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Supplementary Material Available: X-ray structure of compound **16b** and tables of atomic coordinates, bond lengths, and bond angles for **16b** and **1b** (10 pages). Ordering information is given on any current masthead page.

Conformationally Defined Neurotransmitter Analogues. Selective Inhibition of Glutamate Uptake by One Pyrrolidine-2,4-dicarboxylate Diastereomer

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In order to determine the conformational requirements for binding of L-glutamate to the proteins involved in the process of neurotransmission, rigid analogues containing an embedded glutamate moiety have been prepared. These "conformer mimics", the pyrrolidine-2,4-dicarboxylates **4**, **7**, **11**, and **14**, were synthesized from commercially available *trans*-4-hydroxy-L-proline and *cis*-4-hydroxy-D-proline, and then were tested for their ability to inhibit the high-affinity transport of [^{3}H]-L-glutamate into synaptosomes and to block the binding of radioligands to the NMDA (*N*-methyl-D-aspartate), KA (kainate), and QA (quisqualate) glutamate neurotransmitter receptor sites. While none of the four analogues binds effectively to the excitatory receptors, the L-*trans*-isomer **7** is a potent and selective competitive inhibitor of L-glutamate transport. These results delineate a specific structural/conformational preference for binding to the uptake system that is distinct from that required for binding to the NMDA, KA, and QA receptors.

L-Glutamate is one of the major excitatory neurotransmitters in the mammalian brain, mediating much of the synaptic transmission in the central nervous system.[1] The process of synaptic transmission requires the interaction of glutamate with a variety of proteins, including receptors, transport systems, and enzymes. Although the pharmacological specificity of these sites has been characterized to some extent, a detailed understanding of the structural requirements of glutamate binding is lacking.[2] We have therefore attempted to assess preferred binding geometries by preparing a series of conformationally well-defined acidic amino acids that are designed to mimic different glutamate conformers. In this report we describe the enantioselective synthesis of the four diastereomeric pyrrolidine-2,4-dicarboxylates (2,4-PDCs), each of which contains an embedded glutamate moiety, and demonstrate that one is a potent and selective inhibitor of a transport system that acts to remove L-glutamate from the synaptic cleft.

Considerable evidence has firmly established the existence of several classes of postsynaptic receptors[1] through which the excitatory action of L-glutamate is mediated, as well as the presence of a high-affinity transport system[3] that is thought to terminate the resultant excitatory signal. In vivo, endogenous L-glutamate binds to each of these sites, while in vitro, various glutamate analogues exhibit selective affinities and thus can pharmacologically differentiate among individual receptor classes. Specifically, three major types of excitatory amino acid transmitter receptors have been distinguished by their selective interaction with various agonists (Figure 1): *N*-methyl-D-aspartate (NMDA), kainate (KA), and quisqualate (QA).[2] Despite the fact that these analogues have been invaluable in identifying and characterizing the various classes of transmitter receptors empirically, a unified model to explain the observed binding specificities in terms of specific molecular conformations has been elusive.

Unlike systems that depend upon rapid chemical degradation for transmitter signal termination, L-glutamate appears to be removed from the synaptic cleft by high-affinity transport. Again, little is known about the conformational requirements of substrate binding to this transport system, although several competitive inhibitors have been identified.[3] Interest in the functional characteristics of this uptake mechanism has dramatically increased with the recent finding that excessive levels of glutamate (as well as other excitatory agonists) are neurotoxic and may play a significant role in neurological disorders such as ischemia, hypoglycemia, epilepsy, Huntington's disease, and Alzheimer's disease.[4]

The fact that the receptors and the uptake system exhibit selective binding of various glutamate analogues implies that glutamate itself may bind to each in a characteristic conformation that is mimicked by the appropriate analogue. For glutamate in solution there are nine conformers within 2.5 kcal/mol of the ground state,[5] any of which might correspond to the bound form at a specific site.[6] In attempting to relate binding preferences to

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(1) For a review, see: Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 365.
(2) (a) Watkins, J. C.; Olverman, H. J. *Trends Neurosci.* **1987**, *10*, 265. (b) Watkins, J. C.; Krogsgaard-Larsen, P.; Honore, T. *Trends Pharmacol. Sci.* **1990**, *11*, 25.
(3) (a) Balcar, V. J.; Johnston, G. A. R. *J. Neurobiol.* **1972**, *3*, 295. (b) Balcar, V. J.; Johnston, G. A. R. *J. Neurochem.* **1972**, *19*, 2657. (c) Debler, E. A.; Lajtha, A. *Ibid.* **1987**, *48*, 1851.
(4) (a) Meldrum, B. *Clin. Sci.* **1985**, *68*, 113. (b) Wieloch, T. *Science* **1985**, *230*, 681. (c) Rothman, S. M.; Olney, J. W. *Ann. Neurol.* **1986**, *19*, 105. (d) Bridges, R. J.; Geddes, J.; Monaghan, D. T.; Cotman, C. W. In *Excitatory Amino Acids in Health and Disease*, Lodge, D., Ed.; Wiley & Sons, Limited: England, 1987; pp 321-335.
(5) Ham, N. S. *Molecular and Quantum Pharmacology*, Bergman, E., Pullman, B., Eds., D. Reidel Publishing Co.: Dordrecht-Holland, 1974; pp 261-268.
(6) It is of course possible that a substrate would bind in a conformation that does not correspond to a solution energy minimum; however, it is unfavorable to do so because relatively small deviations away from energy minima are energetically costly and would result in low binding energies.

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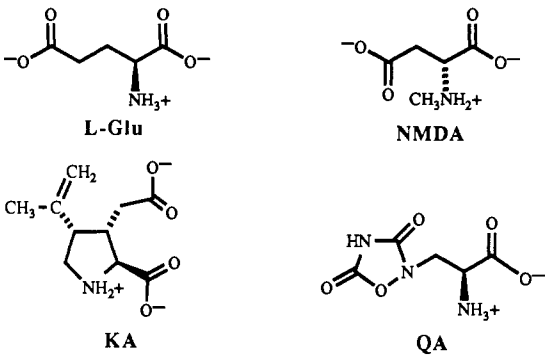


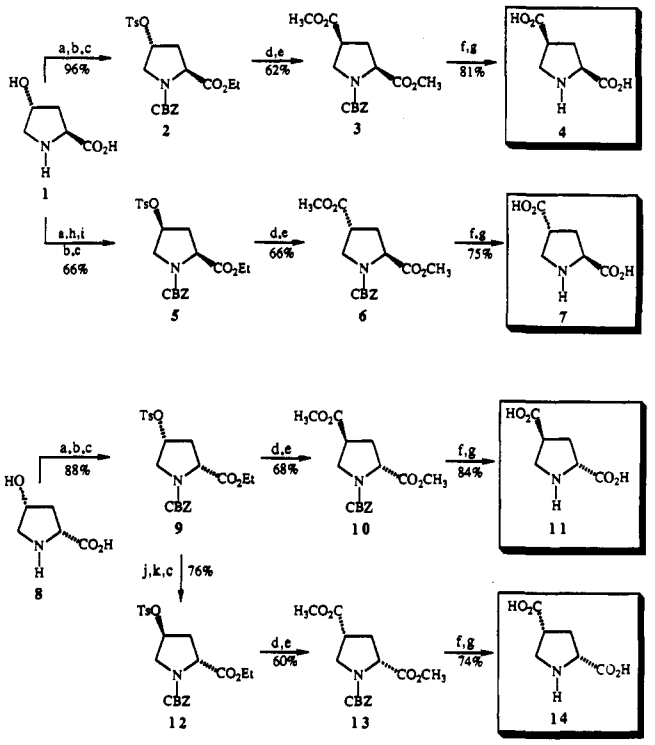
Figure 1. Excitatory amino acids.

specific glutamate conformations, it is therefore necessary to utilize analogues in which the functional groups presumably responsible for binding are in well-defined positions, e.g., within a relatively rigid molecular framework.[7] In this regard, nature has provided a potentially useful molecular prototype, kainic acid (KA), which contains an embedded L-glutamate moiety that presumably mimics the glutamate conformation favored for binding to the KA receptor.[8] Changing the position of the distal carboxyl group in a systematic way would be expected to result in altered binding selectivities,[9] and thus provide specific information on preferred binding geometries. We therefore have prepared a related series of conformationally defined carboxyproline derivatives that mimic distinct glutamate conformations.[10]

Chemistry

cis- and *trans*-L-pyrrolidine-2,4-dicarboxylates (**4** and **7**, respectively) were synthesized from commercially available *trans*-4-hydroxy-L-proline.[11] For the synthesis of **4**, the starting proline derivative **1** was converted into the protected tosylate **2** by sequential reaction with benzyloxycarbonyl (CBZ) chloride, ethanolic tosic acid, and tosyl pyridine. S_N2 displacement of the tosyl group with cyanide gave the *cis*-nitrile ethyl ester, which underwent a Pinner reaction and transesterification in wet methanol to give **3**, followed by saponification of the resultant diester

Scheme I.[a] Synthesis of PDC Diastereomers



[a] (a) $ClCO_2CH_2Ph$, $NaHCO_3$, H_2O, $PhCH_3$; (b) EtOH, *p*-TsOH; (c) TsCl, py; (d) NaCN, DMSO; (e) HCl, wet CH_3OH; (f) NaOH, THF–H_2O 1:1; (g) H_2 (48 psi)/10% Pd–C, CH_3OH; (h) Jones oxidation; (i) $NaBH_4$, CH_3OH; (j) *n*-Bu_4NOAc, acetone; (k) NaOEt, EtOH.

and finally hydrogenolysis to give **4** in an overall yield of 47%. The *cis*-tosylate ethyl ester **5** required for the preparation of *trans*-diacid **7** was readily prepared by Jones oxidation of *N*-CBZ-*trans*-4-hydroxy-L-proline and stereoselective reduction of the resultant ketone with $NaBH_4$. This stereoselective reduction, originally observed by Patchett and Witkop,[12] gives exclusively the cis isomer as confirmed in this case by comparison with the enantiomeric alcohol prepared from authentic *cis*-hydroxyproline (see below). Esterification and tosylation then gave **5** in 66% yield. Conversion of this tosylate into **7** was brought about in 49% yield by the aforementioned four-step displacement/Pinner/deprotection sequence.

cis-4-Hydroxy-D-proline (**8**) serves as starting material for both D-diacids **11** and **14**. Following the procedure for the conversion of **1** into **4**, *cis*-tosylate ethyl ester **9** was prepared in three steps (88%) from **8**, and the four-step conversion of a tosylate ethyl ester (in this case **9**) into the corresponding 2,4-pyrrolidine-dicarboxylate afforded *trans*-4-carboxy-D-proline (**11**; 56%). The alcohol inversion sequence in this case differs from that described above because the available D starting material is the cis diastereomer rather than trans.[11] Hence, the C-4 tosylate group in ester **9** was inverted by S_N2 displacement of the tosyl group with *n*-Bu_4NOAc, hydrolysis of the resultant acetate with NaOEt in ethanol, and tosylation of the inverted alcohol group, giving trans ester **12** in 76% yield. Subjecting **12** to the aforementioned four-step process gave **14** in 44% yield.

Attempts to directly determine the enantiomeric purities of the PDC products were unsuccessful; however, their immediate precursors, N-deprotected dimethyl esters **3**, **6**, **10**, and **13**, were all >95% enantiomerically pure based

(7) For representative recent examples of syntheses and binding studies of rigid glutamate analogues, see: (a) Monahan, J. B.; Hood, W. F.; Compton, R. P.; Cordi, A. A.; Snyder, J. P.; Pellicciari, R.; Natalini, B. *Neurosci. Lett.* **1990**, *112*, 329. (b) Kozikowski, A. P.; Tückmantel, W.; Reynolds, I. J.; Wroblewski, J. T. *J. Med. Chem.* **1990**, *33*, 1561. (c) Tsai, C.; Schneider, J. A.; Lehman, *J. Neurosci. Lett.* **1988**, *92*, 298. (d) Honore, T.; Davies, S. N.; Drejer, J.; Fletcher, E. J.; Jacobsen, P.; Lodge, D.; Flemming, E. N. *Science* **1988**, *241*, 701. (e) Yamanoi, K.; Ohfune, Y. *Tetrahedron Lett.* **1988**, *29*, 1181. (f) Curry, K.; Peet, D. S.; Magnusson, D. S. K.; McLennan, H. *J. Med. Chem.* **1988**, *31*, 864. (g) Curry, K.; Magnuson, D. S. K.; McLennan, H.; Peet, M. J. *Can. J. Physiol. Pharmacol.* **1987**, *65*, 2196. (h) Krogsgaard-Larson, P.; Madsen, U.; Nielsen, B.; Hansen, J. J.; Nielsen, E. O.; Brehm, L.; Curtis, D. R. *Proc. Xth Congr. Pharmacol.* **1987**, 113.

(8) Kainate can mimic more than one glutamate conformation because the ring appendage containing the distal carboxylate is free to rotate.

(9) For examples of synthetic studies on structural modifications of kainate itself, see: (a) Goldberg, O.; Luini, A.; Teichberg, V. I. *Tetrahedron Lett.* **1980**, *21*, 2355. (b) Oppolzer, W.; Robiani, C.; Battig, K. *Helv. Chim. Acta* **1980**, *63*, 2015.

(10) Proline has also served as the basis of conformationally restricted analogues of amino acids other than glutamate: Koskinen, A. M. P.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 1859.

(11) The trans-L, cis-D, and cis-L isomers of 4-hydroxyproline are commercially available, but the latter is prohibitively expensive (>$150/g) as a starting material.

(12) Patchett, A. A.; Witkop, B. *J. Am. Chem. Soc.* **1957**, *79*, 185.

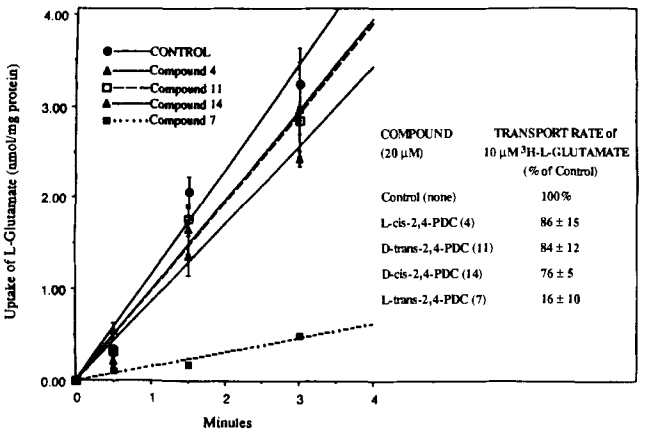


Figure 2. Inhibition of [^{3}H]-L-glutamate transport into synaptosomes by L-*trans*-2,4-PDC (**7**). Synaptosomal uptake of [^{3}H]-L-glutamate (10 μM) was followed at 37 °C in the presence and absence of 20 μM PDC isomers. The curves shown are from representative experiments (n = 2–4 rate determinations) and are reported as mean ±SD. Transport rates were determined by linear-regression analysis and have been corrected for background and nonspecific uptake. The effect of the PDC isomers is summarized in the inset and is reported as percent of control transport rate ±SD (n = 8–16 rate determinations).

on careful ^{1}H NMR analysis of the corresponding Mosher amides.[13] Thus, the only step during which the stereochemical integrity of any of the target PDCs could have been compromised is during the final deprotection step. But racemization in these cases requires inversion at two centers, and it cannot occur without simultaneous epimerization because the epimer is neccessarily an intermediate in racemization process. Since none of the target dicarboxylates showed any sign of the corresponding epimer by careful ^{1}H NMR analysis,[14] *both* stereochemically labile centers in each case survived intact, and the enantiomeric purities of the dicarboxylates are at least 95%. The enantiomeric purities are also consistent with the observed differences in biochemical activities (see below).

Pharmacology

The binding specificities of the four PDC isomers were determined by testing both their ability to inhibit the high-affinity transport of [^{3}H]-L-glutamate into synaptosomes[15] and to block the binding of radioligands to each of the three receptor sites in a synaptic plasma membrane preparation (SPM).[16] When included in the [^{3}H]-L-glutamate uptake assay, as represented in Figure 2, at a 2-fold excess relative to L-glutamate (10 μM of [^{3}H]-L-glutamate vs 20 μM of **7**), the L-*trans*-PDC isomer potently reduced the rate of synaptosomal transport from control levels of 1.13 nmol min^{-1} (mg of protein)$^{-1}$ to 0.16 nmol min^{-1} (mg of protein)$^{-1}$. In contrast, when the L-cis, D-cis, and D-trans isomers were included at similar concentrations, they were found to be substantially less effective. A more detailed kinetic analysis of the action of L-*trans*-PDC is shown in Figure 3. The Lineweaver–Burk plot demonstrates the concentration dependence of the action of L-*trans*-PDC and exhibits a pattern consistent with that of a competitive inhibitor (i.e., an increase in $K_{m_{app}}$ with no change in V_{max}). K_m and V_{max} values for the transport of L-glutamate were found to be 16.6 ± 2.7 μM and 3.41 ± 1.46 nmol/min per mg of protein, respectively, (n = 7) at 25 °C. Slope replots (see Figure 3, inset) indicated a K_i value of 5.06 ± 1.55 μM (n = 5) for L-*trans*-PDC. Thus, a configurationally well-defined competitive transport inhibitor, L-*trans*-PDC, has been identified, clearly delineating a preferred configurational (and/or conformational) relationship for binding to the glutamate uptake protein.

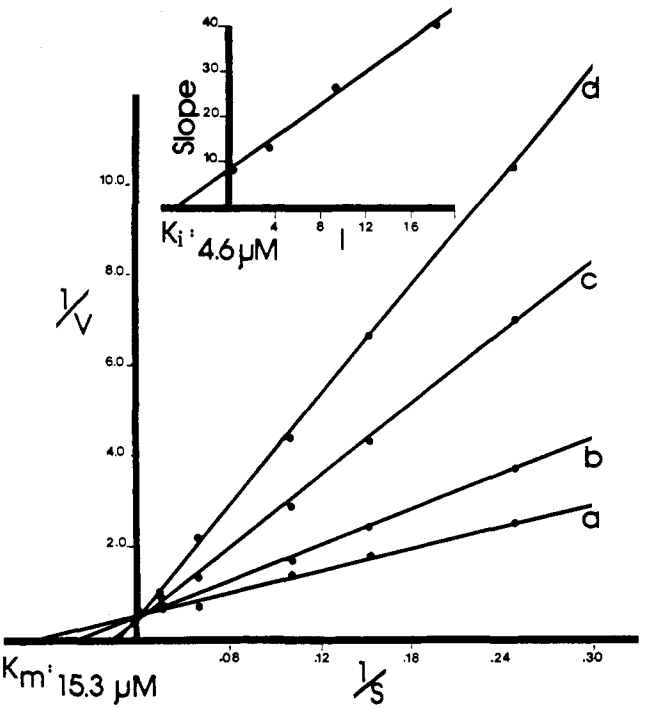


Figure 3. Competitive inhibition of L-glutamate uptake by L-*trans*-2,4-PDC (**7**). Lineweaver-Burk plot of the synaptosomal transport of L-glutamate (V = nmol L-glutamate/min per mg of protein; S = μM L-glutamate. Uptake of [^{3}H]-L-glutamate (4, 6.6, 10, 25, 66 μM) was followed for 2 min at 25 °C in the absence and presence (a, 0; b, 3; c, 9; d, 18 μM) of L-*trans*-2,4-PDC. Lines were determined by linear regression and yielded K_m and V_{max} values for L-glutamate of 15.3 μM and 1.9 nmol/min per mg of protein, respectively. The K_i value for L-*trans*-2,4-PDC was determined by a replot of the slopes and gave a value of 4.6 μM. The plot shown is from a representative experiment, while the values reported in the text were determined from the average of five to seven experiments.

Table I. Comparative Inhibition of L-Glutamate Uptake[a]

compound (μM)	[^{3}H]-L-glutamate (5 μM) uptake, % of control
control	100
L-*trans*-2,4-PDC (**7**) (10)	45 ± 4
D-aspartate (10)	58 ± 5
D,L-β-*threo*-hydroxyaspartate (10)	72 ± 13
dihydrokainate (10)	93 ± 9

[a] The uptake data presented is reported as mean % of control ± SD ($n \geq 6$ sets of triplicates). The uptake assays were performed as described in the Experimental Section and have been corrected for nonspecific uptake and leakage.

Experiments that compared the action of L-*trans*-PDC with that of other known blockers of L-glutamate transport

(13) Dak, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

(14) Each epimer in question of course is identical with one of the other three dicarboxylate products, all of which we obviously have samples of and which are easily distinguishable by NMR.

(15) Synaptosomes were prepared by the procedure of Booth and Clark [(a) Booth, R. F. G.; Clark, J. B. *Biochem. J.* **1978**, *176*, 365.] and assayed for synaptosomal uptake as described by Kuhar and Zarbin [(b) Kuhar, M. J.; Zarbin, M. A. *J. Neurochem.* **1987**, *31*, 251.]

(16) Synaptic plasma membranes were prepared and NMDA receptor binding quantified as described by Monaghan, D. T.; Cotman, C. W. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 2532.

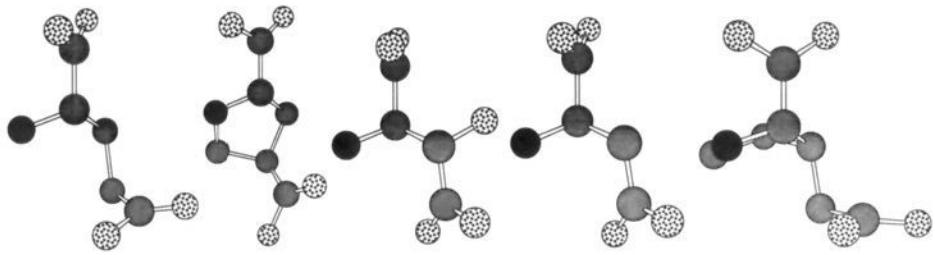


Figure 4. Comparison of L-glutamate, L-*trans*-PDC, *threo*-β-hydroxy-D-aspartate, D-aspartate, and dihydrokainate conformers.

Table II. Competitive Binding Studies for Diacids **4**, **7**, **11**, and **14**[a]

PDC (μM)	% of control [³H]AMPA binding to QA receptors	% of control [³H]KA binding to KA receptors	% of control [³H]-L-Glu binding to NMDA receptors
control	100	100	100
L-*cis* **4** (100)	102 ± 12	100 ± 12	89 ± 13
L-*trans* **7** (100)	109 ± 13	102 ± 10	87 ± 10
D-*trans* **11** (100)	108 ± 11	92 ± 6	95 ± 12
D-*cis* **14** (100)	110 ± 9	101 ± 6	38 ± 12
D-*cis* **14** (20)			76 ± 5

[a] The binding data is reported as mean of % of control binding ± SD ($n \geq 3$ sets of triplicates). The assays were carried out as described in the Experimental Section and have been corrected for nonspecific binding.

are summarized in Table I. The novel inhibitor reduced [³H]-L-glutamate (5 μM) uptake to 45 ± 4% of the control value when it was included in the transport assay at 10 μM. Under the experimental conditions employed, the extent of this inhibition was greater than that observed with either D-aspartate, D,L-β-*threo*-hydroxyaspartate, or dihydrokainate. (Dihydrokainate reduced the levels of L-glutamate transport only at higher concentrations; data not shown.) These findings identify L-*trans*-PDC as one of the most potent glutamate blockers yet identified.

To further characterize the pharmacological specificity of the PDC conformers and identify other potential sites at which the transport inhibitor could bind, the compounds were tested for their ability to inhibit the binding of [³H]-L-glutamate to NMDA receptors, [³H]-KA binding to KA receptors, and [³H]-AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) binding to QA receptors.[17] As is reported in Table II, only D-*trans*-PDC proved to exhibit any activity, weakly inhibiting the binding of [³H]-L-glutamate to the NMDA receptor. Notably, L-*trans*-PDC did not significantly inhibit radioligand binding to any of the three receptors even when present in 10^4-fold excess. This lack of interaction with any of the excitatory receptors is in direct contrast with its potent inhibition of transport and identifies L-*trans*-PDC as a specific probe of the L-glutamate transport system.

Discussion

As an endogenous neurotransmitter, L-glutamate interacts with several different proteins during the course of synaptic transmission. These include the multiple receptors mediating synaptic responses as well as the transport system thought to be responsible for clearing L-glutamate from the synaptic cleft and terminating its excitatory signal. Presumably these proteins each bind a specific L-glutamate conformer(s), since conformationally biased analogues selectively interact with the individual sites and thereby discriminate among receptor subclasses. This hypothesis is further supported by the present results, which show that L-*trans*-PDC is a potent competitive inhibitor of transport, but does not appreciably bind to the three classes of excitatory glutamate receptors. On the basis of this selectivity, L-*trans*-PDC embodies specific structural/conformational characteristics necessary for binding to the uptake system that are distinct from those required for binding to the NMDA, KA, and QA receptors.

If such a conclusion is correct, then both L-glutamate and previously identified transport inhibitors should all be capable of assuming the conformation represented by L-*trans*-PDC. To test this concept, MMX-minimized conformations of L-glutamate, L-*trans*-PDC, and the previously identified transport inhibitors[3] *threo*-β-hydroxy-L-aspartate, D-aspartate, and dihydrokainate were compared by calculating the mean deviation of atoms for the α-carboxyl, α-amino, and distal carboxyl groups for pairs of conformational minima.[18] As shown in Figure 4, there is considerable homology among specific conformations of each of these acidic amino acids. Each conformer is shown without hydrogens for clarity and is aligned such that the α-carboxyl/C_α bond lies along the y axis and the α-carboxyl, α-amino (represented as black), and distal carboxyl groups are in the xy plane (the isopropyl group of dihydrokainate has also been omitted for clarity). L-Glutamate conformer **15** is a local minimum structure that closely resembles an all-staggered conformation reported to be present in approximately 20% abundance in solution.[5] Of all pairwise comparisons between L-glutamate and L-*trans*-PDC, the conformer **15** and **7** overlap most closely. Similar comparisons were also conducted for *threo*-β-hydroxy-L-aspartate, D-aspartate, and dihydrokainate, yielding **16**, **17**, and **18**.

Although these compounds share similar functional groups, the present analysis has helped reveal conformational similarities that were not previously apparent. For example, one instinctively tends to superimpose the pyrrolidine rings when visually comparing dihydrokainate with L-*trans*-PDC (**7**), but a satisfactory match among the functional groups is achieved only when the proline rings themselves are not aligned, as in **18**. All five of the conformations shown are characterized by placement of the two carboxyl groups in a folded array such that the distance separating the carboxyl carbons is 4.2 ± 0.3 Å and the distal carboxyl-to-nitrogen distance is 3.4 ± 0.3 Å. The

(17) Binding to kainate receptors was quantified by the procedures of Simon et al. [(a) Simon, J. R.; Contrera, J. F.; Kuhar, M. J. *Exp. Brain Res.* **1976**, *60*, 323.] and London and Coyle [(b) London, E. D.; Coyle, J. T. *Mol. Pharmacol.* **1979**, *15*, 492.] Binding to quisqualate receptors (QA) was quantified as described by Honore et al. [(c) Honore, T.; Lauridsen, J.; Krogsgaard-Larsen, P. *J. Neurochem.* **1982**, *38*, 173.]

(18) These are MMX-minimized conformations, using PC Model for the MacII (Serena Software). For references to experimental and computational work on glutamate conformations, also see: Lehman, M. S.; Nunes, A. C. *Acta Crystallogr.* **1980**, *B36*, 1621.

functional group array thus defined suggests that the five compounds bind to the transport protein in the conformations shown. Furthermore, since L-*trans*-PDC does not inhibit excitatory amino acid receptor binding, the results support the hypothesis that the identified arrangement of functional groups is specific for the glutamate transport system.

From a physiological perspective, the availability of specific inhibitors of L-glutamate uptake are potentially useful probes for evaluating the role of the transport system in neurotransmission. Previous studies have demonstrated that the excitatory action of L-glutamate applied to neurons is prolonged by coadministration of transport inhibitors.[19] Indeed, a reduction in transport capacity would be expected to result in the accumulation of potentially excitotoxic levels of L-glutamate in the synaptic cleft. In this regard it is interesting that L-*trans*-PDC has been isolated from several species of algae related to those that produce the neurotoxins domoic and kainic acids[20] and thus may provide an alternative defense mechanism that exerts its effect upon predators by preventing the removal of glutamate from the synapse.

These initial studies demonstrate that the strategy of utilizing conformationally well-defined, relatively rigid PDCs as glutamate conformer mimics is a workable one that should provide a new understanding of the chemical basis of selective binding to not only the uptake system but to the excitatory receptors as well. Other PDC derivatives have been prepared, and their interaction with the KA, QA, and NMDA receptors will be the subject of future reports.

Experimental Section

General Methods. Proton and carbon-13 nuclear magnetic resonance (NMR) spectra were measured, as specified, on either a Bruker WM 250 (250 MHz) or a General Electric QE-300 (300 MHz) spectrometer. For spectra measured in organic solvents, data are reported in ppm from internal tetramethylsilane for ^1H NMR and in ppm from the solvent in ^{13}C NMR. For spectra taken in D_2O, data are reported in ppm from internal 3-(trimethylsilyl)propionic acid, sodium salt, for both ^1H NMR and ^{13}C NMR. Data are reported as follows: chemical shift, multiplicity (app = apparent, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant, and integration. Infrared (IR) spectra were taken with a Perkin-Elmer Model 283 spectrophotometer. Mass spectra (MS) were measured on a Finnegan 9610 spectrometer. High resolution mass spectra (HRMS) were determined on a VG analytical 7070E spectrometer. Optical rotations were obtained on a Perkin-Elmer 241 MC polarimeter or a JASCO DIP-360 digital polarimeter. Melting points (mp) were taken on a Laboratory Devices Mel-Temp melting point apparatus and are reported uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and agreed with the calculated values to within ±0.4%.

Dry tetrahydrofuran (THF) and ethyl ether (Et_2O) were distilled from calcium hydride and deperox molecular sieves (Fluka). All inert atmosphere operations were done under argon passed through a Drierite drying tube in oven- or flame-dried glassware. Unless otherwise noted, all organic layers from extractive workups were dried over $MgSO_4$ or Na_2SO_4 and filtered, and the solvent was removed on a rotary evaporator. Thin-layer chromatography (TLC) was performed on 0.25 mm Merck precoated silica gel plates (60 F-254). Flash chromatography was performed on ICN 200–400 mesh silica gel.

***N*-(Benzyloxycarbonyl)-*trans*-4-hydroxy-L-proline.** *trans*-4-Hydroxy-L-proline (1; 10.0 g, 76.3 mmol) and $NaHCO_3$ (16.0 g, 190 mmol) were dissolved in H_2O (165 mL), and a solution of benzyl chloroformate (12.5 mL, 15.0 g, 87.7 mmol) in toluene (40 mL) was added over a period of 15 min. After stirring at room temperature for 16 h, CO_2 evolution had ceased and the two phases were separated. The aqueous phase was extracted with ether (4 × 50 mL), cooled in an ice bath, and acidified to pH 2 with concentrated HCl. The resultant oily product was extracted into ethyl acetate (5 × 50 mL), and the combined organic extracts were dried ($MgSO_4$) and concentrated to a viscous oil, which crystallized upon standing at room temperature to provide 19.9 g (98%) of *N*-CBZ-*trans*-4-hydroxy-L-proline: mp 106–107 °C (lit.[12] mp 106–107 °C); $[\alpha]^{24}_D$ = −75.5° (*c* 1.03, $CHCl_3$) [lit.[12] $[\alpha]^{20}_D$ = −72° (*c* 1.0, $CHCl_3$)].

***N*-(Benzyloxycarbonyl)-*trans*-4-hydroxy-L-proline Ethyl Ester.** *N*-CBZ-*trans*-4-hydroxy-L-proline (5.00 g, 18.8 mmol) and *p*-toluenesulfonic acid monohydrate (0.36 g, 1.9 mmol) were dissolved in ethanol (300 mL), and the reaction mixture was heated at reflux with the collection of wet ethanol in a Dean–Stark trap. After 16 h, the trap was emptied whenever full until the total reaction volume had decreased to about 50 mL, then the tosic acid was neutralized with an excess of $NaHCO_3$ (0.8 g, 10 mmol). Volatiles were removed in vacuo, the residue was diluted with ethyl acetate (100 mL), and the mixture was stirred for 0.5 h or until the salts were free of oil. Solids were removed by filtration through Celite–K_2CO_3, and the filter cake was washed with ethyl acetate (50 mL). Concentration of the combined organic layers in vacuo provided 5.6 g (100%) of the crude *trans*-hydroxy ester as a thick, pale yellow oil of sufficient purity to be used in the next step without further purification: TLC R_f 0.07 (5:3 Et_2O–petroleum ether); $[\alpha]^{25}_D$ = −60° (*c* 1.04, $CHCl_3$); ^1H NMR (250 mHz, $CDCl_3$) δ 7.35–7.27 (m, 5 H), 5.18–4.99 (m, 2 H, $PhCH_2$), 4.52–4.46 (m, 2 H, H-2 and H-4), 4.20 + 4.01 (q, *J* = 7.1 Hz, 2 H, $CO_2CH_2CH_3$, two rotamers), 3.71–3.52 (m, 2 H, H-5), 2.85 + 2.74 (br s, 1 H, O*H*), 2.38–2.23 (m, 1 H, H-3), 2.12–2.00 (m, 1 H, H-3), 1.26 + 1.10 (t, *J* = 7.1 Hz, 3 H, $CO_2CH_2CH_3$, two rotamers); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.74, 172.55, 154.98, 154.60, 136.35, 136.11, 128.38, 128.31, 127.93, 127.77, 127.72, 69.94, 69.20, 67.22, 67.14, 61.27, 61.14, 57.99, 57.76, 55.16, 54.56, 39.09, 38.33, 14.04, 13.92; IR ($CHCl_3$) 3620–3200 (br), 2990, 1737, 1700, 1420, 1355, 1190, 1170 cm^{-1}; LRMS (CI, 70 eV, isobutane) *m/e* 294 (MH^+, 100), 250 (83), 220 (19), 158 (18), 91 (58); HRMS (EI, 70 eV) *m/e* 293.1260 (293.1263 calcd for $C_{15}H_{19}NO_5$).

***N*-(Benzyloxycarbonyl)-*trans*-4-(tosyloxy)-L-proline Ethyl Ester (2).** To a solution of *N*-CBZ-*trans*-4-hydroxy-L-proline ethyl ester (5.6 g, 19 mmol) and anhydrous pyridine (4.6 mL, 4.5 g, 57 mmol) in $CHCl_3$ (30 mL) was added *p*-toluenesulfonyl chloride (7.2 g, 38 mmol) in one portion. After stirring at room temperature for 72 h, the reaction mixture was diluted with CH_2Cl_2 (70 mL) and extracted with 10% HCl (4 × 10 mL). The organic layer was dried ($MgSO_4$) and concentrated to a golden oil which was purified by silica gel flash chromatography (gradient elution, using 1:1, 2:1 ethyl ether–petroleum ether) to yield 8.2 g (98%) of pure **2** as a colorless oil: TLC R_f 0.27 (3:3:1 ethyl ether–petroleum ether–CH_2Cl_2); $[\alpha]^{24}_D$ = −29° (*c* 1.5, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 7.79–7.74 (m, 2 H), 7.37–7.26 (m, 7 H), 5.18–5.00 (m, 3 H, $PhCH_2$ and H-4), 4.44 (app q, *J* = 7.5 Hz, 1 H, H-2), 4.19 + 3.99 (q, *J* = 7.1 Hz, 2 H, $CO_2CH_2CH_3$, two rotamers), 3.76–3.60 (m, 2 H, H-5), 2.62–2.38 (m, 1 H, H-3), 2.45 + 2.43 (s, 3 H, $PhCH_3$), 2.23–2.08 (m, 1 H, H-3), 1.25 + 1.08 (t, *J* = 7.1 Hz, 3 H, $CO_2CH_2CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.59, 171.39, 154.12, 153.64, 145.10, 135.96, 135.81, 133.14, 133.08, 129.88, 129.86, 128.25, 128.17, 127.85, 127.60, 127.48, 78.66, 78.00, 67.14, 67.11, 61.25, 61.17, 57.39, 57.07, 52.25, 51.87, 36.99, 35.86, 21.41, 13.84, 13.69; IR ($CHCl_3$) 1740, 1705, 1420, 1355, 1175 cm^{-1}; LRMS (EI, 70 eV) *m/e* 447 (M^+, 0.23), 374 (2), 202 (2), 158 (24), 91 (100); HRMS (EI, 40 eV) *m/e* 447.1337 (447.1351 calcd for $C_{22}H_{25}NO_7S$).

***N*-(Benzyloxycarbonyl)-*cis*-4-cyano-L-proline Ethyl Ester.** Finely powdered NaCN (0.85 g, 17 mmol) was added to a stirring mixture of tosylate **2** (5.19 g, 11.6 mmol) in DMSO (15 mL). The reaction mixture was heated in an oil bath at 80 °C for 4 h, then cooled to room temperature. Brine (6 mL) and H_2O (7 mL) were added to the orange-red reaction mixture, and the resultant solution was extracted with Et_2O (5 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to give a crude yellow syrup which was purified by flash chromatography (silica gel, using 4:3 ethyl ether–petroleum ether) yielding the

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desired *cis*-nitrile ethyl ester (2.31 g, 66%) as a colorless viscous oil: TLC R_f 0.15 (3:3:1 ethyl ether–petroleum ether–CH_2Cl_2); $[\alpha]^{25}_D$ = −32° (*c* 1.11, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.36–7.32 (m, 5 H), 5.21–5.04 (m, 2 H, PhCH_2), 4.48–4.37 (m, 1 H, H-2), 4.25 (q, *J* = 7.1 Hz, 1 H, $CO_2CH_2CH_3$, one of two rotamers), 4.14–3.96 (m, 2 H, $CO_2CH_2CH_3$ and H-5), 3.80–3.70 (m, 1 H, H-5), 3.21–3.07 (m, 1 H, H-4), 2.78–2.64 (m, 1 H, H-3), 2.41–2.27 (m, 1 H, H-3), 1.29 + 1.16 (t, *J* = 7.1 Hz, 3 H, $CO_2CH_2CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.83, 170.60, 153.84, 153.48, 135.81, 135.73, 128.40, 128.32, 128.13, 128.08, 127.92, 127.82, 118.77, 118.66, 67.44, 67.38, 61.69, 58.22, 57.87, 49.44, 48.93, 34.20, 33.15, 27.02, 26.24, 13.89, 13.77; IR ($CHCl_3$) 2255, 1740, 1710, 1415, 1360, 1195, 1170, 1120 cm^{-1}; LRMS (CI, 70 eV, isobutane) *m/e* 303 (MH^+, 17), 259 (2), 169 (5), 91 (100); HRMS (EI, 40 eV) *m/e* 302.1247 (302.1266 calcd for $C_{16}H_{18}N_2O_4$).

***N*-(Benzyloxycarbonyl)-*cis*-4-carboxy-L-proline Dimethyl Ester (3).** To a solution of the *cis*-nitrile ester (1.91 g, 6.31 mmol) in methanol (15 mL) was added HCl (2.3 g, 63 mmol) in methanol (13 mL). After stirring at room temperature for 3.5 days, the reaction was quenched with $NaHCO_3$ (5.5 g, 65 mmol), concentrated in vacuo, and diluted with THF (30 mL). Solids were removed by filtration, the filter cake was washed with THF, and the combined organic layers were concentrated purified by flash chromatography (silica gel, using 4:3 ethyl ether–petroleum ether) to give 1.9 g (94%) of **3** as a colorless oil: TLC R_f 0.17 (4:3 ethyl ether–petroleum ether); $[\alpha]^{24}_D$ = −38° (*c* 1.09, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.37–7.27 (m, 5 H), 5.22–5.01 (m, 2 H, PhCH_2), 4.45–4.35 (m, 1 H, H-2), 3.98–3.67 (m, 2 H, H-5), 3.75 + 3.57 (s, 3 H, C-2 CO_2CH_3), 3.705 + 3.700 (s, 3 H, C-4 CO_2CH_3), 3.17–3.02 (m, 1 H, H-4), 2.59–2.31 (m, 2 H, H-3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.23, 172.05, 171.98, 154.33, 153.80, 136.19, 136.15, 128.36, 128.30, 127.97, 127.92, 127.87, 127.74, 67.17, 67.06, 58.60, 58.29, 52.28, 52.20, 52.10, 48.83, 48.34, 42.21, 41.39, 32.92, 31.93; IR ($CHCl_3$) 2960, 1740, 1705, 1425, 1360, 1175, 1120 cm^{-1}; HRMS (EI, 24 eV) *m/e* 321.1225 (321.1212 calcd for $C_{16}H_{19}NO_6$).

***N*-(Benzyloxycarbonyl)-*cis*-4-carboxy-L-proline.** Dimethyl ester **3** (0.761 g, 2.37 mmol) was suspended in 1:1 THF–H_2O (7 mL) and, with stirring, 4 M NaOH (1.33 mL, 5.33 mmol) was added dropwise. During the addition, the reaction solution became homogeneous. Stirring was continued for 50 min, after which the reaction mixture was extracted twice with Et_2O (2 × 7 mL). Acidification of the aqueous layer with concentrated HCl caused precipitation of the oily product, which was extracted into ethyl acetate (3 × 8 mL). Before discarding, the ethyl ether layers from the previous extractions were washed with H_2O (2 × 4 mL) and the aqueous washings were added to the acidified aqueous layers. The aqueous solution was extracted again with ethyl acetate (2 × 8 mL) and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated to afford 0.68 g (97%) of the dicarboxylate as a colorless solid which could be recrystallized from CH_3OH–Et_2O, but was sufficiently pure to be used directly in the next step: mp 175–176 °C; $[\alpha]^{25}_D$ = −27° (*c* 1.05, CH_3OH); ^{1}H NMR (250 MHz, CD_3OD) δ 7.40–7.26 (m, 5 H), 5.18–5.03 (m, 2 H, PhCH_2), 4.40–4.31 (m, 1 H, H-2), 3.85 (app dd, *J* = 10.7, 8.2 Hz, 1 H, H-5), 3.75–3.67 (m, 1 H, H-5), 3.23–3.19 (m, 1 H, H-4), 2.66–2.51 (m, 1 H, H-3), 2.38–2.24 (m, 1 H, H-3); ^{13}C NMR (75.5 MHz, CD_3OD) δ 175.65, 175.35, 175.22, 156.35, 156.05, 137.86, 137.73, 129.55, 129.46, 129.13, 129.00, 128.88, 128.64, 68.34, 60.18, 59.85, 50.19, 49.74, 43.42, 42.67, 34.20, 33.27; IR (KBr) 3600–2300 (br), 1740, 1685, 1635, 1450, 1415, 1360, 1240, 1140. Anal. ($C_{14}H_{15}NO_6$) C, H, N.

***cis*-4-Carboxy-L-proline (4).** *N*-CBZ-*cis*-4-carboxy-L-proline (0.455 g, 1.55 mmol) was dissolved in methanol (37 mL) and transferred to a Parr shaker bottle. After addition of 10% Pd–C (0.080 g), the mixture was shaken under an atmosphere of hydrogen at 48–50 psi for 0.5 h. The catalyst was removed by filtration through Celite and washed with water. The combined filtrate and washings were concentrated in vacuo to yield a white solid, which was recrystallized from H_2O–ethanol–acetone to yield 0.206 g (84%) of **4** as colorless prisms: mp 225–226 °C; $[\alpha]^{25}_D$ = −40° (*c* 1.02, H_2O); ^{1}H NMR (250 MHz, D_2O) δ 4.25 (dd, *J* = 8.6, 7.4 Hz, 1 H, H-2), 3.72 (dd, *J* = 12.0, 6.7 Hz, 1 H, H-5), 3.57 (dd, *J* = 12.0, 8.0 Hz, 1 H, H-5), 3.38 (m, 1 H, H-4), 2.71 (app dt, *J* = 13.7, 8.6 Hz, 1 H, H-3), 2.35 (app overlapping dt, *J* = 13.7, 7.4 Hz, 1 H, H-3); ^{13}C NMR (75.5 MHz, D_2O) δ 178.75, 176.25, 63.87, 50.16, 45.43, 34.78; IR (KBr) 3600–2200 (br), 3080, 1690, 1560, 1340, 1230 cm^{-1}; MS (CI, 70 eV, methane) *m/e* 160 (MH^+, 14), 114 (100). Anal. ($C_6H_9NO_4$) C, H, N.

***N*-(Benzyloxycarbonyl)-*cis*-4-hydroxy-L-proline Ethyl Ester.** *N*-CBZ-*trans*-4-hydroxy-L-proline (2.65 g, 10.0 mmol) was dissolved in acetone (20 mL) and cooled to −5 °C in an ice–salt water bath. Jones reagent (7.5 mL, 20.0 mmol) was added, the ice bath was removed, and the reaction was stirred at room temperatue for 2.25 h before quenching the excess Jones reagent with isopropanol (1 mL). After stirring for 2 h, the green solution was decanted from the dark green precipitate, concentrated in vacuo, poured into brine (10 mL), and extracted with ethyl acetate (2 × 10 mL). The aforementioned dark green precipitate was then dissolved in water (50 mL), saturated with NaCl, and extracted with ethyl acetate (5 × 10 mL). All organic layers were combined, dried ($MgSO_4$), and concentrated in vacuo to yield the crude *N*-CBZ-4-keto-L-proline as a yellow oil, which was used without further purification.

This ketone was dissolved in methanol (50 mL) and the reaction flask was cooled to 0 °C in an ice bath. A solution of $NaBH_4$ (1.44 g, 38.0 mmol) in water (5 mL) was added dropwise. The reaction was allowed to sit in the refrigerator (−5 °C) for 20 h and concentrated. The residue was diluted with H_2O (10 mL), acidified to pH 2–3 with concentrated HCl, and extracted with ethyl acetate (2 × 10 mL). The aqueous layer was saturated with NaCl and again extracted with ethyl acetate (2 × 10 mL). The aqueous layer was then concentrated to a white solid, which was diluted with 2:1 ethyl acetate–THF (30 mL) and heated at reflux for 0.5 h. Salts were removed by filtration through Celite. This filtrate and the organic layers from the previous extractions were combined, dried ($MgSO_4$), and concentrated to yield the crude *N*-CBZ-*cis*-4-hydroxy-L-proline as a yellow oil. This residue was dissolved in ethanol (150 mL) containing a catalytic amount of *p*-toluenesulfonic acid monohydrate (0.25 g, 1.31 mmol). The reaction mixture was heated at reflux while wet ethanol collected in a Dean–Stark trap. After 36 h, the trap was emptied whenever full until the total reaction volume was about 50 mL. After neutralizing the tosic acid with $NaHCO_3$ (0.5 g, 6.0 mmol), the solvent was removed in vacuo, the residue was diluted with ethyl acetate (75 mL), and the mixture was stirred vigorously for 0.5 h to extract the product from the sodium salts. Solids were removed by filtration through Celite–K_2CO_3, and the filter cake was washed with ethyl acetate (15 mL). The combined filtrate and washings were concentrated. Purification of the resulting residue by flash chromatography (silica gel, using ethyl ether) provided the *cis*-hydroxy ethyl ester (2.1 g, 83%) as a yellow oil: TLC R_f 0.18 (ethyl ether); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.37–7.27 (m, 5 H), 5.22–5.04 (m, 2 H, PhCH_2), 4.50–4.35 (m, 2 H, H-2 and H-4), 4.25 (q, *J* = 7.1 Hz, 1 H, $CO_2CH_2CH_3$, one of two rotamers), 4.08 (app qd, *J* = 7.1, 1.7 Hz, 1 H, $CO_2CH_2CH_3$), 3.76–3.45 (m, 3 H, H-5 and OH), 2.41–2.26 (m, 1 H, H-3), 2.16–2.08 (m, 1 H, H-3), 1.30 + 1.14 (t, *J* = 7.1 Hz, 3 H, $CO_2CH_2CH_3$); IR ($CHCl_3$) 3650–3250 (br), 2990, 1735, 1705, 1420, 1355, 1200, 1125, 1080 cm^{-1}; LRMS (CI, 70 eV, isobutane) *m/e* 294 (MH^+, 100), 250 (34), 220 (9), 91 (29); HRMS (EI, 22 eV) *m/e* 293.1272 (293.1263 calcd for $C_{15}H_{19}NO_5$).

***N*-(Benzyloxycarbonyl)-*cis*-4-(tosyloxy)-L-proline Ethyl Ester (5).** To a solution of *N*-CBZ-*cis*-4-hydroxy-L-proline ethyl ester (6.4 g, 22 mmol) and anhydrous pyridine (5.3 mL, 5.2 g, 65 mmol) in $CHCl_3$ (35 mL) was added *p*-toluenesulfonyl chloride (8.3 g, 44 mmol) in one portion. After stirring at room temperature for 7 days, the reaction was diluted with CH_2Cl_2 (100 mL) and pyridine removed by extraction with 10% HCl (4 × 13 mL). The organic layer was dried ($MgSO_4$) and concentrated to a yellow oil which was purified by flash chromatography (silica gel, using 5:4 petroleum ether–ether) affording 7.8 g (80%) of **5** as colorless needles: mp 91–92 °C; TLC R_f 0.15 (4:2 ethyl ether–petroleum ether); $[\alpha]^{25}_D$ = −26° (*c* 1.1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.75 (app d, *J* = 8.4 Hz, 2 H), 7.34–7.28 (m, 7 H), 5.18–5.04 (m, 3 H, PhCH_2 and H-4), 4.52–4.41 (m, 1 H, H-2), 4.20–4.02 (m, 2 H, $CO_2CH_2CH_3$), 3.78–3.62 (m, 2 H, H-5), 2.48–2.37 (m, 2 H, H-3), 2.45 + 2.44 (s, 3 H, PhCH_3, two rotamers), 1.22 + 1.14 (t, *J* = 7.1 Hz, 3 H, $CO_2CH_2CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.89, 170.57, 154.20, 153.89, 145.12, 145.09, 136.11, 133.88, 129.91, 129.85, 128.38, 128.32, 128.03, 127.98, 127.86, 127.77, 127.58, 78.62, 77.74, 67.21, 67.15, 61.47, 57.53, 57.30, 52.25, 51.99, 37.07, 35.98, 21.59, 13.92, 13.85; IR ($CHCl_3$) 2995, 1745, 1700, 1415, 1360, 1175, 1120,

1050, 1020 cm^{-1}. Anal. ($C_{22}H_{25}NO_7S$) C, H, N.

***N*-(Benzyloxycarbonyl)-*trans*-4-cyano-L-proline Ethyl Ester.** Finely powdered NaCN (0.49 g, 10 mmol) was suspended in a stirring mixture of **5** (3.0 g, 6.7 mmol) in DMSO (7 mL). The reaction flask was heated in an oil bath at 80 °C for 3 h, during which time the NaCN had completely dissolved and the reaction mixture had turned a deep orange color. After cooling to room temperature, the reaction was diluted with brine (3 mL) and H_2O (4 mL), then extracted with Et_2O (5 × 11 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to a yellow syrup which was purified by flash chromatography (silica gel, using 1:1 ethyl ether–petroleum ether) yielding 1.42 g (70%) of the *N*-CBZ-*trans*-4-cyano-L-proline ethyl ester as a colorless viscous oil: TLC R_f 0.22 (4:2 ethyl ether–petroleum ether); $[\alpha]^{24}_D = -39°$ (*c* 1.05, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 7.38–7.27 (m, 5 H), 5.21–5.03 (m, 2 H, $PhCH_2$), 4.55–4.45 (m, 1 H, H-2), 4.21 (q, *J* = 7.1 Hz, 1 H, $CO_2CH_2CH_3$, one of two rotamers), 4.09–3.94 (m, 2 H, $CO_2CH_2CH_3$ and H-5), 3.80–3.67 (m, 1 H, H-5), 3.35–3.21 (m, 1 H, H-4), 2.62–2.34 (m, 2 H, H-3), 1.28 + 1.13 (t, *J* = 7.1 Hz, $CO_2CH_2CH_3$, two rotamers); IR ($CHCl_3$) 2995, 2900, 2250, 1740, 1705, 1420, 1355, 1195, 1125, 1020 cm^{-1}; LRMS (CI, 70 eV, isobutane) *m/e* 303 (MH^+, 66), 259 (44), 91 (100); HRMS (EI, 21 eV) *m/e* 302.1264 (302.1266 calcd for $C_{16}H_{18}N_2O_4$).

***N*-(Benzyloxycarbonyl)-*trans*-4-carboxy-L-proline Dimethyl Ester (6).** To a solution of *N*-CBZ-*trans*-4-cyano-L-proline ethyl ester (1.19 g, 3.95 mmol) in methanol (8 mL) was added HCl (1.44 g, 39.5 mmol) in methanol (10 mL). After 4 days, the reaction was quenched with $NaHCO_3$ (3.7 g, 44 mmol), then concentrated in vacuo. The residue was diluted with THF (25 mL), filtered, concentrated, and purified by flash chromatography (silica gel, 1:1 ethyl ether–petroleum ether) to afford 1.2 g (94%) of **6** as a colorless oil: TLC R_f 0.23 (4:3 ethyl ether–petroleum ether); $[\alpha]^{27}_D = -43°$ (*c* 1.06, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 7.35–7.28 (m, 5 H), 5.22–5.01 (m, 2 H, $PhCH_2$), 4.55–4.44 (m, 1 H, H-2), 3.92–3.84 (m, 1 H, H-5), 3.77–3.64 (m, 1 H, H-5), 3.75 + 3.58 (s, 3 H, C-2 CO_2CH_3, two rotamers), 3.70 + 3.69 (s, 3 H, C-4 CO_2CH_3), 3.32–3.15 (m, 1 H, H-4), 2.59–2.40 (m, 1 H, H-3), 2.31–2.19 (m, 1 H, H-3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.51, 172.48, 172.37, 172.32, 154.39, 153.79, 136.19, 128.35, 128.28, 127.94, 127.89, 127.78, 127.66, 67.15, 67.03, 58.70, 58.36, 52.34, 52.17, 48.94, 48.29, 41.68, 40.86, 33.29, 32.40; IR ($CHCl_3$) 2960, 1740, 1705, 1420, 1355, 1195, 1180, 1125 cm^{-1}; LRMS (CI, 70 eV, isobutane) *m/e* 322 (MH^+, 100), 278 (42), 262 (4), 186 (17), 91 (65); HRMS (EI, 21 eV) *m/e* 321.1206 (321.1212 calcd for $C_{16}H_{19}NO_6$).

***N*-(Benzyloxycarbonyl)-*trans*-4-carboxy-L-proline.** Dimethyl ester **6** (0.502 g, 1.56 mmol) was suspended in 3:2 THF–H_2O (5 mL), and with stirring, 4 M NaOH (0.90 mL, 3.6 mmol) was added dropwise. After stirring for 55 min, the reaction mixture was extracted with Et_2O (2 × 5 mL). Acidification of the aqueous layer with concentrated HCl caused precipitation of the oily product, which was removed from the aqueous solution by extraction with ethyl acetate (3 × 6 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to afford a thick, sticky, colorless oil which eventually crystallized upon standing at room temperature to yield 0.480 g (100%) of *N*-(benzyloxycarbonyl)-*trans*-4-carboxy-L-proline: mp 98–100 °C; $[\alpha]^{25}_D = -37°$ (*c* 0.84, CH_3OH); ^1H NMR (300 MHz, CD_3OD) δ 7.36–7.28 (m, 5 H), 5.15–5.01 (m, 2 H, $PhCH_2$), 4.45–4.39 (m, 1 H, H-2), 3.81–3.67 (m, 2 H, H-5), 3.27–3.14 (m, 1 H, H-4), 2.59–2.44 (m, 1 H, H-3), 2.30–2.21 (m, 1 H, H-3); ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 173.73, 173.62, 173.38, 155.06, 154.56, 137.89, 137.85, 129.15, 129.04, 128.58, 128.39, 128.07, 67.24, 67.16, 59.61, 59.14, 49.78, 49.17, 42.42, 41.50, 34.15, 33.11; IR ($CHCl_3$) 3600–2300 (br), 1720, 1705, 1420, 1360, 1235, 1135 cm^{-1}; HRMS (EI, 21 eV) *m/e* 293.0901 (293.0899 calcd for $C_{14}H_{15}NO_6$).

***trans*-4-Carboxy-L-proline (7).** *N*-CBZ-*trans*-4-carboxy-L-proline (0.458 g, 1.56 mmol) was dissolved in methanol (50 mL) and transferred to a Parr shaker bottle. After adding 10% PD–C (0.080 g), the mixture was shaken under an atmosphere of hydrogen at 48–50 psi for 0.5 h. The catalyst was removed by filtration through Celite, washed with water, and concentrated in vacuo to yield a white solid, which was recrystallized from H_2O–EtOH–acetone to yield 0.187 g (75%) of **7** as colorless prisms: mp 219–220 °C (lit.[20] mp 223–225 °C); $[\alpha]^{25}_D = -54°$ (*c* 1.04, H_2O) [lit.[20] $[\alpha]^{20}_D = -46.0°$ (*c* 1.0, H_2O)]; ^1H NMR (300 MHz, D_2O) δ 4.28 (dd, *J* = 8.6, 7.3 Hz, 1 H, H-2), 3.68–3.57 (m, 2 H, H-5), 3.38–3.29 (m, 1 H, H-4), 2.59 (ddd, *J* = 13.7, 8.6, 6.0 Hz, 1 H, H-3), 2.42 (ddd, *J* = 13.7, 8.1, 7.3 Hz, 1 H, H-3); ^{13}C NMR (75.5 MHz, D_2O) δ 178.99, 176.30, 63.67, 50.32, 45.24, 35.18; IR (KBr) 3600–2200 (br), 1720, 1610, 1515, 1360, 1335, 1285, 1220, 1185 cm^{-1}; LRMS (EI, 70 eV) *m/e* 159 (M^+, 19), 114 (100), 87 (53), 68 (92). Anal. ($C_6H_9NO_4$) C, H, N.

***N*-(Benzyloxycarbonyl)-*cis*-4-hydroxy-D-proline.** *cis*-4-Hydroxy-D-proline (**8**; 10.0 g, 76.3 mmol) was dissolved in H_2O (165 mL) containing $NaHCO_3$ (16.0 g, 190 mmol). Benzyl chloroformate (12.5 mL, 15.0 g, 87.7 mmol) in toluene (40 mL) was added to this stirred solution at room temperature over 30 min, and stirring was continued for 16 h. By this time, CO_2 evolution had ceased, and the two phases were separated. The aqueous layer was washed with ether (3 × 50 mL), cooled in an ice bath, and acidified to pH 2 with concentrated HCl, causing precipitation of the oily product. This oil was extracted from the aqueous layer by repeated washings with ethyl acetate (5 × 50 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated to a colorless viscous oil, which crystallized upon standing at room temperature to provide 19.42 g (96%) of *N*-CBZ-*cis*-4-hydroxy-D-proline: mp 110–111 °C (lit.[12] mp 110.5–111.5 °C); $[\alpha]^{25}_D = +24.4°$ (*c* 1.0, $CHCl_3$) [lit.[12] $[\alpha]^{25}_D = +26.3°$ (*c* 1.0, $CHCl_3$)].

***N*-(Benzyloxycarbonyl)-*cis*-4-hydroxy-D-proline Ethyl Ester.** *N*-CBZ-*cis*-4-hydroxy-D-proline (5.00 g, 18.8 mmol) was esterified by refluxing in ethanol (300 mL) containing a catalytic amount of *p*-toluenesulfonic acid monohydrate (0.3 g, 1.6 mmol). Wet ethanol was collected in a Dean–Stark trap. After 16 h, the trap was emptied whenever full until the reaction volume had decreased to about 50 mL. The tosic acid was neutralized with an excess of $NaHCO_3$ (0.5 g, 6 mmol), concentrated, diluted with ethyl acetate (100 mL), and then stirred for 0.5 h. Solids were removed by filtration through Celite and the filter cake was washed with ethyl acetate (50 mL). Concentration of the combined filtrate and washings in vacuo provided 5.6 g (100%) of the crude *cis*-hydroxy ester as a colorless oil: ^1H NMR and IR spectra were identical in all respects with those of the enantiomer *N*-(benzyloxycarbonyl)-*cis*-4-hydroxy-L-proline ethyl ester; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.54, 174.35, 154.87, 154.21, 136.24, 136.11, 128.39, 128.31, 127.99, 127.96, 127.83, 127.74, 70.99, 70.01, 67.18, 61.88, 61.72, 58.17, 57.77, 55.90, 55.60, 38.54, 37.64, 13.93, 13.80; LRMS (CI, 70 eV, isobutane) *m/e* 294 (MH^+, 81), 250 (100), 220 (23), 91 (66); HRMS (EI, 50 eV) *m/e* 293.1267 (293.1263 calcd for $C_{15}H_{19}NO_5$).

***N*-(Benzyloxycarbonyl)-*cis*-4-(tosyloxy)-D-proline Ethyl Ester (9).** By the procedure described above for enantiomer **5**, *N*-CBZ-*cis*-4-hydroxy-D-proline (4.7 g, 16 mmol) was converted into 6.6 g (92%) of **9** as colorless needles: mp 91–91 °C; $[\alpha]^{26}_D = +27°$ (*c* 1.1, $CHCl_3$); spectra were identical in all respects with those of **5**. Anal. ($C_{22}H_{25}NO_7S$) C, H, N.

***N*-(Benzyloxycarbonyl)-*trans*-4-cyano-D-proline Ethyl Ester.** Treatment of **9** (1.0 g, 2.23 mmol) with NaCN in DMSO provided *N*-CBZ-*trans*-4-cyano-D-proline ethyl ester (0.48 g, 1.6 mmol, 72%) as a colorless oil: $[\alpha]^{25}_D = +40°$ (*c* 1.0, $CHCl_3$); ^1H NMR and IR spectra were identical in all respects with those of the enantiomer *N*-(benzyloxycarbonyl)-*trans*-4-cyano-L-proline ethyl ester; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.21, 171.13, 153.86, 153.42, 135.77, 135.70, 128.38, 128.29, 128.10, 128.03, 127.86, 127.74, 118.73, 118.61, 67.45, 67.38, 61.65, 61.56, 58.02, 57.61, 49.33, 48.80, 34.39, 33.38, 26.82, 26.15, 13.92, 13.81; LRMS (CI, 70 eV, isobutane) *m/e* 303 (MH^+, 100), 259 (84), 91 (85); HRMS (EI, 70 eV) *m/e* 302.1253 (302.1266 calcd for $C_{16}H_{18}N_2O_4$).

***N*-(Benzyloxycarbonyl)-*trans*-4-carboxy-D-proline Dimethyl Ester (10).** With the procedure for preparation of L enantiomer **6**, *N*-CBZ-*trans*-4-cyano-D-proline ethyl ester (0.47 g, 1.6 mmol) was converted to 0.47 g (94%) of **10** as a colorless oil: $[\alpha]^{28}_D = +41°$ (*c* 1.13, $CHCl_3$); spectra were identical in all respects with those of enantiomer **6**; LRMS (CI, 70 eV, isobutane) *m/e* 322 (MH^+, 100), 278 (48), 262 (4), 186 (16), 91 (28); HRMS (EI, 70 eV) *m/e* 321.1231 (321.1212 calcd for $C_{16}H_{19}NO_6$).

***N*-(Benzyloxycarbonyl)-*trans*-4-carboxy-D-proline.** Dimethyl ester **10** (0.336 g, 1.05 mmol) was saponified as described above to yield 0.294 g (96%) of *N*-CBZ-*trans*-4-carboxy-D-proline as a colorless, extremely viscous oil: $[\alpha]^{24}_D = +39°$ (*c* 0.51, CH_3OH); spectra were identical in all respects with those of the enantiomer *N*-(benzyloxycarbonyl)-*trans*-4-carboxy-L-proline.

***trans*-4-Carboxy-D-proline (11).** Hydrogenation of the crude *N*-CBZ-*trans*-4-carboxy-D-proline (0.294 g, 1.00 mmol) gave 0.138 g (87%) of 11 as colorless prisms: mp 219–220 °C; $[\alpha]^{25}_D$ = +51° (*c* 1.04, H_2O); spectra were identical in all respects with those of enantiomer 7; HRMS (EI, 22 eV) *m/e* 160.0613 (160.0610 calcd for $C_6H_9NO_4 + H^+$), 159.0530 (159.0532 calcd for $C_6H_9NO_4$). Anal. ($C_6H_9NO_4$) C, H, N.

***N*-(Benzyloxycarbonyl)-*trans*-4-acetoxy-D-proline Ethyl Ester.** To a solution of tosylate 9 (4.00 g, 8.94 mmol) in acetone (25 mL) was added *n*-Bu_4NOAc (4.04 g, 13.4 mmol) and the resulting mixture was stirred at room temperature for 20 h. After concentration of the reaction mixture, the resulting yellow oil was diluted with H_2O (5 mL) and extracted with ethyl ether (5 × 11 mL). The combined organic layers were dried ($MgSO_4$), concentrated, and purified by flash chromatography (silica gel, using 4:3 petroleum ether–ether) to give 2.49 g (83%) of the pure acetate as a colorless oil: TLC R_f 0.30 (4:2 ethyl ether–petroleum ether); $[\alpha]^{26}_D$ = +48° (*c* 1.15, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 7.37–7.33 (m, 5 H), 5.32–5.27 (m, 1 H, H-4), 5.21–5.04 (m, 2 H, $PhCH_2$), 4.47 + 4.43 (app t, *J* = 8.0 Hz, 1 H, H-2, two rotamers), 4.22 + 4.02 (q, *J* = 7.1 Hz, 2 H, $CO_2CH_2CH_3$), 3.83–3.63 (m, 2 H, H-5), 2.48–2.36 (m, 1 H, H-3), 2.28–2.15 (m, 1 H, H-3), 2.054 + 2.045 (s, 3 H, CH_3CO_2), 1.28 + 1.11 (t, *J* = 7.1 Hz, 3 H, $CO_2CH_2CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.08, 171.86, 170.28, 170.23, 154.61, 154.07, 136.17, 136.02, 128.36, 128.29, 127.95, 127.78, 72.48, 71.73, 67.20, 67.16, 61.33, 61.22, 57.86, 57.60, 52.47, 52.02, 36.49, 35.46, 20.93, 13.99, 13.85; IR ($CHCl_3$) 1740, 1705, 1420, 1355, 1245, 1200, 1170, 1130, 1070, 1028 cm^{-1}; LRMS (CI, 70 eV, isobutane) *m/e* 336 (MH^+, 100), 292 (36), 91 (33); HRMS (EI, 70 eV) *m/e* 335.1349 (335.1369 calcd for $C_{17}H_{21}NO_6$).

***N*-(Benzyloxycarbonyl)-*trans*-4-hydroxy-D-proline Ethyl Ester.** To a stirred solution of *N*-CBZ-*trans*-4-acetoxy-D-proline (2.45 g, 7.31 mmol) in ethanol (50 mL) was added freshly prepared NaOEt/EtOH (0.017 g, 0.7 mmol of Na dissolved in 1 mL of EtOH). After stirring at room temperature for 20 min, the reaction was quenched with NH_4Cl (0.1 g), and concentrated in vacuo to an oily solid. The desired alcohol was isolated (2.01 g, 94%) as a colorless oil by diluting the residue with ethyl ether (50 mL), filtering the solution through Celite, washing the filter cake with ethyl ether, and concentrating the combined filtrate and washings in vacuo. The alcohol was sufficiently pure to use directly in the next step: $[\alpha]^{25}_D$ = +59° (*c*, 1.04, $CHCl_3$); spectra were identical in all respects with those of the enantiomer *N*-(benzyloxycarbonyl)-*trans*-4-hydroxy-L-proline ethyl ester, LRMS (CI, 70 eV, isobutane) *m/e* 294 (MH^+, 100), 250 (98), 220 (24), 158 (25), 91 (89); HRMS (EI, 50 eV) *m/e* 293.1272 (293.1263 calcd for $C_{15}H_{19}NO_5$).

***N*-(Benzyloxycarbonyl)-*trans*-4-(tosyloxy)-D-proline Ethyl Ester (12).** Tosylate 12 (2.24 g, 97%) was prepared as a colorless oil from *N*-CBZ-*trans*-4-hydroxy-D-proline ethyl ester (1.51 g, 5.16 mmol) by the procedure described previously for making the L enantiomer: $[\alpha]^{24}_D$ = +29° (*c* 1.01, $CHCl_3$); spectra were identical in all respects with those of enantiomer 2; HRMS (EI, 26 eV) *m/e* 447.1331 (447.1351 calcd for $C_{22}H_{25}NO_7S$).

***N*-(Benzyloxycarbonyl)-*cis*-4-cyano-D-proline Ethyl Ester.** Tosylate 12 (2.02 g, 4.53 mmol) was treated with NaCN in DMSO at 80 °C to provide, after workup, the *cis*-nitrile ester (0.88 g, 64%) as a colorless oil: $[\alpha]^{25}_D$ = +32° (*c* 1.04, $CHCl_3$); spectra were identical in all respects with those of the enantiomer *N*-(benzyloxycarbonyl)-*cis*-4-cyano-L-proline ethyl ester; HRMS (EI, 22 eV) *m/e* 302.1255 (302.1266 calcd for $C_{16}H_{18}NO_4$).

***N*-(Benzyloxycarbonyl)-*cis*-4-carboxy-D-proline Dimethyl Ester (13).** The *N*-CBZ-*cis*-4-cyano-D-proline ethyl ester (0.755 g, 2.50 mmol) was treated with wet methanolic HCl and worked up as described above to provide dimethyl ester 13 (0.746 g, 2.32 mmol, 93%) as a colorless oil: $[\alpha]^{26}_D$ = +40° (*c* 1.15, $CHCl_3$); spectra were identical in all respects with those of enantiomer 3; HRMS (EI, 22 eV) *m/e* 321.1226 (321.1212 calcd for $C_{16}H_{19}NO_6$).

***N*-(Benzyloxycarbonyl)-*cis*-4-carboxy-D-proline.** Saponification of *cis*-dimethyl ester 13 (0.502 g, 1.56 mmol) gave 0.44 g (96%) of the desired *cis*-dicarboxylate as colorless prisms: mp 175–176 °C; $[\alpha]^{26}_D$ = +30° (*c* 1.10, CH_3OH); spectra were identical in all respects with those of the enantiomer *N*-(benzyloxycarbonyl)-*cis*-4-carboxy-L-proline. Anal. ($C_{14}H_{15}NO_6$) C, H, N.

***cis*-4-Carboxy-D-proline (14).** Hydrogenation of the crude *N*-CBZ-*cis*-4-carboxy-D-proline (0.398 g, 1.36 mmol) gave 0.167 g (77%) of 14 as colorless prisms: mp 224–225 °C; $[\alpha]^{24}_D$ = +37° (*c* 1.01, H_2O); spectra were identical in all respects with those of enantiomer 4. Anal. ($C_6H_9NO_4$) C, H, N.

General Procedure for Preparation of the Mosher Amides. In a typical preparation, a solution of the *N*-CBZ-4-carboxyproline dimethyl ester (0.059 g, 0.18 mmol) in ethanol (12 mL) was hydrogenated on a Parr apparatus at 50 psi over 10% Pd–C (0.011 g) for 0.5 h. The catalyst was removed by filtration through Celite, and the filter cake was washed with ethanol. The combined filtrate and washings were concentrated in vacuo. The residue was diluted with THF and again concentrated in vacuo. After dissolution of the colorless residue in pyridine (1 mL), the crude 4-carboxyproline dimethyl ester was treated with (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride[13] (0.07 g, 0.28 mmol) and the resulting mixture stirred at room temperature for 16 h. The brownish reaction mixture was quenched with H_2O (1.5 mL), stirred for 0.5 h, and diluted with Et_2O (10 mL). After separation of the layers, the organic layer was washed successively with 10% HCl (2 × 5 mL), saturated $NaHCO_3$ (2 × 5 mL), and H_2O (5 mL), dried ($MgSO_4$), and concentrated to an orange oil.

The diastereomeric purity of the crude Mosher amide derivatives of the *cis*-4-carboxyproline dimethyl esters was determined by analysis with 300-MHz ^1H NMR. For each derivative, the signals corresponding to the H-4 and one H-5 proton were chosen for analysis.

Mosher amide of *cis*-4-carboxy-L-proline dimethyl ester: ^1H NMR δ 3.18 (dd, *J* = 11.4, 7.5 Hz, 1 H, H-5), 2.73–2.63 (m, 1 H, H-4); de >95%.

Mosher amide of *cis*-4-carboxy-D-proline dimethyl ester: ^1H NMR δ 3.01–2.86 (m, 2 H, H-4 and H-5); de >95%.

The diastereomeric purity of the crude Mosher amide derivatives of the *trans*-4-carboxyproline dimethyl esters was determined by analysis with 250-MHz ^1H NMR. For each derivative, the signal corresponding to H-2 was chosen for analysis.

Mosher amide of *trans*-4-carboxy-L-proline dimethyl ester: ^1H NMR δ 4.73 (dd, *J* = 8.5, 6.9 Hz, 1 H, H-2); de >95%.

Mosher amide of *trans*-4-carboxy-D-proline dimethyl ester: ^1H NMR δ 4.66 (dd, *J* = 8.7, 4.7 Hz, 1 H, H-2); de >95%.

Pharmacology. Synaptosomal Uptake of Glutamate. Synaptosomes were prepared by the procedure of Booth et al.,[15a] using Ficoll/sucrose gradient centrifugation. Uptake of [^3H]-L-glutamate was followed essentially as described by Kuhar and Zarbin.[15b] Synaptosomes were suspended in a physiological buffer (Krebs–Ringer phosphate buffer) and preincubated at either 37 or 25 °C (as noted) for 5 min. The uptake assays were initiated by the addition of [^3H]-L-glutamate (0.5–100 μM) and allowed to incubate for 0.5–4 min, as noted. In the inhibition experiments, the [^3H]-L-glutamate and inhibitor (2–18 μM) were added simultaneously. Aliquots were removed at appropriate times, and the assay was terminated by rapidly filtering on GF/F glass-fiber filters. The filters were quickly rinsed with 20 volumes of ice-cold buffer. Radioactivity present on the filters was determined by liquid scintillation counting. Uptake rates were corrected for both background and leakage (i.e., uptake at 0 °C). Within each experiment, uptake rates were determined in triplicate. Protein concentrations were determined by the Pierce BCA (bicinchoninic acid) protein assay (Pierce, Rockford, IL).

Excitatory Amino Acid Receptor Binding. Synaptic plasma membrane (SPMs) were prepared as previously described.[16] Briefly, male, Sprague–Dawley rats (200 g) were decapitated, their forebrains were rapidly removed, and the brain tissue was homogenized in 0.32 M sucrose. Following differential centrifugation, a membrane fraction enriched in synapses, but low in myelin and mitochondria, was obtained. This membrane fraction was washed three times with 200 μM Tris–acetate buffer, pH 7.2, and diluted into the appropriate assay buffer to about 200 μg protein/mL.

Binding to NMDA, KA, and QA receptors was quantitated with [^3H]-L-glutamate, [^3H]-KA, and [^3H]-AMPA, respectively. The binding assays were carried out under optimally selective conditions of time, temperature, and buffer for each of the three receptor classes. [^3H]-L-Glutamate binding was quantified under conditions that selectively label NMDA receptors.[16] The SPMs were incubated with [^3H]-L-glutamate (10 nM, 50.9 Ci/mmol) in 50 mM Tris–acetate, pH 7.0, at 4 °C for 30 min. Nonspecific

binding was determined by the inclusion of 500 μM L-glutamate. KA receptors were quantitated as previously described.[17a,b] [^{3}H]-KA (10 nM, 60 Ci/mmol) binding was determined in 50 mM Tris–citrate buffer, pH 7.0, at 4 °C for 30 min. Nonspecific binding was determined by the inclusion of 100 μM unlabeled KA. QA receptors were measured with [^{3}H]-AMPA binding as described by Honore et al.[17c] Essentially, [^{3}H]-AMPA (10 nM, 27.6 Ci/mmol) is quantified in 50 mM Tris–acetate buffer, pH 7.2, containing 100 mM KSCN for 30 min at 4 °C. Nonspecific binding was determined by the inclusion of 100 μM quisqualic acid. In the inhibition studies, the conformationally defined analogues were included in the assay mixture at the appropriate concentrations (0.1–200 μM). The assays (total volume, 1.08 mL) were initiated by the addition of radiolabel and terminated by centrifugation (Beckman Microfuge, top speed, 3 min). Unbound radioligand in the supernatant was removed by suction and the radioactivity in the pellet quantitated by liquid scintillation counting. All of the binding studies were carried out as two sets of triplicates within a single experiments and each experiment was repeated at least three times.

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Registry No. 1, 51-35-4; *N*-Cbz-1, 13504-85-3; 2, 130830-60-3; 2 (4-*O*-deprotected), 103667-57-8; 3, 130830-69-2; 4, 64927-38-4; *N*-Cbz-4, 130830-73-8; 4 (dimethyl ester, Mosher amide), 130830-79-4; 5, 130830-61-4; 5 (4-*O*-deprotected), 130930-26-6; 6, 130830-70-5; 7, 64769-66-0; *N*-Cbz-7, 130830-74-9; 7 (dimethyl ester, Mosher amide), 130830-81-8; 8, 2584-71-6; *N*-Cbz-8, 130930-25-5; 9, 130830-63-6; 9 (4-*O*-deprotected), 130930-27-7; 10, 130830-71-6; 11, 130830-77-2; *N*-Cbz-11, 130830-75-0; 11 (dimethyl ester, Mosher amide), 130830-82-9; 12, 130830-62-5; 12 (4-*O*-deprotected), 130930-28-8; 12 (4-acetoxy analog), 130830-64-7; 13, 130830-72-7; 14, 130830-78-3; *N*-Cbz-14, 130830-76-1; 14 (dimethyl ester, Mosher amide), 130830-80-7; *N*-(benzyloxycarbonyl)-*cis*-4-cyano-L-proline ethyl ester, 130830-65-8; *N*-(benzyloxycarbonyl)-*trans*-4-cyano-L-proline ethyl ester, 130830-66-9; *N*-(benzyloxycarbonyl)-*trans*-4-cyano-D-proline ethyl ester, 130830-67-0; *N*-(benzyloxycarbonyl)-*cis*-4-cyano-D-proline ethyl ester, 130830-68-1.

Fadrozole Hydrochloride: A Potent, Selective, Nonsteroidal Inhibitor of Aromatase for the Treatment of Estrogen-Dependent Disease

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A new class of potent, selective, nonsteroidal inhibitors of aromatase have been discovered. The most potent member of this series is fadrozole hydrochloride, CGS 16949 A, 4-(5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridin-5-yl)benzonitrile monohydrochloride, **26a**. In addition, the 6,7-dihydropyrrolo[1,2-*c*]imidazole (**21a**) and the 6,7,8,9-tetrahydroimidazo[1,5-*a*]azepine (**21b**) analogues were synthesized and evaluated. CGS 16949 A's ability to selectively inhibit aromatase (IC_{50} = 4.5 nM) over other cytochrome P-450 enzymes and suppress estrogen production when administered orally make it a suitable candidate to test the potential of an aromatase inhibitor in estrogen-dependent diseases including breast cancer.

Estrogen has been implicated in the progression of several diseases. Consequently the direct antagonism of estrogen or the lowering of its circulating level is thought to be relevant to the therapy of disorders such as estrogen-dependent breast cancer, gynecomastia, systemic lupus erythematosus, and premature labor.[1] Currently tamoxifen, an estrogen receptor antagonist, and aminoglutethimide, an aromatase inhibitor, have proven to be efficacious in the treatment of estrogen-dependent breast cancer.[2,3]

The full potential of aromatase inhibitors in the treatment of estrogen-dependent diseases is yet to be fully evaluated. This has been due to the lack of a potent, highly selective, orally active, side-effect-free inhibitor of this enzyme. From a clinical viewpoint aminoglutethimide (**6**) comes closest to satisfying these criteria. However, its relatively low potency and lack of specificity make it less than ideal. The objective of these studies has been to identify a compound which fulfills these criteria and so provide the basis for testing the hypothesis that aromatase inhibition could be beneficial in the treatment of estrogen-dependent breast cancer.

Selectivity Criteria

Aromatase occurs widely in tissues such as adipose tissue, brain, testes, and ovaries. The enzyme is a membrane-bound microsomal complex containing NADPH-cytochrome *c* reductase and cytochrome P-450 units. The mechanism by which androstenedione is converted to estrone has been studied in detail.[4-17] This conversion can

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