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SYNTHESIS AND METABOTROPIC GLUTAMATE RECEPTOR ACTIVITY OF A 2-AMINOBICYCLO[3.2.0]HEPTANE-2,5-DICARBOXYLIC ACID, A MOLECULE POSSESSING AN EXTENDED GLUTAMATE CONFORMATION

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Abstract: A photochemical approach to the synthesis of the aminobicycloheptane **6a** is reported. This compound assumes an extended glutamate conformation, and for this reason was created to further probe the structural features relevant to achieving selectivity for the subtypes of the metabotropic glutamate family of receptors.

Introduction

The amino acid glutamate (Glu) plays a pivotal role in biological processes ranging from memory and learning to neuronal degeneration. This major excitatory amino acid (EAA) acts through disparate Glu receptors, which can be categorized into two distinct types, the ionotropic glutamate receptors and the metabotropic glutamate receptors. The ionotropic Glu receptors, or iGluRs, are associated with integral cation-specific ion channels and include the NMDA [*N*-methyl-D-aspartic acid], AMPA [2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid], and KA (kainic acid) subtypes.[1] On the other hand, the metabotropic Glu receptors (mGluR) are coupled to cellular effectors through GTP-binding proteins.[2]

The mGluRs have been distinguished pharmacologically from the iGluRs by the use of the mGluR-selective agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid [(1*S*,3*R*)-ACPD] generally through measurements involving phosphoinositide hydrolysis or Ca^{2+} mobilization. To date, the use of expression cloning techniques has led to the identification of eight mGluR subtypes which have been placed into three major categories based on their molecular structure, signal transduction mechanisms, and pharmacological properties.[3] Group I mGluRs (mGluR1 and 5) are coupled to phosphoinositide (PI) hydrolysis, whereas group II (mGluR2 and 3) and group III (mGluR4, 6, 7, and 8) are negatively linked to adenylyl cyclase activity. The group I receptors are more sensitive to quisqualic acid than to ACPD; the group II receptors are more sensitive to ACPD than quisqualic acid; and the group III receptors are most sensitive to 2-amino-4-phosphonobutyric acid (L-AP4). While the mGluRs have been shown to play a fundamental role in the modulation of synaptic transmission,

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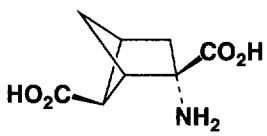
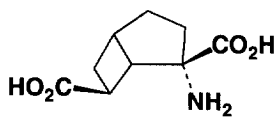
considerable attention has also been given to their role in neurodegenerative diseases, including Alzheimer's disease, cerebral ischemia, Parkinsonism, spinal cord injury, and epilepsy.[4]

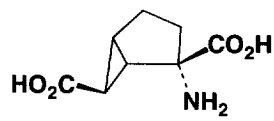
In order to better characterize the roles of GluRs in physiological and pathophysiological processes, there is an important need to identify novel, high affinity ligands that are family and subtype specific. To date, many of the biological studies that have been conducted in the area of mGluR research have made use of the agonist (1*S*,3*R*)-ACPD. This compound has been shown to be an agonist of both the group I and group II receptors. Moreover, (1*S*,3*S*)-ACPD, the cis-isomer, shows negligible activity at group I receptors and is a good agonist of mGluR2.[5] Since ACPD is itself somewhat flexible conformationally, with four distinctive conformations being identified from molecular modeling studies for the trans isomer, and five conformations for the cis isomer,[6] we believed that it would be of interest to examine the activity of various ring constrained analogues of ACPD. Specifically, we have shown previously that the aminobicyclo[2.1.1]hexanedicarboxylic acid analogue ABHxD-I is selective for the mGluR family of receptors, acting with approximately the same selectivity and potency as glutamate.[7] This particular ligand assumes an extended glutamate conformation. Accordingly we felt that it would be valuable to examine the biology of other structures possessing this mGluR preferring conformation. In this paper we report the synthesis and biological study of the aminobicyclo[3.2.0]heptanedicarboxylic acid **6a**, which like ABHxD-I also possesses the extended glutamate conformation. Further, we note that this compound can be considered to be an homologue of 2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740), a molecule recently shown to serve as a potent group II agonist (EC_{50} = 5.1 nM at human mGluR2).[8] The creation of a family of related molecules displaying similar 3-D pharmacophores but differing in steric features will ultimately be useful in obtaining a better understanding of the elements crucial to controlling subtype selectivity.



ABHxD-I



6a



LY354740

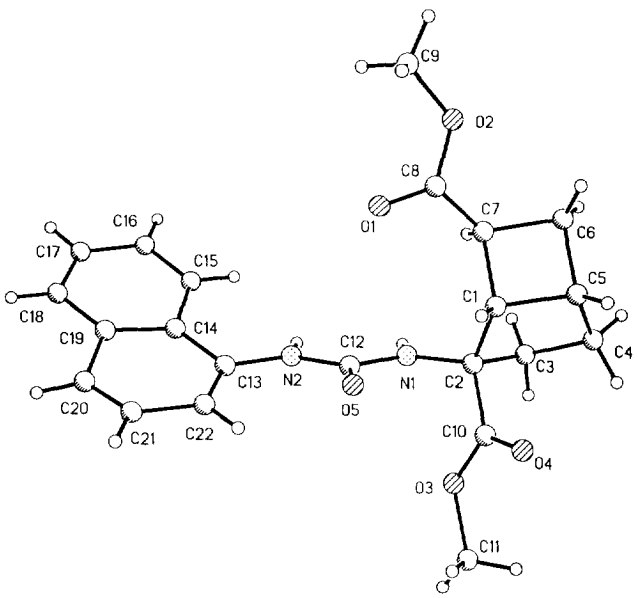
Chemistry

As an obvious synthetic approach to the 2-aminobicyclo[3.2.0]heptane-2,5-dicarboxylic acid **6a**, we considered carrying out an intramolecular photochemical [2+2] cycloaddition reaction of an appropriately functionalized dehydroglutamate **4**. One compelling virtue of this approach was that we could employ L-serine as a chiral educt, thus allowing us to access the required aminodiacid in optically pure form. Further support for this approach is also derived from our previous work, in which we have shown that ABHxD-I could be produced via a related photochemical cycloaddition that proceeds in the criss-cross mode.[7] Accordingly, employing a known procedure,[9] the oxazolidine **1** was generated in good yield and in excellent optical purity. Next, alkylation of **1** with 4-iodo-1-butene[10] was realized in 65% yield using LiHMDS as base.[11] Treatment of **2** with HCl in methanol yielded α-(3-butenyl)serine methyl ester which was protected with benzyl chloroformate to give compound **3**. The alcohol was oxidized under Swern conditions, and the resulting aldehyde was treated with methyl (triphenylphosphoranylidene)acetate to produce the α,β-unsaturated ester **4**, the required precursor for the [2+2] photocycloaddition chemistry.[12] The solution of the diene **4** in acetone was then irradiated using a Pyrex

apparatus to afford an inseparable mixture of two bicyclic products in a ratio of 8/3. The mixture was deprotected with Pd/C under an atmosphere of H_2 to afford the products **5a** and **5b** as two chromatographically separable isomers. Unequivoval proof of the structure of compound **5a** was obtained from single crystal X-ray analysis of its 1-naphthylurea derivative **7**.[13] The ORTEP drawing of **7** is shown in Figure 1.

The diesters **5a** and **5b** were individually treated with 6 N HCl at reflux for 1 h to afford the final products **6a** and **6b**.[14]

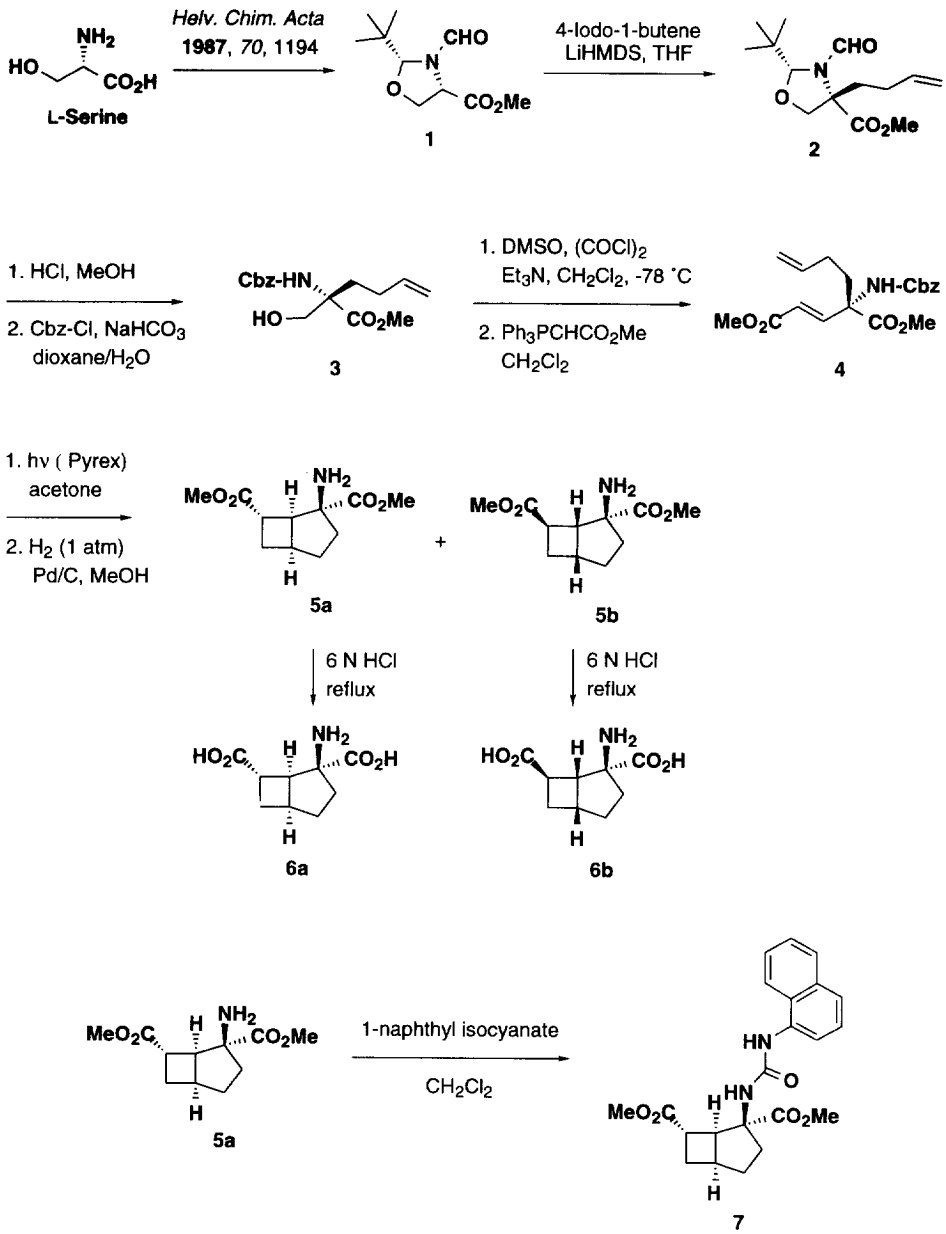
Figure 1. ORTEP drawing of **7**.



Biological Results

Chinese hamster ovary (CHO) cells stably expressing mGluR1a, mGluR5a, mGluR2, mGluR4, or mGluR6 were cultured as described previously[15] and used for measurements of phosphoinositide (PI) hydrolysis or cAMP formation. For measurements of PI hydrolysis, cells expressing mGluR1a or mGluR5a were cultured in 24-well plates and then labeled overnight with 1 μCi/mL of [^{3}H]*myo*-inositol (specific activity 17 Ci/mmol, Amersham). CHO cells expressing mGluR2, mGluR4, or mGluR6 were cultured in 96-well plates. Measurements of PI hydrolysis and of forskolin-induced cAMP formation were performed as described previously.[15] When tested at these six subtypes as an antagonist, compound **6a** was found to exhibit no activity. Also, when tested as an agonist at each of these subtypes, **6a** was found to have relatively weak activity at all of the subtypes. Expressed as a percentage of the maximum agonist activity that can be achieved at the individual subtypes, a 300 μM concentration of compound **6a** gave the following results: 16% at mGluR1a, 19% at mGluR5, 17% at mGluR2, 23% at mGluR4, and 20% at mGluR6. In sharp contrast to these results, we note that LY354740 has been shown to act as a potent group II agonist with an EC_{50} = 5.1 nM at human mGluR2, a finding that we have independently confirmed in our own studies. While the present results were somewhat unexpected, the work clearly shows that even a modest structural change in these rigidified glutamate analogues can have a dramatic effect on compound potency.

Scheme 1


L-Serine
Helv. Chim. Acta
1987, 70, 1194
1
4-Iodo-1-butene
LiHMDS, THF
2
1. HCl, MeOH
2. Cbz-Cl, NaHCO3
dioxane/H2O
3
1. DMSO, (COCl)2
Et3N, CH2Cl2, -78 °C
2. Ph3PCHCO2Me
CH2Cl2
4
1. hν (Pyrex)
acetone
2. H2 (1 atm)
Pd/C, MeOH
5a
5b
6 N HCl
reflux
6a
6b
1-naphthyl isocyanate
CH2Cl2
7

Acknowledgment. We are indebted to the Department of Defense (grant N° DAM D 17-93-V-3018), the Office of Naval Research, and NIDA for their support of these studies.

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13. Crystal structure details: $C_{22}H_{24}N_2O_5$, M_r = 396.43, orthorhombic space group $P2_12_12_1$, a = 10.508(5), b = 17.402(2), c = 24.121(2) Å, V= 4411(2) Å^3, Z = 8. D_X = 1.19g cm^{-3}, Cu Kα radiation (λ = 1.54178 Å), μ

= 0.70 mm^{-1}, $F(000)$ = 1680, T = 293 K. Data were collected on a 1K CCD Siemens area detector system using a Rigaku rotating anode source and Gobel mirrors. Three degree ω rotation scans were used to collect 11908 reflections out to a resolution of ~1 Å. There were 3950 independent reflections, of which 2490 were observed ($I > 2\sigma I$), giving an R_{int} of 0.050. The structure was solved using a combination of Shake n Bake[16] to get a partial structure which was then expanded using SHELXS.[17] The structure was refined on F^2 using all 3950 data and 519 parameters with the program SHELXL.[17] While the conformation of the C1 - C7 fused ring system is the same in both molecules in the asymmetric unit, there are differences in the orientations of the side chains, and there is a disordered side chain on one of the molecules. All nonhydrogen atoms were refined with anisotropic thermal displacements, and H atoms were included using a riding model. Final agreement factors were R(F) = 0.085 and wR(F2) = 0.219 for the observed data, S = 1.11 with maximum and minimum peaks in the final difference map of 0.28 and -0.21 Å. The authors have deposited the atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. They can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EW, UK.

14. The structures and stereochemical assignments for all compounds were determined using ^{1}H NMR, ^{13}C NMR, HETCOR, 2D COSY, and NOESY experiments. Selected data for key compounds: Compound **2**: R_f 0.7 (EtOAc/hexane 3/7); $[\alpha]^{25}_D$ -23.6° (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$; MR = major, mr = minor rotamer) δ 0.91 (s, 9H, MR), 1.02 (s, 9H, mr), 1.80–2.32 [m, 4H (MR) and 3H (mr)], 2.52–2.68 (m, 1H, mr), 3.77 (s, 3H, mr), 3.80 (s, 3H, MR), 3.98 (d, *J* = 8.7 Hz, 1H, mr), 4.30 (d, *J* = 8.7 Hz, 1H, mr), 4.63 (d, *J* = 9.3 Hz, 1H, MR), 4.90–5.10 [m, 4H (MR) and 2H (mr)], 5.31 (br s, 1H, mr), 5.64–5.84 [m, 1H (MR) and 1H (mr)], 8.40 (s, 1H, mr), 8.50 (s, 1H, MR). Compound **4**: $[\alpha]^{25}_D$ +27° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.80–1.96 (m, 1H), 1.96–2.14 (m, 2H), 2.36–2.51 (m, 1H), 3.74 (s, 3H), 3.77 (s, 3H), 4.97 (d, *J* = 9.9 Hz, 1H), 5.00 (d, *J* = 17.1 Hz, 1H), 5.09 (s, 2H), 5.62–5.78 (m, 1H), 5.85 (br s, 1H), 5.98 (d, *J* = 15.9 Hz, 1H), 7.10 (d, *J* = 16.0 Hz, 1H), 7.36 (br s, 5H). Compound **6a**: ^{1}H NMR (D_2O) δ 1.78–1.92 (m, 1H), 1.92–2.06 (m, 1H), 2.14–2.31 (m, 1H), 2.37–2.48 (m, 2H), 2.49–2.62 (m, 1H), 2.91–3.06 (m, 1H), 3.21 (q, 1H, *J* = 8.1 Hz), 3.36 (t, 1H, *J* = 6.6 Hz); ^{13}C NMR (D_2O) δ 27.1, 30.3, 34.1, 35.2, 35.3, 47.4, 67.1, 174.2, 178.3. Compound **6b**: ^{1}H NMR (D_2O) δ 1.76–1.94 (m, 3H), 2.07 (dd, 1H, *J* = 6.6 and 14.4 Hz), 2.33 (dt, 1H, *J* = 9.0 and 12.3 Hz), 2.68 (ddd, 1H, *J* = 9.3, 12.6 and 14.7 Hz), 2.74–2.88 (m, 1H), 3.05 (t, 1H, *J* = 6.3 Hz), 3.21–3.32 (m, 1H).

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