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SYNTHESIS AND NEUROBIOLOGICAL ACTIONS OF PYRROLIDINE-2,3-DICARBOXYLIC ACIDS (PRDA). CONFORMATIONALLY RESTRICTED ANALOGUES OF *L*-ASPARTATE

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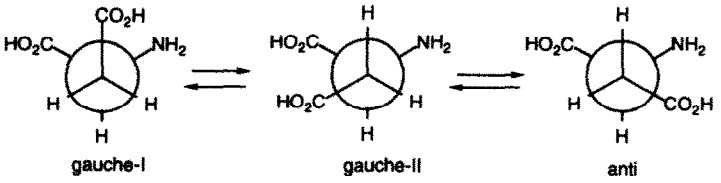
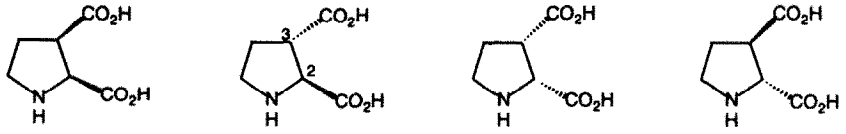
Abstracts: Four stereoisomers of PRDAs **1~4**, a novel aspartate analogue incorporating a pyrrolidine ring, were synthesized in an enantiomerically pure form starting from a chiral synthon, 5-amino-5,6-dihydropyrone. Electrophysiological experiments of **1~4** in newborn rat spinal cords showed a variety of depolarizing activities. The conformational studies of PRDAs and their activity profile suggested that the gauche and the anti conformers about two carboxylic acid groups of Asp could be active forms to NMDA and non-NMDA receptors, respectively.

L-Glutamic acid and *L*-aspartic acid cause depolarization of mammalian central neurons.[1] It is reasonable to assume that the primary binding sites of these excitatory amino acids (EAAs) are their three polar functional groups,[2] and in the case of *L*-aspartate, the spatial arrangement of its functional groups seems essential for activation of NMDA receptors. As shown in Figure 1, *L*-aspartate has three representative rotamers, gauche-I, gauche-II and anti, which equilibrate freely under ambient conditions. Among them, the spatial position of their polar functional groups is varied. In the present study, we describe the syntheses and pharmacological actions of the title compounds **1~4** which partially restrict the free rotation of *L*-aspartate.

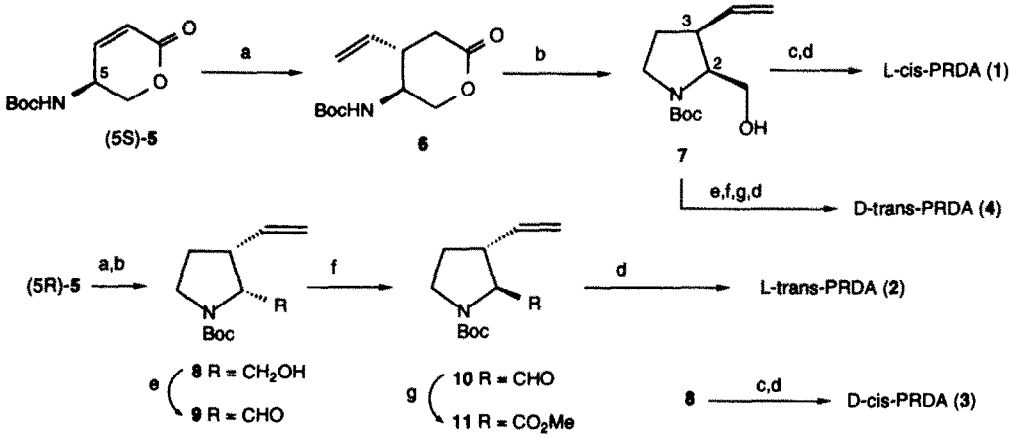
The synthesis of (2*S*,3*R*)-pyrrolidine-2,3-dicarboxylic acid (*L*-cis-PRDA:**1**) was started from (5*S*)-5-(*N*-*tert*-butoxycarbonyl)amino-5,6-dihydro-2-pyrone (**5**), a chiral synthon previously reported by us.[3] This was treated with vinyl magnesium bromide in the presence of CuBr-DMS to give in 78% yield the trans adduct **6**, exclusively. Reduction of **6** with diisobutylaluminum hydride afforded the corresponding lactol, which upon treatment with formic acid followed by adjustment of pH to10~12 with 6 *N* KOH gave the 5-membered ring imine. This was reduced with $LiAlH_4$ and the resulting imino group was protected with di-*tert*-butyl dicarbonate (Boc_2O) to give pyrrolidine **7** where the C2 and C3 substituents are in a *cis*-configuration. Conversion of **7** into *cis*-PRDA (**1**)[4] was performed by the following sequence of reactions: (1) oxidation of the hydroxyl group with pyridinium dichromate (PDC), (2) esterification of the resulting carboxylic acid with CH_2N_2, (3) hydrolysis of the ester group with 1 *N* KOH, and (4) cleavage of the vinyl group by ozonolysis followed by oxidative degradation of the resulting ozonide in formic acid and 30% H_2O_2. The synthesis of (2*S*,3*S*)-PRDA (*L*-*trans*-PRDA, **2**)[5] was performed from (5*R*)-**5**. (2*R*,3*R*)-Pyrrolidine **8** was obtained in the same manner as the preparation of (2*S*,3*S*)-pyrrolidine **7**. After oxidation of **8** with $(COCl)_2$/dimethylsulfoxide (DMSO), exclusive epimerization at C2 was effected by treatment of the resulting aldehyde **9** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the desired *trans* aldehyde **10**. Oxidation of **10** with $NaClO_2$ in phosphate buffer followed by esterification with CH_2N_2 gave ester **11** which was transformed into **2**[4] in the same manner as used for **1**. Using the above synthetic processes, *D*-*cis*-PRDA (**3**)[4] was prepared from **8**, and the *D*-*trans*-isomer (**4**)[4] was synthesized from **7**.

Figure 1



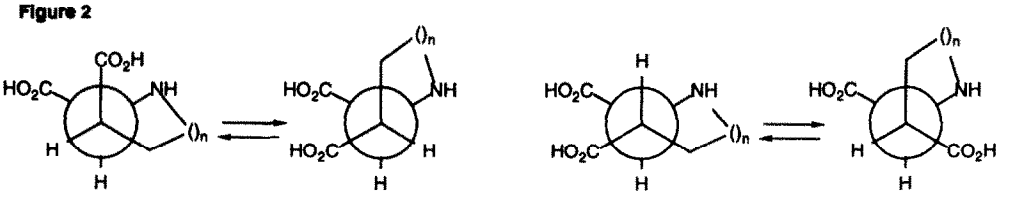


Scheme I[a]



[a](a) Vinylmagnesium bromide, CuBr-SMe_2, THF, -78 °C; (b) (1)$^{i}Bu_2AlH$, (2) HCO_2H; (3) $LiAlH_4$; (4) Boc_2O; (c) (1) PDC, DMF; (2) CH_2N_2; (d) (1) KOH; (2) O_3, MeOH, -78 °C; (3) HCO_2H, H_2O_2; (e) Swern Ox.(f) DBU; (g) (1) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene; (2) CH_2N_2

Figure 2



n = 1 cis-PRDA (1)
n = 2 cis-PDA (12)

n = 1 trans-PRDA (2)
n = 2 trans-PDA (13)

Table 1 Neuropharmacological prophile of PRDAs (1~4) and representative EAAs in the newborn rat spinal motoneurone.[6]

Substrate	Depolarizing activity Relative potency ratio (L-Glu=1)	Receptor subtype(s)
L-glutamate	1	mixed type
NMDA	50	NMDA type
L-cis-PRDA (1)	0.1	NMDA type
L-trans-PRDA (2)	50	mixed type (NMDA & AMPA)
D-cis-PRDA (3)	3	NMDA type
D-trans-PRDA (4)	5	mixed type (NMDA & AMPA)
dl-cis-PDA (12)*	10	NMDA type (ref 7)
dl-trans-PDA (13)*	20	NMDA type (ref 7)

*PDA : piperidine-2,3-dicarboxylic acid[7]

Preliminary accounts of electrophysiological experiments of **1~4** in the newborn rat spinal motoneurone were summarized in Table 1.[6]The depolarizing responses of the cis isomers **1** and **3** were due to the activation of NMDA receptors and were completely depressed by the selective antagonists, Mg^{2+} and CPP (3-(carboxypiperazin-4-yl)-propyl-1-phosphonic acid). The depolarizations induced by the trans isomers **2** and **4** were depressed in part in the presence of the NMDA antagonists, but were completely depressed in the presence of an additional KA & AMPA antagonist, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione).[8] Furthermore, these isomers did not show any depolarizing responses in the dorsal root C-fiber of immature rats.[9] These results suggest that trans isomers **2** and **4** are classified as mixed agonists of NMDA and non-NMDA (putatively, AMPA type agonists) receptors.

Due to a conformationally flexible five-membered ring,[10] *trans*-**2** exists as a mixture of two conformers, gauche-IIb and anti. *cis*-PRDA (**1**) is also present as a mixture of gauche-I and gauche-IIa conformers. The *cis*-**1** and *trans*-**2** form similar conformers, gauche-IIa and IIb, respectively, which have the same arrangement of three polar functional groups like gauche-II of Asp, but the configurations at C3 are opposite each other (Figure 2). Because *cis*-**1** was found to be a markedly weak agonist for NMDA receptors, the gauche-I rotamer of *L*-aspartate could be eliminated as an active rotamer of *L*-aspartate. The C4,5 methylene groups of the gauche-IIa conformer of *cis*-**1** would sterically hinder the binding to the receptor. On the other hand, *trans*-**2** is a potent agonist for NMDA receptors suggesting that the active rotamer of *L*-aspartate for NMDA receptors is either gauche-IIb or anti. The following characteristics of *trans*-piperidine-2,3-dicarboxylic acid (PDA) (**13**) supported the speculation that the gauche-IIb rotamer would be an active rotamer for NMDA receptors: (1) trans-**13** is a selective agonist for NMDA receptors, (2) its 2,3-diequatorial conformation is a rigid and fixed *L*-aspartate fragment in the gauche-II rotamer, and (3) its spatial arrangement of the polar functional groups is almost superimposable on those of gauche-IIb of **2** (Figure 2). However, only the *dl*-form of PDA is available to date, and the possibility that the active form of *trans*-PDA is the *D*-isomer cannot be eliminated . AMPA-type responses to *trans*-**2** would be induced by the anti conformer. The conformers with two carboxylic acids on the same side such as gauche-IIb seem to activate NMDA receptors and the conformers with two carboxylic acids on the opposite side like the anti conformer seem to activate AMPA type receptors.

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References and Notes

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4. Mp for **1**~**4**; >300°C(decomp). $[\alpha]_D$ for **1**; -39.2°(*c* 0.12, H_2O), **2**; +34.0°(*c* 0.15, H_2O), **3**; +37.5°(*c* 0.20, H_2O), **4**; -32.8°(*c* 0.125, H_2O). ^1H NMR for **1**(400MHz, D_2O, HOD=4.8ppm, pD=3.3); 2.32(1H, dddd, *J*=4, 4.5, 8, 13.5Hz) 2.44(1H, dt, *J*=13.5, 8.5Hz) 3.47(1H, ddd, *J*=4.5, 8.5, 11Hz) 3.52(1H, dt, *J*=4, 8Hz) 3.59(1H, dt, *J*=11, 9Hz) 4.36(1H, d, *J*=7.5Hz). **2**(pD=2.7); 2.28(1H, dq, *J*=13, 7Hz) 2.37(1H, dq, *J*=13, 7.5Hz) 3.41(1H, dt, *J*=8.5, 6Hz) 3.45(1H, dt, *J*=12, 7.5Hz) 3.50(1H, dt, *J*=12, 7Hz) 4.50(1H, d, *J*=6Hz).
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   It has been reported that the dorsal root C-fiber of immature rats is depolarized selectively by the kainate agonists but not by NMDA, and high concentrations of AMPA cause a slight depolarization of the C-fibre. In this preparation, trans-2 and 4, NMDA, D-glutamate, D- andL-aspartate did not showed any responses.
10. *cis*-PDA (**12**) can exists as a mixture of rotamers as can *cis*-**1**, and its activity is less than that of *trans*-PDA(**13**). In addition, the cis-compound **12** is known to be an antagonist of EAA receptors.[7] On the other hand, the conformation of *trans*-PDA (**13**) is rather inflexible but is fixed as the chair form of the 6-membered ring with diequatorial 2,3-dicarboxyl moieties which are quite similar to the gauche-IIb type of trans-PRDA (**2**). It is well known that the conformation of 1,2-disubstituted cyclopentane is rather flexible, and the energy barrier for its conformational change (gauche to anti) requires less than several Kcal/mol which is much smaller than that of trans-2,3-PDA. These facts were well elucidated by the ^1H NMR *J* values of **1** and **2** at C2H (*J*=7.5Hz (pD=3.3) and 6Hz (pD=2.7), respectively) which are typical values for the vicinal protons attached to a freely rotating C-C bond. Those of PDAs (**12**; *J*=3.4Hz (pD=2.7), **13**; *J*=9.3Hz (pD=2.9)) showed that they take a rather rigid chair form.

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