Methyl Substitution of 2-Aminobicyclo[3.1.0]hexane 2,6-Dicarboxylate (LY354740) Determines Functional Activity at Metabotropic Glutamate Receptors: Identification of a Subtype Selective mGlu2 Receptor Agonist

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LY354740 (**1**) is a highly potent and selective agonist of metabotropic glutamate (mGlu) receptors 2 and 3. In the present study, we have prepared C3- and C4-methyl-substituted variants of *rac*-**1**, compounds **5**, **9**, and **13**. Each of these racemic methyl-substituted analogues 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 mGlu2 or mGlu3 receptor subtypes. Evaluation of the functional effects of this series on second messenger responses in cells expressing human mGlu2 or mGlu3 receptors revealed C3β-methyl analogue **5** to possess antagonist properties at both mGlu2 and mGlu3 receptors while C4β-methyl analogue **9** acts as a full agonist at each of these targets. Unexpectedly, we found that incorporation of a methyl substituent at the C4α-position as in analogue **13** results in a mixed mGlu2 agonist/mGlu3 antagonist pharmacological profile. All of the mGlu2 agonist and mGlu3 antagonist activity of *rac*-**13** was found to reside in its resolved (+)-isomer.

Introduction

(*S*)-Glutamic acid ((*S*)-Glu) is the primary excitatory neurotransmitter in the mammalian central nervous system (CNS). The neuronal effects of (*S*)-Glu are mediated by two heterogeneous families of cell membrane associated receptors: the ion-channel linked, or ionotropic glutamate (iGlu), receptors; and the G-protein-coupled, or metabotropic glutamate (mGlu), receptors.[1,2] The eight known mGlu receptors (mGlu1−8) are highly heterogeneous with respect to their structure, function, and localization within the central nervous system 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**, Figure 1), a potent and selective agonist for mGlu2 and mGlu3 receptors[11−13] that has been found to possess anxiolytic,[12,14−19] antipsychotic,[20−22] anticonvulsant,[23,24] anti-Parkinsonian,[25,26] analgesic,[27] and neuroprotective[28−31] properties in vivo. As part of our continued interest in this mechanism, we have explored the effect of substitution of the bicyclic ring system of **1**. In this account, we describe the synthesis and in vitro pharmacological profile of methyl-substituted variants of *rac*-**1**. Unexpectedly, we have found that simple methyl substitution at the C3- or C4-positions of the bicyclo[3.1.0]hexane ring system results in a spectrum of functional effects, including dual mGlu2/3 agonist activity, dual mGlu2/3 antagonist activity, and mixed mGlu2 agonist/mGlu3 antagonist activity.

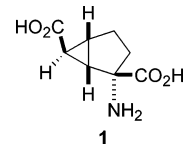

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Figure 1. Chemical structure of LY354740.

Chemistry

The *rac*-C3β-methyl analogue **5** was prepared by the method outlined in Scheme 1. Alkylation of the lithium enolate of **2**[12,32] with methyl iodide at −78 °C afforded C3β-methyl ketone **3**. This was converted to a mixture of C2-spiro-5′-hydantoins by the Bucherer−Bergs method, and the desired diastereomer **4** was isolated directly from the reaction mixture as a precipitate. Exhaustive hydrolysis of **4** followed by ion exchange chromatography then provided the desired zwitterion *rac*-**5**. The *rac*-C4α- and C4β-methyl-substituted analogues of **1** were prepared from the common α,β-unsaturated ketone intermediate **6** (Scheme 2). Dimethyl cuprate addition to **6** yielded the C4β-methyl analogue **7** that was converted to the desired amino acid precursor **8** by the Strecker reaction and in situ acylation followed by chromatographic separation of the C2-acetamidonitrile diastereomers. Hydrolysis of **8** in refluxing aqueous HCl then afforded *rac*-**9**. Alternatively, **7** was converted to the TMS-enol ether and oxidized to the C4-methyl-substituted unsaturated ketones **10** by the Saegusa method.[33] Hydrogenation of **10** (H_2/Pd−C) afforded exclusively the C4α-methyl bicyclic ketone **11**. Conversion to the desired C2-spiro-5′-hydantoin **12** was achieved in low overall yield via the Bucherer−Bergs reaction followed by crystallization from EtOH. Base-mediated hydrolysis of **12** provided the desired C4α-methyl bicyclic amino acid *rac*-**13**. The individual enantiomers of **13** were obtained in an analogous manner from indi-

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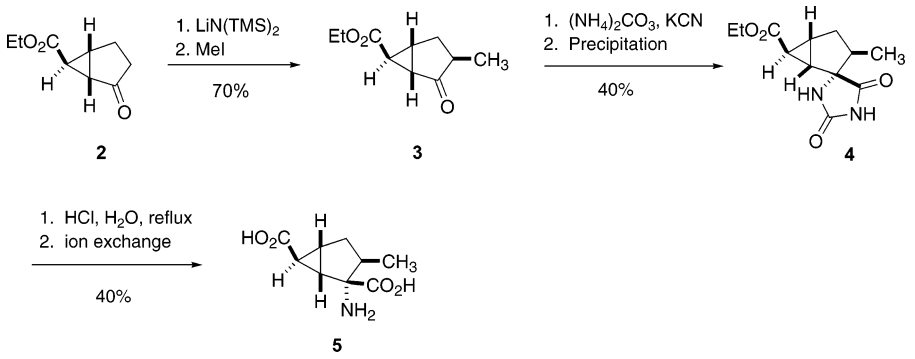
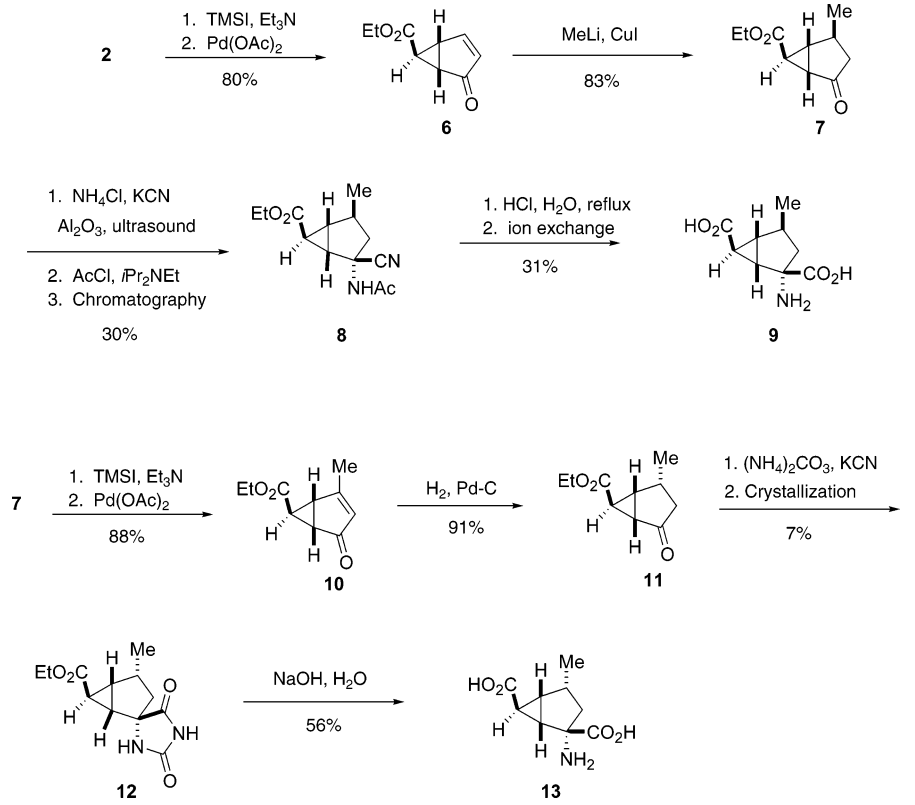
Scheme 1



Scheme 2



vidual enantiomers of **11**, obtained via chiral chromatography (Scheme 3). Assignment of relative stereochemistry for methyl-substituted derivatives **5**, **9**, and **13** was based on analysis of proton−proton nuclear Overhauser effects (NOEs) in hydantoins **4** and **12** and acetamido nitrile **8**. Key NOEs are depicted in Figure 2.

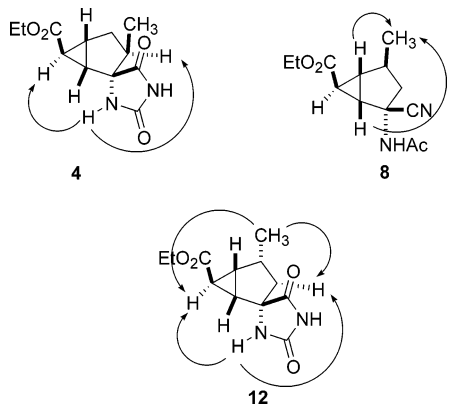


Figure 2. Key NOEs observed for methyl-substituted amino acid precursors.

Biochemical Methods

Test compounds were evaluated for their ability to displace ^{3}H-2*S*-2-amino-2-(1*S*,2*S*-2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid from membranes expressing individual recombinant human mGlu2 and mGlu3 receptor subtypes.[34,35] The K_i values were calculated from the IC_{50} values employing the Cheng−Prusoff equation[36] 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 nonneuronal 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 employing methods previously described.[11,37]

Scheme 3

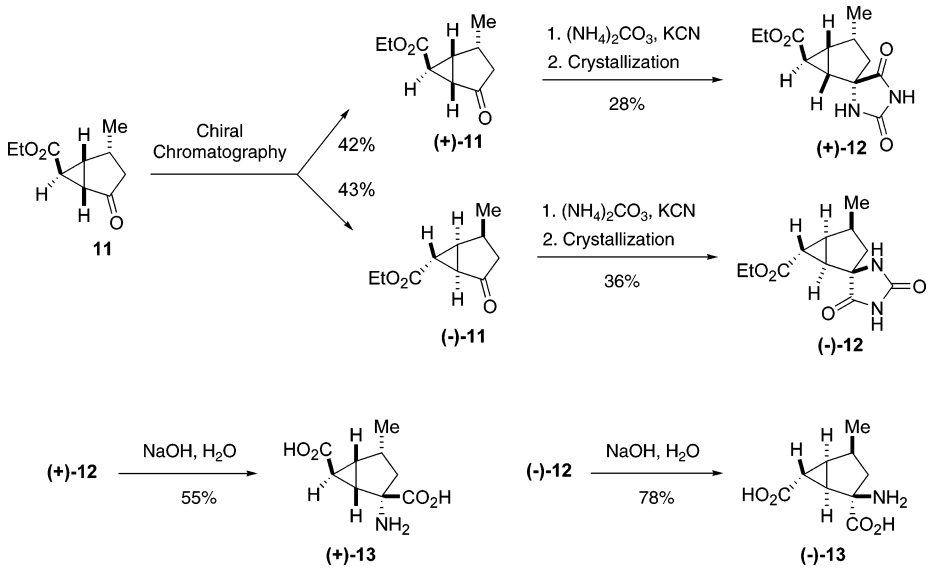


Table 1. Effects of Bicyclic Amino Acids **1**, **5**, **9**, and **13** at Recombinant Human mGlu2 and mGlu3 receptors in Vitro

	displacement of ^{3}H-341495 binding to membranes expressing recombinant mGlu2 or mGlu3[a] K_i (μM)		functional (cAMP) responses in mGlu receptor-expressing cells[b] EC_{50} (μM) or IC_{50} (μM)	
compd	mGlu2	mGlu3	mGlu2	mGlu3
1	0.074 ± 0.009	0.093 ± 0.003	0.010 ± 0.002	0.038 ± 0.003
5	0.983 ± 0.035	0.146 ± 0.056	*1.75 ± 0.62*	*9.83 ± 0.37*
9	0.635 ± 0.097	0.511 ± 0.020	0.045 ± 0.005	0.034 ± 0.004
13	0.409 ± 0.025	0.570 ± 0.049	0.397 ± 0.039	*2.94 ± 0.20*
(+)-**13**	0.165 ± 0.003	0.302 ± 0.048	0.161 ± 0.019	*1.05 ± 0.23*
(−)-**13**	>100	>100	>100	>100

[a] For experimental details, see refs 34 and 35. [b] For experimental details, see ref 11. Roman values are EC_{50} values, and italic values are IC_{50} values. At concentrations up to 100 μM, no agonist or antagonist activity was observed for **5**, **9**, or **13** in cells expressing mGlu1a, mGlu5a, mGlu4a, mGlu7a, or mGlu8 receptors.

Molecular Modeling

The crystal structure coordinates of rat mGlu1 ligand-binding domain with glutamate bound[38] were obtained from the Protein Data Bank (PDB code 1ewk).[39] The molecular modeling program Insight2000[40] was used to manipulate the protein structures studied. The protein sequence of rat mGlu1 ligand-binding domain was aligned with sequences of human mGlu2 and mGlu3 using the Align123 utility in Insight2000. Homology models for each of the human mGlu ligand binding domains were built by replacing the appropriate rat mGlu1 residue with the corresponding residue present in the human form. The models were energy-minimized using the Discover module in Insight2000. By use of the rat mGlu1 crystal structure as a guide, glutamate was manually docked into each homology model. Additional energy minimization was then performed on each complex. The three-dimensional structures of **5**, **9**, and **13** were generated using Concord.[41] Compounds **5**, **9**, and **13** were then manually docked into the glutamate binding site, followed by energy minimization.

Results

Racemic methyl-substituted analogues **5**, **9**, and **13** 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 from membranes expressing recombinant human mGlu2 receptors with K_i values of 0.983 ± 0.035, 0.635 ± 0.097, and 0.409 ± 0.025 μM, respectively (Table). Comparable affinity of these compounds for ^{3}H-antagonist labeled mGlu3 receptors was also observed, with K_i values of 0.146 ± 0.056, 0.511 ± 0.020, and 0.570 ± 0.049 μM for analogues **5**, **9**, and **13**, respectively. Affinity for both mGlu2 and mGlu3 receptors was generally diminished relative to the unsubstituted parent amino acid **1**. Evaluation of the functional effects of this series on cAMP responses in RGT cells (Table) identified the C3β-methyl analogue **5** as a full antagonist at both mGlu2 and mGlu3 receptors (IC_{50} = 1.75 ± 0.62 and 9.83 ± 0.37 μM, respectively). In contrast, C4β-methyl analogue **9** was found to possess full agonist activity at each of these targets with potency (EC_{50} = 0.045 ± 0.005 μM at mGlu2 and 0.034 ± 0.004 μM at mGlu3) comparable to **1**. Methyl substitution at the C4α-position as in analogue **13** resulted in mixed mGlu2 agonist (EC_{50} = 0.397 ± 0.039 μM)/mGlu3 antagonist (IC_{50} = 2.94 ± 0.20 μM) activity. The mGlu activity in *rac*-**13** was found to reside exclusively in the (+)-isomer (for (+)-**13**: mGlu2, K_i = 0.165 μM, EC_{50} = 0.161 ± 0.019 μM; mGlu3, K_i = 0.302 μM, IC_{50} = 1.05 ± 0.23 μM). The other enantiomer, (−)-**13**, was devoid of mGlu2 and mGlu3 activity at concentrations up to 100 μM. For concentrations up to 100 μM, none of the racemic or nonracemic methyl-substituted analogues displayed either agonist or antagonist activity in cells expressing recombinant human mGlu1a, mGlu5a, mGlu4a, mGlu7a, or mGlu8 receptors.

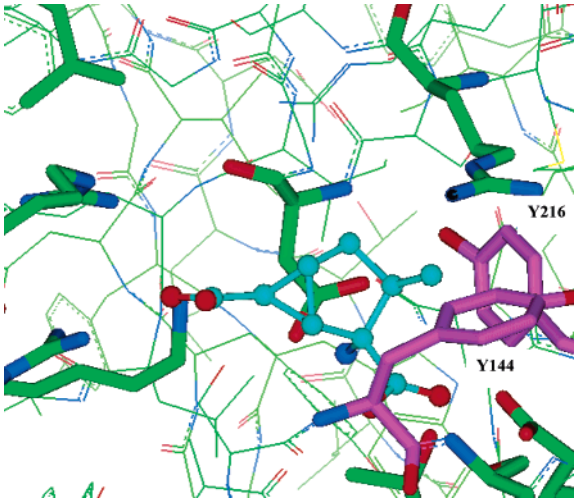


Figure 3. Proposed binding mode for **5** (cyan) in the glutamate binding pocket of mGlu2 (green). The methyl group of **5** fits into a small hydrophobic pocket comprising two tyrosine residues (Y144 and Y216 shown in magenta).

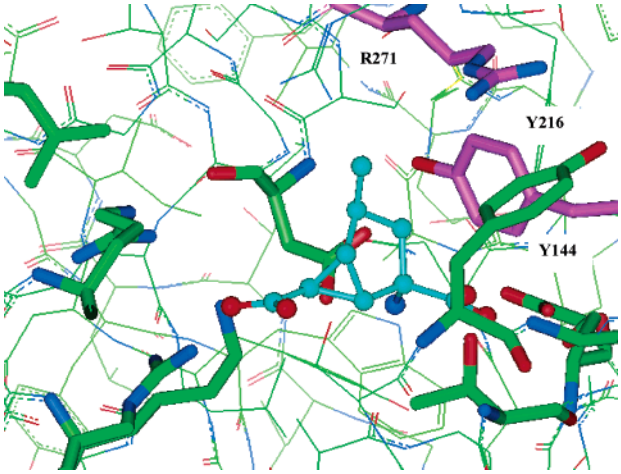


Figure 4. Proposed binding mode for **9** (cyan) in glutamate binding pocket of mGlu2 (green). The methyl group of **9** interacts with a tyrosine residue (Y216, magenta) and the methylene groups of an arginine residue (R271, magenta). Compare this to the proposed binding mode for compound **5** in Figure 3. In this case the methyl interacts with the same tyrosine residue (Y216) and an additional tyrosine residue, Y144.

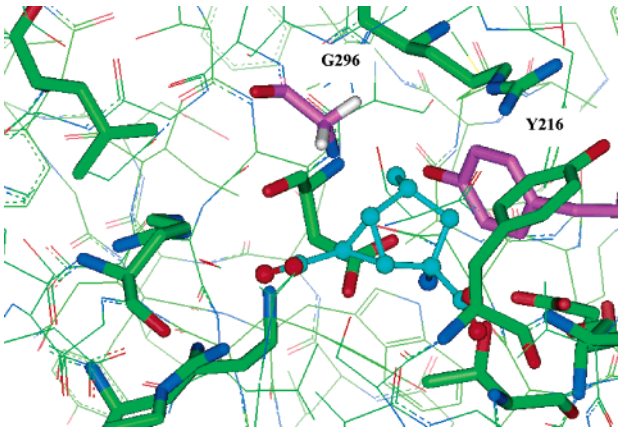


Figure 5. Proposed binding mode for **13** (cyan) in the glutamate binding pocket of mGlu2 (green). In this binding mode the methyl group of **13** is colliding with tyrosine Y216 and glycine G296 (with the α-carbon hydrogens shown in gray).

Discussion

As part of our ongoing efforts to identify novel pharmacological agents to selectively modulate the function of metabotropic glutamate receptor subtypes, we substituted the C3- and C4-positions in the bicyclic ring system of the potent and selective mGlu2/3 receptor agonist **1** with a methyl group. Methyl substitution at the C3β-position (**5**) resulted in an order of magnitude loss of affinity at mGlu2 but no significant loss in affinity at mGlu3 compared to the unsubstituted parent. Moreover, **5** was found to possess antagonist activity at each of these targets, perhaps suggesting that this modification interferes with critical conformational changes in mGlu2 and mGlu3 that are required for receptor signaling. In contrast, while receptor affinity was decreased relative to **1** for C4β-methyl analogue **9**, functional agonist activity at both mGlu2 and mGlu3 receptors was fully maintained in this molecule. Finally, as in the case of β-methyl-substituted analogues **5** and **9**, affinity of C4α-methyl-substituted analogue **13** at both mGlu2 and mGlu3 was diminished compared to the unsubstituted parent. However, **13** unexpectedly produced opposite functional effects in the mGlu2- and mGlu3-expressing cell lines, acting as an agonist at mGlu2 and an antagonist at mGlu3. These functional effects were maintained with an approximately 2-fold increase in potency for the resolved enantiomer (+)-**13**.

By use of the crystal structure of glutamate bound to rat mGlu1[38] as a guide, binding modes for **5**, **9**, and **13** to the ligand-binding pockets of both mGlu2 and mGlu3 were manually generated (Figures 3−6). The proposed binding modes result in a hydrogen-bonding network very similar to that described for the glutamate-mGlu1 crystal structure (see ref 38 and figures therein for details). The methyl group of **5** (mixed mGlu2/mGlu3 antagonist) fits into a hydrophobic region defined by two tyrosine residues (Y144 and Y216) as depicted in Figure 3. Y144 and Y216 arise from different lobes of the glutamate binding site, and mutational studies have shown that each is important for ligand binding to both mGlu2 and mGlu3.[41] It is intriguing to speculate that the methyl group of **5**, by virtue of being situated between these residues, might impede the closure of the two lobes of the glutamate binding site, a conformational change required to elicit an agonist response.[38] Incorporation of the methyl group at the adjacent C4β-position, as in **9** (mixed mGlu2/mGlu3 agonist, Figure 4), results in the methyl substituent interacting with Y216 and the methylene groups of arginine R271, each of which arise from the same lobe of the glutamate binding site. These interactions apparently do not preclude the ability of either mGlu2 or mGlu3 receptors to adopt a fully closed (agonist) conformation. The proposed binding mode for **13**, which displays agonist activity at mGlu2 and antagonist activity at mGlu3, is shown in Figures 5 and 6. The change in stereochemical configuration of the methyl group in **13** compared to **9** leads to steric interactions with Y216 and glycine G296 in both mGlu2 (Figure 5) and mGlu3 (Figure 6) receptors. Energy minimization of **13** bound to each receptor results in an approximately 0.5 Å shift in several residues in the binding site, including Y216, G296, and leucine L300, shown in orange in the upper-left portion of Figure 6. L300 is interesting in that it represents the

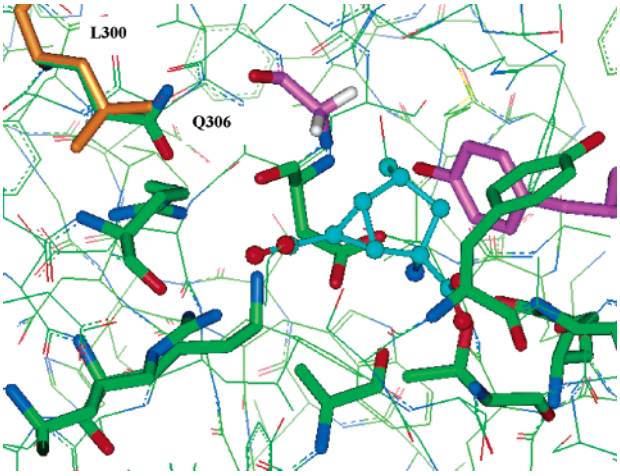


Figure 6. Proposed binding mode for **13** (cyan) in the glutamate binding pocket of mGlu3 (green). Energy minimization of this complex causes movement in several residues, including glutamine Q306. This glutamine is a leucine residue in mGlu2 (L300, orange) and represents the only residue that is different among the residues in the proximity of the glutamate binding site of mGlu2 vs mGlu3. Movement of this key residue may give rise to different conformational changes in the two receptors, which may account for the difference in functional activities observed for **13** at mGlu2 vs mGlu3.

only nonidentical amino acid close to the ligand-binding pocket of mGlu2 and mGlu3 (L300 in the former and glutamine Q306 in the latter, also shown in Figure 6). Recent work has demonstrated that the mutation Q306A in mGlu3 results in an approximate 87% loss in agonist (^{3}H-DCG-IV) binding compared to the wild-type receptor whereas the analogous mutation in mGlu2 (L300A) had a slightly less pronounced effect (67% loss in ^{3}H-DCG-IV binding).[42] The functional consequence of these mutations (e.g., evaluation of agonist/antagonist activity) was not reported. On the basis of our observed activity of **13** as an agonist at mGlu2 and an antagonist at mGlu3, we speculate that while a slight movement of L300 side chain in mGlu2 has no effect on the ability of the mGlu2 glutamate binding site to adopt an agonist conformation, a comparable displacement of the ω-amide functionality in Q306 in mGlu3 may preclude this conformational change, resulting in the observed antagonist effect.

Summary

We have found that substitution at the C3- or C4-positions of **1** by a methyl group results in generally diminished mGlu2/3 receptor affinity and agonist, antagonist, or mixed agonist/antagonist activity in functional assays depending on methyl group regiochemistry and stereochemistry. Importantly, C4α-methyl-substituted analogue **13** is the first example of a glutamate site agonist capable of functionally differentiating between mGlu2 and mGlu3 receptors. Owing to this unique pharmacological profile, **13** may be a useful tool for studying mGlu2 receptor activation in vivo.

Experimental Section

Melting points were obtained using a Thomas-Hoover capilliary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained at 300.15 and 75.48 MHz, respectively, with TMS as an internal standard. Field desorption mass spectrometry (FDMS) was performed using either a VG 70SE or Varian MAT 731 instrument. Preparative HPLC was performed with the Waters Prep LC2000 apparatus using dual silica gel PrepPAK-500 cartridges. Solvent systems employed are given in parentheses for each example. Cation-exchange chromatography was performed with Dowex 50X8-100 ion-exchange resin and anion-exchange chromatography with Bio-Rad AG1-X8 anion-exchange resin (hydroxide form). Compounds for which satisfactory combustion analyses could be obtained are identified as such within the Experimental Section, and a table is provided with detailed elemental analyses as Supporting Information. For compound **3**, analytical HPLC data (normal phase conditions) are included in the experimental part as an indicator of purity (column, Kromasil Si 60, 250 mm × 4.6 mm, 5 μm; isocratic mode, hexane/IPA 5%; flow rate, 1 mL/min; detection with UV−vis diode array detector, 210−400 nM).

(1*SR*,3*RS*,5*RS*,6*SR*)-Ethyl-2-oxo-3-methylbicyclo[3.1.0]-hexane 6-Carboxylate (3). To a solution of **2** (317 mg, 1.88 mmol) in anhydrous THF (30 mL) at −78 °C and under argon was added a 1 M solution of lithium bis-trimethylsilylamide in THF (1.9 mL, 1.88 mL). The reaction mixture was stirred for 45 min at this temperature, and then this solution was cannuled into a solution of methyl iodide (0.35 mL, 5.6 mmol) in THF (10 mL) at −78 °C. The reaction mixture was stirred for 1 h at −78 °C and then overnight at room temperature, quenched with saturated ammonium chloride solution (20 mL), and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated to dryness. Purification of the crude by flash chromatography (hexane/ethyl acetate 4:1) afforded **3** (0.24 g, 1.32 mmol) as an oil in 70% yield. FDMS: $M^+ = 182$. ^{1}H NMR ($CDCl_3$), δ: 4.0 (q, 2H, $J = 7.1$ Hz), 2.32−2.1 (m, 3H), 1.98−1.85 (m, 2H), 1.75−1.6 (m, 1H), 1.15 (t, 3H, $J = 7.1$ Hz), 0.9 (d, 3H, $J = 6.9$ Hz). ^{13}C NMR ($CDCl_3$), δ: 212, 170.26, 60.95, 35.77, 35.21, 31.46, 27.19, 27.07, 13.95, 13.79. Compound was checked on analytical HPLC for quality control. Purity of the desired compound (under normal phase conditions) was >98%.

(1*SR*,2*SR*,3*SR*,5*RS*,6*SR*)-Ethyl-2-spiro-5-hydantoin-3-methylbicyclo[3.1.0]hexane 6-Carboxylate (4). To a solution of **3** (500 mg, 2.7 mmol) in ethanol (1.3 mL) and water (3.3 mL) was added potassium cyanide (195 mg, 3 mmol) and ammonium carbonate (782 mg, 8.1 mmol). The mixture was heated at 55 °C overnight. After the mixture was cooled to room temperature, the precipitated material was filtered and washed with EtOH−H_2O to afford **4** (0.27 g, 1.1 mmol) in 40% yield. FDMS: $M^+ = 252$. ^{1}H NMR (DMSO-d_6), δ: 10.65 (s, 1H), 7.9 (s, 1H), 4.05 (q, 2H, $J = 7.1$ Hz), 2.05−1.6 (m, 5H), 1.2 (t, 3H, $J = 7.1$ Hz), 0.8 (d, 3H, $J = 5.9$). ^{13}C NMR (DMSO-d_6), δ: 175.86, 172.02, 156.75, 71.86, 60.40, 35.76, 33.12, 32.00, 25.97, 20.48, 14.27, 12.18. Anal. ($C_{12}H_{16}N_2O_4$) C, H, N.

(1*SR*,2*SR*,3*SR*,5*RS*,6*SR*)-2-Amino-3-methylbicyclo[3.1.0]-hexane 2,6-Dicarboxylic Acid (5). A mixture **4** (250 mg, 0.99 mmol) in 12 N HCl (10 mL) was refluxed overnight. The resulting solution was evaporated to dryness, yielding a white solid. Compound **5** (78 mg, 0.39 mmol) was isolated in 40% yield as a zwitterion after ion exchange chromatography on Dowex 50X8 50−100 mesh using 10% solution of pyridine in water as the eluent. FDMS: $M^+ = 199$. ^{1}H NMR (D_2O−Pyr-d_5), δ: 2.0−1.7 (m, 6H), 0.9 (d, 3H). ^{13}C NMR (D_2O−Pyr-d_5), δ: 179.95, 173.37, 70.08 36.46, 33.69, 31.79, 26.44, 23.75, 12.18. Anal. ($C_9H_{13}NO_4$) C, H, N.

(1*SR*,5*RS*,6*SR*)-Ethyl-2-oxobicyclo[3.1.0]hex-3-ene 6-Carboxylate (6). Iodotrimethylsilane (75 g, 375 mmol) was added dropwise to a 0 °C solution of **2** (42 g, 250 mmol) and Et_3N (75 g, 750 mmol) in anhydrous CH_2Cl_2 (1 L). The resulting reaction mixture was allowed to warm to ambient temperature as it was stirred overnight. The reaction mixture was washed three times with aqueous NH_4Cl and brine, dried over $MgSO_4$, and concentrated to yield the crude silyl enol ether. This was dissolved in anhydrous CH_3CN (600 mL), treated in one portion with $Pd(OAc)_2$ (61.7 g, 275 mmol), and stirred at ambient temperature overnight. The reaction mixture was diluted with Et_20 (600 mL) and filtered through a pad of Celite. The filtrate was concentrated to yield the crude product that

was purified by HPLC (10% EtOAc/hexanes to 50% EtOAc/hexanes), affording **6** (38.22 g, 230 mmol, 92% yield). Mp = 75−77 °C. FDMS: $M^+ = 166$. ^{1}H NMR ($CDCl_3$) δ: 7.61 (dd, J = 5.5 and 2.4 Hz, 1H), 5.73 (d, J = 5.5 Hz, 1H), 4.15 (q, J = 7.1 Hz, 2H), 2.97−2.94 (m, 1H), 2.64−2.61 (m, 1H), 2.27 (t, J = 2.7 Hz, 1H), 1.27 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 203.24, 167.92, 159.66, 129.60, 61.32, 45.82, 30.01, 28.91, 14.11. Anal. ($C_9H_{10}O_3$) C, H.

(1*SR*,4*SR*,5*RS*,6*SR*)-Ethyl-2-oxo-4-methylbicyclo[3.1.0]-hexane 6-Carboxylate (7). Methyllithium (71 mL of a 1.4 M solution in Et_2O, 100 mmol) was added to a mixture of copper(I) iodide (9.5 g, 50 mmol) in anhydrous Et_2O (10 mL) at 0 °C. The solution was stirred for 30 min at 0 °C and cooled to −78 °C. Compound **6** (1.6 g, 10 mmol) in Et_2O (25 mL) was added dropwise. Upon complete addition, the mixture was stirred for another hour at −20 °C for 1 h and then was quenched with saturated ammonium chloride solution and extracted with Et_2O. The combined organic phases were dried over $MgSO_4$, filtered, and evaporated to dryness. Purification of the crude product by flash chromatography (hexane/ethyl acetate 4:1) gave **7** (1.52 g, 8.3 mmol, 83% yield). FDMS: $M^+ = 182$. ^{1}H NMR ($CDCL_3$), δ: 4.1 (q, 2H, J = 7.15 Hz), 2.5 (m, 1H), 2.35−2.19 (m, 3H), 2.1 (t, 1H, J = 2.60 Hz), 1.65 (d, 1H, J = 18.60 Hz), 1.19 (t, 3H, J = 7.15 Hz), 1.10 (d, 3H, J = 6.90 Hz). ^{13}C NMR ($CDCl_3$), δ: 211.13, 170.16, 61.13, 40.45, 36.09, 34.96, 29.93, 26.92, 21.80, 14.03. Anal. ($C_{10}H_{14}O_3$) C, H.

(1*SR*,2*SR*,4*SR*,5*RS*,6*SR*)-Ethyl-2-acetamido-2-cyano-4-methylbicyclo[3.1.0]hexane 6-Carboxylate (8). A heterogeneous mixture of alumina (14 g, Merck, type 90 for column chromatography, neutral, activity I) and ammonium chloride (26 mmol) in acetonitrile (50 mL) was ultrasonically irradiated for 30 min. Then, a solution of **7** (2.19 mmol) in acetonitrile (5 mL) was added, and after sonication for an addition 2 h, 2.19 mmol of KCN was added. The mixture was sonicated overnight, and then the alumina was filtered off and the filtrate was concentrated to dryness to give a mixture of diastereomeric aminonitriles. To a solution of this mixture (1.25 mmol) in dry CH_2Cl_2 at 0 °C was added ethyl diisopropylamine (1.37 mmol), and the resultant mixture was stirred for 15 min. Acetyl chloride (1.37 mmol) was added, and the mixture was stirred at ambient temperature for 5 h. The reaction was quenched with water and extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and evaporated to dryness. Desired diastereomer **8** (0.16 g, 0.66 mmol) was isolated in 30% yield from the mixture by column chromatography (hexane/ethyl acetate 1:1), using 230−400 mesh silica gel (Merck). FDMS: $M^+ = 250$. ^{1}H NMR ($CDCl_3$), δ: 6.15 (s, 1H), 4.1 (q, 2H, J = 7.1 Hz), 2.7 (dd, 1H, J = 2.8 and 6.2 Hz), 2.55 (d, 1H, J = 15 Hz), 2.45 (m, 1H), 2.15−1.95 (m, 5H), 1.62 (t, 1H, J = 3.5 Hz), 1.55 (dd, 1H, J = 7.8 and 15 Hz), 1.25 (m, 6H). ^{13}C NMR ($CDCl_3$), δ: 171.16, 169.97, 121.09, 61.28, 55.05, 42.26, 34.94, 34.62, 34.29, 23.03, 22.04, 21.15, 14.25. Anal. ($C_{13}H_{18}N_2O_3$) C, H,N.

(1*SR*,2*SR*,4*SR*,5*RS*,6*SR*)-2-Amino-4-methylbicyclo[3.1.0]-hexane 2,6-Dicarboxylic Acid (*rac*-9). A mixture of **8** (0.8 mmol) and 5 N HCl solution (10 mL) was heated under reflux overnight. The resulting solution was evaporated to dryness, yielding a white solid. Amino acid **9** (0.05 g, 0.25 mmol) was isolated in 31% yield as a zwitterion after ion exchange chromatography on Dowex 50X8 50−100 mesh using 10% pyridine−water as eluent. Mp > 300 °C. FDMS: $M^+ = 199$. ^{1}H NMR (D_2O, Pyr-d_5), δ: 2.2 (dd, 1H, J = 3.1 and 6.2 Hz), 1.94 (m, 1H), 1.8−1.6 (m, 3H), 1.52 (t, 1H, J = 3.1 Hz), 0.9 (d, 3H, J = 7.2 Hz). ^{13}C NMR (D_2O, KOD) δ: 181.32, 179.68, 67.05, 41.58, 35.02, 34.77, 33.61, 25.56, 20.24. Anal. ($C_9H_{13}NO_4$) C, H, N.

(1*SR*,5*RS*,6*SR*)-Ethyl-2-oxo-4-methylbicyclo[3.1.0]hex-3-ene 6-Carboxylate (10). A solution of **7** (8.15 g, 44.7 mmol) and Et_3N (10.86 g, 107 mmol) in CH_2Cl_2 at 0 °C was treated dropwise with TMSI (10.74 g, 53.7 mmol) at a rate that maintained the internal temperature below 5 °C. Upon complete addition, the mixture was allowed to stir at this temperature for 2 h. The reaction mixture was diluted with Et_2O and washed with a saturated aqueous solution of NH_4-Cl. The organic phase was dried ($MgSO_4$) and concentrated to dryness. The residue was dissolved in CH_3CN, cooled to 5 °C, and treated with $Pd(OAc)_2$ (11.0 g, 49 mmol) in one portion. The reaction was allowed to continue at room temperature overnight, then was diluted with Et_2O, filtered through a pad of silica gel and Celite, and concentrated to dryness yielding **10** (7.1 g, 35.4 mmol, 88% yield). The product solidified to a low-melting solid on standing, mp = 50−52 °C. FDMS: $M^+ = 180$. ^{1}H NMR ($CDCl_3$) δ: 5.41 (s, 1H), 4.15 (q, J = 7 Hz, 2H), 2.80−2.75 (m, 1H), 2.60−2.55 (m, 1H), 2.22 (t, J = 2.5 Hz, 1H), 2.18 (s, 3H), 1.25 (t, J = 7 Hz, 3H). Anal. ($C_{10}H_{12}O_3.0.05CH_2Cl_2$) C, H.

(1*SR*,4*RS*,5*RS*,6*SR*)-Ethyl-2-oxo-4-methylbicyclo[3.1.0]-hexane 6-Carboxylate (11). To a solution of **10** (5.55 g, 30.8 mmol) in EtOH (100 mL) was added 5% Pd−C (0.6 g). The mixture was subjected to H_2(g) at 40 psi for 1 h. The mixture was diluted with Et_2O and filtered through Celite. The solvent was evaporated, and the crude product was purified by HPLC (hexanes−ethyl acetate 3:1), providing **11** (5.08 g, 28 mmol, 91%). FDMS: $M^+ = 182$. ^{1}H NMR ($CDCl_3$) δ: 4.18−4.08 (m, 2H), 2.70−2.60 (m, 1H), 2.51−2.48 (m, 1H), 2.28−2.25 (m, 1H), 2.18 (ddd, J = 18.4, 8.8, and 1.0 Hz, 1H), 2.03 (dd, J = 3.3 and 2.5 Hz), 1.61 (ddd, J = 18.4, 9.5, and 1.0 Hz, 1H), 1.26 (t, J = 7 Hz, 3 H), 1.15 (d, J = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 210.8, 170.47, 61.22, 39.56, 37.17, 34.79, 29.56, 23.76, 18.07, 14.09. Anal. ($C_{10}H_{14}O_3$) C, H.

(1*SR*,2*SR*,4*RS*,5*RS*,6*SR*)-Ethyl-2-spiro-5-hydantoin-4-methylbicyclo[3.1.0]hexane 6-Carboxylate (12). To a solution of **11** (1.82 g, 10 mmol) in ethanol (20 mL) and water (10 mL) was added KCN (1.0 g, 15 mmol) and $(NH_4)_2CO_3$ (2.0 g, 25 mmol) and the mixture was heated at 50 °C for 48 h. The reaction mixture was diluted with H_2O (30 mL) and cooled in an ice bath, and the resulting solids were filtered to yield 0.5 g of crude product. This was recrystallized from EtOH to yield a single diastereomer **12** (0.18 g, 0.71 mmol, 7.1%). Mp = 222−224 °C. FDMS: $M^+ = 252$. ^{1}H NMR (pyridine-d_5), δ: 12.47 (s, 1H), 9.58 (s, 1H), 4.08 (q, 2H, J = 7.1 Hz), 2.97 (ddd, J = 10.5, 6.8, and 3.8 Hz, 1H), 2.45 (dd, J = 6.3 and 3.2 Hz, 1H), 2.25 (ddd, J = 6.3, 3.2, and 3.2 Hz, 1H), 2.16 (dd, J = 3.2 and 3.2 Hz, 1H), 2.11 (dd, J = 13.7 and 7.9 Hz, 1H), 1.23 (dd, J = 13.7 and 10.5 Hz, 1H), 1.08 (t, J = 7.1 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H). ^{13}C NMR (pyridine-d_5), δ: 178.65, 172.33, 157.97, 70.17, 60.74, 38.43, 34.56, 33.85, 33.39, 18.92, 18.92, 14.20. Anal. ($C_{12}H_{16}N_2O_4$) C, H, N.

(1*SR*,2*SR*,4*RS*,5*RS*,6*SR*)-2-Amino-4-methylbicyclo[3.1.0]-hexane 2,6-Dicarboxylic Acid (13). A mixture of **12** (0.15 g, 0.6 mmol) and 1 N NaOH solution (15 mL) was heated under reflux for 5 days. The pH was adjusted to 1 by addition of 1 N HCl, and the resulting solution was evaporated to dryness, yielding a white solid. The solids were redissolved at pH 12, the solids were removed by filtration, and the filtrate was applied to an anion exchange column (AG1-X8, acetate form). Amino acid **13** (0.067 g, 0.34 mmol, 56%) was isolated following elution with 3 N AcOH. Mp >270 °C (dec). FDMS: M^+ (+H) = 200. ^{1}H NMR (D_2O, KOD), δ: 2.25−2.10 (m, 1H), 1.70−1.43 (m, 3H), 1.22 (br.s, 1H), 0.7−0.67 (m, 3H), 0.52−0.43 (m, 1H). ^{13}C NMR (D_2O, KOD) δ: 180.97, 180.16, 64.26, 40.17, 34.39, 31.46, 31.33, 19.04, 14.47. Anal. ($C_9H_{13}NO_4$) C, H, N.

Separation of the Enantiomers of *rac*-11. The individual enantiomers of *rac*-11 were obtained by chiral column chromatography (Chiralpak AD 8 cm × 28 cm column; 500 mg of racemate loaded each run; elution with 100% CH_3OH at 300 mL/min; detection at 220 nm). Retention times for (+)-**11** and (−)-**11** were 6.22 and 10.07 min, respectively. From 8.5 g (46.6 mmol) of *rac*-**11** was obtained 3.56 g (19.5 mmol, 42% yield, >98.8% ee) of (+)-**11** and 3.66 g (20.0 mmol, 43% yield, 98.8% ee) of (−)-**11**. Optical rotations: (+)-**11**, α_D 78.5 (c 1.07, CH_3OH); (−)-**11**, α_d −83.3 (c 1.08, CH_3OH).

(+)-Ethyl-2-spiro-5-hydantoin-4-methylbicyclo[3.1.0]-hexane 6-Carboxylate ((+)-12). To a solution of (+)-**11** (3.20 g, 17.6 mmol) in ethanol (15 mL) and water (15 mL) was added KCN (1.71 g, 26.3 mmol) and $(NH_4)_2CO_3$ (4.12 g, 52.8 mmol), and the mixture was heated at 50 °C overnight and then at room temperature for an additional 24 h. The resulting solids

were filtered and washed with EtOH and CH_2Cl_2 to yield (+)-**12** (1.25 g, 4.96 mmol, 28%). FDMS: $M^+ = 252$. ^{1}H NMR (pyridine-d_5), δ: 12.43 (s, 1H), 9.53 (s, 1H), 4.08 (q, 2H, J = 7.1 Hz), 2.98 (ddd, J = 10.5, 6.8, and 3.8 Hz, 1H), 2.45 (dd, J = 6.3 and 3.2 Hz, 1H), 2.26 (ddd, J = 6.3, 3.2, and 3.2 Hz, 1H), 2.16 (dd, J = 3.2 and 3.2 Hz, 1H), 2.12 (dd, J = 13.7 and 7.9 Hz, 1H), 1.23 (dd, J = 13.7 and 10.5 Hz, 1H), 1.10 (t, J = 7.1 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H). ^{13}C NMR (pyridine-d_5), δ: 178.66, 172.33, 158.00, 70.20, 60.75, 38.46, 34.57, 33.87, 33.41, 18.95, 17.01, 14.22. α_D 17.82 (c 1.01, CH_3OH). Anal. ($C_{12}H_{16}N_2O_4 \cdot 0.4CH_2Cl_2$) C, H, N.

(−)-Ethyl-2-spiro-5-hydantoin-4-methylbicyclo[3.1.0]-hexane 6-Carboxylate ((−)-12). To a solution of (−)-**11** (3.20 g, 17.6 mmol) in ethanol (15 mL) and water (15 mL) was added KCN (1.71 g, 26.3 mmol) and $(NH_4)_2CO_3$ (4.12 g, 52.8 mmol), and the mixture was heated at 50 °C overnight, then at room temperature for an additional 24 h. The resulting solids were filtered and washed with EtOH to yield (−)-**12** (1.62 g, 6.4 mmol, 36%). FDMS: $M^+ = 252$. ^{1}H NMR (pyridine-d_5), δ: 12.43 (s, 1H), 9.53 (s, 1H), 4.08 (q, 2H, J = 7.1 Hz), 2.98 (ddd, J = 10.5, 6.8, and 3.8 Hz, 1H), 2.45 (dd, J = 6.3 and 3.2 Hz, 1H), 2.26 (ddd, J = 6.3, 3.2, and 3.2 Hz, 1H), 2.16 (dd, J = 3.2 and 3.2 Hz, 1H), 2.12 (dd, J = 13.7 and 7.9 Hz, 1H), 1.23 (dd, J = 13.7 and 10.5 Hz, 1H), 1.10 (t, J = 7.1 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H). ^{13}C NMR (pyridine-d_5), δ: 178.66, 172.33, 158.00, 70.20, 60.75, 38.46, 34.57, 33.87, 33.41, 18.95, 17.01, 14.22. α_D −23.16 (c 0.95, CH_3OH). Anal. ($C_{12}H_{16}N_2O_4 \cdot 0.1H_2O$) C, H, N.

(+)-2-Amino-4-methylbicyclo[3.1.0]hexane 2,6-Dicarboxylic Acid ((+)-13). A mixture of (+)-**12** (1.40 g, 5.5 mmol) and 2 N NaOH solution (28 mL) was heated under reflux for 24 h. The pH was adjusted to 7 by addition of 1 N HCl, and the resulting solution was evaporated to dryness, yielding a white solid. The solids were redissolved at pH 12 and applied to an anion exchange column (AG1-X8, OH form). Amino acid (+)-**13** (0.60 g, 3.0 mmol, 55%) was isolated following elution with 3 N AcOH. Mp >250 °C (dec). FDMS: M^+ (+1) = 200. ^{1}H NMR (D_2O, KOD), δ: 2.60−2.45 (m, 1H), 2.05−1.80 (m, 3H), 1.58−1.52 (m, 1H), 1.00 (t, J = 7 Hz, 3H), 0.78 (dd, J = 13.7 and 10.5 Hz, 1H). α_D 53.61 (c 0.97, 1 N HCl). Anal. ($C_9H_{13}NO_4 \cdot 0.1$AcOH) C, H, N.

(−)-2-Amino-4-methylbicyclo[3.1.0]hexane 2,6-Dicarboxylic Acid ((−)-13). A mixture of (−)-**12** (1.80 g, 7.1 mmol) and 2 N NaOH solution (36 mL) was heated under reflux for 24 h. The pH was adjusted to 7 by addition of 1 N HCl, and the resulting solution was evaporated to dryness, yielding a white solid. The solids were redissolved at pH 12 and applied to an anion exchange column (AG1-X8, OH form). Amino acid (−)-**13** (1.10 g, 5.6 mmol, 78%) was isolated following elution with 3 N AcOH. Mp >250 °C (dec). FDMS: M^+ (+1) = 200. ^{1}H NMR (D_2O, KOD), δ: 2.60−2.45 (m, 1H), 2.05−1.80 (m, 3H), 1.58−1.52 (m, 1H), 1.00 (t, J = 7 Hz, 3H), 0.78 (dd, J = 13.7 and 10.5 Hz, 1H). α_D −54.74 (c 0.95, 1 N HCl). Anal. ($C_9H_{13}NO_4 \cdot 0.1$AcOH) C, H, N.

Supporting Information Available: Results from combustion analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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