Diastereoselective Synthesis of All Four Isomers of 3-(4-Chlorophenyl)glutamic Acid: Identification of the Isomers Responsible for the Potentiation of L-Homocysteic Acid-Evoked Depolarizations in Neonatal Rat Motoneurons

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All four isomers of 3-(4-chlorophenyl)glutamic acid (**5**−**8**) were prepared by diastereoselective synthesis. Addition of (6*S*)-(+)-bis-lactim ether **15** to *cis*-4-chlorocinnamate **12** gave a mixture comprising mainly the (2*R*,3*S*)- and (2*R*,3*R*)-isomers **5** and **6**, respectively (in a ratio of 56:40), while addition of (6*R*)-(−)-bis-lactim ether **16** to 4-chlorocinnamate **12** gave a mixture comprising mainly the (2*S*,3*R*)- and (2*S*,3*S*)-isomers **8** and **7**, respectively (in a ratio of 56:42). The four stereoisomers (**5**−**8**) were therefore conveniently prepared by addition of either 3-lithio-(6*S*)- or -(6*R*)-bis-lactim ether (**15** or **16**, respectively) to 4-chlorocinnamate **12** and separation of the resultant mixtures of diastereoisomers (**23**−**26**) by flash silica gel chromatography. The absolute configurations of **6** and **7** were confirmed by X-ray crystallography. Both the (2*S*,3*S*)- and (2*S*,3*R*)-isomers (**7** and **8**, respectively) at a concentration of 100 μM significantly potentiated depolarizations induced by 10 μM L-homocysteic acid (L-HCA) (% control ± sem: 130.4 ± 3.6, $n = 20$ and 114.5 ± 2.4, $n = 11$, respectively) while the (2*R*,3*S*)-isomer **5** significantly reduced L-HCA response amplitude (94.2 ± 1.4, $n = 9$) and the (2*R*,3*R*)-isomer **6** was inactive. Experiments designed to compare the agonist-potentiating actions of **7** and **8** in the neonatal rat spinal cord with L-*trans*-pyrrolidine-2,4-dicarboxylic acid, the well-known L-Glu uptake inhibitor, provided additional evidence for the selective enhancement of depolarizations due to L-HCA and not those due to L-Glu. This selective action supports the existence of multiple excitatory amino acid uptake sites.

Introduction

Excitatory synaptic transmission in the mammalian central nervous system (CNS) is believed to be mediated predominantly by the excitatory amino acid (EAA) neurotransmitter L-glutamate (L-Glu, **1**). However, various structural analogues of L-Glu have also been proposed as possible excitatory transmitters, and among these is the sulfur-containing amino acid, L-2-amino-4-sulfobutanoic acid (L-homocysteic acid, L-HCA, **2**). Previous studies have demonstrated that L-HCA fulfils several of the classical criteria necessary for neurotransmitter candidature, including its presence in and its stimulus-evoked release from CNS tissue,[1,2] although these are not universal findings.[3,4] Neurotransmitters also require an effective system for terminating their actions following their release into the synaptic cleft and subsequent receptor activation. This usually involves either enzymic degradation of the neurotransmitter or its uptake into neuronal and/or glial elements. In the case of L-Glu, removal is believed to be via high-affinity transporter proteins present in the plasma membrane of glia and neurons.[5] Even though L-HCA does not appear to be transported via a high-affinity carrier, the L-Glu analogue, 3-(4-chlorophenyl)glutamate (**4**), has been shown to selectively enhance the excitatory, depolarizing actions of L-HCA (in comparison with L-Glu) on amphibian and mammalian CNS neurones.[6−8] This has led to the proposal that one or more of the isomers **5**−**8** is an inhibitor of a distinct L-HCA-selective uptake site.[6] If such a site is present in the mammalian CNS, it would provide further support for the neurotransmitter candidature of L-HCA.

The receptors activated by both L-Glu and L-HCA have been implicated in a number of neurological disorders including epilepsy.[9] In addition, it has been suggested that reduced EAA uptake may be responsible for several neuropathologies such as Alzheimer's disease[10] and amyotrophic lateral sclerosis.[11] Other disease states may be characterized by an overactive uptake system. It is therefore plausible that compounds able to selectively interfere with L-HCA uptake processes may modulate excitatory synaptic transmission with resultant clinical benefit.

All earlier studies have utilized **4**, which exists as a mixture of two racemic diastereoisomers. We have undertaken diastereoselective syntheses of the individual isomers of **4** and examined each for their ability to influence some electrophysiological effects of L-Glu and L-HCA on spinal tissue in order to identify the isomers responsible for the potentiating actions previously noted with **4**.

Chemistry

The work of Schöllkopf and co-workers on the stereoselective synthesis of amino acids using the bis-lactim ether method has been reviewed.[12] This method[13,14] has been applied to the stereoselective synthesis of intermediates (**17**−**20**, Scheme 1) which could be potentially converted to the stereoisomers of 3-phenylglutamic acid (**3**), an analogue of 3-(4-chlorophenyl)glutamic acid (**4**) (Chart 1). Thus, Michael addition of 3-lithio-(6*R*)-(−)-bis-lactim ether **14** to methyl *trans*-cinnamate **9** afforded

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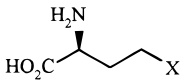
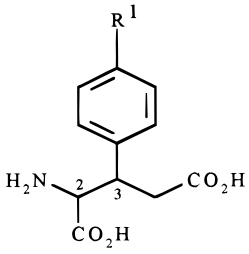
Chart 1

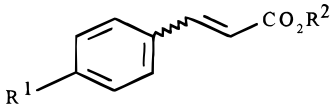


1: X=CO_2H (S)-glutamic acid

2: X=SO_3H (S)-homocysteic acid

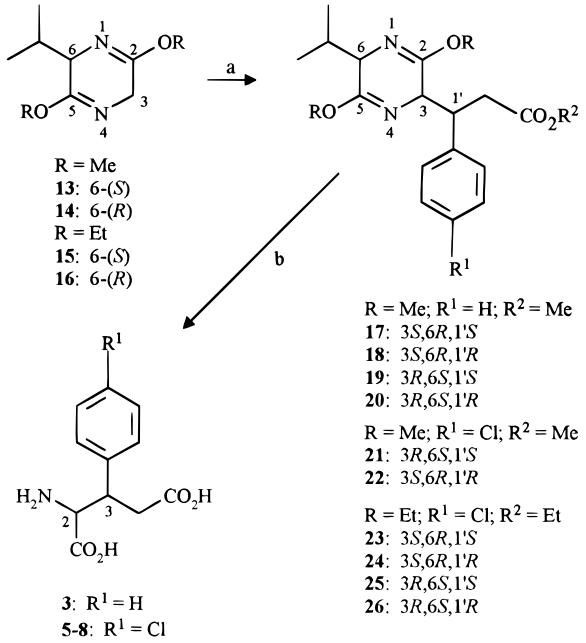


3 R^1 = H
R^1 = Cl
4-(2*RS*,3*RS*)
5-(2*R*,3*S*)
6-(2*R*,3*R*)
7-(2*S*,3*S*)
8-(2*S*,3*R*)



R^1 = H; R^2 = Me
9-*trans*
10-*cis*
R^1 = Cl; R^2 = Me
11-*trans*
R^1 = Cl; R^2 = Et
12-*cis*

Scheme 1[a]



R = Me
13: 6-(*S*)
14: 6-(*R*)
R = Et
15: 6-(*S*)
16: 6-(*R*)

R = Me; R^1 = H; R^2 = Me
17: 3*S*,6*R*,1′*S*
18: 3*S*,6*R*,1′*R*
19: 3*R*,6*S*,1′*S*
20: 3*R*,6*S*,1′*R*

R = Me; R^1 = Cl; R^2 = Me
21: 3*R*,6*S*,1′*S*
22: 3*S*,6*R*,1′*R*

R = Et; R^1 = Cl; R^2 = Et
23: 3*S*,6*R*,1′*S*
24: 3*S*,6*R*,1′*R*
25: 3*R*,6*S*,1′*S*
26: 3*R*,6*S*,1′*R*

3: R^1 = H
5-8: R^1 = Cl

[a] (a) (i) *n*-BuLi/−78 °C; (ii) **9**−**12**; (b) (i) 1 N TFA in THF, room temperature; (ii) 6 N HCl, reflux; (iii) ion-exchange chromatography.

mainly the (3*S*,6*R*,1′*R*)-isomer **18** [ratio of stereoisomers: **18**:**17** = 77:2.8].[13] In contrast, addition of 3-lithio-(6*R*)-(−)-bis-lactim ether **14** to methyl *cis*-cinnamate (**10**) afforded mainly the (3*S*,6*R*,1′*S*)-isomer **17** [ratio of stereoisomers: **17**:**18**:**19**:**20** = 384:70:2.7:1].[13] Schöllkopf and co-workers[14] have reported the ratio of stereoisomers to be **20**:**19**:**18**:**17** = 148:46:<0.5:1 for the intermediates synthesized *via* addition of 3-lithio-(6*S*)-(+)-bis-lactim ether **13** to methyl *cis*-cinnamate (**10**). Neither of these authors attempted to isolate the four stereoisomers of **3** *via* deprotection of the intermediates **17**−**20**. A possible explanation for the diastereoselectivity obtained with methyl *cis*-cinnamate (**10**) relies on the cinnamate approaching the bis-lactim ether from the face opposite the bulky isopropyl group.[13,14] This is likely since, in the transition state, the anti orientation of the isopropyl group and the incoming electrophile would be more favored for steric reasons. This explains the observed stereoselectivity at the α-amino acid stereogenic center but not the observed selectivity at the 1′-position of the isomers **17**−**20**. In order to explain the different diastereoselectivity observed on reaction of either *trans*- or *cis*-cinnamate (**9** and **10**, respectively), it was proposed that the carbonyl group of the cinnamate is oriented above N1 of the bis-lactim ether anion, thus forming a chelate with the lithium cation.[13,14] In addition, it was proposed that in the transition state the two π-systems (the diazapentadienyl anion and the π-system of the cinnamate) form a π-complex which is stabilized by either HOMO−LUMO or charge-transfer interactions.[13,14] The cinnamate molecule is therefore held in a particular orientation relative to the bis-lactim ether, thus explaining the difference in stereochemistry at the 1′-position of **17**−**20** obtained on reaction with either *trans*- or *cis*-cinnamate (**9** and **10**, respectively).

In order to prepare all four stereoisomers **5**−**8**, a method similar to that outlined above was utilized. Our initial strategy was to prepare all four stereoisomers by the stereoselective Michael addition of 3-lithio-(6*S*)- or -(6*R*)-bis-lactim ether (**13** or **14**, respectively) to either *trans*- or *cis*-4-chlorocinnamate (**11** or **12**) (Scheme 1). Thus, Michael addition of 3-lithio-(6*S*)-(+)-bis-lactim ether **13** to 4-chlorocinnamate **11** gave mainly the (2*R*,3*S*)-isomer **5** upon hydrolysis of the intermediate obtained from the reaction mixture and analysis by chiral HPLC (Table 1). Addition of 3-lithio-(6*R*)-(−)-bis-lactim ether **14** to 4-chlorocinnamate **11** gave mainly the (2*S*,3*R*)-isomer **8** (see Table 1). However, addition of 3-lithio-(6*S*)-(+)-bis-lactim ether **15** to 4-chlorocinnamate **12** gave a mixture comprising mainly the (2*R*,3*S*)- and (2*R*,3*R*)-isomers **5** and **6**, respectively (in a ratio of 56:40, see Table 1). Addition of 3-lithio-(6*R*)-(−)-bis-lactim ether **16** to 4-chlorocinnamate **12** gave a mixture comprising mainly the (2*S*,3*R*)- and (2*S*,3*S*)-isomers **8** and **7**, respectively (in a ratio of 56:42, see Table 1). The four stereoisomers **5**−**8** were therefore conveniently prepared by addition of either 3-lithio-(6*S*)- or -(6*R*)-bis-lactim ether (**15** or **16**, respectively) to 4-chlorocinnamate **12** and separation of the resultant mixtures of diastereoisomers (**25**, **26** and **23**, **24**, respectively) by flash silica gel chromatography. Deprotection of the pure adducts (**23**−**26**) was carried out by stirring at room temperature in 1 N TFA in THF overnight followed by hydrolysis in 6 N HCl. Further purification was carried out by ion-exchange chromatography, and crystallization of the resulting solids from water. The

Table 1. Ratio of Stereoisomers[a] of **4** Synthesized by Michael Addition of Bis-Lactim Ether to 4-Chlorocinnamate

reactants		**5**	**6**	**7**	**8**
12	**15**	55.78	40.4	1.72	2.08
12	**16**	0	2.31	41.78	56.05
11	**13**	89.28	5.32	3.78	1.6
11	**14**	1.25	1.86	4.86	92.0

[a] Ratio of stereoisomers was obtained by hydrolyzing the adducts **23**−**26**, obtained directly from the reaction mixture without further purification, in 6 N HCl and analyzing the resultant products by chiral HPLC.[15]

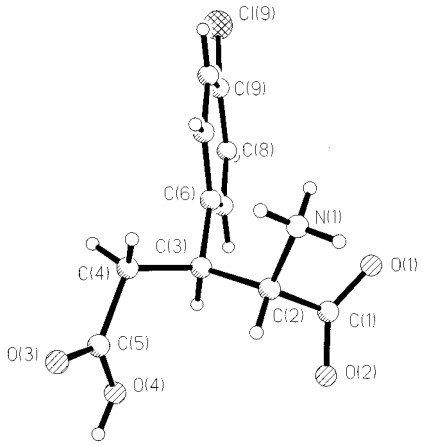


Figure 1. Molecular structure and atom labeling scheme of **7**.

purity of the isomers (**5**−**8**) was established by chiral HPLC (all isomers were >95% pure).[15]

X-ray Crystallographic Analysis of 6 and 7. Both isomers **6** and **7** crystallized in the monoclinic space group $P2_1$ with identical cell dimensions and one molecule in the asymmetric unit. The absolute configuration was clearly determined in both cases, showing **6** to have the configuration (2*R*,3*R*) and **7** to have that of (2*S*,3*S*). Figure 1 shows the molecular structure and atom labeling scheme of **7**. The other two isomers **5** and **8** did not give good enough crystals for X-ray crystallographic analysis.

Both isomers **6** and **7** form hydrogen-bonding networks, involving both N−H···O and O−H···O type interactions. This is illustrated for isomer **7** in Figure 2.

Assignment of Absolute Stereochemistry of 5 and 8. The configurations of the other isomers (**5** and **8**) were assigned by inference from their ^{1}H NMR spectra and by reference to earlier work on the synthesis of 3-phenylglutamic acid derivatives.[13,14] Reaction of (6*R*)-bis-lactim ether **16** and *cis*-4-chlorocinnamate **12** gave two major products. Separation of the resultant products by chromatography and subsequent acid hydrolysis gave two separate amino acids. One of these was found to be the (2*S*,3*S*)-isomer (**7**) by X-ray crystallography. Proton NMR spectra of **7** and the other major product were different, and therefore these amino acids were not enantiomers. Thus, the unidentified product must be either the (2*S*,3*R*)- or (2*R*,3*S*)-isomer (**8** or **5**, respectively). Hartwig and Born[13] observed that on reaction of (6*R*)-(−)-bis lactim ether **14** with *cis*-cinnamate **10** mainly **17** and **18** (corresponding to the (2*S*,3*S*)- and (2*S*,3*R*)-isomers **7** and **8**, respectively) were formed. This strongly suggests that the (2*S*,3*R*)-isomer **8** is the other product obtained by reaction of (6*R*)-bis-lactim ether **16** and *cis*-4-chlorocinnamate **12**. Similarly, reaction of (6*S*)-bis-lactim ether **15** and *cis*-4-chlorocinnamate **12** gave two major products. After chromatographic separation and hydrolysis, one of the amino acids was identified as the (2*R*,3*R*)-isomer **6** by X-ray crystallography. The ^{1}H NMR spectra of **6** and the other major product from the reaction were again different (although the proton NMR spectra of **6** and **7** and **5** and **8**, respectively, were identical), and thus the other amino acid must be the (2*R*,3*S*)-isomer **5**. This assignment is in agreement with the observation of Schollkopf and co-workers[14] who obtained a mixture comprised mainly of **20** and **19** (corresponding to the (2*R*,3*S*)- and (2*R*,3*S*)-isomers **6** and **5**, respectively) on reaction of (6*S*)-bis-lactim ether **13** and *cis*-cinnamate **10**.

It is of interest that in the case of the reactions involving *cis*-4-chlorocinnamate **12** control of the stereochemistry at the C-3 position of **4** is poor compared to the diastereoselectivity observed with *cis*-cinnamate **10**. A possible explanation for the lack of stereocontrol at the 3-position of **4** in reactions using *cis*-4-chlorocinnamate **12** is that the electron-withdrawing chloro group destabilizes either the $\pi-\pi$ interaction of the chlorocinnamate with the bis-lactim ether or the interaction of the carbonyl group and the lithium cation.

Pharmacology[19]

Both the (2*S*,3*S*)- and (2*S*,3*R*)-isomers (**7** and **8**, respectively) at a concentration of 100 μM significantly potentiated depolarizations induced by 10 μM L-HCA (% control ± sem: 130.4 ± 3.6, $n = 20$ and 114.5 ± 2.4, $n = 11$, respectively) while the (2*R*,3*S*)-isomer **5** significantly reduced L-HCA response amplitude (94.2 ± 1.4, $n = 9$) and the (2*R*,3*R*)-isomer **6** was inactive (Figure 3). In contrast, responses induced by L-Glu (50 μM) were not affected by any of the four isomers applied at 100 μM, nor by the (2*S*,3*S*)- or (2*S*,3*R*)-isomers (**7** and **8**, respectively) at 500 μM. The L-Glu uptake inhibitor, L-*trans*-pyrrolidine-2,4-dicarboxylic acid, at a concentration of 500 μM, significantly enhanced the depolarizations evoked by both L-HCA (10 μM) and L-Glu (50 μM), although its potentiating action on L-Glu-evoked responses (+160%) was markedly greater than that on L-HCA-induced responses (+22%).[19]

The depressant activity of the (2*R*,3*S*)-isomer **5** upon L-HCA-evoked depolarizations was likely to be the result of *N*-methyl-D-aspartate (NMDA) receptor antagonism, as responses induced by 10 μM L-HCA under these experimental conditions would be mainly the result of NMDA receptor activation.[16] It was found that the (2*R*,3*S*)-isomer **5** (100 μM) was effective at reversing a sustained depolarization induced by continuous application of 5 μM NMDA, but was approximately 300-fold weaker than D-2-amino-5-phosphonopentanoate in this regard. None of the stereoisomers, **5**−**8** (at a concentration of 100 μM), had any effect on depolarizations induced by either (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ((*S*)-AMPA; 0.4 μM) or kainic acid (2 μM) and only the (2*R*,3*S*)-isomer **5** antagonized NMDA-induced depolarizations (see above).[19]

Discussion

Earlier use of **4** raised the possibility that the individual isomers may have possessed different pharmacological properties which together generated an apparent selectivity of action upon L-HCA-induced responses. The work presented here provides the first evidence that the selective potentiation of L-HCA-induced responses is a property of two isomers, namely, the (2*S*,3*S*)- and (2*S*,3*R*)-isomers (**7** and **8**, respectively). In addition, the weak NMDA receptor antagonist action of **4** previously noted[6] can now be attributed to the (2*R*,3*S*)-isomer **5**. Further use of **4** in electrophysiological studies would seem unwise due to the presence of the (2*R*,3*S*)-isomer **5**.

This study does not produce direct evidence that the actions of the (2*S*,3*S*)- and (2*S*,3*R*)-isomers (**7** and **8**,

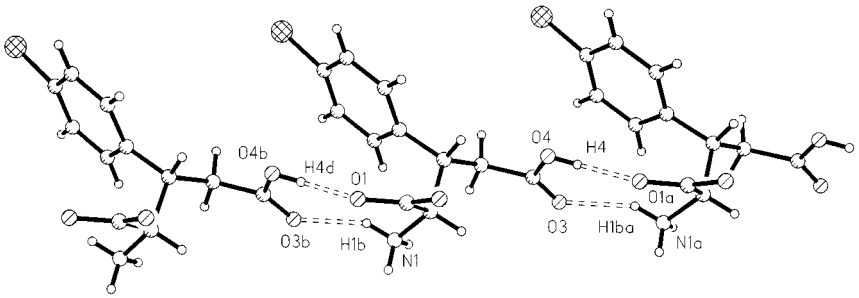


Figure 2. Two of the hydrogen-bonding interactions in **7** between O(3) and H(1) and between O(1) and H(4).

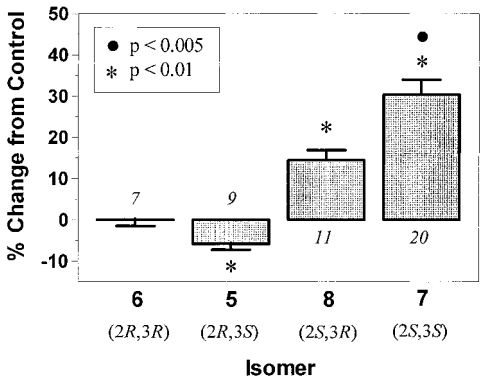


Figure 3. Effects of isomers **5**−**8** (100 μM each) upon 10 μM L-HCA-induced depolarizations. Bars represent mean ± sem of the number of indicated tests. The (2*S*,3*R*)- and (2*S*,3*S*)-isomers (**8** and **7**, respectively) caused potentiations that were significantly different from each other (●, $p < 0.005$, Mann-Whitney *U*-Test). Only the (2*R*,3*R*)-isomer **6** produced a nonsignificant change from control (Wilcoxon signed ranked test; *, $p < 0.01$ for the other isomers).

respectively) are mediated by inhibition of an amino acid uptake site. This seems likely to us, however, in view of the inhibition by **4** of L-[^{35}S]HCA uptake in amphibian spinal neurons.[6] Another possibility warranting consideration is that the observed potentiation of L-HCA responses could be due to allosteric interaction with ionotropic glutamate receptors. However, neither the (2*S*,3*S*)- nor the (2*S*,3*R*)-isomers (**7** and **8**, respectively) influence depolarizations induced by (*S*)-AMPA, NMDA, or kainic acid, suggesting that these isomers do not act directly *via* known ionotropic glutamate receptors. Due to limited compound availability, the actions of isomers **5**−**8** on metabotropic glutamate receptors could not be determined.

The inhibitory action of **4** on L-[^{35}S]HCA uptake reported by Davies and colleagues[6] was apparently selective for the sulfur-containing amino acid relative to L-[^{14}C]Glu or L-[^{14}C]Asp in amphibian spinal neurones. However, in detailed studies on cultured mouse astrocytes and cerebellar granule cells, and rat cerebral cortex synaptosomes, Griffiths *et al.*[17] found **4** to be virtually equieffective as an inhibitor of the uptake of L-HCA and D-Asp (a nonmetabolizable analogue of L-Glu). They proposed that the apparently selective inhibitory action of **4** for L-HCA uptake reported by Davies *et al.*[6] was due principally to its low potency, coupled with the high ratio of L-Glu to L-HCA concentrations used by these authors, rather than to an intrinsic selectivity of uptake blockade. This argument may be of less concern in the present experiments since pure stereoisomers of higher inhibitory potency were used in conjunction with lower relative concentrations of L-Glu to L-HCA. Nevertheless, direct experiments on the relative inhibition of L-HCA and L-Glu uptake will be required to resolve this issue. Experiments designed to compare the agonist-potentiating actions of the isomers (**7** and **8**) in the neonatal rat spinal cord with those of L-*trans*-pyrrolidine-2,4-dicarboxylic acid, the well-known L-Glu uptake inhibitor,[18] provided additional evidence for the selectivity of action of **7** and **8** on L-HCA-induced depolarizations and indirectly supported the existence of multiple excitatory amino acid uptake sites.[19] Whatever the mechanism of action, the identification of **7** (particularly) and **8** as selective enhancers of L-HCA-induced excitation could prove useful for probing sites of proposed L-HCA-mediated synaptic excitation in the CNS.

Experimental Section

Chemistry: General Procedures. Melting points were taken on a digital electrical melting point apparatus and are uncorrected. Proton and carbon NMR spectra were measured with a 270 MHz spectrometer in D_2O/NaOH (at pH 14) unless otherwise stated, using the sodium salt of 3-(trimethylsilyl)-propanesulfonic acid as an internal standard. Thin layer chromatography was performed on 60 Å Whatman precoated silica gel plates. Silica gel for chromatography was Sorbsil C60 (60 Å, 40−60 micron). Elemental analyses were performed at the microanalytical laboratory of the University of Bristol. Ethyl *cis*-4-chlorocinnamate (**12**) was synthesized by an extension of a literature method.[20,21] The bis-lactim ethers **15** and **16** were synthesized by a reported method.[22] Bis-lactim ethers **13** and **14** were obtained from Merck. All other reagents were obtained from the Aldrich Chemical Co., U.K.

(3*S*,6*R*,1′*S*)-2,5-Diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (23) and (3*S*,6*R*,1′*R*)-2,5-Diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (24). A 2.5 N solution of *n*-butyllithium in hexanes (62.26 mL, 0.156 mol) was added dropwise, under a dry nitrogen atmosphere, to a solution of (6*R*)-(−)-2,5-diethoxy-6-isopropyl-3,6-dihydro-1,4-pyrazine (**16**) (30 g, 0.141 mol) in anhydrous THF (480 mL) at −78 °C, and the mixture was stirred for 10 min. Ethyl *cis*-4-chlorocinnamate (**12**) (35.66 g, 0.169 mol) dissolved in dry THF (300 mL) was added and stirring continued for 6 h at −78 °C. A solution of acetic acid (7.8 mL, 0.13 mol) was added, the reaction mixture was allowed to warm to room temperature, the THF was evaporated under reduced pressure, the mixture was extracted with ethyl acetate (3 × 200 mL), and the combined extracts were dried ($MgSO_4$) and evaporated in vacuo to give 60 g of crude **23** and **24**. The residue was flash chromatographed over silica gel. Elution with 20:1 toluene/ethyl acetate gave first (3*S*,6*R*,1′*S*)-2,5-diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (**23**) (6 g, 10%) and then (3*S*,6*R*,1′*R*)-2,5-diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (**24**) (8 g, 13%) as clear oils. A sample of both **23** and **24** were hydrolyzed in 6 N HCl and the hydrolysate analyzed by chiral HPLC in order to assess enantiomeric purity. Once enantiomeric purity (>95%) had been established, **23** and **24** were used in the next step without further characterization.

(3*R*,6*S*,1′*S*)-2,5-Diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (25) and (3*R*,6*S*,1′*R*)-2,5-diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (26) were obtained similarly with 2.5 N *n*-butyllithium (23.76 mL, 0.059 mol), (6*S*)-(−)-2,5-diethoxy-6-isopropyl-3,6-dihydro-1,4-pyrazine (**15**) (11.46 g, 0.054 mol) in dry THF (160 mL), and ethyl *cis*-4-chlorocinnamate (**12**) (13.69 g, 0.065 mol) in dry THF (100 mL). The crude product was flash chromatographed over silica gel. Elution with 20:1 toluene/ethyl acetate gave first (3*R*,6*S*,1′*R*)-2,5-diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (**26**) (6 g, 26%) and then (3*R*,6*S*,1′*S*)-2,5-diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (**25**) (6 g, 26%) as clear oils. Enantiomeric purity (>95%) was verified as described above, and then **25** and **26** were used in the next step without further characterization.

(3*R*,6*S*,1′*S*)-2,5-Dimethoxy-6-isopropyl-3-[2′-(methoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (21) was obtained in an analogous manner from 2.5 N *n*-butyllithium (22.93 mL, 0.057 mol), (6*S*)-(−)-2,5-dimethoxy-6-isopropyl-3,6-dihydro-1,4-pyrazine (**13**) (9.2 g, 0.05 mol) in dry THF (170 mL), and methyl *trans*-4-chlorocinnamate (**11**) (9.83 g, 0.05 mol) in dry THF (170 mL). The crude product was flash chromatographed over silica gel. Elution with 20:1 toluene/ethyl acetate gave (3*R*,6*S*,1′*S*)-2,5-dimethoxy-6-isopropyl-3-[2′-(methoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (**21**) (6 g, 32%) as an oil. Enantiomeric purity (>95%) was verified as described for **23** and **24**.

(3*S*,6*R*,1′*R*)-2,5-Dimethoxy-6-isopropyl-3-[2′-(methoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (22) was obtained in an analogous manner from 2.5 N *n*-butyllithium (21.7 mL, 0.054 mol), (6*R*)-(−)-2,5-dimethoxy-6-isopropyl-3,6-dihydro-1,4-pyrazine (**14**) (10 g, 0.054 mol) in dry THF (200 mL), and methyl *trans*-4-chlorocinnamate (**11**) (10.62 g, 0.054 mol) in dry THF (160 mL). The crude product was flash chromatographed over silica gel. Elution with 20:1 toluene/ethyl acetate gave (3*S*,6*R*,1′*R*)-2,5-dimethoxy-6-isopropyl-3-[2′-(methoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (**22**) (6 g, 29%) as an oil. Enantiomeric purity (>95%) was verified as described for **23** and **24**.

(2*S*,3*S*)-3-(4-Chlorophenyl)glutamic Acid (7). A solution of (3*S*,6*R*,1′*S*)-2,5-diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (**23**) (4.5 g, 0.0106 mol) in 0.25 N HCl (80 mL) and THF (80 mL) was stirred at room temperature overnight. The next day, THF was removed in vacuo and the aqueous layer extracted with diethyl ether (3 × 150 mL). The aqueous layer was separated and evaporated under reduced pressure. The residue was dissolved in 6 N HCl (80 mL), heated under reflux for 4 h, allowed to cool, and evaporated under reduced pressure. The resultant oil was dissolved in water and applied to a bed of AG50 H^+ ion-exchange resin (50 mL). Elution with water, followed by 0.5 N aqueous pyridine and evaporation of the ninhydrin positive fractions of the 0.5 N aqueous pyridine eluate, gave a white solid. The solid was dissolved/suspended in water and the pH brought to 7 by the addition of AG1 OH^- ion exchange resin. The mixture was applied to a bed of AG1 acetate ion-exchange resin (50 mL). Elution with water, 0.01 N acetic acid, 0.05 N acetic acid, 0.1 N acetic acid, and 0.5 N acetic acid and evaporation of the ninhydrin positive fractions of the 0.5 N acetic acid eluate gave a white solid. Crystallization from water gave (2*S*,3*S*)-3-(4-chlorophenyl)glutamic acid (**7**) (0.2 g, 7%) as a white solid: mp 181.9−182.2 °C; $[\alpha]^{20}_D$ + 17.5° (*c* 0.45, 6 N HCl); ^{1}H NMR (D_2O/NaOD, 270 MHz) δ 2.62 (m, 2H), 3.31 (m, 1H), 3.36 (m, 1H), 7.28 (AA′XX′ system, 4H); ^{13}C NMR (D_2O/NaOD, 300 MHz) δ 43.592, 49.382, 63.924, 131.022, 132.684, 134.641, 141.163, 183.316, 184.040. Anal. ($C_{11}H_{12}ClNO_4$) C, H, N.

(2*S*,3*R*)-3-(4-Chlorophenyl)glutamic acid (8) was obtained similarly from (3*S*,6*R*,1′*R*)-2,5-diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (**24**) (8 g, 0.019 mol). Purification by ion-exchange resin chromatography as outlined above and crystallization from water gave (2*S*,3*R*)-3-(4-chlorophenyl)glutamic acid (**8**) (0.68 g, 14%) as a white solid: mp 194.7−194.9 °C; $[\alpha]^{20}_D$ + 21.5° (*c* 0.39, 1 N NaOH); ^{1}H NMR (D_2O/NaOD, 270 MHz) δ 2.64 (m, 2H), 3.26 (m, 1H), 3.36 (d, 1H, J = 7 Hz), 7.28 (AA′XX′ system, 4H); ^{13}C NMR (D_2O/NaOD, 300 MHz) δ 41.684, 49.555, 64.508, 130.891, 132.355, 134.345, 142.748, 183.375, 183.686. Anal. ($C_{11}H_{12}ClNO_4$) C, H, N.

(2*R*,3*R*)-3-(4-Chlorophenyl)glutamic acid (6) was obtained similarly from (3*R*,6*S*,1′*R*)-2,5-diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (**26**) (6 g, 0.0142 mol). Purification by ion-exchange resin chromatography as outlined above and crystallization from water gave (2*R*,3*R*)-3-(4-chlorophenyl)glutamic acid (**6**) (0.92 g, 25%) as a white solid: mp 184.2−185.2 °C; $[\alpha]^{20}_D$ − 18.4° (*c* 0.47, 6 N HCl); ^{1}H NMR (D_2O/NaOD, 330 MHz) δ 2.59 (m, 2H), 3.31 (m, 1H), 3.36 (m, 1H), 7.28 (AA′XX′ system, 4H); ^{13}C NMR (D_2O/NaOD, 300 MHz) δ 43.592, 49.341, 63.899, 130.973, 132.651, 134.575, 141.845, 183.258, 183.982. Anal. ($C_{11}H_{12}ClNO_4$) C, H, N.

(2*R*,3*S*)-3-(4-Chlorophenyl)glutamic acid (5) was obtained similarly from (3*R*,6*S*,1′*S*)-2,5-diethoxy-6-isopropyl-3-[2′-(ethoxycarbonyl)-1′-(4-chlorophenyl)ethyl]-3,6-dihydro-1,4-pyrazine (**25**) (6 g, 0.0142 mol). Purification by ion-exchange resin chromatography as outlined above and crystallization from water gave (2*R*,3*S*)-3-(4-chlorophenyl)glutamic acid (**5**) (0.5 g, 14%) as a white solid: mp 191.4−192.2 °C; $[\alpha]^{20}_D$−21.4° (*c* 0.28, 1 N NaOH); ^{1}H NMR (D_2O/NaOD, 270 MHz) δ 2.67 (m, 2H), 3.26 (m, 1H), 3.36 (d, 1H, J = 7 Hz), 7.28 (AA′XX′ system, 4H); ^{13}C NMR (D_2O/NaOD, 300 MHz) δ 41.749, 49.620, 64.608, 130.923, 132.371, 134.117, 142.295, 183.416, 183.727. Anal. ($C_{11}H_{12}ClNO_4$) C, H, N.

X-ray crystallography. Crystal Data for 6 and 7: $C_{11}H_{12}ClNO_4$, MW 257.67; monoclinic, space group $P2_1$ (No. 4), a = 7.320(1) Å, b = 5.928(1) Å, c = 12.943(3) Å, β = 104.84(3)°, V = 543.0(3) Å^3, Z = 2, D_c = 1.576 Mg m^{-3}. $F(000)$ = 268, μ (Cu Kα) = 3.17 mm^{-1}, T = 150(2) K. Both crystals were clear, colorless needles with dimensions of 0.3 × 0.2 × 0.2 mm and 0.3 × 0.1 × 0.1 mm for **6** and **7**, respectively.

Pharmacology.[19] Amino acid-induced depolarizations were recorded from the ventral roots of isolated hemisected spinal cords from 1−5-day old neonatal rats as previously described.[23] Briefly, the standard superfusion medium consisted of (mM) NaCl 118, $NaHCO_3$ 25, KCl 3, $CaCl_2$ 2.5, and D-glucose 12 and was gassed with 5% CO_2/95% O_2. In order to abolish synaptic activity in the preparation, tetrodotoxin (TTX, 5 μM for 2 min then 0.1 μM continuously) was included in the medium. Applications of L-Glu (50 μM) or L-HCA (10 μM) for 1 min resulted in depolarizations of approximately equal magnitude (0.25−1 mV).

Supporting Information Available: Additional X-ray crystallographic data for **6** and **7** (12 pages). Ordering information is given on any current masthead page.

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