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Absolute configuration of a novel glutamate receptor antagonist kaitocephalin

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Abstract—The absolute configuration of kaitocephalin, a novel glutamate antagonist, was determined by NMR spectral analysis of a cyclic derivative of kaitocephalin and by the modified Mosher's method to be 2*S*,3*S*,4*R*,7*R* and 9*S*.

In the course of our screening for AMPA/kainate (KA) antagonists using chick telencephalic neurons, we isolated a novel AMPA receptor antagonist designated kaitocephalin (**1**) (Fig. 1) from *Eupenicillium shearii*.[1] The planar structure of **1** was elucidated to consist of a dichlorohydroxybenzoate substructure and a pyrrolidine moiety with tricarboxylic acids. The latter moiety was assumed to derive from three amino acids. Compound **1** was proved to be a novel AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor antagonist and it also acted on NMDA receptor. However, the absolute structure of **1**, which was essential to pursue the structure–activity relationship and to develop more potent and selective derivatives, remained to be clarified. Here we describe the establishment of the absolute structure of **1**.

Since the production yield of **1** was considerably poor, chemical degradation of **1** was not a method of choice for determination of its absolute structure. Therefore, we employed NMR techniques on derivatives of **1**, which were easily prepared with high yield by simple chemical manipulations. A secondary hydroxyl group at C-3 was a preferred target for application of the modified Mosher's method. We attempted to prepare cyclic derivatives of **1**, which had been expected to be useful for the relative and absolute stereochemical studies by NMR. At first we assumed that cyclic derivatives

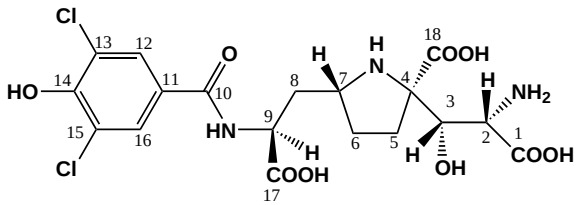
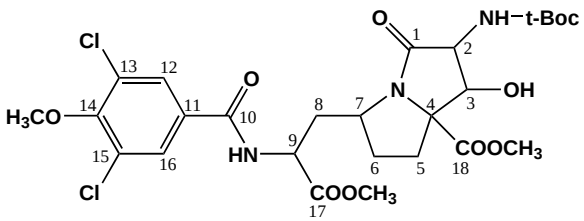


Figure 1.



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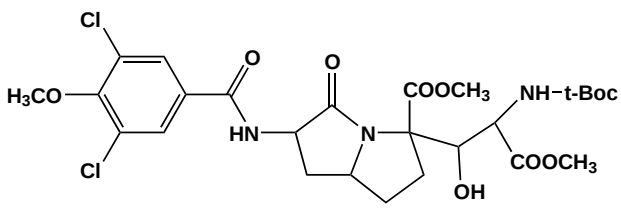

2'

Figure 2.

Keywords: kaitocephalin; absolute structure; AMPA antagonist; NMDA antagonist.

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might be formed by intramolecular condensation between the secondary amine and carboxylic acids. The first attempt to catalyze intramolecular condensation using DEPC[2] (diethyl phosphorocyanidate) in Et_3N/DMF, however, was not successful presumably due to the presence of the free primary amine group. Thus, **1** was selectively protected with di-*t*-butyl-dicarbonate (Boc_2O) in 1,4-dioxane–H_2O to give an *N*-Boc derivative (*m*/*z* 616 $[M+Na]^+$).[3] Introduction of the Boc substituent to the primary amine at C-2 was confirmed by the low field shift of 2-H (3.95 to 4.35 ppm) in the ^1H NMR spectrum. The reaction with Boc_2O of the secondary amine in the pyrrolidine moiety was inhibited by the neighboring bulky substituents. Treatment of the *N*-Boc derivative with trimethylsilyldiazomethane ($TMSCHN_2$) in methanol gave an expected cyclic derivative **2**.[4]

The molecular formula of **2**,[5] which was established as $C_{26}H_{34}Cl_2N_3O_{10}$ by HRFAB-MS $[(M+H)^+$, *m*/*z* 618.1602 (calcd 618.1621)], indicated that **2** was a bicyclic compound, which was presumably produced through an intramolecular ester-exchange reaction of a putative intermediate, a tetramethyl *N*-Boc derivative of **1**. Although formation of two cyclic derivatives **2** and **2′** had been expected, **2** was selectively produced (Fig. 2). The structure of **2** was confirmed by a long-range coupling from 7-H to an amide carbonyl carbon C-1 (δ_C 170.5).

The results of NOESY experiments on the bicyclic compound **2** are summarized in Fig. 3. Since a weak correlation in the NOESY spectrum of **2** was observed between 3-H (δ_H 4.15) and 7-H (δ_H 3.93), the conformation of the bicyclic ring moiety was revealed to be a bent form with 3-H and 7-H approaching each other as shown in Fig. 3. This conformation indicated that relationship between 3-H and the carbomethoxy group at C-4 was *trans*. A NOESY correlation between methoxy protons (δ: 3.74) at C-18 and H-2 (δ: 4.54) indicated that both protons were in *syn* relationship. Thus, the relative configurations at C-2, C-3, C-4 and C-7 in the bicyclic ring of **2** were assigned as *S**,*S**,*R** and *R**, respectively.

The relative stereochemistry at C-9 was determined by analysis of $^3J_{H-H}$ and $^3J_{C-H}$ coupling constants as follows.[6] A large coupling constant (9.8 Hz) between 7-H and 8-H_a (δ: 1.91) revealed that these protons were in *anti* relationship. Both 8-H_a and 8-H_b (δ: 2.24) were coupled to C-6 with a small coupling constant ($^3J_{C-H}$ <2.0 Hz) showing that both protons (8-H_a and 8-H_b) and C-6 were in *gauche* relationship (Fig. 4). Thus, C-6 and C-9 were in *anti* location as shown in Fig. 4. A large coupling constant (7.9 Hz) between H-8_b and H-9, and a small $^3J_{C-H}$ coupling constant (<2 Hz) between H-8_b and a carbonyl carbon C-17 (δ: 171.1) were observed. An intermediate $^3J_{C-H}$ coupling constant between 8-H_a and C-17 ($^3J_{C-H}$=4.9 Hz) hampered to establish the relative stereochemistry at C-9. The stereochemistry at C-9 was assumed to be *S** based on the consideration of a strong NOE between H-7 and amide proton 9-NH (δ: 7.91) (Fig. 4). If the cyclic derivative had the 9*R** configuration, no NOE between these two protons would be expected. The low filed chemical shift of 9-NH was in agreement with its involvement in hydrogen bonding with a carbonyl oxygen at C-1.

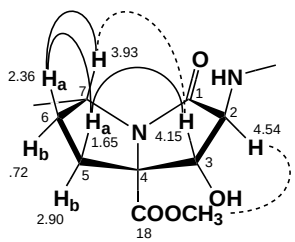

Figure 3.

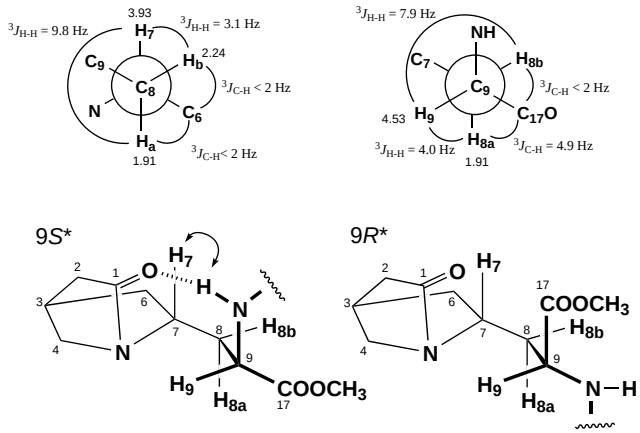

Figure 4.

The absolute stereochemistry of **2** was established by preparation of MTPA esters[7] which were analyzed by the modified Mosher's method.[8] The differences of chemical shift values obtained by subtracting (*R*)-MTPA ester from (*S*)-MTPA ester ($\delta\Delta = \delta$ (*S*)-MTPA-δ (*R*)-MTPA) are shown in Fig. 5. These values indicated the absolute stereochemistry at C-3 as *S*. Therefore, the absolute configuration of **1** was concluded to be 2*S*,3*S*,4*R*,7*R* and 9*S*, as shown in Fig. 1.

Kaitocephalin acts on both AMPA and NMDA receptors. Since the role of kainate receptors in the neuronal system is still unclear, their specific antagonists may be valuable tools for better understanding of the neuronal

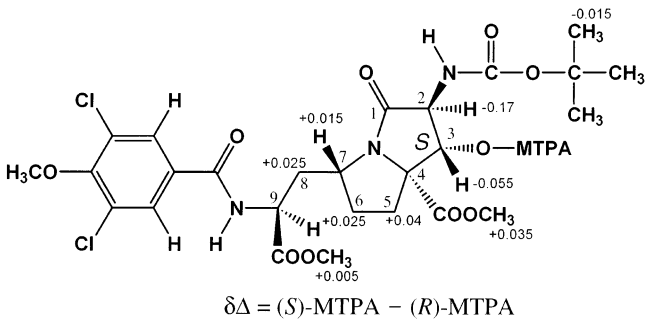

Figure 5.

transmission system. With the stereochemistry of **1** in hand, it may become possible to prepare derivatives of **1** with an antagonistic activity specific to AMPA or NMDA receptor.

Acknowledgements

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3. To a solution of **1** and K_2CO_3 (2 equiv.) in 1,4-dioxane–H_2O (1:1) was added Boc_2O (50 equiv.) at 0°C and stirred at room temperature for 2 h. Then the reaction mixture was purified by preparative ODS TLC (MERCK, RP-18 WF_{254}) developed with 10% MeOH. ^1H NMR (δ_H, CD_3OD at 500 MHz), 7.84 (s, 2H), 4.56 (m), 4.38 (s), 4.28 (s), 3.79 (m), 2.44 (m, 3H), 2.20 (m, 2H), 1.70 (s), 1.42 (s, 9H).
4. To a MeOH solution of **2** was added trimethylsilyldiazomethane (20 equiv.) at room temperature and stirred for 2 h. The reaction mixture was extracted with EtOAc and purified by HPLC (Senshu-Pak, DOCOSIL) using 70% MeOH as a solvent system.
5. ^{13}C NMR (δ_C, $CDCl_3$ at 125 MHz), 170.5 (s, C-1), 62.1 (d, C-2), 83.5 (d, C-3), 75.4 (s, C-4), 34.0 (t, C-5), 33.6 (t, C-6), 52.3 (d, C-7), 36.4 (t, C-8), 51.5 (d, C-9), 163.9 (s, C-10), 130.8 (s, C-11), 128.1 (d, C-12, 16), 129.7 (s, C-13, 15), 155.0 (s, C-14), 171.1 (s, C-17), 170.6 (s, C-18), 157.7 (s, C-19), 81.5 (s, *t*-Boc), 28.2 (q, *t*-Boc methyl), 60.9 (q, 14-OMe), 52.6 (q, 17-OMe), 53.1 (q, 18-OMe). ^1H NMR (δ_H, $CDCl_3$ at 500 MHz), 7.91 (d, 7.5 Hz, NH), 5.45 (s, NH), 5.21 (brs, OH), 4.54 (d, 9.0 Hz, 2-H), 4.15 (d, 9.0 Hz, 3-H), 1.65 (dd, 12.0, 7.5 Hz, 5-H_a), 2.90 (ddd, 12.0, 6.0, 2.0 Hz, 5-H_b), 2.36 (dddd, 12.5, 7.5, 5.0, 2.0 Hz, 6-H_a), 1.72* (m, 12.5, 6.5 Hz, 6-H_b), 3.93 (m, 7-H), 1.90 (ddd, 14.5, 10.0, 4.0 Hz, 8-H_a), 2.24 (ddd, 14.5, 8.0, 3.0 Hz, 8-H_b), 4.53 (ddd, 8.0, 7.5, 4.0 Hz, 9-H), 7.82 (2H, s, 12, 16-H), 1.43 (9H, s, *t*-Boc methyl), 3.93 (3H, s, 14-OMe), 3.76 (3H, s, 17-OMe), 3.74 (3H, s, 18-OMe). * These coupling constants were confirmed by decoupling experiments.
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7. To a solution of **2** containing catalytic amount of DMAP in CH_2Cl_2–pyridine (1:1) was added (*S*)- or (*R*)-MTPA chloride (40 equiv.) and stirred at room temperature for 1 h. Then the reaction mixture was purified by ODS HPLC (PEGASIL) developed with 80% MeOH.
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