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Rational Design, Synthesis, and Pharmacological Evaluation of 2-Azanorbornane-3-*exo*,5-*endo*-dicarboxylic Acid: A Novel Conformationally Restricted Glutamic Acid Analogue

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Received October 2, 2002

The design and synthesis of conformationally restricted analogues of α-amino acids is an often used strategy in medicinal chemistry research. Here we present the rational design, synthesis, and pharmacological evaluation of 2-azanorbornane-3-*exo*,5-*endo*-dicarboxylic acid (**1**), a novel conformationally restricted (*S*)-glutamic acid (Glu) analogue intended as a mimic of the *folded Glu conformation*. The synthesis of **1** was completed in its racemic form in eight steps from commercially available starting materials. As a key step, the first facially selective hydroboration of a 5-methylidene[2.2.1]bicyclic intermediate was investigated. In this transformation, the catalytic methodology of Wilkinson's/catechol borane proved superior to stoichiometric borane or dialkyl borane reagents, in terms of higher diastereomeric excess and chemical yield. To our surprise (±)-**1** did not show affinity in binding studies on native 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)-propionic acid (AMPA) (IC_{50} > 300 μM, [^{3}H]AMPA) or kainic acid (IC_{50} > 160 μM, [^{3}H]kainic acid) receptors nor in binding studies on the cloned iGluR5,6 subtypes (IC_{50} > 300 μM, [^{3}H]kainic acid).

Introduction

(*S*)-Glutamic acid (Glu) is the major excitatory neurotransmitter in the central nervous system (CNS).[1] The Glu receptors are divided into two major classes: the ionotropic (iGlu) and the metabotropic (mGlu) receptors. While iGlu receptors are ion channels and thus mediate a fast response (Na^+, K^+, Ca^{2+} flux), mGlu receptors are classified as G-protein coupled receptors and produce a slower signal transduction through second messenger systems. The iGlu receptors are further divided into subtypes based on pharmacological studies: *N*-methyl-D-aspartic acid (NMDA) receptors (heteromeric receptors comprising the subunits NR1,2A−D,3A−B); 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptors (homo- or heteromeric receptors comprising the subunits iGluR1−4); and kainic acid (KA) receptors (homo- or heteromeric receptors comprising the subunits iGluR5−7 and KA1,2). The mGlu receptors are divided into 8 homodimeric subtypes, mGluR1−8, which are grouped (groups I−III) according to the second messenger system involved, pharmacology, and molecular biology (group I, mGluR1,5; group II, mGluR2,3; group III, mGluR4,6−8).

As a part of our medicinal chemistry research program, we are interested in synthesizing novel rigid carbo- or heterocyclic skeletons which mimic the biologically active conformations of Glu in the CNS. To date, several conformationally restricted Glu analogues have been designed and synthesized. These range from simple 3- to 6-membered carbocyclic analogues, substituted pyrrolidines, and piperidines to highly rigid analogues based on the bicyclo[1.1.1]pentane,[2] spiro[2.2]pentane,[3] bicyclo-[2.1.1]hexane,[4] bicyclo[2.2.1]heptane,[5] and 7-azanorbonane[6] skeletons. On the basis of recent X-ray diffraction studies of the extracellular domain of the iGluR2 receptor subunit in complex with Glu[7] and subsequent SAR studies,[8] it is well-established that Glu binds in a folded

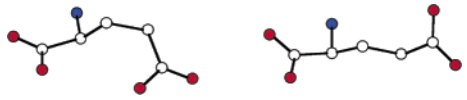

FIGURE 1. Folded and extended binding conformations of (*S*)-glutamic acid.

(1) For a recent review on the glutamate area, see: Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, E.; Madsen, U.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2000**, *43*, 2609−2645 and references therein.

(2) Pellicciari, R.; Raimondo, M.; Marinozzi, M.; Natalini, B.; Costantino, G.; Thomsen, C. *J. Med. Chem.* **1996**, *39*, 2874−2876.

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(4) Kozikowski, A. P.; Steensma, D.; Araldi, G. L.; Tückmantel, W.; Wang, S. M.; Pshenichkin, S.; Surina, E.; Wroblewski, J. T. *J. Med. Chem.* **1998**, *41*, 1641−1650.

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(6) Hart, B. P.; Rapoport, H. *J. Org. Chem.* **1999**, *64*, 2050−2056.

(7) (a) Armstrong, N.; Gouaux, E. *Neuron* **2000**, *28*, 165−181. Brookhaven PDB-file name for (*S*)-Glu crystallized with iGluR2: 1FTJ. (b) Brookhaven PDB-file name for kainate crystallized with iGluR2: 1FTO.

(8) Nielsen, P.; Liljefors, T. *J. Comput. Aided. Mol. Des.* **2001**, *15*, 753−763.

10.1021/jo026509p CCC: $25.00
Published on Web 01/16/2003

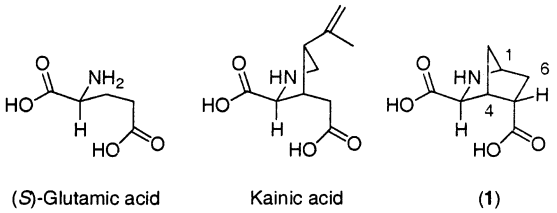

FIGURE 2. Structures of (*S*)-glutamic acid (Glu), kainic acid (KA), and novel conformationally restricted Glu analogue **1**.

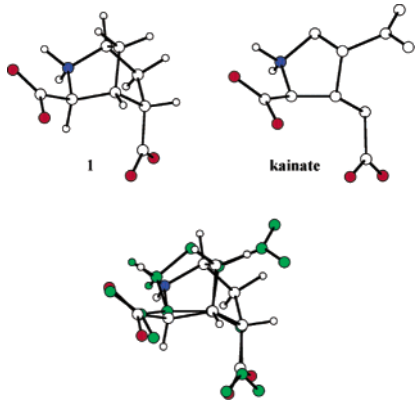

FIGURE 3. Molecular superposition of energy minimized **1** (type code) and the experimentally observed conformation of kainate (green) in complex with iGluR2.

conformation (Figure 1) at iGlu receptors (subunits iGluR1−7 and KA1,2), while it adopts an extended conformation[9] at mGlu receptors (subunits mGluR1−8).

Inspired by the agonist binding conformation of kainate in complex with iGluR2,[7b] the novel highly rigid Glu analogue 2-azanorbornane-3-*exo*,5-*endo*-dicarboxylic acid (**1**) was designed as a potential mimic of the folded Glu conformation (Figure 2).

A study of the molecular superimposition of energy minimized[10] **1** with kainate (Figure 3) shows good overlap of the amino acid moieties and the distal carboxylate groups. A docking study[11] of **1** into the binding pocket of the complex between kainate and the extracellular binding domain construct iGluR2[7] (Figure 4) unequivocally identified **1** as adopting a kainate-like binding mode as the highest scoring (lowest energy) docking pose. This pose is depicted in Figure 4.

The major receptor−ligand interactions required for Glu agonism are fulfilled: the 2-ammonium group can interact with Pro478 (exo NH) and Glu705 (endo NH), the 3-exo-carboxylate group is well-positioned for hydrogen bonding to Arg485, and the orientation of the 5-endo-carboxylate group allows for hydrogen bonding to Thr655 and a receptor H_2O. However, on the basis of our modeling and docking studies we are not able to conclude whether the 6-methylene group is in critical steric conflict

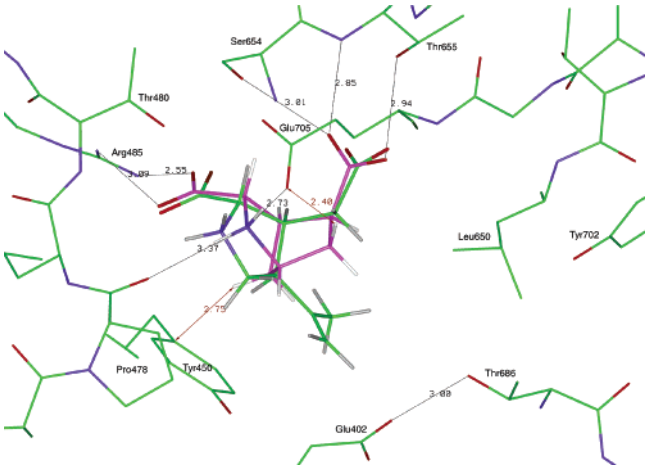

FIGURE 4. Docking of **1** (magenta) and kainate (green) into iGluR2.

with parts of the iGlu receptors (subunits iGluR1−4, iGluR5−7, and KA1,2). In this regard we underline the fact that while superimposition and docking studies can indicate whether a designed structure is consistent with a known pharmacophore or receptor, these techniques are as yet poor predictors of binding affinity. The fact that **1** is highly rigid does not cause concern as other highly rigid Glu agonists have been designed with success.[12]

Chemistry

The 2-azanorbornane skeleton has previously been synthesized via a hetero-Diels−Alder reaction,[13] or by intramolecular strategies: an alkylation reaction,[14] an acylation reaction,[15] electrophilic addition to an in situ formed iminium ion,[16] nucleophilic addition to an in situ formed bromonium ion,[17] or a carbolithiation[18] reaction. In general, the applicability of a given reported synthetic strategy for constructing the 2-azanorbornane skeleton is dependent on the substitution pattern of the target molecule. As the target compound **1** contains a 3-*exo*,5-*endo*-dicarboxy substitution pattern, our retro synthetic analysis suggests the previously described reaction of imine **2** with cyclopentadiene to give Diels−Alder adduct **3** (Scheme 1).[13] This reaction provides the 2-azanorbornane skeleton and will at the same time establish the desired 3-*exo* stereochemistry. The remaining synthetic challenge is then the regio- and stereospecific introduc-

(9) Kunishima, N.; Shimada, Y.; Tsuji, Y.; Sato, T.; Yamamoto, M.; Kumasaka, T.; Nakanishi, S.; Jingami, H.; Morikawa, K. *Nature* **2000**, *407*, 971−977. Brookhaven PDB-file name for (*S*)-Glu crystallized with mGluR1: 1EWK.

(10) Energy minimization of **1** was carried out with MMFF94 for aqueous solution, using the Macromodel program (Schrödinger Inc.).

(11) The commercial docking code Glide 1.8 (Glide 1.8, 2001; Schrödinger, Inc.: 1500 S. W. First Avenue, Suite 1180, Portland, OR 97201) was used with the recommended protein preparation steps and default settings.

(12) Conti, P.; De Amici, M.; De Sarro, G.; Rizzo, M.; Stensbøl, T. B.; Bräuner-Osborne, H.; Madsen, U.; Toma, L.; De Micheli, C. *J. Med. Chem.* **1999**, *42*, 4099−4107.

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(14) (a) Husbands, S. M.; Kline, R. H.; Allen, A. C.; Newman, A. H. *J. Org. Chem.* **1998**, *63*, 418−419. (b) Campell, J. A.; Lee, W. K.; Rapoport, H. *J. Org. Chem.* **1995**, *60*, 4602−4616.

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(16) Klaver, W. J.; Moolenaar, M. J.; Heimstra, H.; Speckamp, W. N. *Tetrahedron* **1988**, *44*, 3805−3818.

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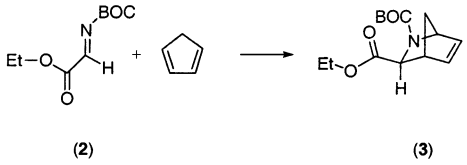
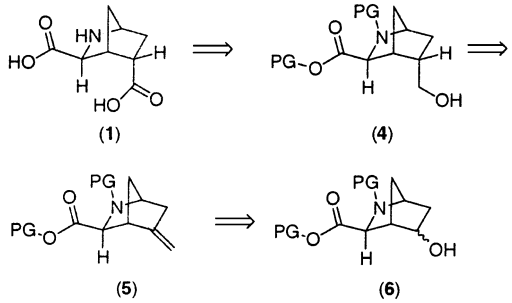
(18) Coldham, I.; Fernandez, J.-C.; Price, K. N.; Snowden, D. J. *J. Org. Chem.* **2000**, *65*, 3788−3795.

SCHEME 1



(2) (3)

SCHEME 2



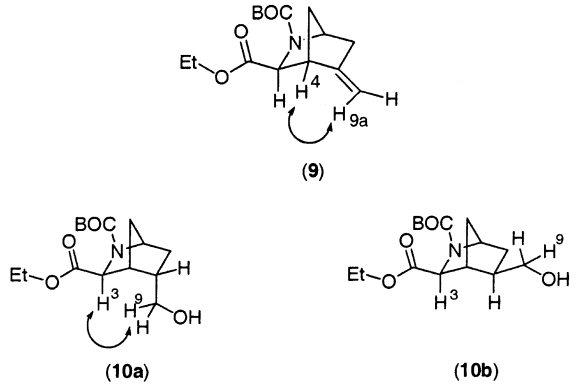
(1) (4) (5) (6)

tion of the 5-*endo*-carboxylic acid group. We envisage solving this problem via a facially selective hydroboration reaction of the 5-methylidene derivative **5** to give alcohol **4** (Scheme 2). To our knowledge, the facially selective reaction of a methylidene-substituted [2.2.1]-bicyclic system has not yet been described. However, based on steric considerations, 5-*exo* hydrogen installation is predicted to be preferred. The synthesis of compound **5** is carried out from alcohol **6**, which is obtained from the hydroboration of Diels−Alder adduct **3**. Although the synthetic strategy presented here only provides a racemic approach to **1**, we find it attractive because it offers an expedient proof of concept with respect to our medicinal chemistry hypothesis.

Results and Discussion

The formation of imine **2** (Scheme 1) from the reaction of ethyl glyoxalate and aza-Wittig reagent *N*-BOC-triphenyliminophosphorane,[19] as well as the subsequent hetero-Diels−Alder reaction with cyclopentadiene to give **3**, were reportedly carried out at 80 °C.[13] However, the reported yield of 55% could not be reproduced. Further experimentation established that both the formation of imine **2**[20] and the pericyclic 4+2 reaction to give **3** proceeded at room temperature. In fact, heating imine **2** prior to reaction resulted in a lower yield of the Diels−Alder product. Because of the rapid polymerization of ethyl glyoxalate, the isolated yield of the Diels−Alder adduct was inconsistent, ranging from 40 to 60%, even when the reaction was carried out at room temperature.

Hydroboration of **3** under standard conditions with BH_3 in THF followed by oxidative workup with H_2O_2/NaOH gave 85% of an approximately 1:1 mixture of the *exo*-alcohols **7a** and **7b** (Scheme 3) which were separated by flash chromatography.[21] By comparison, oxymercuration[22] of **3** gave a 1:3 mixture of **7a** and **7b** in only 50% combined yield. ^{1}H NMR spectroscopy[22] enabled structural assignment of the two regioisomeric alcohols. Oxidation of the 5-OH regioisomer **7a** with Dess−Martin periodinane gave the desired ketone **8** in 91% yield. Conversion of the keto group of **8** into a methylidene group (compound **9**, Scheme 3) was first attempted with use of the standard Wittig reagent. However, only 30% of the desired product was obtained, together with starting material.[23,24] The incomplete reaction is believed to be caused by steric hindrance around the 5-oxo group, leading to competing deprotonation. Alternatively, treatment of **8** with 1.0 equiv of Tebbe's reagent[25] gave **9** as the only product in 71% yield.[26] We also decided to investigate the highly electrophilic and much cheaper reagent CH_2Br_2/Zn/$TiCl_4$. The reagent may be used immediately after mixing (Oshima's protocol)[27] or aged at low temperature (5 °C) for 3 days (Lombardo's reagent).[28] Applying Oshima's protocol at room temperature, **9** was obtained as the major product, but the efficiency of the reaction was low due to formation of byproducts. The use of Lombardo's reagent at 0 °C gave a very slow reaction (5 equiv, 2 h, 25%), but when the reaction was run at room temperature, 60% conversion was seen on ^{1}H NMR after only 5 min. The 5-methylidene derivative **9** could be isolated in 48% yield and ketone **8** was recovered in 35% yield. However, there were persistent problems with inconsistencies in conversion rate and isolated yield of **9,** as well as formation of byproducts.[29] A NOESY NMR experiment of **9** showed NOE effect between H^4 (δ 3.07) and H^{9a} (δ 5.12) (Figure 5), thus



(9) (10a) (10b)

FIGURE 5. NOESY NMR experiments confirming NOE effects in compounds **9** and **10a**.

(19) Calí, P.; Begtrup, M. *Synthesis* **2002**, 63−66.

(20) For examples of aza-Wittig reactions with aldehydes at room temperature, see: Palacios, F.; Alonso, C.; Rubiales, G. *J. Org. Chem.* **1997**, *62*, 1146−1154.

(21) Malpass, J. R.; Cox, C. D. *Tetrahedron Lett.* **1999**, *40*, 1419−1422.

(22) The structural assignment of the 6-OH regioisomer **7b** was done on the basis of an observed coupling (J = 5 Hz) between the H^4 and the H^{5-exo}. For the 5-OH regioisomer **7a**, no coupling from the H^4 was observed. This is in agreement with another report: Cox, C. D.; Malpass, J. R.; Gordon, J.; Rosen, A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2372−2379.

(23) Paquette, L. A.; Brand, S.; Behrens, C. *J. Org. Chem.* **1999**, *64*, 2010−2025.

(24) The yield was not improved when the reaction was carried out with low-temperature addition (−78 °C).

(25) Tebbe, F. N.; Parshall, G. W.; Reddy, G. S. *J. Am. Chem. Soc.* **1978**, *100*, 3611.

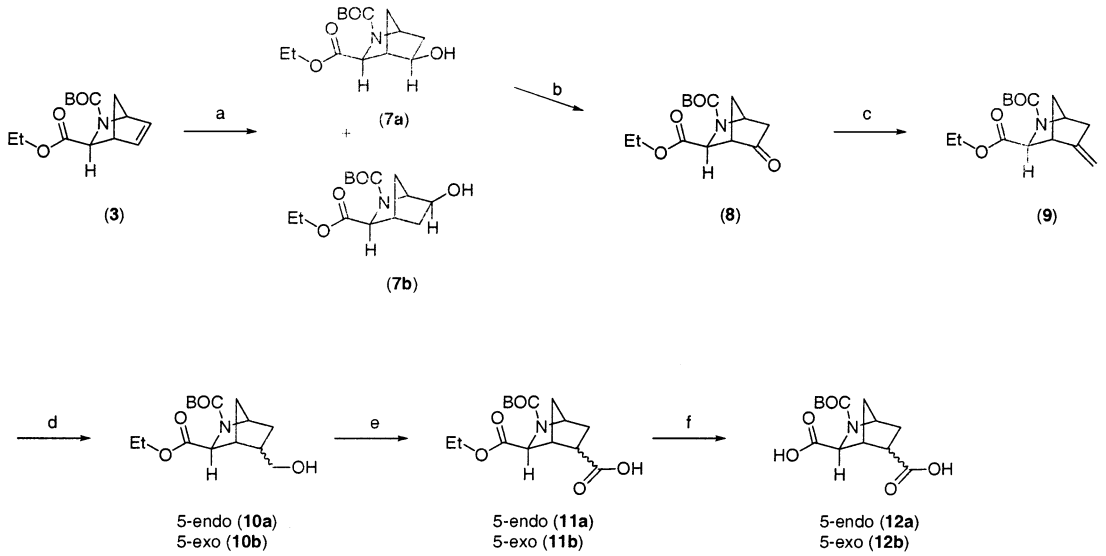
(26) For Tebbe's reagent and ketone/ester chemoselectivity, see: (a) Pine, S. H.; Pettit, R. J.; Geib, G. D.; Cruz, S. G.; Gallego, C. H.; Tijerina, T.; Pine, R. D. *J. Org. Chem.* **1985**, *50*, 1212−1216. (b) Magnus, P.; Mugrage, B.; Deluca, M. R.; Cain, G. A. *J. Am. Chem. Soc.* **1990**, *112*, 5220−5230.

(27) Cossy, J.; Cases, M.; Pardo, D. G. *Tetrahedron* **1999**, *55*, 6153−6166.

(28) Lombardo, L. *Tetrahedron Lett.* **1982**, *23*, 4293−4296.

(29) The quality of the zinc dust used had an impact on the reaction outcome. It is recommended to use only fresh zinc dust.

SCHEME 3[a]



[a] Reagents and conditions: (a) BH_3/THF, −78 °C to room temperature, 1 h, then H_2O_2/NaOH (85%); (b) **7a** only: Dess−Martin, rt, 8 h (91%); (c) 1.0 equiv of Tebbe's reagent, −40 °C to room temperature (71%); (d) $Rh(Cl)(PPh_3)_3$/CatBH, rt, 2 h, then H_2O_2/$NaHCO_3$ (83%); (e) $RuCl_3$/$NaIO_4$, rt, 2 h (70%); (f) LiOH, THF/H_2O, rt (10−15%).

TABLE 1. Hydroboration of 9 To Give Alcohols 10a:10b

reagent[a]	temp (°C)	time (h)	**10a:10b** (%)	combined yield (%)
BH_3	0	1	83:17	n.d.
BH_3	−40	2	90:10	20[b]
BH_3	−78 to 0	3	89:11	50
cHex_2BH	0 to 25	72	50:50	n.d.
9-BBN	25	2		n.r.
9-BBN	65	1	77:23	n.d.
$Rh(Cl)(PPh_3)_3$/CatBH	25	2	95:5	83
$Rh(Cl)(PPh_3)_3$/CatBH	−20	20	95:5	20[b]
$Rh(Cl)(PPh_3)_3$/CatBH	−78	3		n.r.

[a] All reactions were run in dry THF under N_2. [b] Yield based on total ^1H NMR integration of products and starting material. n.d. = not determined; n.r. = no reaction.

confirming the structure of the desired 5-methylidene regioisomer.

Facially selective hydroboration of **9** to give the 5-*endo* methylene alcohol **10a** (Scheme 3) proved to be a major challenge. At first, stoichiometric reactions with borane or dialkyl boranes were investigated (Table 1). Because of the low steric hindrance of **9** and thus the anticipated rapidity of reaction, we expected borane to be less diastereoselective compared with more sterically hindered boranes (dicyclohexylborane (*c*Hex_2BH)[30] and 9-BBN). However, it turned out that borane gave the highest degree of diastereoselectivity, namely 89% of the desired 5-*endo* product **10a** together with 11% of the 5-*exo* product **10b**, in moderate combined yield. An explanation for this unexpected difference in diastereoselectivity may be found in the reaction temperature used. For the dialkyl boranes, the reaction did not proceed at room temperature (Table 1) whereas borane could be employed at low temperature. We next turned to investigate the well-described catalytic hydroboration methodology using $Rh(Cl)(PPh_3)_3$/catecholborane (CatBH).[31,32] An improvement in selectivity, giving 95% of the 5-*endo* product **10a**, was observed when the reaction was run at room temperature. In an attempt to achieve a higher degree of diastereoselectivity, the reaction was run at −25 or −78 °C, giving no improvement in diastereoselectivity and no reaction, respectively. All attempts to separate the diastereomers with flash chromatography failed.[33] The relative stereochemistry (*endo*) at C5 of the major diastereomer formed, **10a**, was confirmed by the identification of a NOE effect between H^3 (δ 3.89, 3.85) and H^9 (δ 3.50−3.34) in a NOESY experiment (Figure 5).

Oxidation of the diastereomeric alcohols **10a**,**b** to the corresponding carboxylic acids **11a**,**b** (Scheme 3) was carried out with use of the improved Sharpless procedure ($RuCl_3$/$NaIO_4$).[34] However, hydrolysis of the ethyl ester functionality of **11a**,**b** with LiOH/THF to give 3,5-diacid **12a**,**b** was accompanied by the formation of significant amounts of retro 1,4-addition products and further hydrolysis thereof. To overcome this problem, the synthetic plan was revised according to Scheme 4. Thus, hydrolysis of the ethyl ester of **10a**,**b** was complete after 24 h, giving **13a**,**b** in 94% yield. The subsequent oxidation of the alcohol moiety with use of the improved Sharpless procedure converted **13a**,**b** smoothly into the 3,5-diacid **12a**,**b** (5-*endo*:5-*exo* 95:5, in agreement with Table 1). However, isolation of compound **12a**,**b** was complicated by the tendency of the 5-*endo* diastereomer **12a** (but not **12b**) to form highly insoluble crystals. After some experimentation this observed difference in the physicochemical property of the two diastereomers **12a** and **12b** was used to our benefit, as it provided an easy pathway

(30) Brown, H. C.; Kanth, J. V. B.; Zaidlewicz, M. *Tetrahedron* **1999**, *55*, 5991−6000.

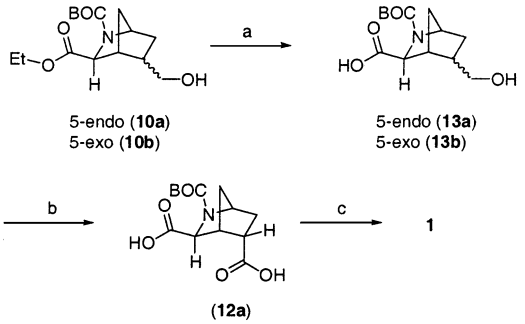
(31) Burgess, K.; Donk, A. van der; Westcott, S. A.; Marder, T. B.; Baker, R. T.; Calabrese, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 9350−9359.

(32) For a recent review on transition metal catalyzed hydroboration, see: Beletskaya, I.; Pelter, A. *Tetrahedron* **1997**, *53*, 4957−5026.

(33) Various solvent mixtures were tried: EtOAc/heptanes, Et_2O/heptanes, Et_2O/EtOH/heptanes, CH_2Cl_2/MeOH, EtOH/toluene, dioxane/heptanes.

(34) Prashad, M.; Lu, Y.; Kim, H.-Y.; Hu, B.; Repic, O.; Blacklock, T. J. *Synth. Commun.* **1999**, *29*, 2937−2942.

SCHEME 4[a]



[a] Reagents and conditions: (a) LiOH, THF/H_2O, rt, 24 h (94%); (b) $RuCl_3/NaIO_4$, rt, 2 h (76%); (c) TFA, rt, 30 min (96%).

to diastereopure **12a**. An X-ray diffraction study of recrystallized **12a** (see Figure S1, Supporting Information) confirmed intermolecular hydrogen bonds between two 3-*exo* carboxylic acid groups, and also between the 5-*endo* carboxylic acid and the *N*-BOC carbonyl group. Finally, the BOC group of 3,5-diacid **12a** was removed by treatment with TFA at room temperature, to give (±)-**1** in 96% yield.

Biological evaluation of re-crystallized (±)-**1** was first carried out in binding studies on native ionotropic Glu receptors. Surprisingly, **1** did not show affinity for AMPA (IC_{50} > 300 μM, [^{3}H]AMPA)[35] or KA (IC_{50} > 160 μM, [^{3}H]-KA)[36] receptors. In light of these results, additional binding studies on cloned iGluR5 and iGluR6 receptor subtypes[37] were carried out but did not reveal any affinity of **1** (IC_{50} > 300 μM, [^{3}H]KA). As expected, **1** showed no affinity for NMDA preferring receptors (IC_{50} > 300 μM, [^{3}H]CGP39653)[38] and was found to exhibit neither agonist nor antagonist behavior in functional assays at cloned metabotropic receptors mGluR1,2,4,[39] representing respectively groups I−III mGlu receptors (EC_{50} > 1000 μM). In light of the biological data presented here, it can be concluded that our rationally designed conformationally restricted Glu analogue **1** is not a ligand at AMPA or KA iGlu receptors. Since **1** was tested as the racemate it can also be concluded that its accompanying enantiomer does not exhibit any biological activity. Thus we do not plan to pursue an enantioselective approach toward the synthesis of **1**.

The question remains as to why **1** fails to meet the requirements for recognition by iGlu receptors, despite presenting the charged pharmacophore elements in a geometry very close to that of kainate and occupying a similar molecular volume apart from one proton of the 6-methylene group (Figure 6). While such comparisons can assist when deciding where to direct synthetic effort, by ruling out structures that will most likely lack activity, one should not lose sight of the fact that any of a number

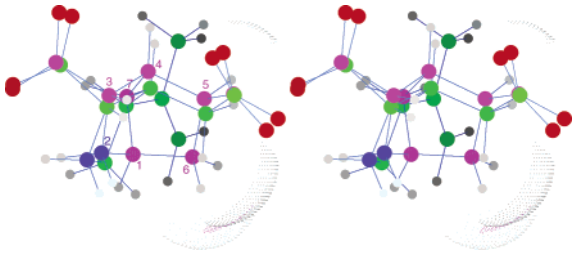


FIGURE 6. Stereoview of the molecular volume of kainate (green) subtracted from the molecular volume of **1** (type code). The 6-hydrogen of **1** protrudes outside the volume occupied by kainate.

of factors can render a target compound inactive. For example, van der Waals interactions are acutely sensitive to close interatomic approach. In the case of **1** the distance between one of the 6-methylene protons and Glu705 is short (marked 2.40 in Figure 4); due to the rigidity of **1**, the ammonium group is attracted to the carboxylate of Glu705 while the proton repels this side chain. This weakens the ion-pair and forces the ligand to adopt a position closer to Tyr450. Here we find another repulsive interaction (marked 2.75 in Figure 4) between the 7-methylene and the face of the phenyl ring in Tyr450. It is worth noting that kainate itself is only a weak partial agonist at iGluR2 inducing only partial domain closure (Glu402-Thr686),[7] and that in the fully agonized state of iGluRs the gap between these two amino acid residues is further decreased. In addition, kainate gains affinity via the exclusion of loosely bound receptor water molecules by its isopropenyl side chain, in hydrophobic contact with Leu650 (valine in iGluR5−7); the C4-unsubstituted analogue 2-carboxy-3-pyrrolidineacetic acid is 160 times less potent than KA,[40] and since **1** partially disrupts the receptor water architecture (cf. Glu) but does not possess a suitably located hydrophobic group, this may contribute to the lack of receptor affinity. As always, this underlines the principle that while conformational constraint may dramatically increase affinity by reducing the conformational entropy and energy penalty, the lines are more sharply drawn than for a flexible ligand—even a slight deviation in atomic position and orientation can abolish all activity for constrained structures.

Conclusion

In conclusion, we have carried out the rational design of **1** on the basis of the known biologically active conformation of kainate in iGluR2. The designed target compound **1** is the first conformationally restricted Glu analogue that contains a 2-azanorbornane skeleton. The synthesis of racemic **1** was carried out in eight steps from commercially available starting materials. As a key step in the synthesis, we have accomplished the first facially selective hydroboration of a methylidene-substituted [2.2.1]-bicyclic skeleton. Surprisingly, pharmacological evaluation of **1** revealed no affinity for the target iGlu receptor subtypes (iGluR1−4, iGluR5−7, and KA1,2), despite the fact that **1** presents the charged pharma-

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cophore elements in a geometry very close to that of kainate and occupies a similar molecular volume apart from one proton of the 6-methylene group (Figure 6).

Experimental Section

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 $CDCl_3$ with $CHCl_3$ as reference, unless otherwise noted. Merck silica gel (35−70 mesh) was used for flash chromatography.

Ethyl (±)-*N*-*tert*-Butyloxycarbonyl-2-azanorborn-5-ene-3-*exo*-carboxylate (3). Commercially available ethylglyoxalate (50% in toluene) was pre-distilled at 110−130 °C.[41] Dicyclopentadiene was cracked at 175 °C, and the cyclopentadiene monomer distilled off at 40−42 °C. To *N*-BOC-triphenyliminophosphorane (11.3 g, 30 mmol) in toluene (100 mL) at room temperature was added freshly cracked cyclopentadiene (5.0 mL, 60 mmol) followed by pre-distilled ethyl glyoxalate (60 mmol). The reaction mixture was allowed to stir overnight at room temperature, then evaporated. Et_2O was added and the precipitate filtered off. The crude product was purified by flash chromatography (heptane/EtOAc 4:1, R_f 0.26) to give **3**, as a clear oil (3.2−4.8 g, 40−60%). ^{1}H NMR (two rotamers) δ 6.43 (br s, $^2/_3$H), 6.33 (br s, $^4/_3$H), 4.73 (br s, 0.4H), 4.60 (br s, 0.6H), 4.14 (q, J = 7 Hz, 2H), 3.42 (br s, 0.4H), 3.34 (br s, 0.6H), 3.21 (br s, 1H), 1.91 (br d, J = 9 Hz, 1H), 1.4 (br s, ca. 5H), 1.31 (s, ca. 5H), 1.22 (t, J = 7 Hz, 3H). ^{13}C NMR (two rotamers) δ 171.60, 137.25, 136.90, 136.30, 80.00, 62.05, 61.20, 60.50, 59.18, 49.17, 48.42, 45.65, 45.40, 28.58, 14.50. Anal. Calcd for $C_{14}H_{21}NO_4$: C, 62.90; H, 7.92; N, 5.24. Found: C, 62.83; H, 7.69; N, 5.14.

Ethyl (±)-*N*-*tert*-Butyloxycarbonyl-5-*exo*-hydroxy-2-azanorbornane-3-*exo*-carboxylate (7a) and Ethyl (±)-*N*-*tert*-Butyloxycarbonyl-6-*exo*-hydroxy-2-azanorbornane-3-*exo*-carboxylate (7b). To a solution of **3** (3.0 g, 11.22 mmol) in THF (45 mL) at −78 °C was added BH_3·THF (11.22 mL, 11.22 mmol) and the reaction mixture was stirred for 15 min at −78 °C. The cooling bath was removed and the reaction mixture allowed to warm to room temperature and stirred for an additional 1 h. The flask was cooled to 0 °C and 2 N NaOH (19.6 mL, 39.27 mmol) and H_2O_2 (5.60 mL, 56.1 mmol) were added sequentially. The mixture was stirred for 30 min at room temperature then quenched with saturated $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was purified by flash chromatography (heptane/EtOAc 1:1, R_f 0.25) to give the regioisomeric alcohols **7a** and **7b**, as a clear oil (2.85 g, 85%). Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.48; H, 7.94; N, 5.18. The mixture of **7a** and **7b** was dissolved in Et_2O and evaporated with silica gel until dryness. The material was then loaded onto on a flash column and run with Et_2O:EtOH:heptane (0.5:1:8.5) as the eluent. The 6-OH regioisomer **7b** was eluted first, then closely followed by the desired 5-OH regioisomer **7a** in average 48% yield from **3**. Compound **7a** (semicrystalline): ^{1}H NMR (two rotamers) δ 4.27 (br s, $^1/_2$H), 4.20−4.09 (m, 2$^1/_2$H), 4.06 (br s, $^1/_2$H), 4.03 (br s, $^1/_2$H), 3.63 (s, $^1/_2$H), 3.54 (s, $^1/_2$H), 2.85 (br s, 1H), 2.55 (s, 1H, H^4), 2.10 (ddd, J = 13, 7, 2 Hz, $^1/_2$H), 2.01 (ddd, J = 13, 7, 2 Hz, $^1/_2$H),1.81 (br d, J = 10 Hz, 1H), 1.67 (br d, J = 10 Hz, 1H), 1.47−1.39 (m, 1H), 1.41 (s, ca. 4H), 1.34 (s, ca. 5H), 1.24 (br t, J = 7 Hz, 1$^1/_2$H), 1.22 (br t, J = 7 Hz, 1$^1/_2$H). ^{13}C NMR (two rotamers) δ 171.00, 170.84, 154.35, 153.35, 80.05, 72.71, 72.50, 61.00, 61.10, 59.94, 59.64, 56.63, 55.38, 42.53, 42.60, 31.58, 30.97, 28.27, 28.12. Anal. Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.29; H, 8.38; N, 5.03. Compound **7b** (semicrystalline): ^{1}H NMR (two rotamers) δ 4.18−4.08 (m, 2H), 3.97 (m, 1H), 3.90 (br s, 1H), 3.60 (s, $^1/_3$H), 3.50 (s, $^2/_3$H), 3.28 (br s, $^2/_3$H), 2.66 (br s, $^1/_3$H), 2.60 (br dd, J = 6, 4 Hz), 1.85 (ddd, J = 14, 6, 3 Hz), 1.77 (br d, J = 10 Hz, 1H), 1.63 (br dd, J = 10, 3 Hz, 1H), 1.52−1.42 (m, 1H), 1.41 (s, 3H), 1.32 (s, 6H), 1.22 (t, J = 7 Hz, ca. 2H), 1.18 (t, J = 7 Hz, ca. 1H). ^{13}C NMR (two rotamers) δ 171.13, 171.00, 154.14, 153.60, 80.27, 80.07, 71.78, 71.21, 63.06, 62.49, 61.27, 60.94, 60.85, 60.44, 40.97, 40.34, 39.84, 39.25, 30.98, 30.24, 28.27, 28.10, 14.11, 13.95.

Ethyl (±)-*N*-*tert*-Butyloxycarbonyl-5-oxo-2-azanorbornane-3-*exo*-carboxylate (8). To a solution of **7a** (1.86 g, 6.52 mmol) in dry CH_2Cl_2 (65 mL) at room temperature was added Dess−Martin periodinane (3.98 g, 9.78 mmol) and the reaction was stirred at room temperature for 8 h. The reaction was quenched by addition of 10% $Na_2S_2O_3$ (65 mL) and stirred until the biphasic system turned clear. Then saturated $NaHCO_3$ (65 mL) was added and the aqueous layer extracted with CH_2Cl_2. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was purified by flash chromatography (heptane/EtOAc 2:1, R_f 0.24) to give **8**, as a clear oil (1.67 g, 91%). ^{1}H NMR (two rotamers) δ 4.67 (br s, $^2/_3$H), 4.55 (br s, $^1/_3$H), 4.22−4.02 (m, 2H), 4.08 (br s, $^1/_3$H), 3.99 (br s, $^2/_3$H), 2.96 (br s, 1H, H^4), 2.42−2.10 (m, 3H), 1.79 (br d, J = 10 Hz, 1H), 1.42 (s, 3H), 1.35 (s, 6H), 1.28−1.17 (m, 3H). ^{13}C NMR (two rotamers) δ 169.38, 169.25 153.72, 152.98, 80.90, 61.76, 58.44, 58.20, 56.82, 55.79, 54.96, 54.14, 45.85, 45.78, 35.23, 34.66, 28.63, 28.45, 14.51, 14.37. Anal. Calcd for $C_{14}H_{21}NO_5$: C, 59.35; H, 7.47; N, 4.94. Found: C, 59.06; H, 7.54; N, 4.96.

Ethyl (±)-*N*-*tert*-Butyloxycarbonyl-5-methylidene-2-azanorbornane-3-*exo*-carboxylate (9). (a) The use of Tebbe's reagent: To as solution of ketone **8** (92 mg, 0.32 mmol) in dry THF (2.9 mL) at −40 °C was added dropwise Tebbe's reagent (Aldrich 0.5 M, 0.64 mL, 0.32 mmol) and the mixture was stirred for 30 min at this temperature then allowed to warm to room temperature over 2 h. The reaction mixture was cooled to −78 °C and quenched by careful addition of 1 M NaOH (0.5 mL), then filtered on Celite. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was purified by flash chromatography (heptane/EtOAc 4:1, R_f 0.23) to give **9**, as a clear oil (65 mg, 71%).

(b) The use of Lombardo's reagent: To Lombardo's reagent (48.95 mL, 25.94 mmol) at room temperature was added ketone **8** (1.47 g, 5.19 mmol) in dry THF (5.0 mL) and the mixture was allowed to stir for 5 min at this temperature. The reaction mixture was then cooled to −78 °C and quenched by careful addition of saturated $NaHCO_3/H_2O$ (2:1) and the aqueous layer was extracted with Et_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was purified by flash chromatography (heptane/EtOAc 4:1, R_f 0.23) to give **9**, as a clear oil (702 mg, 48%). ^{1}H NMR (two rotamers) δ 5.12 (br s, 1H), 4.83 (br s, 1H), 4.42 (br s, $^3/_5$H), 4.30 (br s, $^2/_5$H), 4.24−4.08 (m, 2H), 3.93 (br s, $^2/_5$H), 3.83 (br s, $^3/_5$H), 3.07 (br s, 1H, H^4), 2.43−2.16 (m, 2H), 2.02 (br d, J = 10 Hz, 1H), 1.46−1.40 (m, 1H), 1.42 (s, ca. 4H), 1.36 (s, ca. 5H), 1.26 (br t, J = 7 Hz, $^9/_5$H), 1.24 (br t, J = 7 Hz, $^6/_5$H). ^{13}C NMR (two rotamers) δ 170.70, 153.40, 149.21, 148.70, 107.38, 107.21, 79.79, 63.87, 63.50, 61.00, 60.89, 58.04, 56.90, 50.76, 49.95, 38.48, 38.24, 36.00, 35.41, 28.31, 28.13, 14.17, 14.01. Anal. Calcd for $C_{15}H_{23}NO_4$: C, 64.03; H, 8.24; N, 4.98. Found: C, 64.14; H, 8.25; N, 4.74. Further elution of the column (heptane/EtOAc 2:1) gave ketone **8** (520 mg, 35%).

Ethyl (±)-*N*-*tert*-Butyloxycarbonyl-5-*endo*-(hydroxymethyl)-2-azanorbornane-3-*exo*-carboxylate (10a) and Ethyl (±)-*N*-*tert*-Butyloxycarbonyl-5-*exo*-(hydroxymethyl)-2-azanorbornane-3-*exo*-carboxylate (10b). To a flask containing $Rh(Cl)(PPh_3)_3$ (38 mg, 3 mmol %) was added **9** (386 mg, 1.37 mmol) dissolved in THF (2.74 mL). The reaction mixture was allowed to stir for 5 min, then catecholborane (2.74 mL, 2.74 mmol) was added and stirring was continued for 2 h. The reaction was then cooled to 0 °C and quenched with saturated $NaHCO_3$ (3 mL) followed by 35% H_2O_2 (0.68 mL). Stirring was continued for 30 min at room temperature

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after which the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was purified by flash chromatography (heptane/EtOAc 1:2, R_f 0.25) to give **10a**,**10b** (ratio 95:5), as a clear oil (340 mg, 83%). ^{1}H NMR (major diastereomer **10a**, two rotamers) δ 4.15−4.00 (m, 3H), 3.89 (s, 0.4H), 3.85 (s, 0.6H), 3.70−3.50 (m, 1H), 3.50−3.34 (m, 1H), 3.12−3.06 (br t, $^1/_2$H), 3.03−2.96 (br t, $^1/_2$H), 2.64 (br d, J = 2 Hz, 1H, H^4), 2.27−2.13 (m, 1H), 1.85 (br d, J = 10 Hz, 1H), 1.67 (br d, J = 10 Hz, $^1/_2$H), 1.63 (br d, J = 10 Hz, $^1/_2$H), 1.31 (s, 4H), 1.25 (s, 5H), 1.13 (t, J = 7 Hz, $^3/_2$H), 1.10 (t, J = 7 Hz, $^3/_2$H), 1.08−0.90 (m, 1H). ^{13}C NMR (major diastereomer **10a**, two rotamers) δ 171.88, 171.78, 154.06, 153.20, 80.00, 63.21, 61.30, 61.22, 58.42, 58.21, 57.77, 56.61, 44.15, 43.54, 40.92, 40.75, 36.56, 35.96, 34.23, 33.81, 28.67, 28.55, 14.60, 14.47. Anal. Calcd for $C_{15}H_{25}NO_5$: C, 60.18; H, 8.42; N, 4.68. Found: C, 60.33; H, 8.33; N, 4.47. ^{1}H NMR (minor diastereomer **10b**): δ 2.56 (br s, 1H, H^4).

(±)-*N*-*tert*-Butyloxycarbonyl-5-*endo*-(hydroxymethyl)-2-azanorbornane-3-*exo*-carboxylic Acid (13a) and (±)-*N*-*tert*-Butyloxycarbonyl-5-*exo*-(hydroxymethyl)-2-azanorbornane-3-*exo*-carboxylic Acid (13b). To **10a**,**10b** (ratio 95:5) (310 mg, 1.04 mmol) in THF (4.16 mL) at room temperature was added 2.5 M LiOH (4.16 mL, 10.4 mmol) and the biphasic system was stirred vigorously for 24 h. The flask was cooled to 0 °C, pH adjusted to 2 with 1 M HCl, and the aqueous layer extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH/AcOH 100:6:2, R_f 0.1) to give **13a**,**13b** (ratio 95:5), as a white foam (266 mg, 94%). ^{1}H NMR (major diastereomer **13a**, two rotamers) δ 4.25 (br s, $^1/_3$H), 4.11 (br s, $^2/_3$H), 4.05 (br s, 0.8H), 3.97 (br s, 0.2H), 3.77−3.60 (m, 1H), 3.44 (t, J = 10 Hz, 1H), 2.87 (br s, $^2/_3$H, H^4), 2.77 (br s, $^1/_3$H, H^4), 2.40−2.27 (m, 1H), 1.98−1.82 (m, 1H), 1.77−1.65 (m, 1H), 1.38 (s, 6H), 1.32 (s, 3H), 1.12−0.95 (m 1H). ^{13}C NMR (major diastereomer **13a**, two rotamers) δ 174.84, 173.94, 155.46, 153.53, 128.40, 81.28, 80.61, 62.86, 62.76, 58.47, 58.28, 58.09, 56.75, 44.06, 42.88, 40.76, 40.18, 36.97, 36.02, 33.77, 33.60, 28.69, 28.55. Anal. Calcd for $C_{13}H_{21}NO_5$: C, 57.55; H, 7.80; N, 5.16. Found: C, 56.34; H, 7.73; N, 4.81. ^{1}H NMR (minor diastereomer **13b**, 2 rotamers) δ 2.81 (br s, $^2/_3$H, H^4), 2.70 (br s, $^1/_3$H, H^4).

(±)-*N*-*tert*-Butyloxycarbonyl-2-azanorbornane-3-*exo*,5-*endo*-dicarboxylic Acid (12a). To **13a**,**13b** (ratio 95:5) (100 mg, 0.37 mmol) in CH_3CN (0.8 mL)/H_2O (1.2 mL)/EtOAc (0.8 mL) was added $NaIO_4$ (336 mg) followed by $RuCl_3 \cdot H_2O$ (2−3 mg). The reaction mixture was stirred at room temperature for 2 h then filtered (filter paper) and the filter cake washed with EtOAc. The filtrate was transferred to a separating funnel, brine added, and the aqueous layer extracted with EtOAc. The collective organic layers were dried (Na_2SO_4), filtered (filter paper), and evaporated. The crude product was left to crystallize at room temperature for 24 h. The crystals were triturated with ice-cold H_2O, then Et_2O, and dried to give **12a** in 76% yield. ^{1}H NMR (D_2O, $^-$OH) (two rotamers) δ 4.22 (br s, $^1/_3$H), 4.16 (br s, $^2/_3$H), 3.75 (s, $^2/_3$H), 3.70 ($^1/_3$H), 2.96−2.84 (m, 2H), 1.94−1.72 (m, 3H), 1.51−1.42 (m, 1H), 1.45 (s, 3H), 1.37 (s, 6H). ^{13}C NMR (two rotamers) δ 182.04, 181.93, 179.39, 179.22, 156.50, 156.22, 81.69, 63.90, 63.42, 59.09, 57.88, 47.89, 47.67, 46.90, 46.65, 36.78, 36.51, 34.00, 33.70, 28.56, 28.38. Anal. Calcd for $C_{13}H_{19}NO_6$: C, 54.73; H, 6.71; N, 4.91. Found: C, 54.75; H, 6.64; N, 5.02.

(±)-2-Azanorbornane-3-*exo*,5-*endo*-dicarboxylic Acid (1). Compound **12a** (65 mg, 0.23 mmol) was dissolved in TFA (2 mL) and stirred at room temperature for 30 min, then evaporated. The crude product was evaporated with H_2O (3 × 10 mL) and allowed to crystallize at room temperature overnight. The crystals were then washed with Et_2O and dried to give **1**, as a light gray solid (41 mg, 96% yield). ^{1}H NMR (D_2O) δ 4.25 (br s, 1H), 4.01 (s, 1H), 3.30−3.18 (m, 2H), 2.20−1.96 (m, 2H), 1.90−1.78 (m, 2H). ^{13}C NMR (D_2O) δ 176.51, 172.68, 60.00, 59.57, 44.24, 43.74, 36.00, 28.95. The free amino acid was converted into the HCl salt by treatment with 1 M HCl, evaporated to dryness, and recrystallized from glacial acetic acid/EtOAc. Mp >220 °C. Anal. Calcd for $C_8H_{12}ClNO_4$: C, 43.35; H, 5.46; N, 6.32. Found: C, 43.42; H, 5.60; N, 6.19.

Acknowledgment. We would like to thank Dr. Jesper Langgaard Kristensen for valuable discussions on potential synthetic strategies toward **1**. We gratefully acknowledge the technical assistance of Birgitte Nielsen in determination of iGlu receptor pharmacology, the financial assistance of the Lundbeck Foundation, the Danish Medical Research Council, and the Novo Nordisk Foundation.

Supporting Information Available: X-ray structure determination for compound **12a**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO026509P