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Syntheses of Trans-3'-substituted-CCG-IV Analogs and Their Characterization to Ionotropic Glutamate Receptors

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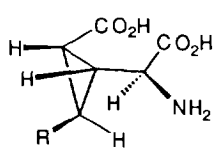
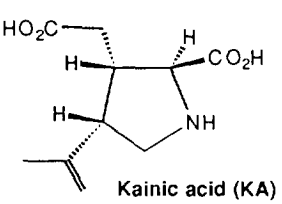
Abstract : Trans-3'-substituted-CCG-IV analogs (**2** and **3**) were efficiently synthesized via an intramolecular cycloaddition of diazoacetamide **4a** using a chiral rhodium catalyst. These analogs evoked marked depolarization through ionotropic glutamate receptors on the spinal motoneurons or the kainate-sensitive dorsal root C-fiber of new born rats even though their binding affinities for the receptors on rat brain synaptic membranes were relatively low. These results suggest that the depolarizing action on C-fiber is not caused by the activation of kainate high affinity sites.

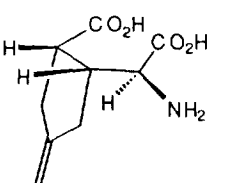
Conformationally constrained glutamate analogs represented by 2-(carboxycyclopropyl)glycines (CCGs) and (2*S*,1'*R*,2'*R*,3'*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) have been used not only as potent and subtype-selective pharmacological reagents but also as useful tools to investigate the physiological functions of glutamate receptors.[1-3] We have proposed that metabotropic glutamate receptors are activated by the extended form of glutamate while ionotropic receptors such as NMDA receptors are activated by the folded form.[1a,3] Moreover, we reported that trans-MCG-IV (**1b**) and trans-BCG-IV (**1c**), the 3'-substituted analogs of CCG-IV (**1a**), caused significant depolarization at the kainate (KA)-sensitive neurons in spite of the fact that **1a** is a selective NMDA agonist.[4] Described herein are our recent efforts on the syntheses and characterization of CCG-IV analogs (**2** and **3**) in the study of the role of the 3'-substituent on KA receptor activation.



1a: CCG-IV R = H
1b: trans-MCG-IV R = CH_2OCH_3
1c: trans-BCG-IV R = CH_2OBzl
2: trans-ethylene-CCG-IV R = $CH{=}CH_2$
3: trans-ethyl-CCG-IV R = CH_2CH_3



Kainic acid (KA)



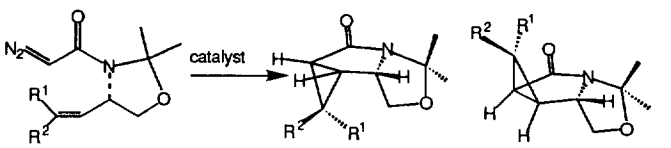
CPG-IV

CCG: 2-(carboxycyclopropyl)glycine
MCG: 2-(2-carboxy-3-methoxymethylcyclopropyl)glycine
BCG: 2-(3-benzyloxymethyl-2-carboxycyclopropyl)glycine
CPG: 2-(2-carboxy-4-methylenecyclopentyl)glycine

Figure 1. Structures of CCG-IV analogs, Kainate, and CPG-IV

Rh-catalyzed cyclopropanation of the diazoacetamide: Syntheses of 2 and 3

Our previous synthesis of trans-MCG-IV (**1b**) and DCG-IV involved intramolecular cyclopropanation of diazoacetamides, **4a** and **4b**, using a palladium catalyst (eq. 1).[3,5] These reactions gave stereoselectively the exo-cycloadducts, **6a** and **6b**, respectively. However, the desired endo-isomer **5a** was the minor product. Multi-step synthetic transformations on the exo-adduct **6b** were required to obtain the amino acids mentioned above. The formation of the exo-cycloadducts was explained by a coordination mechanism where the transition state structure **B** producing an exo-adduct is sterically more favorable than **A** (Scheme 1).[6] Therefore, we re-investigated the cycloaddition process to obtain inverse stereoselectivity providing a straightforward route to these important amino acids. The catalysts to solve this problem were Cu or Rh catalysts which have been known to direct the reaction via the non-chelation carbenoid mechanism. Among the several catalyst systems summarized in Table 1, we found that the reaction of the *E*-diazoacetamide **4a** with Doyle's Rh catalyst [Rh_2(5S- or 5R-MEPY)$_4$] gave the desired endo-cycloadduct **5a** with excellent stereoselectivity.[7] In the case of the *Z*-olefin **4b**, the (5R)-Rh catalyst showed high endo-selectivity to give **5b** in good yield. This stereoselective cycloaddition was assumed to proceed via a transition state structure **C** which is more favorable than structure **D** where severe steric hindrance exists between the substituent R or R' and the Rh ligand.

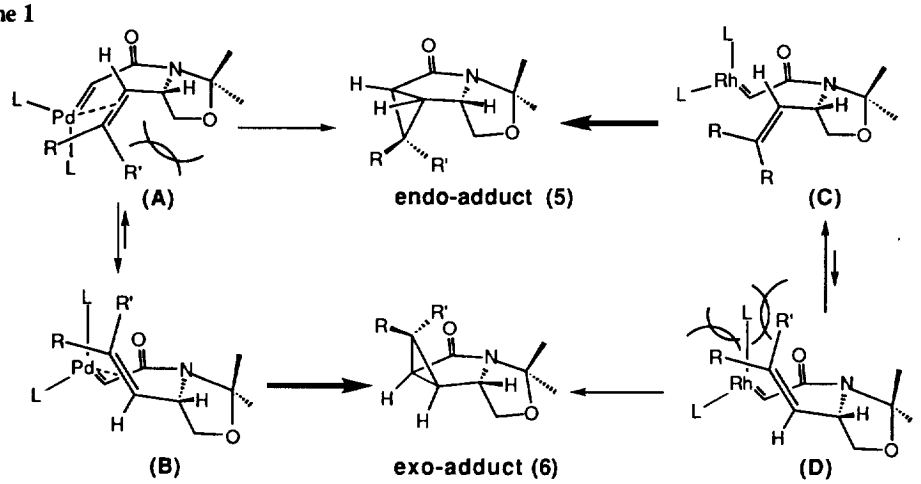


eq.1

4a : R^1 = H, R^2 = $CH_2OTBDMS$ **5** endo-adduct **6** exo-adduct
4b : R^1 = $CH_2OTBDMS$, R^2 = H

TBDMS = *t*-butyldimethylsilyl

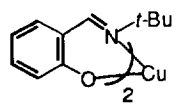
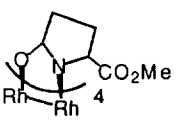
Scheme 1



Compared to the other Cu and Rh catalysts (Table 1), the sterically bulky MEPY ligands seem to play a crucial role in the stereoselectivity; however, the matched or mismatched combination of the ligand with the substrate could not be elucidated.[7d] Since we succeeded in obtaining the desired endo-cycloadducts **5a** and **5b**, trans-substituted CCG-IV analogs such as trans-MCG-IV and DCG-IV could be synthesized from **5a** through a much shorter route compared to the previous synthesis from **6b**.

Table 1. Products ratios and yields by various catalysts

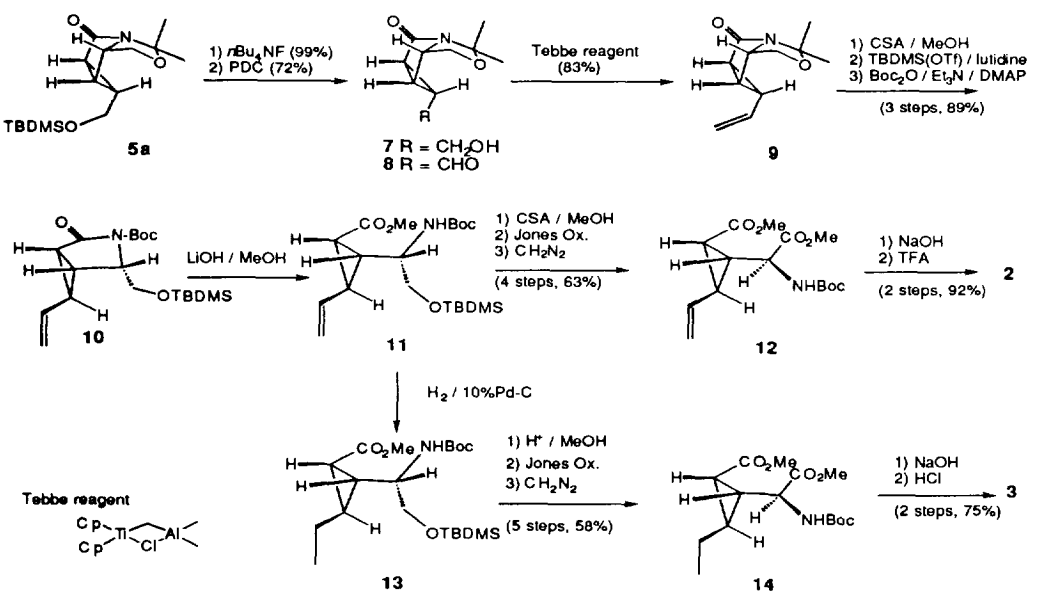
Substrate	Catalyst	Amount (mol%)	Solvent	Products ratio **endo/exo**		Yield (%)
4a	$Pd(OAc)_2$	5	benzene	**5a / 6a**	1 / 3	66
	$Cu(TBS)_2$	5	benzene		3 / 4	79
	$Rh_2(5S\text{-}MEPY)_4$	2	CH_2Cl_2	$R^1 = H$	15 / 1	87
	$Rh_2(5R\text{-}MEPY)_4$	2	CH_2Cl_2	$R^2 = CH_2OTBDMS$	10 / 1	93
	$Rh_2(OAc)_4$	5	CH_2Cl_2		1 / 4	51
4b	$Pd(OAc)_2$	5	benzene	**5b / 6b**	0 / 1	61
	$Cu(TBS)_2$	5	benzene		4 / 1	83
	$Rh_2(5S\text{-}MEPY)_4$	2	CH_2Cl_2	$R^1 = CH_2OTBDMS$	4 / 1	68
	$Rh_2(5R\text{-}MEPY)_4$	2	CH_2Cl_2	$R^2 = H$	20 / 1	74
	$Rh_2(OAc)_4$	5	CH_2Cl_2		4 / 3	53

$Cu(TBS)_2$  $Rh_2(MEPY)_4$ 

TBS: *N*-*t*-butylsalicylamidato

MEPY: methyl 2-oxapyrrolidine-5-carboxylate

Scheme 2



Using the above mentioned new process, we focused on the synthesis of ethyl and ethylene substituted CCG-IV (**2**) and (**3**). These amino acids have attracted significant attention in elucidating the role of the isopropenyl substituent of kainate which is believed to be one of the crucial factors for activating kainate receptors. Our previous studies of CPG-IV also showed the necessity of its exo-methylene group for activation.[8] However, trans-MCG-IV which has no CC double bond as a substituent caused depolarization at the KA-sensitive neurons.[4] These results led us to examine the role of the C3' substituent of trans-MCG-IV using the compounds **2** and **3**. Thus, first the endo-cycloadduct **5a** was converted to aldehyde **8**. Methylenation with Tebbe reagent[9] gave the ethylene-substituted derivative **9** in 83% yield. This was converted to **2** by the use of conventional procedures (Scheme 2). The ethyl derivative **3** was synthesized by hydrogenation of the ethylene group of intermediate **11**, since hydrogenation of **2** was accompanied by the opening of the cyclopropyl ring.

Characterization of 3'-substituted CCG-IV analogs

Neuropharmacological properties of **2** and **3**, together with trans-BCG-IV (**1c**) which was previously classified as a non-NMDA agonist[4], were examined. Electrophysiological assay on the spinal motoneurons showed that the ethylene analog **2** has a potent depolarizing activity.[10] The potency of **2** was almost as high as those of NMDA and KA while that of **3** was 1/4 that of **2**. Their potency values are shown as relative values to KA (KA = 100%); 100% (**2**), 25% (**3**) and 2% (**1c**) (Figure 2A). Relative selectivity to non-NMDA receptors was determined by the following depression ratio using CNQX (non-NMDA type antagonist, 10 μM); AMPA (87%) > **1c** (64%) > KA = **3** (58%) > **2** (45%) (Figure 3).[11] Their depolarizing responses were also depressed in part by the selective NMDA blocker, D-AP5; NMDA (100%) > **2** (80%) >> **3** (48%) > **1c** (32%). Thus, both **2** and **3** were preliminarily classified as mixed agonists of non-NMDA type and NMDA type. In the present assay system, trans-BCG-IV (**1c**) was also found to be a mixed agonist.

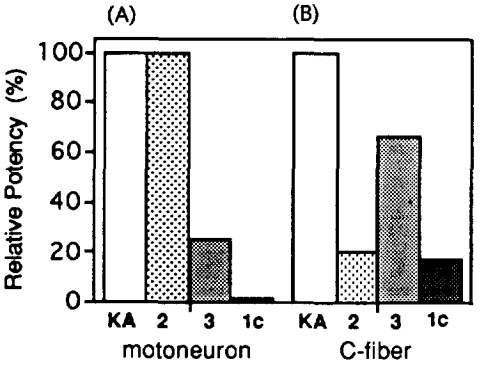


Figure 2. Depolarizing activities of the test compounds in the spinal cord motoneuron (A) and the dorsal root C-fiber (B).

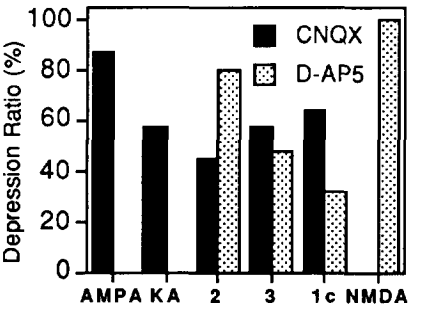


Figure 3. Inhibitory effects of CNQX and D-AP5 on 3'-substituted CCG-IV-induced depolarizing activities in the spinal cord motoneuron.

CNQX inhibits both KA and AMPA type receptors. Thus, an electrophysiological assay using KA-sensitive neurons in the isolated dorsal root C-fiber of new born rats was performed to classify their responses as either KA or AMPA type.[12,13] Relative potencies compared to KA were 20% (**2**), 67% (**3**), and 17% (**1c**),

respectively (Figure 2B). A marked depolarizing activity caused by the ethyl analog **3** indicated that this compound might have a potent KA-like activity. Alternatively, the potency of **2** was much lower than that in the spinal motoneurons.

To further characterize these compounds, we performed radioligand binding assays using rat brain synaptic membranes. All CCG-IV analogs tested here showed weak affinities toward the three ionotropic glutamate receptors; KA ([^{3}H]KA), AMPA ([^{3}H]AMPA), NMDA ([^{3}H]CGS-19755) subtypes.[14] Even the ethyl analog **3** had weaker affinity for binding to KA receptors than glutamate (Table 2). These results are not consistent with the above electrophysiological experiments, suggesting that the KA receptors on C-fiber may be different from that on motoneurons.[12b,15] Our results supported the hypothesis that the depolarization on dorsal roots is not caused by the activation of KA high affinity sites.[16]

Table 2. Inhibitory effects of excitatory amino acids and synthesized CCG-IV analogs on ^{3}H-labelled ligand binding to rat brain synaptic membranes.[a]

Compounds	[^{3}H]KA IC_{50} (μM)	[^{3}H]AMPA IC_{50} (μM)	[^{3}H]CGS-19755 IC_{50} (μM)
Glu	0.12	0.15	0.08
KA	0.0043	2.0	>100
(S)-AMPA	>100	0.0062	>100
NMDA	>100	>100	5.5
2	3.0	4.0	1.2
3	1.3	2.0	2.0
CCG-IV (**1a**)	1.6	0.60	0.0052
t-MCG-IV (**1b**)	4.0	4.0	50
t-BCG-IV (**1c**)	32	>100	21
CPG-IV	10	7.5	>100

[a]Values are the mean of three determinations. Incubation conditions were as follows; [^{3}H]KA, 1nM, 4°C, 1 h, 100 mM Tris-AcOH buffer (pH 7.1)[14a]; [^{3}H]AMPA, 5nM, 4°C, 1 h, 50 mM Tris-AcOH buffer (pH 7.4) containing 100mM KSCN[14b]; [^{3}H]CGS-19755, 10nM, 4°C, 1 h, 50 mM Tris-AcOH buffer (pH 8.0)[14c]. Specific binding was determined from difference in radioactivity in the absence and in the presence of 1mM L-glutamate.

It has been well documented that the saturated analogs of KA and CPG-IV cause significant decrease in the depolarizing activities of both motoneurons and C-fiber.[8,12b,17] However, the present results suggest that the CC double bond of the 3'-substituted CCG-IV analogs is not necessary to activate KA receptors. The structure-activity relationship studies between KA and the present analogs are currently being investigated.

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References and Notes

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10. Electrophysiological studies were performed on a modified version of the neonatal rat hemisected spinal cord preparation (Otsuka, M.; Konishi, S. *Nature*, **1974**, *252*, 733.) using tissue from 2-7 days old Wistar rats. Relative potency values (RP) were calculated as follows from kainate dose-response curve (0.1-10 μM) determined in each experiment. RP(%) = (Concentration of KA to produce the same effect X) / (Concentration of the test compound to produce an effect X) x 100. Each RP value was the mean of values from at least 3 different concentrations (1-10 μM) of the test compound.
11. Depression ratio was represented as average percentage decrease in hight of depolarization compared to pre- or post-antagonist control.
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