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SYNTHESES OF TRANS- AND CIS-α-(CARBOXYCYCLOPROPYL)GLYCINES. NOVEL NEUROINHIBITORY AMINO ACIDS AS L-GLUTAMATE ANALOGUE

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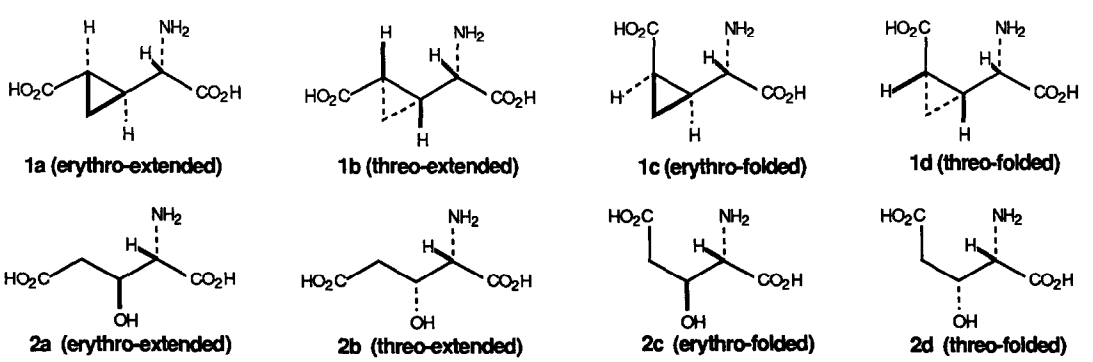
Summary: Four diastereoisomers of α-(carboxycyclopropyl)glycines were synthesized from (2*S*)-2-amino-3-butenol via an inter- or intramolecular cyclopropanation. Results of neurobiological assay using a β-hydroxy-L-glutamate sensitive neuron indicated clear conformation-activity relationship between these synthetic L-glutamate analogues.

Glutamate receptions in the central nervous systems have attracted much attention in the life sciences. Recently, several glutamate analogues such as kainic acid[1] and domoic acid,[1b,2] possessing an L-glutamate moiety as a part of their structures, have become important tools in neuropharmacology because of their potent agonistic behavior. While structure-activity relationship between L-glutamic acid and these agonists cannot yet be clarified to date, it has been proposed that L-glutamic acid adopts a specific conformation (extended or folded) when it interacts with the receptors of some neurons.[3]

Recently, Takeuchi et al. reported pharmacological studies using a variety of L-glutamate analogues on identifiable giant neurons, sensitive to threo and/or erythro β-hydroxy-L-glutamic acid (L-BHGA), of an African giant snail (*Achatina fulica Ferussac*).[4] Through these studies, recognition of the β-configuration of L-BHGA by the receptors was observed. We focused on explaining the role of the hydroxyl group in fixing the conformation and/or as a H^+ donner-acceptor. As shown in **Scheme I**, we believed

Scheme I

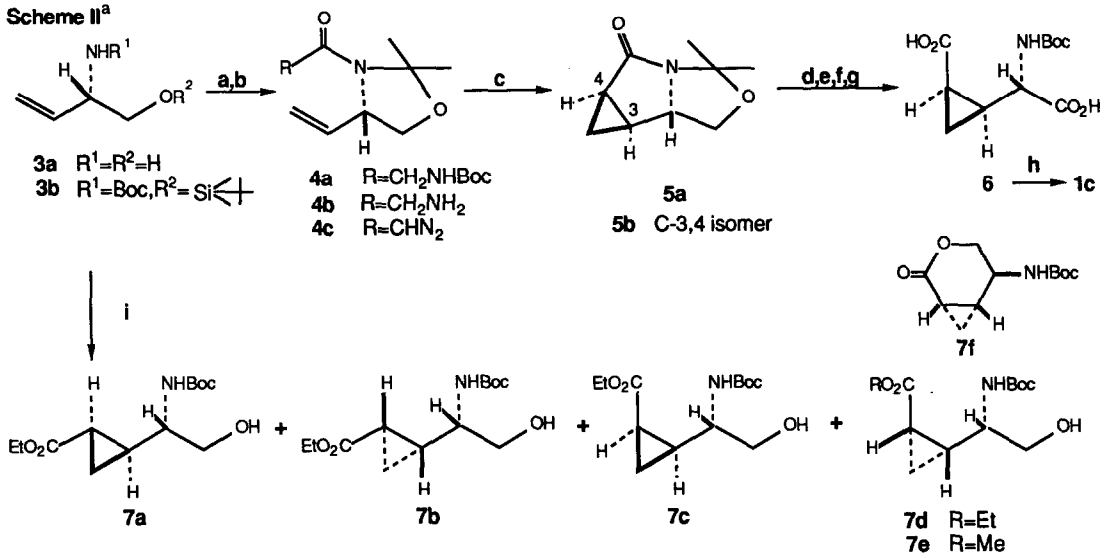


that four BHGA conformational-configurational isomers **2a-2d** would be mimicked by four α-(carboxycyclopropyl)-glycines (CCG) **1a-1d**. CCGs **1a** and **1c** have been isolated from the *Spindaceae* family by Fowden et al.[5,6] We wish to report here the stereoselective synthesis of naturally occurring cis-CCG **1c** and the simple syntheses of the four isomers **1a-1d** from chiral 2-amino-3-butenol **3**.[7] The preliminary bioassay results using L-BHGA sensitive neurons are also described.

For the synthesis of cis-CCG **1c**, we planned to use the intramolecular cyclopropanation of the diazoamide **4c** as the key step. The N-*tert*-butoxycarbonyl (*t*-Boc) group of the dipeptide **4a**, prepared from (2*S*)-2-amino-3-butenol **3a** in two steps [(i) N-*t*-Boc-glycyl-O-succinate and (ii) 2,2-dimethoxypropane/*dl*-camphorsulfonic acid (CSA), 62%], was removed selectively with trimethylsilyl trifluoromethanesulfonate (TMSOTf)/2,6-lutidine[8] to give exclusively the desired amine **4b**. Sequential treatments of the amine with (i) $NaNO_2$/pH 3 buffer and (ii) catalytic palladium (II) acetate yielded the cycloadduct **5a** and **5b** (43%, **5a**/**5b**=6/1). The major isomer, having desired strereochemistry, was purified by SiO_2 column chromatography and was converted to the N-*t*-Boc derivative **6** by the following sequence of reactions: (i) removal of the N,O-acetonide with 60% AcOH, (ii) hydrolysis of the amide with 0.5 N NaOH, (iii) protection of the resulting amine with a *t*-Boc group, and (iv) Jones oxidation (59% from **5a**). Finally, deprotection with trifluoroacetic acid (TFA) provided the desired CCG **1c** (mp 192-197 °C, $[\alpha]_D^{25}$ +20.8° (*c* 0.52, H_2O)), identical in all respects with natural **1c**.[5]

Intermolecular cycloaddition of ethyl diazoacetate with the silyl ether **3b** gave a mixture of cyloadducts **7a-7d** in 41% yield (**7a**/**7b**/**7c**/**7d** =1.2/3.5/1/1; 58% of the starting **3b** was recovered and recycled). After removal of the silyl group with CSA/EtOH, **7b** (Rf 0.46 in ether/hexane=3/1) and **7c** (Rf 0.30) were separated from the above mixture by medium pressure column chromatography. Since **7a** and **7d** were found to have the same Rf value (0.38), these were treated with CSA/CH_2Cl_2 to give a separable mixture of **7a** and δ-lactone **7f** (Rf 0.30 in ether/hexane=3/1). Hydrolysis and esterification of **7f** provided **7e** quantitatively. Thus, each of the isomers **7a-7c** and **7e** were converted to the desired **1a-1d**[9,10] by the following sequence of reactions: (i) Jones oxidation, (ii) 0.5 N NaOH, and (iii) TFA (ca. 80%, 3 steps).

These synthetic CCGs **1a-1d** were submitted for neurobiological assay using the periodically oscillating neuron (PON), which is sensitive to β-hydroxy-L-glutamic acid, of an African giant snail.[4] In PON receptors, inhibitory effects by L-BHGA were observed: the minimum effective concentration (MEC) of erythro-L-BHGA is ca. 10^{-5} M, and that of the threo isomer is ca. 10^{-4} M. The effective potency quotient (EPQ) was calculated: (MEC of erythro-L-BHGA)/(MEC of the substrate). As shown in **Scheme I**, **1a** can be viewed as being conformationally fixed in the erythro-extended conformation **2a**. Likewise, **1b-1d**



[a](a) (1) N-*t*-Boc-Glycyl-O-Succinate, Et_3N, tetrahydrofuran, -20 °C, 3 h; (2) 2,2-dimethoxypropane, acetone, CSA, 80 °C, 16 h; (b) (1) 1.5 equiv TMSOTf, 2.0 equiv 2,6-lutidine, room temperature, 15 min; (2) $NaNO_2$, citric acid, pH 3, 0 °C; (c) 0.05 equiv $Pd(OAc)_2$, toluene, 80 °C, 2h; (d) 60% acetic acid, room temperature, 16 h; (e) 0.5 N NaOH, 70 °C, 4 h; Di-*tert*-butyl dicarbonate, Et_3N, dioxane-H_2O (1:1), room temperature, 16 h; (g) Jones reagent, acetone, 0 °C, 14 h. (h) (1) TFA, 0 °C, 30 min; (2) Dowex 50Wx4 (elution with 3% NH_3; (3) 1 N HCl, pH 3.0; (i) (1) ethyl diazoacetate, 0.05 equiv $Pd(OAc)_2$, room temperature, 4 h; (2) CSA, EtOH, room temperature, 16 h.

corresponds to **2b-2d**. Biossay results are summarized as follows: erythro-L-BHGA **2a** or **2c** (EPQ=1), threo-L-BHGA **2b** or **2d** (0.1), **1a** (30), **1b** (0.03), **1c** (no effect), and **1d** (3). Since the erythro-extended isomer **1a** had marked effect and the threo **1b** and the folded **1c** showed almost no effect, this suggests that the erythro configuration and the extended conformation of **1a** are recognized by the receptor. Therefore, we assume that an active conformation of the erythro-L-BHGA would be the extended **2a** which is mimicked by the erythro-extended **1a**: the role of the β-hydroxyl group of erythro-L-BHGA is to fix the glutamate chain in the extended conformation when it interacts with the receptor. On the other hand, the threo-folded **1d** (EPQ=3) which mimics the threo-folded **2d** exhibited a marked effect compared with that of threo-L-BHGA **2b** or **2d** (EPQ=0.1), while the extended **1b** and the erythro **1c** had almost no effect. We propose the presence of a distinct threo-folded sensitive receptor on PON where the extended conformation is recognized: the β-hydroxyl group of threo-L-BHGA would fix the glutamate chain in the folded conformation (see, **2d**).[11,12]

We hope these compounds will be useful tool to investigate the nature of L-glutamate receptors in view of conformation-activity relationship not only in the molluscan nervous systems but also in mammalian brains. Further studies related this work using **1a-1d** and other glutamate analogues on other neurons are in progress.

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References and Note

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9. The structures **1a** and **1c** were determined by comparison of their spectroscopic data with those of natural **1a** and **1c**, respectively. Since **7d** formed δ-lactone, the structure **1d** can be assigned as depicted. Melting points and $[\alpha]_D$ values of **1a**, **1b**, and **1d**. **1a**: mp 243-247 °C (decomp); $[\alpha]_D^{25}$ +102.0° (*c* 0.5, H_2O). **1b**: mp 255-258 °C (decomp); $[\alpha]_D^{25}$ -20.2° (*c* 0.51, H_2O). **1d**: mp 178-180 °C; $[\alpha]_D^{25}$ +97.1° (*c* 0.52, H_2O).
10. All new compounds exhibited satisfactory ^1H NMR, IR, MS, and elementary analytical or HRMS data.
11. The same EPQ ratio of **1a**/erythro-L-BHGA and **1d**/threo-L-BHGA (30/1) suggests the presence of both erythro- and threo-L-BHGA sensitive receptors on PON in a ratio of 10/1 since these EPQ ratio was 10/1.
12. Detailed results of the neurobiological assay related this work, to be published.

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