Synthesis of the Four Isomers of 4-Aminopyrrolidine-2,4-dicarboxylate: Identification of a Potent, Highly Selective, and Systemically-Active Agonist for Metabotropic Glutamate Receptors Negatively Coupled to Adenylate Cyclase

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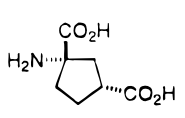
The four isomers of 4-aminopyrrolidine-2,4-dicarboxylate (APDC) were prepared and evaluated for their effects at glutamate receptors in vitro. (2*R*,4*R*)-APDC (**2a**), an aza analog of the nonselective mGluR agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylate ((1*S*,3*R*)-ACPD, **1**), was found to possess relatively high affinity for metabotropic glutamate receptors (mGluRs) (ACPD-sensitive [^{3}H]glutamate binding $IC_{50} = 6.49 \pm 1.21$ μM) with no effects on radioligand binding to NMDA, AMPA, or kainate receptors up to 100 μM. None of the other APDC isomers showed significant mGluR binding affinity, indicating that this interaction is highly stereospecific. Both **1** and **2a** were effective in decreasing forskolin-stimulated cAMP formation in the adult rat cerebral cortex ($EC_{50} = 8.17 \pm 2.21$ μM for **1**; $EC_{50} = 14.51 \pm 5.54$ μM for **2a**); however, while **1** was also effective in stimulating basal tritiated inositol monophosphate production in the neonatal rat cerebral cortex ($EC_{50} = 27.7 \pm 5.2$ μM), **2a** (up to 100 μM) was ineffective in stimulating phosphoinositide hydrolysis in this tissue preparation, further supporting our previous observations that **2a** is a highly selective agonist for mGluRs negatively coupled to adenylate cyclase. Microelectrophoretic application of either **1** or **2a** to intact rat spinal neurons produced an augmentation of AMPA-induced excitation (95 ± 10% increase for **1**, 52 ± 6% increase for **2a**). Intracerebral injection of **1** (400 nmol) produced characteristic limbic seizures in mice which are not mimicked by **2a** (200−1600 nmol, ic). However, the limbic seizures induced by **1** were blocked by systemically administered **2a** in a dose-dependent manner (EC_{50} = 271 mg/kg, ip). It is concluded that (2*R*,4*R*)-APDC (**2a**) is a highly selective, systemically-active agonist of mGluRs negatively coupled to adenylate cyclase and that selective activation of these receptors in vivo can result in anticonvulsant effects.

Introduction

L-Glutamate (Glu) is the principal excitatory amino acid (EAA) neurotransmitter in the mammalian central nervous system (CNS). The effects of Glu are mediated by a variety of presynaptic and postsynaptic neuronal receptors.[1] These are broadly subdivided into two classes based on their mechanism of signal transduction: the ionotropic glutamate receptors (iGluRs) and the metabotropic glutamate receptors (mGluRs).[2−4] Metabotropic glutamate receptors are coupled through G-proteins to intracellular enzymes which regulate the production of second messengers within the target neuron.[3−6] Several mGluRs have been cloned, and these may be classified into three groups based on sequence homology, second-messenger coupling, and pharmacological characteristics. Group 1 mGluRs (mGluR1 and mGluR5) are coupled to phospholipase C (PLC) and are potently activated by quisqualic acid and (1*S*,3*R*)-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD, **1**).[3−8] Agonist activation of group 1 mGluRs causes an increase in phosphoinositide (PI) hydrolysis, resulting in an increase in the intracellular concentrations of inositol triphosphate (IP3) and diacylglycerol (DAG). Group 2 mGluRs (mGluR2, mGluR3, and mGluR8) and group 3 mGluRs (mGluR4, mGluR6, and mGluR7) are negatively coupled to adenylate cyclase (AC). Group 2 mGluRs are also potently activated by **1**,[4−6,8,9] while group 3 mGluRs are insensitive to **1** and selectively activated by L-2-amino-4-phosphonobutyric acid (L-AP4).[4,6] Agonist activation of group 2 or 3 mGluRs produces a decrease in intracellular concentrations of cyclic adenosine monophosphate (cAMP).



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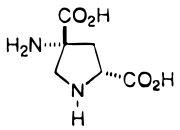

2a

Owing to its favorable pharmacological selectivity for mGluRs versus iGluRs, compound **1** has been the most extensively studied mGluR agonist to date. However, as observed in preparations of cloned and in situ mGluRs, this compound activates both PLC-linked and AC-linked mGluRs in neuronal tissues. Additionally, **1** produces various electrophysiological responses in neuronal tissues which may not involve either PLC or AC but may reflect involvement of voltage-sensitive calcium channels.[5,6]

As part of our ongoing effort to discover subtype selective mGluR agonists and antagonists, we have

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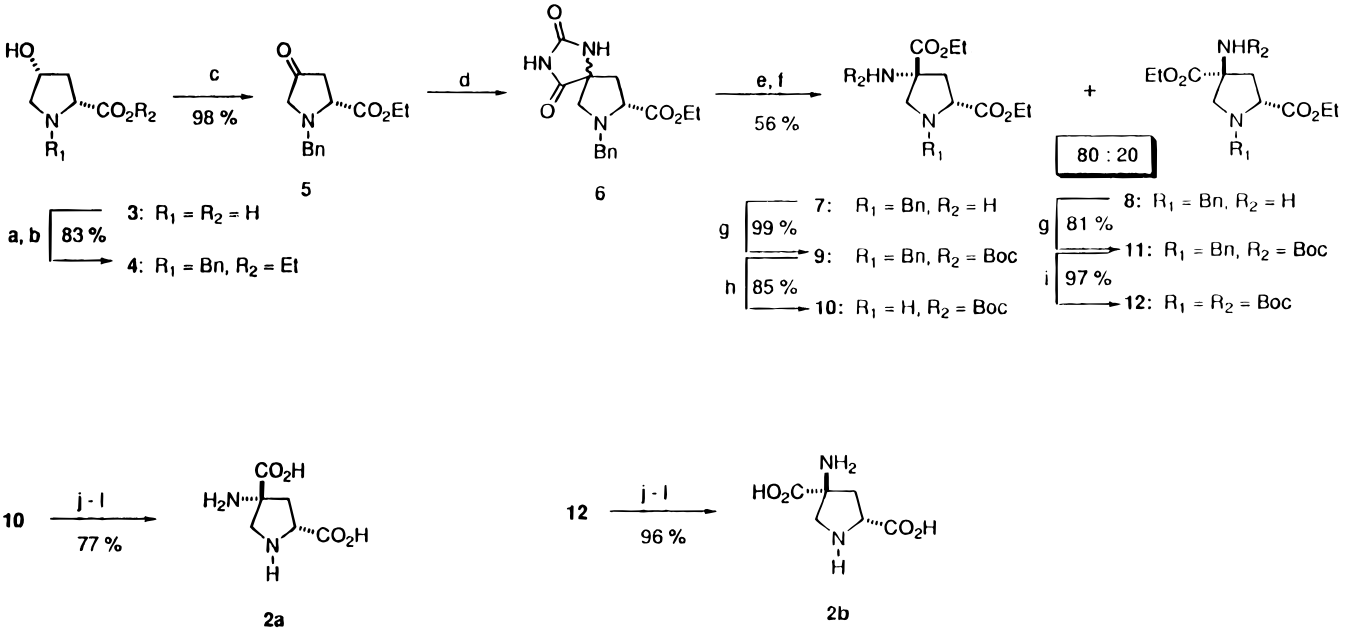
Scheme 1[a]



[a] (a) EtOH, H_2SO_4, reflux; (b) BnBr, *i*-Pr_2NEt, CH_2Cl_2, room temperature; (c) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −78 °C; (d) $NH_2CO_2NH_4$, KCN, EtOH/H_2O, 55 °C; (e) 3 N NaOH, reflux; (f) EtOH, $SOCl_2$, reflux; (g) Boc_2O, CH_2Cl_2, room temperature; (h) 5% PdC/H_2, EtOH, room temperature; (i) Boc_2O, 5% PdC/H_2, EtOH, room temperature; (j) Et_2O·HCl, 0 °C; (k) 1 N NaOH/THF; (l) ion-exchange chromatography.

explored the effect of nitrogen atom substitution at the C4-position of **1** and its isomers on mGluR affinity and selectivity. We have previously reported that (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate ((2*R*,4*R*)-APDC, **2a**) is a potent agonist for mGluRs negatively coupled to AC both in the rat hippocampus and in cells expressing cloned human mGluR2 receptors while possessing no agonist activity at mGluRs positively coupled to PLC either in the rat hippocampus or in cells expressing cloned human mGluR1α receptors.[10] In this account we report (1) the synthesis of **2a** and its isomers **2b**−**d**, (2) the affinities of **2a**−**d** for glutamate receptors in vitro, (3) further biochemical and electrophysiological characterization of **2a** as a selective group 2 mGluR agonist, and (4) the effect of **2a** on limbic seizure activity produced by **1** in mice.

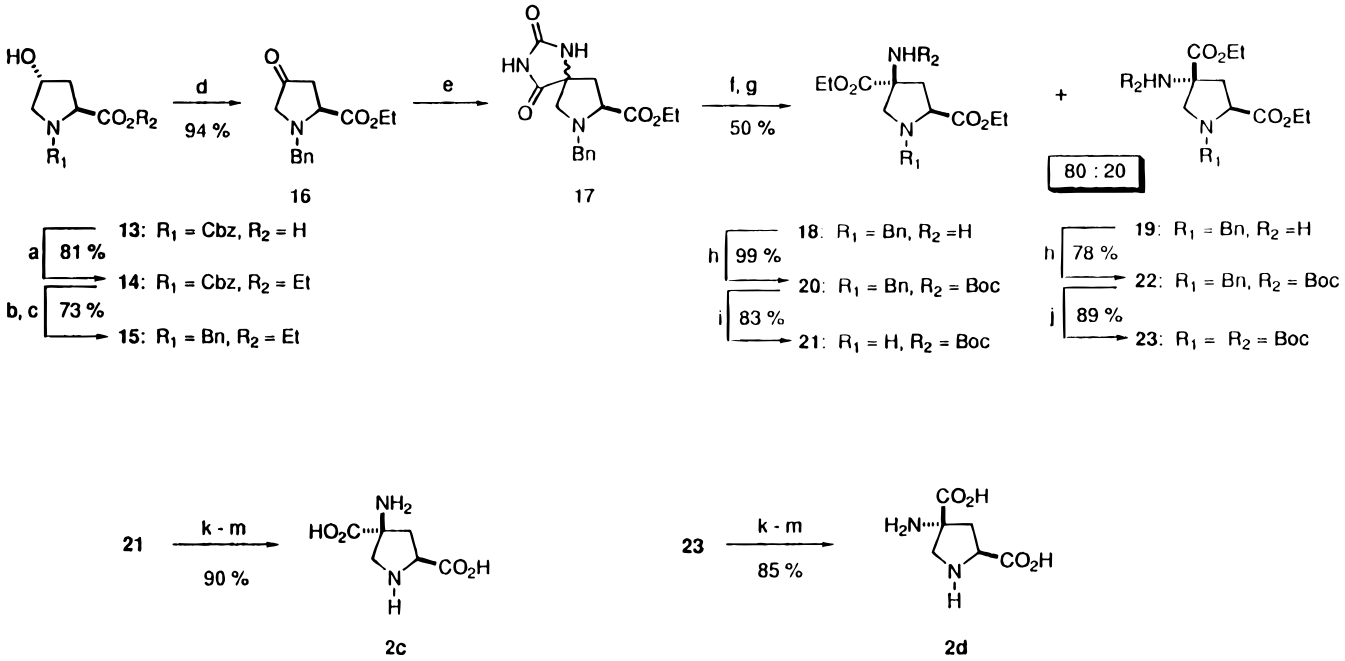
Chemistry

The (2*R*,4*R*)-APDC and (2*R*,4*S*)-APDC derivatives **2a,b** were prepared in a stereochemically controlled fashion starting from D-*cis*-4-hydroxyproline (**3**; Scheme 1).[11] Conversion of **3** to (2*R*)-ethyl 4-oxopyrrolidine-2-carboxylate (**5**) was accomplished in good overall yield by the three-step route shown in Scheme 1. Treatment of **5** with ammonium carbamate and sodium cyanide at 55 °C afforded a mixture of hydantoins **6** which were hydrolyzed in refluxing 3 N NaOH. After acidification with HCl and concentration to dryness, the crude hydrolysate was subjected to Fisher esterification giving rise to a chromatographically separable mixture of diamino diesters **7** and **8**. Products **7** and **8** were then converted in high yields to the corresponding *N*-*tert*-butyloxycarbonyl derivatives **9** and **11** which then underwent hydrogenolysis of the benzyl groups in either the absence (in the case of **9**) or presence (in the case of **11**) of di-*tert*-butyl dicarbonate to yield **10** and **12**, respectively. Final removal of the protecting groups under standard conditions followed by ion exchange chromatography afforded products **2a,b**. In an analogous manner, the (2*S*,4*S*)-APDC and (2*S*,4*R*)-APDC isomers **2c,d** were prepared from *N*-(benzyloxycarbonyl)-L-*trans*-4-hydroxyproline (**13**) as depicted in Scheme 2.

The relative stereochemistry and enantiomeric purity of intermediates arising from the hydantoin formation/hydrolysis/esterification sequence (compounds **7**, **8**, **18**, and **19**) were assigned on the basis of NOE experiments and proton NMR analysis of the corresponding diastereomeric Mosher amides. Thus, for *N*-Boc derivative **20**, irradiation of H_2 produced an enhancement of $H_{3\alpha}$, while irradiation of the NH proton produced an enhancement of $H_{3\beta}$ (Figure 1). Thus, on the basis of these results, the NH and H_2 are confirmed to reside on opposite faces of the pyrrolidine ring for this (major) diastereomer. Determination of the relative stereochemistry of isomeric *N*-Boc derivative **22** was not possible due to overlap of key proton resonances in the 300 MHz spectrum. Thus, **22** was converted to the corresponding trifluoroacetamide **26** (Scheme 3). Irradiation of either the NH or H_2 protons of **26** resulted in an enhancement of both $H_{3\alpha}$ and $H_{5\alpha}$. In addition, an NOE was also observed between H_2 and NH (Figure 1).

These data indicate that the NH and H_2 reside on the same face of the pyrrolidine ring system in this (minor) diastereomer. These results are in good agreement with those previously described.[11] To determine the degree of racemization of ketone **5** or **16** prior to hydantoin formation, **18** was converted to the Mosher amides **24** and **25** by treatment with (*R*)- or (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chlorides, respectively (Scheme 3). Comparison of the 300 MHz proton NMR spectra of **24** and **25** (in conjunction with a proton spectrum of a mixture consisting of **24** and **25**) revealed no trace of enantiomeric contamination. Thus, to the level of detection afforded by this method, the enantiomeric excess of the parent amine **18** is confirmed to be

Scheme 2[a]



[a] (a) EtI, Et_3N, CH_3CN, 55 °C; (b) 5% PdC/H_2, EtOH, room temperature; (c) BnBr, *i*-Pr_2NEt, EtOH, room temperature; (d) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −78 °C; (e) $NH_2CO_2NH_4$, NaCN, $EtOH/H_2O$, 55 °C; (f) 3 N NaOH, reflux; (g) EtOH, $SOCl_2$, reflux; (h) Boc_2O, CH_2Cl_2, room temperature; (i) 5% PdC/H_2, EtOH, room temperature; (j) Boc_2O, 5% PdC/H_2, EtOH, room temperature; (k) Et_2O·HCl, 0 °C; (l) 1 N NaOH/THF; (m) ion-exchange chromatography.

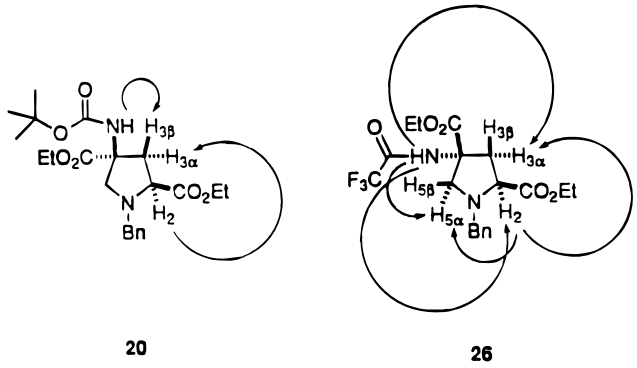


Figure 1. Key NOEs observed for diethyl N^1-benzyl-4-(acylamino)pyrrolidine-2,4-dicarboxylate diastereomers **20** and **26**.

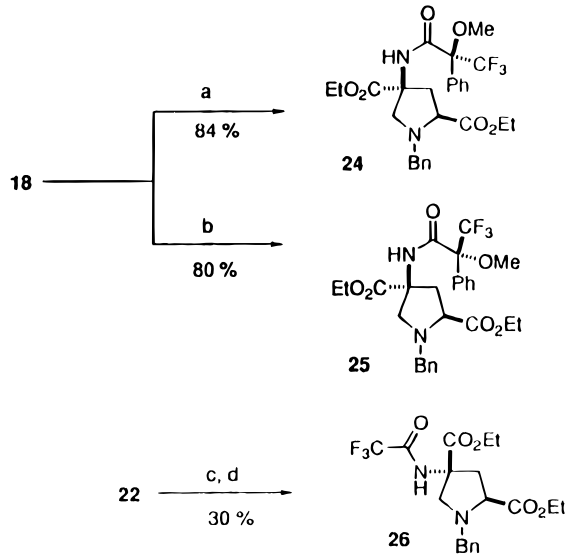
greater than 95%. These results are in full agreement with those previously disclosed.[11]

Pharmacological Methods

Radioligand binding to NMDA, AMPA, kainate, and metabotropic glutamate receptors was performed utilizing [^{3}H]CGS19755, [^{3}H]AMPA, [^{3}H]kainate, and [^{3}H]-glutamate as the radioligands, respectively.[12−15] Measurement of cyclic adenosine monophosphate (cAMP) and tritiated inositol monophosphates ([^{3}H]IP) levels in rat cerebral cortical slices was performed as previously described.[10,16]

Rat Spinal Cord Electrophysiology. The effects of **1** and **2a,c** on responses of spinal neurons in vivo to AMPA were studied using the technique of microelectrophoresis on pentobarbitone-anesthetized rats as described previously.[17] Briefly, female Wistar rats (200−230 g) were anesthetized with pentobarbitone Na (initially 60 mg/kg ip and supplemented ip as required), and a lumbar laminectomy was performed to allow insertion of a seven-barrel glass microelectrode

Scheme 3[a]



[a] (*S*)-PhC(OMe)(CF_3)COCl, pyridine, room temperature; (b) (*R*)-PhC(OMe)(CF_3)COCl, pyridine, room temperature; (c) CF_3CO_2H, room temperature; (d) $(CF_3CO)_2O$, pyridine, room temperature.

into the gray matter of the spinal cord. Action potential firing rate of single neurons was recorded continuously in response to timed intermittent ejection of AMPA (10 mM in 200 mM NaCl, pH 7.4) from one barrel of the electrode. When consistent submaximal responses were established, compounds **1** and **2a,c** (all at 25 mM in 175 mM NaCl, pH 7.4) were ejected sequentially but in different orders on different cells. Changes in response to AMPA were measured as percentage increases from control values and are expressed as mean ± SEM.

Mouse Limbic Seizure Model. Evaluation of the effects of compound **2a** in NIH Swiss mice in the

Table 1. Glutamate Receptor Radioligand Displacement by (1*S*,3*R*)-ACPD (**1**) or APDC Isomers **2a**−**d**

compd	[^{3}H]CGS-19755[a]	[^{3}H]AMPA[b]	[^{3}H]KA[c]	ACPD-sensitive [^{3}H]glutamate[d]
1	>100	>100	>100	10.3 ± 2.06
2a	>100	>100	>100	6.49 ± 1.21
2b	4.40 ± 0.88	>100	>100	>100
2c	>100	>100	>100	>100
2d	23.73 ± 4.76	>100	>100	>100

[a] See ref 12. [b] See ref 13. [c] See ref 14. [d] See ref 15.

presence and absence of **1** was performed as previously described.[18]

Molecular Modeling

Compounds **1** and **2a** were minimized to an energy gradient tolerance of 0.01 using CHARMm 21.3[19] on a Silicon Graphics R4400 Indigo workstation. A constraint of 2.0 ± 0.1 Å was applied between the various heteroatom−proton hydrogen-bonding possibilities to induce an artificial arrangement for a possible hydrogen bond and the appropriate ring pucker. These systems were minimized until the applied constraint was satisfied, the constraint was then removed, and the structures were then allowed to freely relax for 100 steps of ABNR. These structures were submitted to full geometry-optimized density functional calculations using the DZVP basis set and A1 auxiliary through UniChem 2.0[20] on a Cray-2 instrument. Superposition of atomic density (ATSUP) was used as a starting guess for the SCF. Average time to completion was 14 200 CPU s.

Results

Compounds **2a**−**d** were initially evaluated for their ability to displace radioligand binding to ionotropic or metabotropic glutamate receptors in rat forebrain membranes in vitro. As shown in Table 1, compound **2a** ((2*R*,4*R*)-APDC) shows good potency in displacing ACPD-sensitive [^{3}H]glutamate binding (IC_{50} = 6.49 ± 1.21 μM) and high mGluR to iGluR selectivity (IC_{50} values > 100 μM each for [^{3}H]CGS19755, [^{3}H]AMPA, and [^{3}H]-kainate). The other three APDC isomers (**2b**−**d**) were much less potent (IC_{50} values > 100 μM) in binding to mGluRs when compared to **2a** (Table 1). The (2*R*,4*S*)-APDC and (2*S*,4*R*)-APDC isomers, **2b,d**, weakly displaced [^{3}H]CGS19755 binding to the NMDA receptor (IC_{50} values = 4.40 ± 0.88 and 23.73 ± 4.76 μM, respectively). These isomers correspond to the two *cis*-ACPD isomers which are known to be weak NMDA receptor agonists.[16]

Compound **2a** was then evaluated for its ability to influence cAMP (Figure 2A)[10] or [^{3}H]IP formation (Figure 2B)[16] in adult rat cerebral cortical slices. As shown in Figure 2A, both **1** and **2a** are effective in decreasing forskolin-stimulated cAMP formation in the adult rat cerebral cortex (EC_{50} values = 8.17 ± 2.21 and 14.51 ± 5.54 μM for **1** and **2a**, respectively). The agonist effect of **2a** is stereospecific, as its enantiomer **2c** was ineffective in decreasing forskolin-stimulated cAMP formation at 100 μM. Moreover, while **1** is effective in stimulating the formation of [^{3}H]IP in the adult rat cerebral cortex (EC_{50} = 27.7 ± 5.2 μM), the aza analog **2a** (up to 100 μM) does not stimulate PI hydrolysis above basal levels in this tissue (Figure 2B). These results are summarized in Table 2.

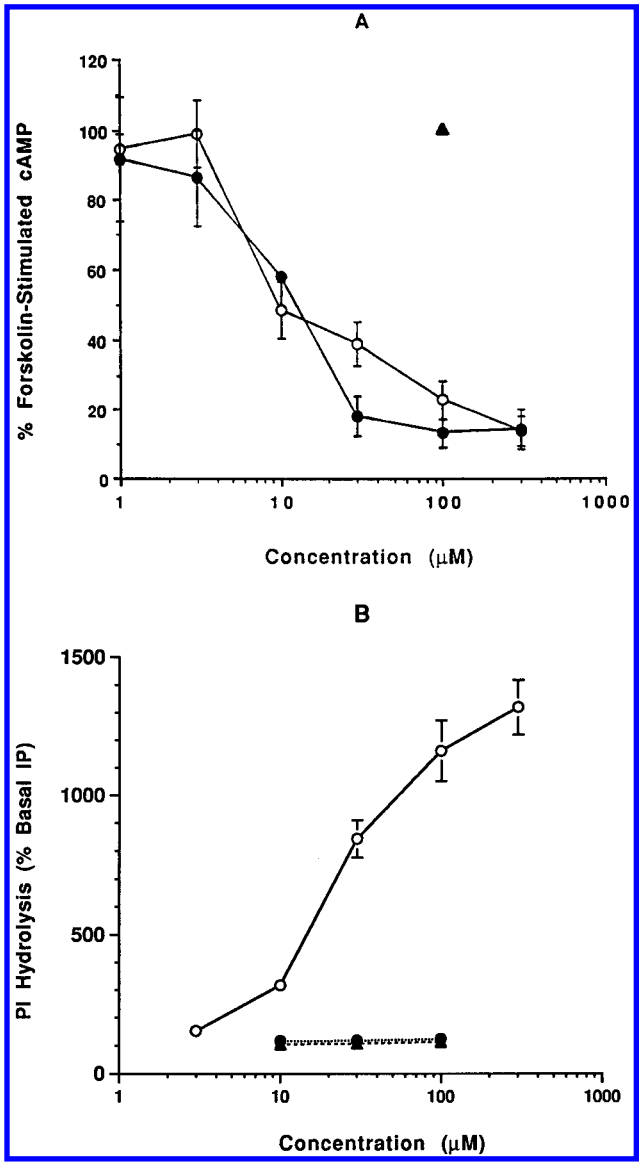


Figure 2. Comparison of (1*S*,3*R*)-ACPD (**1**; ○), (2*R*,4*R*)-APDC (**2a**; ●), and (2*S*,4*S*)-APDC (**2c**; ▲) on forskolin (30 μM)-stimulated cAMP formation in adult rat cerebral cortical slices (A) and on [^{3}H]phosphoinositide hydrolysis in neonatal rat cerebral cortical slices (B). *Denotes $p < 0.05$ when compared to the basal value.

Table 2. Second-Messenger and Spinal Cord Electrophysiological Responses to Metabotropic Glutamate Receptor Agonists **1** and **2a**

	EC_{50} (μM) ± SEM		
compd	inhib forskolin-stim cAMP formation[a]	stimulation of [^{3}H]IP formation[b]	increase in AMPA-induced currents[c] (%)
1	8.17 ± 2.21	27.7 ± 5.2	95 ± 10
2a	14.51 ± 5.54	>100	52 ± 6

[a] Data obtained in cross-chopped slices of the adult rat cerebral cortex.[10] [b] Data obtained in cross-copped slices of the neonata rat cerebral cortex.[16] [c] Data obtained in intact rat spinal neurons (see methods section).

The electrophysiological effects of **1** or **2a** on AMPA-evoked responses in intact rat spinal cord neurons are summarized in Table 2. As has been previously reported, **1** enhances the excitatory effects of AMPA on spinal neurons, ejecting currents of 2−15 nA, causing a 95 ± 10% increase of the responses of six neurons to AMPA.[21] Compound **2a** (25−40 nA, n = 6) also caused

a robust enhancement (52 ± 6%) of AMPA responses. The effects of each compound reached a peak within 5 min of starting ejection, and responses to AMPA recovered to control levels within 5 min of stopping the ejection of the mGluR agonists.

When compound **1** is administered (400 nmol, ic) to NIH Swiss mice, a characteristic limbic seizure is subsequently observed which persists for the entire 10 min observation period (Figure 3). In contrast, administration of **2a** (200−1600 nmol, ic) did not result in limbic seizure activity; rather, **2a** produced only a dose-related (400−1600 nmol) decrease in spontaneous locomotor activity. Furthermore, systemic administration of **2a** (100−600 mg/kg, ip) **30** min prior to direct injection of **1** (400 nmol, ic) produced a dose-related decrease in the number of animals exhibiting limbic seizures over the 10 min observation period (EC_{50} = 271 mg/kg; Figure 3).

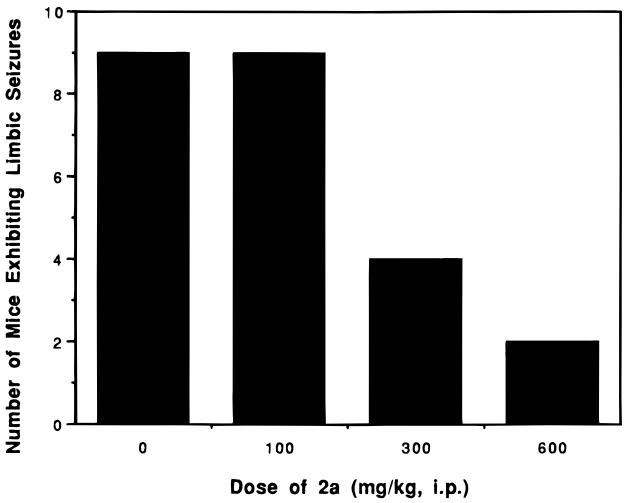
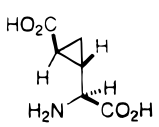


Figure 3. Anticonvulsant effect of **2a** on limbic seizures produced by **1** (400 nmol, ic) in mice. Compound **2a** (100−600 mg/kg) was administered intraperioneally to NIH Swiss mice 30 min prior to the administration of **1**.

Discussion

The functions of mGluRs in mammalian physiology and pathophysiology are not well understood, largely due to the lack of potent, highly selective, and systemically-active agonists and antagonists for these receptors. In particular, while (1*S*,3*R*)-ACPD (**1**) has proven valuable in the study of mGluRs due to its favorable mGluR to iGluR receptor selectivity, this compound does not distinguish between mGluRs positively coupled to PLC (group 1 mGluRs) and those which are negatively coupled to AC (group 2 mGluRs). More recently, (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (L-CCG-1, **27**)[22,23] and (2*S*,1′*R*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV, **28**)[24] have each been established as exceptionally potent agonists for group 2 mGluRs; however, both suffer from agonist activity at other glutamate receptors at somewhat higher concentrations (group 1 mGluRs are activated by **27** and NMDA receptors by **28**, respectively).[22−24]



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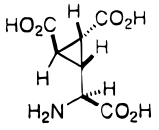

28

In our previous account, we described (2*R*,4*R*)-APDC (**2a**) as a new pharmacological tool for studying mGluRs negatively coupled to AC which does not possess agonist activity at either iGluRs or PLC-linked mGluRs up to 1 mM.[10] In this account we have described the preparation of **2a** and its isomers **2b**−**d**. Evaluation of the ability of isomers **2a**−**d** to displace radioligand binding from iGluRs and mGluRs demonstrates that only the (2*R*,4*R*)-APDC isomer **2a** (the isomer which corresponds to (1*S*,3*R*)-ACPD) is able to potently displace ACPD-sensitive [^{3}H]glutamate binding from rat forebrain homogenates (IC_{50} = 6.49 ± 1.21 μM), an indication of group 2 mGluR affinity.[25] Furthermore, this isomer is devoid of binding affinity (IC_{50} values > 100 μM) for the glutamate recognition site on the NMDA, AMPA, and kainate receptors. In accordance with our previous findings in the adult rat hippocampus and in AV12 cells expressing cloned human mGluR2 or human mGluR1α receptors,[10] **2a** was found in this study to potently activate mGluRs negatively coupled to AC in the rat cerebral cortex while possessing no agonist activity for stimulation of PI hydrolysis in this tissue. In addition, **2a** has no effect on NECA-stimulated cAMP formation in the rat hippocampal slice, a system which is highly sensitive to group 3 (but not group 1 or group 2) mGluR agonists,[26] and does not act as an agonist or antagonist at recombinant human group 3 (mGluR4 or mGluR7) receptors up to 300 μM.[27] These data, in conjunction with those reported elsewhere,[10,27] indicate that **2a** is a highly selective agonist of both cloned and native group 2 mGluRs, having no effects on either cloned or native group 1 or 3 mGluRs.

We have extended our study of **2a** to the evaluation of its effects in vivo. The results on spinal neurons agree with previous observations that agonists of both group 1 and 2 mGluRs (e.g., 1, **27**) facilitate responses to agonists of iononotropic excitatory and inhibitory amino acids.[21] The results are consistent with an mGluR-mediated increase in membrane resistance, for example, decreased potassium conductance.[28] Although absolute potencies cannot be calculated from electrophoresis experiments, it appears that **2c** is approximately 10 times less effective than **2a** in potentiating the actions of AMPA and that **2a** is about 8 times less potent that **1**. In addition, the present results with the selective group 2 mGluR agonist **2a** and those with the selective group 1 agonist (3,5-dihydroxyphenyl)glycine[21] show that this effect can be produced by activating either group 1 or 2 mGluRs. The modulation of responses to AMPA by mGluR agonists suggests an important role for these receptors in spinal processing.

While **1** and **2a** behave similarly in augmenting AMPA-induced excitation in rat spinal cord, they differ considerably in the behavioral effects which they evince when administered directly into the mouse thalamus. Thus, while **1** (400 nmol, ic) produces limbic seizure activity in mice, **2a** (200−1600 nmol, ic) does not. Rather, the only behavioral change which was noted upon direct injection of **2a** (400−1600 nmol, ic) in mice was a decrease in spontaneous locomotor activity which appeared to be dose related. This finding is consistent with the observation that intraventricular administration of low doses (less than 1 nmol) of **28** in rats produces central depressant actions (higher doses of **28**

produced clonic convulsions, presumably due to its agonist effects at NMDA receptors).[29] Importantly, systemic administration of **2a** (100−600 mg/kg, ip) 30 min prior to direct injection of **1** resulted in a dose-related decrease in the number of mice exhibiting limbic seizure activity (ED_{50} = 271 mg/kg). Thus, we conclude that the convulsant effects of centrally administered **1** are likely due to its group 1 (PLC-linked) agonist effects. In accordance with this hypothesis, direct intracerebral injection of the selective group 1 mGluR agonist (3,5-dihydroxyphenyl)glycine[30−32] produces limbic seizures in mice.[33] The mechanism by which (2*R*,4*R*)-APDC (**2a**) produces its anticonvulsant effects in this model is not fully understood at this time. It has been proposed that metabotropic glutamate receptors which are negatively coupled to AC may be presynaptically localized and function in an autoreceptor capacity in certain glutamatergic synapses. Activation of these mGluRs may then inhibit the release of glutamate from presynaptic nerve terminals.[34] An agonist acting selectively at these sites might then be expected to decrease excitatory neurotransmission in the affected synapses which could, in turn, be manifested as an anticonvulsant effect. Alternatively, a direct postsynaptic effect of **2a**, resulting in decreased cAMP levels in the postsynaptic neuron, might also decrease neuronal excitability. While the precise mechanism by which **2a** elicits its anticonvulsant effect in mice remains unclear, it is concluded that activation of group 1 mGluRs in vivo results in enhanced neuronal excitability, while selective activation of group 2 mGluRs appears to result in decreased excitability. This is the first example which documents the effects of a selective group 2 mGluR agonist administered systemically.

Compound **2a** differs from **1** only in the substitution of an amino functionality in **2a** for the C4-methylene unit in **1**. We have investigated the possibility that this structural variation might alter the number and/or types of accessible conformations of the heterocyclic derivative **2a** relative to the carbocyclic parent **1**. For **1**, the low-energy conformer (predicted by both NMR and molecular dynamics simulation at pH 7) is predicted to be folded, whereby intramolecular electrostatic attraction forces the α-amino and γ-carboxyl moieties into axial orientations.[35] However, the proposed bioactive conformation of **1** for interacting with group 2 mGluRs is one in which the glutamate backbone resides in a fully extended orientation.[36] We wondered whether the fully extended (higher energy) conformation of **1** might be more highly stabilized in the aza derivative **2a**. After initial minimization and conformational searching about the carboxyl carbon−ring carbon axes, **1** and **2a** were reminimized with artificial H-bond constraints incorporated. Compounds **1** and **2a** were considered to be in their ground state (CO_2H and NH_2 as opposed to CO_2^- and NH_3^+), and although it was recognized that the most likely hydrogen-bonding combinations involve the nitrogen atom as a H-bond acceptor (Figure 4, structures **1-A**, **2a-A**, **2a-B**, and **2a-C**), the converse case (Figure 4, structure **1-B**, nitrogen as H-bond donor) was also considered. To induce the "inverse" H-bonded conformation in **1-B**, a similar strategy was adopted; a distance constraint of 2.1 Å (representing a reasonable H-bond distance) was applied to the NH proton and the carbonyl oxygen. When this constraint was satisfied

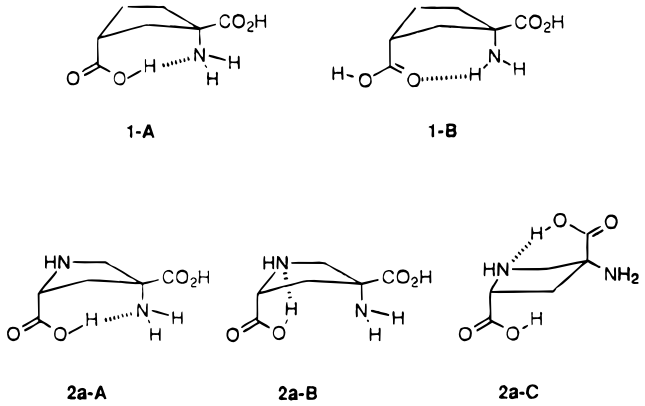

Figure 4. Idealized hydrogen-bonding geometries for **1** and **2a**.

and the required conformation induced, it was turned off and the molecule was then allowed to freely minimize for 100 steps of ABNR, with the "inverse" H-bonded conformation being partially maintained. Both the freely minimized and the H-bonded (constrained) conformations of **1** and **2** were then examined by geometry-optimized density functional and standard Hartree−Fock methods.

Vacuum phase DZVP calculations performed on **1** indicate that there is the possibility of a hydrogen bond in the arrangement wherein the α-NH_2 nitrogen acts as the hydrogen bond acceptor to the γ-CO_2H proton (Figure 4, structure **1-A**). In this case, an ideal hydrogen-bonding distance of 1.86 Å was calculated. When the nitrogen hydrogen is invoked as the hydrogen bond donor (Figure 4, structure **1-B**), an unlikely longer hydrogen-bonding distance of 2.42 Å results. Further data (Table 3) supporting a γ-OH−α-NH_2 hydrogen bond in **1** are evidenced by the fact that structure **1-A** possesses the lowest energy and the greatest Mulliken point charge separation.

In contrast to **1**, the number of possible combinations of intramolecular hydrogen bonds in **2a** is greatly increased due to the fact that there are two possible nitrogen H-bond acceptors in the molecule. Furthermore, the mobility of the pyrrolidine ring is greater than that of a cyclopentane, allowing for facile intramolecular H-bonding. Three potential H-bonded structures of **2a** were examined (**2a-A**, **2a-B**, and **2a-C**, Figure 4) plus the non-hydrogen-bonded form. However, during molecular mechanics minimizations, after the artificial H-bond constraints were turned off, the molecules rapidly reverted to structures unlikely to hydrogen bond. Although the pyrrolidine ring of **2a** is more flexible and less sterically strained than the cyclopentane ring of **1**, it is not as puckered. Consequently, the geometries of the idealized hydrogen bonds could not always be sustained. However, when starting from the constrained molecular mechanics structures, one case was found where an intramolecular H-bond arrangement was sustained during the DZVP calculations. A hydrogen bond of 1.82 Å is created in a stabilized six-membered ringlike conformation (structure **2a-C**, Figure 4) which is energetically favored with respect to the non-hydrogen-bonded structure by 28.6 kJ mol^{-1}. Since this conformation of **2a** involves an interaction between the ring nitrogen (H-bond acceptor) and the C4-carboxylic acid (H-bond donor), it is distinctly different from the hydrogen bond-stabilized conformation of **1**. This

Table 3. Mulliken Point Charges, Hydrogen-Bonding Distances, and Energies (Relative to lowest energy analog) from DZVP Density Functional Calculations on Different H-Bonded Forms of **1**

	Mulliken charges[b]					
structure[a]	α-N nitrogen	α-NH proton	γ-CO oxygen	γ-OH proton	H-bond distance[b] (Å)	energy[b] (kJ mol^{-1})
1 (no H-bond)	−0.688	0.341	−0.312	0.429		0.9
1-A	−0.815	0.368	−0.319	0.506	1.87	0
1-B	−0.709	0.360	−0.325	0.425	2.52	9.9

[a] See Figure 4. [b] See Molecular Modeling section.

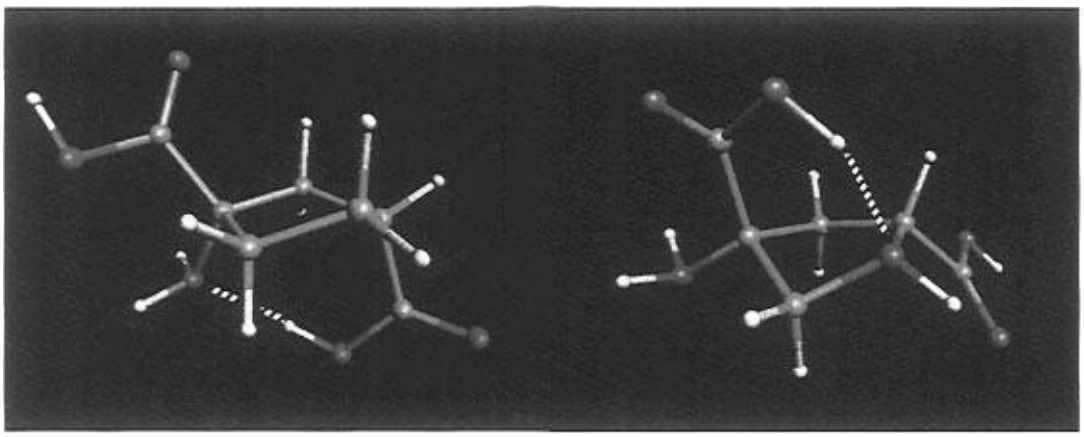

Figure 5. Intramolecular hydrogen-bonded DZVP structures of **1** and **2a**. Dashed lines show hydrogen bonds.

results in a qualitatively different spatial orientation of the acidic and basic functionalities for these two molecules (Figure 5). It is noteworthy that structure **2a-C** possesses the fully extended glutamate backbone conformation predicted to be required for optimal interaction at group 2 mGluRs.[36]

Whether the lack of group 1 mGluR agonist effects by **2a** is indeed due to this conformational bias or to other influences (e.g., negative electrostatic interaction between the ring nitrogen of **2a** at the glutamate recognition site of group 1 mGluRs), this compound is likely to be a useful pharmacological tool for studying the functions of mGluRs negatively coupled to AC. Further evaluation of the effects produced by **2a** in vivo may shed a greater light on the potential therapeutic utilities for compounds of this type.

Summary

The four isomers of 4-aminopyrrolidine-2,4-dicarboxylic acid have been prepared and evaluated for their ability to bind to and activate metabotropic glutamate receptors. From these studies, we have identified the 2*R*,4*R*-isomer **2a** as a potent and highly selective agonist for group 2 mGluRs. Compound **2a** is fully efficatious in displacing ACPD-sensitive [^{3}H]glutamate binding in rat brain homogenates ($IC_{50} = 6.49 \pm 1.21$ μM), indicative of group 2 mGluR affinity. Furthermore, **2a** (up to 100 μM) does not interact with the glutamate binding site on NMDA, AMPA, or kainate receptors. When examined for functional responses in the adult rat cortex, **2a** was found to potently activate mGluRs negatively coupled to adenylate cyclase ($EC_{50} = 14.51 \pm 5.54$ μM) without affecting (up to 100 μM) those mGluRs positively coupled to phospholipase C. In vivo, **2a** was found to augment AMPA currents in the rat spinal cord, an effect shared by the nonselective mGluR agonist (1*S*,3*R*)-ACPD (**1**). However, while **1** (400 nmol, ic) produces characteristic limbic seizures when injected directly into the mouse thalamus, **2a** (up to 1600 nmol, ic) does not. Furthermore, systemically administered **2a** was found to effectively antagonize ($ED_{50} = 271$ mg/kg ip) the limbic seizures produced by **1**. This represents the first example documenting the effects of a systemically-active mGluR agonist. Due to its potency, selectivity, and systemic activity, **2a** should prove to be a useful pharmacological tool for studying the functional consequences of group 2 mGluR activation in vivo.

Experimental Section

General Procedures. Melting points were obtained using a Thomas Hoover capilliary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained at 300.15 and 75.48 MHz, respectively, with TMS as an internal standard. Field desorption mass spectroscopy (FDMS) was performed using either a VG 70SE or Varian MAT 731 instrument. Optical rotations were obtained using the Perkin-Elmer 241 polarimeter and are reported at the sodium D line (589 nm), unless otherwise noted. Preparative HPLC was performed with the Waters Prep LC2000 apparatus using dual silica gel PREPPAK-500 cartridges. Solvent systems employed are given in parentheses for each example. Preparative centrifugal thin layer chromatography (PC-TLC) was performed on a Harrison Model 7924A chromatotron using Analtech silica gel GF rotors. The solvent system employed is indicated in the particular example. Cation-exchange chromtography was performed with Dowex 50X8-100 ion-exchange resin and anion-exchange chromatography with Bio-Rad AG 1-X8 anion-exchange resin (acetate form converted to hydroxide form).

(2*R*,4*R*)-Ethyl *N*^{1}-Benzyl-4-hydroxypyrrolidine-2-carboxylate (4). Concentrated H_2SO_4 (15 mL, 267 mmol) was added dropwise to a 0 °C ethanolic solution (700 mL) of *cis*-4-hydroxy-D-proline (**3**) (29.75 g, 227 mmol). Upon complete addition, the resulting reaction mixture was refluxed for 10 days with removal of H_2O via a Soxhlet condenser filled with 3 Å molecular sieves. The reaction mixture was concentrated under reduced pressure and the resulting oil reconstituted in anhydrous CH_2Cl_2 (400 mL). *N*,*N*-Diisopropylethylamine (77.55 g, 600 mmol) and benzyl bromide (46.6 g, 272 mmol) were consecutively added, and the resulting solution was

stirred at room temperature overnight; 1 N NaOH (200 mL) was added to the reaction mixture and the product extracted with Et_2O (3 × 300 mL). All of the organic phases were combined, washed with brine, dried over K_2CO_3, and concentrated under reduced pressure to afford the crude product, which was purified by HPLC (10% EtOAc/hexanes to 50% EtOAc/hexanes) affording **4** (47.20 g, 189 mmol) in 83% yield: ^{1}H NMR ($CDCl_3$) δ 1.20 (t, J = 7 Hz, 3H), 1.90−2.0 (m, 1H), 2.3−2.42 (m, 1H), 2.65 (dd, J = 3.7, 10 Hz, 1H), 3.02 (d, J = 10 Hz, 1H), 3.20 (br s, 1H), 3.35 (dd, J = 3.3, 10 Hz, 1H), 3.73 (d, J = 13.2 Hz, 1H), 3.90 (d, J = 13.2 Hz, 1H), 4.00−4.20 (m, 2H), 4.20−4.30 (m, 1H), 7.20−7.50 (m, 5H); FDMS M^+ = 249. Anal. ($C_{14}H_{19}NO_3$) C, H, N. $[\alpha]_D$ = +70.3° (c = 0.05, CH_2Cl_2) [lit. +76.2° (CH_3OH)[11]].

(2*R*)-Ethyl *N*^{1}-Benzyl-4-oxopyrrolidine-2-carboxylate (5). Oxalyl chloride (16.0 g, 126 mmol, 11 mL) was added dropwise to a solution of anhydrous CH_2Cl_2 (300 mL) and DMSO (13.12 g, 168 mmol) at −78 °C. The reaction mixture was allowed to equilibrate for 10 min, after which time a solution of **4** (20.90 g, 84 mmol) in CH_2Cl_2 (100 mL) was added dropwise at a rate to keep the reaction temperature below −60 °C. Upon complete addition the reaction mixture was allowed to stir at −78 °C for 2 h; then triethylamine (25.50 g, 252 mmol) was added dropwise. After complete addition, the reaction mixture was allowed to warm to room temperature. H_2O (50 mL) was added to the reaction mixture, the pH was adjusted to 10 with saturated aqueous $NaHCO_3$, and the product was extracted with Et_2O (3 × 500 mL). All organic phases were combined, washed with brine, dried over K_2CO_3, and concentrated *in vacuo* to yield crude product which was purified by HPLC (10% EtOAc/hexanes to 50% EtOAc/hexanes) affording **5** (20.44 g, 82.7 mmol) in 98% yield: ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7 Hz, 3H), 2.55 (dd, J = 7.7, 18.4 Hz, 1H), 2.70 (dd, J = 7.7, 18.4 Hz, 1H), 3.00 (d, J = 17.3 Hz, 1H), 3.35 (d, J = 17.3 Hz, 1H), 3.75 (d, J = 13.2 Hz, 1H), 3.85 (dd, J = 5.5, 7.7 Hz, 1H), 3.95 (d, J = 13.2 Hz, 1H), 4.22 (q, J = 7.7 Hz, 2H), 7.25−7.40 (m, 5H); FDMS M^+ = 247. Anal. ($C_{14}H_{17}NO_3$) C, H, N. $[\alpha]_D$ = +46.6° (c = 0.10, CH_2Cl_2) [lit. +48.8° (CH_3OH)[11]].

(2*R*,4*R*)-Diethyl *N*^{1}-Benzyl-4-aminopyrrolidine-2,4-dicarboxylate (7) and (2*R*,4*S*)-Diethyl *N*^{1}-Benzyl-4-aminopyrrolidine-2,4-dicarboxylate (8). KCN (13.36 g, 205 mmol) was added in one portion to a solution of **5** (20.30 g, 82 mmol) and ammonium carbamate (19.21 g, 246 mmol) in EtOH (500 mL) and H_2O (500 mL). The resulting reaction mixture was heated at 55 °C for 2 days. NaOH (90.0 g, 2.25 mol) was added, and the reaction mixture was warmed under reflux overnight. The reaction mixture was chilled to 0 °C, acidified to pH 1 with concentrated HCl (∼200 mL), and concentrated *in vacuo*. EtOH (500 mL) was added to the crude amino diacid mixture and then concentrated to dryness (5×) so as to remove residual H_2O. The resulting anhydrous amino diacid was then reconstituted in EtOH (1 L), cooled to 0 °C, and treated with $SOCl_2$ (39.02 g, 328 mmol). Upon complete addition the reaction mixture was refluxed for 3 days. The solids were filtered, and the filtrate was concentrated *in vacuo*. The crude product was partitioned between 3 N NaOH, NaCl, and EtOAc. The EtOAc was removed and the aqueous phase extracted with EtOAc (3 × 1 L). All the organic phases were combined, washed with brine, dried over K_2CO_3, and concentrated *in vacuo* to yield a dark red oil which was purified by HPLC (10% EtOAc/hexanes to 90% EtOAc/hexanes) affording **7** (12.14 g, 38 mmol) in 46% yield and **8** (3.05 g, 10 mmol) in 12% yield.

Compound **7**: ^{1}H NMR ($CDCl_3$) δ 1.20−1.30 (m, 6H), 2.04 (dd, J = 5.9, 13.6 Hz, 1H), 2.25 (br s, 2H), 2.78 (dd, J = 9.9, 13.6 Hz, 1H), 2.82 (d, J = 9.6 Hz, 1H), 2.92 (d, J = 9.6 Hz, 1H), 3.45 (dd, J = 5.9, 9.9 Hz, 1H), 3.58 (d, J = 12.9 Hz, 1H), 3.98 (d, J = 12.9 Hz, 1H), 4.10−4.22 (m, 4H), 7.22−7.38 (m, 5H); FDMS M^+ = 320. Anal. ($C_{17}H_{24}N_2O_4$) C, H, N. $[\alpha]_D$ = +60.6° (c = 0.10, CH_2Cl_2).

Compound **8**: ^{1}H NMR ($CDCl_3$) δ 1.20−1.30 (m, 6H), 1.75 (br s, 2H), 2.07 (dd, J = 7.4, 12.5 Hz, 1H), 2.47 (d, J = 9.9 Hz, 1H), 2.60 (dd, J = 8.1, 12.5 Hz, 1H), 3.51 (d, J = 9.9 Hz, 1H), 3.68 (d, J = 12.9 Hz, 1H), 3.76 (t, J = 7.9 Hz, 1H), 4.05 (d, J = 12.9 Hz, 1H), 4.10−4.20 (m, 4H), 7.20−7.40 (m, 5H); FDMS M^+ = 320. Anal. ($C_{17}H_{24}N_2O_4$·0.3EtOAc) C, H, N. $[\alpha]_D$ = +38.8° (c = 0.102, CH_2Cl_2).

(2*R*,4*R*)-Diethyl *N*^{1}-Benzyl-4-[(*tert*-butyloxycarbonyl)-amino]pyrrolidine-2,4-dicarboxylate (9). Di-*tert*-butyl dicarbonate (12.26 g, 56.2 mmol) was added in one portion to a solution of **7** (12.0 g, 37.5 mmol) in CH_2Cl_2 (400 mL), and the resulting reaction mixture was stirred at room temperature overnight; 0.5 N NaOH (100 mL) was added to the reaction mixture, and the product was extracted with Et_2O. All the organic phases were combined, washed with brine, dried over K_2CO_3, and concentrated *in vacuo* to yield the crude product, which was purified by HPLC (10% EtOAc/hexanes to 50% EtOAc/hexanes) affording **9** (15.92 g, 37.5 mmol) in 100% yield: ^{1}H NMR ($CDCl_3$) δ 1.20−1.30 (m, 6H), 1.40 (s, 9H), 2.26 (dd, J = 5.9, 13.6 Hz, 1H), 2.86 (dd, J = 2.7, 9.6 Hz, 1H), 2.91 (d, J = 9.6 Hz, 1H), 3.07 (d, J = 9.6 Hz, 1H), 3.48 (dd, J = 5.9, 9.6 Hz, 1H), 3.57 (d, J = 12.9 Hz, 1H), 4.03 (d, J = 13.2 Hz, 1H), 4.10−4.25 (m, 4H), 5.40 (br s, 1H), 7.25−7.40 (m, 5H); FDMS M^+ = 420. Anal. ($C_{22}H_{32}N_2O_6$) C, N; H: calcd, 6.93; found, 6.52. $[\alpha]_D$ = +30.7° (c = 0.01, CH_2Cl_2).

(2*R*,4*R*)-Diethyl 4-[(*tert*-Butyloxycarbonyl)amino]pyrrolidine-2,4-dicarboxylate (10). The amine **9** (15.80 g, 37.5 mmol) was added to an ethanolic suspension (100 mL) of 5% Pd/C (4.0 g) and exposed to H_2 (60 psi) for 4 h at room temperature. The reaction mixture was filtered through Celite and concentrated *in vacuo* to yield the crude product which was purified by HPLC (20% EtOAc/hexanes to 80% EtOAc/hexanes) affording **10** (10.48 g, 31.7 mmol) in 85% yield: mp = 58−60 °C; ^{1}H NMR ($CDCl_3$) δ 1.25−1.30 (m, 6H), 1.42 (s, 9H), 2.28−2.38 (m, 1H), 2.77 (dd, J = 9.2, 14.2 Hz, 1H), 3.25−3.40 (m, 2H), 3.95 (dd, J = 5.1, 9.2 Hz, 1H), 4.20 (q, J = 7.2 Hz, 2H), 4.22 (q, J = 7.2 Hz, 2H), 5.00 (br s, 1H); FDMS M^+ + 1 = 331. Anal. ($C_{15}H_{26}N_2O_6$) C, H, N. $[\alpha]_D$ = +4.94° (c = 0.053, CH_2Cl_2).

(2*R*,4*R*)-4-Aminopyrrolidine-2,4-dicarboxylate (2a). A solution of **10** (1.00 g, 3.00 mmol) in Et_2O (35 mL) was chilled to 0 °C, purged with anhydrous HCl gas, and allowed to warm to room temperature as it stirred for 1 h. The reaction mixture was concentrated to dryness and stirred in a 1:1 mixture of THF/1 N NaOH (20 mL total volume) at room temperature overnight. The reaction mixture was neutralized, concentrated to dryness, reconstituted in H_2O, and adjusted to pH 2 with 1 N HCl. The title compound was purified by cation-exchange chromatography (5% pyridine/H_2O) affording **2a** (0.40 g, 2.30 mmol) in 77% yield: mp > 250 °C; ^{1}H NMR (D_2O/KOD) δ 2.22 (dd, J = 7.7, 14.0 Hz, 1H), 2.82 (dd, J = 9.2, 14.0 Hz, 1H), 3.47 (d, J = 12.5 Hz, 1H), 3.69 (d, J = 12.5 Hz, 1H), 4.29 (dd, J = 7, 9 Hz, 1H); FDMS M^+ + 1 = 175. Anal. ($C_6H_{10}N_2O_4$) C, H, N. $[\alpha]_D$ = +26.6° (c = 0.10, 1 N HCl), $[\alpha]_D$ = +46.3° (c = 0.10, H_2O) [lit. −24.8° (H_2O)[11,37]].

(2*R*,4*S*)-Diethyl *N*^{1}-Benzyl-4-[(*tert*-butyloxycarbonyl)-amino]pyrrolidine-2,4-dicarboxylate (11). Di-*tert*-butyl dicarbonate (0.91 g, 4.17 mmol) was added in one portion to a solution of **8** (0.89 g, 2.78 mmol) in CH_2Cl_2 (35 mL), and the resulting reaction mixture was stirred at room temperature overnight; 0.5 N NaOH (100 mL) was added to the reaction mixture and the product extracted with Et_2O. All the organic phases were combined, washed with brine, dried over K_2CO_3, and concentrated *in vacuo* to yield the crude product which was purified by PC-TLC (10% EtOAc/hexanes to 20% EtOAc/hexanes) affording **11** (0.95 g, 2.26 mmol) in 81% yield: ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 8 Hz, 3H), 1.28 (t, J = 7 Hz, 3H), 1.42 (s, 9H), 2.40 (dd, J = 8.5, 13.6 Hz, 1H), 2.71 (d, J = 10.3 Hz, 1H), 2.83 (dd, J = 7.7, 13.6 Hz, 1H), 3.48 (d, J = 10.3 Hz, 1H), 3.71 (d, J = 12.9 Hz, 1H), 3.72 (d, J = 7.7 Hz, 1H), 4.03 (d, J = 13.2 Hz, 1H), 4.15−4.25 (m, 4H), 4.92 (br s, 1H), 7.25−7.40 (m, 5H); FDMS M^+ = 420. Anal. ($C_{22}H_{32}N_2O_6$) C, H, N. $[\alpha]_D$ = +41.04° (c = 0.10, CH_2Cl_2).

(2*R*,4*S*)-Diethyl *N*^{1}-(*tert*-Butyloxycarbonyl)-4-[(*tert*-butyloxycarbonyl)amino]pyrrolidine-2,4-dicarboxylate (12). The amine **11** (0.85 g, 2.02 mmol) and di-*tert*-butyl dicarbonate (0.88 g, 4.04 mmol) were consecutively added to an ethanolic suspension (30 mL) of 5% Pd/C (0.25 g) and exposed to H_2 (40 psi) for 4 h at room temperature. The reaction mixture was filtered through Celite and concentrated *in vacuo* to yield the crude product which was purified by PC-TLC (10% EtOAc/

hexanes to 50% EtOAc/hexanes) affording **12** (0.84 g, 1.95 mmol) in 97% yield: ^{1}H NMR ($CDCl_3$) δ 1.20−1.35 (m, 6H), 1.41 (s, 6H), 1.43 (s, 12H), 2.42−2.80 (m, 2H), 3.70−3.81 (m, 2H), 4.15−4.41 (m, 5H), 4.92 (m, 1H); FDMS $M^+ = 430$. Anal. ($C_{20}H_{34}N_2O_8$·0.1hexane) C, H, N. $[\alpha]_D = +42.46°$ (c = 0.08, CH_2Cl_2).

(2*R*,4*S*)-4-Aminopyrrolidine-2,4-dicarboxylate (2b). A solution of **12** (0.75 g, 1.74 mmol) in EtOH (35 mL) was chilled to 0 °C, purged with anhydrous HCl gas, and allowed to warm to room temperature as it stirred for 1 h. The reaction mixture was concentrated to dryness and stirred in a 1:1 mixture of THF/2 N NaOH (20 mL total volume) at room temperature overnight. The reaction mixture was concentrated to dryness, reconstituted in H_2O, and adjusted to pH 10 with 1 N HCl. The title compound was purified by anion-exchange chromatography (3 N acetic acid) affording **2b** (0.29 g, 1.67 mmol) in 96% yield: mp > 240 °C dec; ^{1}H NMR (D_2O/KOD) δ 2.12 (d, J = 8.83 Hz, 2H), 2.74 (d, J = 12.13 Hz, 1H), 3.42 (d, J = 11.76 Hz, 1H), 3.80 (t, J = 8.8 Hz, 1H); FDMS $M^+ = 175$. Anal. ($C_6H_{10}N_2O_4$) C, H, N. $[\alpha]_D = -15.22°$ (c = 0.062, 1 N HCl) [lit. +36.8° (H_2O)[11,37]].

(2*S*,4*R*)-Ethyl *N*^{1}-(Benzyloxycarbonyl)-4-hydroxypyrrolidine-2-carboxylate (14). A solution of *N*-CBZ-4-hydroxy-L-proline (**13**) (25.0 g, 94.23 mmol) and triethylamine (14.30 g, 141.35 mmol) in CH_3CN (250 mL) at room temperature was treated with a solution of iodoethane dropwise (29.39 g, 188.46 mmol) in CH_3CN (50 mL). Upon complete addition the reaction mixture was warmed to 55 °C and stirred overnight. The reaction mixture was concentrated *in vacuo* to a yellow oil and partitioned between 1 N HCl and Et_2O and the product extracted with Et_2O. All organics were combined, washed with 1 N NaOH (3×) and then brine, dried over K_2CO_3, and concentrated *in vacuo* to yield the crude product. Purification by HPLC (10% EtOAc/hexanes to 90% EtOAc/hexanes) afforded **14** (22.50 g, 76.7 mmol) in 81% yield: ^{1}H NMR ($CDCl_3$, doubling due to carbamate rotomers) δ 1.11 and 1.28 (t, J = 7 Hz, 3H), 1.74 (br s, 1H), 2.05−2.18 (m, 1H), 2.23−2.40 (m, 1H), 3.45−3.75 (m, 2H), 3.95−4.05 (m, 1H), 4.18−4.24 (m, 1H), 4.42−4.58 (m, 2H), 5.0−5.2 (m, 2H), 7.25−7.40 (m, 5H); FDMS $M^+ = 293$. Anal. ($C_{15}H_{19}NO_5$) C, H, N. $[\alpha]_D = -58.22°$ (c = 0.101, CH_2Cl_2).

(2*S*,4*R*)-Ethyl *N*^{1}-Benzyl-4-hydroxypyrrolidine-2-carboxylate (15). Benzyl carbamate **14** (22.40 g, 76.4 mmol) was added to an ethanolic suspension of 5% Pd/C (5.6 g in 125 mL) and exposed to H_2 (60 psi) at room temperature for 2.5 h. The reaction mixture was filtered through Celite and the filtrate treated with *N*,*N*-diisopropylethylamine (14.81 g, 114.6 mmol) and then benzyl bromide (15.68 g, 91.6 mmol) and stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* and partitioned between Et_2O and 0.5 N NaOH, and the product was extracted with Et_2O. All organics were combined, washed with brine, dried over K_2CO_3, and concentrated *in vacuo* to yield the crude product. Purification by HPLC (10% EtOAc/hexanes to 90% EtOAc/hexanes) afforded **15** (13.89 g, 55.7 mmol) in 73% yield: ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7 Hz, 3H), 1.90 (br s, 1H), 2.05−2.18 (m, 1H), 2.23−2.37 (m, 1H), 2.45−2.55 (m, 1H), 3.36 (dd, J = 10, 5.7 Hz, 1H), 3.64 (dd, J = 7.7, 7.7 Hz, 1H), 3.71 (d, 12.9 Hz, 1H), 3.97 (d, 13.2, 1H), 4.14 (q, J = 7.4 Hz, 2H), 4.42−4.5 (m, 1H), 7.25−7.40 (m, 5H); FDMS: $M^+ = 249$. Anal. ($C_{14}H_{19}NO_3$) C, H, N. $[\alpha]_D = -65.28°$ (c = 0.102, CH_2Cl_2) [lit. −65.0° (CH_3OH)[11]].

(2*S*)-Ethyl *N*^{1}-Benzyl-4-oxopyrrolidine-2-carboxylate (16). Oxalyl chloride (10.46 g, 82.4 mmol, 7.2 mL) was added dropwise to a solution of anhydrous CH_2Cl_2 (200 mL) and DMSO (8.59 g, 110 mmol) at −78 °C. The reaction mixture was allowed to equilibrate for 10 min, after which time a solution of **15** (13.70 g, 55.0 mmol) in CH_2Cl_2 (50 mL) was added dropwise at a rate to keep the reaction temperature below −60 °C. Upon complete addition the reaction mixture was allowed to stir at −78 °C for 2 h; then triethylamine (16.70 g, 165 mmol) was added dropwise. After complete addition, the reaction mixture was allowed to warm to room temperature. The reaction mixture was partitioned with 1 N NaOH and the product extracted with CH_2Cl_2. All organic phases were combined, washed with brine, dried over K_2CO_3, and concentrated *in vacuo* to yield crude product which was purified by HPLC (10% EtOAc/hexanes to 50% EtOAc/hexanes) affording **16** (12.73 g, 51.5 mmol) in 94% yield: ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7 Hz, 3H), 2.55 (dd, J = 7.7, 18.4 Hz, 1H), 2.70 (dd, J = 7.7, 18.4 Hz, 1H), 3.00 (d, J = 17.3 Hz, 1H), 3.35 (d, J = 17.3 Hz, 1H), 3.75 (d, J = 13.2 Hz, 1H), 3.85 (dd, J = 5.5, 7.7 Hz, 1H), 3.95 (d, J = 13.2 Hz, 1H), 4.22 (q, J = 7.7 Hz, 2H), 7.25−7.40 (m, 5H); FDMS $M^+ = 247$. Anal. ($C_{14}H_{17}NO_3$) C, H, N. $[\alpha]_D = -49.11°$ (c = 0.10, CH_2Cl_2) [lit. −49.3° (CH_3OH)[11]].

(2*S*,4*S*)-Diethyl 1-*N*^{1}-Benzyl-4-aminopyrrolidine-2,4-dicarboxylate (18) and (2*S*,4*R*)-Diethyl 1-*N*^{1}-Benzyl-4-aminopyrrolidine-2,4-dicarboxylate (19). NaCN (3.75 g, 76 mmol) was added in one portion to a solution of **16** (12.6 g, 51 mmol) and ammonium carbamate (11.9 g, 153 mmol) in EtOH (75 mL) and H_2O (75 mL). The resulting reaction mixture was heated at 55 °C overnight. NaOH (5.0 g, 125 mmol) was added, and the reaction mixture was warmed under reflux for 5 days. The reaction mixture was chilled to 0 °C, acidified to pH 1 with concentrated HCl, and concentrated to dryness *in vacuo*. EtOH (500 mL) was added to the crude amino diacid mixture and then concentrated to dryness (3×), so as to remove residual H_2O. The resulting anhydrous amino diacid was then reconstituted in EtOH (125 mL), cooled to 0 °C, and treated with $SOCl_2$ (19.9 g, 165 mmol). Upon complete addition the reaction mixture was refluxed for 3 days. The reaction mixture was concentrated to dryness *in vacuo* and partitioned between 2 N NaOH and EtOAc, and the products were extracted with EtOAc. All the organic phases were combined, washed with brine, dried over K_2CO_3, and concentrated *in vacuo* to yield a crude mixture of C4-diastereomers which were isolated by HPLC (10% EtOAc/hexanes to 100% EtOAc) affording **18** (4.44 g, 13.9 mmol) in 42% yield and **19** (0.88 g, 2.75 mmol) in 8.3% yield.

Compound **18**: ^{1}H NMR ($CDCl_3$) δ 1.20−1.30 (m, 6H), 1.80 (br s, 2H), 2.04 (dd, J = 5.9, 13.6 Hz, 1H), 2.78 (dd, J = 9.9, 13.6 Hz, 1H), 2.82 (d, J = 9.6 Hz, 1H), 2.92 (d, J = 9.6 Hz, 1H), 3.45 (dd, J = 5.9, 9.9 Hz, 1H), 3.58 (d, J = 12.9 Hz, 1H), 3.98 (d, J = 12.9 Hz, 1H), 4.10−4.22 (m, 4H), 7.22−7.38 (m, 5H); FDMS $M^+ = 320$. Anal. ($C_{17}H_{24}N_2O_4$) C, H, N. $[\alpha]_D = -60.40°$ (c = 0.10 CH_2Cl_2).

Compound **19**: ^{1}H NMR ($CDCl_3$) δ 1.20−1.30 (m, 6H), 1.75 (br s, 2H), 2.07 (dd, J = 7.4, 12.5 Hz, 1H), 2.47 (d, J = 9.9 Hz, 1H), 2.60 (dd, J = 8.1, 12.5 Hz, 1H), 3.51 (d, J = 9.9 Hz, 1H), 3.68 (d, J = 12.9 Hz, 1H), 3.76 (t, J = 7.9 Hz, 1H), 4.05 (d, J = 12.9 Hz, 1H), 4.10−4.20 (m, 4H), 7.20−7.40 (m, 5H); FDMS $M^+ = 320$. Anal. ($C_{17}H_{24}N_2O_4$) C, H, N. $[\alpha]_D = -38.61°$ (c = 0.10, CH_2Cl_2).

(2*S*,4*S*)-Diethyl *N*^{1}-Benzyl-4-[(*tert*-butyloxycarbonyl)-amino]pyrrolidine-2,4-dicarboxylate (20). Di-*tert*-butyl dicarbonate (6.04 g, 27.5 mmol) was added in one portion to a solution of **18** (3.95 g, 12.33 mmol) in CH_2Cl_2 (100 mL), and the resulting reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* to yield the crude product, which was purified by HPLC (10% EtOAc/hexanes to 50% EtOAc/hexanes) affording **20** (5.12 g, 12.2 mmol) in 99% yield: ^{1}H NMR ($CDCl_3$) δ 1.20−1.30 (m, 6H), 1.40 (s, 9H), 2.26 (dd, J = 5.9, 13.6 Hz, 1H), 2.86 (dd, J = 2.7, 9.6 Hz, 1H), 2.91 (d, J = 9.6 Hz, 1H), 3.07 (d, J = 9.6 Hz, 1H), 3.48 (dd, J = 5.9, 9.6 Hz, 1H), 3.57 (d, J = 12.9 Hz, 1H), 4.03 (d, J = 13.2 Hz, 1H), 4.10−4.25 (m, 4H), 5.40 (br s, 1H), 7.25−7.40 (m, 5H); FDMS $M^+ = 420$. Anal. ($C_{22}H_{32}N_2O_6$) C, H, N. $[\alpha]_D = -28.11°$ (c = 0.10, CH_2Cl_2).

(2*S*,4*S*)-Diethyl 4-[(*tert*-Butyloxycarbonyl)amino]pyrrolidine-2,4-dicarboxylate (21). The amine **20** (4.90 g, 11.65 mmol) was added to an ethanolic suspension (50 mL) of 5% Pd/C (1.3 g) and exposed to H_2 (60 psi) for 6 h at room temperature. The reaction mixture was filtered through Celite and concentrated *in vacuo* to yield the crude product which was purified by HPLC (10% EtOAc/hexanes to 90% EtOAc/hexanes) affording **21** (3.19 g, 9.7 mmol) in 83% yield: mp = 56−57 °C; ^{1}H NMR ($CDCl_3$) δ 1.25−1.30 (m, 6H), 1.42 (s, 9H), 2.28−2.38 (m, 1H), 2.77 (dd, J = 9.2, 14.2 Hz, 1H), 3.25−3.40 (m, 2H), 3.95 (dd, J = 5.1, 9.2 Hz, 1H), 4.20 (q, J = 7.2 Hz,

2H), 4.22 (q, J = 7.2 Hz, 2H), 5.00 (br s, 1H); FDMS $M^+ + 1$ = 331. Anal. ($C_{15}H_{26}N_2O_6$) C, H, N. $[\alpha]_D = -0.4°$ (c = 0.10, CH_2Cl_2).

(2*S*,4*S*)-4-Aminopyrrolidine-2,4-dicarboxylate (2c). A solution of **21** (1.94 g, 5.87 mmol) in EtOH (50 mL) was chilled to 0 °C, purged with anhydrous HCl gas, and allowed to warm to room temperature as it stirred overnight. The reaction mixture was concentrated to dryness and stirred in a 1:1 mixture of THF/1 N NaOH (20 mL total volume) at room temperature overnight. The pH of the reaction mixture was adjusted to pH = 10 with 1 N HCl, and the mixture was purified by anion-exchange chromatography (3 N acetic acid) affording **2c** (0.92 g, 5.28 mmol) in 90% yield: mp > 250 °C; ^{1}H NMR (D_2O/KOD) δ 1.84 (dd, J = 6.6, 13.6 Hz, 1H), 2.54 (dd, J = 9.5, 14.0 Hz, 1H), 3.07 (d, J = 12.2 Hz, 1H), 3.25 (d, J = 12.1 Hz, 1H), 3.88 (dd, J = 7.1, 9.6 Hz, 1H); FDMS $M^+ + 1$ = 175. Anal. ($C_6H_{10}N_2O_4$) C, H, N. $[\alpha]_D = -29.39°$ (c = 0.079, 1 N HCl) [lit. +25.5° (H_2O)[11,37]].

(2*S*,4*R*)-Diethyl *N*^{1}-Benzyl-4-[(*tert*-butyloxycarbonyl)-amino]pyrrolidine-2,4-dicarboxylate (22). Di-*tert*-butyl dicarbonate (1.61 g, 7.40 mmol) was added in one portion to a solution of **19** (0.79 g, 2.47 mmol) in CH_2Cl_2 (35 mL), and the resulting reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* to yield the crude product, which was purified by PC-TLC (10% EtOAc/hexanes) affording **22** (0.81 g, 1.9 mmol) in 78% yield: ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 8 Hz, 3H), 1.28 (t, J = 7 Hz, 3H), 1.42 (s, 9H), 2.40 (dd, J = 8.5, 13.6 Hz, 1H), 2.71 (d, J = 10.3 Hz, 1H), 2.83 (dd, J = 7.7, 13.6 Hz, 1H), 3.48 (d, J = 10.3 Hz, 1H), 3.71 (d, J = 12.9 Hz, 1H), 3.72 (d, J = 7.7 Hz, 1H), 4.03 (d, J = 13.2 Hz, 1H), 4.15−4.25 (m, 4H), 4.92 (br s, 1H), 7.25−7.40 (m, 5H); FDMS $M^+ + 1$ = 420. Anal. ($C_{22}H_{32}N_2O_6$) C, H, N. $[\alpha]_D = -33.33°$ (c = 0.084, CH_2Cl_2).

(2*S*,4*R*)-Diethyl *N*^{1}-(*tert*-Butyloxycarbonyl)-4-[(*tert*-butyloxycarbonyl)amino]pyrrolidine-2,4-dicarboxylate (23). The amine **22** (0.51 g, 1.2 mmol) and di-*tert*-butyl dicarbonate (0.52 g, 2.4 mmol) were consecutively added to an ethanolic suspension (20 mL) of 5% Pd/C (0.125 g) and exposed to H_2 (60 psi) for 4 h at room temperature. The reaction mixture was filtered through Celite and concentrated *in vacuo* to yield the crude product, which was purified by PC-TLC (10% EtOAc/hexanes to 50% EtOAc/hexanes) affording **23** (0.46 g, 1.07 mmol) in 89% yield: ^{1}H NMR ($CDCl_3$) δ 1.20−1.35 (m, 6H), 1.41 (s, 6H), 1.43 (s, 12H), 2.42−2.80 (m, 2H), 3.70−3.81 (m, 2H), 4.15−4.41 (m, 5H), 4.92 (m, 1H); FDMS M^+ = 430. Anal. ($C_{20}H_{34}N_2O_8$) C, H, N. $[\alpha]_D = -49.43°$ (c = 0.1, CH_2Cl_2).

(2*S*,4*R*)-4-Aminopyrrolidine-2,4-dicarboxylate (2d). A solution of **23** (0.41 g, 0.95 mmol) in EtOH (30 mL) was chilled to 0 °C, purged with anhydrous HCl gas, and allowed to stir at this temperature for 1 h. The reaction mixture was concentrated to dryness and then stirred in a 1:1 mixture of THF/1 N NaOH (20 mL total volume) at room temperature overnight. The pH of the reaction mixture was adjusted to pH = 11 with 1 N HCl, and the mixture was purified by anion-exchange chromatography (3 N acetic acid) affording **2d** (0.14 g, 0.80 mmol) in 85% yield: mp > 250 °C; FDMS $M^+ + 1$ = 175. Anal. ($C_6H_{10}N_2O_4 \cdot 0.5H_2O$) C, H, N. $[\alpha]_D = 20.8°$ (c = 0.05, 1 N HCl) [lit. −37.3° (H_2O)[11,37]].

(*S*)-Mosher's Amide of (2*S*,4*S*)-Diethyl 1-*N*^{1}-Benzyl-4-aminopyrrolidine-2,4-dicarboxylate (24). A solution of **18** (0.16 g, 0.5 mmol) in pyridine (5 mL) was treated at room temperature with (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.13 g, 0.05 mmol). The reaction was allowed to proceed for 1 h; then the mixture was partitioned between saturated aqueous $NaHCO_3$ and Et_2O. The organic phase was separated, dried over Na_2SO_4, and concentrated under reduced pressure. The title compound was purified by PC-TLC (10% EtOAc in hexane to 50% EtOAc in hexane) affording **24** (0.22 g, 0.42 mmol) in 84% yield: ^{1}H NMR ($CDCl_3$) δ 1.19 (t, J = 7.1 Hz, 3H), 2.33 (dd, J = 14.0, 4.4 Hz, 1H), 2.85 (dd, J = 14.0, 9.9 Hz, 1H), 2.98 (d, J = 10.0 Hz, 1H), 3.17 (d, J = 10.0 Hz, 1H), 3.45 (s, 3H), 3.50 (dd, J = 9.9, 4.5 Hz, 1H), 3.60 (d, J = 13.1 Hz, 1H), 4.00−4.22 (m, 5H), 7.22−7.55 (m, 10H), 7.82 (s, 1H); FDMS M^+ = 536. Anal. ($C_{27}H_{31}F_3N_2O_6$) C, H, N.

(*R*)-Mosher's Amide of (2*S*,4*S*)-Diethyl 1-*N*^{1}-Benzyl-4-aminopyrrolidine-2,4-dicarboxylate (25). The title compound was prepared by the method described for the preparation of **24** employing **18** (0.16 g, 0.05 mmol) and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.13 g, 0.05 mmol). After chromatography, **25** (0.21 g, 0.40 mmol, 80% yield) was obtained: mp = 120−121 °C; ^{1}H NMR ($CDCl_3$) δ 1.19 (t, J = 7.2 Hz, 3H), 1.21 (t, J = 7.1 Hz, 3H), 2.21 (dd, J = 14.0, 4.4 Hz, 1H), 2.74 (dd, J = 14, 9.9 Hz, 1H), 3.04 (d, J = 10.0 Hz, 1H), 3.24 (d, J = 10.0 Hz, 1H), 3.47 (s, 3H), 3.50 (dd, J = 10.0, 4.5 Hz, 1H), 3.63 (d, J = 13.1 Hz, 1H), 4.00−4.23 (m, 5H), 7.23−7.61 (m, 10H), 7.83 (s, 1H); FDMS M^+ = 536. Anal. ($C_{27}H_{31}F_3N_2O_6$) C, H, N.

(2*S*,4*R*)-Diethyl 1-*N*^{1}-Benzyl-4-[(α,α,α-trifluoromethyl)-amino]pyrrolidine-2,4-dicarboxylate (26). Compound **22** (0.04 g, 0.1 mmol) was stirred in TFA (3 mL) at room temperature overnight. The volatiles were removed under reduced pressure, affording **19**, which was dissolved in pyridine (5 mL) and treated at 5 °C with trifluoroacetic anhydride (0.5 mL). After stirring at room temperature for 2 h, the reaction mixture was concentrated to dryness, and the title compound was purified by PC-TLC (1 mm SiO_2, 10% EtOAc in hexane to 25% EtOAc in hexane) affording **26** (0.012 g, 0.03 mmol) in 30% yield: ^{1}H NMR ($CDCl_3$) d 1.26 (t, J = 7.2 Hz, 3H), 1.28 (t, J = 7.2 Hz, 3H), 2.54 (dd, J = 13.9, 8.1 Hz, 1H), 2.86 (d, J = 10.4 Hz, 1H), 2.87 (dd, J = 13.9, 7.7 Hz, 1H), 3.43 (d, J = 10.3 Hz, 1H), 3.70 (d, J = 13.2 Hz, 1H), 3.84 (t, J = 7.7 Hz, 1H), 4.05 (d, J = 13.2 Hz, 1H), 4.15−4.29 (m, 4H), 6.86 (s, 1H), 7.26−7.33 (m, 5H); FDMS M^+ = 416. Anal. ($C_{19}H_{23}F_3N_2O_5$) C, H, N.

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(37) The origin of the discrepancies in the sign of rotations exhibited by analogs **2a**−**d** in our work with those previously disclosed[11] is unclear. We have repeated the literature procedure for the preparation of **2a** (chromatographic separation of the hydantoin isomers from the mixture **6**, HCl hydrolysis of the major 2*R*,4*R*-diastereomer, and PdOH-catalyzed hydrogenolysis) obtaining **2a** which is identical in every respect (including optical rotation: $\alpha_D = 44.7°$ ($c = 0.1$, H_2O)) with the material prepared by the method described in Scheme 1.

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