Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 129–133

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

First Enantiospecific Synthesis of a 3,4-Dihydroxy-L-glutamic Acid [(3*S*,4*S*)-DHGA], a New mGluR1 Agonist

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Received 26 May 1999; accepted 15 November 1999

Abstract—The first synthesis of one of the 4 possible stereoisomers of 3,4-dihydroxy-L-glutamic acid ((3*S*,4*S*)-DHGA **3**), a natural product of unknown configuration, is described. The synthesis is based on the Lewis acid catalyzed reaction of benzyl alcohol with a D-ribose-derived 2,3-aziridino-γ-lactone 4-benzyl carboxylate (**6**). Preliminary pharmacological studies showed that (3*S*,4*S*)-**3** is an agonist of metabotropic glutamate receptors of type 1 (mGluR1) and a weak antagonist of mGluR4 but has no discernible activity with respect to mGluR2. This activity profile can be rationalized by fitting extended conformations of (3*S*,4*S*)-**3** in proposed models of each of these receptor subtypes.

Glutamic acid is the major excitatory neurotransmitter of the central nervous system. It acts through four major receptor classes termed NMDA, AMPA, kainate and metabotropic receptors each of which is in turn comprised of several subclasses.[1–5] While the first three types of receptors, collectively referred to as ionotropic glutamate receptors (iGluRs), function via cation (Ca^{2+}, K^+, Na^+) permeable channels, the metabotropic receptors (mGluRs) are coupled to effector systems (e.g. phospholipase C and phosphoinositide (PI) hydrolysis; adenylyl cyclase) through GTP-binding proteins. Eight mGluR subtypes (mGluR1 to mGluR8) have been isolated and these are classified in three groups (groups I, II and III) based on their amino acid sequence homology. Group I, composed of mGluR1 and mGluR5, is positively coupled to phospholipase C and intracellular production of inositol phosphate via a Gq G-protein subtype. On the other hand, groups II (mGluR2 and mGluR3) and III (mGluR4, 6, 7 and 8) are negatively coupled to adenylyl cyclase via Gi/Go G-protein subtypes. The mGluRs are currently therapeutic targets for the treatment of such neurodegenerative syndromes as Parkinson's and Alzheimer's diseases, as well as brain ischemia and epilepsy.[4,5]

In order to delineate the physiological role of each mGluR subtype, there is presently a great need for specific ligands. For this purpose, a large number of analogues of glutamic acid have been synthesized in recent years and some have been shown to exhibit such specificity.[6–17] Thus, (2*S*,4*S*)-4-methylglutamic acid (**1**) is an agonist of the mGluR1 and 2 subtypes[18–20] while (2*S*,4*S*)-4-(ω,ω-diphenylalkyl)glutamic acids **2a** and **2b** are specific mGluR2 antagonists.[14,15] Because naturally-occurring compounds have very often been shown to be biologically active and therapeutically useful and in an effort to discover potentially selective mGluR ligands, we recently undertook a literature survey of natural glutamic acid derivatives. Interestingly, the isolation of 3,4-dihydroxyglutamic acid (DHGA, **3**) from the seeds of *Lepidum sativum* as well as from the leaves of *Rheum rhaponticum* was reported over 40 years ago[21] and this amino acid was subsequently shown to be a constituent of a large variety of flowering plants, ferns, mosses and mushrooms.[22,23] However, nothing is known concerning the relative and, a fortiori, the absolute configurations of the two chiral centers of this natural compound. Moreover, until now, neither chiral nor achiral syntheses of DHGA, or of 3,4-disubstituted glutamic acid derivatives in general, have been reported. In order to evaluate the possible interactions of this class of natural substances with mGluR subtypes, the synthesis of one

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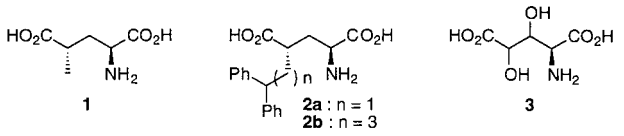
PII: S0960-894X(99)00641-1

of the four possible isomers of **3** was undertaken utilizing our 2,3-aziridino-γ-lactone approach to the enantiospecific synthesis of substituted α- or β-amino acids.[24–29]

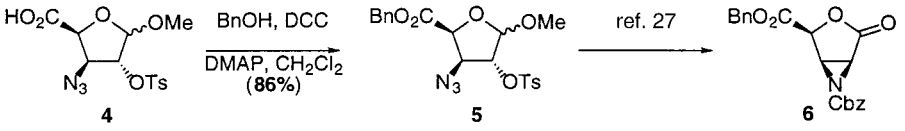


The starting material for our study was the uronic acid derivative **4** (α,β mixture, Scheme 1) which was prepared from D-ribose as previously described.[24] Previous experience has shown that hydroxyglutamic acid derivatives are prone to degradation when subjected to acidic or basic conditions. Our objective was thus to prepare a synthon which would allow complete deprotection of the amino acid derivative simply by hydrogenolysis in the final step. For this reason, compound **4** was first transformed into the benzyl ester **5** in 86% yield using Steglich conditions[30] and then into the 2,3-aziridino-γ-lactone **6** using the general sequence of reactions we have developed for the synthesis of this class of compounds.[27,31]

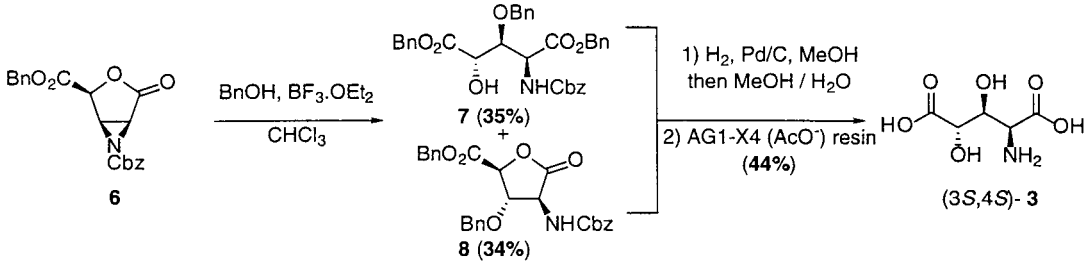
The conversion of aziridino-γ-lactone **6** into (3*S*,4*S*)-DHGA (3*S*,4*S*-**3**) is depicted in Scheme 2. Thus, treatment of a chloroform solution of **6**, with a 10-fold excess of benzyl alcohol and 2 eq of boron trifluoride etherate at 0 °C for 20 h, afforded an equimolar mixture of the protected glutamic acid derivative **7** (resulting from nucleophilic attack of both the aziridine and lactone functionalities by benzyl alcohol) and the lactone **8** (resulting from partial cyclization of **7**). While both these products could be separated by chromatography, their facile interconversion encouraged us to proceed directly with hydrogenolytic deprotection of the mixture. ^{1}H NMR spectroscopy of the crude reaction product showed, as expected, the presence of both (3*S*,4*S*)-DHGA **3** and its lactonized form. Passage of this material through a basic anion exchange column (AG1-X4 resin), using acetic acid (0.2–0.5 M) as the eluent, then led to isolation of a single species, identified as the amino acid **3**.[32]

In preliminary pharmacological studies, the effect of (3*S*,4*S*)-DHGA on mGluRs was determined by measuring IP production relative to glutamate itself, on one representative receptor subtype from each group (i.e. mGluR1 for group I; mGluR2 for group II; mGluR4 for group III) using previously described methodology.[33–35] The coupling of mGluR2 and mGluR4 to PLC was made possible by co-expressing these receptors with the chimeric G-protein Gqi9, as previously described.[33–35] As shown in Figure 1A, (3*S*,4*S*)-DHGA (1 mM) significantly stimulated IP production (IP conversion ratio = 0.29, corresponding to a 4.5-fold increase of the basal IP production) in cells expressing mGluR1 though not as strongly as glutamate (IP conversion ratio = 0.44 at 1 mM, corresponding to a 6.6-fold increase of the basal IP production). The effect was dose-dependent with an EC_{50} of 257 ± 64 μM ($n = 3$) showing that (3*S*,4*S*)-**3** is less potent than Glu ($EC_{50} = 1.05 \pm 0.20$ μM, $n = 5$). In contrast, (3*S*,4*S*)-DHGA did not activate mGluR2 or mGluR4, the measured IP production being in both cases practically identical to basal values (i.e. with no ligands or in the presence of the mGluR specific antagonists). (3*S*,4*S*)-DHGA is thus an agonist of mGluR1. In order to verify for receptor-specific antagonist effects of (3*S*,4*S*)-**3**, IP production resulting from simultaneous application of this compound and glutamate to each receptor subtype was measured (Fig. 1B). As expected for mGluR1, where it acts as an agonist, (3*S*,4*S*)-DHGA did not inhibit the IP production induced by Glu (1 μM). On the other hand, (3*S*,4*S*)-**3** inhibited the glutamate-induced stimulation of mGluR4 (57.0 ± 11.9% inhibition, $n = 3$) but had little effect on mGluR2 (29.0 ± 10.7% inhibition, $n = 3$). (3*S*,4*S*)-DHGA can thus be considered a weak mGluR4 antagonist.

These biological activities of (3*S*,4*S*)-**3** can be tentatively interpreted on the basis of mGluR1, mGluR2, mGluR4 pharmacophore models which have been recently described.[20,37] In all three models, glutamic



Scheme 1.



Scheme 2.

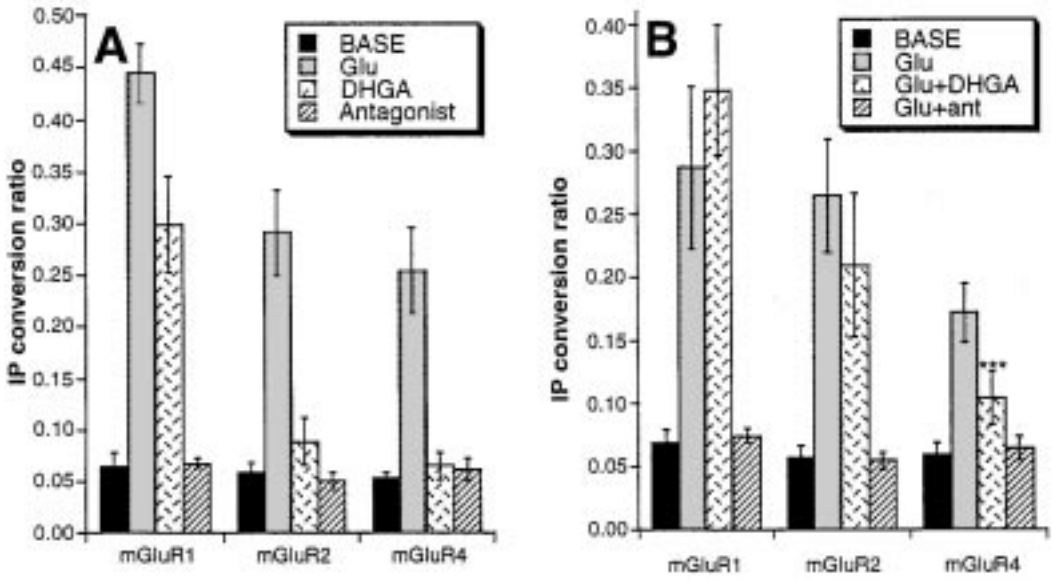


Figure 1. Effects of (3*S*,4*S*)-DHGA on mGluR1, 2 and 4 transiently expressed in HEK 293 cells: HEK 293 cells expressing mGluR1, mGluR2 (plus Gqi9) or mGluR4 (plus Gqi9) were stimulated by the indicated compounds and the intracellular activity of the phospholipase C enzyme was measured by quantifying the production of inositol phosphates (IP). **A**: (3*S*,4*S*)-DHGA is an agonist on mGluR1. HEK 293 cells expressing mGluR1, 2 or 4 are incubated in a medium containing glutamate (Glu, 1 mM), (3*S*,4*S*)-DHGA (1 mM) or the appropriate antagonist[36] (4CPG, MCCG-I or MAP4, respectively; 1 mM). Glu activated all three receptors but (3*S*,4*S*)-DHGA activated only mGluR1. As expected, none of the antagonists induced any agonist effect. **B**: (3*S*,4*S*)-DHGA is a weak antagonist on mGluR4. HEK 293 cells expressing mGlu R1, 2 or 4 were stimulated by Glu (1 μM on mGluR1; 20 μM on mGluR2; 30 μM on mGluR4) in the presence or absence of their respective antagonists (4CPG, MCCG-I and MAP4) or (3*S*,4*S*)-DHGA. (3*S*,4*S*)-DHGA (1 mM) inhibited Glu-induced IP production to a significant level only on mGluR4. As a control, the Glu-induced stimulation of the three receptors was inhibited by their respective antagonists (1 mM). Results are expressed as the amount of IP produced over the radioactivity present in the membranes (IP conversion ratio). $^{***} = P < 0.001$.

acid, which can adopt numerous conformations, lies in an extended conformation (*aa*, g^+a, g^-a)[38] and is bound to the receptor by means of hydrogen bonds or ionic interactions with the amino and carboxylic functions (S1, S2, S3a,b sites). Selective agonists for each of the three mGluR groups such as quisqualic acid (mGluR1), LY354740 (mGluR2), ACPT-I (mGluR4)[39] were shown to bind to these sites and to take advantage of the differing protein environments resulting in specific interactions located in the S4, S5 and S6 regions as depicted in Figure 2.

(3*S*,4*S*)-DHGA conformations were generated by molecular dynamics, minimized and clustered using InsightII/Discover softwares (Molecular Simulations, San Diego, CA) as previously described.[20,37] (3*S*,4*S*)-DHGA was taken in its zwitterionic state so that the protonation of the distal acidic functions allows all conformations to be generated with a dielectric constant of 80 or 5 within 3.5 kcal energy difference. Selected conformers were superimposed to the pharmacophore models. Interestingly, while S1, S2, S3a,b sites are fitted with the amino and carboxylic groups of (3*S*,4*S*)-DHGA, the mGluR1 hydrophilic site in the S4 region is fitted with the 4-hydroxyl group of the g^+a conformation or the 3-hydroxyl of the g^-a conformation. The folded ag^+ conformation accommodates the same 5 sites with the 4-hydroxyl at S3a and the distal carboxylate at S3b and S4. Thus, several (3*S*,4*S*)-DHGA conformations with similar energies display binding analogous to quisqualate, the most potent mGluR1 agonist. However, the apparent weaker affinity of (3*S*,4*S*)-DHGA might result from the additional hydroxyl group located in the restricted S5 region.

In the mGluR2 and mGluR4 models, glutamic acid adopts an *aa* conformation.[20,37] In the analogous (3*S*,4*S*)-DHGA conformation, the 4-hydroxyl group points to the S6 hydrophobic region located in the

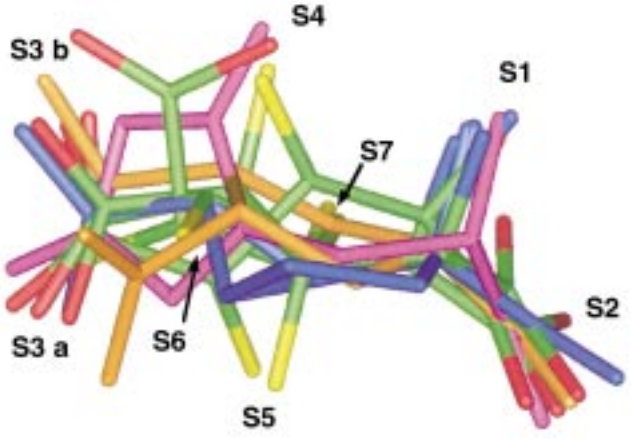


Figure 2. Superposition of the *aa*, g^+a, g^-a and ag^+ (3*S*,4*S*)-DHGA conformations onto quisqualate (magenta), LY354740 (blue), ACPT-I (orange) in their respective mGluR1, mGluR2, mGluR4 pharmacophore model conformations:[20,37] (3*S*,4*S*)-DHGA carbon atoms are colored in green, nitrogen in blue, carboxylic oxygens in red and hydroxylic oxygens in yellow. Common glutamate binding sites S1, S2, S3a,b and selective S4, S7 regions are indicated. S4 and S6 are selective hydrophilic sites of respectively mGluR1 and mGluR4, in contrast S6 is hydrophobic at mGluR2. S5 is a region with a different steric allowance according to each mGluR. S7 is an unexplored region.

foreground region of Figure 2 (lining the LY354740 ring as defined previously).[20] This situation could be responsible for the observed lack of activity of (3*S*,4*S*)-DHGA on mGluR2 compared to the strong potency of (2*S*,4*S*)-4-MeGlu.[20] In contrast, this S6 region is hydrophilic in mGluR4 as shown by ACPT-I binding, so that the antagonist property of (3*S*,4*S*)-DHGA on mGluR4 would be due to the position of the 3-hydroxyl in the unexplored S7 region. However, as mentioned above, the g^+a and g^-a conformations of (3*S*,4*S*)-DHGA position a hydroxyl group in the S4 region which was proposed as a restricted area in mGluR2 and mGluR4.[20,37] It was suggested that a hydrophilic group in this S4 region might be responsible for an antagonist property at mGluR4. Thus, all three extended forms of (3*S*,4*S*)-DHGA can account for the observed biological activities at mGluR2 and mGluR4.

In conclusion, use of 2,3-aziridino-γ-lactone methodology has now been successfully applied to the first enantiospecific preparation of a 3,4-disubstituted glutamic acid, namely (3*S*,4*S*)-dihydroxy-L-glutamic acid [(3*S*,4*S*)-**3**]. The selective activity of this compound on mGluR1 relative to mGluR2 and mGluR4 which can be rationalized in terms of receptor models, suggests that the other isomers of this compound may also constitute a valuable source of specific ligands of the metabotropic glutamate receptors of the central nervous system. This is presently being investigated.

Acknowledgements

We are grateful for fellowships from the French Ministry of Defence (P.D.) and from the Ministry of Higher Education (C.S.-F.). The pharmacological work and molecular modeling was supported by grants from the "Action Incitative Physique et Chimie du Vivant" (PCV97-115), the European Community Biomed 2 (BMH4-CT96-0228) and Biotech 2 (BI04-CT96-0049) programs, the Fondation pour la Recherche Médicale and Bayer Company (France and Germany).

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32. Compound (3*S*,4*S*)-**3**: $[\alpha]_D^{28}$ −0.8 (*c* 1.0, H_2O); ^1H NMR (250 MHz, D_2O) δ 3.80 (s, 1H), 4.24 (d, 1H, $J_{4,3}=3.3$ Hz), 4.48 (d, 1H, $J=3.2$ Hz) ; ^{13}C NMR (75 MHz, D_2O) δ 56.9, 71.2, 76.2, 173.9, 178.3; mass spectrum (FAB) *m*/*z* 180 $(MH)^+$.
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35. As indicated in the introduction, mGluR2 and mGluR4 are negatively coupled to adenylyl cyclase. Because this transduction cascade is more difficult to measure in transient transfection assays than the stimulation of IP formation, the coupling of these receptors to phospholipase C was made possible by co-expressing them with a chimeric G-protein alpha subunit, Gqi9 (ref 36).
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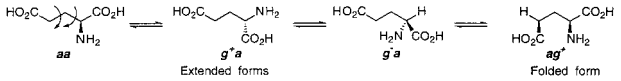
38. The various conformations of L-glutamic acid are described as follows:



39. Structures of the selective agonists of the three mGluR groups depicted in Figure 2.