Synthesis and Metabotropic Glutamate Receptor Activity of S-Oxidized Variants of (−)-4-Amino-2-thiabicyclo-[3.1.0]hexane-4,6-dicarboxylate: Identification of Potent, Selective, and Orally Bioavailable Agonists for mGlu2/3 Receptors

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(−)-4-Amino-2-thiabicyclo-[3.1.0]hexane-4,6-dicarboxylate (LY389795, (−)-**3**) is a highly potent and selective agonist of metabotropic glutamate receptors 2 (mGlu2) and 3 (mGlu3). As part of our ongoing research program, we have prepared S-oxidized variants of (−)-**3**, compounds (−)-**10**, (+)-**11** (LY404040), and (−)-**12** (LY404039). Each of these chiral heterobicyclic amino acids displaced specific binding of the mGlu2/3 receptor antagonist ^{3}H-2*S*-2-amino-2-(1*S*,2*S*-2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid (^{3}H-LY341495) from membranes expressing recombinant human mGlu2 or mGlu3 and acted as potent agonists in cells expressing these receptor subtypes. Docking of the most potent of these derivatives, (+)-**11**, to mGlu2 revealed the possibility of an additional H-bond interaction between the sulfoxide oxygen of (+)-**11** with tyrosine residue Y236. Pharmacokinetic analysis of mGlu active enantiomers (+)-**11** and (−)-**12** in rats showed each to be well absorbed following oral administration. Consistent with their mGlu2/3 agonist potency and pharmacokinetic properties, both (+)-**11** and (−)-**12** blocked phencyclidine-evoked ambulations in a dose-dependent manner, indicating their potential as nonclassical antipsychotic agents.

Introduction

Glutamic acid is the principle excitatory neurotransmitter in the mammalian central nervous system (CNS), influencing neuronal transmission through its activation of both ligand-gated ion channels (ionotropic glutamate [iGlu] receptors,) and G-protein coupled (metabotropic glutamate [mGlu]) receptors.[1,2] MGlu receptors (mGlu1−8) belong to the class 3 GPCR superfamily whose members include a bacterial periplasmic binding protein, as well as the calcium-sensing GABA-b and certain insect pheromone receptors. This unique family of GPCRs is characterized by the presence of a large, bi-lobed extracellular N-terminal domain that constitutes the agonist recognition site. Agonist activation is believed to occur via the closure of the two lobes around the agonist ligand in a manner conceptually similar to a clamshell or venus flytrap mechanism. MGlu receptors are highly heterogeneous with respect to their structure, function, and localization within the CNS and represent promising targets for therapeutic intervention in a multiplicity of CNS disorders.[3−10] We have previously reported the identification of (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate ((+)-**1**) as well as heterocyclic variants of this molecule ((−)-**2** and (-)-**3**, Figure 1) as potent and selective agonists for mGlu2 and mGlu3 receptors.[11−14] Compounds **1** and **2** have been studied extensively both *in vitro* and *in vivo*, providing important target validation data supporting hypotheses that mGlu2/3 receptors are novel targets for the treatment of anxiety,[12,15−20] schizophrenia,[21−23] convulsive disorders,[12,14,24,25] Parkinson's disease,[26,27] pain,[28] and neurodegeneration.[29−32] Recently, we showed that substitution at the C4-position of **1** with a methyl group resulted in mGlu2/3 receptor agonist activity for **4** (Figure 1), but mixed mGlu2 agonist/mGlu3 antagonist activity for **5** (Figure 1), indicating that mGlu2 and mGlu3 receptor space adjacent to the C4-position of bound **1** is highly sensitive to ligand structure, and that differential effects on mGlu2 versus mGlu3 receptor function can be realized depending on C4-substituent stereochemistry.[33] In this account, we continue to investigate the nature of this ligand−receptor interaction through the preparation and pharmacological evaluation of S-oxidized variants of (−)-**3**.

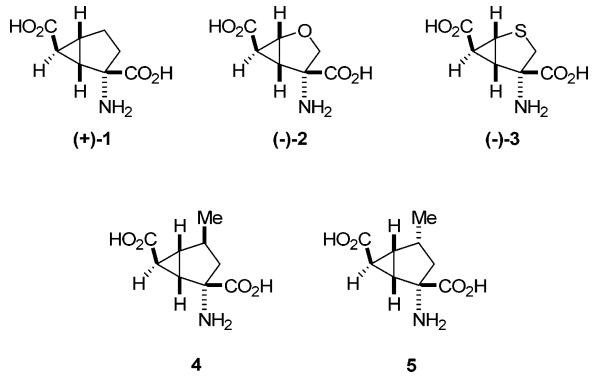

Figure 1. Chemical structures of mGlu2/3 receptor orthosteric ligands.

Results

Chemistry. Analogs (−)-**10,** (+)-**11**, and (−)-**12** were prepared as described in the Scheme from the mGlu2/3 receptor-active (−)-1*R*,4*S*,5*S*,6*S* enantiomer (−)-**3**, itself synthesized as previously described.[14,34] Conversion of the amino diacid to the protected N-Boc amino dimethylester was accomplished under standard conditions affording (−)-**6** in 79% overall yield. Oxidation of (−)-**6** with *m*-CPBA at low temperature afforded a mixture of diastereomeric sulfoxides that were chromatographically separated to provide (−)-**7** and (+)-**8** in 5 and 89% yields, respectively. The predominant sulfoxide diastereomer (+)-**8** was further oxidized to sulfone (−)-**9** with *m*-CPBA at

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Scheme 1. Synthesis of S-Oxidized Variants of (−)-**3**

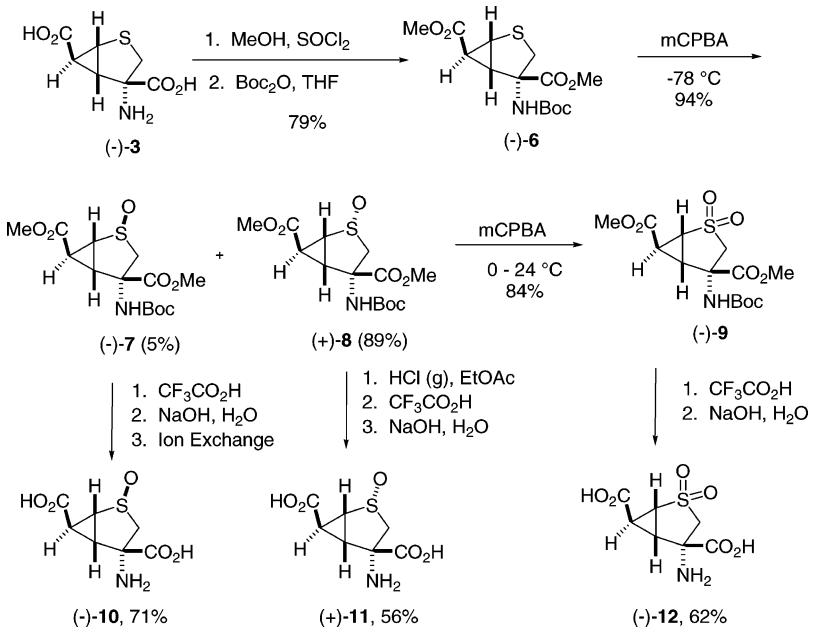

Table 1. Effects of Bicyclic Amino Acids at Recombinant Human mGlu2 and mGlu3 Receptors *In Vitro*

	displacement of ^{3}H-341495 binding to membranes expressing recombinant mGlu2, mGlu3, or mGlu8[a]		functional (cAMP) responses in mGlu receptor-expressing cells[b,c] EC_{50} (nM)		
cmpd	mGlu2	mGlu3	mGlu2	mGlu3	mGlu8
(+)-**1**	74.9 ± 9.1	93.3 ± 2.6	11.1 ± 2.5	38.0 ± 3.1	11470 ± 207
(−)-**2**	14.1 ± 1.4	5.8 ± 0.6	2.7 ± 0.3	4.6 ± 0.04	1690 ± 130
(−)-**3**	40.6 ± 3.7	4.7 ± 1.2	3.9 ± 0.8	7.6 ± 2.1	7340 ± 2720
(−)-**10**	508 ± 9	447 ± 27	18.1 ± 0.1	210 ± 50	>100 000
(+)-**11**	4.4 ± 0.5	0.17 ± 0.02	0.089 ± 0.002	1.4 ± 0.8	3070 ± 770
(−)-**12**	149 ± 11	92 ± 14	23.4 ± 0.9	48 ± 10	47 773 ± 3505

[a] For experimental details, see references 46 and 47. [b] For experimental details, see references 11 and 49. [c] At concentrations up to 100 μM, no agonist or antagonist activity was observed for compounds of the analogs in cells expressing mGlu1a, mGlu5a, mGlu4a, or mGlu7a receptors.

room temperature in 84% isolated yield. Sequential deprotection of the Boc and ester groups under standard conditions provided the final products (−)-**10**, (+)-**11**, and (−)-**12**, each isolated from water at or near the isoelectric point in 71, 56, and 62% yields, respectively (Scheme 1). Unambiguous assignment of the sulfoxide stereochemistry for analogs (−)-**10** and (+)-**11** was achieved by single-crystal X-ray analysis of intermediate (−)-**7** and final product (+)-**11** (Figure 2).[35] In an analogous manner, stereoisomeric (−)-**11** and (+)-**12** were prepared from (+)-**3** (syntheses not shown).

Biochemical Pharmacology. Compounds (−)-**10**, (+)-**11**, and (−)-**12** inhibited specific binding of the mGlu2/3 receptor antagonist ^{3}H-2*S*-2-amino-2-(1*S*,2*S*-2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid from membranes expressing recombinant human mGlu2 receptors, with K_i values of 508 ± 9 nM, 4.4 ± 0.5 nM, and 149 ± 11 nM, respectively (Table 1).[36] Comparable affinities of these compounds for mGlu3 receptors was also observed, with K_i values of 447 ± 27 nM, 0.17 ± 0.02 nM, and 92 ± 14 nM for analogs (−)-**10**, (+)-**11**, and (−)-**12**, respectively. At concentrations up to 100 μM, no appreciable mGlu2 or mGlu3 receptor binding was observed for enantiomers (−)-**11** or (+)-**12** (data not shown). Evaluation of the functional effects of the potent mGlu2/3 ligands on cAMP responses in RGT cells expressing either mGlu2 or mGlu3 receptors demonstrated each to possess potent agonist activity in both mGlu2- and mGlu3-expressing cell lines, with (−)-**10**, (+)-**11**, and (−)-**12** displaying mGlu2 EC_{50} values of 18.1 ± 0.1 nM, 0.089 ± 0.002 nM, and 23.4 ± 0.9 nM and mGlu3

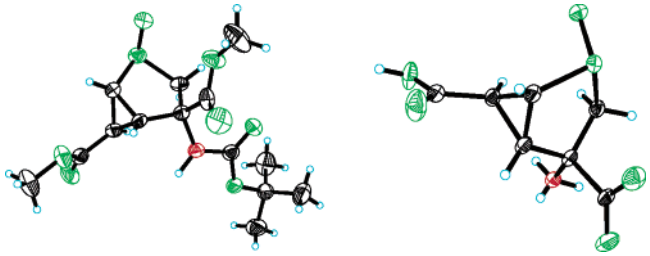

Figure 2. X-ray crystal structures of (left) minor sulfoxide intermediate (−)-**7** and (right) major sulfoxide amino diacid (+)-**11**.

EC_{50} values of 210 ± 50 nM, 1.4 ± 0.8 nM, and 48 ± 10 nM, respectively.[36] At concentrations up to 100 μM, no appreciable agonist or antagonist activity at mGlu1, mGlu4, and mGlu5 was detected. However, as has been observed for (+)-**1**, (−)-**2**, and (−)-**3**,[11,14] compounds (+)-**11** and (−)-**12** demonstrated weak agonist activity in cells expressing recombinant human mGlu8 receptors, with EC_{50} values of 3070 ± 770 nM and 47 773 ± 3505 nM, respectively.

Pharmacokinetics. The pharmacokinetic properties of (+)-**11**, (−)-**11**, and (−)-**12** in rats are summarized in Table 2. The area under the curve (AUC_{0-24h}) determined for (+)-**11** in fasted male Fischer 344 rats was 13 μg-h/mL following both intravenous and oral dosing at 10 mg/kg, resulting in a calculated absolute oral bioavailability of 100%. Peak plasma concentrations (7.5 μg/mL) were achieved at 0.5 h following oral dosing, and plasma levels in excess of 0.5 ug/mL were maintained for 4 h. No detectable (<10 ng/mL) plasma levels of (+)-**11** were

Table 2. Mean Pharmacokinetic Parameters (n = 9/group) for Heterobicyclic Amino Acids[a]

cmpd	route	dose (mg/kg)	AUC_{0-24h} (μg·h/mL)	C_{max} (mg/mL)	T_{max} (h)	F (%)
(+)-**11**	i.v.	10	13	29	0	
(+)-**11**	p.o.	10	13	7.5	0.5	100
(−)-**12**	i.v.	2.5	2.9	7.5	0	
(−)-**12**	p.o.	10	7.2	4.0	1	63
(−)-**11**	p.o.	10	1.1	0.4	1	n.d.

[a] See Experimental Section for details.

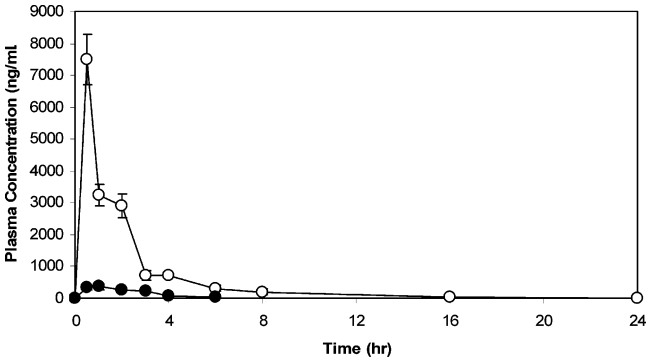

Figure 3. Oral plasma exposure time of 24 h; course data for (+)-**11** (open circles) and (−)-**11** (closed circles) following a single oral dose of 10 mg/kg in male Fisher 344 rats; N = 9 rats/dose group for (+)-**11** and 6 rats/dose group for (−)-**11**; n = 3 rats/time-point). See Experimental Section for details.

apparent 24 h after the 10 mg/kg dose (Figure 3). In contrast, the area under the curve for (−)-**11** following a single 10 mg/kg oral dose was 1.1 μg-h/mL, with a peak plasma concentration of 0.4 μg/mL achieved at 1 h after dosing (Figure 3). For sulfone analog (−)-**12**, the area under the curve (AUC_{0-24h}) was 2.9 μg-h/mL following intravenous dosing, and 7.2 μg-h/mL following oral dosing at 2 mg/kg and 10 mg/kg, respectively, resulting in a calculated absolute oral bioavailability of 63%. The peak plasma concentration for (−)-**12** (4.0 μg/mL) was achieved at 1 h, with plasma levels exceeding 0.5 μg/mL maintained for 3 h and subsequently decreasing to levels below quantification (<10 ng/mL) at 8 h.[36]

Behavioral Pharmacology. Based on preliminary time-course experiments (data not shown), a 2-h time point was chosen to study oral dose response effects of these compounds against a 5 mg/kg, s.c. phencyclidine (PCP) challenge (Figure 4). Compound (−)-**12** had statistically significant effect reducing PCP-induced ambulations at 3 and 10 mg/kg with a 91% maximal inhibition of PCP ambulations resulting in an ED_{50} of 2.5 mg/kg. Compound (+)-**11** had statistically significant effect reducing PCP-induced ambulations at 3 and 10 mg/kg with a 98% maximal inhibition of PCP ambulations, resulting in an ED_{50} of 2.3 mg/kg. The dose−response for rotorod impairment by either (+)-**11** or (−)-**12** was likewise assessed at 2 h post oral dosing (Figure 5). At doses up to 30 mg/kg, (−)-**12** produced no significant effects on motor coordination. In contrast, while (+)-**11** produced no significant motor impairment at 1 mg/kg, it significantly impaired rotorod performance when given at a dose of 3 mg/kg.

Discussion. As part of our ongoing efforts to identify novel pharmacological agents to selectively modulate the function of metabotropic glutamate receptor subtypes, we sought to assess the effect of sulfoxide and sulfone variants of (−)-**3** on mGlu2/3 receptor activity. We had previously demonstrated that methyl-substituted bicyclic amino acids **4** and **5** each show diminished affinity for mGlu2 and mGlu3 receptors compared to their C4-unsubstituted parent **1**.[33] In addition, while both of these methyl-substituted analogs demonstrated agonist activity at mGlu2, **4** functioned as an agonist and **5** as an antagonist at mGlu3. In an attempt to further characterize the effect of substitution at this position of bicyclic amino acids of this type, we prepared and evaluated S-oxidized variants of the potent and selective mGlu2/3 agonist, (−)-**3**.[14] In contrast to our findings for the methyl-substituted derivatives **4** and **5**, each of the S-oxidized variants **10**−**12** acted as full agonists at both mGlu2 and mGlu3 receptors. In addition, a clear stereochemical preference for the sulfoxide isomers was evident, with sulfoxide (+)-**11** being significantly preferred over (−)-**10** both in terms of binding affinity and in terms of functional agonist potency. To the best of our knowledge, (+)-**11** is the most potent ligand (agonist or antagonist) for mGlu2/3 receptors described to date. The selectivity of (+)-**11** and (−)-**12** for mGlu2/3 receptors over other mGlu receptor subtypes is very high, with the next most potent target of action, mGlu8, only activated at concentrations approximately 1000-fold higher than those required for mGlu2/3 activity. In an effort to rationalize the remarkable potency of (+)-**11**, it was manually docked to a homology model of the glutamate recognition site of mGlu2, itself derived from the analogous mGlu1 crystal structure.[37] As can be seen in Figure 6, when (+)-**11** is minimized within this site, a very similar hydrogen-bonding network to that described for the glutamate-mGlu1 crystal structure was observed for the ammonium and carboxylate functionalities (see ref 37 and figures therein for details). Notably, in addition to these interactions, the sulfoxide oxygen of (+)-**11** is oriented within hydrogen-bonding distance to the phenol functionality of tyrosine residue Y236, previously identified as a residue involved in a π-cation interaction with the charged ammonium group of glutamate.[37,38] This additional (sulfoxide oxygen to tyrosine-OH) H-bonding interaction is not geometrically feasible in the case of sulfoxide diastereomer (−)-**10**. The increase in binding affinity of sulfoxide (+)-**11** (mGlu2 K_i = 4.4 nM; mGlu3 K_i = 0.17 nM) over sulfide (−)-**3** (mGlu2 K_i = 40.6 nM; mGlu3 K_i = 4.7 nM) translates to estimated differences in binding energy of 1.2 kcal/mol at mGlu2 and 1.8 kcal/mol at mGlu3.[39] These values are consistent with the formation of a new H-bond interaction as suggested by the homology model.

When given orally to rats at a dose of 10 mg/kg, (+)-**11** and (−)-**12** are rapidly absorbed (C_{max} = 0.5 and 1.0 h, respectively) and result in high peak plasma concentrations (7.5 μg/mL and 4.0 μg/mL, respectively) as well as excellent exposures over 24 h (AUC = 13 μg-h/mL and 7.2 μg-h/mL, respectively). These values compare favorably to those published for (+)-**1** in that an oral dose of 100 mg/kg of this compound was required to provide an AUC comparable to 10 mg/kg (+)-**11** or (−)-**12**.[40] Furthermore, to achieve comparable peak plasma levels to (+)-**11** and (−)-**12**, even higher oral doses of (+)-**1** (between 300 and 1000 mg/kg, p.o.) are required.[40] The calculated bioavailability of (+)-**11** (100%) and (−)-**12** (63%) also compared favorably to that reported for (+)-**1** (10%). The basis of the more optimized oral plasma exposure and bioavailability profiles seen with (+)-**11** or (−)-**12** is not fully understood. It is unlikely that these acidic amino acids are absorbed via passive diffusion. The calculated Log P values for the uncharged forms of (+)-**1**, (+)-**11**, and (−)-**12** are −1.22, −2.61, and −2.02, respectively.[41] On the other hand, the active (carrier-mediated) transport of L-glutamate and L-aspartate across human intestinal (Caco-2) cells is known.[42] We considered the possibility that if constrained bicyclic acidic amino acids (+)-**1**, (+)-**11**, and (−)-**12** are substrates for carrier-mediated transport, a stereochemical preference might be observed. In support of this hypothesis,

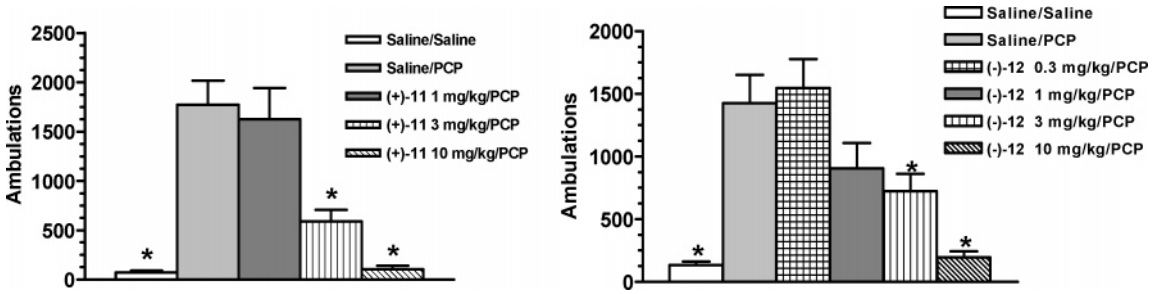

Figure 4. Oral dose−response relationships for reversal of PCP-induced ambulatory activity in rats by (+)-**11** (left) or (−)-**12** (right). Activity was assessed 2 h after a single oral dose of each molecule. Data (ambulations) are expressed as the mean number of horizontal photobeam breaks (n = 8−13 per group) over a 1 h test period. See Experimental Section for details. *p < 0.05 vs saline/PCP.

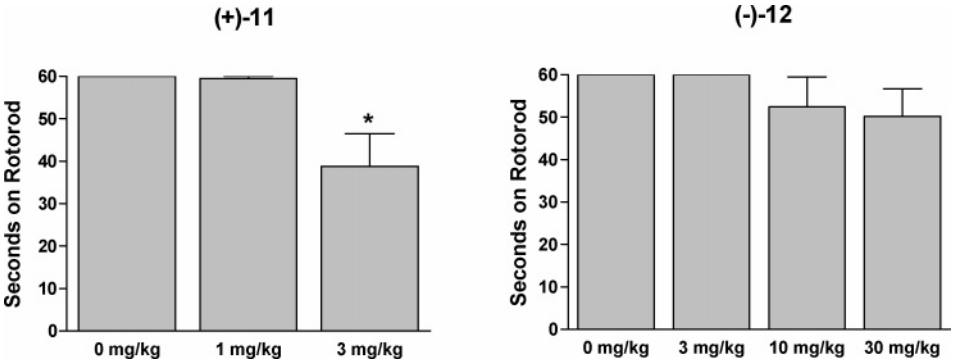

Figure 5. Effect of mGlu2/3 receptor agonists (+)-**11** and (−)-**12** on rotorod motor coordination in rats assessed 2 h following a single oral dose. Data are expressed as the mean number of seconds in which the rats remained on the rotorod apparatus (n = 8 rats per group). See Experimental Section for details. *p < 0.05 vs vehicle.

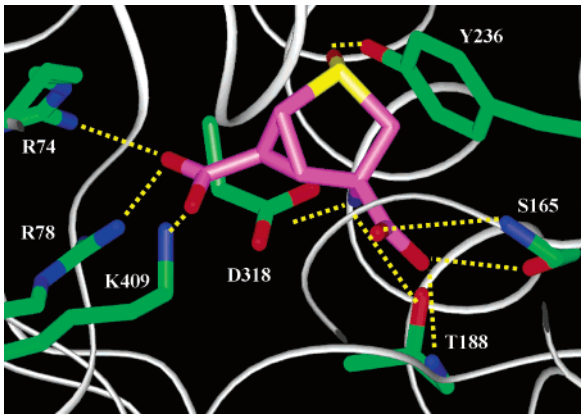

Figure 6. Proposed binding mode for compound (+)-**11** (cyan) in the mGlu2 ligand binding pocket. This configuration of the sulfoxide is capable of forming a hydrogen bond between the sulfoxide oxygen and tyrosine Y236. Amino acids are numbered according to the residue in the mGlu1 crystal structure.

when the mGlu2/3 receptor inactive enantiomer (−)-**11** was orally administered to rats at the same dose as the mGlu-active (+)-antipode, significantly lower peak plasma levels (C_{max} = 0.4 μg/mL; 5% of those arising from (+)-**11**) and 24 h plasma exposures (AUC = 1.1 μg-h/mL; 8% of that arising from (+)-**11**) were observed. These results suggest that (+)-**11** and (by analogy) (−)-**12** may indeed be substrates for one or more transporter proteins in the rat gastrointestinal tract, though we cannot exclude the possibility that differential rates of clearance may play some role in their pharmacokinetic behavior.

Noncompetitive NMDA antagonists such as PCP and ketamine induce a psychosis resembling schizophrenia in normal volunteers and will exacerbate symptoms in schizophrenia patients.[43,44] The ability of a compound to reverse PCP stimulated behavior is an often-used animal model for potential antipsychotic activity. It was reported that the mGlu2/3 receptor agonist (+)-**1** suppressed PCP-induced excitatory behaviors (for example, hyperlocomotion and stereotypy) in rats.[21] The present study shows that (+)-**11** and (−)-**12** significantly block PCP-induced hyperlocomotion with oral ED_{50} values of 2.3 and 2.5 mg/kg, respectively. Though these two analogs displayed comparable efficacy in this model, (−)-**12** did so in the absence of motor coordination effects, displaying no significant rotorod impairment at doses up to 30 mg/kg. Conversely, (+)-**11** displayed significant motor impairment in this assay at a dose of 3 mg/kg, rendering the interpretation of its effects in the PCP ambulation assay ambiguous. It should be noted that tolerance to the effects on motor responses by other mGlu2/3 agonists following acute administration have been observed following repeated dosing,[45] though we have not explored this possibility in the case of (+)-**11**. The collective attributes of (−)-**12**, including mGlu2/3 agonist potency and selectivity, excellent rat oral pharmacokinetics, and behavioral efficacy in the absence of acute motor impairment, suggest that this molecule will be a useful tool for exploring the effects of mGlu2/3 receptor activation in mammals.

Experimental Section

Melting points were obtained using a Thomas Hoover capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on either a Bruker AVANCE 300 MHz or Bruker AMX 500 MHz instrument. Optical rotations were obtained using a Perkin-Elmer 341 polarimeter and are reported at the sodium D-line (589 nm). Mass spectra were obtained using either a VG 70S (FD-MS), Sciex API-100 (ESI-MS) or VG Platform LCZ (ESI-MS) instrument. Preparative HPLC was performed with the Waters Prep LC2000 apparatus using dual silica gel PrepPAK-500 cartridges. Preparative radial chromatography was performed using a Harrison model 7924T chromatotron. Chromatography solvent systems employed are given in parentheses for each example. All intermediates and final products gave satisfactory combustion analyses and are so identified within the experimental section. A

table is provided with detailed elemental analyses (C, H and N) as Supporting Information.

(−)- (1*R*,4*S*,5*S*,6*S*)-Dimethyl 4-(*N*-*tert*-Butyloxycarbonylamino)-2-thiabicyclo-[3.1.0]hexane-4,6-dicarboxylate ((−)-6). Compound (−)-**3** (6.2 g, 30.5 mmol)[14] was stirred in methanol (500 mL) at 0 °C and treated dropwise with thionyl chloride (46.8 g, 393 mmol). The reaction was then warmed to ambient temperature and refluxed for 40 h. The reaction mixture was concentrated, and the residue was partitioned between ethyl acetate and water. The aqueous phase was made basic by the addition of solid $NaHCO_3$ and extracted with ethyl acetate (3 × 300 mL). The combined organic layers were dried over anhydrous K_2CO_3 and concentrated to afford the crude diacid, 5.9 g (84%). Without further purification, this material (5.0 g, theoretically 22 mmol) was dissolved in THF (200 mL). Di-*tert*-butyl dicarbonate (15.9 g, 72.8 mmol) was added, and the reaction mixture was stirred for 24 h at room temperature under nitrogen. The reaction mixture was concentrated under reduced pressure, and the crude product was subsequently purified by HPLC (EtOAc/hexanes), affording 6.7 g (79% overall) of (−)-**6**. ESI-MS (positive ion) $M^+ + H = 332$; (negative ion) $M^+ - H = 330$, $M^+ + CH_3COO^- = 390$. ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.07 (app. t, 1H, J = 3.7 Hz), 2.59 (d, 1H, J = 12.7 Hz), 2.78 (dd, 1H, J = 4.0 and 7.1 Hz), 2.88 (dd, 1H, J = 3.3 and 7.1 Hz), 3.68 (d, 1H, J = 12.7 Hz), 3.70 (s, 3H), 3.83 (s, 3H), 5.48 (s, 1H). Anal. for $C_{14}H_{21}NO_6S$: C, H, N. $[\alpha]_D = -102$ (c = 1.06, CH_2Cl_2, 20 °C).

(−)-(1*R*,2*S*,4*S*,5*S*,6*S*)-Dimethyl 4-(*N*-*tert*-Butyloxycarbonylamino)-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylate-2-oxide ((−)-7) and (−)-(1*R*,2*R*,4*S*,5*S*,6*S*)-Dimethyl 4-(*N*-*tert*-Butyloxycarbonylamino)-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylate-2-oxide ((+)-8). Compound (−)-**6** (3.5 g, 10.6 mmol) was dissolved in anhydrous CH_2Cl_2 (approximately 300 mL) under N_2 and treated at −78 °C with *m*-chloroperoxybenzoic acid (2.40 g, 13.9 mmol). The reaction mixture was allowed to stir for 4 h at −78 °C. TLC analysis indicated incomplete reaction, thus additional *m*-CPBA (0.25 g, 1.5 mmol) was added. The reaction was allowed to proceed for an additional 0.5 h, then quenched with aqueous $NaHSO_3$, concentrated, and partitioned between H_2O and EtOAc. The organic layer was washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated to afford the crude mixture of products (3.4 g), which was purified by HPLC (EtOAc/hexanes), yielding 0.2 g of (−)-**7** (5%) and 3.3 g (89%) of (+)-**8**. Compound (−)-**7**: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.78 (dd, 1H, J = 3.8 and 3.8 Hz), 2.73 (d, 1H, J = 15.5 Hz), 3.29 (dd, 1H, J = 3.8 and 6.0 Hz), 3.31 (ddd, 1H, J = 5.6, 3.8 and 1.7 Hz), 3.74 (s, 3H), 3.87 (s, 3H), 3.91 (dd, 1H, J = 15.5 and 1.7 Hz), 5.47 (br s, 1H). ESI-MS (positive ion): $M^+ + H = 348$; (negative ion): $M^+ - H = 346$, $M^+ + CH_3COO^- = 406$. Anal. for $C_{14}H_{21}NO_7S$: C, H, N. $[\alpha]_D = -120$ (c = 0.884, CH_2Cl_2, 20 °C). Compound (+)-**8**: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.26 (d, 1H, J = 13.7 Hz), 2.64 (dd, 1H, 5.8 and 5.8 Hz), 2.81 (app. t, 1H, J = 5.7 Hz), 3.45 (dd, 1H, 5.7 and 5.5 Hz), 3.75 (s, 3H), 3.81 (s, 3H), 4.35 (br d, 1H, J = 13.7 Hz), 5.67 (br s, 1H). FD-MS: $M^+ + H = 348$. Anal. for $C_{14}H_{21}NO_7S$: C, H, N. $[\alpha]_D = 21$ (c = 1.07, CH_2Cl_2, 20 °C).

(−)-(1*R*,4*S*,5*S*,6*S*)-Dimethyl 4-(*N*-*tert*-Butyloxycarbonylamino)-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylate-2,2-dioxide ((−)-9). Sulfoxide (+)-**8** (0.80 g, 2.3 mmol) dissolved in anhydrous CH_2Cl_2 under N_2 was treated with *m*-chloroperoxybenzoic acid (0.465 g, 2.7 mmol) at 0 °C and stirred for 0.5 h. The cooling bath was removed, and the reaction was stirred for 1 h at room temperature. At this time, the reaction was judged incomplete by TLC, and additional *m*-CPBA (0.20 g, 1.2 mmol) was added. The reaction was allowed to proceed at room temperature overnight, then was diluted with aqueous $NaHSO_3$ and partitioned between H_2O and EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. The crude product was purified by radial chromatography (EtOAc/hexane), yielding 0.76 g (84%) of (−)-**9**; mp = 143−145 °C. ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.50 (dd, 1H, J = 3.9 and 4.3 Hz), 2.92 (dd, 1H, J = 4.3 and 7.3 Hz), 3.01 (d, 1H, J = 14.2 Hz), 3.41 (dd, 1H, J = 7.3 and 3.9 Hz), 3.77 (s, 3H), 3.88 (s, 3H), 4.19 (d, 1H, J = 14.2 Hz), 5.47 (br s, 1H). FD-MS: $M^+ = 363$. Anal. for $C_{14}H_{21}NO_8S$: C, H, N. $[\alpha]_D = -6$ (c = 1.05, CH_2Cl_2, 20 °C).

(−)-(1*R*,2*S*,4*S*,5*S*,6*S*)-4-Amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic Acid 2-Oxide ((-)-10). The title compound was prepared by stirring (−)-**7** (0.45 g, 1.3 mmol) in trifluoroacetic acid for 2 h. The reaction mixture was concentrated to dryness, then reconstituted in THF (5 mL) and 1 N NaOH (5 mL), and stirred overnight at room temperature. The pH was subsequently adjusted to approximately 4 by addition of 1 N HCl, and the mixture was concentrated to remove the THF. The pH of the aqueous phase was further adjusted to 2, and the crude product was applied in H_2O (3−5 mL) to a glass column containing Dowex 50XB-100 ion-exchange resin, hydroxide form. The resin was washed slowly with H_2O until the effluent pH was neutral, then sequentially with H_2O (1 column volume), 1:1 THF/H_2O (1 column volume), and H_2O (1 column volume) before eluting the product with 10% aqueous pyridine. Concentration of product-containing fractions led to isolation of a slightly discolored solid (0.25 g) that was triturated with 2-propanol/H_2O to afford (−)-**10** (0.20 g, 71%); mp > 220 °C (dec). ^{1}H NMR (D_2O/KOD) δ 1.47−1.51 (m, 1H), 2.54 (d, 1H, J = 15.7 Hz), 2.72−2.76 (m, 1H), 2.95−3.15 (m, 1H), 3.40 (d, 1H, J = 15.7 Hz). ^{13}C NMR (D_2O) δ 22.94, 36.51, 43.27, 58.24, 68.77, 176.95, 179.05. ESI-MS (positive ion): $M^+ + H = 220$ (negative ion): $M^+ - H = 218$. Anal. for $C_7H_9NO_5S - 0.6$ equiv H_2O: C, H, N. $[\alpha]_D = -188$ (c = 0.926, 1 N NaOH, 20 °C).

(+)-(1*R*,2*R*,4*S*,5*S*,6*S*)-4-Amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic Acid 2-Oxide ((+)-11). Compound (+)-**8** (15.6 g, 44.9 mmol) in EtOAc (500 mL) at 0 °C was treated with anhydrous HCl (g) over 5 min and allowed to stir at 0 °C for an additional 15 min. The reaction was judged incomplete by TLC. Additional HCl (g) was added, but the reaction did not appear to progress to completion. The reaction mixture was concentrated under reduced pressure, and trifluoroacetic acid was added and concentrated to dryness (repeated twice). The solids were dissolved in THF (100 mL) and 2 N NaOH (125 mL) was added. The reaction was allowed to stir for 2 days at room temperature, then acidified with 1 N HCl to pH = 3−4 and concentrated to dryness. The crude product was purified by trituration from boiling H_2O, affording 5.55 g (56%) of (+)-**11**; mp > 250 °C. ^{1}H NMR (D_2O/KOD) δ 2.18 (d, 1H, J = 13.2 Hz), 2.35 (dd, 1H, J = 4.3 and 6.5 Hz), 2.43 (dd, 1H, J = 3.2 and 4.2 Hz), 3.41 (dd, 1H, J = 3.2 and 6.5 Hz), 3.67 (d, 1H, J = 13.2 Hz). ^{13}C NMR (D_2O/KOD) δ 21.88, 39.27, 42.54, 55.71, 66.88, 178.06, 180.12. ESI-MS (positive ion): $M^+ + H = 220$ (negative ion): $M^+ - H = 218$. Anal. for $C_7H_9NO_5S$: C, H, N. $[\alpha]_D = 16.57$ (c = 1.03, 1 N HCl, 20 °C). $[\alpha]_D = -68$ (c = 1.52, 1 N NaOH, 22.8 °C).

(−)-(1*R*,4*S*,5*S*,6*S*)-4-Amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic Acid 2,2-Dioxide ((−)-12). A solution of (−)-**9** (2.85 g, 7.84 mmol) in trifluoroacetic acid (10 mL) was stirred at room temperature overnight. The reaction mixture was concentrated, diluted in EtOAc, washed sequentially with saturated $NaHCO_3$, H_2O, and brine, and dried over K_2CO_3. Concentration under reduced pressure afforded 1.5 g (73%) of the intermediate amino dimethylester (FD-MS: $M^+ = 263$). A portion of this material (1.37 g, 5.21 mmol) was dissolved in 20 mL of THF, and 20 mL of 1 N NaOH was added to the reaction, which was stirred overnight. HCl (5 N) was added to adjust the pH = 3. After stirring for 2 h, the product was filtered, washed with H_2O, and air-dried to afford 1.05 g of solid, which was recrystallized from water to give 1.04 g of the desired product (−)-**12** in 62% overall yield from (−)-**9**; mp > 250 °C. FD-MS: $M^+ + 1 = 236$. ^{1}H NMR (D_2O, KOD) δ 2.20−2.25 (m, 1H), 2.63−2.66 (m, 1H) 3.08 (d, 1H, J = 14.9 Hz), 3.32−3.40 (m, 1H), 3.66 (d, 1H, J = 14.9 Hz). ^{13}C NMR (D_2O, KOD) δ 24.66, 31.99, 43.01, 54.57, 60.49, 174.16, 174.34. Anal. for $C_7H_9NO_6S$: C, H, N. $[\alpha]_D = -17.58$ (c = 1.02, 1 N HCl, 20 °C). $[\alpha]_D = -76$ (c = 1.52, 1 N NaOH, 22.8 °C).

Biochemical Pharmacology. Test compounds were evaluated for their ability to inhibit the specific binding of ^{3}H-2*S*-2-amino-2-(1*S*,2*S*-2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid to membranes expressing individual recombinant human mGlu2 and mGlu3 receptor subtypes.[46,47] The K_i values were calculated from

the IC_{50} values employing the Cheng−Prusoff equation[48] and represent the mean of at least three separate experiments. Test compounds were also evaluated for their ability to influence the production of second messengers in "RGT" cells. RGT cells are non-neuronal AV12−664 cells coexpressing both GLAST (a recombinant glutamate transporter) to minimize constituent glutamate activity and a recombinant human group I (mGlu1a or mGlu5a), group II (mGlu2 or mGlu3), or group III (mGlu4a, mGlu6, mGlu7a or mGlu8) receptor.[11,49]

Molecular Modeling. The crystal structure coordinates of rat mGlu1 ligand-binding domain with glutamate bound[37] was obtained from the Protein Data Bank (PDB code 1ewk).[50] The molecular modeling program Insight2000[51] was used to manipulate the protein structures studied. The protein sequence of rat mGlu1 ligand-binding domain was aligned with the sequence of human mGlu2 using the Align123 utility (default settings) in the Homology module of Insight2000. The alignment was used as a guide to transform the rat mGlu1 ligand-binding domain structure into a model of the human mGlu2 ligand-binding domain structure. This was accomplished by replacing the appropriate rat mGlu1 residues (using the residue-replace feature in the Biopolymer module of Insight2000) with the corresponding residues present in human mGlu2. The mGlu2 model was energy minimized (conjugate gradient algorithm, 1000 iterations with a derivative setting of 0.001) using the Discover module in Insight2000. Amino acids were numbered according to the residue in the mGlu1 crystal structure. The three-dimensional structure of (+)-**11** was generated using Concord.[52] Using the binding orientation of glutamate in the rat mGlu1 crystal structure as a guide, (+)-**11** was manually docked into the ligand-binding site of the mGlu2 homology model to generate the proposed orientation and hydrogen-bonding interactions depicted in Figure 5. The resulting mGlu2−(+)-**11** complex was energy minimized using the Discover module in Insight2000 (conjugate gradient algorithm, 1000 iterations with a derivative setting of 0.001).

Pharmacokinetic Assessments. Test compounds were evaluated for their oral bioavailability in male Fisher 344 rats (10 mg/kg i.v./10 mg/kg, p.o. for (+)-**11**; 2.5 mg/kg i.v./10 mg/kg p.o. for (−)-**12**). Dose solutions were prepared in MilliQ water titrated to pH 7 with NaOH. Volumes of 2 mL/kg or 5 mL/kg were used for intravenous or oral administrations, respectively. Rats were fasted overnight prior to dosing. Food was restored 4 h after dosing. Water access was ad libitum. Nine rats were used per dose group. Three blood samples were collected from each rat at three different time points, successively from each retroorbital sinus and by cardiac puncture. At each time point, blood was collected from three rats. Blood samples were collected at 0.08, 0.17, 0.5, 1, 2, 4, 8, 16, and 24 h post dose for (+)-**11** and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 24 h post dose for (−)-**12** (n = 3 rats/time point). In the case of (−)-**11**, plasma exposure was assessed following oral (but not i.v.) administration in Fisher 344 rats using essentially the same experimental protocol as described for (+)-**11** and (−)-**12**, with the following exceptions: n = 6 rats/group; blood samples taken at 0.5, 1, 2, 3, 4, and 6 h post dose. Plasma was prepared by centrifugation, and samples were extracted utilizing solid-phase extraction and analyzed by LC/MS/MS. One hundred microliters of a sample was combined with 20 μL of internal standard and 500 μL of water. The internal standards used were (−)-**12** for evaluation of (+)-**11** and (+)-**1** for evaluation of (−)-**12** and (−)-**11**. The sample was slowly extracted at approximately 1 drop/3 s on a SAX 100 mg cartridge (IST) that had been preconditioned with 0.5 mL of methanol and 0.5 mL of water. The cartridge was then washed with 0.5 mL of water and 1 mL of methanol. The compound was eluted from the cartridge with 0.8 mL of 3% trifluoroacetic acid, 5% water, and 93% methanol. The extract was then dried utilizing a Savant Speed Vac concentrator at medium heat and diluted in 150 μL of 1% acetic acid prior to LC/MS/MS analysis using a Sciex API300 instrument (positive ion electospray). LC/MS/MS conditions: (+)-**11**; HPLC column, Inertsil ODS-3 150 × 2.1 mm, 5u - MP; A = 1% acetic acid, B = 1/9/90 acetic acid/H_2O/MeOH, isocratic at 25% B, flow rate = 0.25 mL/min, injection volume = 25 μL. MS transition 220.0 → 185.0. (−)-**11**; HPLC column, Aquasil C-18 100 × 2.1 mm, 5u - MP; A = 1% acetic acid, B = 1% acetic acid in MeOH, isocratic at 20% B, flow rate = 0.18 mL/min, injection volume = 25 μL, MS transition 220.0 → 159.1. (−)-**12**; HPLC column, Inertsil ODS-3 50 × 2.1 mm, 5u - MP; A = 1% acetic acid, B = 1% acetic acid in MeOH, gradient (time, %B) (0 min, 15%), (0.25 min, 15%), (0.26 min, 85%), (2.75 min, 85%), 2.76 min, 15%), (5 min, stop), flow rate = 0.25 mL/min, injection volume = 25 μL, MS transition 236.2 → 190.1. The detection limits for (+)-**11**, (−)-**11**, and (−)-**12** were 10 ng/mL, 100 ng/mL, and 50 ng/mL, respectively. All standard points used were calculated to be within 20% of theoretical. The plasma concentration of (−)-**11** at the 6 h time point was below the level of quantification (71.8 ng/mL, see below); however, this value was used in the AUC calculation rather than setting it to zero. Had a value of 0 ng/mL at the 6 h time point been used, the calculated AUC (1.0 ug*h/mL) would have been slightly lower than that reported in Table 2 (1.1 μg·h/mL).

Behavioral Pharmacology. Test compounds were assessed for their ability to suppress PCP-induced motor activation (ambulations) in rats. Behavioral parameters were monitored in transparent, shoebox cages that measured 45 × 25 × 20 cm, with a 1 cm depth of wood chips on the cage floor and a metal grill on top of the cage. Rectangular photocell monitors (Hamilton Kinder, Poway, CA) with a bank of 12 photocell beams (8 × 4 formation) surrounded each test cage. A lower rack of photocell beams was positioned 5 cm above the cage floor to enable detection of the location of the animal's body, while an upper bank positioned 10 cm above the first tabulated rearing activity. Ambulations (locomotor activity) were recorded by the computer and stored for each test session. Male Sprague−Dawley rats were food-fasted 12−18 h prior to the experiment in all oral dosing studies. On the test day, animals were placed in the test cage for a 30 min habituation period before testing to allow for acclimation to the test cage environment. Following this habituation period, animals were administered challenges of PCP (5 mg/kg s.c.) or 0.9% NaCl vehicle (1 mL/kg), and behavioral assessment began immediately following their administration. Animals were monitored over a 60 min period in all instances. Test drugs or vehicle were orally administered 2 h prior to the PCP challenge.[22] Data are expressed as the mean number of horizontal photobeam breaks (n = 8−13 per group) over a 1 h test period.

An automated rotorod apparatus (Rotor-Rod; San Diego Instruments, Inc., San Diego, CA) was used as a test for motor impairment/ataxia. Ninety minutes before drug administration, rats were trained to stay on the rotorod, rotating at 4 rpm, over four successive trials. Those rats that remained on the rod for a consecutive 60-s period were retested 30 min before drug administration. Rats successful in the retesting session were then given oral doses of vehicle, (+)-**11** and (−)-**12**. After an additional 2 h, these rats were again tested on the rotorod for a period of up to 60 s. Data are expressed as the mean number of seconds in which the animals (n = 8/group) remained on the rotorod apparatus. Statistical analysis was carried out using the GraphPad PRISM statistical/graphing package (GraphPad, San Diego, CA). Data were analyzed using a one-way analysis of variance (ANOVA), and post-hoc comparisons were performed using Newman−Keuls or Dunnett's multiple comparisons test. All experiments were performed in accordance with Eli Lilly and Company animal care and use policies, each animal being used on only one occasion. Male Sprague−Dawley rats (225−274 g) obtained from Harlan Industries, Indianapolis, Indiana. Animals were group housed (maximum 8 rats per cage) under standard laboratory conditions (12 h light/dark cycle) with ad libitum access to water and feed for at least 1 day prior to overnight fasting for use in oral dosing studies.

Supporting Information Available: Combustion analyses, crystal data and structure refinement, and mean rat plasma levels and associated standard error measurements. The material is available free of charge via the Internet at http://pubs.acs.org.

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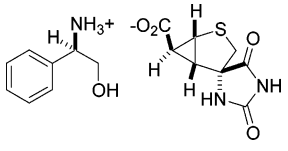
(34) The absolute stereochemistry of (−)-**3** was determined by single crystal X-ray of the *R*-2-phenylglycinol salt of hydantoin precursor shown below. The preparation of this intermediate and its conversion to (−)-**3** are detailed in reference 14. The X-ray structure and coordinates have been filed with the Cambridge Crystallographic Database and are available as Supporting Information.



(35) The X-ray structure and coordinates for (−)-**7** and (+)-**11** have been filed with the Cambridge Crystallographic Database. Coordinate data for each are available as Supporting Information.

(36) More extensive pharmacological and pharmacokinetic characterization of (+)-**12** will be reported in the following: Rorick-Kehn, L.; Johnson, B. G.; Burkey, J. L.; Wright, R. A.; Calligaro, D. O.; Marek, G. J.; Nisenbaum, E. S.; Catlow, J. T.; Kingston, A. E.; Monn, J. A.; McKinzie, D. L.; Schoepp, D. D. Pharmacological and pharmacokinetic properties of a structurally-novel, potent, selective mGlu2/3 receptor agonist: *In vitro* characterization of LY404039. *J. Pharmacol. Exp. Ther.* **2006**, accepted for publication.

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