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PREPARATION OF DIFLUORO ANALOGS OF CCGs AND THEIR PHARMACOLOGICAL EVALUATIONS

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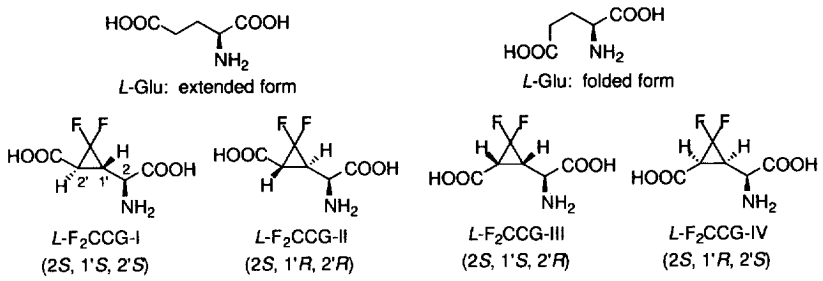
Abstract: All the stereoisomers of 2-(2-carboxy-3,3-difluorocyclopropyl)glycines (F_2CCGs) were synthesized in enantiomerically pure forms using (*R*)-2,3-*O*-isopropylideneglyceraldehyde as a chiral precursor. L-F_2CCG-I, one of the stereoisomers corresponding to an extended form of L-glutamate was found to be a potent agonist for metabotropic glutamate receptors (mGluRs). © 1998 Elsevier Science Ltd. All rights reserved.

2-(2-Carboxycyclopropyl)glycines (CCGs), which can be considered as conformationally restricted analogs of L-glutamate, an excitatory neurotransmitter, have been recognized to serve as useful tools for elucidating the conformational requirement (extended or folded form) for the L-glutamate receptor subgroup specificity.[1,2] In particular, L-CCG-I (2*S*,1'*S*,2'*S*-isomer) corresponding to an extended form was found to be a potent and selective metabotropic glutamate receptor (mGluR) agonist, whereas L-CCG-IV (2*S*,1'*R*,2'*S*-isomer) possessing a folded form was endowed high affinity for NMDA receptor, classified as one of ionotropic glutamate receptors (iGluRs).[1,3,4] On the basis of these neuropharmacological achievements by employing such conformationally restricted analogs, we became interested in introduction of fluorine atom onto the cyclopropane ring of CCGs. It would be expected that introduction of fluorine brings about a little change in steric size of the molecule, but enhancement of acidity of carboxyl group due to the electron-withdrawing nature of fluorine and/or the molecule has a more conformationally restricted structure by the formation of an intramolecular hydrogen bond between fluorine and carboxyl group when these two groups are in a *cis*-configuration.[5] Although we have reported a highly diastereoselective synthesis of *trans*-2-(2-carboxy-3,3-difluorocyclopropyl)glycine (F_2CCG) using 4-bromo-4,4-difluorocrotonate derivative,[6] it was needed to prepare all the stereoisomers in enantiomerically pure forms to examine the fluorine-substituent effect on receptor subtype specificities. In this paper, we report the preparation of all the stereoisomers of 2-(2-carboxy-3,3-difluorocyclopropyl)glycines (F_2CCGs) in enatiomerically pure forms using (*R*)-2,3-*O*-isopropylideneglyceraldehyde as a chiral precursor.



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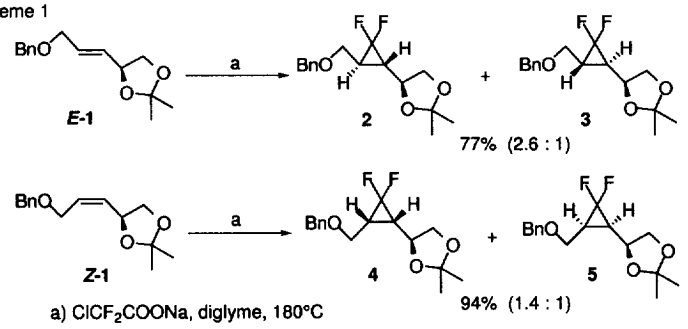
Furthermore, as a preliminary result of pharmacological evaluation of these analogs, it should be noted that L-F_2CCG-I, (2*S*,1'*S*,2'*S*) isomer, one of the stereoisomers corresponding to an extended form of L-glutamate was found to be a potent mGluRs agonist; 2-3 times more active than L-CCG-I in causing deplarization and depression of monosynaptic component of the spinal reflex in the isolated spinal cord of newborn rats.

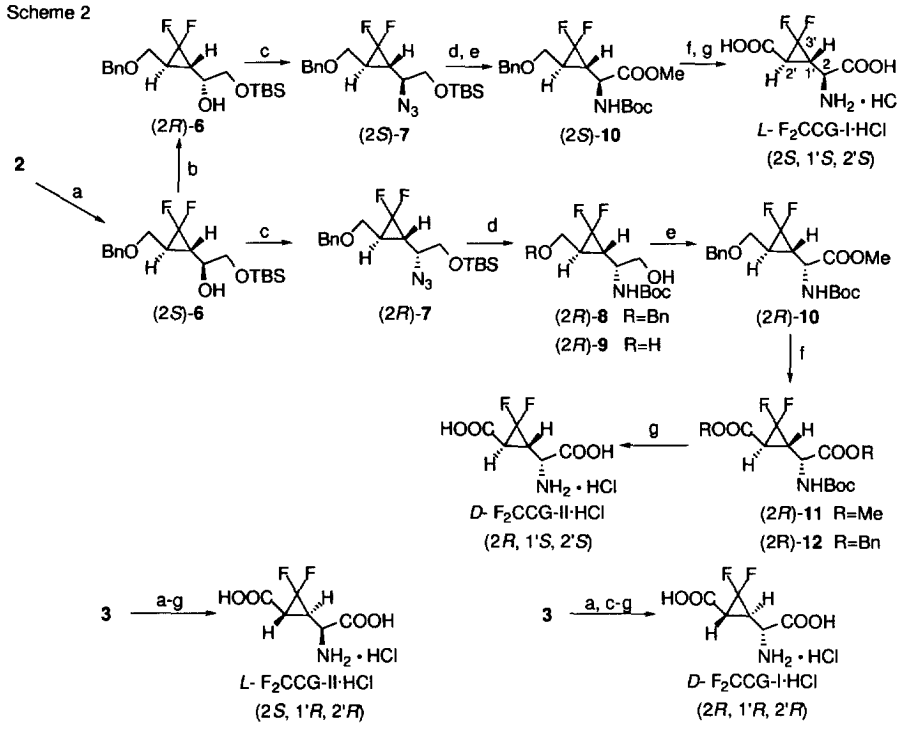
For the synthesis of F_2CCGs, we chose both *E*- and *Z*-isomers of the particular allylic alcohol derivative **1**[7] derived from (*R*)-2,3-*O*-isopropylideneglyceraldehyde due to the suitable functionalities. Difluorocarbene addition to ***E*-1** and ***Z*-1** proceeded in stereospecific manner to give the difluorocyclopropanes; *trans*-isomers **2**, **3** from ***E*-1** and *cis*-isomers **4**, **5** from ***Z*-1**, respectively (Scheme 1).[8] Although, in both cases, diastereoselectivities were low; 2.6 : 1 for ***E*-1** and 1.4 : 1 for ***Z*-1**, separation of each diastereomer could readily carried out by silica gel column chromatography. The absolute stereochemistry of each difluorocyclopropane was determined by X-ray analysis after conversion to an appropriate crystalline derivative.[8,12]

Scheme 1



As a typical procedure, preparation of L-F_2CCG-I or D-F_2CCG-II from **2** and L-F_2CCG-II or D-F_2CCG-I from **3** is illustrated in Scheme 2. For the synthesis of D-series of F_2CCGs, conversion of the secondary hydroxyl group having *S*-configuration to amino functionality in an inversion manner is required. Thus, the mono silylated alcohol **(2*S*)-6**, obtained from **2** by conventional procedure, was converted to the azide **(2*R*)-7** through S*N*2 replacement by employing Shioiri's method.[9] Selective hydrogenation of azide group (H_2, 5% Pd-C, AcOEt) followed by protection of the resulting amino group by Boc and desilylation provided the *N*-Boc alcohol **(2*R*)-8**. Each step proceeded in high yield (averagely over 90% yield). One step oxidation of the two hydroxyl groups in **(2*R*)-9**, obtained by debenzylation (H_2, $Pd(OH)_2$, AcOEt) of **(2*R*)-8**, to the dicarboxylic acid was tried, but resulted in a complex mixture. Therefore, we carried out stepwise conversion to the dicarboxylate using mono-protected alcohol **(2*R*)-8**. Oxidation of **(2*R*)-8** with $RuCl_3$-$NaIO_4$ system[10] or Jones reagent and the subsequent treatment with CH_2N_2 provided the methyl ester **(2*R*)-10** in 50-85% yield, which was, in turn, debenzylated by hydrogenolysis and converted to the dimethyl ester **(2*R*)-11** by oxidation of the ω-hydroxyl group followed by CH_2N_2 treatment. A clean conversion of the dimethyl ester **(2*R*)-11** to the desired D-F_2CCG-II hydrochloride

Scheme 2



a) i) HCl, MeOH, ii) TBDMS-Cl, Imidazole; b) i) PhCOOH, DEAD, Ph_3P, ii) 1*N* KOH; c) $(PhO)_2P(O)N_3$, DEAD, Ph_3P; d) i) H_2, 5% Pd-C, AcOEt, ii) Boc_2O, $NaHCO_3$, iii) 5% HCl, MeOH; e) $RuCl_3$, $NaIO_4$ or Jones reagent then CH_2N_2; f) i) H_2, $Pd(OH)_2$, AcOEt, ii) $RuCl_3$, $NaIO_4$ or Jones reagent then CH_2N_2 (**11** from **10**), iii) $Ti(OBn)_4$, BnOH (**12** from **11**); g) i) H_2, $Pd(OH)_2$, AcOEt, ii) 10% HCl, rt

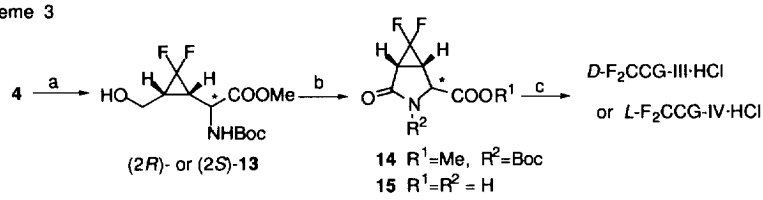
was achieved through the dibenzyl ester **(2*R*)-12** obtained by ester-exchange reaction of **(2*R*)-11** with benzyl alcohol and $Ti(OBn)_4$ (49% yield from **(2*R*)-10**). Hydrogenolysis of the dibenzyl ester and the final deprotection of *N*-Boc group by treating with 5% HCl at room temperature gave D-F_2CCG-II hydrochloride in 64% yield.[11]

In a similar manner and nearly the same yield in each step as above except for the inversion of the *S*-configurated secondary hydroxyl group to the *R*-configuration, L-F_2CCG-I as its hydrochloride form was synthesized from **2**. Thus, the mono silylated alcohol **(2*R*)-6** was obtained in 47% yield by Mitsunobu reaction (benzoate synthesis) of **(2*S*)-6** followed by alkaline hydrolysis.

The *trans*-difluorocyclopropane **3** was converted to D-F_2CCG-I and L-F_2CCG -II by employing the essentially same procedure as above (Scheme 2).[11]

In the synthesis of F_2CCG-III and F_2CCG-IV starting from the *cis*-difluorocyclopropane **4** or **5** (Scheme 3), construction of 2-amino acid ester part leading to **13** was achieved by the similar manner for the preparation of **10** shown in Scheme 2 (ca 90% yield for each step). Oxidation of the ω-hydroxyl group of the alcohol **13** resulted in the formation of the lactam derivative **14** (64-86% yield).[12] Hydrolysis of ester and lactam moieties along with removal of the *N*-Boc group was carried out by treating

Scheme 3



a) i) same as for **10** in Scheme 2, ii) H_2, $Pd(OH)_2$, AcOEt; b) Jones reagent; c) 10% HCl, 50 °C

14 with 10% HCl at 50 ℃ to give a mixture of the desired F_2CCG-III or -IV hydrochloride (29-53 % yield)[11] and the NH-lactam acid **15** (41-13 % yield) accompanied by decomposition in some extent. An attempt was made to complete hydrolysis at higher temperature or prolonged reaction time, but a complex reaction mixture resulted.

Table 1. Depolarization Activities of F_2CCGs [a]

F_2CCGs	Threshold Concentration (μM)	Ratio of Depolarizing activity [b]	Activated Receptors	F_2CCGs	Threshold Concentration (μM)	Ratio of Depolarizing activity [b]	Activated Receptors
L-F_2CCG-I	2	3	mGluR I	*D*-F_2CCG-I	30	1.2	NMDA
L-F_2CCG-II	300	0.5	mGluR I NMDA	*D*-F_2CCG-II	10	0.1	NMDA
L-F_2CCG-III	100	2	NMDA	*D*-F_2CCG-III	1000	<0.1	not determined
L-F_2CCG-IV	1	1.5	NMDA	*D*-F_2CCG-IV	1000	<0.01	not determined

a) In the spinal cord of newborn rats. The depolarization caused by each F_2CCG was estimated in the Mg^{2+}-free, tetrodotoxin (0.5 μM) containing solution. Each compound was administered to perfusing solution for a period of 10 s. b) Relative activity in depolarization to that of the corresponding CCG (CCG = 1) determined by comparing with the threshold concentration.

Neuropharmacological activities of F_2CCGs (used as their hydrochloride forms) were evaluated as compared with those of the corresponding CCG isomers[1] in the isolated spinal cord of newborn rats.[13] Their depolarization activities are shown as their threshold concentrations and relative potencies to those of the corresponding CCG isomers (Table 1). The depolarization responses were measured at various concentrations (10^{-7}-10^{-3} M)[1a] to reveal that the incorporation of geminal fluorines on the cyclopropane ring of CCGs did not alter these responces markedly and the relative potency of each isomer was comparable to that of the corresponding parent CCG except for those of D-F_2CCG-III and D-F_2CCG-IV, which are remarkably less active than D-CCG-III and D-CCG-IV, respectively. The depolarization response caused by L-F_2CCG-I was only sightly decreased by the selective NMDA receptor antagonist (100 μM), D-AP5 (D-2-amino-5-phosphonovaleric acid)[14] and was not affected by the non-NMDA receptor antagonist (blocker) (100 μM), CNQX (6-cyano-7-nitroquinoxaline-2,3-dione).[15] The depolarization action of L-F_2CCG-II was weak (threshold concentration about 300 μM), with the depolarization reduced to about 55% of the control by D-AP-5 (100 μM) and the residual depolarization unaffected by CNQX (100 μM). On the other hand, those of the other isomers, L-F_2CCG-III, L-F_2CCG-IV, D-F_2CCG-I and D-F_2CCG-II, were almost completely depressed by D-AP-5. The depolarization responses of L-F_2CCG-I and L-

F_2CCG-II were depressed by a relatively non-selective mGluR antagonist (1mM), MCPG (α-methyl-4-carboxyphenylglycine).[16] It seems likely that L-F_2CCG-I activates mGluRs including group I mGluRs, L-F_2CCG-II acts as both NMDA receptor and mGluRs agonist, while L-F_2CCG-III, L-F_2CCG-IV, D-F_2CCG-I, D-F_2CCG-II can be classified as NMDA-type iGluR agonist. It should be noted, as one of the most marked pharmacological changes induced by the geminal fluorination on the cyclopropane ring, that L-F_2CCG-III did not possess the inhibitory action of Na^+-dependent uptake of L-glutamate.[17]

To determine the activation of group II mGluR which is coupled to the inhibition of forskolin-stimulated cyclic AMP formation, the depressions of spinal reflex induced by F_2CCGs were measured.[1a] The potency of L-F_2CCG-I to depress the spinal reflex (threshold concentration 0.03 μM) was found to be 3 times more active than that of L-CCG-I. This response induced by L-F_2CCG-I was inhibited by a group II mGluR antagonist (0.3-1.0 mM), MCCG-I [(2*S*,3*S*,4*S*)-α-methyl-2-(2-carboxycyclopropyl)glycine][18] and a group III mGluR antagonist (0.3 mM), MAP4 ((*S*)-2-amino-2-methyl-4-phosphonobutanoic acid).[19] These results possibly suggest that L-F_2CCG-I is classified as mGluRs agonist.[20]

Although the effect of fluorine-substitution on the pharmacological activities cannot be definitely explained at this moment, these fluoro analogs would be useful pharmacological tools for the study of GluRs.[21]

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11. All the F_2CCG hydrochlorides, which were synthesized in pure forms, were fully characterized by the ^{1}H-, ^{13}C- and ^{19}F-NMR spectra and HPLC analysis (ODS column). Their $[\alpha]_D$ values (in H_2O at 27 ℃) are as follows: L-F_2CCG-I, +50.6; L-F_2CCG-II, +40.7; L-F_2CCG-III, -13.9; L-F_2CCG-IV, +70.8.
12. To confirm the absolute stereochemistry of the *cis*-series of F_2CCGs, X-ray crystallographic analysis of one of the lactam derivatives, (2*R*,1'*S* , 2'*R*)-**14**, was carried out.
13. Details will be reported by Saitoh *et al.* Saitoh, T.; Ishida, M.; Shinozaki, H. *Br. J. Pharmacol.* in press.
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20. It should be also noted that L-F_2CCG-I showed the "priming effect" related to the inhibitory action on the monosynaptic spinal reflex. See ref. 13.
21. As expected, introduction of fluorine atom onto the cyclopropane ring of CCG brings about increases in acidities of the carboxyl group and the conjugate acid of amino group as measured the p*K*a values by ^{1}H-NMR titration experiment in D_2O, in particular pK_2 and pK_3 values are markedly lowered.[4e] pK_1, pK_2 and pK_3 Values are as follows: 2.1, 2.6, 9.2 for L-F_2CCG-I; 2.2, 4.3, 9.7 for L-glutamic acid and 1.9, 4.2, 9.7 for CCG-I, respectively.