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Asymmetric Synthesis and Receptor Pharmacology of the Group II mGlu Receptor Ligand (1*S*,2*R*,3*R*,5*R*,6*S*)-2-Amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic Acid—HYDIA

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*The asymmetric synthesis and receptor pharmacology of (1S,2R,3R,5R,6S)-2-amino-3-Hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic Acid (+)-**9** (HYDIA) and a few of its O-alkylated derivatives are described. The key step of the synthesis utilizes Sharpless' asymmetric dihydroxylation (AD-β) for the kinetic resolution of a bicyclic racemic precursor olefin. In contrast to the bicyclic glutamate analogue LY354740, which is a potent and selective agonist for the group II metabotropic glutamate receptors (mGluRs), these new conformationally restricted and also hydroxylated or alkoxylated glutamate analogues are potent and selective antagonists for the group II mGluRs.*

Introduction

Among all excitatory amino-acids (EAA) L-glutamic acid (L-Glu) is the major neurotransmitter in the mammalian central nervous system (CNS). L-Glu activates two major classes of glutamate receptors termed ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors.[1] Whereas iGluRs mediate fast synaptic transmission through ligand-gated ion channels, the G-protein coupled mGluRs play a more modulatory role. The family of mGluRs consists of at least eight subtypes, grouped according to their sequence homology, agonist pharmacology, and second messenger coupling.[2] The primary transduction mechanism of group I receptors (mGluR1 and mGluR5) is the stimulation of phosphoinositide (PI) hydrolysis, whereas group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, and mGluR8) receptors evoke an inhibition of forskolin-stimulated cyclic AMP accumulation.[3] In recent years the mGluRs were recognized as valuable therapeutic targets mainly in psychiatric disorders.[4]

Conformationally restricted L-glutamate analogues have been synthesized and used to determine and alter the biological function of these receptors. Within the group of (carboxycyclopropyl)glycines (CCG), the first generation monocyclic L-Glu analogues such as (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine **1** (L-CCG-I),[5] (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine **2** (DCG-IV),[6] and (2*S*,1′*S*,2′*S*,3′*R*)-2-(2′-carboxy-3′-methoxymethylcyclopropyl)glycine **3** (*cis*-MCG-I)[7] were further developed to the second generation bicyclic L-Glu analogues, with (1*S*,2*R*,5*R*,6*S*)-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (+)-**4** (LY354740)[8] and its 4-oxygen and 4-sulfur derivatives **5** (LY379268), **6a** (LY389795),[9] and **6b** (LY404039)[10] being the most prominent examples of potent and selective group II mGluR agonists (Scheme 1). Selectivity and agonistic biological properties are also the main features of **7** and (+)-**8** (MGS0008), the corresponding 3-fluoro derivatives of **4**.[11]

The very rigid bicyclo[3.1.0]hexane system fixes the two carboxylates in an extended conformation—beneficial for binding to the mGluR2/3 receptors as demonstrated by its selectivity versus the group I mGluRs and the iGluRs, where a more hairpin-type arrangement of the carboxylates is required for high-affinity binding.[12]

Thus, this ring system appeared to be a good starting point in our quest to find selective ligands of mGluR2/3. We have based our initial structural considerations on a 3D model of the essential amino acid residues involved in the interaction of (+)-**4** (LY354740) with mGluR2/3 generated using site directed mutagenesis. We especially wanted to explore the effect(s) of the substitution at the 3-position in the cyclopentane-ring,

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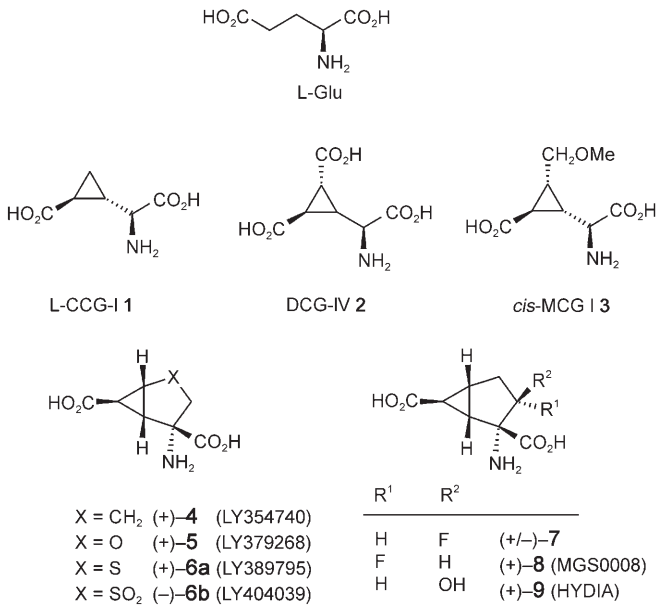
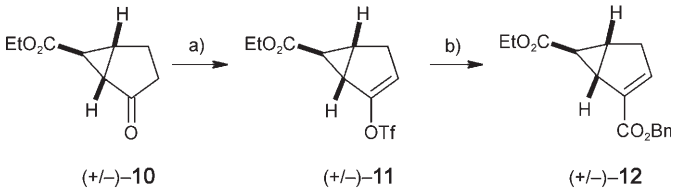
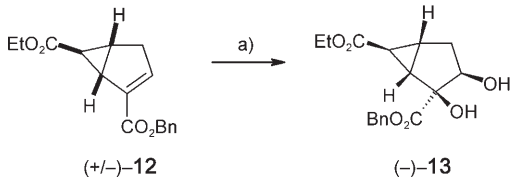


Scheme 1.
L-Glutamate (L-Glu) and conformationally restricted analogues prepared as ligands for the group II metabotropic glutamate receptors.



Scheme 2. Synthesis of the precursor (+/−)-**12** for the asymmetric dihydroxylation. a) LDA, $PhNTf_2$, −78 to 23 °C, 87%; b) BnOH, Et_3N, CO (1 atm), cat. $Pd(OAc)_2$, PPh_3, DMF, 23 °C, 75%.



Scheme 3. The key step: kinetic resolution of the racemic (+/−)-**12** by Sharpless asymmetric dihydroxylation to the chiral diol (−)-**13**. a) cat. $(DHQD)_2PHAL$, cat. $K_2[OsO_2(OH)_4]$, $K_3Fe(CN)_6$, $MeSO_2NH_2$, *t*BuOH/H_2O, 4 °C, 46% (63% *ee*); after two recrystallizations from EtOAc/Et_2O/hexane, 26%, >99% *ee*.

which is proximal to the region of the molecule interacting with amino acid residues common to all mGluRs and possibly responsible for receptor activation (Y168-Y216 and D295 in mGluR2).[13]

From our ongoing effort to identify new ligands for the mGluR2/3 receptors, we report herein the asymmetric synthesis of the selective mGluR2/3 antagonist (1*S*,2*R*,3*R*,5*R*,6*S*)-2-amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (+)-**9** (HYDIA) and a few of its *O*-alkylated derivatives.[14]

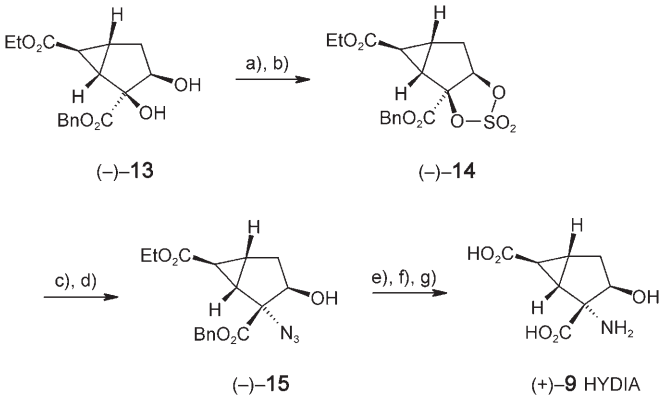
Chemistry

The synthesis started from the known ketoester (+/−)-**10**[15] which was converted to the vinyl triflate (+/−)-**11** by generation of the enolate with LDA at −78 °C and reacting it with $PhNTf_2$. The vinyl triflate (+/−)-**11** was converted to the α,β-unsaturated ester (+/−)-**12** in high yield by a Palladium catalyzed carboxybenzylation reaction using only 2 equiv benzyl alcohol and CO at atmospheric pressure and ambient temperature (Scheme 2).

The key step of the synthesis used the Sharpless asymmetric dihydroxylation (AD-β)[16] to kinetically resolve the racemic precursor (+/−)-**12**. The preferred top face addition in bicyclo-[3.1.0]-systems together with the use of the β-ligand $(DHQD)_2PHAL$ are the matched combination to produce the desired enantio-enriched product (63% *ee*) in good yield (46%), which could be easily recrystallized to give the enantiopure diol (−)-**13** (Scheme 3).

Treatment of (−)-**13** with thionyl chloride in DCM at reflux and subsequent oxidation of the intermediate cyclic sulfite (not shown) with $RuCl_3/NaIO_4$ furnished the cyclic sulfate (−)-**14** in excellent yield.[17] The ring opening of the tricyclo[4.3.0.02,4]-system 6 with sodium azide had to come from the concave side of the bicyclo[3.1.0]-system, but was overruled with 3:1 selectivity by the convex side of the bicyclo-[3.3.0]-system and the α-directing electronic effect of the benzyloxycarbonyl-group.[18] The intermediate sulfate (not shown) was hydrolyzed with aqueous sulfuric acid to the 2-azido-3-hydroxy diester (−)-**15**. This sequence has been described by Shao and Goodman for the preparation of 2-amino-3-hydroxy acids.[19]

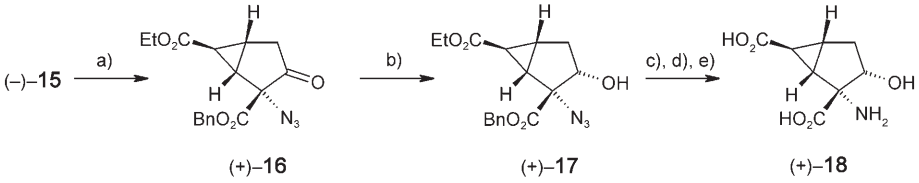
The final steps towards the desired amino acid (Scheme 4) made use of concomitant catalytic hydrogenation of the benzyl ester and the azide, acid hydrolysis of the ethyl ester by simple refluxing in 10% HCl and isolation of the acid free (1*S*,2*R*,3*R*,5*R*,6*S*)-2-amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-di-



Scheme 4. Final steps for the synthesis of (+)-**9** HYDIA: a) $SOCl_2$, DCM, 40 °C; b) cat. $RuCl_3$, $NaIO_4$, CCl_4/MeCN/H_2O, 23 °C, 98% over two steps; c) NaN_3, aq. acetone, 50 °C; d) H_2SO_4, Et_2O, 23 °C, 62% over two steps; e) H_2, Pd/C, aq. AcOH, 23 °C; f) 10% HCl, reflux; g) propylene oxide, EtOH, reflux, 87% over three steps.

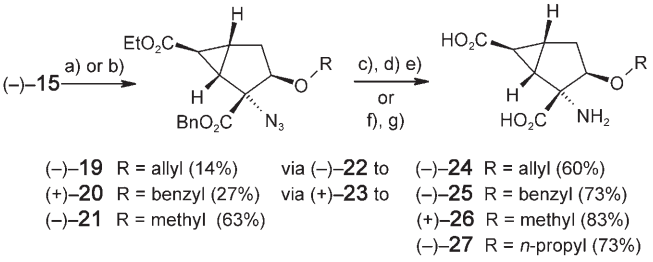
carboxylic acid (+)-**9** (HYDIA) by treatment with propylene oxide as HCl scavenger in refluxing EtOH. Using this methodology (+)-**9** HYDIA was obtained as a white powder, mp $>250\,^{\circ}C$; $[\alpha]_D^{20}=+7.4$ ($c=1.01$ in H_2O).

To obtain a full picture regarding the influence of the newly introduced 3-hydroxy group, we also wanted to gain insight on the influence of the configuration at this center. Therefore the *S*-configured epimer at this position was prepared by first oxidizing the free OH-group of (−)-**15** with PCC on silica gel and then reducing the newly formed ketone (+)-**16** with $NaBH_4$ at low temperature (−50 °C). Although only moderate yield (51%, residual starting material could not be recovered and other products could not be identified) was achieved, the hydride delivery occurred exclusively from the top face of the bicyclo[3.1.0]system giving rise to a single diastereomer. Deprotection followed the same sequence as described before yielded the desired *S*-configured 3-hydroxy amino acid (+)-**18** (Scheme 5).



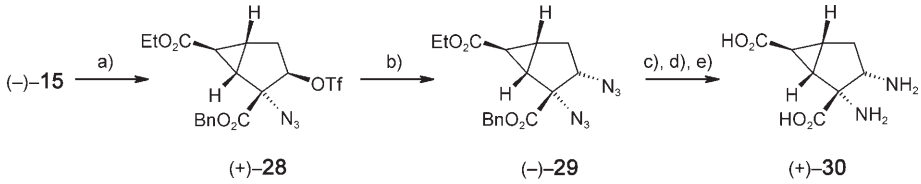
Scheme 5. Inversion of 3-hydroxy configuration of HYDIA: a) PCC, DCM, 23 °C, 67%; b) $NaBH_4$, EtOH/THF, −50 °C, 51%; c) H_2, Pd/C, aq. AcOH, 23 °C; d) 10% HCl, reflux; e) propylene oxide, EtOH, reflux, 86% over three steps.

The intermediate 2-azido-3-hydroxy diester (−)-**15** also served as starting material to explore the activity of some *O*-alkylated HYDIA-derivatives. Either acid catalyzed etherification with trichloroacetimidates[20] (allyl or benzyl) or alkylation with methyl triflate in the presence of a sterically very hindered base produced the desired *O*-alkyl products (−)-**19**, (+)-**20** and (−)-**21** in moderate to good yields. To obtain the *O*-propyl and the *O*-methyl derivatives (+)-**26** and (−)-**27** the final transformations followed the same protocols as for (+)-**9** HYDIA itself. For the *O*-allyl and the *O*-benzyl derivatives (−)-**24** and (−)-**25** azide reduction was performed under Staudinger conditions with trimethyl phosphine[21] in aqueous THF and subsequently both esters were saponified with LiOH to give the desired amino acids (−)-**24** and (−)-**25** (Scheme 6).



Scheme 6. Synthesis of *O*-alkylated HYDIA derivatives: a) ROC(=NH)CCl_3, cat. TfOH, cyclohexane/DCM, 23 °C; b) MeOTf, 2,6-di-*t*Bu-pyridine, DCM, 23 °C; c) H_2, Pd/C, aq. AcOH, 23 °C; d) 10% HCl, reflux; e) propylene oxide, EtOH, reflux; f) Me_3P, aq. THF, 23 °C; g) $LiOH\cdot H_2O$, THF/MeOH/H_2O, 23 °C.

Finally the introduction of a second amino group was established by transforming the OH-group of the very versatile intermediate (−)-**15** into the corresponding triflate (+)-**28** under standard conditions. The S_N2 reaction with sodium azide in DMF was accompanied by the elimination side product, which could be easily removed by simple dihydroxylation with OsO_4/NMO producing the pure diazide (−)-**29** in moderate yield (49%) as a single diastereomer. The final deprotection sequence as described before for (+)-**9** revealed the diaminodiacid (+)-**30** (Scheme 7).

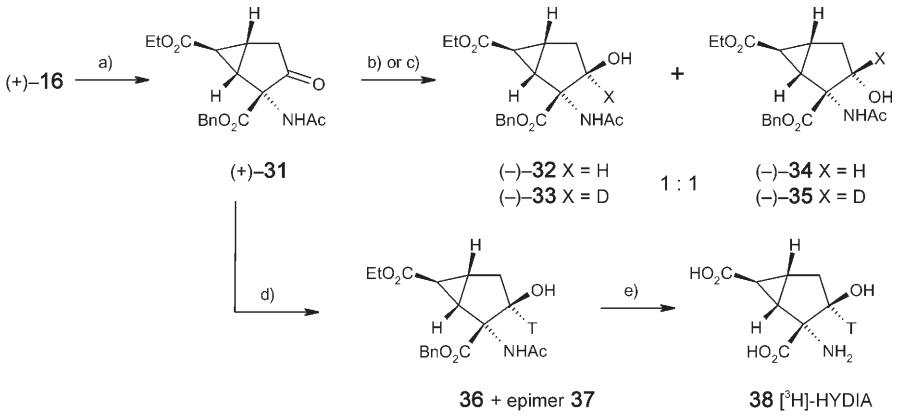


Scheme 7. Introduction of a 3-amino group: a) Tf_2O, pyridine, −78 to 0 °C, 86%; b) NaN_3, DMF, 80 °C, 49%; c) H_2, Pd/C, aq. AcOH, 23 °C; d) 10% HCl, reflux; e) propylene oxide, EtOH, reflux, 79% over three steps.

We also developed a synthesis of the stably tritiated **38** [^{3}H]-HYDIA (Scheme 8). As the face-selective reduction of the azidoketone (+)-**16** with $NaBH_4$ only occurred from the convex side and therefore gave the epimeric azido-α-alcohol (−)-**17** (Scheme 5), we had to alter the substrate to affect hydride delivery from the concave side. The azido group in ketone (+)-**16** was reduced and concomitantly acetylated by thioacetic acid yielding the *N*-acetamidoketone (+)-**31**.[22] Using the newly introduced acetamido group as a potential coordination site for the hydride reagent, the ketone (+)-**31** could be reduced with either $LiBH_4$ or $NaBD_4$ in good yield to the easily separable epimeric alcohols (−)-**32** and (+)-**34**, respectively (−)-**33** and (+)-**35**, wherein at least half of the reduction took place from the concave side. $LiBT(OMe)_3$—prepared from reaction of *n*BuLi, TMEDA with tritium gas to obtain LiT, then reacted with $B(OMe)_3$—was used for the tritiation of ketone (+)-**31** which afforded alcohol **36** (and its epimer **37**). Final deprotection was effected by refluxing **36** in aqueous HCl and after purification by ion exchange chromatography and HPLC **38** [^{3}H]-HYDIA was obtained in 98% radiochemical purity.

Results and Discussion

(+)-**9** HYDIA concentration-dependently inhibited the binding of [^{3}H]-LY354740 to rat mGlu2 and mGlu3 receptors[23] with K_i



Scheme 8. Synthesis of [^{3}H]-HYDIA: a) thioacetic acid, neat, 70 °C, 71%; b) $LiBH_4$ EtOH/THF, −50 °C, 88%; c) $NaBD_4$, EtOH/THF, −50 °C, 90%; d) $LiBT(OMe)_3$ from *n*BuLi, TMEDA, T_2, $B(OMe)_3$, THF, −60 °C; e) 10% HCl, reflux.

values of 52 and 80 nM, respectively (Table 1, Figure 1). All the *O*-alkylated derivatives (−)-**24**, (−)-**25**, (+)-**26**, and (−)-**27** also exhibited moderate to good affinity in binding to the rat mGlu2 receptor with K_i values of 65 nM, 20 nM, 80 nM, and 55 nM, respectively (Table 1).

Table 1. [^{3}H]-LY354740 binding study: indirect measurement of the affinities of synthesized glutamate analogues and reference compounds (+)-**4** and **39** for mGluR2/3.

compound	mGluR2 binding K_i [nM]	mGluR3 binding K_i [nM]
(+)-**9** HYDIA	52	80
(+)-**18**	48	NT[a]
(−)-**24**	65	NT[a]
(−)-**25**	20	NT[a]
(+)-**26**	80	NT[a]
(−)-**27**	55	NT[a]
(+)-**30**	155	NT[a]
(+)-**4** (LY354740)	13[b]	51[b]
39 (LY341495)	9[b]	10[b]

[a] Not tested. [b] see also Schweitzer et al.[23]

These compounds were tested for functional activities at mGlu2 receptor using GTPγ^{35}S binding. Interestingly, (+)-**9** HYDIA and its *O*-alkylated derivatives (−)-**25**, (+)-**26**, and (−)-**27** appeared to be antagonists with IC_{50} values of 1000 nM, 120 nM, 580 nM, and 200 nM, respectively (Figure 2) when tested in cells expressing the recombinant mGluR2 in the presence of 10 μM (1*S*,3*R*)-ACPD.

The competitive nature of the antagonism was demonstrated for (+)-**9** HYDIA by binding studies (Figure 1B, Schild analysis) and efficacy studies evaluating its effect on the maximal response of 1*S*,3*R*-ACPD on the native mGluRs using measurements of forskolin-stimulated cAMP production in adult rat striatal slices (Figure 3).[24]

The antagonist properties of (+)-**9** HYDIA were also evaluated electrophysiologically in CHO cells stably expressing the human Kir3.1 and Kir3.2c GIRK subunits and transiently transfected with either rat mGlu2 or rat mGlu3 receptors. In these cells, glutamate induced an inward K^+ current that was reversible and dependent on the glutamate concentration (Figure 4). The pEC_{50} values for glutamate were 5.70 ± 0.17 (n = 5) and 7.21 ± 0.03 (n = 6) for the rat mGlu2 and mGlu3 receptor, respectively. Glutamate concentration-response curves were also generated in the presence of 10 μM (+)-**9** HYDIA (Figure 4C,D). The pEC_{50} values for glutamate in the presence of (+)-**9** HYDIA were smaller, being 4.29 ± 0.11 (n = 4) and 5.25 ± 0.03 (n = 4) for the rat mGlu2 and mGlu3 receptor expressing cells, respectively.

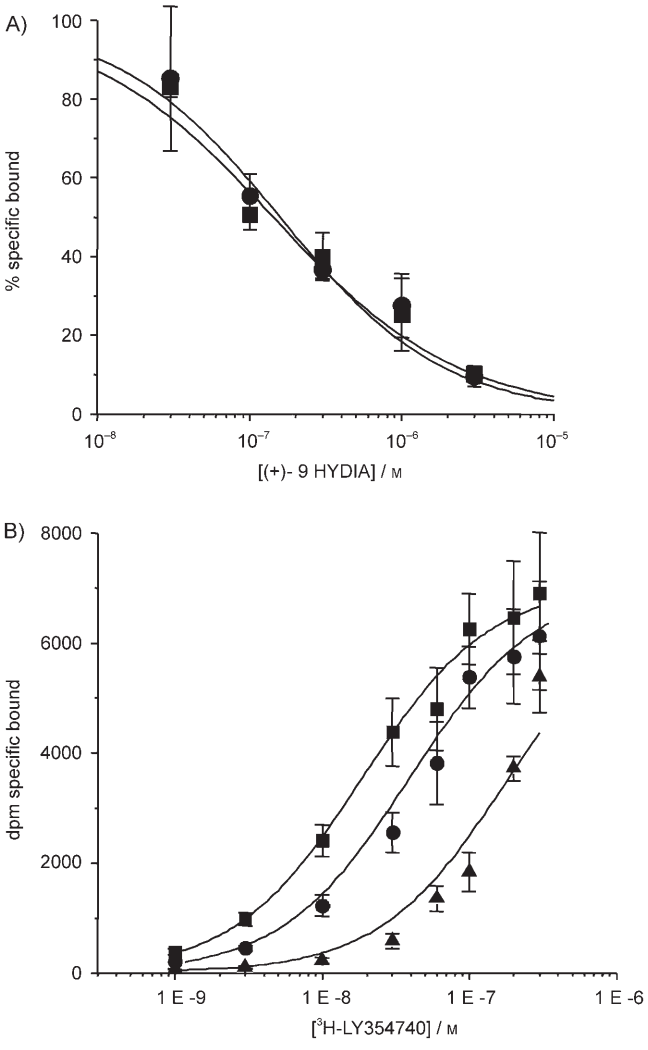


Figure 1. Displacement of [^{3}H]-LY354740 binding to rat mGlu2 and mGlu3. A) Concentration dependent effect of (+)-**9** HYDIA (■: mGluR2; Hill coeff. 0.98; ●: mGluR3). In analogue experiments carried out using [^{3}H]-**2** DCGIV as ligand to the mGlu2 receptor the K_i was 0.6 μM (data not shown). B) Parallel shift to the right of the [^{3}H]-LY354740 specific binding curve in the presence of increasing concentrations of (+)-**9** HYDIA (■: control; ●: + 100 nM (+)-**9** HYDIA; ▲: + 1 μM (+)-**9** HYDIA).

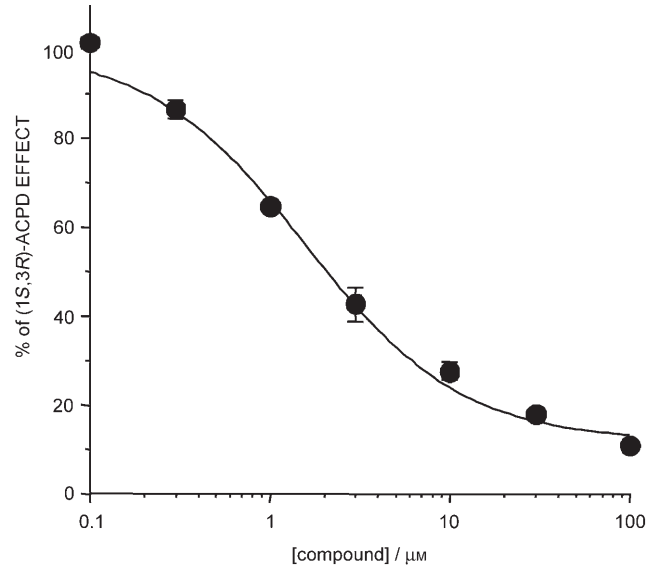


Figure 2. Concentration dependent effect of (+)-**9** HYDIA on the maximal GTPγ^{35}S binding induced by 1*S*,3*R*-ACPD (10 μM) (membranes from cells expressing rat mGluR2).

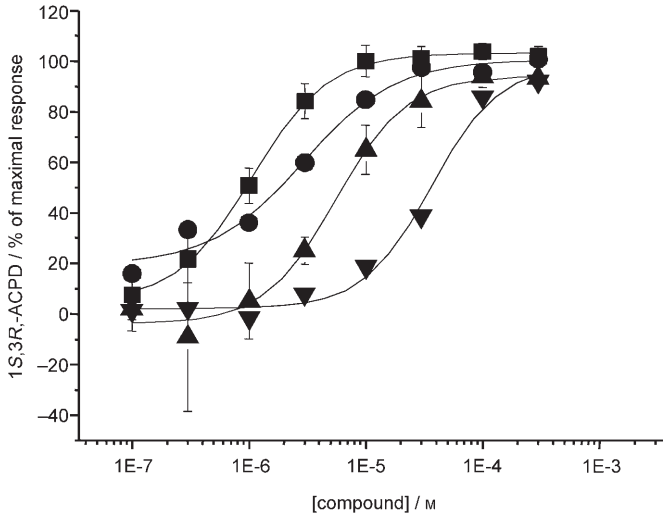


Figure 3. Shild plot analysis of (+)-**9** HYDIA's antagonism on the concentration dependent effect of (1*S*,3*R*)-ACPD on forskolin stimulated cAMP levels in adult rat striatal slices (■: (1*S*,3*R*)-ACPD, $n=9$; ●: (1*S*,3*R*)-ACPD + (+)-**9** HYDIA 100 nM, $n=3$; ▲: (1*S*,3*R*)-ACPD + (+)-**9** HYDIA 300 nM, $n=4$; ▼: (1*S*,3*R*)-ACPD + (+)-**9** HYDIA 1 μM, $n=2$).

In rat hippocampal slices, (+)-**4** (LY354740) inhibited fEPSPs evoked by stimulation of the medial perforant path input and recorded in the dentate gyrus mid-moleculare (mainly mGlu2 receptors are expressed in the terminal fields of the perforant path). The inhibition by (+)-**4** (LY354740) was antagonized competitively by (+)-**9** HYDIA with a pK_B value of 6.04 (Figure 5). In the same assay the pK_B of **39** (LY341495)[25] was 7.92 (data not shown).

(+)-**9** HYDIA (10 μM) was also tested in freshly dissociated Golgi cells from rat cerebellum (these cells express both mGluR2 and mGluR3 mRNA) and the antagonistic properties were compared to those of **39** (LY341495—1 μM). Both compounds shifted the (+)-**4** (LY354740) inhibition curve to the right in a parallel fashion suggestive of competitive antagonism. Measured pK_B values were 7.0 for **39** (LY341495) and 6.3 for (+)-**9** HYDIA, respectively. The difference in potency (about 5.6-fold) between these two antagonists in Golgi cells is consistent with that observed in displacement studies at the mGlu2 receptor (Table 1) whereas the difference in potency detected in hippocampal slices is larger and possibly suggestive of the involvement of additional receptors.

The selectivity of (+)-**9** HYDIA was further assessed and tested at a final concentration of 100 μM. This compound did not activate or inhibit glutamate-stimulated rat mGlu1a, mGlu5a, and mGlu7a receptors in a Ca^{2+} mobilization functional assay. However, it concentration-dependently inhibited the binding of [^{3}H]-L-AP4 to rat mGlu4a and mGlu8a receptors with K_i values of 22 and 15 μM, respectively (Figure 6). Finally, it was devoid of any affinity at the ionotropic glutamate receptors (Table 2). No clear evidence of specific interaction with other receptors was obtained during a specificity screening with indirect affinity studies on mainly monoaminergic receptors (data not shown).

The antagonist properties of (+)-**9** HYDIA were also demonstrated in vivo in mice. (+)-**4** (LY354740) produces a dose-dependent decrease of the horizontal activity after intraperitoneal (1–30 mg kg^{-1}) and intracerebroventricular (3-100 nmol/2 μL/mouse) administration in mice. The experimental conditions of this test have been validated for the contribution of mGlu2 receptor using mGlu2 null mutant mice.[26] The i.c.v. administration of (+)-**9** HYDIA (30-300 nmol/2 μL/mouse) was able to completely antagonize the hypoactivity caused by the administration of (+)-**4** (LY354740) (Figure 7).

The 3*S*-configurated epimer of (+)-**9** HYDIA—(+)-**18**—as well as the 3*S*-configurated diamino acid (+)-**30** both inhibited [^{3}H]-LY354740 binding to rat mGlu2 receptor with K_i values of 48 and 155 nM, respectively (Table 1). However, when these compounds were tested for functional activities at mGlu2 receptors using GTPγS^{35} binding they appeared to be agonists with (+)-**18** showing an EC_{50} value of 98 nM and (+)-**30** an EC_{50} value of 200 nM, respectively. Interestingly (+)-**18** and (+)-**30** exhibit partial agonist activity and weak partial agonist activity, respectively when compared to (+)-**4** (LY354740) (data not shown).

The enantiomer of (+)-**9** HYDIA, that is, (1*R*,2*S*,3*S*,5*S*,6*R*)-2-amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid, also exhibited partial agonist properties causing only 28% stimulation when tested at 100 μM concentration in the GTPγS^{35} binding assay.

In summary, the addition of a simple hydroxy group could allow the potent agonist (+)-**4** (LY354740) to turn into the competitive antagonist (+)-**9** HYDIA. The 3*R*-configuration of the newly introduced OH-group appears to be crucial for functional activity, as the 3*S*-configurated epimer (+)-**18** still exhibits agonist properties. By etherification of the 3*S*-hydroxy function a small set of ethers, which are all potent mGlu2 receptor antagonists, was generated with the most lipophilic benzyl ether (−)-**25** being the most potent one ($K_i=20$ nM).

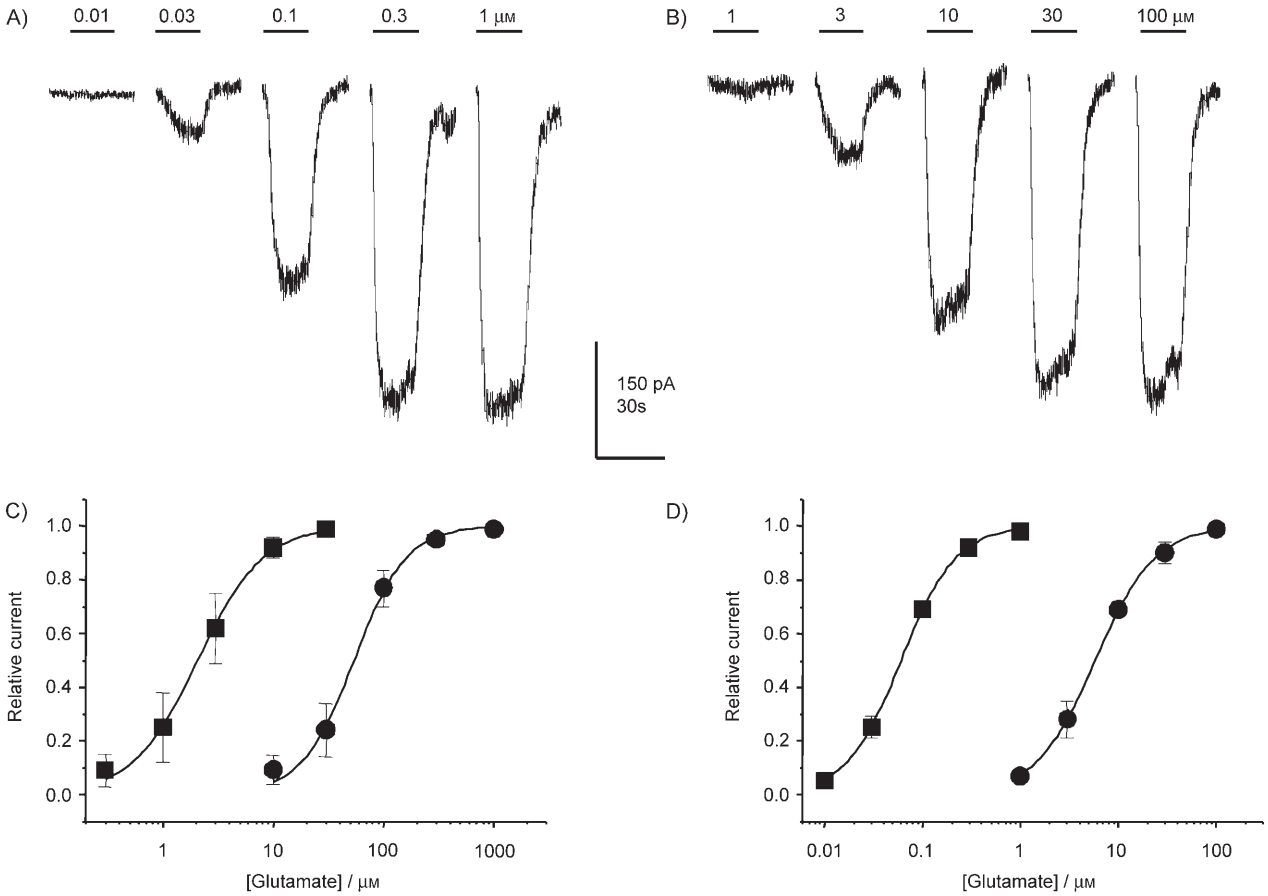


Figure 4. (+)-**9** HYDIA competitively antagonizes the glutamate-induced current in CHO cells stably expressing GIRKs and transiently co-transfected with mGlu2 or mGlu3 receptors. Glutamate was applied at the indicated concentrations and for the time indicated by the bars in the A) absence and B) presence of 10 μM (+)-**9** HYDIA to a cell expressing mGlu3 receptors. Glutamate concentration-response curves were generated in the absence and presence of 10 μM (+)-**9** HYDIA for c) mGluR2 and D) mGluR3. (■: control; ●: + 10 μM (+)-**9** HYDIA). Data points are mean ± SE maximum current amplitude, the curves were generated with the Hill equation.

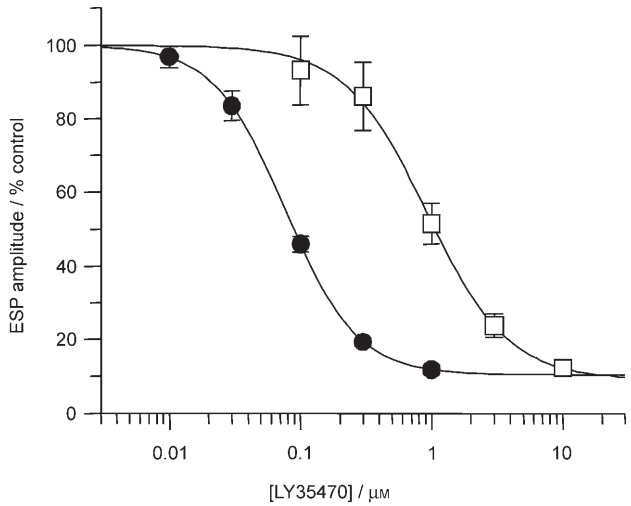


Figure 5. Concentration-dependent inhibition of fEPSPs evoked by stimulation of the medial perforant path input and recorded in the dentate gyrus mid-moleculare by (+)-**4** (LY354740) in the presence (□) and absence (●) of (+)-**9** HYDIA. Data points are mean ± SE fEPSP amplitudes expressed as a percentage of the predrug control value. Fitted curves yielded IC_{50} values of 0.077 μM and 0.92 μM for LY354740 alone (●) and LY354740 + 10 μM (+)-**9** HYDIA, respectively.

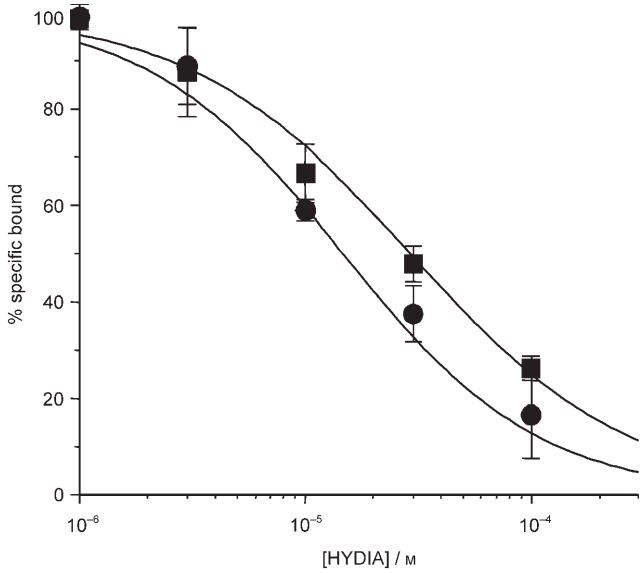


Figure 6. Displacement of ^{3}H-LAP4 binding to rat mGlu4 and mglu8a Concentration dependent effect of (+)-**9** HYDIA (■: rat mGluR4 K_i 22 μM; ●: rat mGluR8a K_i 15 μM).

Table 2. Selectivity of (+)-**9** HYDIA towards other glutamate receptors.

Metabotropic glutamate receptor	IC_{50} [μM]	Ionotropic glutamate receptor	K_i [μM]
rat mGluR1a	>100	NMDA[a]	>100
rat mGluR5a	>100	AMPA[b]	>100
rat mGluR7a	>100	Kainate[c]	>100

[a] ^{3}H-MK801 binding. [b] ^{3}H-RO0488587 binding. [c] ^{3}H-Kainate binding assays.[29]

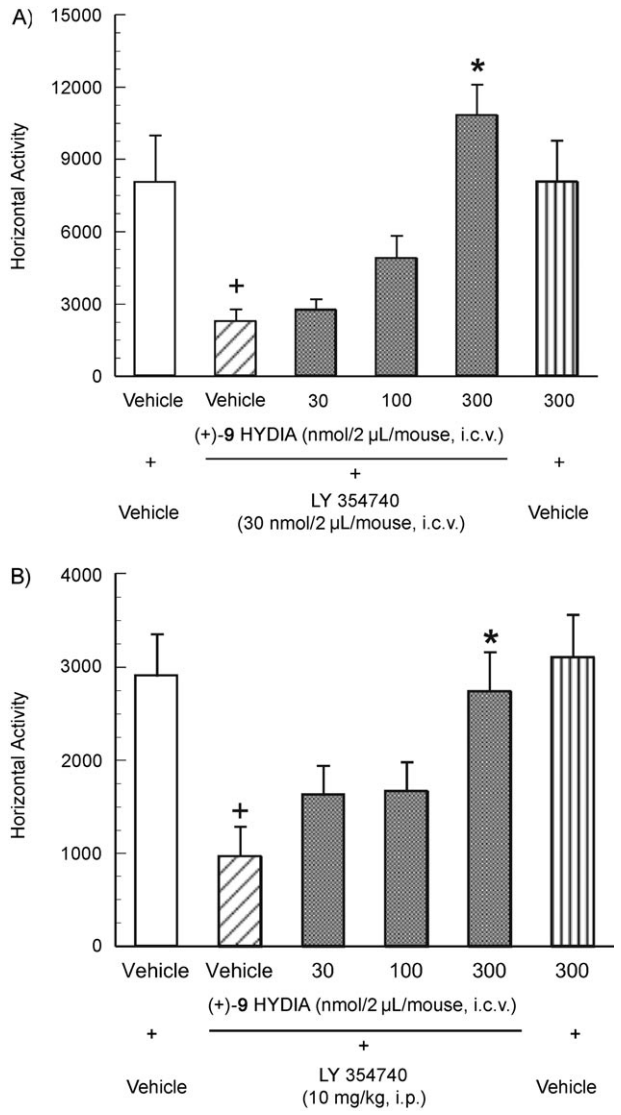


Figure 7. Effect of (+)-**9** HYDIA on (+)-**4** LY354740-induced decrease of Horizontal Activity in mice. A) (+)-**9** HYDIA and (+)-**4** LY354740 were administered i.c.v. simultaneously. B) (+)-**9** HYDIA and (+)-**4** LY354740 were administered i.c.v. and i.p., respectively but simultaneously. Bar graphs represent the cumulative values for 120 min and 20 min, respectively. Eight mice were used per group. $^+$ $P<0.05$ compared to vehicle + vehicle-treated mice and * $P<0.05$ compared to vehicle + (+)-**4** LY354740-treated mice (Anova followed by Dunnett's t-test).

These new ligands represent very useful tools to establish the molecular mechanisms responsible for the selective activation of group II mGluR. Our group has indicated in the past the essential residues involved in the glutamate binding pocket of the group II metabotropic glutamate receptor using [^{3}H]-LY354740 binding and site-directed mutagenesis.[12c] Similar studies are on going using **38** [^{3}H]-HYDIA to obtain more information about the molecular events associated with response to stimuli in metabotropic glutamate receptors 2 and 3.

Conclusions

We were able to stereoselectively introduce a 3-hydroxy function into the well-known scaffold of the 2-amino-bicyclo-[3.1.0]hexane-2,6-dicarboxylic acid by an asymmetric synthesis and also show the feasibility of further alkylation of the newly generated OH-group to produce the corresponding ethers.

Surprisingly, the introduction of the *R*-configured 3-hydroxy-group turned the mGluR2/3 agonist into an mGluR2/3 antagonist, and attachment of more lipophilic residues onto the oxygen further enhanced the affinity of these antagonists. This knowledge was further developed and transferred to the 6-fluorinated derivative and led eventually to the very potent mGluR2/3 antagonist (−)-**40** (MGS0039) (Figure 8).[27]

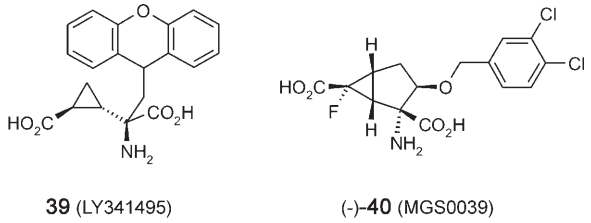


Figure 8. Structures of **39** (LY341495) and (−)-**40** (MGS0039).

In contrast, the *S*-configured 3-hydroxy-group—as well as an *S*-configured 3-amino-group—did not affect the agonist properties, as both corresponding compounds are still mGlu2/3 agonists, albeit with reduced intrinsic activity.

The described molecules and the **38** [^{3}H]-HYDIA in particular may serve as very useful tools for the study of the molecular determinants of activity in the glutamate binding pocket of mGluR2 possibly further supporting the definition of the structural changes required for the signal transduction mechanism of these receptors. The possibility to use these ligands in drug development is also currently under assessment (Figure 9).

Experimental Section

Chemistry:

General methods and materials: ^{1}H NMR spectra were recorded on a Bruker AC-250 spectrometer at 25 °C with TMS (tetramethylsilane) or residual ^{1}H of the given deuterated solvents as internal

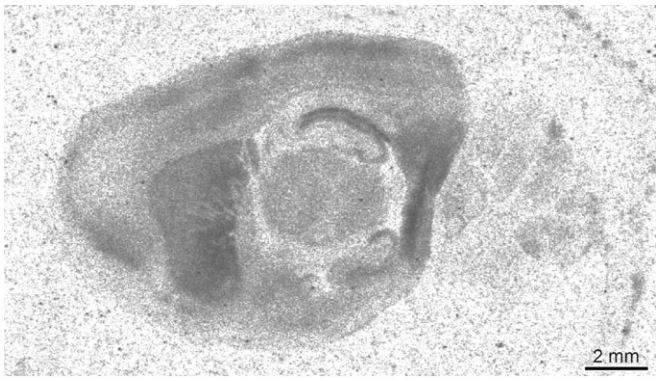


Figure 9. In vitro radioautography of **38** [^{3}H]-HYDIA (50 nM) in sagittal section of Roro rat brain. Regional distribution consistent with binding to both mGluR2 and mGluR3 and high density binding sites in hippocampus - lacunosum moleculare (LMol) and caudate putamen (CPu).

standards. Mass spectra (MS) were measured either with ion spray positive or negative (ISP or ISN) method on a Perkin–Elmer SCIEX API 300 or with electron impact method (EI, 70 eV) on a Finnigan MAT SSQ 7000 spectrometer. High resolution mass spectra (HRMS) were measured with nanospray positive (ISP) method on a Finnigan LTQ-FTMS spectrometer (7 Tesla) and the average of 7 scans is reported. Optical rotations were measured with a Perkin–Elmer 341 polarimeter. Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected. Enantiomeric excess was determined by chiral HPLC using a Chiralpak AS (250×4.6 mm) column with 10% EtOH in hexane as eluent. Elemental analysis was done by Solvias AG, Basel, Switzerland. Column chromatography was performed on Merck silica gel 60 (230–400 mesh). Analytical thin-layer chromatography was performed using Merck silica gel 60 F_{254} precoated glass-backed plates and visualized by cerium(IV)molybdophosphate or ninhydrin. Solvents and reagents were purchased from Fluka AG, Merck KGaA, Aldrich or Acros Organics and used without further purification.

*(1*RS,*5*SR,*6*RS*)-2-Trifluoromethanesulfonyloxy-bicyclo[3.1.0]hex-2-ene-carboxylic acid ethyl ester (+/−)-**11***: *n*BuLi (47.6 mL, 76.1 mmol, 1.6 M solution in hexane) at 0 °C was added dropwise to a solution of diisopropylamine (11.94 mL, 84.6 mmol) in THF (77 mL) and stirred for 10 min at 0 °C. After cooling to −78 °C a solution of (1*RS*,5*SR*,6*RS*)-2-oxo-bicyclo[3.1.0]hexane-6-carboxylic acid ethyl ester (+/−)-**10** (11.86 g, 70.5 mmol) in THF (39 mL) was added dropwise within 25 min. Stirring was continued at −78 °C for 1 h, whereupon a solution of *N*-phenyl-bis(trifluoromethylsulfonyl)imine (27.7 g, 77.5 mmol) in THF (83 mL) was added and then stirred for 90 min at 23 °C. Aqueous workup with ether, sat. $NaHCO_3$ solution, brine, drying over Na_2SO_4, removal of the solvent under vacuum left an orange-brown oil, which was purified by silica gel column chromatography with hexane/ethyl acetate 9:1 to give (+/−)-**11** (18.47 g, 87%) as a slightly brown oil. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.27$ (t, ^3J(H,H) = 7.1 Hz, 3H), 1.46 (m, 1H), 2.28 (m, 1H), 2.55 (m, 2H), 2.79 (m, 1H), 4.15 (q, ^3J(H,H) = 7.1 Hz, 2H), 5.41 ppm (m, 1H); MS(EI): *m/z* 300 [M^+].

*(1*RS,*5*SR,*6*RS*)-Bicyclo[3.1.0]hex-2-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (+/−)-**12***: A solution of (1*RS*,5*SR*,6*RS*)-2-trifluoromethanesulfonyloxy-bicyclo[3.1.0]hex-2-ene-carboxylic acid ethyl ester (+/−)-**11** (14.48 g, 48.2 mmol), $Pd(OAc)_2$ (326 mg, 1.45 mmol), PPh_3 (760 mg, 2.9 mmol), benzyl alcohol (10.0 mL, 96.5 mmol), and Et_3N (13.5 mL, 96.5 mmol) in DMF (195 mL) was purged with CO for 10 min and then stirred for 5 h at 23 °C under a balloon with CO. Aqueous workup with ether, 1 N HCl solution, sat. $NaHCO_3$ solution and brine was followed by drying over $MgSO_4$. Removal of the solvent under vacuum left a dark brown oil, which was purified by silica gel column chromatography with hexane/ethyl acetate 9:1 to yield (+/−)-**12** (10.36 g, 75%) as a yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.13$ (m, 1H), 1.26 (t, ^3J(H,H) = 7.1 Hz, 3H), 2.25 (m, 1H), 2.64 (m, 1H), 2.79–2.91 (m, 2H), 4.10 (q, ^3J(H,H) = 7.1 Hz, 2H), 5.18 (d, ^2J(H,H) = 12 Hz, 1H), 5.24 (d, ^2J(H,H) = 12 Hz, 1H), 6.58 (bs, 1H), 7.30–7.40 ppm (m, 5H); MS(EI): *m/z* 286 [M^+].

*(1*S*,2*S*,3*R*,6*S*)-2,3-Dihydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (−)-**13***: A solution of (+/−)-**12** (11.69 g, 40.4 mmol), $K_2[OsO_2(OH)_4]$ (99 mg, 0.27 mmol), $(DHQD)_2PHAL$ (1.05 g, 1.35 mmol), $K_3Fe(CN)_6$ (26.6 g, 80.8 mmol), K_2CO_3 (11.2 g, 80.8 mmol), and $MeSO_2NH_2$ (11.53 g, 121.2 mmol) in *tert*-butanol (140 mL) and H_2O (140 mL) was stirred vigorously at 4 °C for 24 h. After addition of Na_2SO_3 (40.4 g) and stirring for 30 min at 23 °C the mixture was diluted with water (300 mL) and extracted with ethyl acetate (3×300 mL). The combined organic layers were washed with 2 N NaOH solution (200 mL) and brine (200 mL) followed by drying over Na_2SO_4. Removal of the solvent under vacuum left a dark brown solid (13.51 g), which was subjected to silica gel column chromatography with hexane/ethyl acetate 2:1→3:2→1:1 to yield the residual starting material as a yellow oil (3.58 g, 31%, 65% *ee*), the undesired diastereomeric diol as a yellow oil (1.30 g, 10%) and the crystalline diol (−)-**13** (5.91 g, 45.7%, 63% *ee*) as a pale yellow solid. The latter material was twice recrystallized from ethyl acetate/ether/hexane to give enantiopure (−)-**13** (3.36 g, 26%, >99% *ee*) as white needles. mp: 112–114 °C; $[\alpha]_D^{20} = -73.35$ (*c* = 1.17 in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.24$ (t, ^3J(H,H) = 7.1 Hz, 3H), 1.80–1.92 (m, 2H), 1.99 (m, 1H), 2.08 (dd, ^3J(H,H) = 6.9, 2.9 Hz, 1H), 2.30–2.38 (m, 2H), 3.82 (s, 1H), 4.10 (q, ^3J(H,H) = 7.1 Hz, 2H), 5.20 (d, ^2J(H,H) = 12.3 Hz, 1H), 5.34 (d, ^2J(H,H) = 12.3 Hz, 1H), 7.36 ppm (bs, 5H); MS(ISP): *m/z* 321 [M^++H]; ISP HRMS [M^++Na]: calcd. 343.11521; found 343.11525; Anal. (−)-**13** ($C_{17}H_{20}O_6$) C calcd. 63.74%, found 63.50%, H calcd. 6.29%, found 6.09%, H_2O found < 0.1%.

*(1*S*,1a*S*,1b*S*,4a*R*,5a*R*)-3,3-Dioxo-tetrahydro-2,4-dioxa-6-thia-cyclopropa[a]pentalene-1,1b-dicarboxylic acid 1b-benzyl ester 1-ethyl ester (−)-**14***: $SOCl_2$ (1.21 mL, 16.64 mmol) was added to a solution of (−)-**13** (2.66 g, 8.32 mmol) in CH_2Cl_2 (14 mL) at 0 °C and stirring was continued at 40 °C until TLC indicated complete conversion to the cyclic sulfite. The solvent and excess $SOCl_2$ were removed under vacuum, the residual oil was dissolved in CCl_4 (8.3 mL), CH_3CN (8.3 mL), and H_2O (12.5 mL) and cooled to 0 °C. $NaIO_4$ (2.67 g, 12.5 mmol) and $RuCl_3$ hydrate (33 mg) were added and the mixture was stirred at 23 °C for 30 min. Aqueous workup with ether, water, and brine was followed stirring of the organic phase with $MgSO_4$ and a spatula tip of activated carbon. After filtration through celite the solvent was removed under vacuum to yield the crude cyclic sulfate as a brown oil (3.31 g). An analytical sample was obtained by silica gel column chromatography with hexane/ethyl acetate 2:1 to yield (−)-**14** (98%) as a colorless oil. $[\alpha]_D^{20} = -36.08$ (*c* = 1.13 in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.25$ (t, ^3J(H,H) = 7.2 Hz, 3H), 1.67 (t, ^3J(H,H) = 3.5 Hz, 1H), 2.28 (m, 1H), 2.52–2.62 (m, 3H), 4.11 (m, 2H), 5.32 (s, 2H), 7.38 ppm (s, 5H); MS(ISP): *m/z* 400 [M^++NH_4].

*(1*S*,2*R*,3*R*,5*R*,6*S*)-2-Azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (−)-**15***: The crude (−)-**14** was dissolved in acetone (45 mL) and H_2O (4.5 mL), NaN_3 (720 mg, 11.1 mmol) was added and the mixture was stirred at 50 °C until TLC indicated complete conversion of the cyclic sulfate. The solvent was removed under vacuum, the residue partitioned between ether (160 mL) and water (4.5 mL), cooled to 0 °C, whereupon 20% H_2SO_4 (13.5 mL) was added dropwise. The mixture was stirred vigorously at 23 °C for 37 h, the layers were separated, the organic

layer washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$. After removal of the solvent under vacuum, the residual oil (2.78 g, 97%) was purified by silica gel column chromatography with hexane/ethyl acetate 9:1→5:1 to give (−)-**15** (1.79 g, 62%) as a colorless oil. $[\alpha]_D^{20} = -48.43$ ($c = 1.09$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 1.26$ (t, ^3J(H,H) = 7.1 Hz, 3H), 1.81 (t, ^3J(H,H) = 3.1 Hz, 1H), 2.04–2.20 (m, 3H), 2.25 (dd, ^3J(H,H) = 6.9, 2.9 Hz, 1H), 2.34 (dd, ^3J(H,H) = 7.7, 3.9 Hz, 1H), 3.80 (bq, ^3J(H,H) = 9 Hz, 1H), 4.12 (q, ^3J(H,H) = 7.1 Hz, 2H), 5.27 (d, ^2J(H,H) = 12.2 Hz, 1H), 5.34 (d, ^2J(H,H) = 12.2 Hz, 1H), 7.36–7.40 ppm (m, 5H); MS(ISN): *m/z* 404 [M^-+OAc]; ISP HRMS [M^++Na]: calcd. 368.12169; found 368.12149.

*(1S,2R,3R,5R,6S)-2-Amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (+)-**9** HYDIA*: A solution of (−)-**15** (1.55 g, 4.49 mmol) in HOAc (20 mL) and H_2O (5 mL) was hydrogenated in the presence of Pd/C (100 mg, 10% Pd/C) at 23 °C for 18 h. The catalyst was removed by filtration, the filter cake washed with 50% aqueous acetic acid. After removal of the solvent in vacuum, the beige residue was refluxed in 10% HCl (55 mL) for 4 h. The solution was cooled to 23 °C, filtered, washed with water, and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (45 mL) and propylene oxide (24 mL) and refluxed for 15 min, whereupon the amino acid precipitated. After cooling to 23 °C, the product was filtered off, washed with ether, and dried to give (+)-**9** HYDIA (784 mg, 87%) as a white solid. mp: >250 °C; $[\alpha]_D^{20} = +7.41$ ($c = 1.01$ in H_2O); ^{1}H NMR (250 MHz, D_2O): $\delta = 1.87$ (t, ^3J(H,H) = 3.1 Hz, 1H), 2.15–2.26 (m, 3H), 2.38 (dd, ^3J(H,H) = 13.0, 7.6 Hz, 1H), 3.97 ppm (dd, ^3J(H,H) = 8.6, 7.4 Hz, 1H); MS(ISP): *m/z* 202 [M^++H]; ISP HRMS [M^++H]: calcd. 202.07100; found 202.07097; Anal. (+)-**9** ($C_8H_{11}NO_5 \cdot 1.04\,H_2O$) C calcd. 43.69%, found 43.64%, H calcd. 5.99%, found 5.86%, N calcd. 6.37%, found 6.35%, H_2O found 8.55%.

*(1S,2R,5R,6S)-2-Azido-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (+)-**16***: PCC (2.40 g, 50% on silica gel) at 0 °C was added to a solution of (−)-**15** (960 mg, 2.78 mmol) in DCM (18 mL) and stirring was continued at 23 °C for 20 h. The reaction mixture was placed on a silica gel column and the product was eluted with DCM to yield (+)-**16** (746 mg, 78%) as a white solid. mp: 46–48 °C; $[\alpha]_D^{20} = +210.91$ ($c = 1.07$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.27$ (t, ^3J(H,H) = 7.2 Hz, 3H), 1.62 (t, ^3J(H,H) = 3.4 Hz, 1H), 2.29 (m, 1H), 2.44 (dd, ^3J(H,H) = 7.7, 3.1 Hz, 1H), 2.55 (d, ^2J(H,H) = 19.2 Hz, 1H), 2.98 (dd, $^{2,3}J$(H,H) = 19.2, 5.6 Hz, 1H), 4.13 (q, ^3J(H,H) = 7.2 Hz, 2H), 5.29 (s, 2H), 7.36–7.40 ppm (m, 5H); MS-(ISP): *m/z* 361 [M^++NH_4].

*(1S,2R,3S,5R,6S)-2-Azido-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (−)-**17***: $NaBH_4$ (22 mg, 0.58 mmol) at −50 °C was added to a solution of (+)-**16** (100 mg, 0.29 mmol) in EtOH (1.7 mL) and THF (0.5 mL) and stirring was continued at −50 °C for 4 h. The reaction mixture was poured on ice, acidified with 1 N HCl, and extracted with ether. After washing with sat. $NaHCO_3$ solution, brine, and drying over $MgSO_4$ the crude product was purified by silica gel column chromatography with hexane/EtOAc 5:1 to give (−)-**17** (51 mg, 51%) as a colorless oil. $[\alpha]_D^{20} = 0.61$ ($c = 0.54$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.26$ (t, ^3J(H,H) = 7.2 Hz, 3H), 2.03–2.18 (m, 3H), 2.31–2.48 (m, 2H), 2.53 (t, ^3J(H,H) = 4 Hz, 1H), 4.13 (q, ^3J(H,H) = 7.2 Hz, 2H), 4.21 (bs, 1H), 5.26 (s, 2H), 7.36–7.40 ppm (m, 5H); MS(EI): *m/z* 300 [M^+−OEt].

*(1S,2R,3S,5R,6S)-2-Amino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (+)-**18***: A solution of (−)-**17** (50 mg, 0.145 mmol) in HOAc (4 mL) and H_2O (1 mL) was hydrogenated in the presence of Pd/C (11 mg, 10% Pd/C) at 23 °C for 23 h. The catalyst was removed by filtration, the filter cake washed with 50% aqueous acetic acid. After removal of the solvent in vacuum, the beige residue was refluxed in 10% HCl (6.75 mL) for 4 h. The solution was cooled to 23 °C, filtered, washed with water, and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (5 mL) and propylene oxide (2 mL) and refluxed for 15 min, whereupon the amino acid precipitated. After cooling to 23 °C, the product was filtered off, washed with ether, and dried to give (+)-**18** (24 mg, 86%) as a white solid. mp: 208 °C (dec.); $[\alpha]_D^{20} = +27.52$ ($c = 1.03$ in H_2O); ^{1}H NMR (250 MHz, D_2O): $\delta = 2.05$ (d, ^3J(H,H) = 15 Hz, 2H), 2.10–2.20 (m, 2H), 2.12 (m, 1H), 2.61 (m, 1H), 4.20 ppm (d, ^3J-(H,H) = 7.2 Hz, 1H); MS(ISP): *m/z* 202 [M^++H]; ISP HRMS [M^++H]: calcd. 202.07100; found 202.07095.

*(1S,2R,3R,5R,6S)-3-Allyloxy-2-azido-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (−)-**19***: TfOH (29 μL) was added to a solution of (−)-**15** (200 mg, 0.58 mmol) and allyl 2,2,2-trichloroacetimidate (0.18 mL, 1.16 mmol) in cyclohexane (0.7 mL), whereupon the solution became hot and stirring was continued at 23 °C for 1 h. The reaction mixture was poured on ice, diluted with ether, washed with sat. $NaHCO_3$ solution, brine, and dried over $MgSO_4$. The crude product was purified by silica gel column chromatography with hexane/EtOAc 4:1 to give (−)-**19** (31 mg, 14%) as a yellow oil. $[\alpha]_D^{20} = -17.85$ ($c = 0.50$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.26$ (t, ^3J(H,H) = 7.1 Hz, 3H), 1.76 (t, ^3J(H,H) = 3.5 Hz, 1H), 2.03–2.26 (m, 3H), 2.32 (dd, ^3J(H,H) = 12, 7 Hz, 1H), 3.58 (dd, ^3J-(H,H) = 9, 7 Hz, 1H), 3.92 (m, 1H), 4.02 (m, 1H), 4.13 (q, ^3J(H,H) = 7.1 Hz, 2H), 5.10–5.22 (m, 2H), 5.25 (d, ^2J(H,H) = 12 Hz, 1H), 5.34 (d, ^2J(H,H) = 12 Hz, 1H), 5.75 (m, 1H), 7.30–7.42 ppm (m, 1H); MS(EI): *m/z* 284 [M^+−CO_2Et−N_2]; ISP HRMS [M^++Na]: calcd. 408.15299; found 408.15301.

*(1S,2R,3R,5R,6S)-2-Azido-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (+)-**20***: TfOH (0.05 mL) was added to a solution of (−)-**15** (305 mg, 0.88 mmol) and benzyl 2,2,2-trichloroacetimidate (0.2 mL, 1.06 mmol) in cyclohexane (2.4 mL) and DCM (1.2 mL) , whereupon the solution became hot and stirring was continued at 23 °C for 5 h. The reaction mixture was poured on ice, diluted with ether, washed with sat. $NaHCO_3$ solution, brine, and dried over $MgSO_4$. The crude product was purified by silica gel column chromatography with hexane/EtOAc 4:1 to give (+)-**20** (102 mg, 27%) as a yellow oil. $[\alpha]_D^{20} = +2.03$ ($c = 0.99$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.25$ (t, ^3J(H,H) = 7.1 Hz, 3H), 1.75 (t, ^3J(H,H) = 3.5 Hz, 1H), 2.06 (m, 1H), 2.17–2.31 (m, 3H), 3.60 (bt, ^3J(H,H) = 8 Hz, 1H), 4.12 (q, ^3J(H,H) = 7.1 Hz, 2H), 4.42 (d, ^2J-(H,H) = 12 Hz, 1H), 4.57 (d, ^2J(H,H) = 12 Hz, 1H), 5.23 (d, ^2J(H,H) = 12 Hz, 1H), 5.34 (d, ^2J(H,H) = 12 Hz, 1H), 7.15–7.42 ppm (10H, m); MS(ISP): *m/z* 408 [M^++H−N_2]; ISP HRMS [M^++Na]: calcd. 458.16864; found 458.16861.

*(1S,2R,3R,5R,6S)-2-Azido-3-methoxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (−)-**21***: A solution of (−)-**15** (345 mg, 1.0 mmol), 2,6-di-*tert*-butylpyridine (1.35 mL, 6.0 mmol) and methyl triflate (0.55 mL, 5.0 mmol) in DCM (2 mL) was stirred at 23 °C for 4 days. The reaction mixture was poured on ice, acidified with 1 N HCl and extracted with ether. After washing with sat. $NaHCO_3$ solution, brine, and drying over $MgSO_4$ the crude product was purified by silica gel column chromatography with hexane/EtOAc 4:1 to give (−)-**21** (226 mg, 63%) as a yellow oil. $[\alpha]_D^{20} = -48.02$ ($c = 1.11$ in $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.26$ (t, ^3J-(H,H) = 7.1 Hz, 3H), 1.77 (t, ^3J(H,H) = 3.5 Hz, 1H), 2.05–2.18 (m, 2H), 2.25 (dd, ^3J(H,H) = 7, 3.5 Hz, 1H), 2.34 (dd, ^3J(H,H) = 12, 7 Hz, 1H), 3.28 (s, 3H), 3.42 (bt, ^3J(H,H) = 8 Hz, 1H), 4.12 (q, ^3J(H,H) = 7.1 Hz, 2H), 5.25 (d, ^2J(H,H) = 12 Hz, 1H), 5.34 (d, ^2J(H,H) = 12 Hz, 1H), 7.30–7.40 ppm (m, 5H); MS(EI): *m/z* 258 [M^+−CO_2Et−N_2]; ISP HRMS [M^++Na]: calcd. 382.13734; found 382.13715.

*(1S,2R,3R,5R,6S)-3-Allyloxy-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (−)-**22***: Me_3P (0.29 mL, 0.29 mmol, 1 M sol. in THF) was added to a solution of (−)-**19** (101 mg, 0.262 mmol) in THF (3.9 mL) and H_2O (0.4 mL) and stirring was continued at 23 °C for 3 h. The reaction mixture was diluted

with ether, washed with sat. $NaHCO_3$ solution, brine, and dried over Na_2SO_4. The crude product was purified by silica gel column chromatography with hexane/EtOAc 1:1 (+ small amount of Et_3N) to give (−)-**22** (47 mg, 50%) as a light brown oil. $[\alpha]_D^{20} = -10.67$ ($c = 1.00$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.24$ (t, $^3J(H,H) = 7.1$ Hz, 3H), 1.76 (t, $^3J(H,H) = 3.5$ Hz, 1H), 1.93 (bs, 2H), 1.99 (m, 1H), 2.07–2.18 (m, 2H), 2.31 (dd, $^3J(H,H) = 12.5$, 7 Hz, 1H), 3.45 (bt, $^3J(H,H) = 8$ Hz, 1H), 3.93 (bd, $^3J(H,H) = 6$ Hz, 2H), 4.12 (q, $^3J(H,H) = 7.1$ Hz, 2H), 5.10–5.35 (m, 4H), 5.78 (m, 1H), 7.28–7.38 ppm (m, 5H); MS(ISP): *m/z* 360 [M^++H].

*(1S,2R,3R,5R,6S)-2-Amino-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (+)-**23***: Me_3P (0.30 mL, 0.30 mmol, 1 M sol. in THF) was added to a solution of (+)-**20** (120 mg, 0.276 mmol) in THF (4 mL) and H_2O (0.4 mL) and stirring was continued at 23 °C for 4 h. The reaction mixture was diluted with ether, washed with sat. $NaHCO_3$ solution, brine, and dried over Na_2SO_4. The crude product was purified by silica gel column chromatography with hexane/EtOAc 1:1 (+ small amount of Et_3N) to give (+)-**23** (58 mg, 51%) as a yellow oil. $[\alpha]_D^{20} = +3.54$ ($c = 0.90$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.23$ (t, $^3J(H,H) = 7.1$ Hz, 3H), 1.72 (t, $^3J(H,H) = 3.5$ Hz, 1H), 1.93 (bs, 2H), 2.00 (m, 1H), 2.10 (dd, $^3J(H,H) = 7$, 3.5 Hz, 1H), 2.19 (m, 1H), 2.27 (dd, $^3J(H,H) = 12$, 7 Hz, 1H), 3.46 (dd, $^3J(H,H) = 8$, 7 Hz, 1H), 4.10 (q, $^3J(H,H) = 7.1$ Hz, 2H), 4.47 (s, 2H), 5.20 (d, $^2J(H,H) = 12$ Hz, 1H), 5.30 (d, $^2J(H,H) = 12$ Hz, 1H), 7.18–7.42 ppm (m, 10H); MS(ISP): *m/z* 410 [M^++H]; ISP HRMS [M^++H]: calcd. 410.19620; found 410.19603.

*(1S,2R,3R,5R,6S)-3-Allyloxy-2-amino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (−)-**24***: A solution of (−)-**22** (47 mg, 0.131 mmol) and $LiOH{\cdot}H_2O$ (15 mg, 0.357 mmol) in THF (4 mL), H_2O (2 mL), and MeOH (0.4 mL) was stirred at 23 °C for 36 h. The solution was acidified with conc. HCl and evaporated to dryness. The remaining pale yellow solid was suspended in EtOH, filtered, washed with more EtOH, and the filtrate was evaporated to dryness. The residue was dissolved in EtOH (1 mL) and propylene oxide (1 mL) and refluxed for 3 min, whereupon the amino acid precipitated. After cooling to 23 °C, the product was filtered off, washed with ether, and dried to give (−)-**24** (19 mg, 59%) as a white solid. mp: >250 °C; $[\alpha]_D^{20} = -10.62$ ($c = 0.40$ in H_2O); ^1H NMR (250 MHz, D_2O) $\delta = 1.48$ (t, $^3J(H,H) = 3.5$ Hz, 1H), 1.78 (m, 2H), 1.98 (ddd, $^3J(H,H) = 12.5$, 8, 3.5 Hz, 1H), 2.27 (dd, $^3J(H,H) = 12.5$, 7 Hz, 1H), 3.44 (bt, $^3J(H,H) = 7.5$ Hz, 1H), 3.97 (m, 2H), 5.13–5.26 (m, 2H), 5.82 ppm (ddt, $^{2,3}J(H,H) = 18$, 11, 6 Hz, 1H); MS(ISN): *m/z* 240 [M^-−H].

*(1S,2R,3R,5R,6S)-2-Amino-3-benzyloxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (−)-**25***: A solution of (+)-**23** (50 mg, 0.122 mmol) and $LiOH{\cdot}H_2O$ (13 mg, 0.30 mmol) in THF (4 mL), H_2O (2 mL) and MeOH (0.4 mL) was stirred at 23 °C for 36 h. The solution was acidified with conc. HCl and evaporated to dryness. The remaining pale yellow solid was suspended in EtOH, filtered, washed with more EtOH, and the filtrate was evaporated to dryness. The residue was dissolved in EtOH (1 mL) and propylene oxide (1 mL) and refluxed for 3 min, whereupon the amino acid precipitated. After cooling to 23 °C, the product was filtered off, washed with ether, and dried to give (−)-**25** (26 mg, 72%) as a white solid. mp: >250 °C; $[\alpha]_D^{20} = -5.69$ ($c = 0.25$ in H_2O); ^1H NMR (250 MHz, D_2O): $\delta = 1.57$ (t, $^3J(H,H) =$ Hz, 1H), 1.97 (m, 2H), 2.13 (ddd, $^3J(H,H) = 12.5$, 8, 3.5 Hz, 1H), 2.35 (dd, $^3J(H,H) = 12.5$, 7 Hz, 1H), 3.73 (dd, $^3J(H,H) = 8$, 7 Hz, 1H), 4.51 (s, 2H), 7.36 ppm (bm, 5H); MS(ISN): *m/z* 290 [M^-−H].

*(1S,2R,3R,5R,6S)-2-Amino-3-methoxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (−)-**26***: A solution of (−)-**21** (214 mg, 0.56 mmol) in HOAc (8 mL) and H_2O (2 mL) was hydrogenated in the presence of Pd/C (35 mg, 10% Pd/C) at 23 °C for 18 h. The catalyst was removed by filtration, the filter cake washed with 50% aqueous acetic acid. After removal of the solvent in vacuum, the beige residue was refluxed in 10% HCl (15 mL) for 4 h. The solution was cooled to 23 °C, filtered, washed with water, and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (10 mL) and propylene oxide (5.6 mL) and refluxed for 15 min, whereupon the amino acid precipitated. After cooling to 23 °C, the product was filtered off, washed with ether, and dried to give (−)-**26** (100 mg, 74%) as a white solid. mp: >250 °C; $[\alpha]_D^{20} = -13.84$ ($c = 1.01$ in H_2O); ^1H NMR (250 MHz, D_2O): $\delta = 1.86$ (t, $^3J(H,H) = 3.5$ Hz, 1H), 2.04–2.23 (m, 3H), 2.50 (dd, $^3J(H,H) = 12$, 7 Hz, 3H), 3.29 (s, 3H), 3.62 ppm (bt, $^3J(H,H) = 8$ Hz, 1H); MS(ISN): *m/z* 214 [M^-−H]; ISP HRMS [M^++H]: calcd. 216.08665; found 216.08661; Anal. (−)-**26** ($C_9H_{13}NO_5{\cdot}0.41\ H_2O$) C calcd. 48.56%, found 48.46%, H calcd. 6.26%, found 6.17%, N calcd. 6.29%, found 6.22%, H_2O found 3.22%.

*(1S,2R,3R,5R,6S)-2-Amino-3-propoxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (−)-**27***: A solution of (−)-**19** (28 mg, 0.073 mmol) in HOAc (0.75 mL) and H_2O (0.25 mL) was hydrogenated in the presence of Pd/C (3 mg, 10% Pd/C) at 23 °C for 18 h. The catalyst was removed by filtration, the filter cake washed with 50% aqueous ethanol. After removal of the solvent in vacuum, the beige residue was refluxed in 10% HCl (1.25 mL) for 4 h. The solution was cooled to 23 °C and evaporated to dryness. The remaining pale yellow solid was dissolved in EtOH (1 mL) and propylene oxide (0.5 mL) and refluxed for 10 min, whereupon the amino acid precipitated. After cooling to 23 °C, the product was filtered off, washed with ether, and dried to give (−)-**27** (13 mg, 72%) as a white solid. mp: >250 °C; $[\alpha]_D^{20} = -3.72$ ($c = 0.19$ in H_2O); ^1H NMR (250 MHz, D_2O): $\delta = 0.81$ (t, $^3J(H,H) = 7.5$ Hz, 1H), 1.48 (m, 2H), 1.84 (m, 1H), 2.16 (m, 3H), 2.26 (dd, $^3J(H,H) = 12$, 7 Hz, 1H), 3.43 (m, 2H), 3.73 ppm (bt, $^3J(H,H) = 8$ Hz, 1H); MS(ISN): *m/z* 242 [M^-−H]; ISP HRMS [M^++H]: calcd. 244.11795; found 244.11789.

*(1S,2R,3R,5R,6S)-2-Azido-3-trifluoromethanesulfonyloxy-bicyclo[3.1.0]-hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (−)-**28***: Trifluoromethanesulfonic anhydride (0.37 mL, 2.25 mmol) in DCM (1.4 mL) was added to a solution of (−)-**15** (518 mg, 1.5 mmol) and pyridine (0.36 mL, 4.5 mmol) in DCM (19 mL) at −78 °C and the mixture was allowed to reach 0 °C. The reaction mixture was diluted with ether, poured on ice, extracted with sat. $CuSO_4$ solution, and brine, followed by drying over Na_2SO_4. After removal of the solvent in vacuum, the crude product was purified by silica gel column chromatography with hexane/EtOAc 4:1 to give (−)-**28** (574 mg, 86%) as a colorless oil. $[\alpha]_D^{20} = -17.30$ ($c = 1.09$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.27$ (t, $^3J(H,H) =$ Hz, 3H), 1.84 (t, $^3J(H,H) = 3.5$ Hz, 1H), 2.17 (m, 1H), 2.34 (dd, $^3J(H,H) = 7$, 3.5 Hz, 1H), 2.53 (m, 2H), 4.15 (q, $^3J(H,H) = 7.1$ Hz, 2H), 4.58 (bt, $^3J(H,H) = 8$ Hz, 1H), 5.26 (d, $^2J(H,H) = 12$ Hz, 1H), 5.41 (d, $^2J(H,H) = 12$ Hz, 1H), 7.39 ppm (s, 5H); MS(ISP): *m/z* 495 [M^++NH_4]; ISP HRMS [M^++Na]: calcd. 500.07098; found 500.07070.

*(1S,2R,3S,5R,6S)-2,3-Diazido-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (−)-**29***: NaN_3 (187 mg, 2.88 mmol) was added to a solution of (−)-**28** (200 mg, 0.48 mmol) in DMF (0.8 mL) and the mixture was stirred at 80 °C for 1 h. After cooling to 23 °C, the reaction was poured onto ice, extracted with ethyl acetate, washed with brine, and dried over $MgSO_4$. Removal of the solvent in vacuum left the crude (−)-**29**, contaminated with ca. 12% of (*1S,2R,5R,6S*)-2-azido-bicyclo[3.1.0]hex-3-ene-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester, which was removed by reacting the mixture with OsO_4 (3 drops of a 2.5% solution in *t*BuOH), NMO (18 mg, 0.162 mmol) in acetone (1.5 mL), and H_2O (3 mL) at 23 °C for 24 h. After quenching with sodium sulfite (approximately 150 mg) the reaction was poured onto ice, extracted with EtOAc (3×50 mL), and dried over $MgSO_4$. After removal of the solvent in vacuum, the residue was purified by silica gel column chromatography with hexane/ethyl acetate 4:1 to give (−)-**29** (81 mg, 49%) as a colorless oil. $[\alpha]_D^{20} = -56.71$ ($c = 1.11$ in $CHCl_3$); ^1H NMR

(250 MHz, $CDCl_3$): $\delta = 1.27$ (t, $^3J(H,H) = 7.2$ Hz, 3H), 1.97 (m, 1H), 2.02 (d, $^3J(H,H) = 15.2$ Hz, 1H), 2.32–2.40 (m, 2H), 2.48 (ddd, $^3J(H,H) = 15$, 7.5, 5 Hz, 1H), 4.14 (q, $^3J(H,H) = 7.2$ Hz, 2H), 4.20 (d, $^3J(H,H) = 7.4$ Hz, 1H), 5.28 (s, 2H), 7.39 ppm (s, 5H); MS(ISP): *m/z* 388 [$M^+ + NH_4$]; ISP HRMS [$M^+ + Na$]: calcd. 393.12817; found 393.12805.

*(1S,2R,3S,5R,6S)-2,3-Diamino-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (+)-**30***: A solution of (−)-**29** (45 mg, 0.122 mmol) in HOAc (3.8 mL) and H_2O (0.95 mL) was hydrogenated in the presence of Pd/C (12 mg, 10% Pd/C) at 23 °C for 18 h. The catalyst was removed by filtration, the filter cake washed with water. After removal of the solvent in vacuum, the brown residue was refluxed in 10% HCl (8.9 mL) for 4 h. The solution was cooled to 23 °C, filtered, washed with water, and evaporated to dryness. The remaining beige solid was dissolved in EtOH (7.1 mL) and propylene oxide (3.8 mL) and refluxed for 15 min, whereupon the amino acid precipitated. After cooling to 23 °C, the product was filtered off, washed with ether, and dried to give (+)-**30** (19 mg, 79%) as a beige solid. mp: > 250 °C; $[\alpha]_D^{20} = +9.50$ ($c = 0.29$ in H_2O); ^1H NMR (250 MHz, D_2O): $\delta = 1.80$ (t, $^3J(H,H) = 3$ Hz, 1H), 2.00 (dd, $^3J(H,H) = 15$, 5 Hz, 1H), 2.10 (m, 1H), 2.15 (dd, $^3J(H,H) = 5$, 3 Hz, 1H), 2.82 (ddd, $^3J(H,H) = 15$, 10, 5 Hz, 1H), 4.07 ppm (dd, $^3J(H,H) = 10$, 5 Hz, 1H); MS(ISN): *m/z* 199 [M^- − H].

*(1S,2R,5R,6S)-2-Acetylamino-3-oxo-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (+)-**31***: A solution of (+)-**16** (974 mg, 2.84 mmol) in thioacetic acid (6.5 mL) was stirred at 70 °C for 2 days. The reaction mixture was concentrated in vacuum and subjected to silica gel column chromatography with hexane/ethyl acetate 3:2→1:1 to yield (+)-**31** (726 mg, 71%) as a pink oil. $[\alpha]_D^{20} = +44.33$ ($c = 0.97$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta =$ 1.25 (t, $^3J(H,H) = 7.1$ Hz, 3H), 1.63 (t, $^3J(H,H) = 3.5$ Hz, 1H), 2.05 (s, 3H), 2.32 (ddd, $^3J(H,H) = 7$, 6, 3.5 Hz, 1H), 2.54 (d, $^2J(H,H) = 19$ Hz, 1H), 2.90 (dd, $^{2,3}J(H,H) = 19$, 6 Hz, 1H), 3.06 (dd, $^3J(H,H) = 7$, 3.5 Hz, 1H), 4.12 (m, 2H), 5.16 (d, $^2J(H,H) = 12$ Hz, 1H), 5.25 (d, $^2J(H,H) =$ 12 Hz, 1H), 7.24–7.35 ppm (m, 5H); MS(ISP): *m/z* 360 [$M^+ + H$].

*(1S,2R,3R,5R,6S)-2-Acetylamino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (−)-**32** and (1S,2R,3S,5R,6S)-2-Acetylamino-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic 2-benzyl ester 6-ethyl ester (+)-**34***: $LiBH_4$ (5 mg, 0.23 mmol) at −50 °C was added to a solution of (+)-**31** (77 mg, 0.214 mmol) in EtOH (2 mL) and THF (1 mL) and the mixture was stirred at −50 °C for 45 min. The reaction was quenched by addition of 1 N HCl (approximately 0.5 mL), warmed to 23 °C, and stirred for 10 min. After dilution with ethyl acetate and extraction with sat. $NaHCO_3$ solution, brine, and dried over $MgSO_4$ the crude product was purified by silica gel column chromatography with toluene/acetone 3:1 to give (−)-**32** (33 mg, 43%, less polar product) as a colorless oil and (+)-**34** (35 mg, 45%, more polar product) as a white solid. (−)-**32**: $[\alpha]_D^{20} = -11.24$ ($c = 1.18$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.26$ (t, $^3J(H,H) = 7.2$ Hz, 3H), 1.76 (t, $^3J(H,H) =$ 3.5 Hz, 1H), 2.07 (m, 1H), 2.10 (s, 3H), 2.16 (dd, $^3J(H,H) = 6.1$, 3.0 Hz, 1H), 2.30–2.41 (m, 3H), 3.97–4.13 (m, 3H), 5.24 (bs, 2H), 6.19 (bs, 1H), 7.26–7.38 ppm (m, 5H); MS(ISP): *m/z* 362 [$M^+ + H$]. (+)-**34**: mp: 145–147 °C; $[\alpha]_D^{20} = +42.44$ ($c = 1.01$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.25$ (t, $^3J(H,H) = 7.1$ Hz, 3H), 1.98 (m, 1H), 2.05–2.11 (m, 1H), 2.06 (s, 3H), 2.18 (t, $^3J(H,H) = .5$ Hz, 1H), 2.27–2.41 (m, 2H), 2.59 (ddd, $^{2,3}J(H,H) = 13$, 7, 5 Hz, 1H), 4.12 (m, 2H), 4.68 (dd, $^3J(H,H) = 7$, 5 Hz, 1H), 5.17 (s, 2H), 6.30 (bs, 1H), 7.26–7.40 ppm (m, 5H); MS(ISP): *m/z* 362 [$M^+ + H$].

*(1S,2R,3R,5R,6S)-2-Acetylamino-3-deutero-3-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester (−)-**33** and (1S,2R,3S,5R,6S)-2-Acetylamino-3-deutero-3-hydroxy-bicyclo[3.1.0]-hexane-2,6-dicarboxylic 2-benzyl ester 6-ethyl ester (+)-**35***: Sodium borodeuteride ($NaBD_4$) (24.5 mg, 0.584 mmol) at −50 °C was added to a solution of (+)-**31** (105 mg, 0.292 mmol) in EtOH (1.9 mL) and THF (1.0 mL) and the mixture was stirred at −50 °C for 90 min. The reaction was quenched by addition of AcOH (ca. 0.5 mL), warmed to 23 °C, and stirring for 10 min. After dilution with ethyl acetate, 1 N HCl was added, followed by washings with sat. $NaHCO_3$ solution, brine, and dried over $MgSO_4$. After removal of the solvent in vacuum the crude product was purified by silica gel column chromatography with toluene/acetone 3:1 to give (−)-**33** (45 mg, 43%, less polar product) as a colorless oil and (+)-**35** (50 mg, 47%, more polar product) as a white solid. (−)-**33**: $[\alpha]_D^{20} = -11.18°$ ($c = 0.90$, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.26$ (t, $^3J(H,H) = 7.2$ Hz, 3H), 1.98 (m, 1H), 1.74 (t, $^3J(H,H) = 3.5$ Hz, 1H), 2.04–2.10 (m, 1H), 2.09 (s, 3H), 2.17 (dd, $^3J(H,H) = 7$, 2.9 Hz, 1H), 2.35–2.40 (m, 3H), 4.10 (m, 2H), 5.24 (s, 2H), 6.33 (bs, 1H), 7.26–7.37 ppm (m, 5H); MS(ISP): *m/z* 363 [$M^+ + H$]. (+)-**35**: mp: 145–146 °C; $[\alpha]_D^{20} = +44.33$ ($c = 1.03$ in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.24$ (t, $^3J(H,H) = 7.1$ Hz, 3H), 1.97 (ddd, $^3J(H,H) = 8$, 6.5, 3 Hz, 1H), 2.04 (s, 3H), 2.06 (dd, $^3J(H,H) =$ 13.5, 3.7 Hz, 1H), 2.17 (t, $^3J(H,H) = 3.1$ Hz, 1H), 2.32 (dd, $^3J(H,H) =$ 6.6, 3.0 Hz, 1H), 2.56 (dd, $^3J(H,H) = 13.5$, 5 Hz, 1H), 2.58 (s, 1H), 4.09 (m, 2H), 5.23 (s, 2H), 6.42 (bs, 1H), 7.28–7.40 ppm (m, 5H); MS(ISP): *m/z* 363 [$M^+ + H$].

*(1S,2R,3R,5R,6S)-2-Acetylamino-3-hydroxy-3-tritio-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid 2-benzyl ester 6-ethyl ester **36** and (1S,2R,3S,5R,6S)-2-Acetylamino-3-hydroxy-3-tritio-bicyclo[3.1.0]hexane-2,6-dicarboxylic 2-benzyl ester 6-ethyl ester **37***: Radiochemical samples were counted in a wallac WinSpectral 1414 Liquid Scintillation Counter using OptiPhase "HiSafe"3 as scintillation cocktail. *n*-Butyllithium (200 μL, 0.312 mmol, 1.56 M in *n*-hexane) and *N,N,N′,N′*-tetramethylethylenediamine (52 μL, 0.347 mmol) were transferred into a 7.5 mL two-necked-flask mounted to the tritiation apparatus under argon. The stopcock of the side neck was closed and the mixture was stirred under an atmosphere of tritium gas for 2 h 43 min, while the tritium gas pressure dropped from 646 mbar to 559 mbar. Excess tritium gas was reabsorbed onto the uranium bed and the volatile components were lyophilized off. The residue was dried at 10^{-3} mbar for about 5 min. The flask was filled with dry nitrogen and the LiT was suspended in THF (250 μL), which was added by syringe through the silicon septum (Hamilton #76005) of the side neck. Then trimethyl borate (35 μL, 0.314 mmol) was added and the mixture was stirred for 10 min. The two-necked-flask was disconnected from the tritiation apparatus and equipped with a balloon filled with argon. (+)-**31** (118.7 mg, 0.33 mmol) in THF (250 μL) was added at −60 °C. After stirring for 30 min the reaction was quenched by adding 1 N HCl (0.31 mL). The reaction mixture was partitioned between EtOAc and H_2O. The organic phase was washed once with saline and dried over Na_2SO_4. The total ^3H-activity of the crude product was 5.18 Ci. Column chromatography using 15 g of Lichroprep Si60 25–40 μm (Merck Art.1.09390) with toluene/acetone 5:1 afforded 1.152 Ci of **36** with 99% radiochemical purity according to TLC (toluene/acetone 3:1). The specific activity was 23.9 Ci mmol^{-1} according to mass spectrometry. In addition about 2 Ci of **37** were isolated.

*(1S,2R,3R,5R,6S)-2-Amino-3-hydroxy-3-tritio-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid **38** [^3H]-HYDIA*: **36** (576 mCi) was refluxed in 10% HCl (5 mL) for 6 h. The reaction mixture was diluted with H_2O (5 mL) and applied onto a Dowex 50WX8 100–200 mesh cation exchange column (7.5×70 mm). The column was rinsed with H_2O (20 mL). Elution with 2 N NH_4OH (20 mL) afforded **38** (280 mCi). The radiochemical purity was 91.7% according to TLC (*n*BuOH/HOAc/H_2O 3:1:1). The crude product (56 mCi) was purified by HPLC on a μBondapak C18 column (3.9×300 mm) using H_2O/acetonitrile 95:5 (*v/v*) as mobile phase with a flow rate of 0.8 mL min^{-1} and UV-detection at 220 nm. The total ^3H-activity obtained was 43 mCi and the radiochemical purity was 98.1% according to TLC (*n*BuOH/HOAc/H_2O 3:1:1).

Biology

Materials: Six day old and adult Sprague Dawley rats were obtained from Biological Research Laboratories (BRL) (Füllinsdorf, Switzerland). L-2-amino-4-phosphonobutanoate (L-AP4), (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD), and (*S*)-3,5-dihydroxyphenylglycine ((*S*)-DHPG), were all obtained from Tocris Cookson (Bristol, U.K.). LY354740 ((1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo-[3.1.0]-hexane-2,6-dicarboxylate) was synthesized in our laboratory by Dr. G. Adam. [^{3}H]-LY354740 (s.a. 35 Ci mmol^{-1}) was synthesized by Dr. P. Huguenin at the Roche chemical and isotope laboratories according to a procedure developed by Dr H. Stadler. [^{3}H]-L-AP4 was purchased from Tocris Cookson (Bristol, U.K.). [^{3}H]-MK801 and [^{3}H]-Kainate were purchased from NEN Radiochemicals; [^{3}H]-RO0488587 was synthesized at Amersham International. Glycine, *N*-[4-[6-[(acetyloxy)methoxy]-2,7-dichloro-3-oxo-3H-xanthen-9-yl]-2-[2-[2-[bis[2-[(acetyloxy)methoxy]-2-oxyethyl]amino]-5-methylphenoxy]-ethoxy]phenyl]-*N*-[2-[(acetyloxy)methoxy]-2-oxyethyl]-(acetyloxy)-methyl ester, (Fluo-3 AM) was purchased from Molecular Probes, Inc. (Eugene, OR, USA). The cAMP measurement kit (RPN 225) was purchased from Amersham (Zürich, Switzerland). Forskolin 7β-deacetyl-7β-[γ-(*N*-methyl-piperazino)-butyryl] dihydrochloride, a highly water-soluble form of forskolin, was obtained from Calbiochem (Lucerne, Switzerland).

Methods: *Transfections:* cDNAs encoding for rat mGlu1a, mGlu2, mGlu3, mGlu4, mGlu5a, and mGlu7a receptors were generously provided by Prof. S. Nakanishi (Kyoto, Japan). cDNA encoding for rat mGlu8a was cloned by C. Kratzeisen at Hoffmann La-Roche. cDNA for mGlu2, mGlu3, mGlu4, and mGlu8a receptors was subcloned into the Semliki Forest Virus (SFV) expression vector and in vivo packaging of recombinant SFV particles carried out. CHO cells were infected with the virus and the cells were harvested after overnight incubation. cDNA encoding for rat mGlu1a and 5a receptors was transiently transfected into HEK-293 cells using RO 1539, a transfection reagent developed at Hoffmann La-Roche. HEK-293 cells coexpressing rat mGlu7a and the promiscuous G-protein Gα15 were developed for Hoffmann La-Roche at Aurora Bioscience Corporation. For electrophysiological experiments, the 1537 bp hGIRK1 cDNA (corresponding to nt. 1343 to 2880 of AC:U39196)and the 1450 bp hGIRK2C cDNA (corresponding to nt. 582 to 2032 of AC:L78480) were amplified by PCR from a superscript human adult brain cDNA library (Life-Technologies). PCR reactions were performed using the Expand high fidelity PCR system (Roche Molecular Biochemicals, RMB). To construct hGIRK1-GIRK2 dimer, two unique restriction enzyme sites were introduced by site-directed mutagenesis as follows (the nucleotide numbering starts with initial ATG = 1 to 3): hGIRK1, 3′ NheI, introduced by T1489→G, G1492→A, A1493→G, T1494→C and hGIRK2C, 5′ AvrII, introduced by G4→C, C6→T, A8→G. The dimer hGIRK1-GIRK2 was generated by ligation of the 3′-end NheI of hGIRK1 to the 5′-end AvrII of hGIRK2C (AvrII and NheI have compatible overhangs) The translation of the concatenated hGIRK1-GIRK2 cDNAs produces a protein in which the amino acid Asn 496 of hGIRK1 (5 amino acids removed) is connected to Leu 4 of hGIRK2 (3 amino acids removed) through two new amino acids Ala-Arg. The hGIRK1-GIRK2 dimer was subcloned into the expression vector pcDNA3.1/Hygro (Invitrogen). Chinese Hamster Ovary (CHO) cells were co-transfected with a 1:1:1 (*w*/*w*/*w*) mixture of cDNAs encoding the rat mGlu2 and mGlu3 receptors in pBlueScript II : GIRK1-GIRK2 : EGFP (enhanced green fluorescent protein) plasmids using FuGENE™6 transfection reagent (RMB).

Membrane preparation: Membranes from cells infected with mGlu2, mGlu3, mGlu4, and mGlu8a receptors containing SFV were prepared by homogenization of the cells with a polytron (Kinematica AG, Littau, Switzerland) for 10 s at 10000 rpm in a HEPES-NaOH 20 mM, EDTA 10 mM pH 7.4 buffer. After centrifugation at 48.000 g for 30 min at 4 °C the pellet was washed once in a HEPES-NaOH 20 mM, EDTA 0.1 mM pH 7.4 buffer and then resuspended in a Tris-HCl 50 mM, $MgCl_2$ 2 mM pH 7.4 buffer for mGlu2 and mGlu3 receptor containing membranes or in a HEPES-NaOH 30 mM, $MgCl_2$ 1.2 mM, NaCl 110 mM, $CaCl_2$ 2.5 mM, cystine 100 μM, pH 8 buffer for mGlu4 and mGlu8 receptor containing membranes. The membrane suspension was frozen at −80 °C before use.

Binding experiments: [^{3}H]-LY354740 binding on mGlu2 and mGlu3 receptor have been performed according to Schweitzer et al.[23] ^{3}H-L-AP4 binding on mGlu4 and mGlu8a was performed according to Malherbe et al.[28] ^{3}H-MK 801 (NMDA),[^{3}H]-RO0488587 (AMPA), and ^{3}H-kainate binding were performed according to Mutel et al.[29]

2nd messenger measurements: (1*S*,3*R*)-ACPD-stimulated GTPγS^{35} binding was performed using mGlu2 receptor transfected cell membranes according to Cartmell et al.[23a] [Ca^{2+}]i measurement were performed on mGlu1a, 5a, and 7a transfected HEK-293 cells after incubation of the cells with Fluo-3 AM for 1 h and four washes with assay buffer (DMEM supplemented with Hank's salt and 20 mM HEPES). [Ca^{2+}]i measurement were done using a fluorometric imaging plate reader (FLIPR, Molecular Devices Corporation, La Jolla, CA, USA). cAMP accumulation in adult rat striatal slices was measured according to Cartmell et al.[23b] cAMP levels were quantified using a commercially available enzyme immunoassay kit (RPN 225, Amersham). PI hydrolysis in 6 day old rat cerebellar slices was performed according to Schaffhauser et al.[30] Protein concentration was measured after solubilization with 1% of SDS using the Pierce method. Concentration effect curves analysis were carried out using a four parameter logistic equation fitting with the computer program Origin (Microcal software Inc., Northampton, MA, USA). The experiments were performed in triplicate, three times.

Electrophysiology: Experiments were performed with the whole-cell configuration of the patch-clamp technique. Pipettes were filled with a solution containing (in mM): KCl 130, HEPES 10, K-BAPTA 5, $MgCl_2$ 1, Na_2-GTP 0.3, Na_2-ATP 3, pH 7.2 with KOH, osmolarity adjusted to 310 mOsm/L with sucrose. The cells were superfused with a solution that contained (in mM): NaCl 122.25, KCl 30, $CaCl_2$ 2, $MgCl_2$ 2, HEPES 10, D-Glucose 11, pH 7.4 with NaOH, osmolarity adjusted to 340 mOsm/L with sucrose. The pipette resistances ranged from 2 to 3 MΩ. Currents were amplified with an Axopatch 200 A amplifier, filtered at 5 KHz and digitized at 10 KHz with a Digidata 1200 A acquisition board for subsequent storage on a Dell Optiplex PC. The data acquisition and analysis were performed with the pClamp 8 software package.

Hippocampal slices (400 μm) were cut from the brain of 90–140 g male Roro rats, placed in a submerged chamber and perfused with a solution containing (in mM): NaCl 124, KCl 2.5, $MgSO_4$ 2, $CaCl_2$ 2.5, KH_2PO_4 1.25, $NaHCO_3$ 26, D-Glucose 10, sucrose 4, gassed with 95% O2/5% CO2 (pH 7.4, 307 mOsm) with sucrose at a rate of 2 mL min^{-1} and maintained at 35 °C. fEPSPs were evoked by stimulation of the medial perforant path input to the dentate gyrus (0.033 Hz, 100 μs, 40% relative maximum amplitude) and recorded in the dentate gyrus mid-moleculare. Drugs were applied by bath perfusion. LY354740 was synthesized at F. Hoffmann-La Roche. Inhibition curves were fitted according to the Hill equation.[31]

Radioligand binding to tissue sections:[32] Brains were rapidly dissected from anesthetized (5% Fluothane for 30 s) Roro rats (a Wistar strain bred at RCC Ltd., Itingen, Switzerland) and immediately frozen in dry ice. Parasagittal cryostat-cut sections (10 μm thick) were mounted on precleaned slides and stored at −20 °C until use. Assay buffer: 50 mM Tris-HCl buffer, pH 7.0. Binding conditions: Preincubation in assay buffer + 10 mM EDTA (22 °C, 10 min), fol-

lowed by a further preincubation in assay buffer alone (10 min). Incubation with 50 nM **38** [^{3}H]-HYDIA in assay buffer + 2 mM $CaCl_2$ + 2 mM $MgCl_2$ (22 °C, 60 min). Nonspecific binding was determined in the presence of 10 μM (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine **2** (DCG-IV). The incubation was followed by three washes (2×30 s + 1 min) in ice cold assay. The sections were dipped in ice cold distilled water to remove buffer salts before being dried in an air stream (4 °C).

Spontaneous locomotor activity studies in mice were carried out following the experimental conditions described by Spooren et al.[26] The experimental procedures used in the present investigation received prior approval from the City of Basel Cantonal Animal Protection Committee based on adherence to federal and local regulations.

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