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(2*S*,1′*S*,2′*R*,3′*R*)-2(2′-Carboxy-3′-hydroxymethyl-cyclopropyl)glycine-[^{3}H], a potent and selective radioligand for labeling group 2 and 3 metabotropic glutamate receptors

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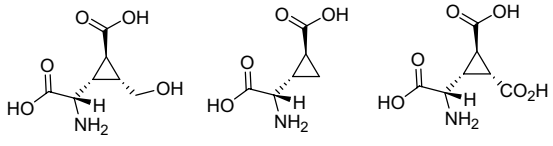
Abstract—We report herein the synthesis of the tritium labeled isotopomer of **1** and its use as a radioligand to label mGlu8 receptors in rat forebrain membranes as well as cloned human recombinant mGlu receptors. **[^{3}H]-1** was synthesized by the $NaBT_4$ reduction of an activated analog of **5**. **[^{3}H]-1** bound appreciably to recombinant human mGlu2, mGlu3 and mGlu8 receptors and to rat forebrain membranes and was displaced by *L*-glutamate and *L*-(+)-2 amino-4-phosphonobutyric acid. The results indicate that **[^{3}H]-1** should be a useful ligand for the study of mGluR2, 3, and 8 receptors in cloned cell lines and possibly brain tissue.

L-Glutamate is the major excitatory amino acid in the mammalian central nervous system. Glu receptors are subdivided into ionotropic (i-Glu's) and metabotropic receptors (mGlu's).[1] Eight distinct mGlu receptor proteins (mGlu1-8) have been cloned, which have been divided into three distinct groups based on amino acid homology, signal transduction mechanisms, and agonist pharmacology.[2] Various members of the carboxycyclopropylglycines (CCG's) have been synthesized and possess both agonist and antagonist activity depending on their substitution patterns. L-CCG-I ((2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine, **2**) is a selective, potent agonist for Group 2 and some Group 3 mGlu receptors.[3] DCG-IV (**3**, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine) is a potent Group 2 agonist, but it also has agonist activity at the NMDA receptor and antagonist activity at Group 3.[4] The synthesis and biological evaluation of (2*S*,1′*S*,2′*R*,3′*R*)-2(2′-carboxy-3′-hydroxymethylcyclopropyl)glycine (**1**), which is a highly potent agonist at mGlu2, 3, 6, and 8 receptors has recently been described.[5] In addition to low nanomolar EC_{50}'s (measured by its ability to influence forskolin-stimulated c-AMP formation) to mGlu2, 3, 6, and 8 receptors, **1** has shown potent oral activity in animal models.[5] In this letter, we report on the synthesis of the tritium labeled isotopomer of **1** and its use as a radioligand to label mGlu8 receptors in rat forebrain membranes as well as cloned human recombinant mGlu receptors.



1 **2**, *L*-CCG-1 **3**, DCG-IV

It was envisioned that RuO_4 oxidation of **1**, which was suitably protected would afford a protected analog of **3**. Subsequent reduction of the resulting carboxylic acid with tritiated borane would then provide **1**-[^{3}H] with two tritium labels. Reaction of **4** with $RuCl_3/NaIO_4/CCl_4/H_2O$ yielded not only the desired carboxylic acid **5** but also **6** (Scheme 1).

The products of the reaction (**5**, **6**) were readily separated by flash chromatography on silica gel.

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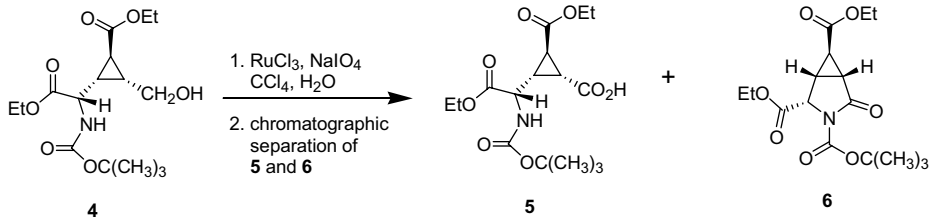
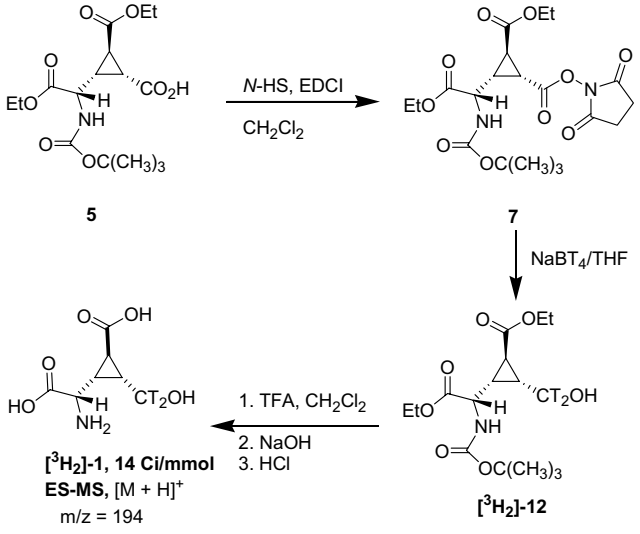


Scheme 1.



Scheme 2.

In model studies, reduction of ***ent*-5** with BD_3/THF afforded ***ent*-4-[$^2H_2$]** (which with the exception of the deuterium, was identical to **4** by TLC and NMR) in 27% yield after chromatography.[6] Reaction of **5** with BT_3/THF (generated in situ by the reaction of $NaBT_4$ with BF_3) yielded only unreacted starting material (presumably because of the hydrolysis of BT_3 by adventitious water). Cordova et al. had previously synthesized the δ-*N*-hydroxysuccinimide (*N*-HS) ester of *N*-*t*-Boc-glutamic acid α-benzyl ester, which allowed the regiospecific reduction of the δ-carboxyl with $NaBH_4$.[7] Treatment of **5** with *N*-HS in CH_2Cl_2 in the presence of EDCI, yielded *N*-hydroxysuccinimide ester **7** after work-up ($[M+H]^+$, $m/z = 457$) (Scheme 2). Subsequent reduction of **7** with $NaBT_4$/THF (5 Ci, 60 Ci/mmol, with acidic work-up) yielded the desired alcohol **8**.[8] This material was not characterized, but treated directly with TFA/CH_2Cl_2 followed by saponification of the esters and extensive HPLC purification to yield **[^{3}H]-1**.[9] This material co-eluted with authentic **1** on HPLC;[10] the specific activity was 14 Ci/mmol. The ^{3}H NMR in D_2O showed resonances at δ 3.68 and 3.87 ppm, which correspond to tritium labeling in the hydroxymethyl functionality. ES-MS showed a protonated mass ion ($[M+H]^+$) at $m/z = 194$.

Rat forebrain membranes were prepared from dissected brain tissue, washed multiple times by centrifugation, and then frozen.[11,12] Membranes from cells expressing recombinant human mGlu receptors were prepared in the same manner. The frozen membrane pellets were thawed on the day of the assay, suspended in ice cold 30 mM Tris HCl buffer (plus 5 mM $ZnCl_2$) at pH 7.6, homogenized, and washed twice by centrifugation at 50,000*g* for 10 min. The washed tissue (0.1–0.2 mg of protein) was then added to deep well micro-titer plates containing **[^{3}H]-1** at appropriate concentrations for saturation curves and at 5 nM for testing across the clones. The final assay volume was 0.5 mL. Nonspecific binding of **[^{3}H]-1** was defined as the amount bound in the presence of 1 mM L-glutamate. Assay plates were incubated for 60 min at room temperature and then the reaction was terminated using ice cold assay buffer. Bound and free radioligand were separated by rapid filtration.

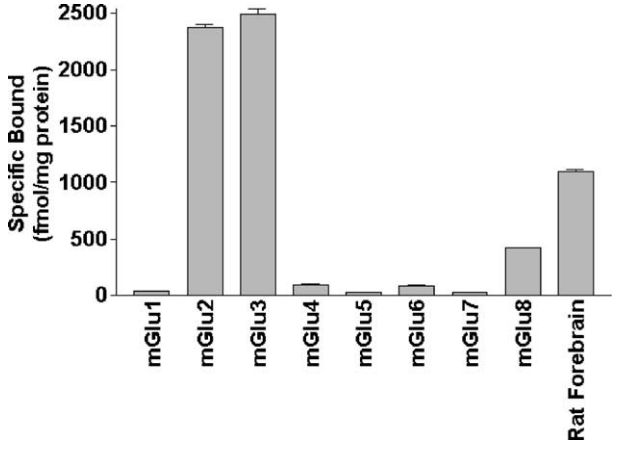


Figure 1. **[^{3}H-1]** binding to human mGluR's and to rat forebrain.

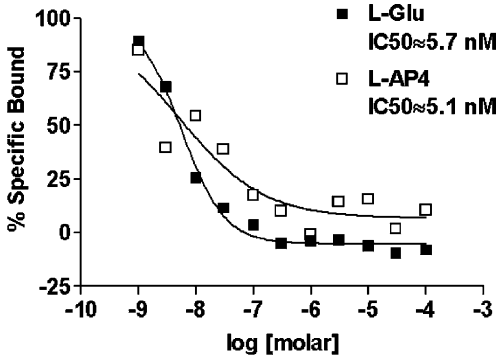


Figure 2. Displacement of **[^{3}H-1]** binding to mGlu8 receptors by L-glutamate and L-AP4.

[^{3}H]-1 bound appreciably to recombinant human mGlu2, mGlu3, and mGlu8 receptors and to rat forebrain membranes (Fig. 1). Specific binding to mGlu1, 4, 5, 6 and 7 receptors was very low by comparison. The binding of **[^{3}H]-1** to the mGlu8 receptor is of high affinity and was displaced by the endogenous ligand L-glutamate (IC_{50} = 5.7 nM) and the selective Group III mGlu receptor agonist L-(+)-2 amino-4-phosphonobutyric acid (L-AP4, IC_{50} = 5.1 nM) (Fig. 2). **[^{3}H]-1** bound with a $K_d = 6.3 \pm 0.9$ nM and a $B_{max} = 1217 \pm 132$ fM/mg protein. These data indicate that **[^{3}H]-1** should be a useful ligand for the study of mGluR2, 3, and 8 receptors in cloned cell lines and possibly brain tissue.

References and notes

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6. All compounds were evaluated by NMR, HPLC, and HR-ES-MS.
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8. The titration was conducted at Amersham Biosciences, Cardiff, Wales, UK using methods developed by the authors.
9. The material required extensive purification by HPLC using a Hypersil column eluting with a H_2O/CH_3CN/TFA gradient, followed by Partisil ODS column eluting with H_2O/TFA and finally a Hydrobond Aquapore column eluting with H_2O/HOAc.
10. HPLC chromatography: Zorbax SB-C18 (4.6 × 250 mm) with gradient elution at 1 mL/min and UV detection at 200 nm as well as radiochemical detection; Solvent A: 2.3 g/L octanesulfonic acid, sodium salt in 0.1% TFA, Solvent B: 0.1% TFA in acetonitrile, Gradient: 3% B for 15 min then to 50% B over 30 min. Radiochemical purity was 92.8%.
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