(2*R*,1′*S*,2′*R*,3′*S*)-2-(2′-Carboxy-3′-phenylcyclopropyl)glycine (PCCG-13), the First Potent and Selective Competitive Antagonist of Phospholipase D-Coupled Metabotropic Glutamate Receptors: Asymmetric Synthesis and Preliminary Biological Properties

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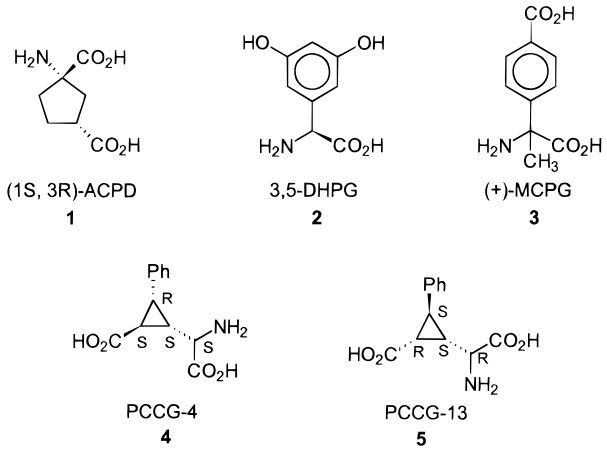
Received March 18, 1999

The asymmetric synthesis of (2*R*,1′*S*,2′*R*,3′*S*)-2-(2′-carboxy-3′-phenylcyclopropyl)glycine (PCCG-13), a trisubstituted carboxycyclopropylglycine endowed with unusual stereochemical features, is described. Preliminary biological evaluation demonstrates PCCG-13 as a very potent and selective competitive antagonist for the novel class of metabotropic glutamate (mGlu) receptors coupled to the activity of phospholipase D (PLD). PCCG-13 is therefore a useful tool for the exploration of the physiopathological role of this novel class of receptors.

Introduction

A variety of physiological functions of glutamate, the major excitatory neurotransmitter in the mammalian central nervous system (CNS), are mediated by two families of receptors: namely, ionotropic glutamate receptors (iGlu), which contain integral cation-specific ion channels and are divided into NMDA, AMPA, and KA receptors, and metabotropic glutamate receptors (mGlu), which are coupled, through G-proteins, to a number of effector systems. On the basis of sequence homology, agonist pharmacology, and signal transduction mechanisms, the eight cloned mGlu receptors are classified into three groups: group I (mGlu1 and mGlu5), group II (mGlu2 and mGlu3), and group III (mGlu4, mGlu6, mGlu7, and mGlu8).[1] When expressed in heterologous systems, cloned mGlu receptors couple to two main signal transduction pathways, namely, to the activation of phospholipase C (PLC) (group I mGlu receptors) which hydrolyzes phosphatidylinositols to inositol triphosphate and diacylglycerol, and to the inhibition of the activity of adenylyl cyclase (groups II and III mGlu receptors) thus decreasing the intracellular concentration of cAMP. Recent reports have demonstrated that other signal transduction mechanisms may be operative upon stimulation of mGlu receptors. Indeed, it has recently been shown that the nonselective mGlu receptor agonist (1*S*,3*R*)-ACPD (**1**; Chart 1) stimulates in neonate and adult hippocampal slices the activity of phospholipase D (PLD).[2,3] This novel transduction mechanism (Scheme 1) involves the hydrolysis of membrane phosphatidylcholine (PC) leading to the formation of choline and phosphatidic acid (PA). PA, itself a potential second messenger, can be converted by a phosphohydrolase to a diacylglycerol (DG) structurally different from that produced by PLC-activated metabolism of phosphoinositides. These distinct DG structural species may activate different phosphokinase C (PKC) isoforms, among the variety that is known to exist in the brain.[4] Interestingly, in the presence of primary alcohols, such as ethanol, PLD activates a transphosphatidyl reaction leading to the formation of the corresponding phosphatidyl alcohols (Scheme 1, pathway b).

Chart 1



In neonate tissue, the glutamatergic activation of PLD appears to be indirectly mediated by group I mGlu receptors via activation of PKC,[5,6] whereas in adult hippocampus the pharmacology of PLD-coupled mGlu receptors does not correspond to that of any of the known subtypes coupled to PLD or AC,[7,8] thus pointing out the possibility of the existence of a new, not yet molecularly characterized, receptor subtype. PLD-coupled mGlu receptors share pharmacological characteristics with group I mGlu receptors (i.e., quisqualate is the most potent agonist) but also bear a number of unique features, such as the selective activation of L-cysteinesulfinic acid[7] and the nonselective and noncompetitive antagonism by 3,5-DHPG (**2**),[8,9] which is also known to be a selective agonist of group I mGlu

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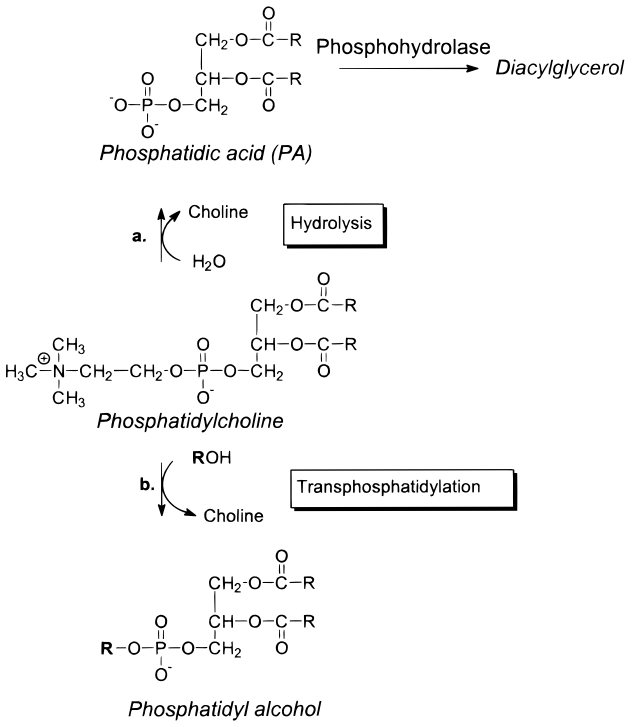
10.1021/jm990128v CCC: $18.00
Published on Web 06/18/1999

Scheme 1



receptors coupled to PLC activation.[10,11] The widely used group I/group II mGlu receptor antagonist (+)-MCPG (**3**) also displays peculiar features, in that it is a mixed agonist/antagonist on PLD-coupled mGlu receptors in adult hippocampal slices.[8]

The clarification of the physiological role and the possible involvement in pathological conditions of this novel class of PLD-coupled receptors requires new ligands, much more potent and selective than those currently available. We have previously reported the synthesis and activity on a variety of mGlu receptor subtypes of 16 diastereoisomeric 2-(2′-carboxy-3′-phenylcyclopropyl)glycines (PCCG-1−PCCG-16), among which (2*S*,1′*S*,2′*S*,3′*R*)-PCCG-4 (**4**) was identified as a potent and selective antagonist of mGlu2 receptors.[12,13]

With the aim to find new tools for the characterization of the intriguing PLD-coupled class of mGlu receptors, this stereolibrary of constrained L-glutamate analogues was tested on the PLD-specific transphosphatidylation reaction (see pathway b, Scheme 1) that is stimulated by (1*S*,3*R*)-ACPD (**1**) and results in the formation of [^{3}H]-phosphatidylethanol ([^{3}H]PEt), a commonly employed assay for detecting PLD activity in intact cells.[14,15] It was discovered, as a result, that the (2*R*,1′*S*,2′*R*,3′*S*)-2-(2′-carboxy-3′-phenylcyclopropyl)glycine (PCCG-13, **5**) antagonizes the formation of [^{3}H]PEt induced by 100 μM (1*S*,3*R*)-ACPD (**1**) in adult rat hippocampal slices in a dose-dependent manner and with a much lower IC_{50} (25 nM) when compared to 3,5-DHPG (**2**), thus revealing compound **5** to be the first potent, selective, and competitive antagonist of PLD-coupled mGlu receptors.[9] Because of its relevance as a new tool for the characterization of PLD-coupled mGlu receptors, it became of interest to devise a practical, enantioselective synthesis of PCCG-13 (**5**). The results are herein described.

Chemistry

Garner's aldehyde (**6**; Scheme 2),[16] a chiral building block easily obtained in four steps from (*S*)-serine,[17] was submitted to Wittig reaction with benzyltriphenylphosphonium bromide to give a 2.3:1 mixture of (*E*)-olefin **7** and (*Z*)-olefin **8**, respectively, which were separated by medium-pressure chromatography. Dirhodium(II) tetraacetate-catalyzed addition[18] of ethyl diazoacetate to (*E*)-olefin **7**, followed by flash chromatography of the reaction mixture, afforded, as the only product, *tert*-butyl 4(*R*)-[(1′*S*,2′*R*,3′*S*)-2′-(ethoxycarbonyl)-3′-phenylcyclopropyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (**9**) as the only cyclopropane derivative (GC−MS) in 26% isolated yield. **9** was then submitted to acidic hydrolysis (*p*-TSA, MeOH, room temperature) and the alcohol **10** thus obtained was oxidized with 8 N Jones reagent (acetone, room temperature) followed by acidic treatment (6 N HCl, 80 °C). Ion-exchange chromatography (Dowex 8×2−200, 1 N AcOH) afforded in 78% yield (2*R*,1′*S*,2′*S*,3′*R*)-PCCG-13 (**5**), with analytical data (^{1}H NMR, ^{13}C NMR, mp, and $[\alpha]_D^{20}$) identical with that of an authentic sample.[12] NMR spectra (Bruker DRX400) of a Mosher amide derived from (*R*)-(+)-α-methoxy-(trifluoromethyl)phenylacetyl cloride (*R*-(+)-MPTA) revealed an enantiomeric enrichment of >95%.[19]

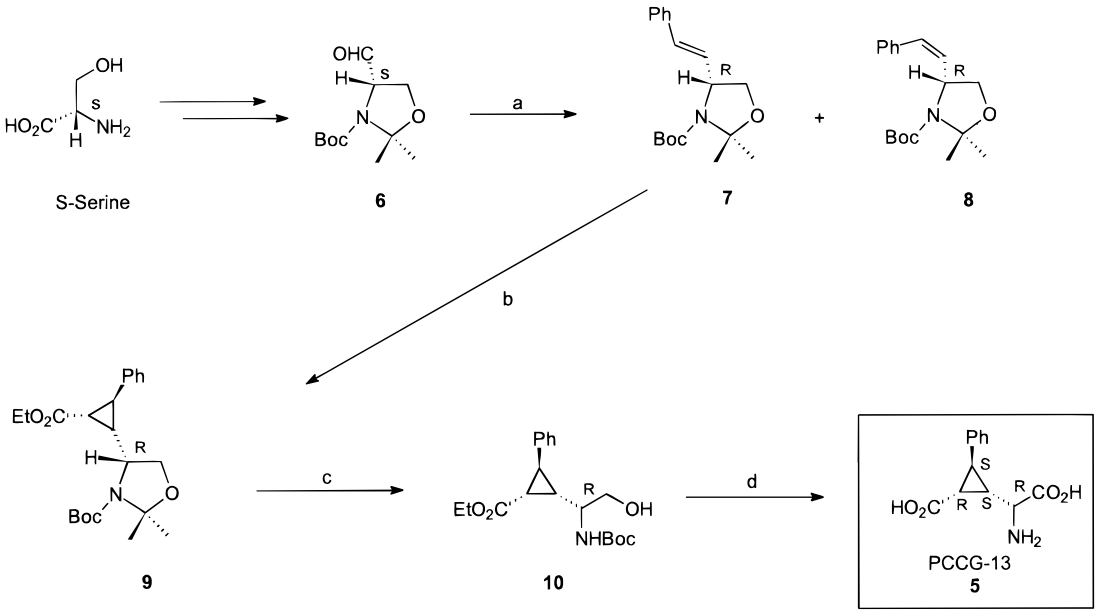
The remarkably high selectivity of the key rhodium(II)-catalyzed cyclopropanation of olefin **7** leading to intermediate **9**, in which three new chiral centers are generated in a single step, was a most gratifying, unpredicted result that can be rationalized according to the following considerations (Figure 1). In our case, the stereochemical outcome is consistent with a diastereofacial attack of the rhodium(II) carbethoxy carbenoid on the more vulnerable (1*si*-2*si*)-face of conformer **a**, the (*E*)-**7** conformer shown to be the most stable according to molecular mechanics calculations (see Figure 1). In this case, the (1*re*-2*re*)-face of the double bond is faced toward and hindered by the bulky N-Boc moiety, and the cyclopropane with (1′*S*,2′*S*)-configuration is the only product. It is worth to note, however, that the energy difference between conformer **a** and conformer **b** is very low and the highly selective stereochemical outcome could not be predicted solely on these bases.

The exclusive formation of the *exo*-isomer **b** over the *endo*-**a** ((*R*)-configuration at C′3, see Figure 1), on the other hand, can be rationalized with the steric effect exerted by the phenyl ring. It is known that less encumbered cyclopropanes are highly favored products of rhodium(II)-catalyzed cyclopropanations. In our case, the resulting cyclopropane is substituted by two groups, the phenyl ring and the oxazolidine moiety, both of them exerting a strong steric effect. The exclusive formation of isomer **9-a** (*exo*-isomer), where the phenyl ring is *trans* to the carbethoxy moiety, infers that is the aromatic portion to play a more demanding steric role.

Pharmacology

Determination of PLC and PLD Activity. PLC and PLD activities were determined in adult rat hippocampal slices (350 μm thick) as previously described.[8] Briefly, phosphoinositide hydrolysis was monitored by measuring the agonist-induced formation of [^{3}H]inositol phosphates (IP1, IP2, and IP3) from slices preincubated with [^{3}H]inositol. Following 10-min stimulation, water-soluble [^{3}H]IPs were transferred to Dowex AG 1×8

Scheme 2[a]



[a] (a) i. $Ph(CH_2)PPh_3Br$, *n*-BuLi, hexane, rt, ii. mpc; (b) EDA, $Rh_2(OAc)_4$, CH_2Cl_2, rt; (c) *p*-TSA, MeOH, rt; (d) i. 8 N Jones reagent, acetone, rt, ii. 6 N HCl, 80 °C, iii. Dowex 1X8-200, 1 N AcOH.

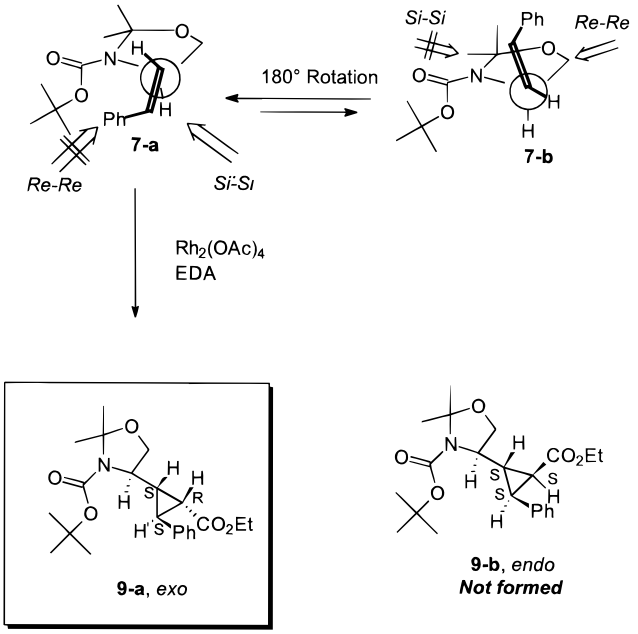


Figure 1. Olefin **7** can exist in two main conformations. Conformer **a** is predicted by molecular mechanics calculation (Tripos force field) to be more stable by about 1 kcal/mol. In conformer **a** the (1*si*,2*si*)-side of the double bond is less hindered by the bulky N-Boc group (top). Attack of the ethyl diazoacetate−dirhodium(II) tetraacetate complex leads preferentially to the *exo* isomer **b**, endowed with a less encumbered cyclopropane ring (bottom).

anion-exchange resin columns and eluted with 1 M ammonium formate, 0.1 M formic acid. The dpm was determined by liquid scintillation spectrometry, and data are expressed as percentage of incorporation of label into [^{3}H]IPs occurring under agonist-free conditions. PLD activity was determined by making use of the transphosphatidylation reaction between phosphatidylcholine and primary alcohols specifically catalyzed by PLD. Thus, in the presence of exogenously added ethanol, PLD preferentially transfers the alcohol rather than water to the phosphatidyl moiety of phosphatidylcholine, producing PEt in place of PA. Slices were incubated with [^{3}H]glycerol for 2 h and then stimulated with agonists [(1*S*,3*R*)-ACPD (**1**) or 3,5-DHPG (**2**)] for 1 h in the presence of 170 mM ethanol. The lipid phase was extracted and spotted on silica gel plates. [^{3}H]PEt was separated from major phospholipids by thin-layer chromatography using the upper phase of the solvent system ethyl acetate/2,2,4-trimethylpentane/acetic acid/water (12:5:1:10). Spots were visualized with iodine vapor, and [^{3}H]PEt was identified by comparison with the PEt standard. The region corresponding to [^{3}H]PEt was scraped off, and radioactivity was counted by liquid scintillation spectrometry. The formation of [^{3}H]PEt was expressed as the percentage of radioactivity incorporated into the total lipids present in the organic phase. Agonist dose−response and antagonist inhibition curves were analyzed by nonlinear regression, and EC_{50} and IC_{50} values were calculated with the PRISM software package (GraphPad Software, San Diego, CA).

Table 1. Summary of EC_{50} and IC_{50} Values of the Agonist and Antagonist Activities of (1*S*,3*R*)-ACPD (**1**), 3,5-DHPG (**2**), (+)-MCPG (**3**), and PCCG-13 (**5**) on PLC and PLD Activity in Adult Rat Hippocampal Slices

	PLC activity		PLD activity	
compd	EC_{50} (μM) agonist	IC_{50} (μM) antagonist	EC_{50} (μM) agonist	IC_{50} (μM) antagonist
(1*S*,3*R*)-ACPD (**1**)	50 ± 2		30 ± 2	
(+)-MCPG (**3**)		150 ± 10	110 ± 10	530 ± 60
3,5-DHPG (**2**)	65 ± 2			70 ± 3
PCCG-13 (**5**)	>300	>300		0.025 ± 0.002

Results and Discussion

Table 1 shows that (1*S*,3*R*)-ACPD (**1**) is an agonist on mGlu receptors coupled to both PLC and PLD, whereas (+)-MCPG (**3**) is an antagonist on receptors coupled to phosphoinositide hydrolysis but a mixed agonist/antagonist on PLD-coupled mGlu receptors.[8] Consistent with previous findings,[11] the group I mGlu

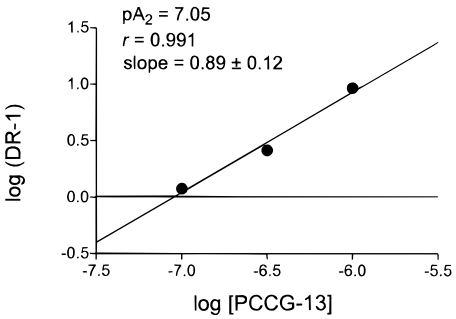


Figure 2. Schild plot of PCCG-13's competitive antagonism upon mGlu receptors coupled to PLD in adult rat hippocampus. Slices were labeled with [^{3}H]glycerol, washed, and incubated for 1 h at 37 °C in buffer with 170 mM ethanol in the presence of increasing concentrations of quisqualate. Quisqualate dose−response curves for [^{3}H]PEt formation were performed in the absence and presence of PCCG-13 (0.1, 0.3, and 1 μM), which was added 10 min before the agonist. EC_{50} values calculated from these curves were used to construct the Schild plot, where DR = (EC_{50} for quisqualate in the presence of PCCG-13)/(EC_{50} for quisqualate alone). The pA_2, r, and slope values were calculated by linear regression analysis.

receptor antagonist 3,5-DHPG (**2**) induced a dose-dependent accumulation of [^{3}H]IPs in adult rat hippocampal slices, with an EC_{50} of 65 ± 2 μM (Table 1). On the contrary, PCCG-13 (**5**) was unable to elicit a PLC response up to a concentration of 300 μM (see also ref 13). When tested on the formation of [^{3}H]PEt, neither 3,5-DHPG (**2**) nor PCCG-13 (**5**) stimulated the formation of [^{3}H]PEt up to 300 μM, but both inhibited the activation of PLD induced by the broad-spectrum mGlu receptor agonist (1*S*,3*R*)-ACPD (**1**) (100 μM). PCCG-13 (**5**) not only displayed pure antagonist activity but was also much more potent than 3,5-DHPG (**2**): their IC_{50}'s were 0.025 ± 0.002 and 70 ± 3 μM, respectively (Table 1).

Moreover, PCCG-13 (**5**) clearly produced a parallel rightward shift in the dose−response curve obtained by stimulating the formation of [^{3}H]PEt with quisqualate.[9] The inhibition of quisqualate-induced PLD activation was surmounted by increasing the concentration of the agonist. The Schild plot analysis of PCCG-13 (**5**) revealed a pA_2 value of 7.05 (r = 0.991) with a slope of 0.89 ± 0.12, suggesting a competitive antagonism for this compound (Figure 2).

These results indicate that PCCG-13 (**5**) is the first competitive antagonist of PLD-coupled mGlu receptors, endowed with a remarkably high potency and selectivity. Despite the presence of the pharmacophoric elements usually required for the interaction with glutamate receptors, PCCG-13 (**5**) bears some structural features that make it unique among the so far described mGlu receptor ligands. First of all, PCCG-13 (**5**) is endowed with an unnatural (*R*)-configuration at the amino acidic center. While the (*R*)-configuration at the amino acidic center is a common motif of competitive NMDA antagonists, it appears as absolutely unusual for metabotropic glutamate receptor. The other point of interest is the presence of a *cis* disposition of the amino acidic moiety and the distal carboxylate group. Again, while this topological arrangement is required for interaction with the NMDA receptor site, no other significant examples are found among mGlu receptor ligands. The absence of other competitive PLD-coupled mGlu receptor antagonists prevents us from defining a clear structure−activity relationship for PLD-coupled mGlu receptor antagonism. The unusual structural features and the high potency of PCCG-13 (**5**), however, permit to anticipate that the recognition site of PLD-coupled mGlu receptors is endowed with a peculiar environment among mGlu receptors that can be instrumental in the design of structurally novel ligands. In particular, it can be speculated that the (*R*)-configuration at the amino acidic center projects the distal carboxylate groups toward an accessory site inaccessible to quisqualate or (1*S*,3*R*)-ACPD (**1**) and characterized by a hydrophobic pocket able to accommodate the phenyl ring. Whether the presence of the phenyl ring is necessary for antagonism (in analogy with group II mGlu antagonists) or the (*R*)-configuration is sufficient to this aim is not predictable at the moment and will be the object of further studies. In conclusion, mGlu receptors coupled to PLD are likely to play an important role in the CNS. Developmental plasticity, synaptogenesis, long-lasting production of diacylglycerol, and activation of PKC are among the events that could be promoted by glutamatergic activation of these receptors. The potent, selective, and competitive antagonist PCCG-13 (**5**) should provide an excellent tool to explore their function in future studies.

Experimental Section

General Methods. Melting points were determined by the capillary method on a Büchi 535 electrothermal apparatus. ^{1}H and ^{13}C NMR spectra were taken on a Brucker AC 200 spectrometer unless otherwise stated. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; br s, broad signal. Mass spectra were recorded on a Hewlett-Packard 6890/5973 MSD instrument equipped with a GC−mass coupling (30-m × 0.25-mm, 0.25-μm capillary HP-1 column): injection temperature, 250 °C; detection temperature, 230 °C; column temperature, 70 °C (1 min), 70−150 °C (7 °C/min), 150−300 °C (5 °C/min), 300 °C (10 min). Specific rotations were recorded on a Jasco Dip-360 digital polarimeter.

***tert*-Butyl (*E*)- and (*Z*)-2,2-Dimethyl-4-(2′-phenylvinyl)-3-oxazolidinecarboxylates (7) and (8).** To a suspension of benzyltriphenylphosphonium bromide (12.70 g, 29.30 mmol) in dry hexane (49 mL) was added 2.5 M *n*-butyllithium in hexane (12 mL) dropwise in 20 min. Stirring was continued for 30 min after which a solution of **6** (6.60 g, 28.80 mmol) in dry hexane (29 mL) was added dropwise in 30 min. Stirring was continued overnight after which the reaction mixture was diluted with water (30 mL), the organic phase was separated, and the aqueous layer was extracted with diethyl ether (3 × 60 mL). The combined organic phases were washed with brine (50 mL) and dried (Na_2SO_4), and after evaporation of the solvent, the residue was submitted to flash chromatography; elution with light petroleum ether−AcOEt (80:20) afforded **7** (42%): mp 78−80 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s, *t*-Bu), 1.60 (3H, s, Me), 1.77 (3H, s, Me), 3.57 (1H, dd, J = 2.6, 8.8 Hz, oxazolidine), 3.79 (1H, dd, J = 6.2, 8.8 Hz, oxazolidine), 4.30 (1H, br s, oxazolidine), 6.12 (1H, dd, J = 7.8, 15.8 Hz, =CH), 6.53 (1H, d, J = 15.8 Hz, =CH−Ph), 7.00−7.30 (5H, m, aromatics); $[\alpha]^{20}_D$ −50° (*c* 2.0, $CHCl_3$). Elution with the same solvents gave **8** (18%): mp 72−4 °C; ^{1}H NMR ($CDCl_3$) δ 1.34 (9H, s, *t*-Bu), 1.56 (3H, s, Me), 1.73 (3H, s, Me), 3.61 (1H, dd, J = 3.4, 8.5 Hz, oxazolidine), 3.81 (1H, dd, J = 6.6, 8.5 Hz, oxazolidine), 4.80 (1H, br s, oxazolidine), 5.69 (1H, dd, J = 9.2, 11.7 Hz, =CH), 6.37 (1H, d, J = 11.7 Hz, =CH−Ph), 7.10−7.20 (5H, m, aromatics); $[\alpha]^{20}_D$ +40° (*c* 1.2, $CHCl_3$).

***tert*-Butyl 4(*R*)-[(1′*S*,2′*R*,3′*S*)-2′-(Ethoxycarbonyl)-3′-phenylcyclopropyl]-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (9).** A solution of ethyl diazoacetate (3.83 g, 33.50

mmol) in dry dichloromethane (220 mL) was added via syringe pump over 30 h to a magnetically stirred solution of the olefin **7** (2.55 g, 8.40 mmol) and $Rh_2(OAc)_4$ (0.41 g, 0.92 mmol) in dry dichloromethane (50 mL). The reaction mixture was evaporated to dryness and the residue submitted to flash chromatography; elution with light petroleum ether−EtOAc (70:30) gave the title compound **9** (26%): ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.0 Hz, $CO_2CH_2CH_3$), 1.40 (9H, s, *t*-Bu), 1.50−1.70 (1H, m, 1′-CH), 1.90−2.00 (1H, m, 2′-CH), 3.00 (1H, m, 3′-CH), 3.75 (1H, d, J = 9.5 Hz, oxazolidine), 3.95 (1H, dd, J = 6.1, 9.5 Hz, oxazolidine), 4.15 (2H, q, J = 7.0 Hz, $CO_2CH_2CH_3$), 4.30 (1H, m, 4-CH), 7.00−7.40 (5H, m, aromatics); ^{13}C NMR ($CDCl_3$) δ 14.2, 23.1, 27.4, 28.4, 32.8, 56.3, 60.6, 68.5, 79.9, 94.0, 126.6, 126.8, 127.3, 139.5, 152.0, 171.6; GC−MS *m*/*z* (%) 289, 333 (7), 274 (36), 189 (100), 115 (27), 57 (20).

Ethyl (1*R*,2*S*,3*S*)-2-(1′-*tert*-Butoxycarbonylamino-2′-hydroxyethyl)-3-phenyl-1-cyclopropanecarboxylate (10). *p*-Toluensulfonic acid monohydrate (0.01 g, 0.06 mmol) was added to a magnetically stirred solution of **9** (0.30 g, 0.77 mmol) in methanol (20 mL). After 5 h, sodium acetate (0.01 g) was added and the stirring was continued for 30 min. The reaction mixture was concentrated in vacuo, and the residue was submitted to flash chromatography; elution with light petroleum ether−EtOAc (1:1) afforded the alcohol **10** (57%): ^{1}H NMR ($CDCl_3$) δ 1.30 (3H, t, J = 7.0 Hz, $CO_2CH_2CH_3$), 1.45 (9H, s, *t*-Bu), 1.85 (1H, m, 2-CH), 2.10 (1H, dd, J = 5.7, 9.0 Hz, 1-CH), 2.85 (1H, t, J = 5.7 Hz, 3-CH), 3.55−3.70 (2H, m, 2′-CH_2), 4.00 (1H, m, 1′-CH), 4.15 (2H, q, J = 7.0 Hz, $CO_2CH_2CH_3$), 5.10 (1H, br s, NH), 7.00−7.30 (5H, m, aromatics); $[\alpha]^{20}_D$ + 46.9° (*c* 1.0, CH_2Cl_2).

(2*R*,1′*S*,2′*R*, 3′*S*)-2-(2′-Carboxy-3′-phenylcyclopropyl)-glycine (5). 8 N Jones' reagent (1.5 mL) was added to a magnetically stirred ice-cold solution of **10** (0.15 g, 0.64 mmol) in acetone (3 mL). The reaction mixture was allowed to come to room temperature and stirred for 1 h. 2-Propanol (1 mL) was added, and after 10 min, the mixture was filtered through Celite washing with ethyl acetate. The solution was evaporated in vacuo to give an oil which was treated with 6 N HCl (10 mL) and stirred at 80 °C for 4 h. The reaction mixture was washed with CH_2Cl_2 (5 mL) and evaporated to dryness. The residue was submitted to ion-exchange resin chromatography (Dowex 1×8, 1 N AcOH) to give the title compound **5** (78%): 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 28.3, 30.8, 51.0, 126.6, 127.5, 128.9, 137.5, 170.5, 174 3; $[\alpha]^{20}_D$ +48.5° (*c* 1.0, 2.5 N HCl).

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JM990128V