# Expedited Articles

## Resolution, Absolute Stereochemistry, and Pharmacology of the *S*-(+)- and *R*-(−)-Isomers of the Apparent Partial AMPA Receptor Agonist (*R,S*)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic Acid [(*R,S*)-APPA]

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*Received December 21, 1993*®

(*R,S*)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid ((*R,S*)-APPA) is the only partial agonist at the (*R,S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) subtype of excitatory amino acid receptors so far described. In light of the pharmacological interest in partial agonists, we have now accomplished the resolution of (*R,S*)-APPA. (*S*)-(+)-APPA (**5**) and (*R*)-(−)-APPA (**6**) were obtained in high enantiomeric purity using (*R*)-(+)- and (*S*)-(−)-1-phenylethylamine, respectively, as resolving agents. The absolute stereochemistry of **6** was established by X-ray analysis of **6**·HCl·0.25$H_2O$. Compounds **5** and **6** were tested electropharmacologically using the rat cortical wedge preparation and in receptor-binding assays using [$^3$H]-AMPA, [$^3$H]kainic acid, and the *N*-methyl-D-aspartic acid (NMDA) receptor ligands [$^3$H]CPP, [$^3$H]MK-801, and [$^3$H]glycine. Whereas **6** did not significantly affect the binding of any of these ligands ($IC_{50}$ > 100 μM), compound **5** revealed affinity for only the [$^3$H]AMPA-binding site ($IC_{50}$ = 6 μM). In electropharmacological tests, **5** showed full AMPA receptor agonism ($EC_{50}$ = 230 μM). This effect of **5** was insensitive to the NMDA antagonist CPP but was inhibited competitively by the non-NMDA antagonist NBQX ($pK_i$ = 6.30). Compound **6**, on the other hand, turned out to be a non-NMDA receptor antagonist, inhibiting competitively depolarizations induced by AMPA ($pK_i$ = 3.54), kainic acid ($pK_i$ = 3.07), and **5** ($pK_i$ = 3.57).

### Introduction

(*S*)-Glutamic acid ((*S*)-Glu, **1**) is the major excitatory amino acid (EAA) neurotransmitter in the central nervous system and operates through multiple ionotropic and G-protein-coupled (metabotropic) receptors.[1-9] The former class of EAA receptors comprises three different heterogeneous families of homo- and/or heteropentameric receptors, named *N*-methyl-D-aspartic acid (NMDA), (*R,S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), and kainic acid receptors.

The NMDA receptor complex, where NMDA is a selective agonist and [(*R,S*)-3-(2-carboxypiperazin-4-yl)-propyl]phosphonic acid (CPP) is a selective competitive antagonist, also contains binding sites for the cotransmitter, glycine, and the noncompetitive antagonist 5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine (MK-801).[10] AMPA-preferring receptors are stereospecifically activated by (*S*)-AMPA (**2**)[11-15] and (*S*)-2-amino-3-(3-hydroxy-4-bromoisoxazol-5-yl)propionic acid ((*S*)-Br-HIBO, **3**)[11,16] (Figure 1). (*S*)-2-Amino-3-[3-(carboxymethoxy)-5-methylisoxazol-4-yl]propionic acid ((*S*)-AMOA, **4**) is a competitive non-NMDA receptor antagonist with some selectivity for the AMPA receptor.[17,18] Like the very potent non-NMDA antagonist 6-nitro-7-sulfamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX),[19,20] **4** also reduces depolarizations induced by kainic acid.

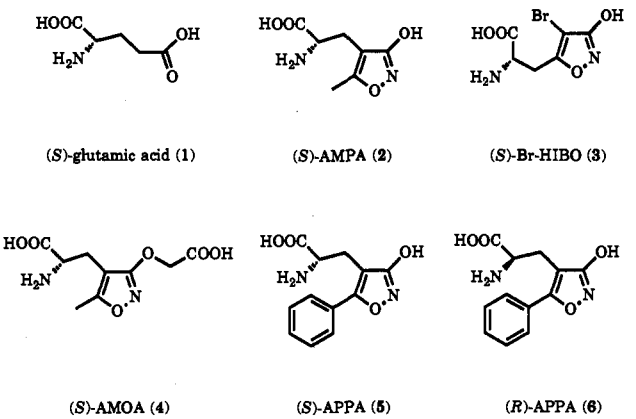


**Figure 1.** Structures of (*S*)-glutamic acid (**1**), (*S*)-AMPA (**2**), (*S*)-Br-HIBO (**3**), (*S*)-AMOA (**4**), (*S*)-(+)-APPA (**5**), and (*R*)-(−)-APPA (**6**).

It is generally agreed that (*S*)-Glu neurotoxicity plays a role in brain damages following hypoxia, hypoglycemia, and status epilepticus.[2,6,7,21] Furthermore, there is growing evidence that imbalance(s) in the (*S*)-Glu neurotransmitter system is a contributing factor in the pathogenesis of certain neurodegenerative diseases such as Huntington's chorea and Alzheimer's disease.[2,6,7,21-23] In Alzheimer's disease, (*S*)-Glu hyperactivity is thought to be involved in

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® Abstract published in *Advance ACS Abstracts*, March 1, 1994.

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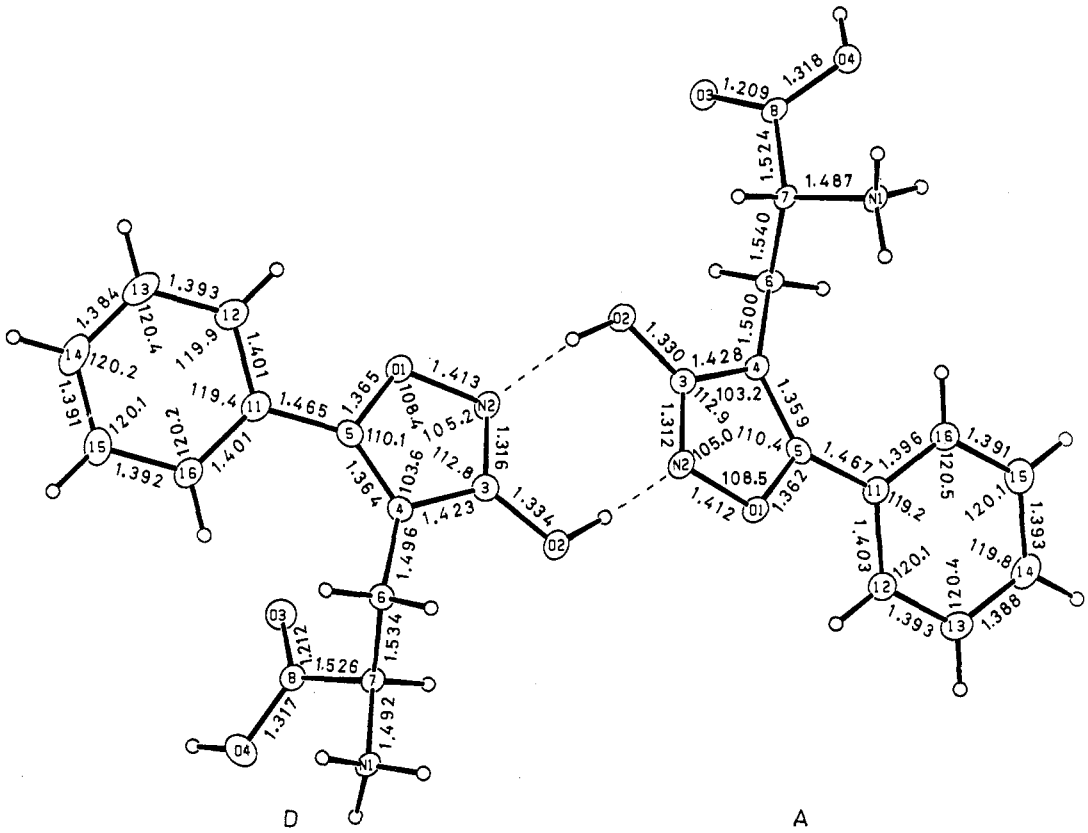


**Figure 2.** Perspective drawings[46,52] with labeling of two of the four crystallographically independent (*R*)-APPA molecules (molecule A and D), illustrating the two different conformational isomers (A/B and C/D) of the (*R*)-APPA molecule and the structural dimers (A/D and B/C) found in the crystalline state. Intermolecular hydrogen bonds are indicated by dashed lines. The bond lengths and angles shown are mean values of molecules A/B and C/D, respectively. esd's: 0.003–0.004 Å and 0.2°–0.3°. Thermal ellipsoids enclose 50% probability; H-atoms are drawn as circles of arbitrary radius.

the irreversible damage of neurons, whereas hypoactivity at (*S*)-Glu-operated synapses may contribute to the clinical manifestations (impaired learning and memory).[22-24] Furthermore, hypofunction of (*S*)-Glu synaptic mechanisms may play a role in schizophrenia.[25-27]

The nature of these apparent abnormalities of (*S*)-Glu neurotransmission mechanisms in Alzheimer's disease or schizophrenia and the relative importance of different types of EAA receptors remain to be established. In principle, however, partial EAA receptor agonists with a certain partial activity have therapeutic interest in these diseases.[23,24,28] (*R,S*)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid ((*R,S*)-APPA) is the only partial AMPA receptor agonist so far described.[29] In light of this pharmacological interest, we have now accomplished the resolution of (*R,S*)-APPA and the determination of the absolute stereochemistry of (*S*)-(+)-APPA (**5**) and (*R*)-(−)-APPA (**6**) (Figure 1). These results as well as the in vitro pharmacology of **5** and **6** are described in this paper.

## Results

**Resolution of (*R,S*)-APPA.** (*R,S*)-APPA was synthesized as described previously.[29] The chemical resolution of zwitterionic (*R,S*)-APPA to give **5** and **6** was achieved via diastereomeric salt formations using (*R*)-(+)- and (*S*)-(−)-1-phenylethylamine, respectively. Compounds **5** and **6** were obtained with an enantiomeric excess (ee) of 99.0% and 99.8%, respectively, as determined by chiral HPLC using a chiral crown ether column [Crownpak CR(−)]. In agreement with earlier observations[30] using this type of column for the separation of α-amino acids of known absolute stereochemistry, (*S*)-APPA (**5**) eluted before the corresponding *R*-form (**6**).

**X-ray Crystallographic Analysis of (*R*)-APPA·HCl·0.25$H_2O$ (6·HCl·0.25$H_2O$).** The asymmetric unit of the crystal structure of 6·HCl·0.25$H_2O$ consists of one molecule of water and the hydrochlorides of four independent molecules of **6**, identified by suffixes A, B, C, and D. Perspective drawings of two of the four molecules of **6** with atom-labeling schemes are shown in Figure 2. Drawings of the molecular packing are depicted in Figures 3 and 4.

This crystallographic analysis affords the assignment of the absolute configuration of (−)-APPA (**6**) as the *R*-configuration. The corresponding bond lengths (Figure 2) in the four independent molecules of **6** in the asymmetric unit are the same within 3σ except for the C6–C7 bond (A, 1.537(3) Å; B, 1.543(3) Å; C, 1.529(3) Å; and D, 1.538(3) Å]. Larger differences are found, however, in corresponding bond angles (Table 1). The selected torsion angles in Table 1 and Figure 2 exemplify the differences with regard to the conformation of the α-aminopropionic acid moieties; the molecules A and B adopt $g^{(+)}/t$ (C3–C4–C6–C7/C4–C6–C7–C8) conformations, while the molecules C and D adopt $g^{(-)}/g^{(-)}$ conformations. The mutual orientations of the isoxazole and benzene rings of molecules A and B are different from those of molecules C and D (see torsion angles in Table 1).

The crystal structure is stabilized by a complex system of H-bonds which involves two-center and three-center H-bond interactions (Table 2, Figures 2, 3, and 4).[31] The H-bonding systems for the four symmetry-independent molecules of **6** show pronounced similarities. All of the

Table 1. Selected Bond and Torsion Angles (degrees) for (*R*)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic Acid·HCl·0.25$H_2O$[a]

| molecules | A | B | C | D |
|---|---|---|---|---|
| | Bond Angles | | | |
| N2–C3–O2 | 123.2(2) | 123.4(2) | 123.8(2) | 123.3(2) |
| C4–C3–O2 | 123.8(2) | 123.8(2) | 123.6(2) | 123.8(2) |
| C3–C4–C6 | 122.9(2) | 122.3(2) | 123.4(2) | 124.1(2) |
| C5–C4–C6 | 133.4(2) | 134.5(2) | 133.1(2) | 132.3(2) |
| C4–C5–C11 | 135.7(2) | 135.6(2) | 134.7(2) | 135.5(2) |
| O1–C5–C11 | 114.2(2) | 113.8(2) | 115.2(2) | 114.3(2) |
| C4–C6–C7 | 113.5(2) | 113.1(2) | 112.0(2) | 113.8(2) |
| C6–C7–N1 | 112.1(2) | 112.1(2) | 110.0(2) | 108.9(2) |
| C6–C7–C8 | 109.8(2) | 110.7(2) | 114.2(2) | 114.2(2) |
| N1–C7–C8 | 110.6(2) | 109.5(2) | 108.3(2) | 109.4(2) |
| C7–C8–O3 | 120.1(2) | 121.3(2) | 123.3(2) | 121.3(2) |
| C7–C8–O4 | 113.6(2) | 112.6(2) | 111.4(2) | 112.4(2) |
| O3–C8–O4 | 126.3(2) | 126.0(2) | 125.2(2) | 126.3(2) |
| C5–C11–C12 | 119.4(2) | 119.6(2) | 118.6(2) | 119.8(2) |
| C5–C11–C16 | 121.6(2) | 120.9(2) | 121.8(2) | 121.0(2) |
| | Torsion Angles | | | |
| C5–O1–N2–C3 | 0.4(3) | −0.6(3) | −0.5(3) | −0.4(3) |
| O1–N2–C3–C4 | −1.2(3) | 0.8(3) | 0.6(3) | −0.1(3) |
| N2–C3–C4–C5 | 1.6(3) | −0.7(3) | −0.5(3) | 0.5(3) |
| C3–C4–C5–O1 | −1.3(3) | 0.3(3) | 0.2(3) | −0.7(3) |
| C4–C5–O1–N2 | 0.6(3) | 0.2(3) | 0.2(3) | 0.7(3) |
| O1–N2–C3–O2 | 178.0(2) | −179.4(2) | −178.7(2) | 179.4(2) |
| N2–C3–O2–H2 | −11(3) | 9(2) | 5(3) | −5(2) |
| C3–C4–C6–C7 | 62.7(3) | 71.9(3) | −65.4(3) | −73.2(3) |
| C4–C6–C7–N1 | 70.6(3) | 84.4(2) | 173.4(2) | 171.7(2) |
| C4–C6–C7–C8 | −166.1(2) | −153.0(2) | −64.6(3) | −65.7(3) |
| N1–C7–C8–O4 | 4.2(3) | 30.0(3) | −37.8(3) | −3.0(3) |
| C7–C8–O4–H4 | 180(3) | 176(3) | −178.(2) | 176(3) |
| C4–C5–C11–C16 | 23.8(4) | 24.4(4) | −23.3(4) | −21.2(4) |

[a] Estimated standard deviations are given in parentheses.

Table 2. Hydrogen-Bond Geometries (Å, degrees) for (*R*)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic Acid·HCl·0.25$H_2O$[a,b]

| X–H···Y | X–H | H···Y | X···Y | <XHY |
|---|---|---|---|---|
| $O2_A$–$H2_A$···$N2_D$[ii] | 0.90(4) | 1.81(4) | 2.704(3) | 169(3) |
| $O4_A$–$H4_A$···$Cl_A$[i] | 0.78(4) | 2.21(4) | 2.982(2) | 173(4) |
| $N1_A$–$H1_{1A}$···$Cl_C$[i] | 0.89(4) | 2.57(3) | 3.127(2) | 121(2) |
| $N1_A$–$H1_{1A}$···$Cl_D$[iii] | 0.89(4) | 2.60(3) | 3.291(2) | 135(3) |
| $N1_A$–$H1_{2A}$···$Cl_B$[i] | 0.87(3) | 2.42(3) | 3.266(2) | 165(3) |
| $N1_A$–$H1_{3A}$···$O3_B$[i] | 0.88(3) | 2.01(3) | 2.863(3) | 162(3) |
| $O2_B$–$H2_B$···$N2_C$[iv] | 1.02(4) | 1.72(4) | 2.706(3) | 162(3) |
| $O4_B$–$H4_B$···$Cl_B$[i] | 0.82(4) | 2.34(4) | 3.130(2) | 161(3) |
| $N1_B$–$H1_{1B}$···$OW$[v] | 0.92(3) | 1.94(3) | 2.790(3) | 152(3) |
| $N1_B$–$H1_{1B}$···$Cl_A$[vi] | 0.92(3) | 2.75(3) | 3.124(2) | 106(3) |
| $N1_B$–$H1_{2B}$···$Cl_D$[i] | 0.92(3) | 2.34(3) | 3.244(2) | 168(3) |
| $N1_B$–$H1_{3B}$···$O3_A$[vi] | 0.92(3) | 1.95(3) | 2.854(3) | 168(3) |
| $O2_C$–$H2_C$···$N2_B$[vii] | 0.90(4) | 1.81(4) | 2.697(3) | 169(3) |
| $O4_C$–$H4_C$···$Cl_B$[v] | 0.88(4) | 2.16(4) | 3.036(2) | 174(3) |
| $N1_C$–$H1_{1C}$···$Cl_C$[i] | 0.92(3) | 2.26(3) | 3.108(2) | 153(3) |
| $N1_C$–$H1_{1C}$···$Cl_D$[iii] | 0.92(3) | 2.80(3) | 3.088(2) | 100(2) |
| $N1_C$–$H1_{2C}$···$O3_D$[iii] | 0.85(4) | 2.09(4) | 2.916(3) | 165(3) |
| $N1_C$–$H1_{3C}$···$Cl_A$[v] | 0.91(3) | 2.38(3) | 3.177(2) | 147(3) |
| $N1_C$–$H1_{3C}$···$Cl_D$[iii] | 0.91(3) | 2.84(3) | 3.088(2) | 97(2) |
| $O2_D$–$H2_D$···$N2_A$[viii] | 0.97(4) | 1.76(4) | 2.718(3) | 172(3) |
| $O4_D$–$H4_D$···$Cl_D$[i] | 0.87(4) | 2.06(4) | 2.924(2) | 174(4) |
| $N1_D$–$H1_{1D}$···$Cl_A$[ix] | 0.83(4) | 2.39(4) | 3.198(2) | 164(3) |
| $N1_D$–$H1_{2D}$···$O3_C$[x] | 0.88(4) | 2.05(3) | 2.822(3) | 147(3) |
| $N1_D$–$H1_{3D}$···$Cl_B$[i] | 0.89(4) | 2.41(4) | 3.277(2) | 163(3) |
| $OW$–$HW_1$···$Cl_B$[i] | 0.84(4) | 2.42(4) | 3.224(2) | 161(4) |
| $OW$–$HW_2$···$Cl_C$[x] | 0.86(4) | 2.23(4) | 3.094(2) | 179(4) |

[a] Symmetry code: (i) $x, y, z$; (ii) $1-x, y+1/2, 1-z$; (iii) $x, y, 1+z$; (iv) $-x, y+1/2, 1-z$; (v) $x-1, y, z$; (vi) $x-1, y, z-1$; (vii) $-x, y-1/2, 1-z$; (viii) $1-x, y-1/2, 1-z$; (ix) $x, y, z-1$; and (x) $1+x, y, z$. [b] Estimated standard deviations are given in parentheses.

five hydrogen atoms bonded to oxygen and nitrogen atoms per molecule of 6·HCl are utilized in the formation of hydrogen bonds. By virtue of intermolecular NH···O=C hydrogen bonds, two hydrogen-bonded chains are formed consisting of alternating A and B molecules and alternating

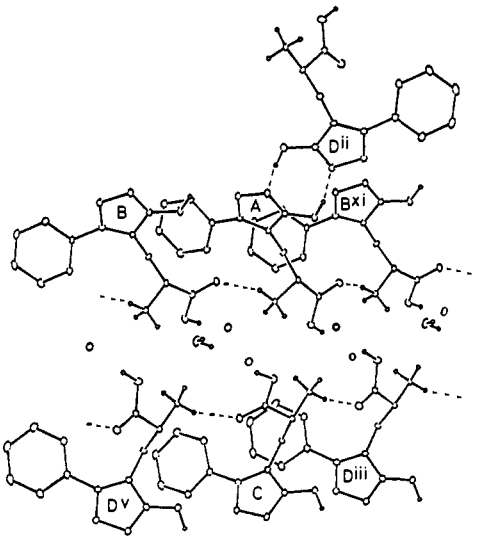


**Figure 3.** Packing diagram of (*R*)-APPA·HCl·0.25$H_2O$ seen in the direction of the *a* axis with horizontal *c* and vertical *b* axes. Only intermolecular H-bonds (dashed lines) between (*R*)-APPA molecules are shown. Thermal ellipsoids enclose 20% probability; H-atoms are drawn as circles of arbitrary radius. The C-bonded H-atoms are omitted for clarity.[46,52] Symmetry code: (ii) $1-x, y+1/2, 1-z$; (iii) $x, y, 1+z$; (v) $x-1, x, y$; and (xi) $x+1, y, z$.

Table 3. Receptor-Binding Data for Excitatory Amino Acid Receptor Ligands

| | inhibition ($IC_{50}$, μM) of the binding of | | | | |
|---|---|---|---|---|---|
| compound | [$^3$H]-AMPA | [$^3$H]kainic acid | [$^3$H]-CPP | [$^3$H]-MK-801[a] | [$^3$H]-glycine |
| (*R,S*)-AMPA | 0.04 ± 0.01 | >100 | >100 | >100 | >100 |
| (*R,S*)-APPA | 35 ± 10 | >100 | >100 | >100 | >100 |
| (*S*)-APPA (**5**) | 6 ± 2 | >100 | >100 | >100 | >100 |
| (*R*)-APPA (**6**) | >100 | >100 | >100 | >100 | >100 |
| kainic acid | 6 ± 2 | 0.020 ± 0.003 | >100 | >100 | >100 |

[a] Fully stimulated membranes.

C and D molecules. They are separated from each other by an infinite sheet of water molecules and chloride anions parallel to the *ac* plane, thus forming a sandwichlike structure. All of the α-amino acid moities of the molecules of **6** are directed toward this sheet. The packing of the "sandwich units" related by the 2-fold screw axis is stabilized by H-bonds involving the 3-hydroxyisoxazole moieties. Molecule A is linked to molecule $D^{ii}$ (Figure 2) and molecule B to molecule $C^{iv}$ in a dimeric manner resembling the common H-bonding motif found for carboxylic acids.[32]

**In Vitro Pharmacology.** Receptor-binding studies were performed using [$^3$H]AMPA,[33] [$^3$H]kainic acid,[34] and the NMDA receptor complex ligands [$^3$H]CPP,[35] [$^3$H]MK-801,[36] and [$^3$H]glycine.[37] In the [$^3$H]AMPA-binding assay, an $IC_{50}$ value of 6 ± 2 μM for **5** was determined. (*R,S*)-APPA inhibited the binding of [$^3$H]AMPA with an $IC_{50}$ value of 35 ± 10 μM, in agreement with earlier observations,[29] whereas **6** was inactive ($IC_{50}$ > 100 μM). In all other binding assays, **5** as well as **6** was inactive ($IC_{50}$ > 100 μM) (Table 3).

The pharmacological effects of **5** and **6** were studied by use of the rat cortical wedge preparation.[38,39] Compound **5** quite effectively depolarized this brain tissue preparation and was shown to be a full AMPA receptor agonist with an $EC_{50}$ value of 230 μM ($pEC_{50}$ = 3.64 ± 0.05) (Figure 5a). This effect of **5** was insensitive to the competitive NMDA antagonist CPP (5 μM) (data not shown) but was markedly

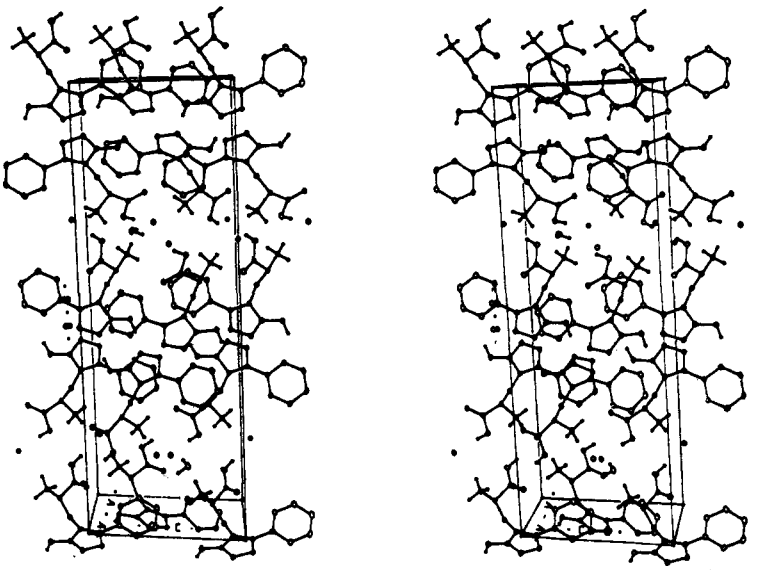

**Figure 4.** Stereoscopic view of the molecular packing of the unit cell of (*R*)-APPA·HCl·0.25$H_2O$ seen in the direction of the *a* axis with horizontal *c* and vertical *b* axes. Thermal ellipsoids enclose 20% probability; H-atoms are drawn as circles of arbitrary radius. The C-bonded H-atoms are omitted for clarity.[46,52]

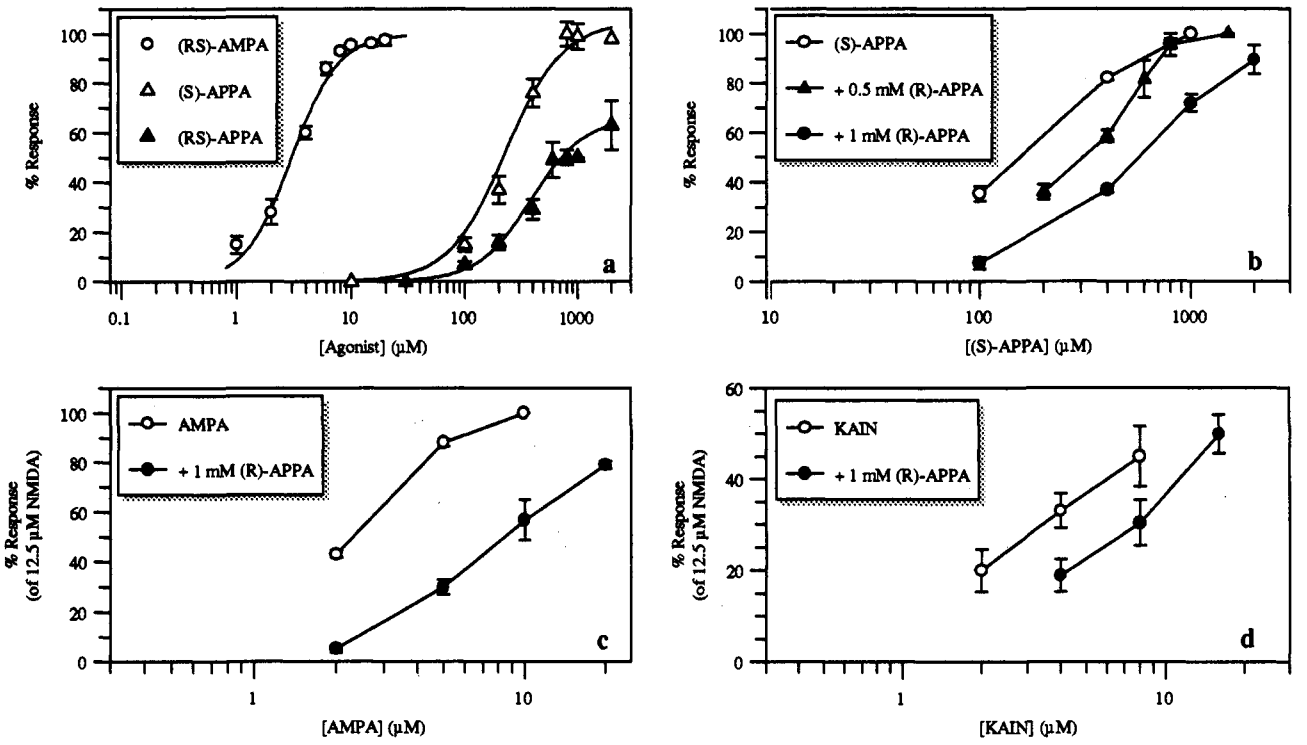


**Figure 5.** Pharmacological profile of (*R*)-APPA (**6**) and (*S*)-APPA (**5**) in the rat cortical wedge preparation: (a) concentration–response curves for (*R,S*)-AMPA, (*S*)-APPA, and (*R,S*)-APPA, (b) effect of 0.5 and 1 mM (*R*)-APPA on (*S*)-APPA responses, (c) effect of 1 mM (*R*)-APPA on (*R,S*)-AMPA responses, and (d) effect of 1 mM (*R*)-APPA on kainic acid (KAIN) responses. Each data point is the mean value ± SEM of at least three individual experiments.

reduced by the non-NMDA antagonist NBQX. This antagonist (0.5 μM) shifts, in a parallel fashion, the dose–response curve for **5**, giving a p$K_i$ value of 6.30 ± 0.02. This value is close to the p$A_2$ values determined for the inhibition by NBQX of AMPA-induced depolarizations (6.56 ± 0.14;[40] 7.03 ± 0.01[41]) but significantly different from that measured for inhibition of kainic-acid-induced depolarizations (5.42 ± 0.14) by NBQX[40] (Table 4).

In the rat cortical wedge preparation, **6** did not show detectable agonist effects at concentrations up to 2 mM. Compound **6** did, however, reduce AMPA and kainic acid responses, whereas NMDA responses were not significantly affected. A concentration of 1 mM **6** parallel-shifts the dose–response curve of AMPA 4.5 ± 0.3 times (p$K_i$ = 3.54 ± 0.03; Figure 5c) and the dose–response curve for kainic acid 2.1 ± 0.1 times (p$K_i$ = 3.07 ± 0.08; Figure 5d).

**Table 4.** Electrophysiological Data (rat cortical wedge preparation) for Excitatory Amino Acid Receptor Ligands

| | AMPA | | agonist sensitivity (p$K_i$) to antagonists | |
|---|---|---|---|---|
| compound | antagonism ($K_i$, μM) | agonism ($EC_{50}$, μM) | NBQX | (*R*)-APPA (**6**) |
| (*R,S*)-AMPA | | 3.5 ± 0.5 | 6.56 ± 0.14 | 3.54 ± 0.03 |
| (*R,S*)-APPA | | 385 ± 59 | NT[a] | NT[a] |
| (*S*)-APPA (**5**) | | 230 ± 12 | 6.30 ± 0.02 | 3.57 ± 0.06 |
| (*R*)-APPA (**6**) | 286 ± 24 | | | |
| kainic acid | | NT[a] | 5.42 ± 0.14 | 3.07 ± 0.08 |

[a] Not tested.

The dose–response curves for **5** in the presence of 0.5 and 1 mM **6** were parallel-shifted without reducing the maximum response (Figure 5b). The determined $pK_i$ value of 3.57 ± 0.06 is significantly different from the $pK_i$ value for kainic acid but not significantly different from the $pK_i$ value for AMPA.

## Discussion

The apparent partial AMPA receptor agonist (*R,S*)-APPA[29] has been resolved via diastereomeric salt formations using the enantiomers of 1-phenylethylamine. The absolute stereochemistry of (*R*)-(−)-APPA (**6**) was established by an X-ray analysis of **6**·HCl·0.25$H_2O$.

On the basis of electropharmacological studies using the rat cortical wedge preparation, (*S*)-APPA (**5**) was shown to be a full AMPA receptor agonist. This stereostructure–activity relationship is in agreement with the earlier observations that the agonistic effects of AMPA[11] and Br-HIBO[11] reside exclusively in the *S*-enantiomers[12–16] (Figure 1). Similarly, (*S*)-AMOA is an AMPA and kainic acid antagonist, whereas (*R*)-AMOA is inactive.[18] The non-NMDA antagonist NBQX[19] blocks with similar potency AMPA receptor agonism induced by AMPA ($pA_2$ = 6.56) and **5** ($pK_i$ = 6.30), whereas the $pA_2$ value (5.42) determined for the blockade of kainic-acid-induced depolarization by NBQX is significantly different (Table 4). These data may indicate that the depolarizations produced by **5** in the cortical wedge preparation are mediated by AMPA receptors and not, or only to a limited extent, by kainic acid receptors, in agreement with the binding data for **5** (Table 3).

In light of the lack of affinity of (*R*)-AMOA[18] and the low affinity of the *R*-enantiomers of AMPA[12–15] and Br-HIBO[16] for AMPA receptors, the AMPA antagonistic effect of **6** is surprising. Compound **6** is one of the few examples of an *R*-α-amino acid showing an effect at AMPA receptors.[13] The similarity of the potencies of **5** as an AMPA agonist ($EC_{50}$ = 230 μM) and **6** as an AMPA antagonist ($K_i$ = 286 μM) is consistent with **5** and **6** interacting with the AMPA receptor with approximately the same affinity. However, compound **6**, in contrast to **5**, does not affect the binding of [$^3$H]AMPA (Table 3). Thus, **5** may interact with an *S*-configuration-preferring site or a conformation of the AMPA receptor, whereas **6** may bind to a different conformation of this receptor.

The dose–response curves for **5** in the presence of 0.5 and 1 mM **6** are parallel-shifted to the right (Figure 5b). Thus, **6** seems to interact as a competitive inhibitor of **5**-induced responses in the rat cortical wedge preparation. These findings show that the apparent partial AMPA receptor agonist (*R,S*)-APPA comprises the full AMPA agonist **5** and the competitive non-NMDA antagonist **6**.

## Experimental Section

**Chemistry.** Melting points were determined in capillary tubes (Büchi SMP-20 apparatus) and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AC 250 spectrometer at 250.13 and 62.90 MHz, respectively. Deuterated dimethyl sulfoxide (DMSO-$d_6$) was used as solvent. Chemical shift values (δ) are determined relative to the internal standard TMS. Elemental analyses, as indicated by elemental symbols, were performed by the Analytical Research Department, H. Lundbeck A/S, and are within ±0.4 of the theoretical values. (*R,S*)-APPA was prepared according to a previously described method.[29]

**Determination of Enantiomeric Purity.** Chiral HPLC was performed on a 150- × 4-mm Crownpak CR(−) column (Daicel) eluted at ambient temperature with 0.4 mL/min of aqueous perchloric acid, pH 2. The instrumentation used consisted of a Jasco 880 pump, a Rheodyne 7125 injector, and a Waters 480 UV detector, set at 210 nm, connected to a Merck-Hitachi D-2000 Chromato-Integrator. The enantiomeric purities were calculated from peak areas.

**(*S*)-(+)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)-propionic Acid ((*S*)-APPA, 5).** A solution of (*R,S*)-APPA·0.5$H_2O$ (11.0 g, 43 mmol) in EtOH (700 mL) was heated to 50 °C. (*R*)-(+)-1-phenylethylamine (5.1 g, 42 mmol) and water (50 mL) were added, and the suspension was heated to reflux temperature. After filtration, the solution was evaporated in vacuo, and the remaining oil was dissolved in EtOH (300 mL). At 0 °C, the salt of (*S*)-(+)-APPA and (*R*)-(+)-1-phenylethylamine precipitated. The crystals were dissolved in water (100 mL), and the solution was acidified to pH 2.5 using 0.1 M hydrochloric acid. The resulting crystals were collected by filtration and dried in vacuo (1.5 g, 27%). Because only partial resolution was accomplished, the procedure was repeated. Crystalline **5** was collected by filtration (320 mg, 6%): mp 251–253 °C; $[\alpha]^{20}_D$ +35.3° (*c* = 0.25, 1 M HCl); $^1$H NMR (DMSO-$d_6$) δ 7.67–7.59 (m, 2H), 7.58–7.46 (m, 3H), 3.74–3.66 (m, 1H), 3.05–2.76 (m, 2H); $^{13}$C NMR (DMSO-$d_6$) δ 171.36 (2C), 164.59, 129.77, 129.03 (2C), 128.45, 127.15 (2C), 102.56, 52.87, 24.96. Anal. ($C_{12}H_{12}N_2O_4$) C, H, N.

**(*R*)-(−)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)-propionic Acid, Monohydrate ((*R*)-APPA·$H_2O$, 6·$H_2O$).** The procedure was analogous to the method described for **5**, using (*R,S*)-APPA·0.5$H_2O$ (11.0 g, 43 mmol) and (*S*)-(−)-1-phenylethylamine (5.1 g, 42 mmol), but the diastereomeric salt of (*R*)-(−)-APPA and (*S*)-(−)-1-phenylethylamine was precipitated only once. Crystalline **6**·$H_2O$ was collected by filtration (1.4 g, 26%): mp 252–254 °C; $[\alpha]^{20}_D$ −37.8° (*c* = 1, 1 M HCl). Anal. ($C_{12}H_{14}N_2O_5$) C, H, N. A Karl Fisher analysis showed 7.7% water content (the theoretical value for **6**·$H_2O$ is 6.8%). The $^1$H NMR (DMSO-$d_6$) and $^{13}$C NMR (DMSO-$d_6$) spectra of **6**·$H_2O$ were identical with those of **5**.

**(*R*)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic Acid, Hydrochloride, 0.25 Hydrate ((*R*)-APPA·HCl·0.25$H_2O$).** (*R*)-(−)-APPA·$H_2O$ (100 mg) was dissolved in aqueous 0.1 M HCl (10 mL). This solution was placed in a desiccator with potassium hydroxide at 760 mmHg for 2 weeks. The crystals formed were collected by filtration and dried in vacuo: mp 212–215 °C dec; $^1$H NMR (DMSO-$d_6$) δ 7.78–7.66 (m, 2H), 7.61–7.49 (m, 3H), 4.04–3.94 (m, 1H), 3.25–3.00 (m, 2H); $^{13}$C NMR (DMSO-$d_6$) δ 170.21 (2C), 165.36, 130.36, 129.38 (2C), 127.97, 126.82 (2C), 100.22, 50.90, 23.49.

**X-ray Crystallographic Analysis of (*R*)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic Acid, Hydrochloride, 0.25 Hydrate ((*R*)-APPA·HCl·0.25$H_2O$).** Crystal data: $C_{12}H_{12}N_2O_4$·HCl·0.25$H_2O$, $M_r$ = 289.21; colorless needles, mp 212–215 °C dec; monoclinic, space group $P2_1$ (No. 4), *a* = 8.5514(9) Å, *b* = 29.335(3) Å, *c* = 10.272(1) Å, β = 100.244(9)°, *V* = 2535.7(4) Å$^3$, *Z* = 8, $D_c$ = 1.515 Mg m$^{-3}$, *F*(000) = 1212, μ(Cu Kα) = 2.83 mm$^{-1}$, *T* ~ 122 K; crystal dimensions 0.08 × 0.10 × 0.30 mm$^3$.

**Data Collection.** Diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer using graphite monochromated Cu Kα radiation (λ = 1.5418 Å). The crystal was cooled to 122 ± 0.5 K in a stream of $N_2$ gas. Unit-cell dimensions were determined by least-squares refinement of 22 reflections with θ values in the range 39°–44°. One hemisphere of data was collected in the ω/2θ scan mode up to θ < 75° ($-10 \le h \le 10$, $0 \le k \le 36$, $-12 \le l \le 12$). Intensities of three reflections monitored every $10^4$ s showed an average decrease in intensity of 3.4%. Data were reduced by a procedure including detailed peak profile analysis using the programs of Blessing (DREADD).[42] Absorption corrections were applied using the numerical program ABSORB[43] ($T_{min}$ = 0.616; $T_{max}$ = 0.813). A total of 10 429 reflections were averaged ($R_{int}$ = 0.021 on $F_o^2$) according to the point-group symmetry 2, resulting in 5321 unique reflections.

The structure was solved by direct methods using the programs MULTAN11/82[44] and DIRDIF[45] in the Enraf-Nonius Structure Determination Package (SDP).[46] Full-matrix least-squares refinements (SHELXL-93)[47] were performed on $F^2$, minimizing $\sum w(F_o^2 - F_c^2)^2$, with anisotropic thermal parameters for non-H-atoms. The positions of all the H-atoms were shown clearly in a Δρ map. The positional parameters of OH and NH H-atoms

were refined with fixed isotropic temperature factors set to 1.5 $U_{eq}$ of the parent atom. The $CH_2$, CH, and aromatic ring H-atoms were included in idealized positions (riding model) with $U_{iso}$ set to $1.2U_{eq}$ of the parent atom. Refinement was carried out on all reflections except for a small number of reflections (three $F_o \gg F_c$ and one $F_c \ll F_o$) which were excluded from the final cycles of refinement. The refinement (760 parameters, 5317 reflections) with the APPA molecule exhibiting an *R* configuration converged at $R_F = 0.0254$, $wR_{F^2} = 0.0706$ ($w^{-1} = (\sigma^2(F_o^2) + (0.0471P)^2 + 0.5835P)$, where $P = (F_o^2 + 2F_c^2)/3$, GOOF = 1.09, 5249 reflections with $F_o \geq 4\sigma(F_o)$). In the final refinement, the largest parameter shift/esd = 0.001 and the residual electron density varied between −0.32 and 0.23 e Å$^{-3}$. Refinement of the Flack absolute structure parameter $\epsilon$ in the final structure factor calculation indicated the *R* enantiomer to have the correct configuration, $\epsilon = 0.001(8)$.[48] Complex atomic scattering factors ($Cl^-$, O, N, C, H) were used.[49]

**In Vitro Pharmacology. Binding Assays.** The membrane preparation used in [$^3$H]AMPA, [$^3$H]kainic acid, [$^3$H]CPP, [$^3$H]-glycine, and [$^3$H]MK-801 binding assays was prepared according to the method of Ransom and Stec.[50] [$^3$H]AMPA binding was performed following a published procedure.[33] [$^3$H]Kainic acid binding was performed as described by Braitman and Coyle[34] with the following modifications: the concentration of [$^3$H]kainic acid was 5 nM rather than 1 nM and the reaction was terminated by filtration through Whatman GF/B filters followed by washing with ice-cold 50 mM Tris-HCl buffer (2 × 5 mL, pH 7.1). [$^3$H]-CPP binding was studied following a published procedure,[35] where termination of the assays was accomplished by filtration through Whatman GF/B filters (presoaked in 0.1% polyethyleneimine) rather than by centrifugation. [$^3$H]Glycine binding was performed as described by Kemp et al.[37] [$^3$H]MK-801 binding to fully stimulated membranes was performed essentially as described earlier,[36] although the incubation time was increased from 1 to 4 h and a concentration of 5 nM of radioactive ligand was used instead of 2 nM.

**Electrophysiology in Vitro.** A rat cortical wedge preparation for the determination of EAA-evoked depolarizations described by Harrison and Simmonds was used in a modified version.[38,39] Wedges (500-μm thick) of rat brain containing cerebral cortex and corpus callosum were placed with the corpus callosum part between two layers of nappy liner and constantly superfused with a magnesium- and calcium-free oxygenated Krebs buffer solution at room temperature. The cortex part was placed like the corpus callosum part and constantly superfused with a magnesium-free oxygenated Krebs buffer solution at room temperature. The two parts were electrically insulated with a grease gap. Ag/AgCl electrodes were placed in contact with the nappy liner on each side of the grease gap, and the potential difference between the electrodes was recorded on an ABB SE120 chart recorder. Standard and test compounds were dissolved in magnesium-free oxygenated Krebs buffer solution and applied to the cortex part of the wedges for 90 s with 10–15-min intervals.

**Curve Fit.** Obtained data were analyzed using nonlinear iterative curve fitting in the Grafit 3.0 program.[51]

**Acknowledgment.** This work was supported by grants from the Alfred Benzon Foundation, the Lundbeck Foundation, and the Danish Natural, Medical and Technical Research Councils. We thank Ms. Lena Tagmose for assistance with the chiral HPLC determinations and Daicel Chemical Industries for a university discount on the Crownpak CR(−) column. The assistance of Mr. F. Hansen with the X-ray data collection and the secretarial assistance of Mrs. Anne Nordly are gratefully acknowledged.

**Supplementary Material Available:** Tables listing final atomic coordinates for (*R*)-APPA·HCl·0.25$H_2O$ and equivalent isotropic or isotropic thermal parameters, anisotropic thermal parameters of the non-hydrogen atoms, and a full list of bond lengths and angles (11 pages); a list of structure factors (13 pages). Ordering information is given on any current masthead page.

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