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Radiosynthesis of PET radiotracer as a prodrug for imaging group II metabotropic glutamate receptors in vivo

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ABSTRACT

Group II metabotropic glutamate receptors (mGluRs) have been implicated in a variety of neurological and psychiatric disorders in recent studies. As a noninvasive medical imaging technique and a powerful tool in neurological research, positron emission tomography (PET) offers the possibility to visualize and study group II mGluRs in vivo under physiologic and pathologic conditions. We synthesized a PET tracer, (*S*,*S*,*S*)-2-(2-carboxycyclopropyl)-2-(3-[^{11}C]methoxyphenethyl) glycine dimethyl ester ([^{11}C]CMGDE), as a prodrug for group II mGluRs, and studied its preliminary biological properties in Sprague-Dawley rats to visualize group II mGluRs. The microPET studies demonstrated that [^{11}C]CMGDE readily penetrated into the brain and the radiotracer generated from [^{11}C]CMGDE had fast reversible binding in the group II mGluRs rich regions including striatum, hippocampus and different cortical areas. Blocking studies with LY341495 showed 20–30% decrease of binding of the radiotracer generated from [^{11}C]CMGDE in all brain areas with the highest decrease in the striatum 31.5 ± 3.2%. The results show [^{11}C]CMGDE is the first PET tracer that is brain penetrating and can be used to image group II mGluRs in vivo.

Glutamate is a major neurotransmitter in the central nervous system (CNS) and acts on diverse sets of receptors. The mGluRs are G-protein coupled receptors (GPCRs). Based on sequence similarity, signal transduction mechanism and pharmacological profiles, mGluRs are classified into three groups. Group II mGluRs (including mGluR2 and mGluR3 subtypes) modulate glutamate transmission by second messenger activation to negatively regulate the activity of adenylyl cyclase. Group II mGluRs have been implicated in a variety of neurological and psychiatric disorders in recent studies, such as anxiety disorders,[1] depression, schizophrenia,[2,3] chronic pain syndromes,[4] seizure disorders,[5,6] Parkinson's disease,[7] and substance abuse.[8,9] Thus, compounds that are potent and selective for mGluR2/3 could provide a valuable tool to investigate the involvement of these receptors in various diseases.

A number of extremely potent group II mGluR selective agonists and antagonists had been developed as competitive orthosteric ligands (examples in Fig. 1).[10] LY354740 (EC_{50} = 11.1 nM for mGluR2; 38.0 nM for mGluR3),[11] LY379268 (EC_{50} = 2.69 nM for mGluR2; 4.58 nM for mGluR3),[12] and MGS0028 (EC_{50} = 0.57 nM for mGluR2; 2.07 nM for mGluR3)[13] are the agonists for group II mGluRs, in which LY354740 (Eglumegad) was developed as a research drug for its potential in the treatment of anxiety and drug addiction.[14] The cyclopropane amino-diacid structures were also further modified to generate selective antagonists for group II mGluR, such as LY341495 (IC_{50} = 21 nM for mGluR2; 14 nM for mGluR3)[15,16] and MGS0039 (IC_{50} = 20 and K_i = 2.2 nM for mGluR2; IC_{50} = 24 nM and K_i = 4.5 nM for mGluR3).[17] LY341495 has been

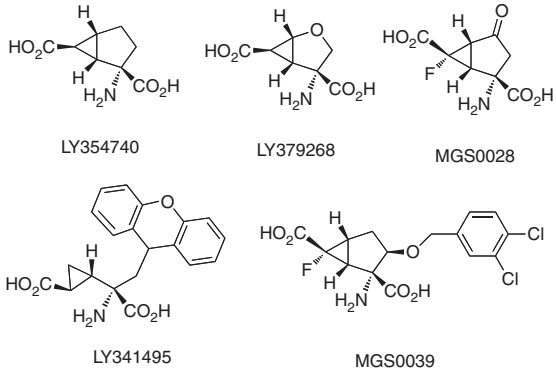

Figure 1. Structures of selected agonists and antagonists for group II mGluRs.

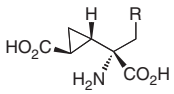

Figure 2. The *S*,*S*,*S*,-isomer of compounds **1–4**.

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Table 1
Binding affinity of the substituted carboxycyclopropylglycines[15]

Compound	R (see Fig. 2)	Stereochemistry	IC_{50}(nM) ± SEM
1	9-Xanthenyl	Mixture	10 ± 1
1-SSS		(*S,S,S*)-Isomer	2.9 ± 0.6
2	3-Methylbenzyl	Mixture	89 ± 36
2-SSS		(*S,S,S*)-Isomer	12 ± 3
3	2,2-Diphenylethyl	Mixture	240 ± 80
3-SSS		(*S,S,S*)-Isomer	35 ± 5
4	3-Methoxybenzyl	Mixture	94 ± 33

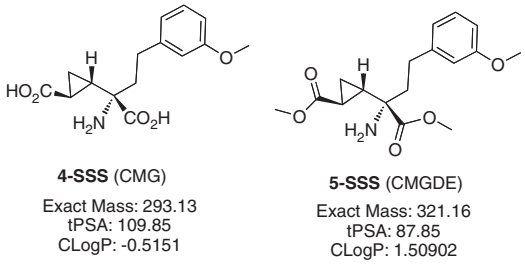
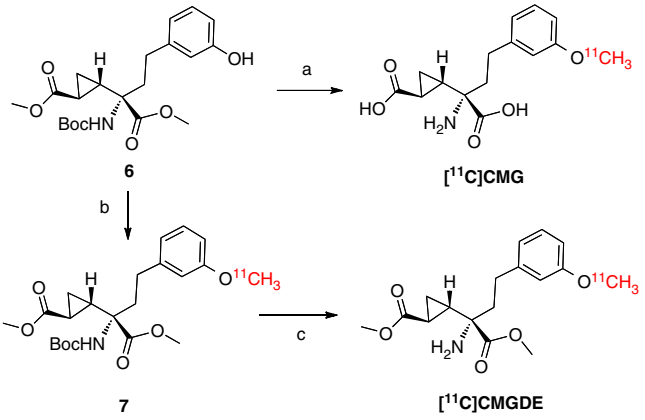


Figure 3. Structures and calculated properties of CMG and CMGDE.

studied for its antidepressant effect and other neurological effects in animal models.[18,19]



Scheme 1. Reagents and conditions: (a) (1) [^{11}C]CH_3I, DMSO, 5 N NaOH, 100 °C; (2) 6 N HCl, 100 °C; (3) 5 N NaOH. (b) [^{11}C]CH_3I, aq. K_2CO_3, DMSO, 120 °C; (c) (1) TFA, CH_2Cl_2, rt; (2) aq. K_2CO_3.

To better understand group II mGluRs and their role in disease, we developed an mGluR2/3 selective radiotracer for in vivo study. As a noninvasive medical imaging technique and a powerful tool in neurological research, PET offers the possibility to visualize and study mGluR2/3 under physiologic and pathologic conditions. Many PET radioligands have been developed for group I mGluRs (mGluR1 and mostly mGluR5),[20] however, no PET tracer is available for imaging group II mGluRs. Here we report on the

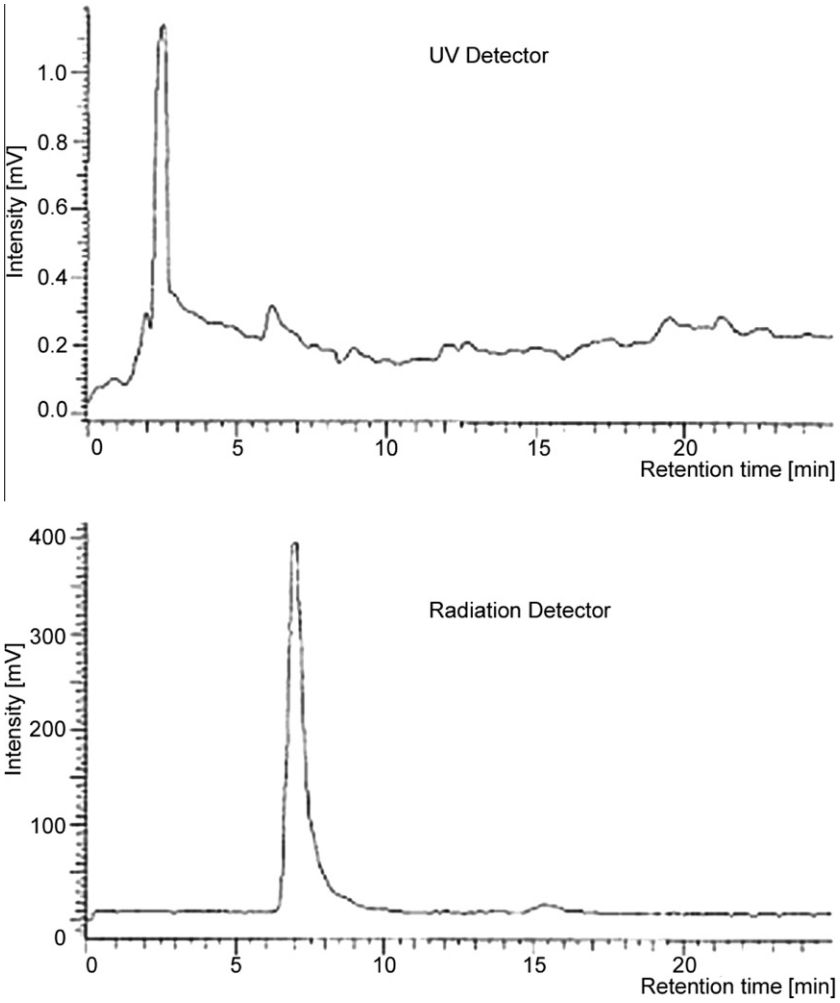


Figure 4. HPLC chromatogram of [^{11}C]CMGDE.

radiosynthesis of a prodrug, (*S*,*S*,*S*)-2-(2-carboxycyclopropyl)-2-(3-[^{11}C]methoxyphenethyl) glycine dimethyl ester ([^{11}C]CMGDE), as a PET tracer for group II mGluRs and its preliminary biological evaluation in Sprague–Dawley rats to imagine group II mGluRs.

Ornstein et al. had reported a series of aryl-substituted carboxycyclopropylglycine derivatives as the potent and selective antagonists of group II mGluRs,[15] in which three of these compounds, **1**, **2**, and **3** (Fig. 2 and Table 1), were resolved into their four constituent isomers (*R*,*S*,*S*-, *S*,*S*,*S*-, *S*,*R*,*R*- and *R*,*R*,*R*-isomer). They found that affinity and functional activity for group II mGluRs reside solely in the *S*,*S*,*S*-isomers of compounds **1–3**, in which the binding affinity of the *S*,*S*,*S*-isomer surpass that of the corresponding isomer mixture by factors of 3.4, 7.4 and 6.9, respectively. LY341495 (**1-SSS**) was the most potent compound in this series and readily penetrated into the brain, however, it is impossible to introduce carbon-11 or fluorine-18 radiolabel into it (along with compounds **2** and **3**) without structural alterations. We therefore selected compound **4** as a lead to PET ligand based on its affinity and easy radiolabelling, in which its *S*,*S*,*S*-isomer will be radiolabelled with carbon-11. Although the IC_{50} value was available only for the mixture of **4**, it was anticipated the radiolabeled *S*,*S*,*S*-isomer of **4** would be more potent than its isomeric mixtures by a similar factor as observed for compounds **1–3**. Moreover, since the amino diacid analog is very polar we also adopted a prodrug approach to improve CNS exposure and pharmaceutical properties of the PET tracer. As Figure 3 shows that the designed prodrug as methyl esters **5-SSS** (CMGDE) has better calculated physical properties compared to the parent compound **4-SSS** (CMG), in which the tPSA value drops from 109.85 to 87.85 and the c log*P* value increase from −0.52 to 1.51.

Radiosynthesis of [^{11}C]CMG and [^{11}C]CMGDE is shown in Scheme 1. [^{11}C]CMG was synthesized in one pot.[21] Carbon-11 methylation of the phenolic hydroxyl in **6**[22] was achieved by heating **6** and [^{11}C]methyl iodide at 100 °C in DMSO and 5 N NaOH, in which two ester groups were also hydrolyzed. In situ addition of hydrochloric acid removed the Boc group, followed by neutralization with NaOH. The crude product was purified by semi-preparative HPLC[23] to give 76 mCi of pure [^{11}C]CMG (radiochemical purity >95%). Total synthesis time was about 45 min. Identity of the labeled compound was confirmed by co-injection of the product [^{11}C]CMG with the cold compound **4-SSS** (CMG). Radiosynthesis of the prodrug [^{11}C]CMGDE was carried out in two steps.[24] Compound **6** was reacted with [^{11}C]methyl iodide in DMSO at 120 °C in the presence of aqueous K_2CO_3 to afford the labeled intermediate **7**. It was critical to use K_2CO_3 as the base in this reaction, which promoted the reaction but not hydrolyzed the ester groups. The labeling reaction was monitored by the analytical HPLC.[23] The intermediate **7** was purified by semi-preparative HPLC, which was then deprotected with trifluoroacetic acid (TFA) in CH_2Cl_2 at room temperature for 12 min. After neutralized by K_2CO_3 solution, the solvent was removed under reduced pressure. The reaction mixture was purified by the semi-preparative HPLC to give 4.4 mCi of [^{11}C]CMGDE with a radiochemical purity of more than 96%. Figure 4 exhibits the HPLC chromatogram of the purified product [^{11}C]CMGDE. The two-steps synthesis took about 70 min.

MicroPET imaging studies in the male Sprague–Dawley rats[25] showed that there was no brain penetration when injected with [^{11}C]CMG (Fig. 5). However, the studies showed that the prodrug [^{11}C]CMGDE readily penetrated into the brain and upon entering into brain the radiotracer[26] generated from [^{11}C]CMGDE had fast reversible binding in several cortical areas, hippocampus and striatum, the sites, which are known to express group II mGluRs[27] (Figs. 6 and 7). The maximum accumulation (2.5–3.5% of the injected dose per cm^3) was observed 2 min after administration (Fig. 6). To confirm the binding specificity, a selective antagonist for group II mGluRs, LY 341495[28], was used as a block agent. The results showed 20–30% decrease of [^{11}C]CMGDE binding in several brain areas excluding olfactory area, which might be affected by the nonspecific accumulation in the Harderian glands (Fig. 7). The highest activity averaged at the time interval 20–40 min was in the striatum, where the highest "blocking" of 31.5 ± 3.2% was also observed. Pre-injection of LY341495 induced 29.5 ± 4.2%

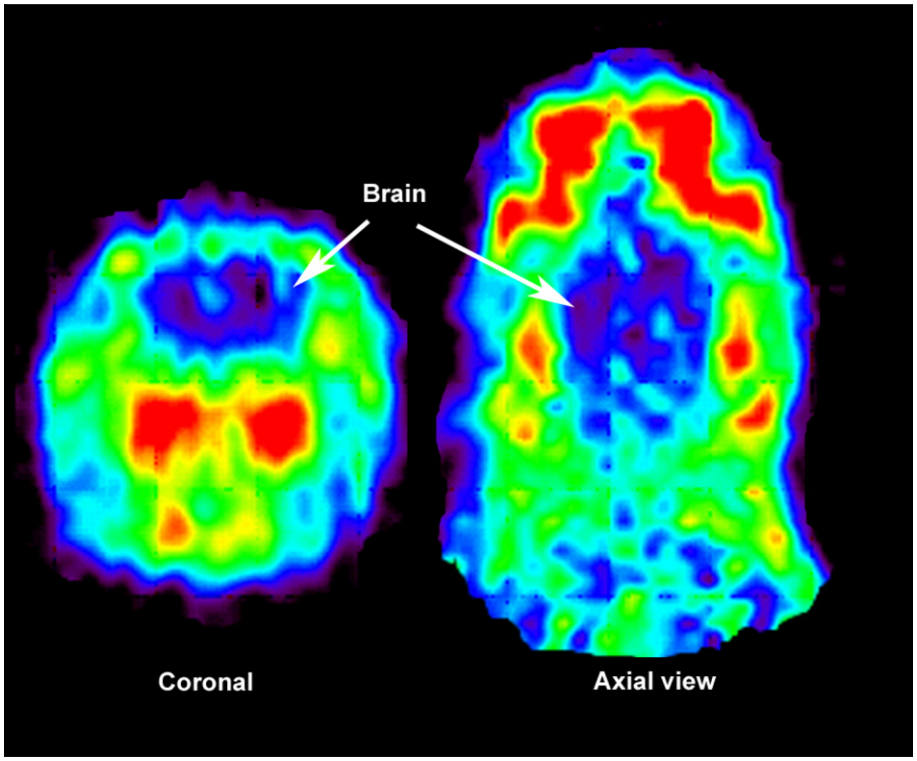


Figure 5. Distribution of [^{11}C]CMG (0.4 mCi) in a Sprague–Dawley rat at 20–25 min after administration.

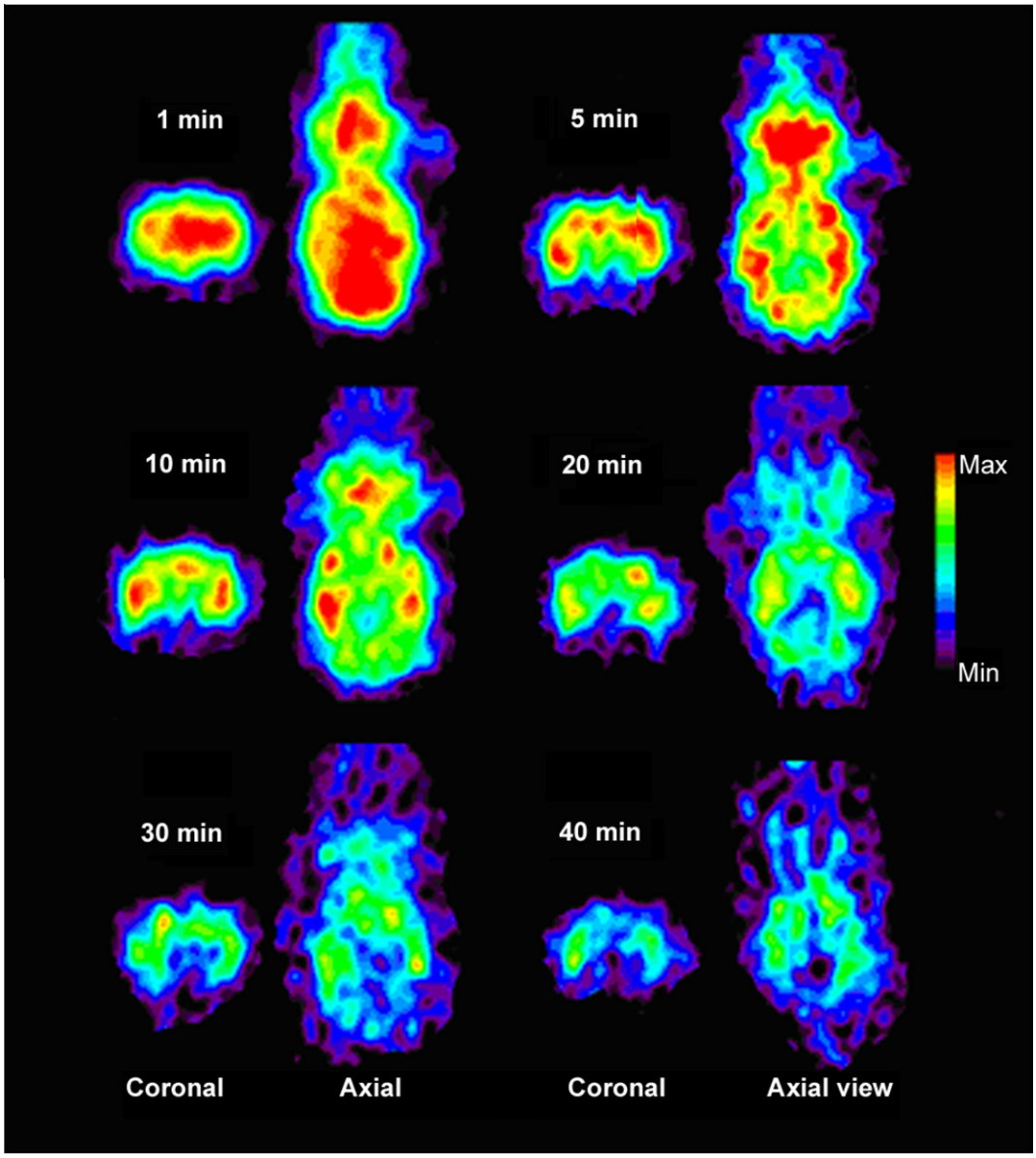


Figure 6. Coronal and axial slices of [^{11}C]CMGDE (0.4 mCi) distribution in the rat brain from 1 min till 40 min after administration. Color-coded images are normalized to each other and correspond the acquisition time of 1 min at the same midbrain level (coronal slice at bregma −1.6 mm; axial slice at bregma −5.4 mm).

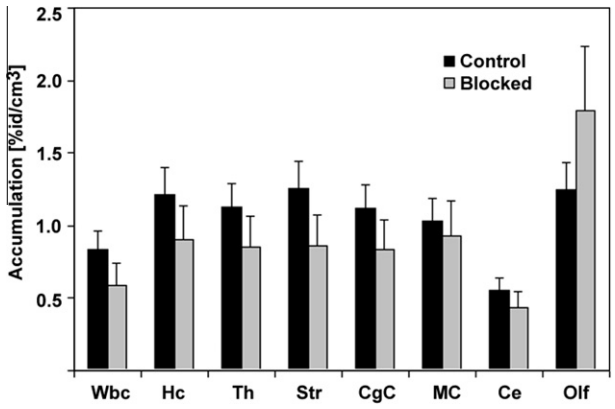


Figure 7. Control PET imaging studies illustrate high accumulation of [^{11}C]CMGDE in several areas of rat brain. Blocking studies with LY341495 verify specific binding. Mean and SEM of the accumulation are presented for 6 control rats and 5 rats after blocking with LY341495 at the time interval of 20–40 min after administration of radioactivity. Wbr = whole brain, Hc = hippocampus, Th = thalamus, Str = striatum, CgC = cingulate cortex, MC=motor cortex, Ce = cerebellum, Olf = olfactory area.

decrease in the accumulation of [^{11}C]CMGDE in the whole brain determined at 20–40 min after administration of radioactivity.

In summary, this is the first successful approach to develop PET imaging ligand for group II mGluRs. The prodrug approach had greatly improved brain penetration and the receptor targeting. These data might provide a foundation for future development of specific PET imaging ligands for group II mGluRs and other subtype selective radioligands. Optimization of the reaction conditions for preparation of [^{11}C]CMGDE is under way to reduce total synthesis time.

Acknowledgment

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21. $^{11}CH_3I$ was bubbled through a 5-ml reaction vial charged with **6** (0.15 ml, 2.7 mg) in DMSO (0.25 ml) and 5 N NaOH (5 μl) at room temperature. The mixture was heated at 100 °C for 7 min and then 0.7 ml of 6 N HCl was added, and the mixture was heated at 100 °C for 5 min. After the reaction was cooled to ambient temperature, 0.9 ml of 5 N NaOH was added to neutralize the mixture. The reaction mixture was then subject to semi-preparative HPLC purification with MeCN and water (v/v 50:50) as the mobile phase. The product was collected between retention time of 3 min and 6 min monitored by a radiation detector, the solvents were evaporated, and the product was then formulated with saline containing 5% ethanol. After filtration the activity was 76 mCi (radiochemical purity >95%).
22. The precursor **6** was obtained from Acenta Discovery Inc., Tucson, Arizona.
23. Analysis and purification were performed on Hitachi L-7100a with a Hitachi UV detector (L-7400), and a Packard radiation detector by using μ-Bondapak C-18 column (7.8 × 300 mm, waters) at a flow of 4 ml/min.
24. $^{11}CH_3I$ was bubbled through a 5 ml V-vial charged with 6 (0.15 ml, 2.7 mg) in DMSO (0.25 ml) and aqueous K_2CO_3 solution (20 μl, 9%) for 3 min at room temperature. The mixture was heated at 120 °C for 7 min and then cooled for 2 min. To the mixture 1.5 ml of MeOH and phosphate buffer (7:3) was added, and the reaction mixture was purified by HPLC using 70% methanol and 30% phosphate buffer (pH 7.4, 20 mM) as the mobile phase. The labeled intermediate **7** was collected at about 6 min (retention time). After the solvent was removed at reduced pressure, TFA, (0.2 ml) in CH_2Cl_2 (3 ml) was added to the flask, which was rotated for 12 min at room temperature. After the mixture was neutralized by slowly adding K_2CO_3 solution (1.5 ml, 14%). The solvent was removed under reduced pressure at room temperature, followed by adding 3 ml ethanol. The crude product was purified by HPLC using 55% MeOH and 45% phosphate buffer (pH 7.4, 20 mM) as the mobile phase. The product was collected at about 4 min (retention time). After the solvent was removed under reduced pressure, the product was formulated with saline containing 5% ethanol to give 4.4 mCi of [^{11}C]CMGDE (radiochemical purity >96%).
25. microPET imaging studies were conducted in male Sprague–Dawley rats (weight 250–300 g, Charles River Laboratories, Wilmington, MA). [^{11}C]CMG was studied in 5 rats. [^{11}C]CMGDE was tested on eleven rats including six control studies and five blocking. Care was performed in accordance with the guidelines of the Committee of Animals of The Massachusetts General Hospital. After the rat was anesthetized (1.0–1.5% isoflurane with 1 L/min of O_2 flow), the tail vein was catheterized for administration of the radiotracer and/or blocking agent. The rat was placed ventrally into the imaging position at the center of the imaging field, where the spatial resolution is 1.8 mm (microPET scanner, P4, Concorde Microsystems, Knoxville, TN). Heart rate was monitored over the whole imaging time using a Heska Vet/Ox Plus 4800 monitor. Transmission imaging (9 min) was done using a rotating point source of cobolt-57 before administration of the radioactivity to obtain data for attenuation correction. Subsequently, [^{11}C]CMGDE (0.4–0.5 mCi) was injected into the tail vein and volumetric dynamic data were acquired for 60 min. To investigate specific binding the mGluR2/3 antagonists, LY341495 (1–1.50 mg/kg, iv) was injected 1 min before administration of the radiotracer.
26. The active species were not identified, in which [^{11}C]CMG or/and the corresponding monoesters were expected to form after the ester groups were hydrolyzed in vivo.
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28. LY341495 was purchased from Tocris Cookson, Inc. The powder was dissolved into the sterile water with a little 0.1 N NaOH. The pH was adjusted to 7 with 0.1 N HCl.