Synthesis and Pharmacological Characterization of All Sixteen Stereoisomers of 2-(2′-Carboxy-3′-phenylcyclopropyl)glycine. Focus on (2*S*,1′*S*,2′*S*,3′*R*)-2-(2′-Carboxy-3′-phenylcyclopropyl)glycine, a Novel and Selective Group II Metabotropic Glutamate Receptors Antagonist

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All 16 2-(2′-carboxy-3′-phenylcyclopropyl)glycine (PCCGs) stereoisomers **32**−**47** have been prepared from the corresponding racemic aldehydes **12**−**15** following an enantiodivergent synthetic protocol. Compounds **32**−**47** were evaluated by a number of binding and functional experiments as potential ligands for several classes of excitatory amino acid receptors, including metabotropic glutamate receptors (mGluR1a, mGluR2, mGluR4) and ionotropic glutamate receptors (NMDA, KA, AMPA) as well as sodium-dependent and calcium/chloride-dependent glutamate transport systems. The stereolibrary of compounds **32**−**47** appears to be endowed with a peculiar pharmacological profile. PCCG-2 (**33**) and PCCG-3 (**34**) displaced labeled kainate at low micromolar concentration; PCCG-9 (**40**) and PCCG-11 (**42**) weakly interacted with the NMDA site; PCCG-5 (**36**), PCCG-10 (**41**), and PCCG-12 (**43**) showed to be potent inhibitors of Ca^{2+}/Cl^{-}-dependent glutamate transport system. Most interestingly, PCCG-4 (**35**) has been shown to be able to antagonize (IC_{50} = 8 μM) the effects of glutamate on forskolin-stimulated cAMP formation in BHK cells expressing mGluR2. Uneffective at mGluR1, **35** is a weak mGluR4 agonist (EC_{50} = 156 μM) and has no effect on either ionotropic receptors or glutamate transport systems, thus demonstrating to be a novel selective mGluR2 antagonist with a 6-fold increase in potency over previously reported antagonists.

Introduction

The continuing quest for selective ligands acting at excitatory amino acid (EAA) receptors is fostered by the important role that these receptors play in areas such as development plasticity, learning, and neurodegenerative and acute diseases and by the constant disclosure of new receptor subtypes whose pharmacological and physiological characterization is a key step on route to the discovery of clinically useful agents.[1] Synaptically released glutamic acid (L-Glu) interacts with a variety of proteins, including ionotropic receptors, metabotropic receptors, transport systems, and metabolizing enzymes. The recent years have seen, in particular, a growing interest in the field of metabotropic glutamate receptor (mGluRs) family,[2] due to the intriguing therapeutic opportunities offered by the modulation of its members. Indeed, mGluRs have been shown to play important roles in the induction of long term potentiation (LTP) or long term depression (LTD) of synaptic transmission,[3] regulation of the baroceptive reflex,[4] spatial learning,[5] preprogramming of rapid movements, motor learning, and the process of postural-kinetic integration[6] as well as in the pathogenesis of either acute (*e.g.*, ischemia, epilepsy, hipoxia) or chronic (*e.g.*, Alzheimer's disease, Parkinsonism, AIDS-related dementia) diseases. After the independent discovery of the first mGluR by Sladeczeck *et al.* in 1985,[7] and by Nicoletti *et al.* in 1986,[8] the multiplicity of this class has been disclosed by expression cloning studies.[9] Currently, eight mGluRs (and several splice variants) have been isolated and subdivided in three groups according to sequence homology, signal transduction, and pharmacology as indicated in Chart 1. Briefly, the first group includes mGluR1 and mGluR5 which are coupled

Chart 1. Classification of Cloned Metabotropic Glutamate Receptors

Group	Receptor Subtype	Splice Variants	Effector System
I	mGluR1	a	PLC / IP3 ↑ ; AC / cAMP ↑
		b	PLC / IP3 ↑
		c	PLC / IP3 ↑
		d	PLC / IP3 ↑
		e	?
	mGluR5	a	PLC / IP3 ↑
		b	PLC / IP3 ↑
II	mGluR2		AC / cAMP ↓
	mGluR3		AC / cAMP ↓
III	mGluR4	a	AC / cAMP ↓
		b	AC / cAMP ↓
	mGluR6		AC / cAMP ↓
	mGluR7		AC / cAMP ↓
	mGluR8		AC / cAMP ↓

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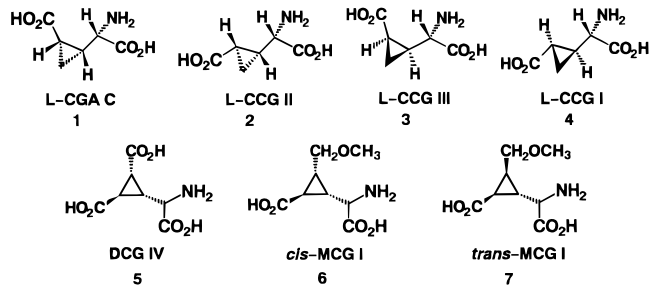
Chart 2



to IP_3/Ca^{2+} signal transduction *via* activation of phospholipase C (PLC), whereas the members of group II, mGluR2 and mGluR3, as well as those of group III, mGluR4, mGluR6 mGluR7, and mGluR8, are negatively linked to the activity of adenylyl cyclase, thus resulting in the inhibition of cAMP accumulation.

Several classes of glutamate analogs have been used in the past as chemical probes for characterizing EAA receptors: among them, (carboxycyclopropyl)glycines (CCGs) have represented a valuable source of potent and selective ligands for the various members of the glutamate receptor family, including ionotropic receptors, metabotropic receptors, and uptake carrier proteins. The introduction of a cyclopropyl moiety on the glutamate skeleton induces chirality, partially reduces the conformational flexibility thus allowing its selective interaction with a reduced number of recognition sites, and, most importantly, defines foreseeable orientations of the ω-carboxylate group with respect to the α-amino acidic moiety, thus allowing the assessment of the conformational requirements of L-Glu acting at its receptor subtypes. Thus, among the eight possible CCG diastereoisomers, we first reported that (2*S*,1′*R*,2′*S*)-(carboxycyclopropyl)glycine [L-CGAC (CCG IV), **1**][10] is a potent and selective NMDA agonist, while the corresponding isomers (2*S*,1′*R*,2′*R*)-CCG II (**2**) and (2*S*,1′*S*,2′*R*)-CCG III (**3**) have been reported to be L-Glu uptake inhibitors[11] (Chart 2). In 1990, Shinozaki's group first described the (2*S*,1′*S*,2′*S*)-CCG isomer (CCG I, **4**)[12] as a potent and rather selective group II mGluRs agonist. 1,2,3-Trisubstituted CCG derivatives have also been evaluated as ligands for glutamatergic pathways. Among the derivatives of this class so far reported, (2*S*,1′*R*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG IV, **5**)[13] has revealed to be a particularly interesting compound, being a potent mGluR2 agonist endowed with neuroprotective properties,[14] also active as an agonist at the NMDA receptor site. Attempts to increase the selectivity of these molecules toward metabotropic receptors have resulted in the substitution of the 3′-carboxy group of **5** with a methoxymethyl group thus leading to (2*S*,1′*R*,2′*R*,3′*R*)-[2-carboxy-3-(methoxymethyl)cyclopropyl]glycine (*cis*-MCG I, **6**),[15] also a group II mGluRs agonist, characterized by lower activity and increased selectivity over ionotropic receptors with respect to **5**. Interestingly, *trans*-MCG (**7**), an epimer of **6** and **5** at C-3′, is also a group II mGluRs agonist, with the same order of activity of **5**.[16] In view of the limited exploitation of trisubstituted CCGs as potential EAA receptor ligands and as a continuation of our research in the field of EAA receptors and in particular in the metabotropic one,[17] we reasoned that the introduction of a hydrophobic moiety, such as a phenyl ring, in position 3′ of 2-(2′-carboxycyclopropyl)glycine can be proved useful for mapping the presence of still unexplored accessory areas in the recognition site of members of the glutamate receptor family.

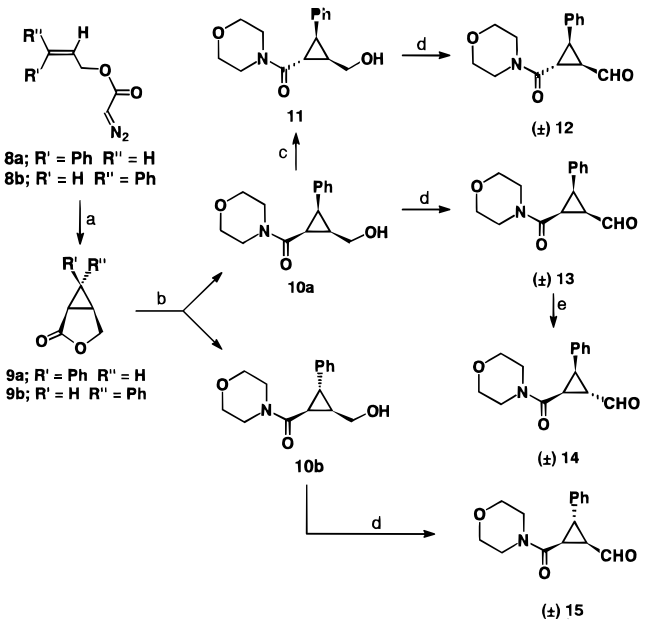
While previous knowledge of conformational requirements of ligands acting at various members of EAA[17,20,21] receptors could have addressed the synthetic efforts toward the preparation of 2-(2′-carboxy-3′-phenylcyclopropyl)glycines (PCCGs) with definite stereochemistry, expected to interact with particular members of EAA receptors, we decided to follow an enantiodivergent synthetic protocol with the aim of producing a complete stereolibrary of (phenylcarboxycyclopropyl)glycines. The availability of this set of compounds will be useful for either a better pharmacological definition of already known or the characterization of still unknown members of the metabotropic glutamate receptor family and, evidently, also for further characterization of ionotropic receptors and glutamate transport systems.

Chemistry

The problem of synthesizing all 16 (phenylcarboxycyclopropyl)glycine stereoisomers **32**−**47** consisted of two different tasks. The first one, the synthesis of the four racemic aldehydes **12**−**15** chosen as precursors for further enantiodivergent synthetic elaborations, was accomplished essentially by utilizing the synthetic protocol developed by Martin *et al*.,[22] involving, as a key step, the intramolecular cyclopropanation of an allylic diazo ester precursor.[23] By this route, the enantioselective synthesis of (+)- and (−)-**13** and (+)- and (−)-**14** has recently been reported.[24] As racemates, aldehydes (±)-**13** and (±)-**14** have been previously reported by the same author as uncharacterized intermediates,[22] while (±)-**12** and (±)-**15** are still unreported. Briefly, the first step is the bis(*N*-*tert*-butylsalicylaldimine)-copper(II)-catalyzed[25] decomposition in refluxing toluene of (*Z*)-cinnamoyl diazo ester **8a** to give the racemic *endo*-lactone **9a** (87% yield), which was then converted[26] into the corresponding hydroxymethyl morpholine amide **10a** in 90% yield (Scheme 1). Selective inversion of the configuration at C-1 of alcohol **10a** as previously described[22,24] affords the isomeric alcohol **11**, which, jointly with **10a**, was oxidized (PCC, CH_2Cl_2, room temperature) to give aldehydes (±)-**13** and (±)-**12**, respectively. The third aldehyde, (±)-**14**, was quantitatively obtained by methanolic potassium carbonate-induced isomerization at C-2 of aldehyde (±)-**13**. Aldehyde (±)-**15**, finally, was synthesized starting from (*E*)-cinnamoyl diazo ester **8b**[23] which was submitted to bis(*N*-*tert*-butylsalicylaldimine)copper(II)-catalyzed[25] decomposition to give *exo*-lactone **9b** (96% yield), which was then converted into aldehyde (±)-**15** by sequential conversion into the corresponding morpholine amide **10b** (99% yield) and oxidation (PCC, CH_2Cl_2, room temperature).

With all four racemic aldehydes **12**−**15** in hand, the enantiodivergent transformation of each one of them into the corresponding four possible enantiopure PCCGs was addressed. The key step in the route (Schemes 2 and 3) was a diastereoselective Strecker synthesis involving the nucleophilic addition of a cyanide ion to the Schiff bases formed by condensing each racemic aldehyde (**12**−**15**) with optically active α-phenylglycinol.[27] Since two new contiguous chiral centers are present, each aldehyde was expected to yield the four corresponding diastereoisomeric (2*R*)- and (2*S*)-N-

Scheme 1[a]



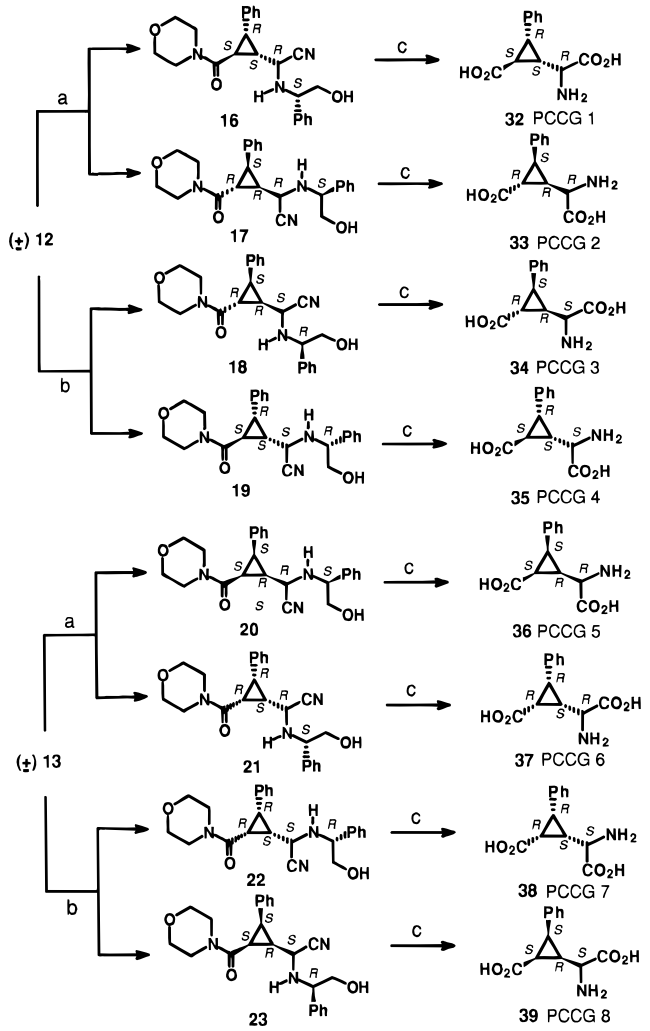
[a] (a) $Cu(TBS)_2$, PhMe, reflux; (b) morpholine, $AlMe_3$, CH_2Cl_2, reflux; (c) Li-HMDS, THF, room temperature; (d) PCC, CH_2Cl_2, room temperature; (e) K_2CO_3, MeOH, room temperature.

substituted α-amino nitrile derivatives in varied amounts according to the configuration of the α-phenylglycinol employed.[27] (*R*)- And (*S*)-α-phenylglycinol preferentially induce opposite chirality in the newly formed asymmetric center. Accordingly, treatment of racemic aldehyde **12** with (*S*)-α-phenylglycinol in MeOH at room temperature for 2 h, followed by reaction of the Schiff base with TMSCN for 12 h at room temperature, yielded a mixture of the expected four α-amino nitriles, as two major and two minor components in *ca*. 8:2 ratio. The diastereoisomeric content of each isomeric pair was *ca*. 1:1 (GC−MS). Medium pressure chromatography (MPC) of the reaction mixture allowed only the separation of the two more abundant constituents. From (*S*)-α-phenylglycinol these were identified as (2*R*)-[(*S*)-(phenylglycinyl)amino] nitrile **16** and (2*R*)-[(*S*)-(phenylglycinyl)amino nitrile **17** in 30% and 15% yields, respectively (for the absolute configuration assignment, see the following section). To isolate the two remaining (2*S*)-α-amino nitriles, aldehyde (±)-**12** was again submitted to the diastereoselective Strecker synthesis utilizing (*R*)-α-phenylglycinol for the formation of the Schiff base. Similarly, the reaction mixture containing the four corresponding α-amino nitriles was submitted to MPC affording (2*S*)-[(*R*)-(phenylglycinyl)amino] nitrile **18** and (2*S*)-[(*R*)-(phenylglycinyl)amino] nitrile **19** in 21% and 32% yields, respectively.

The two (2*R*)-α-amino nitriles **16** and **17** and the two (2*S*)-α-amino nitriles **18** and **19** thus obtained were then submitted to oxidative cleavage with lead tetraacetate,[28] acidic hydrolysis (6 N HCl), and ion exchange chromatography on Dowex 50X2-200 to afford respectively (2*R*,1′*S*,2′*S*,3′*R*)-PCCG-1 (**32**), (2*R*,1′*R*,2′*R*,3′*S*)-PCCG-2 (**33**), (2*S*,1′*R*,2′*R*,3′*S*)-PCCG-3 (**34**), and (2*S*,1′*S*,2′*S*,3′*R*)-PCCG-4 (**35**) with 86%, 74%, 70%, and 73% yields.

The synthetic protocol above described—diastereoselective Strecker synthesis with both (*S*)- and (*R*)-α-phenylglycinol, oxidative cleavage followed by acidic hydrolysis of the corresponding imine, and purification by ion exchange resin chromatography—was

Scheme 2[a]



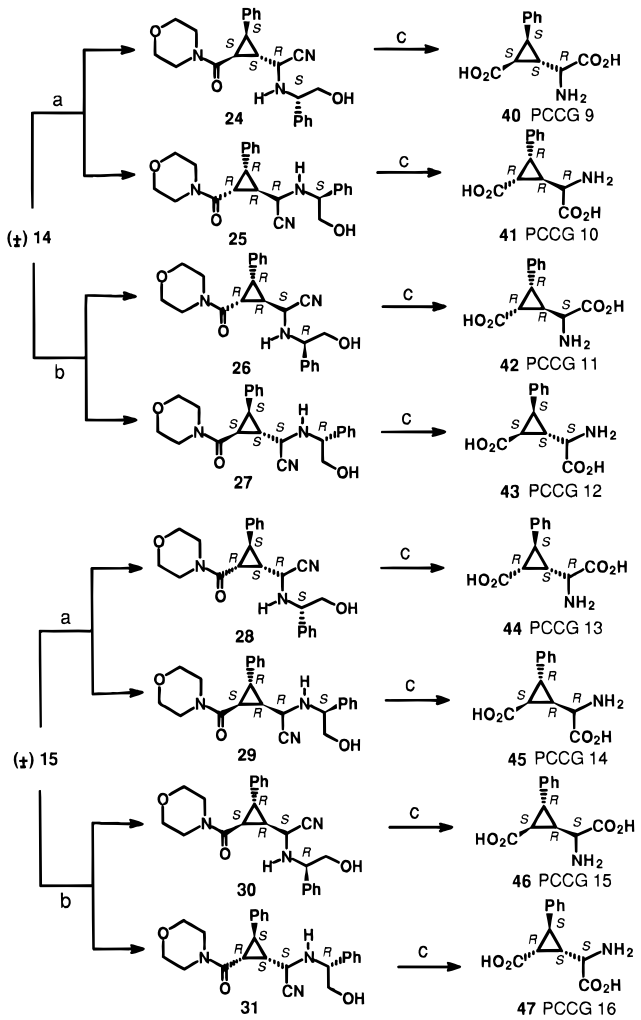
[a] (a) i. (*S*)-α-Phenylglycinol, TMSCN, MeOH, room temperature, 14 h, ii. MPC; (b) i. (*R*)-α-phenylglycinol, TMSCN, MeOH, room temperature, 14 h, ii. MPC; (c) i. $Pb(OAc)_4$, MeOH−CH_2Cl_2, ii. 6 N HCl, reflux, iii. Dowex 50WX2-200.

then applied to the three remaining racemic aldehydes **13**−**15**, thus obtaining: from aldehyde (±)-**13**, (2*R*,1′*R*,2′*S*,3′*S*)-PCCG-5 (**36**), (2*R*,1′*S*,2′*R*,3′*R*)-PCCG-6 (**37**), (2*S*,1′*S*,2′*R*,3′*R*)-PCCG-7 (**38**), and (2*S*,1′*R*,2′*S*,3′*S*)-PCCG-8 (**39**) in 48%, 53%, 48%, and 55% yields, respectively; from aldehyde (±)-**14**, (2*R*,1′*S*,2′*S*,3′*S*)-PCCG-9 (**40**), (2*R*,1′*R*,2′*R*,3′*R*)-PCCG-10 (**41**), (2*S*,1′*R*,2′*R*,3′*R*)-PCCG-11 (**42**), and (2*S*,1′*S*,2′*S*,3′*S*)-PCCG-12 (**43**) in 62%, 62%, 75%, and 63% yields, respectively; and from aldehyde (±)-**15**, (2*R*,1′*S*,2′*R*,3′*S*)-PCCG-13 (**44**), (2*R*,1′*R*,2′*S*,3′*R*)-PCCG-14 (**45**), (2*S*,1′*R*,2′*S*,3′*R*)-PCCG-15 (**46**), and (2*S*,1′*S*,2′*R*,3′*S*)-PCCG-16 (**47**) in 49%, 75%, 50%, and 55% yields, respectively.

Absolute Configuration Assignment

The absolute configuration assignment to the 16 diastereoisomeric amino acids **32**−**47** was based upon the single-crystal X-ray analysis performed on suitably selected compounds chosen among the N-substituted α-amino nitriles or the corresponding final amino acids derived from the three racemic aldehydes (±)-**12**, (±)-**13**, and (±)-**15**, depending on the quality of their

Scheme 3[a]



[a] (a) i. (*S*)-α-Phenylglycinol, TMSCN, MeOH, room temperature, 14 h, ii. MPC; (b) i. (*R*)-α-phenylglycinol, TMSCN, MeOH, room temperature, 14 h, ii. MPC; (c) i. $Pb(OAc)_4$, $MeOH-CH_2Cl_2$, ii. 6 N HCl, reflux, iii. Dowex 50WX2-200.

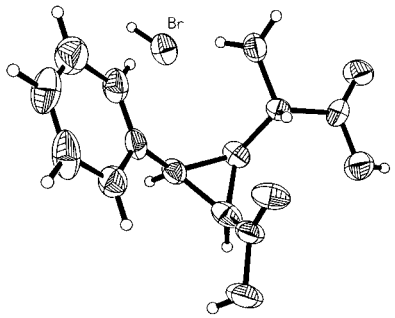


Figure 1. SHELXTL[41] drawing of compound **39**. The non-H atom ellipsoids enclose 50% probability.

crystals, and on the independent, enantioselective synthesis of α-amino nitrile **26** which disclosed the absolute stereochemistry of the four α-amino nitriles derived from aldehyde (±)-**14**. Thus, the N-substituted α-amino nitrile **19** was selected among the compounds derived from aldehyde (±)-**12** and submitted to X-ray analysis which confirmed the *S*-chirality of the α-amino nitrilic center and allowed to fix the absolute configuration at the three cyclopropylic carbon atoms [(1′*S*,2′*S*,3′*R*), Figure 1]. Conversely, the N-substituted α-amino nitrile **18**, proceeding from the same racemic aldehyde **12**, resulted endowed with the same absolute configuration

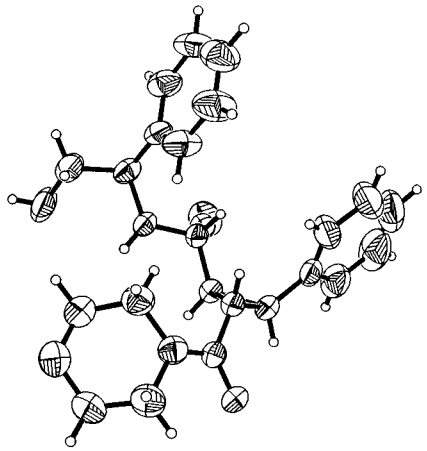


Figure 2. SHELXTL[41] drawing of compound **19**. The non-H atom ellipsoids enclose 50% probability.

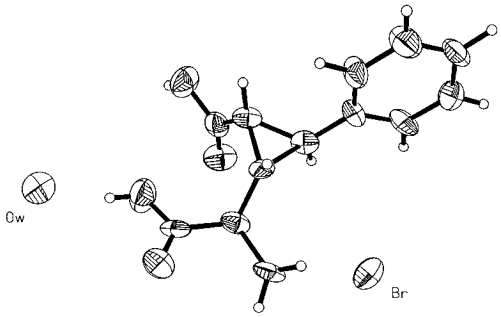


Figure 3. SHELXTL[41] drawing of compound **44**. The non-H atom ellipsoids enclose 50% probability.

(*S*) at the α-amino nitrilic carbon and opposite (1′*R*,2′*R*,3′*S*) at the three cyclopropylic carbons. Moreover, a comparison of the specific rotatory powers of **18** and **19** with those of the two N-substituted α-amino nitriles obtained from the reaction with (*S*)-phenylglycinol (**16** and **17**) revealed the corresponding enantiomeric couples.

The full stereochemical characterization of the compounds derived from aldehyde (±)-**13** was obtained by submitting to X-ray analysis the hydrobromic salt of **39** (Figure 2). Also in this case, the *S*-chirality of the α-amino acidic center was confirmed, and the analysis of the specific rotatory power of the four amino acids **20**−**23** disclosed the enantiomeric couples.

Analogously, the absolute stereochemistry of the compounds derived from aldehyde (±)-**15** was obtained by submitting to X-ray analysis the hydrobromic salt of **44** (Figure 3). In this case, the *R*-chirality of the α-amino acidic center was confirmed, and the analysis of the specific rotatory power of the four corresponding amino acids **44**−**47** revealed the enantiomeric couples.

In the impossibility to obtain suitable crystals for X-ray analysis with compounds derived from aldehyde (±)-**14**, we decided to synthesize the chiral aldehyde (+)-**14** [(1*R*,2*R*,3*R*)-1-(4-morpholinylcarbonyl)-2-formyl-3-phenylcyclopropane] by following Martin's procedure[24] in order to obtain, from the subsequent diastereoselective Strecker synthesis, only two α-amino nitriles. According to the above considerations on the inductive effect of (*R*)-α-phenylglycinol, a more abundant (2*S*,1′*R*,2′*R*,3′*R*)-*N*-[(*R*)-α-phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile was obtained. A comparison of the spectroscopic data as well as the specific rotatory power values of this compound

Table 1. Glutamate Receptor Selectivity of the Stereoisomers of 2-(2′-Carboxy-3′-phenylcyclopropyl)glycine (**32**−**47**)[a]

	glutamate receptor binding K_i (μM)					glutamate uptake IC_{50} (μM)	
	AMPA	NMDA	KA	mGluR1	mGluR4a	Na^+-dep	Ca^{2+}/Cl^--dep
PCCG-1 (**32**)	212 ± 32	193 ± 38	>190	>270	>280	>300	>300
PCCG-2 (**33**)	125 ± 15	129 ± 23	15 ± 3	135 ± 21	>280	>300	>300
PCCG-3 (**34**)	124 ± 25	107 ± 15	25 ± 3	60 ± 15	>280	>300	41 ± 7
PCCG-4 (**35**)	>220	>200	>190	>270	50 ± 6	>300	>300
PCCG-5 (**36**)	>220	>200	117 ± 14	>270	>280	>300	32 ± 6
PCCG-6 (**37**)	251 ± 24	>200	122 ± 10	44 ± 3	>280	>300	143 ± 23
PCCG-7 (**38**)	>220	>200	155 ± 25	>270	>280	>300	270 ± 38
PCCG-8 (**39**)	>220	>200	>190	260 ± 31	>280	>300	233 ± 29
PCCG-9 (**40**)	>220	29 ± 5	>190	>270	>280	>300	138 ± 19
PCCG-10 (**41**)	>300	>200	>190	>270	>280	>300	23 ± 2
PCCG-11 (**42**)	>220	25 ± 5	>190	205 ± 29	>280	>300	85 ± 11
PCCG-12 (**43**)	>220	192 ± 21	126 ± 18	>270	>280	>300	7 ± 2
PCCG-13 (**44**)	>220	>200	>190	>270	>280	>300	>300
PCCG-14 (**45**)	160 ± 30	123 ± 24	155 ± 22	>270	>280	>300	64 ± 5
PCCG-15 (**46**)	141 ± 24	>200	144 ± 13	>270	>280	>300	213 ± 45
PCCG-16 (**47**)	285 ± 41	>200	221 ± 29	>270	>280	>300	185 ± 35

[a] The values (mean ± SEM) are the inhibitory constants (K_i) for displacement of radioligand binding or potencies (IC_{50}) for inhibiting uptake from rat cortical homogenates. IC_{50} values were calculated from at least three data points which were fitted to a sigmoidal curve by a nonlinear regression analysis using the GraphPad Prism program (ISI, Philadelphia). Competition binding experiments were performed as described in the Experimental Section, and K_i values were calculated from at least two individual experiments performed in triplicate using the equation $K_i = IC_{50}/(1 + [L])/K_d$, where [L] is the concentration of the radioligand.[31,46] In experiments ($n = 2$ in triplicate) measuring inhibition of [^{3}H]glutamate uptake (sodium-dependent glutamate uptake) or [^{3}H]-L-AP4 (calcium/chloride-dependent glutamate uptake) into rat cortical synaptosomes, nonspecific uptake was determined by the uptake in the presence of 1 mM glutamate.

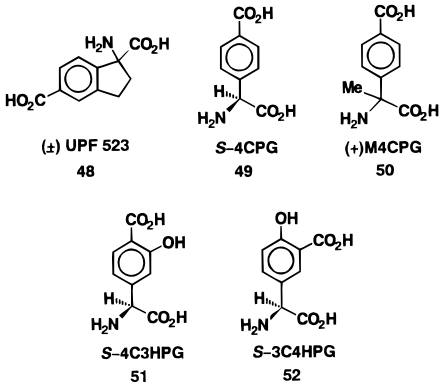
with those derived from α-amino nitriles **26** and **27** enabled us to assign to nitrile **26** the same absolute configuration of the above enantioselectively synthesized α-amino nitrile.

Biological Results

The pharmacological profiles of the 16 stereoisomers of 2-(2′-carboxy-3′-phenylcyclopropyl)glycine (**32**−**47**) were examined by evaluating (1) the inhibition of the binding of selective ligands for AMPA, NMDA, and kainate receptors to rat brain membranes,[29] (2) the inhibition of the binding of labeled glutamate to membranes prepared from baby hamster kidney (BHK) cells expressing mGluR1a[30] or of the binding of labeled L-AP4 to membranes obtained from cells expressing mGluR4,[31] (3) the inhibition of the sodium-dependent glutamate transport into synaptosomes[32] and the inhibition of the calcium/chloride-dependent glutamate uptake,[31] and (4) the antagonism of glutamate effects on PI hydrolysis or forskolin-stimulated cAMP formation[33] in BHK cells expressing mGluR1a, mGluR2, or mGluR4.

As shown in Table 1, some of the 16 stereoisomers displaced the selective ligands from the ionotropic glutamate receptors. Thus, low micromolar concentrations of **33** and **34** displaced labeled kainate, but while **33** was a relatively selective displacer, **34** also interacted with mGluR1 and the Ca^{2+}/Cl^--dependent glutamate uptake sites. Similarly, **40** and **42** interacted with the NMDA recognition sites and the Ca^{2+}/Cl^--dependent uptake sites. Concentrations of up to 300 μM of the 16 stereoisomers did not significantly reduce the sodium-dependent glutamate uptake. On the contrary, the Ca^{2+}/Cl^--dependent glutamate uptake was significantly reduced by low micromolar concentrations of **43**, **41**, and **36**. (2*S*,1′*S*,2′*S*,3′*S*)-PCCG-12 (**43**) in particular, with an IC_{50} of 7 μM, is the most potent and selective inhibitor of this class so far reported. As far as the mGluRs are concerned, Tables 1 and 2 show that **37**, with an IC_{50} of 140 μM, had an acceptable affinity for mGluR1a, and functional measurements revealed that **37** was an antagonist at this receptor subtype. However, **37** was less potent and selective than other mGluR antagonists such as 1-aminoindan-1,5-dicarboxylic acid (UPF 523, **48**)[18] or (*S*)-(4-carboxyphenyl)glycine (*S*-4CPG, **49**) (Chart 3).[30,34]

Chart 3



One of the stereoisomers we considered interesting for the goals of the present study, was PCCG-4 (**35**) which was not able to interact with the ionotropic glutamate receptors or the uptake sites (see Table 1) but which was able to antagonize, with an IC_{50} of 8 μM (see Table 2), the effects of glutamate on forskolin-induced cAMP formation in BHK cells expressing mGluR2. **35** was also a weak agonist of mGluR4a (EC_{50} = 156 μM) but was ineffective at mGluR1a (Tables 1 and 2) and mGluR5a.[35] This compound seems therefore quite selective for mGluRs negatively coupled to adenylate cyclase and in particular for mGluR2. Currently, the most potent mGluR2 antagonist is (+)-α-methyl-(4-carboxyphenyl)glycine [(+)-M4CPG, **50**] (IC_{50} = 50 μM), which is not selective because it has a similar affinity for mGluR1a.[34] Thus, **35** is about 6-fold more potent than **50** and much more selective since it does not interact with mGluR1a or mGluR5.

Table 2 shows also that larger concentrations of **35** (EC_{50} = 156 μM) activate mGluR4, thus indicating that the affinity of the compound toward this receptor is *ca.* 1 order of magnitude lower than that toward mGluR2. We have no information on whether or not **35** interacts with mGluR3 receptors. However cloned mGluR2 and

Table 2. Summary of the Potencies for Subtypes mGluR1a, mGluR2, and mGluR4 of the Stereoisomers of 2-(2′-Carboxy-3′-phenylcyclopropyl)glycine (**32**−**47**)[a]

	mGluR1a		mGluR2		mGluR4	
	IC_{50} (μM)	EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)
PCCG-1 (**32**)	>300	>300	>300	>300	>300	>300
PCCG-2 (**33**)	>300	>300		122 ± 8	>300	>300
PCCG-3 (**34**)	>300	>300	>300	>300	>300	>300
PCCG-4 (**35**)	>300	>300	8 ± 2			156 ± 25
PCCG-5 (**36**)	>300	>300	>300	>300	>300	>300
PCCG-6 (**37**)	140 ± 11		>300	>300	>300	>300
PCCG-7 (**38**)	>300	>300	>300	>300	>300	>300
PCCG-8 (**39**)	>300	>300	>300	>300	>300	>300
PCCG-9 (**40**)	>300	>300	>300	>300	>300	>300
PCCG-10 (**41**)	>300	>300	>300	>300	>300	>300
PCCG-11 (**42**)	>300	>300	>300	>300	>300	>300
PCCG-12 (**43**)	>300	>300	>300	>300	>300	>300
PCCG-13 (**44**)	>300	>300	>300	>300	>300	>300
PCCG-14 (45)	>300	>300	>300	>300	>300	>300
PCCG-15 (**46**)	>300	>300	>300	>300	>300	>300
PCCG-16 (**47**)	>300	>300	>300	>300	>300	>300

[a] The concentrations for half-maximal inhibition (IC_{50}) or stimulation (EC_{50}) of PI hydrolysis (mGluR1a) or cAMP formation (mGluR2 and mGluR4) were determined from two to four experiments performed in triplicate. Compounds were tested for agonist and antagonist activity as described previously.[31]

mGluR3 are considered to possess a similar pharmacology,[36] and it is certainly possible that **35** does not discriminate between these group II mGluR subtypes. It is also not certain whether **35** is able to interact with the other group III mGluR subtypes (mGluR6, mGluR7, and mGluR8).

Discussion

The results of the testing of the 16 diastereoisomeric 2-(2′-carboxy-3′-phenylcyclopropyl)glycines **32**−**47** for binding affinity and functional activity at ionotropic and metabotropic EAA receptors and for glutamate uptake inhibition have indicated that some of them possess an interesting pharmacological profile. Among the active compounds, we have chosen (2*S*,1′*S*,2′*S*,3′*R*)-PCCG-4 (**35**), the most potent and selective group II mGluRs antagonist so far reported, to carry out a research study aimed at evaluating the structure and conformational features underlying its pharmacological properties. As a general consideration, the introduction of the phenyl ring into the (carboxycyclopropyl)glycine core reduces the already limited conformational freedom of the molecule. In fact, a Monte Carlo random search analysis performed on **35** gave only four stable conformers in comparison with the eight obtained for the parent compound L-CCG I (**4**). The most stable conformer is characterized by a 178° torsional angle around the H−C(2)−C(1′)−H rotatable bond. This is consistent with the X-ray structure of the parent α-amino nitrile derivative **19**, and it is also worth noticing that this conformation is the preferred one in solution, as indicated by the H−C(2)−C(1′)−H proton vicinal coupling constant of 11.2 Hz which, within the limitation of the modified Karplus−Altona equation,[37] corresponds to a torsional angle of 172° (Figure 4). This preferred conformation matches the full extended disposition of α-amino acidic moiety and *ω*-carboxylate group that has been proposed by us[17] and others[21] as the bioactive conformation of L-Glu when acting at group II mGluRs.[38]

The structural features displayed by **35**, together with its interesting profile of the first relatively selective

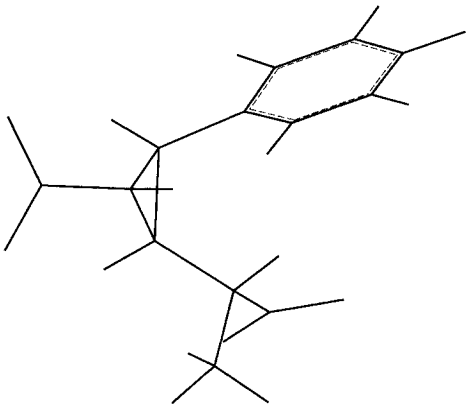


Figure 4. Solution, NMR-derived, and molecular mechanics preferred conformation of PCCG-4 (**35**).

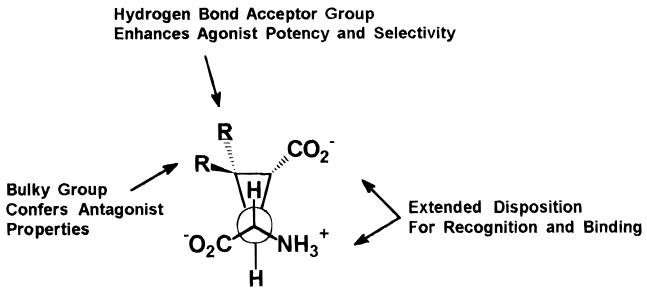


Figure 5. Proposed pharmacophoric requirements for mGluR2 recognition site.

mGluR2 antagonist, can be used to gain some light on the pharmacophore requirements for antagonist at mGluR2 receptor subtype. First of all, the equivalence of spatial disposition of α-amino acidic moiety and *ω*-carboxylate group between **35** and **4** (or **5**) implies that there exists a unique recognition site on mGluR2 where both agonists and antagonists bind. The antagonist profile is then given by the phenyl group of **35** that is likely to occupy an accessory site on the binding pocket. When superimposed, both the phenyl ring of **35** and the methoxymethyl group of **6** showed to occupy the same region of space. The observation that **6** retains the agonist profile of **4** (with lower potency) may be explained by assuming that the methoxymethyl group simply enters the pocket without making additional bonds and without occupying accessory areas responsible for antagonist activity. On the other hand, the 3′ epimerization of **6** yields **7** which displays a higher potency as an agonist.[16] The corresponding epimer on our PCCGs series is completely inactive, thus suggesting that in the case of **7**, the methoxymethyl moiety interacts (probably *via* hydrogen bond) with the binding site but larger substituents cannot be accommodated.

This allows for a preliminary mapping of steric requirements of mGluR2 binding site (Figure 5), being assumed the full extended conformation as the bioactive one. The extended disposition of α-amino acidic moiety and *ω*-carboxylate group defines the spatial requirements for the mGluR2 binding site. A bulky group *trans* to the *ω*-carboxylate can occupy an accessory region on the receptor site and confers the antagonist profile. A hydrogen-bonding acceptor group, such as methoxymethyl, *cis* to the *ω*-carboxylate increases the agonist potency and confers improved selectivity toward other mGluR subtypes. Nevertheless, it should be mentioned that other structures than (carboxycyclopropyl)glycines

have been reported to have both agonist or antagonist profile at mGluR2, although with lower potency, namely, (carboxyphenyl)glycines **49**−**52**.[34] For example, (*S*)-(4-carboxy-3-hydroxyphenyl)glycine [(*S*)-4C3HPG, **51**] is reported to be an agonist at mGluR2 with an EC_{50} = 30 μM, while **50** is an antagonist with an IC_{50} = 50 μM. Since there is apparently no way to superimpose (carboxyphenyl)glycines to (carboxycyclopropyl)glycines either in their proposed bioactive conformations or in otherwise energetically accessible conformations, one should argue that there exist at least two binding sites on mGluR2 receptors.

Conclusion

This study reports the synthesis and preliminary pharmacological characterization of a stereolibrary of conformationally constrained glutamate analogs, useful probes for studies on the structural requirements of glutamate binding to carriers and ionotropic or metabotropic receptors. Low micromolar concentrations of several of the above-mentioned compounds were able to interact with the NMDA (**40** and **42**) or kainate (**33** and **34**) receptors or with the Ca^{2+}/Cl^--dependent glutamate uptake sites (**43**). While studies on the structural profile and the precise pharmacological characterization of these compounds are in progress, we report here that PCCG-4 (**35**) is a potent and relatively selective group II mGluRs antagonist having an IC_{50} of 8 μM. Although larger concentrations of **35** interact also with mGluR4, where the compound behaves as an agonist (EC_{50} = 156 μM), its lack of activity on the uptake sites, ionotropic receptors, and mGluRs linked to the IP_3/Ca^{2+} transduction pathways should facilitate the studies on the role that group II mGluRs have in physiology or pathology.

Experimental Section

General Methods. Melting points were determined by the capillary method on a Büchi 535 electrothermal apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were taken on a Bruker AC 200 spectrometer as solutions in $CDCl_3$ unless otherwise indicated. Carbon magnetic resonance of the final amino acids was performed on the corresponding hydrochloric salts. Proton chemical shifts are reported in ppm downfield from tetramethylsilane, except with D_2O which was also used as an internal standard. Carbon chemical shifts are reported in ppm using MeOH as an internal standard (δ 49.0). The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; td, triple doublet; br s, broad signal. GC−MS was performed on a Hewlett-Packard HP 5890 gas chromatograph (column and conditions: HP-1, 12 m, 0.20 mm i.d., 0.33 μm ft, 150(1′)/280 °C, 10 °C/min) equipped with a mass detector (HP 5971). Flash chromatography was performed on Merck silica gel (0.040−0.063 mm). Medium pressure chromatography (MPC) was performed on Merck LiChroprep Si 60 and LiChroprep RP-8 (reversed phase) lobar columns. Specific rotations were recorded on a Jasco Dip-360 digital polarimeter.

***endo*-6-Phenyl-3-oxabicyclo[3.1.0]hexan-2-one (9a).** A solution of *cis*-3-phenyl-2-propen-1-yl diazoacetate[22] (**8a**; 1.00 g, 4.95 mmol) in dry toluene (165 mL) was added *via* syringe pump to a refluxing solution of bis(*N*-*tert*-butylsalicylaldiminato)copper(II) (0.104 g, 0.25 mmol) in dry toluene (165 mL) magnetically stirred under argon over a period of 12 h. After cooling, the reaction mixture was evaporated to dryness and the residue submitted to flash chromatography; elution with light petroleum containing 10−40% AcOEt afforded the lactone **9a** (0.75 g, 87%): mp 112−3 °C; ^{1}H-NMR ($CDCl_3$) δ 2.60 (2H, m, 1-CH, 5-CH), 2.78 (1H, t, J = 8.8 Hz, 6-CH), 4.05 (1H, dd, J = 0.6, 9.8 Hz, 4-CH_a), 4.35 (1H, dt, J = 2.7, 9.8 Hz, 4-CH_b), 7.20−7.35 (5H, m, aromatics).

1-(4-Morpholinylcarbonyl)-2-(hydroxymethyl)-3-phenylcyclopropane (10a). A 2.0 M solution of trimethylaluminum in hexane (22.35 mL) was added dropwise in 20 min to a solution of morpholine (3.9 g, 44.8 mmol) in dry CH_2Cl_2 (108 mL) magnetically stirred under argon at room temperature. Stirring was continued for 20 min after which a solution of **9a** (2.59 g, 14.88 mmol) in dry CH_2Cl_2 (67 mL) was added dropwise in 20 min, and the resulting mixture was then heated at 40 °C for 20 h. The reaction mixture was carefully acidified with 1 N HCl, the organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 60 mL). The combined organic phases were dried over anhydrous Na_2SO_4, and after evaporation of the solvent, the residue (3.7 g) was submitted to flash chromatography; elution with CH_2Cl_2−MeOH (95:5) gave **10a** (3.50 g, 90%): mp 102−3 °C; ^{1}H-NMR ($CDCl_3$) δ 1.95 (1H, m, 2-CH), 2.15 (1H, t, J = 9.4 Hz, 1-CH), 2.60 (1H, t, J = 9.4 Hz, 3-CH), 3.15 (1H, m, CH_aOH), 3.40−4.00 (9H, m, morpholine ring, CH_bOH), 4.20 (1H, br, OH), 7.15−7.40 (5H, m, aromatics).

Epimerization of the Amidic Function of 10a (11). A solution of **10a** (3.40 g, 13.03 mmol) in dry THF (200 mL) was added dropwise in 30 min to a magnetically stirred solution of lithium hexamethyl disilazide [from addition of 1.5 M butyllithium in hexane (26 mL) to a solution of dry 1,1,1,3,3,3-hexamethyldisilazane (7.0 g, 43.5 mmol) in THF (200 mL)] kept under argon at room temperature. Stirring was continued for 1 h after which the reaction mixture was diluted with saturated NH_4Cl (500 mL) and extracted with CH_2Cl_2 (3 × 200 mL). The combined organic extracts were then dried (Na_2SO_4) and evaporated to give a residue (3.4 g) which was submitted to flash chromatography; elution with CH_2Cl_2−MeOH (95:5) yielded **11** (3.00 g, 88%): ^{1}H-NMR ($CDCl_3$) δ 2.05 (2H, m, 2-CH, OH), 2.25 (1H, t, J = 5.0 Hz, 1-CH), 2.85 (1H, dd, J = 5.0, 12.0 Hz, 3-CH), 3.35 (1H, dd, J = 6.7, 12 Hz, CH_aOH) 3.50−3.90 (9H, m, morpholine ring, CH_bOH), 7.15−7.40 (5H, m, aromatics).

1-(4-Morpholinylcarbonyl)-2-formyl-3-phenylcyclopropane (12). PCC (4.20 g, 19.48 mmol) was added to a solution of **11** (3.00 g, 11.49 mmol) in dry CH_2Cl_2 (130 mL), and the resulting mixture was stirred at room temperature in an argon atmosphere for 16 h. The reaction mixture was then diluted with Et_2O and filtered and the solvent evaporated off. Flash chromatography of the residue (2.5 g) and elution with AcOEt−light petroleum (8:2) afforded **12** (1.90 g, 64%): mp 89−91 °C; ^{1}H-NMR ($CDCl_3$) δ 2.85 (1H, m, 2-CH), 3.12 (1H, t, J = 6.4 Hz, 1-CH), 3.35 (1H, dd, J = 6.4, 9.4 Hz, 3-CH), 3.50−3.90 (8H, m, morpholine ring), 7.18−7.40 (5H, m, aromatics), 9.20 (1H, d, J = 5.1 Hz, CHO).

1-(4-Morpholinylcarbonyl)-2-formyl-3-phenylcyclopropane (13). PCC (4.76 g, 22.08 mmol) was added to a solution of **10a** (3.40 g, 13.03 mmol) in dry CH_2Cl_2 (130 mL), and the resulting mixture was stirred at room temperature in an argon atmosphere for 48 h. The reaction mixture was then diluted with Et_2O and filtered and the solvent evaporated off. Flash chromatography of the residue (3 g) and elution with AcOEt−light petroleum (6:4) afforded **13** (2.20 g, 65%): mp 155−7 °C; ^{1}H-NMR ($CDCl_3$) δ 2.30 (1H, m, 2-CH), 2.72 (1H, t, J = 9.0 Hz, 1-CH), 3.05 (1H, t, J = 9.0 Hz, 3-CH), 3.45−3.90 (8H, m, morpholine ring), 7.20−7.40 (5H, m, aromatics), 9.60 (1H, d, J = 8.1 Hz, CHO).

1-(4-Morpholinylcarbonyl)-2-formyl-3-phenylcyclopropane (14). K_2CO_3 (1.51 g, 10.96 mmol) was aded to a solution of **13** (0.710 g, 2.74 mmol) in MeOH (30 mL, degassed with nitrogen for 30 min), and the resulting suspension was stirred under argon at room temperature for 24 h. The reaction mixture was then diluted with saturated NH_4Cl (20 mL), extracted with Et_2O (4 × 30 mL), and dried (Na_2SO_4). Evaporation of the solvent yielded the aldehyde **14** (0.690 g, 97%): ^{1}H-NMR ($CDCl_3$) δ 2.70−2.90 (2H, m, 1-CH, 2-CH), 2.99 (1H, dd, J = 5.6, 9.1 Hz, 3-CH), 3.10−3.80 (8H, m, morpholine ring), 7.10−7.40 (5H, m, aromatics), 9.90 (1H, d, J = 2.7 Hz, CHO).

***trans*-3-Phenyl-2-propen-1-yl Diazoacetate (8b).** *p*-Tolylsulfonylhydrazone of glyoxylic acid chloride (22.8 g, 87.7

mmol) was added to a cold (0 °C) solution of (*E*)-cinnamic alcohol (10.0 g, 74.6 mmol) in anhydrous CH_2Cl_2 (380 mL). *N*,*N*-Dimethylaniline (10.17 g, 84.1 mmol) was then added, and the resulting mixture was stirred at 0 °C for 15 min, after which Et_3N (39.2 g, 388 mmol) was added dropwise in 40 min. After addition was complete, stirring was continued for 15 min at 0 °C and for 3.5 h at room temperature. The reaction mixture was then diluted with water (300 mL), the organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 60 mL). The combined organic phases were dried (Na_2SO_4), and after evaporation of the solvent, the residue was submitted to flash chromatography; elution with light petroleum−AcOEt (95:5) afforded **8b** (14.5 g, 96%): IR (film) 2110, 1700 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.80 (3H, d, J = 6.4 Hz, CHN_2, CH_2), 6.25 (1H, dt, J = 16.0, 6.5 Hz, 2-CH), 6.65 (1H, d, J = 16.0 Hz, 3-CH), 7.35 (5H, m, aromatics); ^{13}C-NMR ($CDCl_3$) δ 45.73, 64.82, 122.84, 126.22, 127.67, 128.19, 133.76, 135.79, 166.06.

***exo*-6-Phenyl-3-oxabicyclo[3.1.0]hexan-2-one (9b)**: prepared as for **9a** starting from **8b** with 81% yield; mp 93−4 °C; ^{1}H-NMR ($CDCl_3$) δ 2.30 (2H, m, 1-CH, 5-CH), 2.50 (1H, m, 6-CH), 4.45 (2H, m, 4-CH_2), 7.00−7.40 (5H, m, aromatics).

1-(4-Morpholinylcarbonyl)-2-(hydroxymethyl)-3-phenylcyclopropane (10b): prepared as for **10a** starting from **9b** with 99% yield; ^{1}H-NMR ($CDCl_3$) δ 2.00 (2H, m, 1-CH, 2-CH), 2.50 (1H, t, J = 6.3 Hz, 3-CH), 3.40−3.75 (8H, m, morpholine ring), 4.00−4.15 (2H, m, CH_2OH), 7.00−7.35 (5H, m, aromatics).

1-(4-Morpholinylcarbonyl)-2-formyl-3-phenylcyclopropane (15). PCC (2.00 g, 9.20 mmol) was added to a solution of **10b** (1.60 g, 6.10 mmol) in dry CH_2Cl_2 (62 mL), and the resulting mixture was stirred at room temperature in an argon atmosphere for 7 h. Et_2O (180 mL) was then added, the reaction mixture was filtered, and the solvent was evaporated off. Flash chromatography of the residue (1.2 g) and elution with AcOEt−light petroleum (6:4) afforded **15** (1.00 g, 62%): ^{1}H-NMR ($CDCl_3$) δ 2.40 (1H, ddd, J = 9.0, 6.2, 6.0 Hz, 2-CH), 2.70 (1H, dd, J = 9.0, 6.2 Hz, 1-CH), 3.40 (1H, t, J = 6.0 Hz, 3-CH), 3.50−3.75 (8H, m, morpholine ring), 7.10−7.35 (5H, m, aromatics), 9.35 (1H, d, J = 6.2 Hz, CHO).

General Procedure for the Stereoselective Strecker Synthesis To Give Substituted α-Amino Nitriles. (*S*)- Or (*R*)-α-phenylglycinol (3.86 mmol) was added to a solution of the aldehyde (3.86 mmol) in MeOH (38 mL), and the resulting solution was magnetically stirred at room temperature for 2 h. After cooling to 0 °C, TMSCN (7.72 mmol) was added, and the resulting mixture was stirred for 12 h at room temperature. Evaporation of the solvent gave a residue which was submitted to medium pressure chromatography using AcOEt−light petroleum mixtures as eluent to give the corresponding N-substituted α-amino nitriles.

(2*R*,1′*S*,2′*S*,3′*R*)-*N*-[(*S*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (16): AcOEt−light petroleum (8:2); 30% yield as a white solid; mp 120−1 °C; ^{1}H-NMR ($CDCl_3$) δ 2.20 (2H, m, 1′-CH, 2′-CH), 2.75 (1H, d, J = 9.8 Hz, 2-CH), 3.10 (2H, m, 3′-CH, OH), 3.40−3.90 (10H, m, morpholine, CH_2OH), 3.95 (1H, dd, J = 2.9, 8.8 Hz, CHCH_2OH), 6.70−7.40 (10H, 2 × m, aromatics); ^{13}C-NMR ($CDCl_3$) δ 20.97, 30.37, 42.55, 45.96, 46.50, 62.83, 66.37, 66.85, 119.09, 127.12, 127.26, 127.65, 128.53, 133.71, 137.32, 168.83; $[\alpha]^{20}_D$ −30° (*c* 0.90, CH_2Cl_2).

(2*R*,1′*R*,2′*R*,3′*S*)-*N*-[(*S*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (17): AcOEt−light petroleum (8:2); 15% yield as a white solid; mp 148−9 °C; ^{1}H-NMR ($CDCl_3$) δ 2.20 (2H, m, 1′-CH, 2′-CH), 2.61 (1H, d, J = 8 Hz, 2-CH), 2.90 (2H, m, 3′-CH, OH), 3.50−4.05 (11H, m, morpholine, CH_2OH, CHCH_2OH), 6.90−7.40 (10H, 2 × m, aromatics); ^{13}C-NMR ($CDCl_3$) δ 22.94, 29.63, 29.89, 42.77, 46.15, 63.05, 66.67, 118.52, 127.24, 127.62, 128.17, 128.34, 128.56, 128.73, 133.89, 137.99, 169.19; $[\alpha]^{20}_D$ +155° (*c* 0.80, CH_2Cl_2).

(2*S*,1′*R*,2′*R*,3′*S*)-*N*-[(*R*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (18): 21% yield as a white solid; mp 124−5 °C; $[\alpha]^{20}_D$ +25° (*c* 0.70, CH_2Cl_2).

(2*S*,1′*S*,2′*S*,3′*R*)-*N*-[(*R*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (19): 32% yield as a white solid; mp 145−8 °C; $[\alpha]^{20}_D$ −158° (*c* 0.80, CH_2Cl_2).

(2*R*,1′*R*,2′*S*,3′*S*)-*N*-[(*S*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′phenylcyclopropyl]glycinonitrile (20): AcOEt−light petroleum (8:2); 21% yield as an oil; ^{1}H-NMR ($CDCl_3$) δ 1.75 (1H, q, J = 10 Hz, 1′-CH), 2.12 (1H, br s, OH), 2.35 (1H, t, J = 10 Hz, 2′-CH), 2.58 (1H, t, J = 10 Hz, 3′-CH), 3.25−3.80 (11H, m, morpholine, CH_2OH, CHCH_2OH), 4.30 (1H, d, J = 10.5 Hz, 2-CH), 7.20−7.40 (10H, m, aromatics); ^{13}C-NMR ($CDCl_3$) δ 22.95, 26.93, 27.66, 41.98, 45.77, 46.10, 62.78, 65.64, 66.54, 120.60, 127.21, 127.35, 127.75, 128.46, 128.72, 134.23, 140.42, 167.11; $[\alpha]^{20}_D$ +105° (*c* 0.82, CH_2Cl_2).

(2*R*,1′*S*,2′*R*,3′*R*)-*N*-[(*S*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′phenylcyclopropyl]glycinonitrile (21): AcOEt−light petroleum (8:2), 33% yield as an oil; ^{1}H-NMR ($CDCl_3$) δ 1.93 (1H, q, J = 10 Hz, 1′-CH), 2.20 (1H, br s, OH), 2.30 (1H, t, J = 10 Hz, 2′-CH), 2.68 (1H, t, J = 10 Hz, 3′-CH), 3.15−3.78 (10H, m, morpholine, CH_2OH), 3.85 (1H, d, J = 10.5 Hz, 2-CH), 4.08 (1H, dd, J = 4.5, 8.8 Hz, CHCH_2-OH), 6.80−7.30 (10H, 2 × m, aromatics); ^{13}C-NMR ($CDCl_3$) δ 23.04, 26.56, 27.61, 41.88, 44.93, 46.02, 62.89, 66.47, 67.01, 120.44, 126.79, 127.32, 127.54, 128.42, 133.88, 137.98, 167.00; $[\alpha]^{20}_D$ −59° (*c* 1.35, CH_2Cl_2).

(2*S*,1′*S*,2′*R*,3′*R*)-*N*-[(*R*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (22): 23% yield as an oil; $[\alpha]^{20}_D$ −97° (*c* 0.90, CH_2Cl_2).

(2*S*,1′*R*,2′*S*,3′*S*)-*N*-[(*R*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (23): 36% yield as an oil; $[\alpha]^{20}_D$ +56° (*c* 1.35, CH_2Cl_2).

(2*R*,1′*S*,2′*S*,3′*S*)-*N*-[(*S*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (24): AcOEt−light petroleum (8:2); 31% yield as a white solid; mp 161−2 °C; ^{1}H-NMR ($CDCl_3$) δ 2.35 (1H, dd, J = 5.6, 10.4 Hz, 2′-CH), 2.50−2.80 (3H, m, 1′-CH, 3′-CH, 2-CH), 3.00−3.80 (11H, 2 × m, morpholine, CH_2OH, OH), 4.10 (1H, dd, J = 4.5, 10.4 Hz, CHCH_2OH), 7.00−7.40 (10H, 2 × m, aromatics); ^{13}C-NMR ($CDCl_3$) δ 25.01, 28.11, 42.28, 45.70, 49.87, 63.28, 66.48, 67.18, 117.77, 127.09, 127.30, 127.71, 128.23, 128.39, 128.83, 135.33, 138.41, 165.90; $[\alpha]^{20}_D$ + 56° (*c* 0.30, CH_2Cl_2).

(2*R*,1′*R*,2′*R*,3′*R*)-*N*-[(*S*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (25): AcOEt−light petroleum (8:2); 29% yield as a white solid; mp 149−51 °C; ^{1}H-NMR ($CDCl_3$) δ 2.20 (1H, dd, J = 4.8, 9.7 Hz, 2′-CH), 2.65 (3H, m, 1′-CH, 3′-CH, 2-CH), 3.00−3.80 (11H, 2 × m, morpholine, CH_2OH, OH), 4.10 (1H, dd, J = 3.2, 8.1 Hz, CHCH_2OH), 7.10−7.40 (10H, 2 × m, aromatics); ^{13}C-NMR ($CDCl_3$) δ 24.96, 28.11, 28.44, 42.10, 45.47, 50.12, 62.99, 66.35, 66.95, 117.77, 126.97, 127.31, 127.61, 128.26, 128.73, 135.19, 138.13, 165.47; $[\alpha]^{20}_D$ +40° (*c* 0.10, CH_2Cl_2).

(2*S*,1′*R*,2′*R*,3′*R*)-*N*-[(*R*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (26): 30% yield as a white solid: mp 161−2 °C; $[\alpha]^{20}_D$ −59° (*c* 0.45, CH_2Cl_2).

(2*S*,1′*S*,2′*S*,3′*S*)-*N*-[(*R*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (27): 31% yield as a white solid: mp 146−8 °C; $[\alpha]^{20}_D$ −42° (*c* 0.10, CH_2Cl_2).

(2*R*,1′*S*,2′*R*,3′*S*)-*N*-[(*R*)-αPhenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (28): AcOEt−light petroleum (55:45); 23% yield as an oil; ^{1}H-NMR ($CDCl_3$) δ 2.01 (1H, td, J = 4.7, 9.4, 15.6 Hz, 1′-CH), 2.18 (1H, dd, J = 4.7, 9.4 Hz, 2′-CH), 2.55 (1H, br s, OH), 2.68 (1H, t, J = 4.7 Hz, 3′-CH), 3.30−4.30 (11H, m, morpholine, CH_2OH, 2-CH), 4.05 (1H, dd, J = 4.7, 9.4 Hz, CHCH_2OH), 7.05−7.40 (10H, m, aromatics); ^{13}C-NMR ($CDCl_3$) δ 26.79, 29.79, 31.06, 42.43, 46.04, 46.94, 62.95, 66.34, 67.04, 119.52, 126.24, 126.73, 127.20, 127.93, 128.50, 128.55, 138.31, 138.65, 167.03; $[\alpha]^{20}_D$ +105° (*c* 1.69, CH_2Cl_2).

(2*R*,1′*R*,2′*S*,3′*R*)-*N*-[(*R*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (29): AcOEt−light petroleum (55:45); 24% yield as an oil; ^{1}H-NMR ($CDCl_3$) δ 2.00 (1H, m, 1′-CH), 2.18 (1H, dd, J = 5.5, 9.2 Hz, 2′-CH), 2.84 (1H, t, J = 5.5 Hz, 3′-CH), 3.07 (1H, br s, OH), 3.35−3.80 (11H, m, morpholine, CH_2OH, 2-CH), 4.05 (1H,

dd, J = 3.0, 7.7 Hz, *CH*CH_2OH), 7.05−7.35 (10H, m, aromatics); ^{13}C-NMR ($CDCl_3$) δ 26.85, 27.58, 31.23, 42.41, 46.02, 46.36, 62.86, 66.38, 67.07, 119.15, 126.51, 126.70, 127.28, 127.46, 128.01, 128.41, 137.68, 138.29, 167.17; $[\alpha]^{20}_D$ +24° (*c* 1.00, CH_2Cl_2).

(2*S*,1′*R*,2′*S*,3′*R*)-*N*-[(*R*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (30): 23% yield as an oil; $[\alpha]^{20}_D$ −120° (*c* 1.40, CH_2Cl_2).

(2*S*,1′*S*,2′*R*,3′*S*)-*N*-[(*R*)-α-Phenylglycinyl]-2-[2′-(4-morpholinylcarbonyl)-3′-phenylcyclopropyl]glycinonitrile (31): 20% yield as an oil; $[\alpha]^{20}_D$ −19° (*c* 0.79, CH_2Cl_2).

General Procedure for the Hydrolysis of the N-Substituted α-Amino Nitriles. Lead(IV) acetate (1.63 mmol) was added to a cold (0 °C), magnetically stirred solution of the nitrile (1.48 mmol) in anhydrous MeOH−CH_2Cl_2 (0.12 M, 1:1); after 10 min, water (10 mL) was added and the resulting mixture was filtered with the aid of Celite. After evaporation of the solvent, the residue was refluxed in 6 N HCl (30 mL) for 6−12 h. The reaction mixture was washed with CH_2Cl_2 (2 × 10 mL) and evaporated to dryness. The residue was submitted to ion exchange resin chromatography.

(2*R*,1′*S*,2′*S*,3′*R*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (32, PCCG-1): Dowex 50WX2-200 (10% pyridine); 86% yield; mp 240−1 °C; ^{1}H-NMR (D_2O) δ 1.95 (1H, td, J = 5.4, 8.9, 10.8 Hz, 1′-CH), 2.55 (1H, t, J = 5.4 Hz, 2′-CH), 2.85 (1H, dd, J = 5.4, 8.9 Hz, 3′-CH), 3.00 (1H, d, J = 10.8 Hz, 2-CH), 7.20−7.35 (5H, m, aromatics); ^{13}C-NMR (D_2O + DCl) δ 23.90, 27.78, 29.97, 50.40, 128.03, 128.43, 129.17, 132.76, 170.81, 175.24; $[\alpha]^{20}_D$ −74° (*c* 0.30, 2.5 N HCl).

(2*R*,1′*R*,2′*R*,3′*S*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (33, PCCG-2): Dowex 50WX2-200 (10% pyridine); 74% yield; mp 221−3 °C; ^{1}H-NMR (D_2O) δ 2.10 (1H, dt, J = 5.0, 10.5 Hz, 1′-CH), 2.40 (1H, t, J = 5.0 Hz, 2′-CH), 3.00 (2H, m, 3′-CH, 2-CH), 7.30 (5H, m, aromatics); ^{13}C-NMR (D_2O + DCl) δ 22.91, 27.83, 31.39, 51.49, 127.69, 128.59, 128.96, 133.28, 169.70, 175.25; $[\alpha]^{20}_D$ +100° (*c* 0.20, 2.5 N HCl).

(2*S*,1′*R*,2′*R*,3′*S*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (34, PCCG-3): 70% yield; mp 237−8 °C; $[\alpha]^{20}_D$ +72° (*c* 0.30, 2.5 N HCl).

(2*S*,1′*S*,2′*S*,3′*R*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (35, PCCG-4): 73% yield; 217−8 °C; $[\alpha]^{20}_D$ −108° (*c* 0.15, 2.5 N HCl).

(2*R*,1′*R*,2′*S*,3′*S*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (36, PCCG-5): Dowex 50WX2-200 (10% pyridine); 48% yield; mp 219−220 °C; ^{1}H-NMR (D_2O) δ 1.80 (1H, dt, J = 9.4, 11.9 Hz, 1′-CH), 2.49 (1H, t, J = 9.4 Hz, 2′-CH), 2.75 (1H, t, J = 9.4 Hz, 3′-CH), 4.10 (1H, d, J = 11.9 Hz, 2-CH), 7.10−7.30 (5H, m, aromatics); ^{13}C-NMR (D_2O + DCl) δ 23.99, 24.54, 28.51, 49.00, 127.86, 129.20, 129.71, 133.33, 171.27, 175.24; $[\alpha]^{20}_D$ +20° (*c* 0.50, 2.5 N HCl).

(2*R*,1′*S*,2′*R*,3′*R*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (37, PCCG-6): Dowex 50WX2-200 (10% pyridine); 53% yield; mp 231−3 °C; ^{1}H-NMR (D_2O) δ 1.90 (1H, dt, J = 8.8, 11.5 Hz, 1′-CH), 2.55 (1H, t, J = 8.8 Hz, 2′-CH), 2.80 (1H, t, J = 8.8 Hz, 3′-CH), 4.15 (1H, d, J = 11.5 Hz, 2-CH), 7.10−7.30 (5H, m, aromatics); ^{13}C-NMR (D_2O + DCl) δ 23.67, 24.29, 28.40, 49.00, 127.65, 128.59, 129.00, 133.07, 171.05, 173.98; $[\alpha]^{20}_D$ −17° (*c* 0.60, 2.5 N HCl).

(2*S*,1′*S*,2′*R*,3′*R*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (38, PCCG-7): 48% yield; mp 218−9 °C; $[\alpha]^{20}_D$ −21° (*c* 0.50, 2.5 N HCl).

(2*S*,1′*R*,2′*S*,3′*S*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (39, PCCG-8): 55% yield; mp 235−6 °C; $[\alpha]^{20}_D$ +18° (*c* 0.40, 2.5 N HCl).

(2*R*,1′*S*,2′*S*,3′*S*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (40, PCCG-9): Dowex 50WX2-200 (10% pyridine) followed by reversed phase (RP-8) MPC and elution with H_2O−MeOH (75:25); 62% yield; mp 222−4 °C; ^{1}H-NMR (D_2O) δ 2.30 (2H, m, 1′-CH, 2′-CH), 2.80 (1H, dd, J = 6.8, 9.8 Hz, 3′-CH), 3.45 (1H, d, J = 8.7 Hz, 2-CH), 7.25 (5H, m, aromatics); ^{13}C-NMR (D_2O + DCl) δ 24.40, 27.61, 31.10, 54.98, 127.61, 128.59, 128.95, 134.45, 170.44, 172.86; $[\alpha]^{20}_D$ −93° (*c* 0.25, 2.5 N HCl).

(2*R*,1′*R*,2′*R*,3′*R*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (41, PCCG-10): Dowex 50WX2-200 (10% pyridine) followed by reversed phase (RP-8) MPC and elution with H_2O−MeOH (75:25); 62% yield; mp 240−2 °C; ^{1}H-NMR (D_2O) δ 2.20 (2H, m, 1′-CH, 2′-CH), 2.85 (1H, t, J = 8.6 Hz, 3′-CH), 3.30 (1H, d, J = 8.6 Hz, 2-CH), 7.25 (5H, m, aromatics); ^{13}C-NMR (D_2O + DCl) δ 25.05, 26.70, 31.45, 55.35, 127.57, 128.57, 128.95, 134.60, 170.57, 173.05; $[\alpha]^{20}_D$ + 38° (*c* 0.40, 2.5 N HCl).

(2*S*,1′*R*,2′*R*,3′*R*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (42, PCCG-11): 75% yield; mp 222−3 °C; $[\alpha]^{20}_D$ +85° (*c* 0.22, 2.5 N HCl).

(2*S*,1′*S*,2′*S*,3′*S*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (43, PCCG-12): 63% yield; mp 237−8 °C; $[\alpha]^{20}_D$ −33° (*c* 0.30, 2.5 N HCl).

(2*R*,1′*S*,2′*R*,3′*S*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (44, PCCG-13): Dowex 50WX2-200 (10% pyridine) followed by Dowex 1X8 (1 N AcOH); 49% yield; mp 199−200 °C; ^{1}H-NMR (D_2O) δ 2.05 (1H, td, J = 5.6, 9.1, 10.4 Hz, 1′-CH), 2.30 (1H, dd, J = 5.4, 9.1 Hz, 2′-CH), 2.70 (1H, t, J = 5.6 Hz, 3′-CH), 4.00 (1H, d, J = 10.4 Hz, 2-CH), 7.10−7.30 (5H, m, aromatics); ^{13}C-NMR (D_2O + DCl) δ 28.30, 30.80, 51.02, 126.57, 127.47, 128.93, 137.45, 170.53, 174.32; $[\alpha]^{20}_D$ +48.5° (*c* 0.90, 2.5 N HCl).

(2*R*,1′*R*,2′*S*,3′*R*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (45, PCCG-14): Dowex 50WX2-200 (10% pyridine) followed by Dowex 1X8 (1 N AcOH); 75% yield; mp 184−5 °C; ^{1}H-NMR (D_2O) δ 2.05 (1H, td, J = 6.1, 7.8, 10.7 Hz, 1′-CH), 2.25 (1H, dd, J = 6.1, 7.8 Hz, 2′-CH), 2.85 (1H, t, J = 6.1 Hz, 3′-CH), 4.45 (1H, d, J = 10.7 Hz, 2-CH), 7.10−7.30 (5H, m, aromatics); ^{13}C-NMR (D_2O + DCl) δ 25.99, 28.61, 32.02, 51.12, 126.70, 127.50, 128.86, 137.39, 170.98, 174.65; $[\alpha]^{20}_D$ −59.4° (*c* 0.90, 2.5 N HCl).

(2*S*,1′*R*,2′*S*,3′*R*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (46, PCCG-15): 50% yield; mp 202−4 °C; $[\alpha]^{20}_D$ −47.4° (*c* 0.55, 2.5 N HCl).

(2*S*,1′*S*,2′*R*,3′*S*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (47, PCCG-16): 55% yield; mp 184−5 °C; $[\alpha]^{20}_D$ +65° (*c* 0.80, 2.5 N HCl).

Strecker Synthesis from Aldehyde (+)-14. (*R*)-α-Phenylglycinol (0.068 g, 0.5 mmol) was added to a solution of (1*R*,2*R*,3*R*)-1-(4-morpholinylcarbonyl)-2-formyl-3-phenylcyclopropane [(+)-**14**; 0.130 g, 0.5 mmol] in MeOH (5 mL), and the resulting solution was magnetically stirred at room temperature for 2 h. After cooling to 0 °C, TMSCN (0.13 mL, 1.0 mmol) was added and the resulting mixture was stirred for 12 h. Evaporation of the solvent gave a residue which was submitted to MPC using AcOEt−light petroleum (6:4) as eluent to give (2*S*,1′*R*,2′*R*,3′*R*)-N-substituted amino nitrile **26** (0.040 g, 20%): mp 168−70 °C.

X-ray Analysis. Single crystals of **19**, **39**, and **44** were obtained by dissolution in AcOEt (**19**) or H_2O−48% HBr (10:1) (**39** and **44**) and slow evaporation at room temperature. Colorless prisms of **39**, **19**, and **44** of approximate dimensions 0.10 × 0.25 × 0.15, 0.20 × 0.30 × 0.25, and 0.10 × 0.25 × 0.15 mm, respectively, were used for data collection. Lattice parameters were determined by least-squares refinement on 36, 25, and 30 (for **39**, **19**, and **44**, respectively) randomly selected and automatically centered reflections. Data were collected on a Siemens P4 four-circle diffractometer with graphite monochromated Mo Kα radiation, in the ω scan mode for 5 ≤ 2Θ ≤ 50° (**39**), 4 ≤ 2Θ ≤ 50° (**19**), and 2 ≤ 2Θ ≤ 45° (**44**) scan ranges. Scan widths equal to 0.80°, 0.84°, and 1.3° and constant scan speeds equal to 2.5, 2.4, and 2.9 deg min^{-1} were used for the data collections of compounds **39**, **19**, and **44**, respectively. A total of 2240 (**39**), 3964 (**19**), and 1962 (**44**) independent reflections were collected at 22 °C. Three standard reflections measured in each data collection every 60 min showed no variations. Absorption correction based on Ψ-scan were applied for compounds **39** and **44**. The structures were solved by direct methods of the SHELXTL PC package.[41] Refinements were carried out by full-matrix anisotropic least-squares on F^2 for all reflections for non-H atoms by using the SHELXL-93 program.[42] Minimized function $\sum w(F_0^2 - F_c^2)^2$ with weighting scheme $w = 1/[\sigma^2(F_o)^2 + aP^2 + bP]$, where $P = (F_o^2 + 2F_c^2)/3$, a = 0.0546 (**39**), 0.0610 (**19**), or 0.0766 (**44**), and b = 0.0000 (**39**), 0.2282 (**19**), or 0.0000 (**44**). Since **44** crystallizes in a polar space group, polar axis restraints were applied by the method of Flack and Schwarzenbach.[43] Atomic scattering factors including f' and f'' were taken from ref 44. The absolute configurations of the four chiral centers were

assigned by Flack parameter[45] for compounds **39** and **44**, while for **19** it was derived by the presence of the known absolute configuration at the phenylglycinyl moiety. Most of the hydrogen atoms were located on the final difference Fourier synthesis, while the others were placed in calculated positions. The C−H, N−H, and O−H distances were constrained to 0.96, 0.90, and 0.82 Å, respectively. Conventional R factor values are 0.061, 0.048, and 0.070, while R_w values, based on F^2, are 0.108, 0.109, and 0.143 for **39**, **19**, and **44**, respectively. R factors based on F^2 (R_w) are statistically about 2 times as large as those (R) based on F.[42]

Biological Assays. Receptor binding experiments to rat cerebral cortical membranes using [^{3}H]-α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) (47 Ci/mmol), [^{3}H]kainate (47 Ci/mmol), and [^{3}H]-3-(2-carboxypiperazin-4-yl)propane-1-phosphonic acid (CPP) (58 Ci/mmol) all from New England Nuclear) were performed as previously described.[29] Receptor binding experiments using [^{3}H]glutamate (specific activity 50 Ci/mmol; Amersham) to membranes prepared from baby hamster kidney cells (BHK) expressing mGluR1a were conducted as previously reported.[30] Sodium-dependent [^{3}H]glutamate uptake into rat cortical synaptosomes was determined according to the method by Fletcher and Johnston.[32] Calcium/chloride-dependent glutamate uptake was measured as described[46] using [^{3}H]-L-2-amino-4-phosphonobutyrate ([^{3}H]-L-AP4) binding to rat cortical membranes in the presence of 2.5 mM $CaCl_2$ at 37 °C (specific activity of [^{3}H]-L-AP4 50 Ci/mmol; Tocris Cookson, U.K.). [^{3}H]-L-AP4 binding was also used to label mGluR4a when expressed in BHK cells.[31] In brief, membranes from mGluR4a-transfected cells were incubated with 30 nM [^{3}H]-L-AP4 and test compounds in a buffer (30 mM HEPES-Na, pH 8.0, 110 mM NaCl, 1.2 mM $MgCl_2$, 5 mM KCl, 2.5 mM $CaCl_2$, and 0.1 mM phenylmethanesulfonyl fluoride) for 30 min at 0 °C. Bound and free radioactivity was separated by centrifugation (40000g, 3 min, 0 °C). The pellets were quickly rinsed with ice cold buffer (2 × 2 mL), solubilized in 2 M NaOH (0.5 mL), and transferred to scintillation vials. Nonspecific binding was defined as the binding in the presence of 0.1 mM L-serine *O*-phosphate.

BHK cells stably expressing mGluR1a, mGluR2, or mGluR4 were used for measurements of phosphoinositide (PI) hydrolysis or cAMP formation.[33] For measurements of PI hydrolysis, cells expressing mGluR1a were cultured for 2 days in 24-well plates (Costar) and then incubated with 4 μCi/mL [^{3}H]myoinositol (specific activity 17 Ci/mmol; Amersham). After 24 h of prelabeling with [^{3}H]myoinositol, the cells were washed twice with a Krebs-Henseleit buffer (pH 7.4) supplemented with 2.5 mM $CaCl_2$ and 10 mM LiCl and subsequently incubated with test compounds for 30 min in a similar buffer. The incubation was stopped by quickly washing each well with ice cold buffer (1 mL) and adding ice cold 10% perchloric acid (1 mL). Fractions of inositol monophosphates were separated from the neutralized extracts on ion exchange minicolumns (Amersham, RPN 1908). Measurements of cAMP formation were performed as described previously.[33] The protein contents were determined using a BioRad assay with γ-globulin as a standard.

Supporting Information Available: X-ray crystallographic data (16 pages). Ordering information is given on any current masthead page.

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