heated at 80 °C

for 24 h. The suspension was neutralized with aqueous H_2SO_4 and the pH of the mixture was adjusted to 9 with $NaHCO_3$. The insoluble material was filterd and the filtrate was concentrated to 2 mL *in vacuo*. A solution of the residue, Boc_2O (202 mL, 0.88 mmol), and 1,4-dioxane (2 mL) was stirred at room temperature for 12 h. The reaction mixture was washed with Et_2O. The pH of the aqueous layer was adjusted to 1 with 1 *M* aqueous HCl and was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give **39b** (105 mg, 65%) as an oil: ^{1}H NMR (100 MHz, CD_3OD) δ 1.40 (m, 1 H), 1.41 (s, 9 H), 1.68 (dd, 1 H, J = 6, 9 Hz), 1.90 (m, 1 H), 3.46 (m, 2 H), 3.78 (m, 1 H), 4.49 (s, 2 H), 7.29 (s, 5 H). To a solution of **39b** in methanol was added a solution of diazoacetate in Et_2O to give the methyl ester of **39b** as an oil: $[\alpha]^{23}_D$ -28.3° (*c* 0.30, $CHCl_3$); IR ($CHCl_3$ soln.) 3438, 2932, 2864, 2359, 1712 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.42 (s, 9 H), 1.46 (m, 1 H), 1.76 (br s, 1 H), 1.96 (dd, 1 H, J = 6, 12 Hz), 2.79 (br s, 1 H), 3,42 (m, 2 H), 3.68 (s, 3 H), 3.5-3.9 (m, 3 H), 4.49 (s, 2 H), 4.98 (d, 1 H, J = 8 Hz), 7.30 (s, 5 H); HRMS (FAB) *m/z* Calcd for $C_{20}H_{30}NO_6$ $(M+H)^+$: 380.2073; Found: 380.2087.

(2*S*,1'*S*,2'*S*,3'*S*)-*N*-*tert*-butoxycarbonyl-2-(3-benzyloxymethyl-2-methoxycarboxycyclopropyl)glycinol (39a). In a manner similar to that used to prepare **39b**, **39a** (140 mg, 71%) was prepared from **38b** (150 mg, 0.52 mmol). **39a**: Colorless crystals; mp 94.0-95.5 °C; $[\alpha]^{23}_D$ +14.3° (*c* 1.26, $CHCl_3$); IR (neat) 3460, 3012, 2988, 2876, 1726 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$) δ1.38 (m, 1 H), 1.41 (s, 9 H), 1.76 (dd, 1 H, J =

6, 10 Hz), 1.88 (ddd, 1 H, J = 2, 6, 11 Hz), 2.90 (m, 1 H), 3.17 (dd, 1 H, J = 8, 10 Hz), 3.50-3.85 (m, 4 H), 3.69 (s, 3 H), 4.50 (s, 2 H), 4.80 (br s, 1 H), 7.30 (s, 5 H). Anal Calcd for $C_{20}H_{29}NO_6$: C, 63.31; H, 7.70; N, 3.69. Found: C, 63.14; H, 7.62; N, 3.64.

(1*R*, 5*S*, 6*R*, 7*R*)-7-Benzyloxymethyl-5-*N*-(*tert*-butoxycarbonyl)amino-3-oxabicyclo[3.1.0]heptan-2-one (40b). A solution of **39b** (32 mg, 0.084 mmol) and 5 mg of CSA in CH_2Cl_2 (2 mL) was stirred at room temperature for 24 h. The reaction mixture was washed with water and was dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue. This was purificated by column chromatography on silica gel (AcOEt/benzene, 1 : 1) to give **40b** (24 mg, 83%) as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9 H), 1.87 (m, 1 H), 1.92 (dd, 1 H, J = 4.0, 8.0 Hz), 1.98 (m, 1 H), 3.42 (dd, 1 H, J = 6.0, 10.0 Hz), 3.56 (dd, 1 H, J = 5.0, 10.0 Hz), 4.08 (dd, 1 H, J = 2.5, 12.5 Hz), 4.2 (m, 1 H), 4.26 (dd, 1 H, J = 1.5, 12.5 Hz), 4.50 (s, 2 H), 4.94 (d, 1 H, J = 8 Hz), 7.31 (m, 5 H); Anal Calcd for $C_{19}H_{25}NO_5$: C, 65.69; H, 7.25; N, 4.03. Found: C, 65.30; H, 7.02; N, 3.99.

(1*S*, 5*S*, 6*S*, 7*S*)-7-Benzyloxymethyl-5-*N*-(*tert*-butoxycarbonyl)amino-3-oxabicyclo[3.1.0]heptan-2-one (40a). In a manner similar to that used to prepare **40b**, **40a** (21 mg, 16%) was prepared from **39a** (140 mg, 0.40 mmol). **40a**: Colorless crystals; mp 119.5-121.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9 H), 1.85 (ddd, 1 H, J = 4.5, 4.5, 8.0 Hz), 1.92 (dd, 1 H, J = 4.0, 8.0 Hz), 2.13 (dddd, 1 H, J = 4.0, 4.5, 4.5, 7.0 Hz), 3.26 (dd, 1 H, J = 7.0, 10.5 Hz), 3.60 (t, 1 H,

J = 11.0 Hz), 3.65 (dd, 1 H, J = 4.5, 10.5 Hz), 4.26 (m, 1 H), 4.34 (m, 1 H), 4.51 (s, 2 H), 4.63 (d, 1 H, J = 8 Hz), 7.32 (m, 5 H); Anal Calcd for $C_{19}H_{25}NO_5$: C, 65.69; H, 7.25; N, 4.03. Found: C, 65.44; H, 7.24; N, 4.04.

(2*S*,1'*R*,2'*R*,3'*R*)-2-(3-benzyloxymethyl-2-carboxycyclopropyl)glycine (42: t-BCG-IV). To a solution of **39b** (105 mg, 0.287 mmol) in acetone (2 mL) under N_2 atmosphere was added degassed Jones reagent at 0 °C. The reaction mixture was stirred at 0 °C for 3 h, then at room temperature for 3 h. The reaction was quenched with 2-propanol and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give a residue. This was esterified by adding a solution of diazomethane in Et_2O. The dimethyl ester was purified by column chromatography on silica gel (EtOAc/benzene, 1 : 4). To a solution of dimethyl ester in EtOH (1 mL) was added 1 *M* aqueous NaOH and the solution was stirred at 0 °C for 3 h. The pH of the solution was adjusted to 1 with 1 *M* aqueous HCl and extracted with EtOAc. The organic layer was dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give a 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, then was concentrated *in vacuo*. The residue was passed through a column of Dowex 50Wx4 (100-200 mesh) ion exchange resin (H_2O, then 1 *M* aqueous NH_3) to give a solution of the ammonium salt of **42**. The eluate was concentrated *in vacuo* and then was dissolved in water (2 mL). The pH of the solution was adjusted to 3 with 1 *M* aqueous HCl. The crystals that precipitated from solution

were collected by filtration. They were recrystallized from water to give **42** (18 mg, 24%) as colorless crystals: mp 188-189.0 °C (decomp.); $[\alpha]^{25}_{D}$ +40.2° (*c* 0.45, 2 *M* HCl); IR (KBr) 3431, 3049, 2855, 2610, 2084, 1699, 1631, 1510 cm^{-1}; ^1H NMR (500 MHz, 5% DCl/D_2O) δ 1.83 (ddd, 1 H, *J* = 6.5, 9.0, 11.0 Hz), 2.06 (dddd, 1 H, *J* = 5.5, 6.0, 6.5, 7.5 Hz), 2.08 (dd, 1 H, *J* = 5.5, 9.0 Hz), 3.44 (dd, 1 H, *J* = 7.5, 11.5 Hz), 3.76 (dd, 1 H, *J* = 6.0, 11.5 Hz), 4.24 (d, 1 H, *J* = 11.0 Hz), 4.61 (s, 2 H), 7.43 (m, 5 H). Anal. Calcd for $C_{14}H_{17}NO_5$: C, 60.20; H, 6.14; N, 5.02. Found: C, 60.20; H, 6.14; N, 4.95.

(2*S*,1'*S*,2'*S*,3'*S*)-2-(3-benzyloxymethyl-2-carboxycyclopropyl)glycine (41: t-BCG-III). In a manner similar to that used to prepare **42**, **41** (84 mg, 41%) was prepared from **39a** (162 mg, 0.44 mmol). **41**: Colorless crystals; mp 195.0-196.0 °C; $[\alpha]^{25}_{D}$ +63.5° (*c* 0.55, 2 *M* HCl); IR (KBr) 3129, 2860, 1958, 1692, 1616, 1568, 1486 cm^{-1}; ^1H NMR (500 MHz, 5% DCl/D_2O) δ 1.81 (ddd, 1 H, J = 6.0, 8.0, 11.0 Hz), 2.01 (dd, 1 H, *J* = 5.5, 8.0 Hz), 2.11 (dddd, 1 H, *J* = 5.5, 6.0, 6.0, 7.5 Hz), 3.53 (dd, 1 H, *J* = 7.5, 11.5 Hz), 3.72 (dd, 1 H, *J* = 6.0, 11.5 Hz), 4.42 (d, 1 H, *J* = 11.0 Hz), 4.59 (d, 1 H, *J* = 11.0 Hz), 4.63 (d, 1 H, *J* = 11.0 Hz), 7.43 (m, 5 H); MS (SIMS) *m/z* 280 $(M+H)^+$. Anal. Calcd for $C_{14}H_{17}N_5$: C, 60.20; H, 6.14; N, 5.02. Found: C, 59.61; H, 6.05; N, 4.92.

(1*S*,4*S*,5*S*,6*R*)-3-Aza-3-*N*-*tert*-butoxycarbonyl-4,6-bis-(*tert*-butyldimethylsilyloxymethyl)bicyclo[3.1]-hexane-2-one (48). A mixture of **43** (150 mg, 0.48 mmol), Dowex 50Wx4 (50 mg), and MeOH (5 mL) was stirred at room temperature for 14 h. The resine was removed and the filtrate was

concentrated to give an oily residue. To remove MeOH completely, this was dissolved in THF and DMF and the solvent was evaporated *in vacuo*. To the solution of the residue in DMF (5 mL) were added *t*-$BuMe_2SiCl$ (218 mg, 1.44 mmol) and imidazole (131 mg, 1.93 mmol). The reaction mixture was stirred at room temperature for 16 h. This was extracted with EtOAc and the organic layer was dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give an oily residue. The mixture of the residue, Et_3N (134 μL, 0.96 mmol), Boc_2O (166 μL, 0.72 mmol), DMAP (12 mg, 0.096 mmol), and THF (2 mL) was stirred at room temperature for 16 h. The reaction mixture was extracted with EtOAc and 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 a residue. This was subjected to column chromatography on silica gel (ether/hexane, 1 : 3) to give **48** (162 mg, 69%) as colorless crystalls: 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), 1.4-1.55 (m, 1 H), 1.95-2.15 (m, 2 H), 3.45 (dd, 1 H, *J* = 8, 11 Hz), 3.7-3.9 (m, 3 H), 4.05 (m, 1 H).

(1*S*, 2*R*, 3*R*, 1'*S*)-2-(1-*N*-*tert*-Butoxycarbonylamino-2-*tert*-butyldimethylsilyloxyethyl)-3-*tert*-butyldimethylsilyloxymethylcyclopropan-1-carboxylic acid methyl ester (49). A solution of **48** (162 mg, 0.33 mmol) and LiOH (8 mg, 0.33 mmol) in MeOH (3 mL) was stirred at room temperature for 16 h. The reaction mixture was 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 **49** (112 mg, 65%) as an oil: $[\alpha]^{23}_D$ -35.6° (*c* 0.88, CHCl3); 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). Anal. Calcd. for $C_{25}H_{51}NO_6Si_2$: C, 57.98; H, 9.92; N, 2.70; Found: C, 58.08; H, 10.02; N, 2.81.

(1*R*, 2*R*, 3*S*, 1'*S*)-1-(1-*N*-*tert*-butoxycarbonylamino-2-*tert*-butyldimethylsilyloxyethyl)-2-*tert*-butyldimethylsilyloxymethyl-3-methoxypropane (51). To a solution of **49** (620 mg, 1,20 mmol) in CH_2Cl_2 (12 mL) was added DIBAL (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 quenched with ice. To this mixture was added $MgSO_4$ and the insoluble material was filtered. The filtrate was washed successively with 1 *M* aqueous HCl and brine and was dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give a residue. This was subjected to column chromatography on silica gel (Et_2O/hexane, 1 : 1) to give **50** (584 mg, 99%). To a solution of **50** in a mixture of THF/Et_2O (1:1) mixed solvent (5 mL) were added *n-BuLi* (1.6 *M* hexane solution: 352 μL, 0.56 mmol), methyl fluorosulfonate (48 μL, 0.61 mmol) at -78 °C and the reaction mixture was stirred at -78 °C for 2 h. To this were added n-BuLi (1.6 *M* hexane solution: 60 μL, 0.094 mmol), methyl fluorosulfonate (11 μL, 0.14 mmol) at -78 °C and the reaction mixture was stirred at -78 °C for additional 2 h. The reaction mixture was quenched with LiOH (54 mg, 2.26 mmol) in

MeOH (2 mL). This was extracted with Et_2O and washed with water. The organic layer was dried over $MgSO_4$. The solvent was removed under reduced pressure to give a residue. This was purified by column chromatography on silica gel to give **51** (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, 3.46), 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. Calcd for $C_{25}H_{53}NO_5Si_2$: C, 59.59; H, 10.60; N, 2.78. Found: C, 59.66; H, 10.70; N, 2.77.

(2*S*,1'*R*,2'*R*,3'*S*)-*N*-*tert*-butoxycarbonyl-2-(2-carboxy-3-methoxymethyl)cyclopropyl glycine dimethyl ester (54). To a solution of **51** (150 mg, 0.30 mmol) in MeOH was added Dowex 50Wx4 (30 mg) and the reaction mixture was stirred at room temperature for 18 h. The resin was filterd and the filtrate was concentrated in vacuo to give a residue. This 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. This was quenched with 2-propanol and was extracted with EtOAc. The organic layer was washed with brine and was dried over $MgSO_4$. The solvent was evaporated *in vacuo* to give a residue. This was esterificated by diazomethane in Et_2O. The solvent was removed *in vacuo* and the solution of the residue and LiOH (5 mg) in MeOH (2 mL) was stirred at room temperature for 10 min, then was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give a residue. This

was purificated by column chromatography in silica gel (Et_2O) to give **54** (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 (t, 1 H, *J* = 9 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. Calcd for $C_{15}H_{25}NO_7$: C, 54.37; H, 7.60; N, 4.23. Found: C, 54.43; H, 7.64; N, 4.19.

(2*S*,1'*R*,2'*R*,3'*R*)-2-(2-carboxy-3-methoxymethylcyclopropyl)glycine (27: c-MCG-IV). To a solution of **54** (40 mg, 0.127 mmol) in THF (1 mL) was added 0.5 *M* aqueous NaOH (870 μL, 0.435 mmol) at 0 °C and the reaction mixture was stirred at 0 °C for 14 h and then at room temperature for 1 h. The pH of the reaction mixture was adjusted to 1 with 2 *M* aqueous 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 50Wx4 (100-200 mesh) ion exchange resin (H_2O, then 1 *M* aqueous NH_3) to give a solution of the ammonium salt of **27**. The eluate was concentrated *in vacuo* and then was dissolved in water (2 mL). The pH of the solution was adjusted to 3 with 1 *M* aqueous HCl. The crystals that precipitated from the solution were collected by filtration. They were recrystallized from water to give **27** (14 mg, 55%) as colorless crystals: mp 147.0-151.0 °C (decomp.); $[\alpha]^{25}_D$ +83.3° (*c* 0.52, H_2O); IR (KBr) 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 (t, 1 H, *J* = 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). HRMS (FAB) m/z Calcd for $C_8H_{14}NO_5$ $(M+H)^+$: 204.0872; Found: 204.0872.

Chapter 4

The methods of the neurobiological test were described in the following references.

Electrophysiological assay.

See refs 16, 19, 50.

Receptor binding assay.

See ref 48 (Details will be submitted to *Eur. J. Pharmac.*). Preparation of the membrane suspension and binding assays were performed according to the published methods; (a) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmam, J.; Williams, M. *J. Pharmacol. Exp. Thera.* **1987**, *240*, 778. (b) Loo, P. S.; Braunwarder, A. F.; Lehmann, J; Williams, M; Sills, M. A. *Mol. Pharmacol.* **1987**, *32* 820. (c) Honoré, T.; Nielsen, M. *Neurosci.* **1976**, *26*, 1007. (d) Simon, J. R; Contrera, J. F.; Kuhar, M. J. *J. Neurochem.*, **1976**, *26*, 141.

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Abbreviations

Ac	acetyl
Asp	aspartic acid
BCG	2-(3-benzyloxy-2-carboxycyclopropyl)glycine
Boc	*tert*-butoxycarbonyl (t-C_4H_9OCO)
Bu	butyl
Bzl	benzyl
CCG	L-2-(carboxycyclopropyl)glycine
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CNS	central nervous system
CPP	(±)-3-(carboxypiperazine-4-yl)propyl-1-phosphonoic acid
CSA	(±)-camphor-10-sulfonic acid
D-APV	D-2-amino-5-phosphonovaleric acid
DCC	dicyclohexylcarbodiimide
DIBAL	diisobutylaluminum hydride
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
Et	ethyl
Gly	glycine
HOBt	1-hydroxybenzotriazole
HOSu	*N*-hydroxysuccinimide
IP_3	inositol triphosphate
KA	kainic acid
LDA	lithium diisopropylamide
LiHMDS	lithium hexamethyldisilazide
L-Glu	L-glutamic acid

LTP	long term potentiation
MCG	2-(2-carboxy-3-methoxymethylcyclopropyl)glycine
Me	methyl
NMDA	*N*-methyl-D-aspartic acid
OTf	trifluoromethanesulfonate (CF_3SO_3)
Pr	propyl
QA	quisqualic acid
t-ACPD	*trans-dl*-1-amino-1,3-cyclopentanedicarboxylic acid
TBS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	trimethylsilyl
WSCD	water solble carbodiimide [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide]

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