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# [$^3$H]LY341495, A HIGHLY POTENT, SELECTIVE AND NOVEL RADIOLIGAND FOR LABELING GROUP II METABOTROPIC GLUTAMATE RECEPTORS

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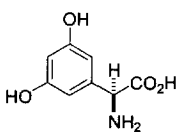
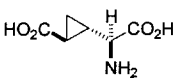
**Abstract**. We report herein the synthesis and pharmacological characterization of a tritiated version of the potent and selective cyclopropyl amino acid LY341495 as a radioligand to label group II metabotropic glutamate receptors in rat brain homogenates. © 1998 Elsevier Science Ltd. All rights reserved.

Metabotropic glutamate receptors (mGluRs) are a class of excitatory amino acid receptors that are coupled to G-proteins and transduce signals through the production of second messengers. Three subclasses of mGluRs are distinguished based on the second messenger system to which they are coupled, their pharmacology, and their molecular sequence homology.[1,2] Group I mGluRs (mGluR1 and mGluR 5), which are selectively activated by S-3,5-DHPG (**1**),[3] are coupled to phospholipase C, so signals are transduced by inositol trisphosphate and diacyl glycerol. Group II mGluRs (mGluR2 and mGluR3) are negatively coupled to adenyl cyclase, so signals are transduced through cyclic-adenosine monophosphate (c-AMP). They are selectively activated by compounds such as L-CCG 1 (**2**)[4] and 2*R*,4*R*-APDC (**3**).[5] Group III mGluRs (mGluR4, mGluR6, mGluR7 and mGluR8) are also negatively coupled to adenyl cyclase, and are selectively activated by L-AP4 (**4**).




**1**

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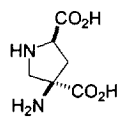


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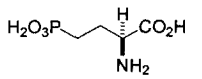


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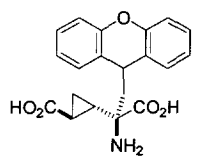


**5**

Our research program was targeted to the development of selective antagonists for group II mGluRs. We hoped to identify effective research tools that would allow us to understand the pharmacology of this class of compounds and better define their therapeutic potential. We recently reported the synthesis and characterization of a series of carboxycyclopropylglycines in which an arylalkyl substituent was appended onto the amino acid carbon.[6,7] This yielded a series of potent, selective and systemically bioavailable group II mGluR antagonists.

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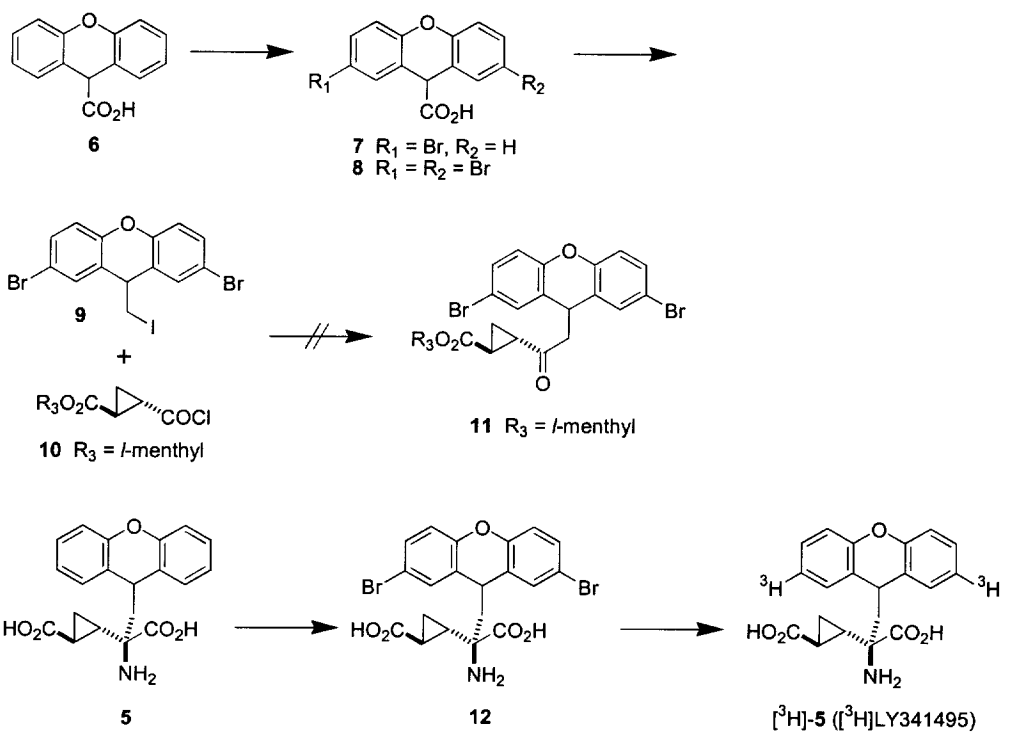
The best compound of this series was the 9-xanthylmethyl substituted amino acid **5** (LY341495),[8] which displaced ACPD-sensitive [$^3$H]glutamate binding in rat forebrain membranes[9,10] with an $IC_{50}$ = 2.9 ± 0.6 nM. Under the conditions that this binding assay is done, glutamate selectively labels group II mGluRs. However, the modest affinity of glutamate ($K_i$ = 0.092 ± 0.050 μM) necessitated the use of a cocktail of compounds to block its binding to ionotropic glutamate sites and centrifugation is required to separate bound from free ligand. The high affinity of **5** made it an attractive target to radiolabel as a ligand for group II mGluRs, and it may well allow for the use of filtration techniques for the binding assay. In this paper, we report the successful tritiation of **5** and its use as a radioligand to label group II mGluRs in rat forebrain membranes.

The synthesis of the radiolabel is shown in the Scheme. We envisioned introduction of a halogen onto each aromatic ring of the xanthyl moiety followed by hydrogenolysis with tritium to introduce the radiolabel. It was our goal to introduce two halogens so that reduction would not produce another asymmetric center. We originally investigated bromination of xanthene-9-carboxylic acid **6**. At room temperature, selective monobromination to **7** was observed. Upon heating, one could force the reaction to near completion to deliver the desired dibromide **8**, however, careful recrystallization was required to remove unwanted **7**. Compound **8** was converted to the iodide **9** by our traditional procedures,[6,7] but this route was abandoned when we found that conversion of **9** to the zincate followed by palladium mediated coupling with **10** afforded little to none of the desired keto-ester **11**. We next turned our attention to direct bromination of **5** to provide the precursor for radiolabeling. We were gratified to find that bromination was efficient at 55 °C in acetic acid with an excess of bromine. Cooling and addition of water at the end of the reaction provided analytically pure crystalline dibromide **12**. Reduction of silylated **12** (*O,N*-bis-(trimethylsilyl)amide, $CH_3CN$) was carried out using 10% palladium on carbon in DMF under one atmosphere of tritium gas to afford after HPLC purification[11] [$^3$H]-**5**.[12] This compound was found to have a specific activity of 50 Ci/mmol, and was determined to be analytically pure by HPLC.[11]

Binding of [$^3$H]-**5** to rat brain homogenates was carried out using assay conditions we have shown previously to produce the selective labeling of group II mGluRs by [$^3$H]glutamate.[9,10] Washed rat forebrain membranes (100-200 μg tissue protein) were incubated on for 30 min with [$^3$H]-**5** in 10 mM phosphate buffer containing 100 mM potassium bromide. Nonspecific binding of [$^3$H]-**5** was determined using 1 mM L-glutamate. Bound and free ligand were separated by rapid filtration, and data are mean ± S.E. of three determinations in triplicate. A high percentage of specific binding of [$^3$H]-**5** (0.1-100 nM) was observed that represented >90% of total [$^3$H]-**5** binding. [$^3$H]-**5** bound with a $K_d$ = 0.844 ± 0.110 nM and Bmax = 3.91 ± 0.65 pmol/mg protein, comparing favorably with its mGluR antagonist potency at mGluR2 and mGluR3 receptors,[8] and the number of ACPD-sensitive [$^3$H]glutamate binding sites reported previously in rat forebrain membranes under similar conditions.[9,10] Specific [$^3$H]-**5** (1 nM) binding was potently displaced by nonlabeled **5** ($IC_{50}$ = 1.4 ± 0.6 nM); 1*S*,3*R*-ACPD ($IC_{50}$ = 28.8 ± 1.2 μM); the group II selective mGlu receptor agonist **3** ($IC_{50}$ = 5.7 ± 0.2 μM),[13] but not by the ionotropic glutamate receptor agonists NMDA, AMPA, or kainate ($IC_{50}$s > 1 mM). These

data indicate that [$^3$H]-**5** binds selectively to group II mGluRs in the rat brain under these conditions. This is also the first demonstration of filtration binding for a radiolabeled mGluR antagonist ligand. [$^3$H]-**5** should be a useful ligand to study and further characterize receptor interactions at cloned and native mGluRs.

Scheme

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11. HPLC Conditions: Sorbax SB-phenyl (3 X 250 mm) column with gradient elution of acetonitrile:water:2% aqueous acetic acid (pH 4.9) from 10:80:10 to 70:20:10 over 25 minutes at 0.6 mL/min. Retention time for **5** was 19.5 min, with a radiochemical purity of ≥98%.
12. Tritiation was conducted at Chemsyn Science Laboratories, Lenexa, Kansas, 66215.
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