N-Methyl-D-aspartic Acid Receptor Agonists: Resolution, Absolute Stereochemistry, and Pharmacology of the Enantiomers of 2-Amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic Acid

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(*R,S*)-2-Amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid [(*R,S*)-AMAA, **4**] is a potent and selective agonist at the *N*-methyl-D-aspartic acid (NMDA) subtype of excitatory amino acid receptors. Using the Ugi "four-component condensation" method, the two diastereomers (2*R*)- and(2*S*)-2-[3-(benzyloxy)-5-methyl-4-isoxazolyl]-*N*-*tert*-butyl-2-[*N*-[(*S*)-1-phenylethyl]benzamido]-acetamide (**16** and **17**, respectively) were synthesized and separated chromatographically. The absolute stereochemistry of **16** was confirmed by an X-ray analysis. Deprotection of these intermediates did, however, provide (*R*)- (**8**) and (*S*)- (**9**) AMAA, respectively, in extensively racemized forms. *N*-BOC-protected (*R,S*)-AMAA (**21**) was successfully resolved via diastereomeric salt formation using cinchonidine. The stereochemical purity and stability of **8** and **9** obtained via this resolution were determined using chiral HPLC. (*R*)-AMAA (**8**) showed weak affinity for [^{3}H]AMPA receptor sites (IC_{50} = 72 ± 13 μM) and was shown to be a more potent inhibitor of [^{3}H]CPP binding (IC_{50} = 3.7 ± 1.5 μM) than (*S*)-AMAA (**9**) (IC_{50} = 61 ± 6.4 μM). Neither enantiomer of AMAA affected [^{3}H]kainic acid receptor binding significantly. In electrophysiological studies using rat brain tissue, **8** (EC_{50} = 7.3 ± 0.3 μM) was 1 order of magnitude more potent than **9** (EC_{50} = 75 ± 9 μM) as an NMDA receptor agonist.

Introduction

(*S*)-Glutamic acid (**1**) is the major excitatory amino acid (EAA) neurotransmitter in the central nervous system. Multiple EAA receptor subtypes have been identified, including both ionotropic and G protein-coupled (metabotropic) receptors.[1−4] The naturally occurring analog of glutamic acid, ibotenic acid (**2**) (Figure 1), is a potent excitotoxin and an agonist at all classes of EAA receptors, notably the *N*-methyl-D-aspartic acid (NMDA) receptors and subtypes of the metabotropic receptors.[5,6] Compound **2** has been extensively used as an experimental neurotoxic agent and as a lead structure for the design of a variety of EAA receptor ligands, e.g., the specific AMPA receptor agonist (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid [(*S*)-AMPA, **3**][5,7] and the selective NMDA receptor agonist (*R,S*)-2-amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid [(*R,S*)-AMAA, **4**].[8] The nonselectivity of ibotenic acid (**2**) and other EAA agonists seems to indicate that the recognition sites at EAA receptors share some structural characteristics. On the othe r hand, the distinctly different pharmacology of the two agonists, AMPA and AMAA, show that even minor structural changes can have dramatic effects on affinity for and agonistic effects at different EAA receptors.

(*S*)-Glutamic acid is the endogenous ligand at NMDA receptors, whereas the standard agonist NMDA (**5**) has *R*-configuration (Figure 1) and is more potent than the *S*-enantiomer. This is opposite to what is observed for the enantiomers of glutamic acid and aspartic acid. Another unusual feature concerning NMDA is its high potency in spite of the *N*-methyl substituent. *N*-Methylation of other NMDA agonists normally results in a marked decrease in activity, as observed for *N*-methyl-glutamic acid[9] and *N*-methyl-(*R,S*)-AMAA.[8] A limited number of stereostructure−activity studies on NMDA agonists have disclosed a relatively low degree of stereoselectivity. Some agonists are most active in the *R*-form, whereas the *S*-form of other agonists is selectively recognized by the NMDA receptors.[10,11] Thus, (1*R*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid[12] (**6**) and (2*S*,3*R*,4*S*)-(2-carboxycyclopropyl)glycine[13] (**7**), having opposite configuration at the α-amino acid carbon, have been shown to be the more active stereoisomers of these amino acids at NMDA receptors. Although a low stereoselectivity is normally observed

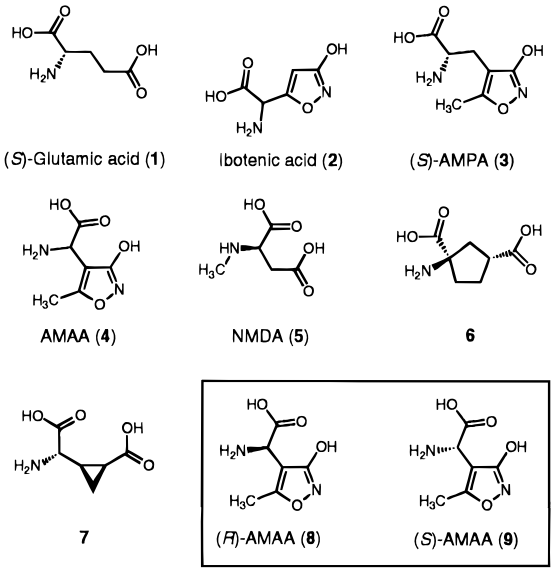

Figure 1. Structures of a number of excitatory amino acids including the enantiomers of AMAA.

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at the NMDA receptors, the orientation of functional groups as well as the molecular volume is of importance for receptor activation. In relation to the latter aspect, an agonist pocket, being able to accommodate extra volume of NMDA agonists, has been suggested,[11] and this may be a determining factor for the stereoselectivity observed for the various agonists. With a very few exceptions, the NMDA receptor affinity of chiral competitive antagonists resides in the enantiomer having *R*-configuration at the α-amino acid carbon.[14,15]

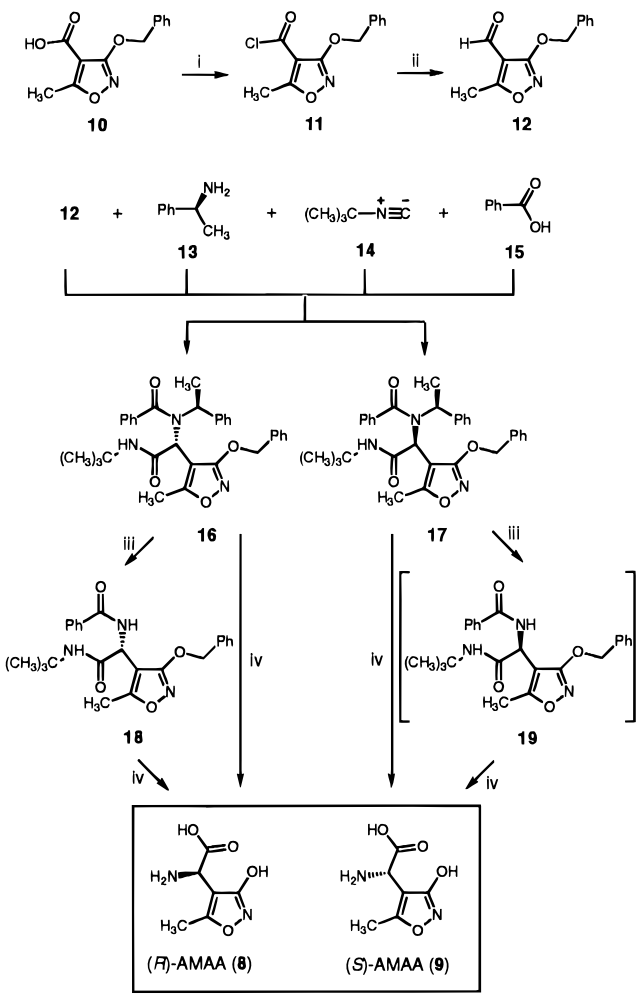
The NMDA agonist (*R,S*)-AMAA (**4**) has previously been shown to be slightly more potent than NMDA in in vitro electrophysiological experiments.[8] Furthermore, (*R,S*)-AMAA has recently been shown to possess substituting effects in a drug discrimination animal model toward NMDA, being more potent than NMDA itself.[16] This activity indicates that (*R,S*)-AMAA, at least to some extent, can penetrate the blood−brain barrier after systemic administration. In light of these observations, we decided to synthesize and pharmacologically characterize the enantiomers of (*R,S*)-AMAA (**4**).

Results

Asymmetric Synthesis. The synthesis of the stereoisomers of (*R,S*)-AMAA (**4**) was attempted using the Ugi "four-component condensation" reaction.[17] This method for asymmetric synthesis of α-amino acids affords both enantiomers through the formation of diastereomeric intermediates, using (*R*)- or (*S*)-1-phenylethylamine as the chiral auxiliary. 3-(Benzyloxy)-5-methyl-4-isoxazolecarbaldehyde (**12**) was synthesized from the carboxylic acid **10**,[18] via the acid chloride **11**, which by reduction with lithium tri-*tert*-butoxyaluminum hydride afforded the desired aldehyde **12** (Scheme 1). The "four-component condensation" of aldehyde **12**, (*S*)-1-phenylethylamine (**13**), *tert*-butylisonitrile (**14**), and benzoic acid (**15**) afforded the two diastereomeric intermediates **16** and **17**, which were easily separated on a silica gel column. The assignment of the absolute stereochemistry of **16** and **17** was based on ^{1}H NMR chemical shift values of especially the *tert*-butyl groups. Empirically, the chemical shift values for the *tert*-butyl groups of *R,S*- and *S,R*-forms of this class of compounds are in the range of δ 1.3−1.4, whereas the corresponding values for the respective *R,R*- and *S,S*-diastereomers are ca. δ 1.1.[19,20] Deprotection of the two diastereomeric intermediates was carried out either by a two-step procedure or in one step (Scheme 1). Following the two-step procedure, **16** or **17** was *N*-debenzylated by treatment with formic acid to give compound **18** or **19**, respectively. Subsequent deprotection in 6 M HCl under reflux afforded (*R*)- (**8**) and (*S*)-(**9**) AMAA, respectively. The deprotection could also be carried out in one step by reflux of **16** or **17** in 6 M HCl to give **8** or **9**, respectively. In both cases, **8** or **9** was isolated as the zwitterion after recrystallization from water. The optical rotation measurements for the final products from different deprotection experiments gave varying and generally very low values. These results were interpreted as being indicative of racemization during the deprotection reactions, and chiral HPLC analyses confirmed that the final products were extensively racemized.

Resolution by Diastereomeric Salt Formation. Resolution was subsequently carried out by diastereomeric salt formation using cinchonidine as the resolving base. For this purpose, a more facile synthesis of (*R,S*)-AMAA[21] (**4**) was developed. Ethyl (*R,S*)-2-amino-2-(3-methoxy-5-methyl-4-isoxazolyl)acetate hydrobromide[22] (**20**) was deprotected by reflux in concentrated hydrobromic acid to give (*R,S*)-AMAA (**4**), which could be isolated in the zwitterionic form (Scheme 2).

Scheme 1[a]



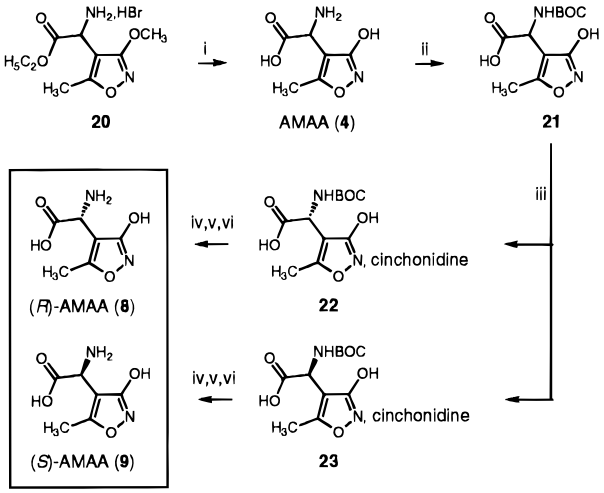
[a] Reagents: (i) $SOCl_2$; (ii) $LiAl[OC(CH_3)_3]_3H$; (iii) HCOOH; (iv) 6 M HCl.

Solutions of *N*-BOC-(*R,S*)-AMAA (**21**) and cinchonidine in ethyl acetate were mixed, and a salt primarily consisting of **22** precipitated. After three recrystallizations, no change in optical rotation values of **22** was observed. Evaporation of the mother liquor from the first precipitation of **22** followed by three recrystallizations gave **23**, again with no change in optical rotation values after the third recrystallization. The two diastereomeric salts **22** and **23** were deprotected to give (*R*)-AMAA (**8**) and (*S*)-AMAA (**9**), respectively.

Chiral HPLC Analysis. The stereochemical purity of **8** and **9** was investigated by chiral HPLC analyses using an (*S*)-proline-derivatized and Cu^{2+}-chelated column. Initial experiments showed very poor resolution of (*R,S*)-AMAA (**4**), whereas a satisfactory resolution of the enantiomers of *N*-BOC-protected AMAA (**21**) was observed. Thus, **8** and **9** were *N*-BOC-protected, and the chiral HPLC analyses disclosed enantiomeric excess (ee) values of 95.0% and 96.0%, respectively.

Due to the stereochemical instability of the enantiomers of AMAA observed during the deprotection

Scheme 2[a]



[a] Reagents: (i) HBr/H_2O; (ii) BOC-O-BOC; (iii) cinchonidine; (iv) AcOH; (v) TFA; (vi) TEA.

experiments on the products from the Ugi synthesis (**16** and **17**), a stability experiment on a sample of **8**, obtained from the diastereomeric salt resolution, was carried out. The stereochemical stability of (*R*)-AMAA (**8**) was investigated in the Krebs buffer used for electrophysiological studies of the compounds (see In Vitro Pharmacology). Compound **8** was dissolved in the buffer, and samples were taken after 0, 2, and 24 h. The samples were *N*-BOC-protected and analyzed by chiral HPLC as described above. These experiments showed slight racemization after 2 h (ee value decreasing from 93.5% to 93.0% of the sample analyzed) and increased racemization after 24 h (ee value decreasing from 93.0% to 89.5%).

X-ray Crystallographic Analysis of Compound 16. An X-ray crystallographic analysis established the assignment of the absolute configuration of compound **16**, which was found to have the *R*,*S*-configuration. The chiral center with *S*-configuration originates from (*S*)-1-phenylethylamine, used for the synthesis of compound **16**. The configuration of the protected α-amino acid moiety was determined relative to this and shown to be *R*. The deprotection experiments performed on compound **16** (see Asymmetric Synthesis) afforded partially racemized AMAA showing negative optical rotation. This qualitatively corresponds to the rotation observed for compound **8**, obtained via diastereomeric salt formation. Thus, the absolute configuration of **8** was established to be *R*. This absolute stereochemistry confirms the stereochemistry predicted from the ^1H NMR experiments performed on the Ugi products **16** and **17**. In agreement with this, the *N*-BOC-protected form of **8** was shown to elute first from the HPLC column, thus showing lower affinity than the *S*-form (**9**) for the (*S*)-proline-derivatized column. This order of elution is analogous to what is normally seen for underivatized α-amino acids on the same type of ligand exchange columns.[23]

The asymmetric unit of the crystal structure of compound **16** consists of two independent molecules of **16** and one molecule of diethyl ether. Perspective drawings of the two independent molecules of **16** (labeled A and B), the former with the crystallographic atom-labeling scheme, are shown in Figure 2. The corresponding bond lengths (Table 1) of the two independent molecules of **16** in the asymmetric unit are the same within three standard deviations, except for C41−N42 [A, 1.469(3) Å; B, 1.485(3) Å] and N42−C43 [A, 1.501(3) Å; B, 1.483(4) Å]. Larger deviations are found, however, for the corresponding bond angles (Table 1). The greatest deviations were observed for the noncyclic parts of the molecules, which may be due to the differences in the conformation of the two independent molecules. In Figure 2, the isoxazole rings of the two molecules (A and B) are oriented in the same way in order to illustrate the conformational differences. The orientations of the three different phenyl groups are different for molecules A and B. For two of the phenyl groups, the changes (10−15°) are of minor importance for the overall conformations of the two molecules, while for the third one (C71−C76), the orientation is changed by 99° (Figure 2). For molecule B, the two phenyl groups are almost parallel, but no stacking effect is observed (Figure 3). The phenyl groups of the benzoyl moieties (C81−C86) of molecules A and B have large displacement parameters. The groups are disordered and can adopt slightly different orientations. The orientations of the carbonyl groups of the amide moieties of molecules A and B, C46═O46, differ by 30°, and this affects the spatial position of the *tert*-butyl moieties. The orientation of the carbonyl group in molecule A results in a favorable intermolecular close contact (C−H···O═C) (Table 1).

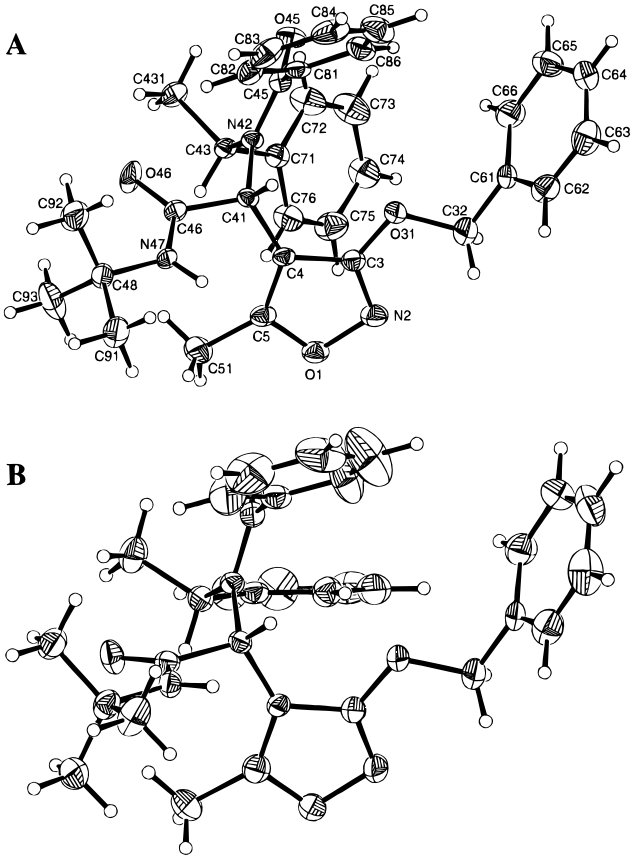


Figure 2. Perspective drawing of the two independent molecules of **16** (ORTEP[24]), A and B. The crystallographic atom labeling is shown on A. Displacement ellipsoids represent 50% probability; H atoms are drawn as circles of arbitrary radius.

The crystal packing (Figure 3) can be described as a collection of columns, packed along the *a*-axis. The alternating columns, consisting of either A or B molecules, interact with one another through van der Waals

Table 1. Selected Bond Lengths (Å), Bond Angles (Deg), Torsion Angles (Deg), and Hydrogen Bond Dimensions (Å, Deg) for Compound **16**[a]

molecule	A	B	molecule	A	B
O1−C5	1.361(4)	1.350(4)	N42−C45	1.349(4)	1.359(4)
O1−N2	1.417(3)	1.423(3)	N42−C43	1.501(3)	1.483(4)
N2−C3	1.307(4)	1.303(4)	C43−C431	1.529(4)	1.525(4)
C3−O31	1.347(3)	1.350(3)	C43−C71	1.524(4)	1.527(4)
C3−C4	1.424(4)	1.427(4)	C45−O45	1.238(3)	1.237(4)
C4−C5	1.354(4)	1.362(4)	C45−C81	1.501(3)	1.493(3)
C4−C41	1.512(4)	1.500(4)	C46−O46	1.221(3)	1.225(3)
C5−C51	1.483(4)	1.483(4)	C46−N47	1.336(4)	1.340(4)
O31−C32	1.464(4)	1.460(4)	N47−C48	1.481(4)	1.489(4)
C32−C61	1.492(4)	1.494(4)	C48−C91	1.522(4)	1.524(5)
C41−N42	1.469(3)	1.485(3)	C48−C92	1.518(5)	1.524(4)
C41−C46	1.542(4)	1.544(4)	C48−C93	1.525(5)	1.528(5)
		Bond Angles			
C5−O1−N2	109.2(2)	109.5(2)	N42−C43−C431	111.9(2)	111.1(3)
C3−N2−O1	103.6(2)	103.3(2)	N42−C43−C71	115.2(2)	113.0(2)
N2−C3−O31	122.2(3)	122.9(3)	C71−C43−C431	112.4(2)	113.6(3)
N2−C3−C4	114.2(3)	114.6(3)	C76−C71−C43	120.1(3)	119.4(3)
O31−C3−C4	123.6(3)	122.5(3)	C72−C71−C43	122.4(3)	122.6(3)
C5−C4−C3	102.9(2)	102.3(3)	O45−C45−N42	120.8(3)	121.3(3)
C5−C4−C41	132.7(3)	133.2(3)	O45−C45−C81	118.2(2)	119.7(3)
C3−C4−C41	124.3(2)	124.5(3)	N42−C45−C81	120.9(2)	119.0(2)
C4−C5−O1	110.1(3)	110.3(3)	C82−C81−C45	121.9(2)	121.0(2)
C4−C5−C51	135.5(3)	135.0(3)	C86−C81−C45	117.6(2)	118.9(2)
O1−C5−C51	114.4(3)	114.7(3)	O46−C46−N47	124.5(3)	125.3(3)
C3−O31−C32	113.4(2)	114.4(2)	O46−C46−C41	120.3(3)	120.7(3)
O31−C32−C61	108.8(3)	107.3(3)	N47−C46−C41	115.1(2)	114.0(2)
C66−C61−C32	122.2(3)	120.2(3)	C46−N47−C48	124.6(2)	124.0(3)
C62−C61−C32	119.5(3)	121.6(3)	N47−C48−C91	106.5(2)	106.0(3)
N42−C41−C4	110.4(2)	111.9(2)	N47−C48−C92	110.2(3)	109.8(3)
N42−C41−C46	110.8(2)	110.6(2)	N47−C48−C93	110.6(3)	109.9(3)
C4−C41−C46	115.8(2)	113.1(2)	C92−C48−C91	109.6(3)	109.4(3)
C45−N42−C41	121.7(2)	121.8(2)	C92−C48−C93	110.9(3)	111.4(3)
C45−N42−C43	120.9(2)	118.9(2)	C91−C48−C93	108.9(3)	110.2(3)
C41−N42−C43	117.4(2)	119.3(2)			
		Torsion Angles			
O1−N2−C3−O31	178.6(3)	177.5(3)	N42−C43−C71−C72	−83.7(4)	15.7(4)
N2−C3−O31−C32	−5.9(4)	−0.1(5)	C41−N42−C43−C431	124.9(3)	119.7(3)
C3−O31−C32−C61	−176.3(3)	−173.6(3)	C41−N42−C45−O45	170.6(3)	173.9(3)
O3−C32−C61−C62	115.2(3)	102.5(4)	C41−N42−C45−C81	−10.2(4)	−6.1(4)
N2−O1−C5−C51	−178.1(3)	−177.9(3)	N42−C45−C81−C82	−59.1(3)	−71.0(3)
N2−C3−C4−C41	−178.3(3)	−178.3(3)	C4−C41−C46−O46	−123.8(3)	−93.9(3)
C3−C4−C41−N42	68.7(3)	79.7(3)	C4−C41−C46−N47	59.0(3)	86.4(3)
C3−C4−C41−C46	−164.3(3)	−154.7(3)	C41−C46−N47−C48	174.7(3)	178.5(3)
C4−C41−N42−C43	59.3(3)	52.8(3)	C46−N47−C48−C91	−179.4(3)	−172.8(3)
C4−C41−N42−C45	−120.2(3)	−128.4(3)	C46−N47−C48−C92	−60.6(4)	−54.7(4)
C41−N42−C43−C71	−105.1(3)	−111.3(3)	C46−N47−C48−C93	62.4(4)	68.1(4)

Hydrogen Bond Dimensions and Close Contact

D−H···X	(symmetry code)	D···X	H···X	<DHX
A, N47−H47---O45	(x−0.5,0.5−y,−z)	2.792(3)	1.944(3)	161.3(1)
B, N47−H47···O45	(x−0.5,1.5−y,−z)	2.941(3)	2.064(3)	173.9(1)
A, C62−H62···O46	(x−0.5,0.5−y,−z)	3.269(4)	2.378(4)	156.0(1)

[a] Estimated standard deviations are given in parentheses.

forces. Molecules within each column, consisting of either A or B molecules, are linked to each other by N−H···O═C hydrogen bonds (Table 1). As mentioned above, another close contact (C−H···O═C), which may be classified as a hydrogen bond,[25] is observed within the columns containing A molecules. The diethyl ether molecules are situated between the columns with no favorable contacts to the molecules of **16**. The large displacement parameters observed for the atoms of the diethyl ether molecule suggest that the molecule is disordered.

In Vitro Pharmacology. The affinities of **8** and **9** for EAA receptors were investigated in receptor-binding assays using [^{3}H]-(*R,S*)-[3-(2-carboxy-4-piperazinyl)-prop-1-yl]phosphonic acid ([^{3}H]CPP), [^{3}H]AMPA, and [^{3}H]kainic acid as ligands for NMDA, AMPA, and kainic acid receptors, respectively.[26−28] The results obtained are listed in Table 2 together with similar data obtained for (*R,S*)-AMAA (**4**), (*R*)-AMPA **2**, (*S*)-AMPA (**3**), and NMDA (**5**). (*R*)-AMAA (**8**) showed high affinity for the [^{3}H]CPP-binding site, slightly higher than (*R,S*)-AMAA (**4**), whereas the *S*-form, **9**, was markedly weaker. Compound **8** showed very veak affinity for the AMPA-binding site, whereas neither enantiomer showed affinity for kainic acid receptor sites.

In vitro electrophysiological experiments were performed using the rat cortical slice model (Table 2).[29] (*R*)-AMAA (**8**) showed an EC_{50} value of 7.3 μM compared to 12 μM for (*R,S*)-AMAA (**4**), whereas (*S*)-AMAA (**9**) was much weaker (EC_{50} = 75 μM). Thus, the *R*-form, **8**, is ca. 10 times more potent than the *S*-form, **9**. The activity of both enantiomers could be fully antagonized by the NMDA antagonist CPP. Dose−response curves for (*R*)- and (*S*)-AMAA are shown in Figure 4. The enantiomeric excess for (*S*)-AMAA was determined to be 96.0% (see Chiral HPLC Analysis), meaning that it

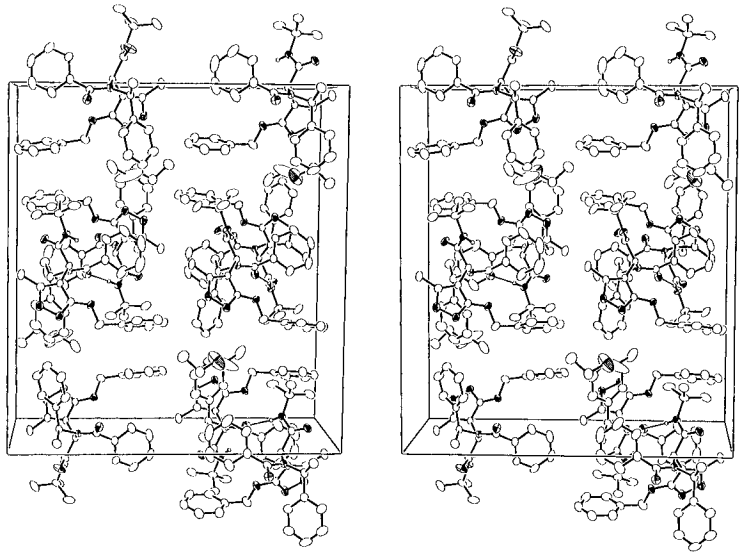


Figure 3. Stereoscopic view of the molecular packing of compound **16**, seen in the direction of the *a*-axis, with horisontal *b*- and vertical *c*-axes. Displacement ellipsoids represent 50% probability for non-hydrogen atoms. For clarity only hydrogen atoms connected to N atoms are shown. Hydrogen bonds are indicated by thin lines.

Table 2. Receptor-Binding and Electrophysiological Data (Mean ± SEM, $n = 3-6$)

compound	IC_{50} (μM) [^{3}H]CPP	[^{3}H]AMPA	[^{3}H]kainic acid	cortical slice, EC_{50} (μM)
(*R*,*S*)-AMAA[a] (**4**)	4.5	>100	>100	12
(*R*)-AMAA (**8**)	3.7 ± 1.5	72 ± 13	>100	7.3 ± 0.3
(*S*)-AMAA (**9**)	61 ± 6.4	>100	>100	75 ± 9
NMDA[a] (**5**)	35	>100	>100	15
(*R*)-AMPA[b] **2**	>100	76	>100	3.8
(*S*)-AMPA[b] (**3**)	>100	0.02	>100	580

[a] Reference 8. [b] Reference 30.

contains 2.0% of (*R*)-AMAA. This content of impurity of the more potent enantiomer **8** is too low to explain the excitatory activity measured for (*S*)-AMAA (**9**). The estimated activity ($EC_{50} = 75$ μM) thus seems to reflect a true activity of (*S*)-AMAA (**9**), considering the degree of stereochemical stability determined in the test buffer (see Chiral HPLC Analysis).

Discussion

Stereostructure−activity studies on NMDA receptor ligands have not yet produced a coherent picture of the stereochemical requirements for activation or blockade of this class of EAA receptors. For a majority of competitive NMDA receptor antagonists, the *R*-form shows markedly greater effect than the *S*-form.[10] In contrast, a low degree of stereoselectivity for NMDA receptor agonists is normally observed, and whereas the *S*-form of some NMDA agonists show higher activity than the *R*-form, the opposite relative potency of such compounds is frequently observed.[11]

Attempts to produce enantiomerically pure (*R*)-AMAA (**8**) and (*S*)-AMAA (**9**) by Ugi reaction were unsuccessful due to extensive racemization during the final deprotection. This stereochemical lability of the AMAA enantiomers is similar to, but less pronounced than, that observed for the analog ibotenic acid[18] (Figure 1). Successful resolution was carried out by diastereomeric salt formation, which did not involve treatment with strong acid or base.

The electrophysiological studies showed (*R*)-AMAA (**8**) to be the more potent enantiomer, ca. 10 times more

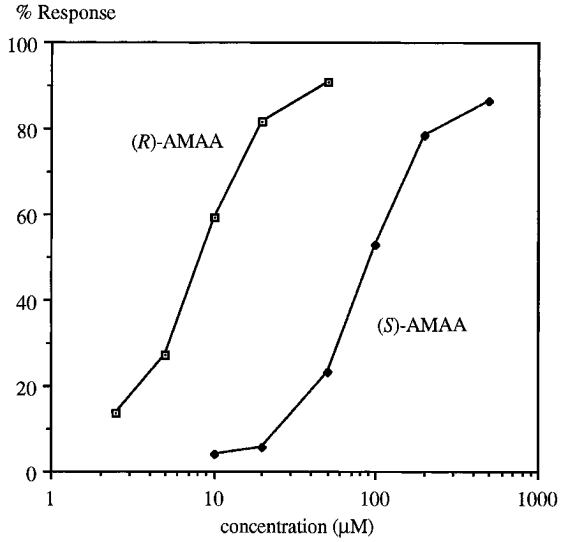


Figure 4. Dose−response curves for (*R*)- (**8**) and (*S*)- (**9**) AMAA obtained from the cortical slice model. The curves represent one experiment, which has been repeated three times.

potent as an NMDA agonist than (*S*)-AMAA (**9**). The enantiomeric impurity of (*S*)-AMAA was determined to be 2.0%. On the basis of this and the result of stability experiments, it is concluded that the observed activity of (*S*)-AMAA (**9**) primarily is reflecting its own activity rather than the activity of the small impurity of (*R*)-AMAA (**8**). The stereochemical preference of NMDA receptors for (*R*)-AMAA is analogous to what has been observed for compounds like NMDA (**5**) and (1*R*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (**6**),[12] whereas (*S*)-glutamic acid[9] and (2*S*,3*R*,4*S*)-(2-carboxycyclopropyl)glycine[13] (**7**) have been shown to be the more potent enantiomers. Molecular modeling studies on a number of NMDA agonists, including AMAA, have predicted (*R*)-AMAA to be the more active enantiomer.[11] The 10-fold higher potency determined for (*R*)-AMAA (**8**) as compared to the *S*-enantiomer **9** is relatively low compared to the degree of stereoselectivity exhibited by the AMPA receptor subtype of EAA receptors. Thus (*S*)-AMPA (**3**) shows a 3800 times higher affinity than the enantiomer

(*R*)-AMPA **2** in [^{3}H]AMPA-binding experiments (Table 2). Glutamic acid also shows high stereoselective affinity for the [^{3}H]AMPA-binding site, the *S*-form having an IC_{50} value of 0.50 μM compared to 47 μM for the *R*-form.[31] A similar degree of stereoselectivity is observed for the binding of (*S*)- and (*R*)-glutamic acid to NMDA receptor sites, whereas the enantiomers of aspartic acid show equal affinity for NMDA receptors.[32]

In conclusion, the enantiomers of the NMDA agonist AMAA have been synthesized, their absolute stereochemistry and stereochemical stability have been determined, and the *R*-form (**8**) has been shown to be 1 order of magnitude more active than (*S*)-AMAA (**9**).

Experimental Section

Chemistry. General Procedures. Melting points were determined in capillary tubes and are uncorrected. Optical rotations were determined on a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by Mr. G. Cornali, Microanalytical Laboratory, LEO Pharmaceutical Products, Denmark, or Mr. P. Hansen, Department of General and Organic Chemistry, University of Copenhagen, Denmark, and are within ±0.4% of the calculated values unless otherwise stated. ^{1}H NMR spectra were recorded on a Varian EM 360L (60 MHz), a Jeol FX 90Q (90 MHz), or a Bruker AC-200F (200 MHz) instrument using TMS or 1,4-dioxane as internal standard for spectra recorded in $CDCl_3$ or D_2O, respectively. IR spectra were recorded from KBr disks or for oils as liquid sandwiches between NaCl disks on a Perkin-Elmer 781 grating infrared spectrophotometer. Column chromatography (CC) was performed on Merck silica gel 60 (0.060−0.200 mm). Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} plates. Compounds containing the 3-hydroxyisoxazole moiety were visualized on TLC plates using UV light and a $FeCl_3$ spraying reagent (yellow color). Compounds containing amino groups were visualized using a ninhydrin spraying reagent, and all compounds under study were also detected on TLC plates using a $KMnO_4$ spraying reagent. Evaporations were performed under vacuum on a rotary evaporator at 15 mmHg.

3-(Benzyloxy)-5-methyl-4-isoxazolecarbonyl Chloride (11). To a suspension of 3-(benzyloxy)-5-methyl-4-isoxazolecarboxylic acid[18] (**10**) (1.0 g, 4.3 mmol) in thionyl chloride (5 mL) was added DMF (1 drop), and the mixture was refluxed for 15 min. After evaporation and re-evaporation from CH_2Cl_2, Kugelrohr distillation (0.2 mmHg, 200−225 °C) afforded **11** (990 mg, 92%) as a colorless oil, which crystallized when stored at 5 °C: IR 3070 (w), 3030 (w), 2960 (w), 2930 (m), 1750 (s), 1590 (s) cm^{-1}. Anal. ($C_{12}H_{10}ClNO_3$) C, H, N; Cl: calcd, 14.12; found, 13.24.

3-(Benzyloxy)-5-methyl-4-isoxazolecarbaldehyde (12). To a solution of compound **11** (400 mg, 1.6 mmol) in dry THF (7 mL) at −78 °C was added, during a period of 5 min, a solution of lithium tri-*tert*-butoxyaluminum hydride (450 mg, 1.8 mmol) in dry THF (5 mL). After stirring at −78 °C for 30 min and slow return to room temperature, H_2O (10 mL) was added. The mixture was acidified with AcOH and extracted with Et_2O (3 × 15 mL). The combined extracts were dried and evaporated, and the residue was subjected to CC [tol−EtOAc (9:1)]. Recrystallization (tol−petroleum ether) afforded **12** (190 mg, 55%): mp 68−69 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 9.8 (1H, s), 7.4 (5H, s), 5.3 (2H, s), 2.3 (3H, s); IR 3015 (w), 2915 (w), 2850 (w), 2760 (w), 1675 (s), 1610 (s), 1510 (s) cm^{-1}. Anal. ($C_{12}H_{11}NO_3$) C, H, N.

(2*R*)- and (2*S*)-2-[3-(Benzyloxy)-5-methyl-4-isoxazolyl]-*N*-*tert*-butyl-2-[*N*-[(*S*)-1-phenylethyl]benzamido]acetamide (16 and 17). A solution of **12** (350 mg, 1.6 mmol) and (*S*)-1-phenylethylamine (200 μL, 1.6 mmol) in MeOH (4 mL) was refluxed for 30 min. After cooling to room temperature, benzoic acid (200 mg, 1.6 mmol) and *tert*-butylisonitrile (180 μL, 1.6 mmol) were added, and the reaction mixture was stirred over night. Evaporation followed by CC [CH_2Cl_2−EtOAc (9:1)] afforded after recrystallization **16** (380 mg, 45%) (tol−petroleum ether): mp 170−171 °C. $[\alpha]^{21}_D = -4.9°$, $[\alpha]^{21}_{365} = +8.8°$ (c =1.0, MeOH); ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.5−7.1 (15H, m), 5.8 (1H, s), 5.1 (1H, d, J = 12 Hz), 4.9 (1H, d, J = 12 Hz), 4.8 (1H, br), 4.7 (1H, s), 2.2 (3H, s), 1.8 (3H, d, J = 7 Hz), 1.3 (9H, s); IR 3250 (m), 3050 (m), 3020 (w), 2955 (m), 2920 (w), 1680 (s), 1615 (s) cm^{-1}. Anal. [$C_{32}H_{35}N_3O_4 \cdot {}^1/_2C_7H_8$(tol)] C, H, N.

Further elution afforded after recrystallization **17** (205 mg, 24%) (tol−petroleum ether): mp 116.5−117.5 °C; $[\alpha]^{21}_D = -2.5°$, $[\alpha]^{21}_{365} = -5.8°$ (c = 1.0, MeOH); ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.6−7.2 (15H, m), 6.0 (1H, s), 5.3 (2H, s), 5.0 (1H, q, J = 7 Hz), 4.8 (1H, s), 2.4 (3H, s), 1.5 (3H, d, J = 7 Hz), 1.1 (9H, s); IR 3250 (w), 3050 (w), 3020 (w), 2970 (m), 2920 (w), 1670 (s), 1635 (s), 1625 (s) cm^{-1}. Anal. ($C_{32}H_{35}N_3O_4$) C, H, N.

(*R*)-2-Benzamido-2-[3-(benzyloxy)-5-methyl-4-isoxazolyl]-*N*-*tert*-butylacetamide (18). A solution of compound **16** (100 mg, 0.2 mmol) in concentrated formic acid (95%, 5 mL) was stirred for 30 min at room temperature and then at 60 °C for 30 min. Evaporation followed by CC [CH_2Cl_2−EtOAc (9:1)] afforded after recrystallization **18** (55 mg, 68%) (tol−petroleum ether): mp 83−84 °C; $[\alpha]^{24}_{365} = -251°$ (c = 0.6, MeOH); ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.5−7.3 (10 H, m), 5.7 (1H, br), 5.35 (2H, br), 5.3 (2H, s), 2.45 (3H, s), 1.25 (9H, s); IR 3280 (s), 3050 (m), 3015 (w), 2960 (m), 2920 (w), 1645 (s), 1630 (s) cm^{-1}. Anal. ($C_{24}H_{27}N_3O_4$) H, N; C: calcd, 68.36; found, 66.92.

(*S*)-2-Benzamido-2-[3-(benzyloxy)-5-methyl-4-isoxazolyl]-*N*-*tert*-butylacetamide (19). Compound **19** (42 mg, 52%) was obtained from **17** (100 mg, 0.19 mmol) by a method similar to that described for the synthesis of **18**. IR and ^{1}H NMR spectra were virtually identical with those of compound **18**. Compound **19** did not crystallize and could not be completely purified due to coelution with the starting material when chromatographed [R_f = 0.25, CH_2Cl_2−EtOAc (9:1)].

(*R*)- and (*S*)-2-Amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic Acid [(*R*)-AMAA (8) and (*S*)-AMAA (9)]. Method A: A suspension of compound **18** (47 mg, 0.11 mmol) was refluxed in 6 M HCl (3 mL) for 2 h. The reaction mixture was evaporated and the residue dissolved in H_2O (5 mL) and extracted with CH_2Cl_2 (2 × 5 mL). After evaporation of the aqueous phase, recrystallization (H_2O) afforded **8** (8.2 mg, 39%): $[\alpha]^{24}_{365} = -15.8°$ (c = 0.20, 0.16 M HCl). Anal. ($C_6H_8N_2O_4 \cdot H_2O$) C, H, N. Compound **9** (6.5 mg, 41%) was synthesized by the same method from **19** (35 mg, 0.083 mmol): $[\alpha]^{24}_{365} = +12.9°$ (c = 0.13, 0.16 M HCl).

Method B: Compound **8** (10.3 mg, 27%) was synthesized as described in method A using compound **16** (105 mg, 0.20 mmol) as the starting material and a reflux time of 4 h: $[\alpha]^{24}_{365} = -2.8°$ (c = 0.16, 0.16 M HCl). Similarly was compound **9** (12 mg, 33%) synthesized from compound **17** (100 mg, 0.19 mmol): $[\alpha]^{24}_{365} = +3.4°$ (c = 0.17, 0.16 M HCl). For further characterization of **8** and **9**, see the later synthesis of these enantiomers from compounds **22** and **23**, respectively.

(*R,S*)-2-Amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic Acid [(*R,S*)-AMAA (4)] Monohydrate. A solution of **20**[22] (430 mg, 1.5 mmol) in concentrated HBr (7 mL, 48%) was refluxed for 30 min and evaporated. The residue was re-evaporated twice from H_2O and recrystallized (H_2O) to give (*R,S*)-AMAA (**4**) monohydrate (211 mg, 76%): mp 184−186 °C dec; ^{1}H NMR (90 MHz, D_2O) δ 4.64 (1H, s), 2.34 (3H, s); IR 3260 (s), 3200−2200 (multiple, w-m), 1645 (s), 1620 (s), 1585 (m), 1530 (s), 1510 (s) cm^{-1}. Anal. ($C_6H_8N_2O_4 \cdot H_2O$) C, H, N.

(*R,S*)-2-[(*tert*-Butoxycarbonyl)amino]-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic Acid (21). To a solution of **4** (380 mg, 2 mmol) in triethylamine (1 mL, 7 mmol) and H_2O (10 mL) was added di-*tert*-butyl dicarbonate (0.5 mL, 22 mmol) dissolved in THF (10 mL). The reaction mixture was stirred at room temperature for 2.5 h, after which the THF was evaporated. After acidification with 4 M HCl, the aqueous phase was quickly extracted with EtOAc (3 × 10 mL), and the organic phases were dried and evaporated. Recrystallization (CH_2Cl_2) afforded **21** (465 mg, 85%) as colorless crystals: mp 99−104 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 5.07 (1H, s), 2.32 (3H, s), 1.45 (9H, s); IR 3500−2400 (multiple bands, m-s), 3260 (s), 1650 (s), 1620 (s), 1585 (s), 1530 (s), 1515 (s), 1505 (s) cm^{-1}. Anal. ($C_{11}H_{16}N_2O_6 \cdot {}^1/_4H_2O$) C, H, N.

Cinchonidinium (*R*)-2-[(*tert*-Butoxycarbonyl)amino]-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetate (22) and Cinchonidinium (*S*)-2-[(*tert*-Butoxycarbonyl)amino]-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetate (23). A solution of **21** (500 mg, 1.8 mmol) in EtOAc (10 mL) was added to a solution of cinchonidine (540 mg, 1.8 mmol) in EtOAc (50 mL), both dissolved by heating. Upon standing at room temperature and then at 5 °C, a precipitate, primarily consisting of **22** (435 mg), was collected; $[\alpha]^{29}_{365} = -332.4°$ ($c = 0.5$, MeOH). These crystals were recrystallized three times from EtOAc (the optical rotation did not change significantly for the crystals obtained from second and third recrystallizations) to give **22** (94 mg, 18%): mp 186.5−187.0 °C dec; $[\alpha]^{25}_{365} = -399.0°$ ($c = 0.6$, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) (aromatic region) δ 8.80 (1H, d, $J = 5$ Hz), 7.98 (1H, d, $J = 8$ Hz), 7.89 (1H, d, $J = 8$ Hz), 7.67 (1H, d, $J = 5$ Hz), 7.55 (1H, t, $J = 8$ Hz), 7.38 (1H, t, $J = 8$ Hz); IR 3320 (m, br), 3290 (m), 2980 (m), 2940 (w), 2560 (w, br), 1715 (s), 1615 (s) cm^{-1}. Anal. ($C_{30}H_{28}N_4O_7$) C, H, N.

The mother liquor from the first precipitation of **22** was evaporated. The residue consisted primarily of **23** (605 mg); $[\alpha]^{29}_{365} = -233.8°$ ($c = 0.5$, MeOH). Three consecutive recrystallizations from EtOAc (the optical rotation did not change significantly for the crystals obtained from second and third recrystallizations) afforded **23** (102 mg, 20%): mp 189.5−190.5 °C dec; $[\alpha]^{25}_{365} = -173.4°$ ($c = 0.5$, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) (aromatic region) δ 8.79 (1H, d, $J = 5$ Hz), 7.96 (2H, m), 7.65−7.45 (3H, m); IR 3420 (m), 3250 (m, br), 2970 (m), 2940 (w), 2560 (w, br), 1715 (s), 1615 (s) cm^{-1}. Anal. ($C_{30}H_{28}N_4O_7$) C, H, N.

(*R*)-(−)-2-Amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)-acetic Acid [(*R*)-AMAA (8)] Monohydrate. Compound **22** (85 mg, 0.15 mmol) was dissolved in H_2O (5 mL) containing AcOH (5 drops), and the aqueous phase was extracted with EtOAc (3 × 5 mL). The organic phases were dried and evaporated to give crude *N*-BOC-protected **8**. The crude product was treated with trifluoroacetic acid (0.5 mL) in H_2O (2 mL) for 1 h at room temperature. After evaporation, the residue was dissolved in H_2O (2 drops) and EtOH (1 mL) and pH adjusted to 4.5 by addition of triethylamine to precipitate crude **8**. The crude product was recrystallized from H_2O to give **8** (14.6 mg, 51%): mp 180.5−182.5 °C; $[\alpha]^{25}_{365} = -357.0°$ ($c = 0.26$, MeOH); ^{1}H NMR spectrum was virtually identical with that of the racemate **4**; IR 3380 (m), 3300−2200 (multiple, w-m), 1670 (s), 1610 (m, br), 1585 (m, br), 1495 (s) cm^{-1}.

(*S*)-(+)-2-Amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)-acetic Acid [(*S*)-AMAA (9)] Monohydrate. (*S*)-AMAA (**9**) (15.0 mg, 47%) was obtained from compound **23** (95 mg, 0.17 mmol) by a method similar to that used for compound **8**: mp 180.5−182.5 °C; $[\alpha]^{25}_{365} = +328.0°$ ($c = 0.22$, MeOH); ^{1}H NMR was virtually identical with that of the racemate **4** and IR with that of the enantiomer **8**.

Determination of Enantiomeric Purity. Chiral HPLC was performed on a Waters HPLC Model 510, Waters UK6 injector, LKB 2155 HPLC column oven, Waters UV detector Model 480 (210 nm), Hitachi D-2000 Chromato-Integrator, and an analytical column (length, 120 mm; i.d., 4.6 mm) containing a silica-based packing material with (*S*)-proline chemically bound via a 3-glycidoxypropyl spacer and Cu^{2+} as a complexing agent.[33] Chromatography was performed with the column at 50 °C and isocratic elution with an aqueous buffer (50 mM KH_2PO_4, pH = 4.6, 1 mL/min) as the mobile phase. The enantiomeric composition was determined from peak areas.

Very poor separation of the enantiomers (*R*)- (**8**) and (*S*)- (**9**) AMAA was observed on the chiral column, whereas good enantiomeric separation was obtained when analyzing the *N*-BOC-protected derivative **21**. Thus, an analytical sample of the obtained crystals of (*R*)- (**8**) and (*S*)- (**9**) AMAA was *N*-BOC-protected with di-*tert*-butyl dicarbonate, by a method similar to that described for the synthesis of **21**, and the products were analyzed by chiral HPLC. For (*R*)-AMAA (**8**) the ee, after *N*-BOC-protection, was determined to 95.0% and for (*S*)-AMAA (**9**), 96.0%.

Determination of Enantiomeric Stability. An analytical sample of (*R*)-AMAA (**8**) was dissolved in the Krebs buffer used for the in vitro electrophysiological studies (see In Vitro Pharmacology). Samples were taken out at 0, 2, and 24 h and then treated with di-*tert*-butyl dicarbonate to obtain the *N*-BOC-protected product, which was analyzed by chiral HPLC, as described above. The ee of the sample taken out at the beginning (0 h) was determined to be 93.5%; after 2 and 24 h in the buffer solution, the ee was reduced to 93.0% and 89.5%, respectively.

X-ray Crystallographic Analysis of (2*R*)-2-[3-(Benzyloxy)-5-methyl-4-isoxazolyl]-*N*-*tert*-butyl-2-[*N*-[(*S*)-1-phenylethyl]benzamido]acetamide (16). Crystal data: $2(C_{32}H_{35}N_3O_4)$, $CH_3CH_2OCH_2CH_3$, $M_r = 1125.4$, colorless needles; recrystallized from Et_2O, mp 168−170 °C [161−163 °C (rearrangment)]; orthorhombic, space group $P2_12_12_1$ (no. 19); $a = 12.443(3)$ Å, $b = 21.161(1)$ Å, $c = 23.271(2)$ Å, $V = 6127(1)$ Å^3, $Z = 4$, $D_c = 1.220$ Mg m^{-3}; $F(000) = 2408$, μ(Cu Kα) = 0.649 mm^{-1}, $T = 122$ K, crystal dimensions = 0.07 × 0.24 × 0.38 mm^3.

Data collection and processing: Diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer, using graphite monochromated Cu Kα radiation ($\lambda = 1.5418$ Å). The crystal was cooled to 122 ± 0.5 K in a stream of nitrogen gas. Unit cell dimensions were determined by least-squares refinement of 20 reflections with θ values in the range 36.42−38.52°. Two octants of data were collected in the $\omega/2\theta$ scan mode ($\theta < 75°$: $0 \le h \le 15$; $0 \le k \le 26$; $-29 \le l \le 29$). Data were reduced by a procedure including detailed peak profile analysis using the programs of Blessing (DREADD).[34,35] Intensities of five reflections were monitored every 10^4 s, and they showed a decrease in intensity of 4.6%. Appropriate scaling was performed using the program SCALE3.[34,35] Absorption corrections were applied using the program ABSORB[36] ($T_{min} = 0.830$; $T_{max} = 0.957$). A total of 14 368 reflections were averaged ($R_{int} = 0.011$ on F_o^2) according to the point group symmetry 222, resulting in 12 621 unique reflections.

Structure determination and refinement: The structure was solved by direct methods using the program SHELXS-86.[37,38] Full-matrix least-squares refinements (SHELXL-93[39]) were performed on F^2, minimizing $\Sigma w(F_o^2 - F_c^2)^2$ with anisotropic displacement parameters for non-hydrogen atoms. The positions of some of the hydrogen atoms were observed in $\Delta\rho$ maps, but all hydrogen atoms were included in idealized positions (riding atoms) with U_{iso} set to $1.2U_{eq}$ of the parent atom ($1.5U_{eq}$ for H atoms of methyl groups). The phenyl groups (C81−C86) of the two (crystallographically) independent molecules were during the refinements treated as rigid groups, in order to keep bond lengths and bond angles of equal magnitude. These phenyl groups are slightly disordered and were observed with large displacement parameters. Refinement was carried out on 12 617 unique reflections. Four reflections were suppressed as the intensities were observed negative. The refinement (736 parameters, 12 617 reflections) with the molecule **16** having the *R,S*-configuration converged at $R_F = 0.060$, $R_{wF}^2 = 0.128$ [$w^{-1} = (\sigma^2(F_o^2) + (0.0521P)^2 + 3.6171P$, where $P = (F_o^2 + 2F_c^2)/3$, GOOF = 1.06, 10 092 reflections with $F_o \ge 4\sigma(F_o)$]. In the final refinement, the largest parameter shift/esd = −0.047, and the residual electron density varied between 0.50 and −0.66 e Å^{-3} (observed in the area in which the diethyl ether molecule is located). The *S*-configuration of the *N*-(1-phenylethyl) moiety was known from the asymmetric synthesis, and the *R*-configuration of the protected α-amino acid moiety was determined relative to this. The absolute configuration was supported by refinement of the Flack absolute structure factor ϵ in the final structure factor calculation, $\epsilon = -0.09(21)$.[40] The large standard deviation of ϵ is due to the atoms present in this compound (C, H, N, O), which produce minor anomalous scattering. Complex atomic scattering factors (C, H, N, O) were used.[41]

Receptor Binding. Affinity for NMDA, AMPA, and kainate receptors were determined using the ligands [^{3}H]CPP, [^{3}H]-AMPA, and [^{3}H]kainic acid, respectively.[26−28] The membrane preparations used in all the receptor-binding experiments were prepared according to the method of Ransom and Stec.[42]

In Vitro Electrophysiology. A rat cortical slice preparation for determination of EAA-evoked depolarizations described by Harrison and Simmonds[29] was used in a slightly modified version. Wedges (500 μm thick) of rat brain, contain-

ing cerebral cortex and corpus callosum, were placed through a grease barrier for electrical isolation with each part in contact with an Ag/AgCl pellet electrode. The cortex and corpus callosum parts were constantly superfused with a Mg^{2+}-free oxygenated Krebs buffer at room temperature, the test compounds were added to the cortex superfusion medium, and the potential difference between the electrodes was recorded on a chart recorder.

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Supporting Information Available: Tables listing final atomic coordinates for compound **16** and diethyl ether, equivalent isotropic displacement parameters, anisotropic displacement parameters for the non-hydrogen atoms, and a full list of bond lengths and bond angles (8 pages); a list of structure factors (31 pages). Ordering information is given on any current masthead page.

References

(1) Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. The excitatory amino acid receptors. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 365−402.
(2) Watkins, J. C.; Krogsgaard-Larsen, P.; Honoré, T. Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends Pharmacol. Sci.* **1990**, *11*, 25−33.
(3) Simon, R. P., Ed. *Excitatory Amino Acids*; Thieme: New York, 1992.
(4) Hollmann, M.; Heinemann, S. Cloned Glutamate Receptors. *Annu. Rev. Neurosci.* **1994**, *17*, 31−108.
(5) Krogsgaard-Larsen, P.; Honoré, T.; Hansen, J. J.; Curtis, D. R.; Lodge, D. New class of glutamate agonist structurally related to ibotenic acid. *Nature (London)* **1980**, *284*, 64−66.
(6) Schoepp, D. D.; Johnson, B. G. Excitatory amino acid agonist-antagonist interactions at 2-amino-4-phosphonoburytic acid-sensitive quisqualate receptors coupled to phosphonoinositide hydrolysis in slices of rat hippocampus. *J. Neurochem.* **1988**, *50*, 1605−1613.
(7) Hansen, J. J.; Lauridsen, J.; Nielsen, E.; Krogsgaard-Larsen, P. Enzymic resolution and binding to rat brain membranes of the glutamic acid agonist α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid. *J. Med. Chem.* **1983**, *26*, 901−903.
(8) Madsen, U.; Ferkany, J. W.; Jones, B. E.; Ebert, B.; Johansen, T. N.; Holm, T.; Krogsgaard-Larsen, P. NMDA receptor agonists derived from ibotenic acid. Preparation, neuroexcitation and neurotoxicity. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1990**, *189*, 381−391.
(9) Watkins, J. C.; Olverman, H. J. In *Exctiatory Amino Acids in Health and Disease*; Lodge, D., Ed.; J. Wiley & Sons: Chichester, England, 1988; pp 13−45.
(10) Watkins, J. C., Collingridge, G. L., Eds. *The NMDA receptor*; Oxford University Press: Oxford, U.K., 1989.
(11) Kyle, D. J.; Patch, R. J.; Karbon, E. W.; Ferkany, J. W. NMDA receptors: heterogeneity and agonism. In *Excitatory Amino Acid Receptors: Design of Agonists and Antagonists*; Krogsgaard-Larsen, P., Hansen, J. J., Eds.; Ellis Horwood: Chichester, England, 1992; pp 121−161.
(12) Curry, K.; Peet, M. J.; Magnuson, D. S. K.; McLennan, H. Synthesis, resolution, and absolute configuration of the isomers of the neuronal excitant 1-amino-1,3-cyclopentanedicarboxylic acid. *J. Med. Chem.* **1988**, *31*, 864−867.
(13) Shinozaki, H.; Ishida, M.; Shimamoto, K.; Ohfune, Y. A conformationally restricted analogue of L-glutamate, the (2*S*,3*R*,4*S*) isomer of L-α-(carboxycyclopropyl)glycine, activates the NMDA-type receptor more markedly than NMDA in the isolated rat spinal cord. *Brain Res.* **1989**, *480*, 355−359.
(14) Watkins, J. C. In *Excitatory Amino Acid Antagonists*; Meldrum, B. S., Ed.; Blackwell Science Publishers: London, 1991; pp 84−100.
(15) Ornstein, P. L.; Klimkowski, V. J. Competitive NMDA receptor antagonists. In *Excitatory Amino Acid Receptors: Design of Agonists and Antagonists*; Krogsgaard-Larsen, P., Hansen, J. J., Eds.; Ellis Horwood: Chichester, England, 1992; pp 183−200.
(16) Arnt, J.; Sánches, C.; Lenz, S. M.; Madsen, U.; Krogsgaard-Larsen, P. Differentiation of in vivo effects of AMPA and NMDA receptor ligands using drug discrimination methods and convulsant/anticonvulsant activity. *Eur. J. Pharmacol.* **1995**, in press.
(17) Gokel, G.; Lüdke, G.; Ugi, I. Four-component condensations and related reactions. In *Isonitrile Chemistry*; Ugi, I., Ed.; Academic Press: New York, 1971; pp 145−199.
(18) Madsen, U.; Brehm, L.; Krogsgaard-Larsen, P. Excitatory amino acids. Improved synthesis of ibotenic acid and X-ray analysis of an unexpected reaction product, (*RS*)-N-[2-(3-benzyloxyisoxazol-5-yl)-1-phenylethyl]-3-oxobutyramide. *J. Chem. Soc., Perkin Trans. I* **1988**, 359−364.
(19) Marquarding, D.; Hoffman, P.; Heitzer, H.; Ugi, I. Stereoselective four-component condensations of α-ferrocenylethylamine and its absolute configuration. *J. Am. Chem. Soc.* **1970**, *92*, 1969−1971.
(20) Semple, J. E.; Wang, P. C.; Lysenko, Z.; Joullié, M. M. Total synthesis of (+)-furanomycin and stereoisomers. *J. Am. Chem. Soc.* **1980**, *102*, 7505−7510.
(21) Christensen, S. B.; Krogsgaard-Larsen, P. Structural analogues of ibotenic acid. Synthesis of (±)-α-amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid and derivatives thereof. *Acta Chem. Scand.* **1978**, *B32*, 27−30.
(22) Johansen, T. N.; Frydenvang, K.; Ebert, B.; Krogsgaard-Larsen, P.; Madsen, U. Synthesis and structure-activity studies on acidic amino acids and related diacids as NMDA receptor ligands. *J. Med. Chem.* **1994**, *37*, 3252−3262.
(23) Gübitz, G. Direct separation of enantiomers by high performance ligand exchange chromatography on chemically bonded chiral phases. *J. Liq. Chromatogr.* **1986**, *9*, 519−535.
(24) Johnson, C. K. ORTEP II. Report ORNL-5138; Oak Ridge National Laboratory: Oak Ridge, TN, 1976.
(25) Jeffrey, G. A.; Saenger, W. *Hydrogen bonding in biological structures*; Springer-Verlag: Berlin, 1991; pp 29−30.
(26) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, M. Binding of [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid to rat brain membranes. A selective high-affinity ligand for N-methyl-D-aspartate receptors. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 778−784.
(27) Honoré, T.; Nielsen, M. Complex structure of quisqualate-sensitive glutamate receptors in rat cortex. *Neurosci. Lett.* **1985**, *54*, 27−32.
(28) Braitman, D. J.; Coyle, J. T. Inhibition of [^{3}H]Kainic acid receptor binding by divalent cations correlates with ion affinity for the calcium channel. *Neuropharmacology* **1987**, *26*, 1247−1251.
(29) Harrison, N. L.; Simmonds, M. A. Quantitative studies on some antagonists of N-methyl-D-aspartate in slices of rat cerebral cortex. *Br. J. Pharmacol.* **1985**, *84*, 381−391.
(30) Nielsen, B.; Fisker, H.; Ebert, B.; Madsen, U.; Curtis, D. R.; Krogsgaard-Larsen, P.; Hansen, J. J. Enzymatic resolution of AMPA by use of α-chymotrypsin. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 107−114.
(31) Hansen, J. J.; Nielsen, B.; Krogsgaard-Larsen, P.; Brehm, L.; Nielsen, E. Ø.; Curtis, D. R. Excitatory amino acid agonists. Enzymic resolution, X-ray structure and enantioselective activities of (*R*)- and (*S*)-bromohomoibotenic acid. *J. Med. Chem.* **1989**, *32*, 2254−2260.
(32) Fagg, G. E.; Baud, J. Characterization of NMDA receptor ionophore complexes in the brain. In *Excitatory Amino Acids in Health and Disease*; Lodge, D., Ed.; J. Wiley & Sons: Chichester, England, 1988; pp 63−90.
(33) Gübitz, G.; Jellenz, W.; Santi, W. Resolution of the optical isomers of underivatized amino acids on chemically bonded chiral phases by ligand exchange chromatography. *J. Liq. Chromatogr.* **1981**, *4*, 701−712.
(34) Blessing, R. H. Data reduction and error analysis for accurate single crystal diffraction intensities. *Crystallogr. Rev.* **1987**, *1*, 3−58.
(35) Blessing, R. H. DREADD - Data reduction and error analysis for single-crystal diffractometer data. *J. Appl. Crystallogr.* **1989**, *22*, 396−397.
(36) DeTitta, G. T. ABSORB: an absorption correction program for crystals enclosed in capillaries with trapped mother liquor. *J. Appl. Crystallogr.* **1985**, *18*, 75−79.
(37) Sheldrick, G. M. *SHELXS-86. Program for the Solution of Crystal Structure*; University of Göttingen: Göttingen, Germany, 1986.
(38) Sheldrick, G. M. Phase annealing in SHELX-90: Direct methods for larger structures. *Acta Crystallogr.* **1990**, *A46*, 467−473.
(39) Sheldrick, G. M. *SHELXL-93. Program for Crystal Structure Refinement*; University of Göttingen: Göttingen, Germany, 1993.
(40) Flack, H. D. On enantiomorph-polarity estimation. *Acta Crystallogr.* **1983**, *A39*, 876−881.
(41) *International Tables for Crystallography*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1992; Vol. C, Tables 4.2.6.8 and 6.1.1.4.
(42) Ransom, R. W.; Stec, N. L. Cooperative modulation of [^{3}H]MK-801 to the N-methyl-D-aspartate receptor ion channel complex by L-glutamate, glycine and polyamines. *J. Neurochem.* **1988**, *51*, 830−836.

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