Rational Design and Enantioselective Synthesis of (1*R*,4*S*,5*R*,6*S*)-3-Azabicyclo[3.3.0]octane-4,6-dicarboxylic Acid − A Novel Inhibitor at Human Glutamate Transporter Subtypes 1, 2, and 3

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The natural product kainic acid is used as template for the rational design of a novel conformationally restricted (*S*)-glutamic acid (Glu) analogue, (1*R*,4*S*,5*R*,6*S*)-3-azabicyclo[3.3.0]octane-4,6-dicarboxylic acid (**1a**). The target structure **1a** was synthesized from commercially available (*S*)-pyroglutaminol, in an enantioselective fashion, in 14 steps. Pharmacological characterization of **1a** at human glutamate transporter subtypes 1, 2, and 3 yielded K_i values of 127, 52, and 46 μM, respectively. Furthermore, binding studies at native ionotropic Glu (iGlu) receptors revealed low affinity for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-preferring iGlu receptors (IC_{50} > 100 μM), whereas affinities for the KAIN-preferring iGlu receptors and the *N*-methyl-D-aspartate (NMDA)-preferring group of iGlu receptors were in the low micromolar range (IC_{50} = 14 and 2.9 μM, respectively). At metabotropic Glu receptors (mGluR), EC_{50} values for **1a** were >1000 μM for mGluR1 and 4, representing group I and III, respectively, and ∼1000 μM (agonist) for mGluR2, representing group II.

Introduction

The design and synthesis of novel rigid carbo- or heterocyclic skeletons that mimic the biologically active conformations of the major excitatory neurotransmitter (*S*)-glutamate (Glu) in the central nervous system (CNS) continues to be an important topic of research. In the healthy CNS, activation of Glu receptors is involved in important neurophysiological processes such as memory and learning, motor functions, and neural plasticity and development.[1] However, under conditions of metabolic stress and oxygen deprivation, Glu is a neurotoxic agent. Thus, it is believed that neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), epilepsy, and cerebral stroke may be directly related to disordered glutamatergic neurotransmission originating from dysfunction of either the Glu receptors, ionotropic receptors (iGluR), or metabotropic receptors (mGluR) or the Glu reuptake system, excitatory amino acid transporters (EAATs).[1]

To this date, several conformationally restricted Glu analogues, such as simple three- to six-membered carbocyclic analogues, substituted norprolines,[2] pyrrolidines and piperidines, as well as highly rigid analogues based on the bicyclo[1.1.1]-pentane,[3] spiro[2.2]pentane,[4] bicyclo[2.1.1]hexane,[5] bicyclo-[3.1.0]hexane,[6] bicyclo[2.2.1]heptane,[7] the 7-azanorbonane,[8] or the 2-azanorbonane[9] skeletons, have been designed and synthesized. Subsequent pharmacological characterization of these Glu analogues has provided important information about the binding mode of Glu to Glu receptors and transporters, as well as the structural requirements for Glu receptor agonist/antagonist activity, Glu transporter substrate/inhibitor activity, and subtype selectivity. Considering the essential role played by the EAATs in the maintenance of synaptic Glu concentrations below neurotoxic levels, surprisingly little attention has been paid to the Glu transporters as potential drug targets in the treatment of the plethora of neurotoxic and neurodegenerative disorders mentioned above.[10,11] Recently, it has been suggested that the EAATs may exhibit reverse-transport of Glu under ischemic conditions and that selective EAAT inhibitors may prevent this.[12] However, at present only a few selective EAAT inhibitors have been described, none of which, apart from dihydrokainic acid (DHK), displaying significant EAAT subtype selectivity.[13] In this paper we describe the rational design and enantioselective synthesis of a novel conformationally restricted Glu analogue **1a** (Figure 1), whose chemical structure holds the small, highly rigid heterocyclic skeleton, 3-azabicyclo[3.3.0]octane. The compound is characterized pharmacologically at human glutamate transporters EAAT1, EAAT2, and EAAT3, and iGlu receptors.

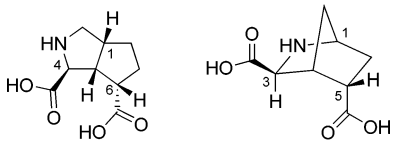

Figure 1. Rationally designed novel EAAT inhibitors **1a** and **2**.

Rational Design of 1a. It has been suggested by us[14] and others[10,15,16] that the inhibitory binding conformation at EAAT1−3 is similar to the *folded* Glu conformation also observed for iGluR agonist activity,[17] while EAAT substrates bind in an *extended* Glu conformation, however, different from the conformation observed for mGluR agonist activation.[18] The natural product kainic acid (KAIN), as well as its close structural analogue DHK, are both selective inhibitors at the EAAT2 subtype (K_i = 60 and 31 μM, respectively) (Table 1). However, the C-4-dihydro KAIN/DHK-analogue CPAA[19] is a non-subtype-selective EAAT inhibitor with 4−8-fold lower potency at EAAT1, -2, and -3 (K_i > 250 μM) (Table 1). To investigate the role of the C-4 substituent in these observations and with the aim of designing a small rigid skeleton that could potentially be a non-subtype-selective EAAT1−3 inhibitor, we performed a superimposition study of the low-energy *folded* conformations of KAIN, DHK, and CPAA (Figure 2). As expected, the isopropylene group of KAIN and the isopropyl group of DHK align

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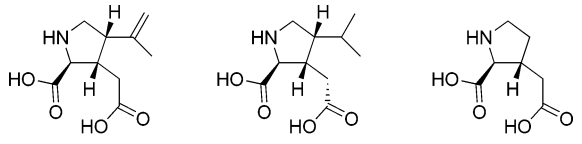
Table 1. Reported EAAT Inhibitors Kainic Acid (KAIN), Dihydrokainic Acid (DHK), and 2-Carboxy-3-pyrrolidineacidic Acid (CPAA)



	K_i (μM)		
	EAAT1	EAAT2	EAAT3
KAIN[a]	>3000	60	>3000
DHK[a]	>3000	31	>3000
CPAA[b]	>250	>250	>250

[a] Data taken from ref 21. [b] Data taken from ref 19.

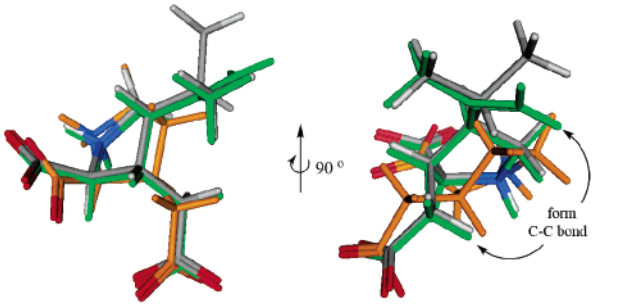


Figure 2. Superimposition by fitting the ammonium nitrogen and the two carboxylate groups of calculated low-energy, folded conformations of dihydrokainic acid (DHK) (type code), kainic acid (KAIN) (green), and 2-carboxy-3-pyrrolidineacetic acid (CPAA) (orange).

well; thus, unfavorable van der Waals interactions of these substituents must be responsible for the observed lack of potency at EAAT1 and EAAT3. On the other hand, the C-4-dihydro analogue, CPAA, is a nonselective EAAT inhibitor. This can be understood from the fact that no structural features of this compound project into the area in space occupied by the isopropylene or the isopropyl groups. However, from this superimposition study it is not clear to us why CPAA is at least 4-fold less potent than KAIN and at least 8-fold less potent than DHK. We speculate that the observed 4−8-fold decreased potency of CPAA is solely due to the amplified desolvation and entropic energies of CPAA compared with KAIN/DHK. We also take note of a reported KAIN analogue that restricts the orientation of the isopropylene group.[20] However, in a binding study on native rat neurons, the compound exhibited significantly less affinity (10−20-fold) than KAIN itself, and no data as to its characteristics as an EAAT inhibitor were presented.

On the basis of the modeling considerations described above, we predicted that truncation of the isopropylene group in KAIN, linking the remaining methyl group to the α-carbon of the acetic acid moiety, would offer a new small rigidified Glu analogue that is a potential non-subtype-selective EAAT inhibitor. The compound, (1*R*,4*S*,5*R*,6*S*)-3-azabicyclo[3.3.0]octane-4,6-dicarboxylic acid (**1a**) (Figure 1) was subjected to a stochastic conformational search, which revealed that the low-energy conformation of **1a** mimics the *folded* Glu conformation very well and that the area probing selectivity for EAAT2 is not affected (Figure 3). However, methylene groups C^7H_2 and C^8H_2 in **1a** do reside in a volume not occupied by KAIN/DHK. This could result in unfavorable van der Waals interactions and thus decreased or abolished potency. With this ambiguity in mind, we predicted **1a** to be a candidate as a novel nonselective EAAT1−3 inhibitor. In addition to newly designed structure

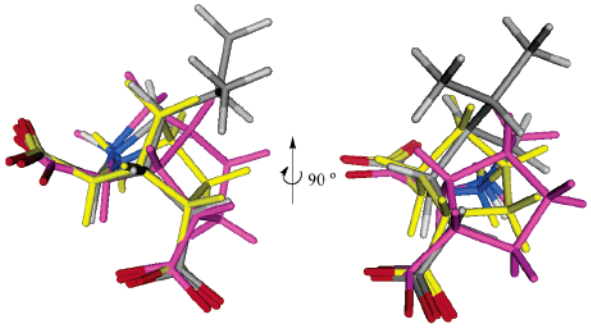
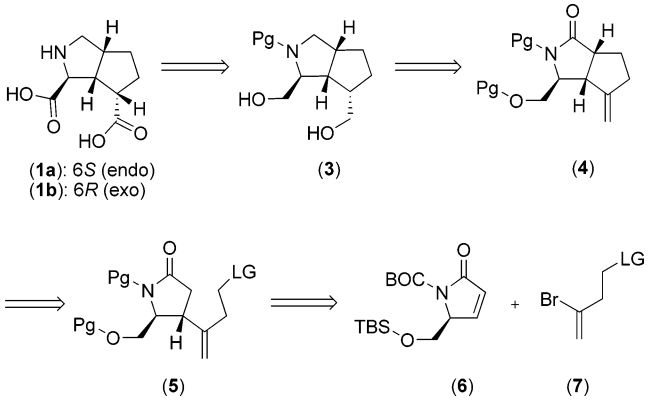


Figure 3. Superimposition by fitting the ammonium nitrogen and the two carboxylate groups of calculated low-energy, folded conformations of dihydrokainic acid (DHK) (type code), **1a** (magenta), and **2** (yellow).

Scheme 1



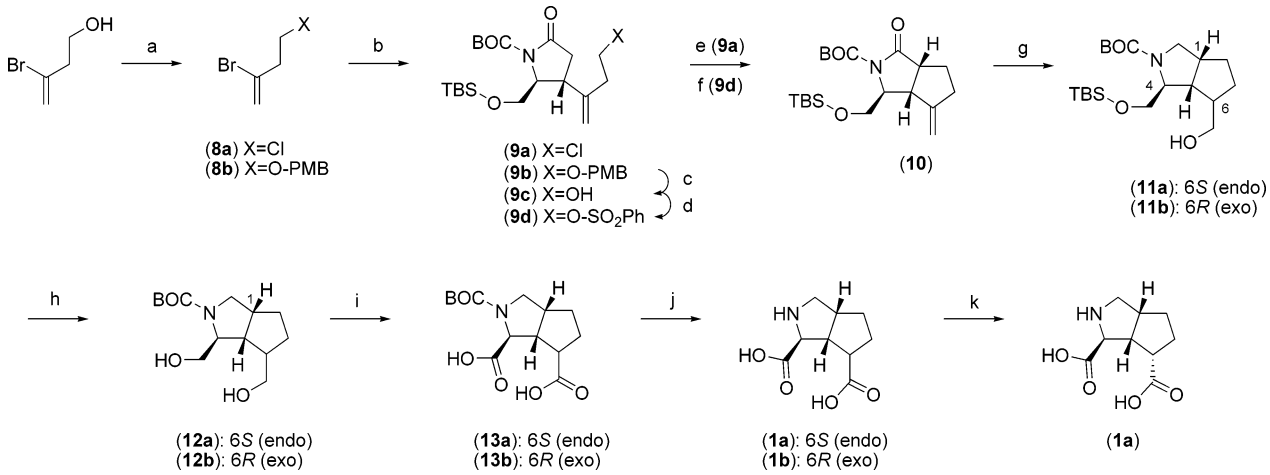
1a, conformationally restricted bicyclic Glu analogue **2** (Figure 1), previously synthesized in our group,[9] also fulfills the before mentioned structural requirements and exerts the same ambiguity with respect to the total volume occupied (Figure 3). Thus, both compounds **1a** and **2** are candidates as novel non-subtype-selective EAAT1−3 inhibitors.

Retrosynthetic Analysis of 1a. The 4,6-disubstituted 3-azabicyclo[3.3.0] heterocarbon skeleton is an unexplored target structure in synthetic organic chemistry. Given the importance of stereochemistry in medicinal chemistry, we sought to follow an eantioselective route to **1a**. Thus, a retrosynthetic analysis of **1a** (Scheme 1) suggests the synthesis of suitably protected diol **3**, which may be obtained from key intermediate **4** by hydroboration of the alkene from the less sterically hindered face. The identification of a pyroglutaminol moiety in **4** suggests its synthesis from the reaction of readily available enantiopure protected 3,4-didehydropyroglutaminol **6**,[22] with the cuprate formed from vinyl bromide **7**. This reaction sets the desired absolute stereochemistry at C^5 in **5**, which again allows for cyclization into the cis fused ring system in **4** via generation of the enolate. The reaction may be explored as a one-pot annulation reaction (LG = Cl), but since **6** has been shown to be sensitive in cuprate addition reactions and the corresponding vinyllithio anion of vinyl bromide **7** (LG = Cl) is unstable at temperatures above −50 °C,[23] the reaction may necessarily be carried out in two steps (LG being a masked leaving group, e.g. O−PMB) with isolation and appropriate functional group transformation of **5**.

Results and Discussion

The enantioselective synthesis of **1a** (Scheme 2) commenced with the conversion of the alcohol group in 3-bromo-3-buten-1-ol (available from Sigma-Aldrich) to its corresponding chloride, **8a**. Copper(I)-mediated conjugate addition to **6** has

Scheme 2[a]



[a] Reagents and conditions: (a) for **8a**, NCS, PPh_3, DCM, rt, 20 h (49%); for **8b**, PMB−TCA, La(OTf)$_3$, toluene, rt, 5 min (77%); (b) 2.5 equiv of **8a** or **8b**, *t*-BuLi, CuCN, TMSCl, THF, −78 to −50 °C, then **6**, −50 °C, 1 h (**9a**, 24−38%; **9b**, 86%); (c) DDQ, H_2O, DCM, rt, 1 h (90%); (d) $PhSO_2Cl$, Et_3N, DMAP, DCM, rt, 4 h (92%); (e) 1.03 equiv of KHMDS, THF, −78 °C to room temperature, 1 h, then rt, 1 h (90%); (f) 1.03 equiv LHMDS, THF, −78 to 0 °C, 18 h (92%); (g) 3% $RhCl(PPh_3)_3$, CatBH, THF, rt, 1 h, then 4 equiv of BH_3·THF, rt, 20 h, then 35% H_2O_2, NaOH, rt, 30 min (one-pot 85%, 80% de); (h) TBAF, rt, 30 min (97%); (i) $RuCl_3$, $NaIO_4$, H_2O, MeCN, EtOAc, rt, 1 h (77%); (j) 20 equiv of HCl/EtOAc, rt, 1−2 h (83%, 84% de); (k) Recrystallization from glacial AcOH (70%, 99% ee, 96% de).

previously been investigated by us.[24] However our optimized protocol for this specific reaction failed on larger scales (2 mmol) giving inconsistent yields (24−38%) of the desired product **9a**. We believe this observation is due to problems with metal−halogen exchange of **8a**. Even though **9a** was readily cyclized into key intermediate **10** in high yield, we decided to use a more traditional synthetic pathway by the use of protecting group chemistry. The *p*-methoxybenzyl (PMB) group was chosen because of its possibly selective removal and following conversion of the alcohol to a leaving group. Thus, **8b** was prepared by the use of a very mild method for the introduction of a PMB group,[25] and using our protocol, conjugate addition of its corresponding cuprate to **6** gave **9b** in high yield (86%). Selective removal of the PMB group followed by conversion of the free alcohol **9c** to its corresponding phenylsulfonic ester **9d** allowed for cyclization into key intermediate **10** in high yield. Subsequent hydroboration of the methylidene group in compound **10** gave a high degree of facial selectivity (ratio 9:1) by the use of Wilkinson's catalyst/catecholborane.[9] In one pot, the *endo*-carbonyl group was subsequently reduced by the addition of borane to give alcohols **11a**/**11b** (85% yield). Cleavage of the O-TBS silyl ether by treatment with tetrabutylammonium fluoride gave diols **12a**/**12b** in 97%, which were oxidized to their corresponding diacids **13a**/**13b** via the modified Sharpless procedure[26] ($RuCl_3$/$NaIO_4$). Removal of the BOC group with HCl(g)/EtOAc gave the target compound **1a**/**1b** as their HCl salts in 83% yield, de = 84%. Recrystallization of this crude diastereomeric mixture from glacial AcOH gave **1a**, in 70% yield, de = 96%.

The conformationally restricted Glu analogues **1a**, synthesized as described in Scheme 2, and **2**, synthesized as described earlier by us,[9] were characterized pharmacologically at the glutamate transporter subtypes EAAT1, EAAT2, and EAAT3, using a FLIPR membrane potential (FMP) assay (see Experimental Section for details). The assay was performed essentially as described previously,[21] and results are summarized in Table 2. Compound **1a** was found to be an inhibitor at the three EAAT subtypes tested here, with modest potency at EAAT1 (K_i = 127 μM) and midrange potencies, comparable with KAIN and DHK, at EAAT2 and EAAT3 (K_i = 52 and 46 μM, respectively). On the other hand, compound **2** was found to be inactive as substrate and inhibitor at EAAT1, EAAT2 and EAAT3 with K_m and K_i values >400 μM. Furthermore, **1a** was characterized in binding studies at native ionotropic Glu (iGlu) receptors, and results are summarized in Table 3. The novel Glu analogue **1a** was inactive at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-preferring iGlu receptors (IC_{50} >100 μM), while affinities for the KAIN-preferring iGlu receptors and the *N*-methyl-D-aspartate (NMDA)-preferring group of iGlu receptors were in the low micromolar range (IC_{50} = 14 and 2.9 μM, respectively). Pharmacological characterization of **1a** in a functional assay[27] at the metabotropic Glu receptor subtypes mGluR1, mGluR2, and mGluR4, representing groups I, II, and III, respectively, gave EC_{50} values of >1000 μM for mGluR1 and -4, and ∼1000 μM (agonist) for mGluR2.

Table 2. Pharmacological Characterization of Conformationally Restricted Glu Analogues **1a** and **2** at Human EAAT1−3

	K_i (μM)		
	EAAT1	EAAT2	EAAT3
1a	127	52	46
2	>400[a]	>400[a]	>400[a]

[a] Also inactive when tested as a substrate.

Table 3. Pharmacological Characterization of Conformationally Restricted Glu Analogues **1a** and **2** at iGlu receptors

	IC_{50} (μM)		K_i (μM):
	[^{3}H]AMPA	[^{3}H]KAIN	[^{3}H]CGP39653
1a	>100	14	2.9
2[a]	>300	>160	>300

[a] Data taken from ref 9.

From the pharmacological data presented above for **1a**, we can conclude that the two methylene groups C^7H_2 and C^8H_2 are not in critical conflict with parts of the glutamate transporter proteins EAAT1, EAAT2, and EAAT3. Thus, this volume can be viewed as allowed in the future design of EAAT inhibitors. Furthermore, as predicted in the design phase, **1a** is characterized as a non-subtype-selective EAAT inhibitor. The fact that the highly rigid heterocyclic skeleton locks the Glu backbone in the *folded* Glu conformation underlines that this conformation indeed is the biologically active one for EAAT inhibitors.

The question remains as to why activity at EAAT1−3 is *not* observed for 2-azanorborane analogue **2**. We propose that the origin for this observation is the development of disfavored van der Waals interactions between **2** and the EAAT protein. In detail, C^7 in **2** overlaps well with C^4 in KAIN, and C^6 in **2** is included in the acceptable volume defined by C^7 and C^8 of **1a**. However, these two positive conclusions for **2** do not combine. In this context, it is important to point out that C^1 in **1a** is *not* overlapping with C^7 in **2**. Thus, we suggest that the lack of EAAT activity for **2** can be ascribed to the compound's inability to maneuver in the EAAT protein because of the strict relative position in space of C^1 and C^6.

Conclusions

In conclusion, we have carried out the rational design and enantioselective synthesis of a novel conformationally restricted Glu analogue **1a**. The enantioselective synthesis was carried out in 14 steps from commercially available (*S*)-pyroglutaminol. As predicted in the design phase, **1a** was characterized as a non-subtype-selective EAAT inhibitor (EAAT1, EAAT2, and EAAT3). Furthermore, we have characterized the 2-azanorbornane analogue **2** previously synthesized in our group. However, against our prediction, this compound was not found to be a ligand at EAAT1−3. To meet the requirements from the scientific community for subtype-selective EAAT inhibitors, we advocate for the design and synthesis of substituted analogues of **1a**. This work is currently ongoing in our group.

Experimental Section

Chemistry. All reagents were obtained from commercial suppliers and used without further purification. THF was distilled from sodium/benzophenone. NMR (300 MHz) spectra were recorded in $CDCl_3$ using $CHCl_3$ as reference, unless otherwise noted. Melting points are uncorrected. Merck silica gel (35−70 mesh) was used for flash chromatography.

2-Bromo-4-chlorobut-1-ene (8a). To a solution of triphenylphosphine (9.45 g, 36 mmol) in dry dichloromethane (100 mL) was added 3-bromo-3-buten-1-ol (3.0 mL, 30 mmol). The flask was then cooled to 0 °C and a solution of *N*-chlorosuccinimide (4.80 g, 36 mmol) in dry dichloromethane (50 mL) was added dropwise via a funnel. The reaction was allowed to warm to room temperature and stirring continued overnight. The organic layer was concentrated on a rotary evaporator (50 °C, 1 atm) and freezing cold diethyl ether was added. The slurry was filtered and the filtrate concentrated on a rotary evaporator (50 °C, 1 atm). The crude product was distilled (50 °C, 20 mmHg) to give **8a**, as a clear oil (2.5 g, 49%): ^{1}H NMR δ 5.74−5.70 (m, 1H), 5.56 (d, 1H, J = 2 Hz), 3.69 (br t, 2H, J = 7 Hz), 2.84 (br t, 2H, J = 7 Hz); ^{13}C NMR δ 129.12, 119.71, 44.11, 41.84. Anal. (C_4H_6BrCl) C, H.

1-((3-Bromobut-3-enyloxy)methyl)-4-methoxybenzene (8b). To a solution of 3-bromo-3-buten-1-ol (760 mg, 5.0 mmol) and *p*-methoxybenzyl trichloroacetimidate[28] (2.1 g, 7.5 mmol) in dry toluene (25 mL) was added $La(OTf)_3$ (147 mg, 0.25 mmol). The reaction mixture was stirred for 5 min and then filtered on silica gel. The silica gel cake was washed with toluene (3 × 25 mL), and the collective organic layers were concentrated. The crude product was purified using flash chromatography (heptane/EtOAc 19:1, R_f = 0.18) to give **8b**, as a colorless oil (1.04 g, 77%): ^{1}H NMR δ 7.26 (d, 2H, J = 9 Hz), 6.88 (d, 2H, J = 9 Hz), 5.67 (dd, 1H, J = 3 & 2 Hz), 5.48 (d, 1H, J = 2 Hz), 4.48 (s, 2H), 3.81 (s, 3H), 3.64 (t, 2H, J = 6 Hz), 2.71 (br t, 2H, J = 6 Hz); ^{13}C NMR δ 159.32, 130.92, 130.36, 129.49, 118.57, 113.98, 73.01, 67.67, 55.59, 42.05. Anal. ($C_{12}H_{15}BrO_2$) C, H.

(4*R*,5*S*)-1-*tert*-Butoxycarbonyl-5-(*tert*-butyldimethylsilyloxymethyl)-4-(4-chlorobut-1-en-2-yl)pyrrolidin-2-one (9a). To a solution of **8a** (850 mg, 5.0 mmol) in dry THF (16 mL) at −78 °C was added *t*-BuLi (5.88 mL, 1.7 M in hexanes, 10.0 mmol). After stirring for 15 min, a slurry of CuCN (225 mg, 2.50 mmol) in dry THF (2 mL) was added and the reaction mixture allowed to warm to −50 °C (clear solution). After 5 min, the flask was recooled to −78 °C and **6** (655 mg, 2.0 mmol) dissolved in dry THF (2.0 mL) was added, followed by TMSCl (0.65 mL, 5.0 mmol). The reaction mixture was allowed to warm to −50 °C and stirred at this temperature for 1 h. The reaction was quenched with saturated NH_4-Cl and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Purification of the crude product by flash chromatography (heptane/EtOAc 6:1, R_f = 0.23) gave **9a**, as a colorless oil (321 mg, 24−38%): $[\alpha]^{20}_{589}$ = −51.91 (c = 1.0, $CHCl_3$); ^{1}H NMR δ 4.96 (br s, 1H), 4.92 (br s, 1H), 3.98 (m, 1H), 3.84 (dd, 1H, J = 10 & 5 Hz), 3.74 (dd, 1H, J = 10 & 3 Hz), 3.64 (t, 2H, J = 7 Hz), 2.94 (dd, 1H, J = 17 & 8 Hz), 2.82 (br d, 1H, J = 10 Hz), 2.51 (br t, 2H, J = 7 Hz), 2.34 (dd, 1H, J = 17 & 2 Hz), 1.51 (s, 9H), 0.88 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ^{13}C NMR δ 173.94, 150.21, 146.76, 112.02, 83.54, 64.22, 64.21, 42.75, 38.80, 38.16, 38.10, 28.60, 26.36, 18.72, −4.93. Anal. ($C_{20}H_{36}ClNO_4Si$) C, H, N.

(4*R*,5*S*)-1-*tert*-Butoxycarbonyl-5-(*tert*-butyldimethylsilyloxymethyl)-4-(4-(4-methoxybenzyloxy)but-1-en-2-yl)pyrrolidin-2-one (9b). To a solution of **8b** (2.72 g, 10.0 mmol) in dry THF (34 mL) at −78 °C was added *t*-BuLi (11.8 mL, 1.7 M in hexanes, 20.0 mmol). After stirring for 15 min, a slurry of CuCN (450 mg, 5.0 mmol) in dry THF (4 mL) was added and the reaction mixture allowed to warm to −50 °C (clear solution). After 5 min, the flask was recooled to −78 °C and **6** (1.31 g, 4.0 mmol) dissolved in dry THF (4.0 mL) was added followed by TMSCl (1.30 mL, 10.0 mmol). The reaction mixture was allowed to warm to −50 °C and stirred at this temperature for 1 h. The reaction was quenched with saturated NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Purification of the crude product by flash chromatography (heptane/EtOAc 4:1, R_f = 0.20) gave **9b**, as a colorless oil (1.78 g, 86%): $[\alpha]^{20}_{589}$ = −40.70 (c = 1.0, $CHCl_3$); ^{1}H NMR δ 7.22 (d, 2H, J = 10 Hz), 6.85 (d, 2H, J = 9 Hz), 4.87 (br s, 1H), 4.84 (br s, 1H), 4.42 (s, 2H), 3.97 (m, 1H), 3.87 (dd, 1H, J = 10 & 4 Hz), 3.78 (s, 3H), 3.66 (dd, 1H, J = 10 & 2 Hz), 3.56 (t, 2H, J = 7 Hz), 2.90 (dd, 1H, J = 17 & 9 Hz), 2.80 (br d, 1H, J = 10 Hz), 2.38−2.30 (m, 3H), 1.50 (s, 9H), 0.87 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H); ^{13}C NMR δ 173.87, 158.97, 149.81, 147.50, 130.05, 129.15, 113.65, 110.46, 82.80, 72.65, 68.36, 63.88, 63.82, 55.24, 38.75, 37.93, 34.98, 28.12, 25.88, 18.23, −5.43, −5.44. Anal. ($C_{28}H_{45}NO_6Si$) C, H, N.

(4*R*,5*S*)-1-*tert*-Butoxycarbonyl-5-(*tert*-butyldimethylsilyloxymethyl)-4-(4-hydroxybut-1-en-2-yl)pyrrolidin-2-one (9c). To a solution of **9b** (1.78 g, 3.43 mmol) in dichloromethane (40 mL) and H_2O (2 mL) was added DDQ (1.56 g, 6.86 mmol). The reaction mixture was stirred at room temperature for 1 h and then quenched with saturated $NaHCO_3$. The aqueous phase was extracted with dichloromethane, and the collective organic layers were washed with brine, dried (Na_2SO_4), and concentrated. Purification of the crude product by flash chromatography (heptane/EtOAc 3:2, R_f = 0.25) gave **9c**, as a colorless oil (1.23 g, 90%): $[\alpha]^{20}_{589}$ = −50.37 (c = 1.0, $CHCl_3$); ^{1}H NMR δ 4.90 (br s, 1H), 4.86 (br s, 1H), 3.94 (m, 1H), 3.82 (dd, 1H, J = 10 & 5 Hz), 3.72 (t, 2H, J = 6 Hz), 3.68 (dd, 1H, J = 10 & 2 Hz), 2.88 (dd, 1H, J = 17 & 9 Hz), 2.79 (br d, 1H, J = 9 Hz), 2.36−2.24 (m, 3H), 1.48 (s, 9H), 0.83 (s, 9H), 0.01 (s, 3H), 0.0 (s, 3H); ^{13}C NMR δ 174.03, 150.09, 147.20, 111.41, 83.30, 64.14, 64.10, 60.84, 38.79, 38.07, 38.03, 28.44, 26.18, 18.55, −5.10. Anal. ($C_{20}H_{37}NO_5Si$) C, H, N.

(4*R*,5*S*)-1-*tert*-Butoxycarbonyl-5-(*tert*-butyldimethylsilyloxymethyl)-4-(4-(phenylsulfonyloxy)but-1-en-2-yl)pyrrolidin-2-one (9d). To a solution of **9c** (1.23 g, 3.09 mmol), *N*,*N*-(dimethylamino)-pyridine (38 mg, 0.31 mmol), and triethylamine (0.85 mL, 6.17 mmol) in dry dichloromethane (20 mL) at 0 °C was added dropwise phenylsulfonyl chloride (0.57 mL, 4.32 mmol). The ice bath was removed and the reaction mixture allowed to stir at room temperature for 4 h. Dichloromethane (100 mL) was added and the organic phase washed with aqueous HCl (25 mL, 1 M), saturated $NaHCO_3$ (25 mL), brine; dried (Na_2SO_4); and then concentrated. Purification of the crude product by flash chromatography (heptane/EtOAc 2:1,

R_f = 0.28) gave **9d**, as a colorless oil (1.54 g, 92%): $[\alpha]^{20}_{589}$ = −32.54 (*c* = 1.0, $CHCl_3$); ^{1}H NMR δ 7.87 (m, 2H), 7.64 (m, 1H), 7.54 (m, 2H), 4.86 (br s, 1H), 4.78 (br s, 1H), 4.16 (br t, 2H, *J* = 7 Hz), 3.90 (m, 1H), 3.84 (dd, 1H, *J* = 10 & 4 Hz), 3.68 (dd, 1H, *J* = 10 & 2 Hz), 2.87 (dd, 1H, *J* = 17 & 9 Hz), 2.72 (br d, 1H, *J* = 9 Hz), 2.39 (dt, 2H, *J* = 7 & 2 Hz), 2.23 (dd, 1H, *J* = 17 & 2 Hz), 1.51 (s, 9H), 0.87 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H); ^{13}C NMR δ 173.83, 150.17, 145.32, 136.22, 134.24, 129.65, 128.19, 112.30, 83.58, 68.73, 64.27, 64.12, 39.03, 38.09, 34.29, 28.58, 26.35, 18.71, −4.95. Anal. ($C_{26}H_{41}NO_7SSi$) C, H, N.

(1*R*,4*S*,5*R*)-*N*-*tert*-Butoxycarbonyl-3-aza-4-(*tert*-butyldimethylsilyloxymethyl)-6-methylidene-2-oxobicyclo[3.3.0]octane (10). To a solution of **9d** (632 mg, 1.17 mmol) in dry THF (23 mL) at −78 °C was added LHMDS (1.21 mL, 1.21 mmol, 1 M in hexanes). The reaction mixture was stirred for 30 min, warmed to −50 °C, and left overnight, reaching 10 °C as the end temperature. The reaction was quenched with saturated NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. Purification of the crude product by flash chromatography (heptane/EtOAc 9:1, R_f = 0.24) gave **10**, as a white solid (410 mg, 92%): mp = 65−66 °C; $[\alpha]^{20}_{589}$ = −164.75 (*c* = 1.0, $CHCl_3$); ^{1}H NMR δ 5.06 (d, 1H, *J* = 2 Hz), 5.01 (d, 1H, *J* = 2 Hz), 3.99 (m, 1H), 3.92 (dd, 1H, *J* = 10 & 4 Hz), 3.78 (dd, 1H, *J* = 10 & 2 Hz), 3.16 (br t, 1H, *J* = 8 Hz), 3.00 (br d, 1H, *J* = 8 Hz), 2.42−2.18 (m, 3H), 1.97−1.80 (m, 1H), 1.52 (s, 9H), 0.88 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H); ^{13}C NMR δ 176.65, 154.73, 149.68, 107.72, 82.78, 65.79, 64.28, 49.10, 43.52, 32.28, 28.94, 28.13, 25.88, 18.22, −5.39. Anal. ($C_{20}H_{35}NO_4Si$) C, H, N.

(1*R*,4*S*,5*R*,6*S*)-*N*-*tert*-Butoxycarbonyl-3-aza-4-(*tert*-butyldimethylsilyloxymethyl)-6-(hydroxymethyl)bicyclo[3.3.0]octane (11a) and (1*R*,4*S*,5*R*,6*R*)-*N*-*tert*-Butoxycarbonyl-3-aza-4-(*tert*-butyldimethylsilyloxymethyl)-6-(hydroxymethyl)bicyclo[3.3.0]octane (11b). To a solution of **10** (740 mg, 1.94 mmol) in dry THF (8 mL) was added $RhCl(PPh_3)_3$ (56 mg, 0.058 mmol) dissolved in dry THF (14 mL) and the reaction mixture stirred for 5 min. A solution of catecholborane (3.82 mL, 3.82 mmol, 1 M in THF) was added and the reaction mixture stirred for 1 h. A solution of borane (7.64 mL, 7.64 mmol, 1 M in THF) was the added and stirring continued for 20 h. The flask was then cooled to 0 °C and H_2O (1.0 mL) was added carefully followed by NaOH (15.7 mL, 2 N) and H_2O_2 (4.85 mL, 35 w/w%). The reaction mixture was then stirred at room temperature for 1 h and quenched with saturated $NaHCO_3$. The aqueous phase was extracted with EtOAc, and the collective organic layers were washed with brine, dried (Na_2SO_4), and concentrated. Purification of the crude product by flash chromatography (heptane/diethyl ether 2:3, R_f = 0.23) gave **11a/11b** (ratio 9:1), as a colorless oil (639 mg, 85%): $[\alpha]^{20}_{589}$ = −76.87 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (two diastereomers) δ 4.00−3.20 (m, 7H), 2.69 (br s, 2H), 2.30 (m, 1H), 1.92 (m, 1H), 1.64−1.40 (m, 2H), 1.46 (s, 9H), 1.20 (m, 1H), 1.93 (s, 9H), 0.13 (s, 6H); ^{13}C NMR (two diastereomers) δ 154.26, 79.84, 65.94, 65.31, 63.93, 57.46, 53.87, 53.23, 49.84, 48.94, 47.58, 43.10, 42.23, 32.40, 29.00, 26.50, 27.88, 19.02, −4.92. Anal. ($C_{20}H_{39}NO_4Si$) C, H, N.

(1*R*,4*S*,5*R*,6*S*)-*N*-*tert*-Butoxycarbonyl-3-aza-4,6-bis(hydroxymethyl)bicyclo[3.3.0]octane (12a) and (1*R*,4*S*,5*R*,6*R*)-*N*-*tert*-Butoxycarbonyl-3-aza-4,6-bis(hydroxymethyl)bicyclo[3.3.0]-octane (12b). To a solution of **11a/11b** (ratio 9:1) (642 mg, 1.66 mmol) in dry THF (14 mL) was added tetrabutylammonium fluoride (2.66 mL, 2.66 mmol, 1 M in THF). The reaction mixture was stirred for 30 min then quenched with half-saturated $NaHCO_3$. The aqueous phase was extracted with EtOAc, and the collective organic layers were washed with brine, dried (Na_2SO_4), and concentrated. Purification of the crude product by flash chromatography (EtOAc, R_f = 0.20) gave **12a/12b** (ratio 9:1), as a colorless oil (435 mg, 97%): $[\alpha]^{20}_{589}$ = −99.43 (*c* = 0.52, $CHCl_3$); ^{1}H NMR (two diastereomers) δ 3.90−3.30 (m, 7H), 2.60 (br s, 2H), 2.33 (m, 1H), 1.92 (m, 1H), 1.63 (m, 1H), 1.45 (s, 9H), 1.40−1.20 (m, 2H); ^{13}C NMR (two diastereomers) δ 156.05, 80.47, 65.76, 64.84, 63.64, 58.60, 57.48, 53.47, 52.95, 50.34, 49.60, 46.93, 42.78, 31.40, 28.97, 27.32. Anal. ($C_{14}H_{25}NO_4$) C, H, N: calcd, 61.97, 9.29, 5.16; found, 59.51, 9.29, 4.69.

(1*R*,4*S*,5*R*,6*S*)-*N*-*tert*-Butoxycarbonyl-3-azabicyclo[3.3.0]octane-4,6-dicarboxylic Acid (13a) and (1*R*,4*S*,5*R*,6*R*)-*N*-*tert*-Butoxycarbonyl-3-azabicyclo[3.3.0]octane-4,6-dicarboxylic Acid (13b). To **12a/12b** (ratio 9:1) (353 mg, 1.30 mmol) dissolved in MeCN (10.0 mL) and EtOAc (10.0 mL) was added a solution of $RuCl_3 \cdot H_2O$ (5.4 mg, 0.026 mmol) and $NaIO_4$ (2.28 g, 10.66 mmol) in H_2O (15.0 mL). The reaction mixture was stirred for 1 h and then filtered on filter paper, and the filter cake was washed with EtOAc. The aqueous phase was extracted with EtOAc, and the collective organic layers were washed with brine, dried (Na_2SO_4), and concentrated. Purification of the crude product by flash chromatography (dichloromethane:MeOH:AcOH 100:8:2, R_f = 0.23) gave **13a/13b** (ratio 9:1), as a white foam (300 mg, 77%): $[\alpha]^{24}_{589}$ = −63.67 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (major diastereomer **13a**, two rotamers) δ 10.40 (br s, 2H), 4.44 (br s, $^1/_2$H), 4.32 (br s, $^1/_2$H), 3.90−3.50 (br m, 2H), 3.20 (br s, 2H), 2.94 (br s, 1H), 2.10 (br s, 3H), 1.76 (br s, 1H), 1.61 (br s, 5H), 1.57 (br s, 4H); ^{13}C NMR (major diastereomer **13a**, two rotamers) δ 178.90, 177.72, 176.35, 155.56, 154.48, 81.64, 81.27, 61.63, 61.11, 53.63, 53.27, 52.03, 50.46, 48.85, 43.12, 42.54, 31.64, 31.38, 28.87, 28.70, 27.59, 27.19. Anal. ($C_{14}H_{21}NO_6$) C, H, N: calcd, 56.18, 7.07, 4.68; found, 54.66, 6.88, 4.32.

(1*R*,4*S*,5*R*,6*S*)-3-Azabicyclo[3.3.0]octane-4,6-dicarboxylic Acid (1a) and (1*R*,4*S*,5*R*,6*R*)-3-Azabicyclo[3.3.0]octane-4,6-dicarboxylic Acid (1b). To **13a/13b** (ratio 9:1) (300 mg, 1.0 mmol) in EtOAc (15 mL) at 0 °C was added HCl(g)/EtOAc (2 mL, 12 mmol, 6 M). The reaction mixture was stirred at room temperature for 1 h and then concentrated. The solid was triturated with freezing cold diethyl ether to give the HCl salt of **1a/1b** (ratio 92:8), as a white solid (202 mg, 83%). Recrystallization from glacial acetic acid gave **1a**, as white crystals (141 mg, 60%, diastereomeric ratio 98:2, ee > 99%): TLC (butanol/EtOAc/AcOH/H_2O 1:1:1:1) R_f = 0.57; mp = 186−188 °C; $[\alpha]^{25}_{589}$ = +14.40 (*c* = 0.25, H_2O); ^{1}H NMR (D_2O) δ 4.25 (d, 1H, *J* = 7 Hz), 3.64 (q, 1H, *J* = 11 & 7 Hz), 3.25 (q, 1H, *J* = 16 & 8 Hz), 3.14−3.00 (m, 3H), 2.05−1.75 (m, 3H), 1.72−1.60 (m, 1H); ^{13}C NMR (D_2O) δ 177.77, 171.99, 62.07, 51.23, 48.77, 47.73, 42.58, 29.49, 28.16. Anal. ($C_9H_{14}ClNO_4$) C, H, N: calcd, 45.87, 5.99, 5.94; found, 45.35, 5.68, 4.96. Characteristic ^{1}H NMR of **1b** (D_2O) δ 4.17 (d, 1H, *J* = 7 Hz).

Molecular Modeling Study of Glu Analogues. The modeling study was performed using the software package MOE (Molecular Operating Environment, v2004.03, Chemical Computing Group, 2004) using the build-in mmff94x force field and the GB/SA continuum solvent model. Each compound was submitted to a stochastic conformational search, and with respect to its global minimum returned (ΔG in kcal/mol), conformations above +7 kcal/mol were discarded. For all compounds, the γ-carboxylate group was protonated prior to execution of the conformational search, as this gave a larger and thus more reliable number of output conformations. Superimpositions of ligands were carried out using the built-in function in MOE, by fitting the ammonium group and the two carboxylate groups.

Determination of Diastereomeric Excess/Diastereomeric Ratio. Chiral HPLC was performed using a Sumichiral OA-5000 column (4.6 × 150 mm, Sumika Chemical Analysis Service). The column was eluted at 1.0 mL/min with an aqueous solution of ammonium acetate (10 mM) containing copper(II) acetate (0.1 mM), adjusted to pH 4.7, and 2-propanol (9:1 v/v). The column was connected to a TSP HPLC system consisting of a P2000 pump, an AS3000 autoinjector equipped with a column oven (60 °C), and an SM5000 PDA detector. For data handling, TSP PC1000 software was used. On the basis of peak areas at 240 nm, the diastereomeric excess/diastereomeric ratio were determined.

Pharmacological Characterization of 1a at iGluR. Rat brain membrane preparations used in the receptor binding experiments were prepared according to the method described by Ransom and Stec.[29] Affinity for AMPA,[30] KAIN,[31] and NMDA[32] receptor sites was determined using 5 nM [^{3}H]AMPA, 5 nM [^{3}H]KAIN, and 2 nM [^{3}H]CGP 39653 with some modifications previously described.[22]

Pharmacological Characterization of 1a and 2 at Human EAATs. The pharmacological properties of **1a** and **2** at human EAAT1, EAAT2, and EAAT3 were determined in the FLIPR membrane potential (FMP) assay. The construction of human embryonic kidney 293 (HEK293) cell lines stably expressing human EAAT1, EAAT2, and EAAT3 has been reported previously, and the pharmacological characterization was performed essentially as described here.[21] Briefly, cells were split into poly-D-lysine-coated black-walled clear-bottom 96-well plates in Dulbecco's modified Eagle medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), 10% dialyzed fetal bovine serum, and 1 mg/mL G-418. Then 16−24 h later the medium was aspirated, and the cells were washed with 100 μL of Krebs buffer (140 mM NaCl/4.7 mM KCl/2.5 mM $CaCl_2$/1.2 mM $MgCl_2$/11 mM HEPES/10 mM D-glucose, pH 7.4). Then 50 μL of Krebs buffer was added to each well (in the characterization of nonsubstrate inhibitors, the inhibitors were added to this buffer). Krebs buffer (50 μL) supplemented with FMP assay dye was then added to each well, and the plate was incubated at 37 °C for 30 min. The plate was assayed at 30 °C in a NOVOstar plate reader measuring emission at 560 nm caused by excitation at 530 nm before and up to 1 min after addition of 25 μL of substrate solution. The experiments were performed in triplicate at least three times for each compound. For the characterization of nonsubstrate inhibitors, 30 μM Glu was used as substrate concentration. IC_{50} values were converted to K_i values by the use of the Cheng−Prusoff equation.[33]

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

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