

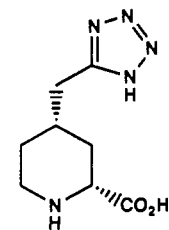
NMDA Antagonist Activity of (±)-(2SR,4RS)-4-(1H-Tetrazol-5-ylmethyl)piperidine-2-carboxylic Acid Resides with the (-)-2R,4S-Isomer

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The tetrazole-substituted amino acid (±)-(2SR,4RS)-4-(1H-tetrazol-5-ylmethyl)piperidine-2-carboxylic acid (LY233053, (±)-1) was resolved into its constituent enantiomers by treatment of a key intermediate in the synthesis of the racemic amino acid, ethyl (±)-cis-4-(cyanomethyl)-N-allylpiperidine-2-carboxylate, with either 2S,3S- or 2R,3R-di-p-toluoyltartaric acid. These resolved amines were then converted as for the racemate to the amino acids (-)-1 and (+)-1. The activity of this potent and selective NMDA antagonist was found to reside with the (-)-isomer of 1 (LY235723). X-ray crystallographic analysis of the 2S,3S-di-p-toluoyltartaric acid salt of ethyl cis-4-(cyanomethyl)-N-allylpiperidine-2-carboxylate showed that the resolved amine, and thus (-)-1, possessed the 2R,4S absolute stereochemistry. Affinity for the NMDA receptor was determined using the specific radioligand [³H]-(2SR,4RS)-4-(phosphonomethyl)piperidine-2-carboxylic acid ([³H]CGS 19755; IC₅₀ = 67 ± 6 nM), and selective NMDA antagonist activity was determined using a cortical slice preparation (IC₅₀ versus 40 μM NMDA = 1.9 ± 0.24 μM). This compound also demonstrated potent NMDA antagonist activity in vivo following systemic administration through its ability to block NMDA-induced convulsions in neonatal rats, NMDA-induced lethality in mice, and NMDA-induced striatal neuronal degeneration in rats.

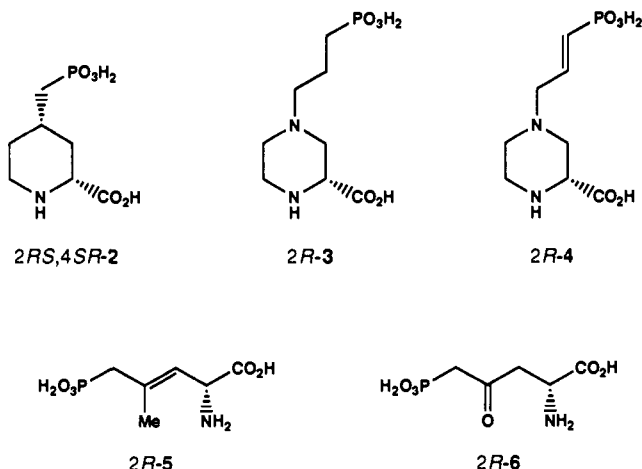
Competitive antagonists of the NMDA subtype of excitatory amino acid (EAA) receptors¹ appear to be potentially useful for treating a wide variety of acute and chronic neurodegenerative diseases, where overstimulation of NMDA receptors (i.e., excitotoxicity) may play a role in the pathophysiology of these diseases. For example, recent evidence indicates that NMDA antagonists may find use in the treatment of cerebral ischemia² and head³ and spinal cord trauma,⁴ as anticonvulsants,⁵ and in the treatment of Alzheimer's disease⁶ and Parkinson's disease.⁷ We recently described the synthesis⁸ and pharmacological characterization⁹ of (±)-(2SR,4RS)-4-(1H-tetrazol-5-ylmethyl)piperidine-2-carboxylic acid (LY233053, (±)-1), a tetrazole-substituted NMDA antagonist with a more rapid onset and a shorter duration of action⁹ when compared to the corresponding phosphonate, (±)-(2SR,4RS)-4-(phosphonomethyl)piperidine-2-carboxylic acid (CGS 19755, (±)-2).¹⁰ We believe that this profile may bode well for the use of such a compound to treat acute excitotoxic disorders, such as cerebral ischemia.



(±)-1 (LY233053)
(-)-1 (LY235723)

In cases where NMDA antagonists have been resolved, such as 2,¹⁰ (2SR)-4-(3-phosphonoprop-1-yl)piperazine-2-carboxylic acid (3, CPP),¹¹ (2SR)-4-(3-phosphonoprop-2-en-1-yl)piperazine-2-carboxylic acid (4, CPP-ene),¹¹ (2SR)-2-amino-4-methyl-5-phosphonopentanoic acid (5,

Chart I



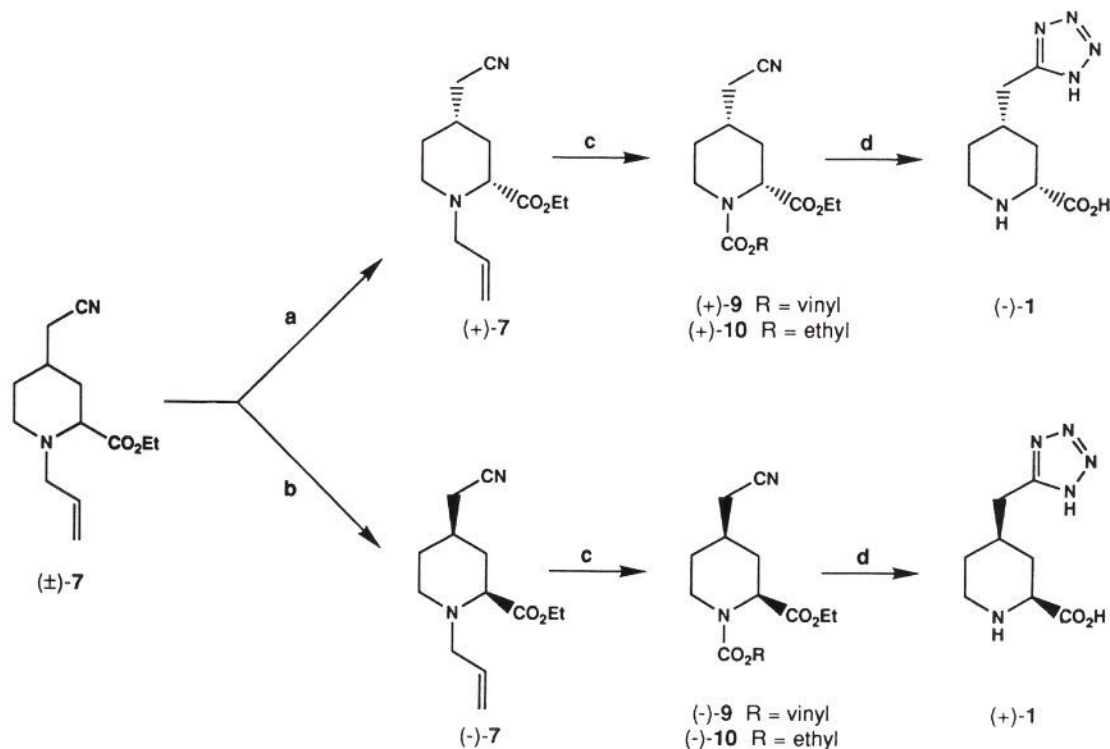
CGP 37849),¹² and (2SR)-2-amino-4-oxo-5-phosphonopentanoic acid (6, MDL 100,453)¹³ (Chart I), one enan-

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Scheme I^a

^a(a) (2*S*,3*S*)-Di-*p*-toluoyltartaric acid, EtOAc, H₂O; aqueous NaHCO₃, CH₂Cl₂. (b) (2*R*,3*R*)-Di-*p*-toluoyltartaric acid, EtOAc, H₂O; aqueous NaHCO₃, CH₂Cl₂. (c) Vinyl chloroformate, ClCH₂CH₂Cl, reflux; 60 psi H₂, EtOH, room temperature. (d) *n*-Bu₃SnN₃, 80 °C; 6 N HCl, reflux; Dowex 50-X8, 10% aqueous pyridine.

tiomer retains the NMDA antagonist activity, while the other is weakly active or inactive. Except for CGS 19755, where the absolute stereochemistry has not been reported for the active isomer, all of these amino acids possess the *R* absolute stereochemistry at the α -amino acid center. For the development of 1 as a therapeutic agent, we believed it was essential to resolve this compound in order to determine if it demonstrated a similar profile of stereoselectivity. In this paper we describe the resolution of 1,

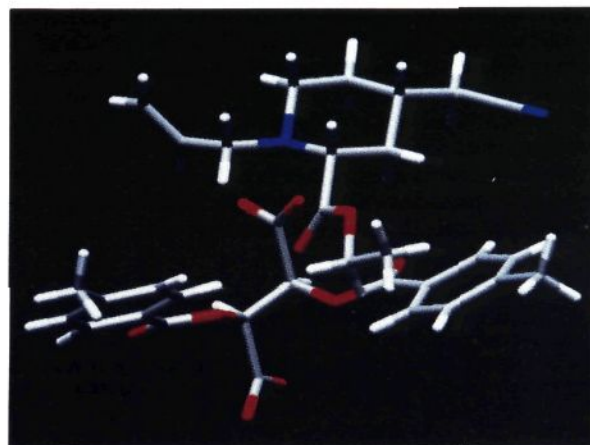


Figure 1. Plot of the X-ray crystal structure of (+)-8. The four waters of hydration that crystallized with the salt have been omitted for clarity.

demonstrate that the NMDA receptor antagonism resides with the isomer (-)-1 (LY235723), and show that this isomer has the *R* absolute stereochemistry at the α -amino acid center.

Chemistry

The synthetic approach to the isomers of 1 is shown in Scheme I. The *cis*-*N*-allylamine (\pm)-7, a key intermediate in the preparation of racemic 1,⁸ was resolved into (+)- and (-)-7 by formation of the salts (+)- and (-)-8 with either (2*S*,3*S*)- or (2*R*,3*R*)-di-*p*-toluoyltartaric acid, respectively, in ethyl acetate containing 1 equiv of water. After one recrystallization of each salt from ethyl acetate, the amines were liberated and shown by ¹H NMR to be $\geq 97\%$ one

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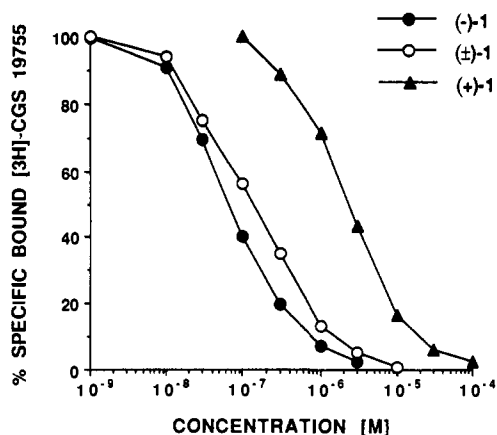


Figure 2. Displacement of [³H]CGS 19755 binding by (-)-1, (±)-1 and (+)-1. Affinities for the NMDA receptor, as determined by IC₅₀ values (nM), are as follows: (-)-1, 67 ± 6; (±)-1, 107 ± 7; and (+)-1, 2393 ± 368.

enantiomer using the chiral shift reagent (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol in benzene-*d*₆.¹⁴ While the allyl group of (+)- or (-)-7 could be removed with ethyl chloroformate and 1,8-bis(dimethylamino)naphthalene (proton sponge) in refluxing dichloroethane to directly afford the ethyl carbamates (+)- or (-)-10, respectively, this reaction was sluggish, taking 2–3 days and large excesses of reagents to go to completion. Instead, we found it more expeditious to first remove the allyl group with vinyl chloroformate and proton sponge in refluxing dichloroethane¹⁵ and then hydrogenate the vinyl carbamates (+)- or (-)-9 to the ethyl carbamates (+)- or (-)-10, respectively. Conversion of (+)- or (-)-10 to the tetrazole with azido tri-*n*-butylstannane neat at 80 °C was followed by exhaustive hydrolysis with 6 N HCl and then purification by cation exchange chromatography on Dowex 50-X-8 (100–200 mesh, elution with 10% pyridine in water) to afford (-)- and (+)-1, respectively.

A crystal of (+)-8 (a 1:1 salt) suitable for X-ray diffraction studies was grown from 2-propanol. The bond distances and angles within the ions are all within the normal ranges for such bonds. (A full listing of interatomic distances and angles is presented in the supplementary material.) Since the absolute stereochemistry is known for the tartaric acid derivative that was used [(2*S*,3*S*)-di-*p*-toluoyltartaric acid], we determined the absolute stereochemistry for (+)-7 to be 2*R*,4*S*. Figure 1 shows a plot of (+)-8, clearly indicating the absolute stereochemistry of the C-2 and C-4 carbons. We know from the synthesis of racemic 1 that no epimerization occurs in the deallylation or deprotection steps. Therefore, the stereochemistry of the amino acids derived from (+)- or (-)-7 should be (-)-(2*R*,4*S*)- or (+)-(2*S*,4*R*)-1, respectively, as shown in Scheme I.

- (14) When (±)-2 was evaluated by 300-MHz ¹H NMR with 1 equiv of the chiral shift reagent (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol in benzene-*d*₆ at room temperature, two absorptions were well resolved. We observed two multiplets at δ 5.81 and 5.59 and two doublets of doublets at δ 3.16 or 2.97. To determine the optical purity of our resolved amines, a small amount of (+)- or (-)-2 was evaluated in the same manner. For (+)-2, we observed only the absorptions at δ 5.59 and δ 2.97; for (-)-2, we observed only the absorptions at δ 5.81 and δ 3.16. On the basis of integration of the ¹H NMR spectra, we determined these amines to be ≥97% one enantiomer.
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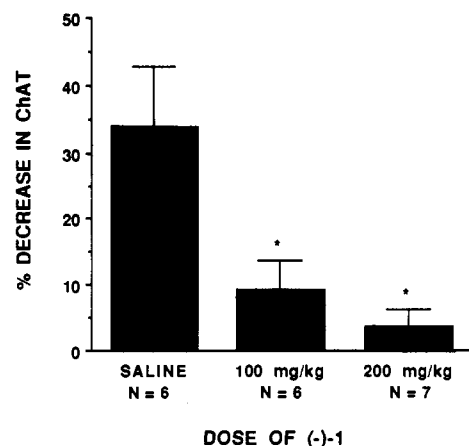


Figure 3. Dose-dependent protection afforded by (-)-1 against the neurotoxic effects of 300 nmol of NMDA injected into the striatum of rats, as measured by losses in choline acetyltransferase (ChAT) activity relative to saline-injected control animals.

Pharmacology: In Vitro

These resolved amino acids were examined for their ability to displace [³H]CGS 19755 binding¹⁶ as a measure of their affinity for the NMDA receptor (Figure 2). The isomer (-)-1 displaced [³H]CGS 19755 binding with an IC₅₀ of 67 ± 6 nM, while the IC₅₀ for the isomer (+)-1 was 2393 ± 368 nM. The racemate displaced binding with an IC₅₀ of 107 ± 7 nM, so as one might expect, the affinity of the active isomer was nearly twice that of the racemate. As was observed for the racemate, no significant displacement of radioligand binding by (-)-1 to AMPA or kainic acid receptors was observed (IC₅₀'s > 10000 nM).

NMDA antagonist activity for these resolved amino acids was determined by evaluating their ability to inhibit depolarizations due to NMDA, quisqualic acid (QUIS) and kainic acid (KA) in a cortical slice preparation.¹⁷ The isomers (-)- and (+)-1 showed IC₅₀'s versus NMDA-induced depolarizations of 1.9 ± 0.24 and 39.1 ± 3.0 μM (compare to an IC₅₀ of 4.2 ± 0.4 μM for (±)-1), with no effects on depolarizations due to QUIS or KA at concentrations up to 100 μM.

Pharmacology: In Vivo

The isomers of 1 were evaluated both in a model of NMDA-induced convulsions in neonatal rats¹⁸ and for their ability to block NMDA-induced lethality in mice.¹⁹ In these models, (±)-1 demonstrated potent in vivo NMDA antagonist activity.⁹ The minimum effective dose (MED; this is the dose where > 50% of the animals tested survived) for (-)-1 to antagonize NMDA-induced convulsions in neonatal rats was 20 mg/kg (ip) while (+)-1 was inactive at doses up to 200 mg/kg (ip). (-)-1 was also effective at blocking NMDA-induced lethality in mice, with an MED of 2.5 mg/kg (ip), whereas (+)-1 showed no protective

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effects up to doses as high as 160 mg/kg (ip). (Compare to (\pm)-1, with MED's of 20 mg/kg (ip) versus NMDA-induced convulsions in neonatal rats and 5 mg/kg (ip) versus NMDA-induced lethality in mice).

When NMDA (300 nmol) is stereotactically injected into the striatum of rats, there is neuronal degeneration mediated through NMDA receptor activation.²⁰ The loss of cholinergic neurons that occurs following this insult is quantified 7 days after treatment with NMDA by measuring levels of choline acetyl transferase (ChAT).²⁰ One useful aspect of this assay is the ability to evaluate the potential of a novel NMDA antagonist, such as (-)-1, to attenuate an excitotoxic CNS injury that occurs following overstimulation of NMDA receptors. We found that single doses of (-)-1 (as for (\pm)-1⁹) given ip 30 min prior to treatment with NMDA provided a dose-dependent protection from striatal injury in rats, with the highest dose tested providing complete neuroprotection (Figure 3). In saline pretreated (control) rats, NMDA induced a $34 \pm 9\%$ ($n = 6$) decrease in ChAT activity. When animals were treated with doses of 100 or 200 mg/kg of (-)-1 we observed decreases in ChAT activity of $9 \pm 4\%$ or $4 \pm 3\%$, respectively ($p < 0.05$ when compared to the saline control group). The 2-fold difference in potency for (-)-1 relative to (\pm)-1 that was observed in the protection of mice from NMDA-induced lethality was also observed for neuroprotection in rats.

Conclusions

We have successfully resolved the potent and selective NMDA antagonist (\pm)-1 and found that like other competitive NMDA antagonists, the activity of this amino acid resides within a single enantiomer, (-)-(2*R*,4*S*)-1. We were able to demonstrate that this amino acid, like 3-6, has the 2*R* absolute stereochemistry at the α -amino acid carbon. This compound [(-)-1] has high affinity for the NMDA receptor and demonstrated selective NMDA antagonism in a cortical slice preparation. This compound also showed potent NMDA antagonist activity in vivo following systemic administration as evidenced by its ability to block NMDA-induced convulsions in neonatal rats and NMDA-induced lethality in mice. In addition, a single dose of this amino acid administered intraperitoneally was effective in dose-dependently blocking NMDA-induced striatal neuronal degeneration in rats. This combination of potent and selective NMDA antagonism in vitro and in vivo, combined with the rapid onset and short duration of action that is observed for tetrazole-substituted NMDA antagonists,⁹ bodes well for the therapeutic use of such a compound to treat acute neurotrauma such as cerebral ischemia.

Experimental Section

General. All experiments were run under a positive pressure of dry nitrogen. Dichloroethane was dried over 4-Å molecular sieves prior to use. All other solvents and reagents were used as obtained. ¹H and ¹³C NMR spectra were obtained on a GE QE-300 spectrometer at 300.15 and 75.48 MHz, respectively, with tetramethylsilane as an internal standard. Where indicated, a small amount of 40% aqueous KOD was added to aid solution of NMR samples run in D₂O.

Ethyl (+)-(2*S*,4*R*)-4-(Cyanomethyl)-*N*-allylpiperidine-2-carboxylate (2'*S*,3'*S*)-Di-*p*-toluoyltartaric Salt [(+)-8] and (-)-(2*R*,4*S*)-4-(Cyanomethyl)-*N*-allylpiperidine-2-

carboxylate (2'*R*,3'*R*)-Di-*p*-toluoyltartaric Salt [(-)-8]. A mixture of 10.6 g (44.7 mmol) of racemic amine 7, 17.3 g (44.7 mmol) of (2*S*,3*S*)-di-*p*-toluoyltartaric acid, and 0.81 mL (0.81 g, 44.4 mmol) of water were dissolved in ethyl acetate with heating and then filtered; most of the ethyl acetate removed to give a final volume of ≈ 50 mL. The mixture was cooled to room temperature, and the crystals that formed were collected and washed with ethyl acetate, ether, and pentane, and dried in vacuo to afford 13.5 g (48%). This material was recrystallized from ethyl acetate to afford 9.7 g (35%) of the desired (+)-8: mp 142–142.2 °C; $[\alpha]_D = +106.8^\circ$ ($c = 1$, methanol). Anal. (C₃₃H₃₈N₂O₁₀) C, H, N. The mother liquors from both recrystallizations were combined and concentrated in vacuo. The residue was partitioned between 100 mL of dichloromethane and 200 mL of saturated aqueous sodium bicarbonate. The organic layer was separated, the aqueous layer was extracted with 100 mL of dichloromethane and 100 mL of ether, and then the combined organic extracts were dried (MgSO₄), filtered, and concentrated in vacuo to afford 7.0 g (65%) of 2. This material was dissolved in ethyl acetate with 12.0 g (29.6 mmol) of (2*R*,3*R*)-di-*p*-toluoyltartaric acid monohydrate and crystallized as above to afford 9.7 g (35%) of the desired (-)-8: mp 144–144.3 °C; $[\alpha]_D = -105.6^\circ$ ($c = 1$, methanol). Anal. (C₃₃H₃₈N₂O₁₀) C, H, N. A small portion of (+)-8 and (-)-8 were free based as above, and ¹H NMR in benzene-*d*₆ with 1 equiv of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol showed each compound to be $\geq 97\%$ one enantiomer.¹⁴ ¹H NMR (DMSO-*d*₆) δ 7.84 (d, $J = 8.0$ Hz, 4 H), 7.34 (d, $J = 8.0$ Hz, 4 H), 5.75 (s, 2 H), overlapping 5.75 (m, 1 H), 5.11 (d, $J = 17.1$ Hz, 1 H), 5.10 (d, $J = 10.4$ Hz, 1 H), 4.07 (q, $J = 7.1$ Hz, 2 H), 3.14 (dd, $J = 13.7, 4.9$ Hz, 1 H), 2.93 (m, 2 H), 2.77 (dd, $J = 13.7, 8.1$ Hz, 1 H), 2.47 (d, $J = 6.3$ Hz, 2 H), 2.35 (s, 6 H), 2.03 (t, $J = 11.5$ Hz, 1 H), 1.80 (m, 1 H), 1.65 (m, 2 H), 1.30 (m, 3 H), 1.14 (t, $J = 7.1$ Hz, 3 H), 0.80 (t, $J = 6.7$ Hz, 1 H); ¹³C NMR (DMSO-*d*₆) δ 171.8, 167.3, 164.6, 144.4, 133.6, 129.4, 125.9, 119.0, 118.7, 71.4, 64.6, 60.4, 57.7, 50.4, 34.2, 31.4, 29.7, 22.3, 21.2, 13.9.

Ethyl (+)-(2*R*,4*S*)- and (-)-(2*S*,4*R*)-(Cyanomethyl)-*N*-allylpiperidine-2-carboxylate [(+)- and (-)-7]. To a flask was added 9.6 g (15.4 mmol) of the (+)-8, 100 mL of dichloromethane, and 100 mL of saturated aqueous sodium bicarbonate, the mixture was stirred for 10 min at room temperature, the organic layer then was separated, and the aqueous layer was extracted three times with 100 mL each of dichloromethane and 1 time with 75 mL of ether. The combined organic extracts were dried (Na₂SO₄), filtered, and concentrated in vacuo to afford 3.5 g (96%, 34% from (\pm)-7) of (+)-7: $[\alpha]_D = +70.3^\circ$ ($c = 1$, dichloromethane). Anal. (C₁₃H₂₀N₂O₂) C, H, N. Similarly, 9.6 g (15.4 mmol) of (-)-8 gave 3.5 g (96%, 34% from (\pm)-7) of (-)-7: $[\alpha]_D = -67.5^\circ$ ($c = 1$, dichloromethane). Anal. (C₁₃H₂₀N₂O₂) C, H, N.

Ethyl (+)-(2*R*,4*S*)- and (-)-(2*S*,4*R*)-4-(Cyanomethyl)-*N*-[(vinylloxy)carbonyl]piperidine-2-carboxylate [(+)- and (-)-9]. As for (\pm)-7,⁸ 2.3 g (9.8 mmol) of (+)-7 with 1.7 mL (2.1 g, 19.6 mmol) of vinyl chloroformate and 4.2 g (19.6 mmol) of 1,8-bis(dimethylamino)naphthalene in 40 mL of dichloroethane gave 2.0 g (80%) of (+)-9: $[\alpha]_D = +22.6^\circ$ ($c = 1$, dichloromethane). Anal. (C₁₃H₁₈N₂O₄) C, H, N. And, as for (\pm)-7,⁸ 3.3 g (13.8 mmol) of (-)-7 with 2.4 mL (3.0 g, 27.7 mmol) of vinyl chloroformate and 5.9 g (27.7 mmol) of 1,8-bis(dimethylamino)naphthalene in 50 mL of dichloroethane gave 3.0 g (81%) of (-)-9: $[\alpha]_D = -21.6^\circ$ ($c = 1$, dichloromethane). Anal. (C₁₃H₁₈N₂O₄) C, H, N.

Ethyl (+)-(2*R*,4*S*)- and (-)-(2*S*,4*R*)-4-(Cyanomethyl)-*N*-(ethoxycarbonyl)piperidine-2-carboxylate [(+)- and (-)-10]. (+)-9 (1.3 g, 4.9 mmol) was hydrogenated with 0.2 g of 5% Pd/C in 95 mL of ethanol at room temperature and 60 psi for 1 h. The mixture was filtered through Celite and concentrated in vacuo. The residue was chromatographed on 70 g of silica gel eluting with 40% ethyl acetate/hexane to afford 1.0 g (77%) of (+)-10: $[\alpha]_D = +11.2^\circ$ ($c = 1$, dichloromethane). Anal. (C₁₃H₂₀N₂O₄) C, H, N. Similarly, 4.8 g (18.2 mmol) of (-)-10 was hydrogenated with 0.8 g of 5% Pd/C in 95 mL of ethanol at room temperature and 60 psi for 1 h. Workup and chromatography (200 g of silica gel) as for (+)-3 gave 3.8 g (78%) of (-)-10: $[\alpha]_D = -13.6^\circ$ ($c = 1$, dichloromethane). Anal. (C₁₃H₂₀N₂O₄) C, H, N.

(-)-(2*R*,4*S*)-4-(1*H*-Tetrazol-5-ylmethyl)piperidine-2-carboxylic Acid and (+)-(2*S*,4*R*)-4-(1*H*-Tetrazol-5-ylmethyl)piperidine-2-carboxylic Acid [(-)- and (+)-1]. (+)-4

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(6.0 g, 20.9 mmol) and azidotri-*n*-butylstannane (13.9 g, 41.8 mmol) was heated at 80 °C for 3 days and then heated overnight at 100 °C with 125 mL of 6 N aqueous hydrochloric acid. The mixture was cooled and extracted four times with 200 mL each of ether, and then the aqueous layer was concentrated in vacuo. Ion exchange chromatography (Dowex 50-X8 [100-200 mesh], 4 × 18-cm column, loaded in water, eluted with 10% pyridine/water) afforded a solid (after concentration of the product containing fractions⁸) which was washed with water, acetone, and ether and dried in vacuo at 80 °C to afford 3.5 g (74%) of (-)-1 monohydrate: $[\alpha]_D = -20.4^\circ$ ($c = 1, 1 \text{ N HCl}$), mp 199-201 °C. Anal. ($\text{C}_8\text{H}_{13}\text{N}_5\text{O}_2\cdot\text{H}_2\text{O}$) C, H, N. Similarly, 2.6 g (9.5 mmol) of (-)-4 and 6.3 g (19.0 mmol) of azidotri-*n*-butylstannane gave 1.2 g (55%) of (+)-1 monohydrate: $[\alpha]_D = +19.9^\circ$ ($c = 1, 1 \text{ N HCl}$), mp 200-202 °C. Anal. ($\text{C}_8\text{H}_{13}\text{N}_5\text{O}_2\cdot\text{H}_2\text{O}$) C, H, N.

X-ray Experimental Data for (+)-8. (+)-8 ($\text{C}_{33}\text{H}_{38}\text{N}_2\text{O}_{10}$) crystallized in the orthorhombic space group $P2_12_12_1$, with a unit cell having the dimensions $a = 13.932$ (4) Å, $b = 29.450$ (9) Å, $c = 9.862$ (5) Å and a calculated density of 1.022 g cm^{-3} . The unit cell contained four molecules of water. A total of 3150 unique reflections with 2θ less than 116.0° were measured on an automated four-circle diffractometer (Siemens R3m/V) using monochromatic copper radiation ($\text{Cu K}\alpha$, $\lambda = 1.54178$ Å). The structure was solved using direct methods using the Siemens SHELXTL PLUS (VMS) system²¹ and was refined by the full-matrix least-squares method with anisotropic temperature factors for all

(21) Sheldrick, G. M. Shelxtl, Rev 4, Instrument Corporation, 1983.

atoms except hydrogen. All hydrogen atoms were included with isotropic temperature factors at calculated positions. The final R factor was 0.0997 for 2677 observed reflections. Maximum peak height in final difference Fourier map is $0.71 \text{ e}\text{\AA}^{-3}$. Figure 1 in the supplementary material gives the structure of (+)-8 showing the numbering of the non-hydrogen atoms. Tables 1-5 in the supplementary material give the atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, and H-atom coordinates.

[³H]CGS 19755 Binding. The method for this assay has been published. See ref 16.

Cortical Wedge Assay. The method for this assay has been published. See ref 17.

NMDA-Induced Convulsions in Neonatal Rats. The method for this assay has been published. See ref 18.

NMDA-Induced Lethality in Mice. The method for this assay has been published. See ref 19.

NMDA-Induced Striatal Neurotoxicity. The method for this assay has been published. See ref 20.

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Supplementary Material Available: X-ray crystallographic data (structure, atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients and H-atom coordinates) for (+)-8 (6 pages). Ordering information is given on any current masthead page.

1,4-Dihydropyridines as Antagonists of Platelet Activating Factor. 1. Synthesis and Structure-Activity Relationships of 2-(4-Heterocyclyl)phenyl Derivatives

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A novel class of 2-(4-heterocyclylphenyl)-1,4-dihydropyridines (2-38) possessing antagonist activity against platelet activating factor (PAF) was prepared by the Hantzsch synthesis from a variety of ethyl 4'-heterocyclic-substituted benzoylacetates, aryl or heteroaryl aldehydes, and substituted 3-aminocrotonamides or 3-aminocrotonate esters. Structure-activity relationships were evaluated where PAF antagonist activity was measured in vitro by determining the concentration of compound (IC_{50}) required to inhibit the PAF-induced aggregation of rabbit washed platelets, and in vivo by determining the oral dose (ED_{50}) which protected mice from a lethal injection of PAF. The nature of the substituent at the dihydropyridine 2-position was found to be important for both in vitro and in vivo activity, whereas there was greater flexibility for structural variation at the 4- and 5-positions. The most potent compound was 4-(2-chlorophenyl)-1,4-dihydro-3-(ethoxycarbonyl)-6-methyl-2-[4-(2-methylimidazo[4,5-*c*]pyrid-1-yl)phenyl]-5-[*N*-(2-pyridyl)carbamoyl]pyridine (17, UK-74,505), $\text{IC}_{50} = 4.3 \text{ nM}$, $\text{ED}_{50} = 0.26 \text{ mg/kg po}$, which was found to be approximately 33 times more potent in vitro (rabbit platelet aggregation) and about 8 times more potent in vivo (murine lethality) than WEB2086. Compound 17 also exhibited a long duration of action in the dog (inhibition of PAF-induced whole blood aggregation *ex vivo* was maintained for >24 h following a single oral dose of 75 $\mu\text{g/kg}$) and was highly selective as a PAF antagonist, showing only weak affinity ($\text{IC}_{50} = 6600 \text{ nM}$) for the [³H]nitrendipine binding site. As a result of its high oral potency, selectivity, and duration of action, UK-74,505 has been selected for clinical evaluation.

Platelet activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is an ether phospholipid which exhibits, in addition to potent platelet aggregating activity, a wide spectrum of biological activities elicited directly or via the release of other mediators.¹ Research studies have implicated PAF as a potential mediator in a number of pathophysiological conditions.² Particular interest has focussed on the role of PAF in bronchial hyperreactivity and the delayed bronchospasm following allergen chal-

lenge, and it is thought that PAF antagonists will be especially useful for controlling asthma,³ although results from definitive clinical trials have yet to be reported. Other disease states in which PAF may play a role include inflammatory conditions such as rhinitis, psoriasis and

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- (3) Chung, K. F.; Barnes, P. J. PAF Antagonists: Their Potential Therapeutic Role in Asthma. *Drugs* 1988, 35, 93-103.

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