Stereoselective Synthesis of 4-Amino-3-hydroxy-4,5,6,6a-tetrahydro-3aH-cyclopenta[d]isoxazole-4-carboxylic Acid, a Conformationally Constrained Analogue of Aspartic Acid

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Abstract: An alternative synthesis of 4-amino-3-hydroxy-4,5,6,6a-tetrahydro-3aH-cyclopenta[d]isoxazole-4-carboxylic acid, a conformationally constrained analogue of aspartic acid, is described. The synthetic strategy is based on a regioselective 1,3-dipolar cycloaddition to give the cyclopenta[d]isoxazoline framework; subsequent condensation of this 4-oxocyclopenta[d]isoxazoline with 4-methoxybenzylamine gives a 4-imino derivative, which undergoes a highly stereoselective nucleophilic attack by the cyanide ion. This gives a 4-(methoxybenzylamino)-4-cyano derivative, which is oxidized to the corresponding 4-amino-4-cyano derivative, which itself is transformed into the target 4-carboxylic acid. This amino acid is obtained in 27% overall yield in five steps, whereas the previously described synthetic strategy gave the target derivative in only 0.5% overall yield over 15 steps.

Key words: aspartate analogues, heterocycles, 1,3-dipolar cycloaddition, stereoselective synthesis, glutamate transporters

L-Glutamate (Glu) is considered to be the primary excitatory amino acid neurotransmitter in the mammalian central nervous system. It is involved in many important physiological functions such as development, synaptic plasticity, learning and memory, cognition, pain, and nociception.1 Glu exerts its signaling role by acting on ionotropic and metabotropic glutamate receptors. An overactivation of these receptors causes cell death.2 Consequently, the extracellular concentration of Glu is kept below toxic levels by suitable transporters, the excitatory amino acid transporters (EAATs).3 The EAATs remove Glu from the synaptic cleft by using, as driving forces, the electrochemical gradients across the plasma membranes.4 Five subtypes of EAATs have so far been identified and characterized by molecular cloning.5

An imbalance of the Glu uptake machinery is associated with several acute and chronic neurodegenerative pathological conditions such as ischemia, amyotrophic lateral sclerosis, Alzheimer’s disease, epilepsy, and traumatic brain injury.6 In some pathological events, e.g. ischemia, EAATs reverse their mode of action, causing the release of Glu from the glia and the consequent increase of the Glu concentration up to neurotoxic levels.7

The nontransportable inhibitors of EAATs so far described affect both Glu uptake and EAAT-mediated reverse transport. The EAAT blockers could therefore be useful drugs to prevent cell death caused by the EAAT-mediated release; on the other hand, they could be neurotoxic in physiological conditions. Thus, ideal drugs acting on EAATs should leave the uptake of Glu unaffected while preventing its release in pathological conditions.

A number of ligands capable of interacting with the Glu transporters have been reported; most of them share structural similarities with agonists of the Glu receptors.3,8 In this context, the interaction of 3-hydroxy-4,5,6,6a-tetrahydro-3aH-pyrrolo[3,4-d]isoxazole-4-carboxylic acid (HIP-A, Figure 1),6 a conformationally constrained aspartate analogue, with the EAATs expressed in rat brain cortex synaptosomes was pharmacologically characterized.10 It turned out to be a potent noncompetitive inhibitor and behaved as an EAAT blocker, but, unexpectedly, it inhibited EAAT-mediated reverse transport with IC50 values 10 times lower than those required to inhibit Glu uptake. Thus, there is a concentration window in which this amino acid is effective in blocking EAAT-mediated release of Glu, without affecting its uptake.

Figure 1 Model and target compounds

To further investigate the relationship between the structure of HIP-A and its peculiar pharmacological profile, we planned the synthesis of its conformationally constrained analogue 1A (Figure 1). This novel derivative differs from the lead compound HIP-A in the spatial arrangement of the amino acidic moiety.

Amino acid 1A and its three stereoisomers 1B, 1C, and 1D (Figure 1) have been prepared previously, and their af-
finity for Glu receptors has been tested. The synthetic strategy, based on a 1,3-dipolar cycloaddition between dibromoformaldehyde oxime and a suitable unsaturated amino acid derivative, yielded a mixture of the four possible regio/isomers. Compound 1A was obtained in 10% yield after a difficult chromatographic separation of the mixture of the four cycloadducts followed by appropriate elaborations of the functional groups. In addition, the synthesis of the dipolarophile is rather complicated (ten steps) and produces the desired compound in 5% overall yield.

Since we were interested in testing 1A as a ligand of EAATs, we designed a novel and simplified synthesis. We here describe an alternative synthetic strategy, which allowed us to obtain amino acid 1A stereoselectively and in higher yield.

To improve the synthesis of amino acid 1A, we investigated an alternative strategy associated with more favorable regioselectivity. The 1,3-dipolar cycloaddition between cyclopent-2-en-1-one and dibromoformaldehyde oxime afforded the two regioisomeric cycloadducts 3-bromo-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazol-4-one (2) and 3-bromo-6H-3a,4,5,6a-tetrahydro-cyclopenta[d]isoxazol-6-one (3) in a 6:1 ratio (Scheme 1). The pericyclic reaction was carried out in ethyl acetate with an excess of solid sodium bicarbonate, and 100% conversion was reached after four days at room temperature. Microwave irradiation of the reaction mixture resulted in complete conversion within 40 minutes. Compound 2, the suitable precursor of amino acid 1, could be separated from its regioisomer 3 by crystallization. Compound 2 was then reacted with 4-methoxybenzylamine to yield the corresponding imino derivative 4 (Scheme 2). The condensation was carried out in a Dean–Stark apparatus, with benzene used as solvent, and in the presence of a catalytic amount of p-toluenesulfonic acid. Under these conditions, 100% conversion was reached in 30 minutes. The progress of the reaction was monitored by 'H NMR spectroscopy, by following the change of the chemical shift of the hydrogen in the 3a-position. After evaporation of the solvent, the imino derivative 4 was directly submitted to the next step without any purification. The crude material, dissolved in anhydrous methanol, was reacted with trimethylsilyl cyanide in the presence of zinc(II) chloride to yield derivative 5. The reaction mixture was stirred at room temperature overnight. The nucleophilic attack of the cyanide takes place solely at the less hindered face of the imino group, affording derivative 5 as the only detectable stereoisomer (Scheme 2).

Intermediate 5 was then reacted with an excess of cerium(IV) ammonium nitrate to produce 4-amino-4-cyano derivative 6 (Scheme 2). Compound 6 was purified by flash chromatography and then transformed into the corresponding amino acid 1A by treatment with sodium hydroxide in a water–dioxane mixture at 60 °C. Under these conditions, we achieved both the hydrolysis of the nitrile group and the replacement of the 3-bromo moiety by the hydroxy group (Scheme 2). The zwitterionic form of amino acid 1A was then obtained by ion-exchange chromatography, as described earlier. The physical and spectroscopic properties of the amino acid obtained in this way were compared with those of amino acid 1A previously reported. The perfect correspondence confirmed that the nucleophilic attack of the cyanide was completely stereoselective and occurred on the Si face of the carbonyl group.

The synthetic strategy described here allowed us to obtain amino acid 1A in five synthetic steps in 27% overall yield. The interaction of amino acid 1A with the EAATs will be evaluated in rat brain cortex synaptosomes, as described for HIP-A, and reported in due course along with the results of structurally related analogues.

Scheme 1 Synthesis of 3-bromo-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazol-4-one (2). Reagents and conditions: (a) NaHCO₃, EtOAc, r.t., 4 d; (b) NaHCO₃, EtOAc, MW, 40 min.

Scheme 2 Stereoselective synthesis of 4-amino-3-hydroxy-4,5,6,6a-tetrahydro-3aH-cyclopenta[d]isoxazole-4-carboxylic acid (1A). Reagents and conditions: (a) TsOH, benzene, heat; (b) TMSCN, ZnCl₂, MeOH, r.t.; (c) CAN, MeCN–H₂O; (d) 1 M NaOH, dioxane, heat; (e) 2 M HCl; (f) Amberlite IR-120 MS, 10% aq py.
Dibromoformaldehyde oxime was prepared by a literature procedure.\(^1\) All reagents and solvents were purchased from Aldrich. \(^1\)H and \(^13\)C NMR spectra of samples in CDCl\(_3\) at 20 °C were recorded on a 300-MHz Varian spectrometer. TLC was performed on commercially available silica gel 60 F254 aluminum sheets; spots were further made visible by spraying with dilute alkaline KMnO\(_4\) solution or with ninhydrin. Melting points were determined by a capillary method on a Büchi B 540 apparatus and are uncorrected. Microanalyses of new compounds agreed with theoretical values (±0.3%).

Compounds 2 and 3
Dibromoformaldehyde oxime (12.2 g, 60 mmol) and an excess of solid NaHCO\(_3\) (10 g) were added to a soln of cyclopent-2-en-1-one (2.0 mL, 24 mmol) in EtOAc (40 mL).

Method a: The mixture was stirred at r.t. for 4 d. The progress of the reaction was monitored by TLC (PE–EtOAc, 7:3). The solids were removed by filtration, H\(_2\)O was added, and the two phases were separated. The organic layer was dried (Na\(_2\)SO\(_4\)) and the solvent was concentrated. Compound 2 (3.02 g, 14.8 mmol) spontaneously crystallized; compound 3 (504 mg, 2.47 mmol) was purified by flash chromatography (PE–EtOAc, 8:2). Overall yield: 72%.

Method b: The mixture was irradiated with MW (150 W) for 40 min in a sealed vessel to reach 100 °C. The progress of the reaction was monitored by TLC (PE–EtOAc, 7:3). After workup, compound in a sealed vessel was added to a soln of PMBNH\(_2\) (0.763 mL, 5.88 mmol) and TsOH (42 mg, 0.24 mmol) and the mixture was refluxed in a Dean–Stark apparatus for the azeotropic removal of H\(_2\)O. The progress of the reaction was monitored by \(^1\)H NMR spectroscopy. After 30 min from the beginning of the distillation the transformation was complete. The solvent was evaporated and the crude material was directly submitted to the next step.

3-Bromo-3a,5,6,6a-tetrahydro-4H-cyclopenta[d]isoxazol-4-one (5)

Amine acid 1A was obtained from 6 as previously described.\(^1\) Its physical and spectroscopic properties matched those reported.\(^1\)

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References


