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Drug Design, in Vitro Pharmacology, and Structure−Activity Relationships of 3-Acylamino-2-aminopropionic Acid Derivatives, a Novel Class of Partial Agonists at the Glycine Site on the *N*-Methyl-D-aspartate (NMDA) Receptor Complex

Stephan Urwyler,[§] Philipp Floersheim, Bernard L. Roy, and Manuel Koller*

Novartis Institutes for BioMedical Research, Neuroscience, 4056 Basel, Switzerland. [§]*Current affiliation: Department of Chemistry and Biochemistry, University of Berne, Switzerland.*

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Retaining agonistic activity at the glycine coagonist site of the NMDA receptor in molecules derived from glycine or D-serine has proven to be difficult because in the vicinity of the α-amino acid group little substitution is tolerated. We have solved this problem by replacing the hydroxy group of D-serine with an amido group, thus keeping the hydrogen donor function and allowing for further substitution and exploration of the adjacent space. Heterocyclic substitutions resulted in a series of 3-acylamino-2-aminopropionic acid derivatives, with high affinities in a binding assay for the glycine site. In a functional assay assessing the activation of the glycine site, these compounds displayed a wide range of intrinsic efficacies, from antagonism to a high degree of partial agonism. Structure−activity relationships reveal that lipophilic substituents, presumably filling an additional hydrophobic pocket, are accepted by the glycine site, provided that they are separated from the α-amino acid group by a short linker.

Introduction

The *N*-methyl-D-aspartate (NMDA[a]) receptor, a subtype of the ionotropic glutamate receptor family, is involved in key physiological functions, such as learning, memory and developmental processes on the one hand, but also in a variety of disease states (epilepsy, acute and chronic neurotoxicity) on the other hand. It is therefore believed to have a considerable potential as a site for therapeutic intervention.[1] The receptor comprises multiple functional domains interacting with each other, thus allowing a subtle regulation of receptor activity and efficacy. In particular, glycine has been shown to be a mandatory coagonist necessary for the opening of NMDA receptor ion channels.[2,3] The glycine recognition site, located on the NR1 subunit of the NMDA receptor complex, can be distinguished from the inhibitory glycine receptor by its insensitivity to strychnine. It has attracted considerable interest as a potential drug target, as an alternative to the NMDA recognition site itself.[1,4,5] A great number of glycine site antagonists from different structural classes have been described in recent years.[6−17] However, serious side effects (e.g., memory impairment, neurotoxic or psychotomimetic effects) have been observed with the use of competitive or channel blocking NMDA receptor antagonists in animal models and/or in human clinical trials.[18] Since glycine is an obligatory coagonist, one would expect the same to happen from an antagonist blocking the glycine site. Therefore, the use of partial agonists at this site might be more promising than that of "silent" antagonists, since such compounds would tune down receptor activity to a certain level rather than completely blocking it. Moreover, the concept of partial agonism might be useful not only in clinical indications requiring inhibition of NMDA receptor function (e.g., epilepsy, neurodegeneration) but also in others, such as possibly schizophrenia, where a certain stimulation might be desirable (for reviews, see refs 19 and 20).

Retaining agonistic activity in molecules derived from glycine **1** (Figure 1) has proven to be difficult because of severe space restrictions for substituents at or in the immediate vicinity of the amino acid function.[21−23] D-Alanine **2**,[21] aminocyclopropanecarboxylic acid (ACC, **3**), and D-serine **4** are among the few examples of full agonists at the glycine site. However, substitution at the β-C atom of D-alanine **2** destroys its affinity for the glycine site (**2d**−**f**) (with the noteworthy exception of some E-γ-substituted vinylglycine derivatives, **2a**−**c**;[24] see below). Similarly, stepwise expansion of the ring size from ACC **3** to aminocyclohexanecarboxylic acid drastically reduces not only the affinity (**3a**−**c**) but also the intrinsic efficacy of compounds at the glycine site.[23] Also, no substitution is tolerated at or in the vicinity of the hydroxy group of D-serine **4** (**4a**−**c**).

The E-γ-substituted vinylglycine derivatives **2a**−**c**[24] retain agonistic activity at the glycine site very likely because of the slender shape of the 3,4-double bond in the close neighborhood of the α-C atom (compare with the inactive compound **2e** with a 4,5-double bond!). The activity of these compounds suggests the existence of an extra hydrophobic binding pocket at the glycine site, in addition to the hydrophilic pocket with which the amino acid function interacts. On the other hand, it seems clear that the inactivity of the *O*-methyl derivative of

*To whom correspondence should be addressed. Phone: +41 61 324 66 08. Fax: +44 61 696 24 55. E-mail: manuel.koller@novartis.com.

[a] Abbreviations: ACBC, 1-aminocyclobutane carboxylic acid; ACC, 1-aminocyclopropanecarboxylic acid; 5,7-DCK, 5,7-dichlorokynurenic acid; DCS, D-cycloserine; NMDA, *N*-methyl-D-aspartate; Z, benzyloxycarbonyl.

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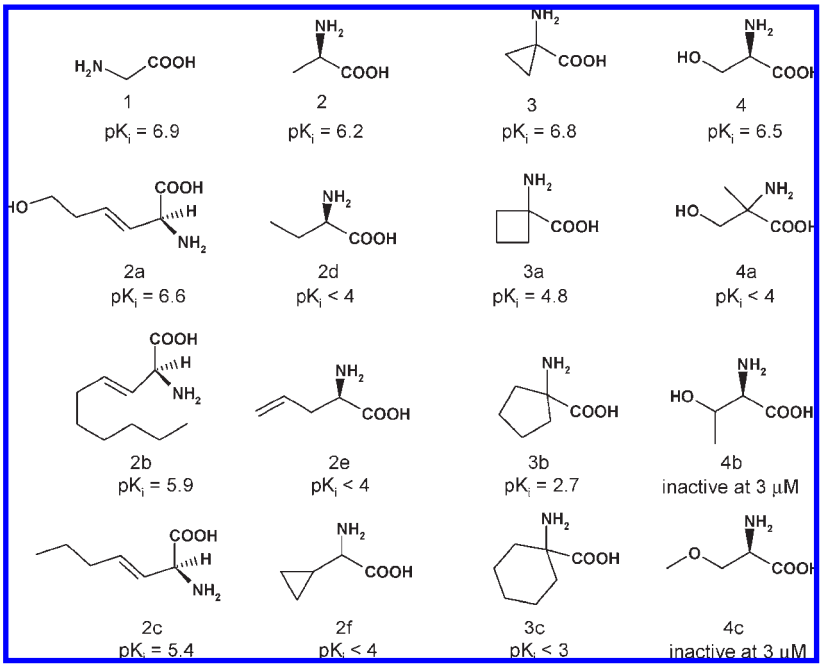
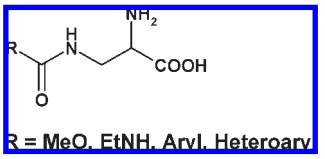


Figure 1. Affinities (pK_i values) of different α-substituted derivatives of glycine **1**, D-alanine **2**, 1-aminocyclopropanecarboxylic acid (ACC, **3**), and D-serine **4** for the glycine recognition site on the NMDA receptor. The values were obtained from a [^{3}H]glycine binding assay performed as described in the Experimental Section, or they were drawn from ref 24 (in the case of compounds **2a**−**c**) or from ref 22 (in the cases of compounds **4b** and **4c**).

Chart 1



D-serine (**4c** in Figure 1) is not due to steric hindrance but to the removal of the hydrogen bond donor function of the hydroxy group, which is involved in the agonism by D-serine **4** at the glycine site.[22,25] Therefore, we started a synthetic program based on the replacement of the hydroxy group in D-serine **4** by an amido group, leading to 3-acylamino-2-aminopropionic acid derivatives (Chart 1) and thus preserving its potential for hydrogen bond formation and allowing for further substitution to explore the space adjacent to this functional group.

Chemistry

Most of the 3-acylamino-2-aminopropionic acid derivatives (**III**) were prepared as depicted in Scheme 1. Condensation of the appropriate carboxylic acid or acid chloride (**I**) with 3-amino-2-benzyloxycarbonylaminopropionic acid methyl ester **5b** (either racemic or, for the optically active target molecules (**III**), as the enantiomers **R-5b**[26] and **S-5b**[27]) gave the esters (**II**). Deprotection was accomplished by saponification with aqueous base followed by removal of the Z-group either by hydrogenolysis or by reaction with iodotrimethylsilane.

The racemic diaminopropionic acid ***rac*-5a** and its methyl ester ***rac*-5b** were prepared starting from *rac*-asparagine and using the procedure described for **R-5b**[26] (structures shown in Chart 2).

Compounds **6** and **7** (Table 1) were obtained by reacting ***rac*-5a** with methyl chloroformate and ethyl isocyanate, respectively, followed by deprotection.

Several starting materials RCOX (Scheme 1) were commercial, such as the acid chlorides (X = Cl) for target compounds **8**, **10**, **11** or the carboxylic acids (X = OH) used for target compounds **17**, **19**−**22**, **38** (Tables 1−3). Most others were known from literature and synthesized following the published procedures, as for compounds **9**, **12**−**16**, **18**, **24**, **25**, **30**, **32**, **34**−**37**, **46**, **51** (Tables 1−4). For the target compounds **33**, **47**, **48**, and **50**, the intermediate indole carboxylic acids **33c**, **47c**, **48b**, and **50d** were not known in the literature but could be prepared from the hydrazones **33a**, **47a** (Scheme 2), and **50b** (obtained in four steps from 1-chloro-4-iodo-2-nitrobenzene as depicted in Scheme 3), respectively, by the Fischer indole synthesis. The cyclization of **50b** delivered the desired product **50c**, together with the abnormal Fischer indolization product[28] **50c′** (Scheme 3).

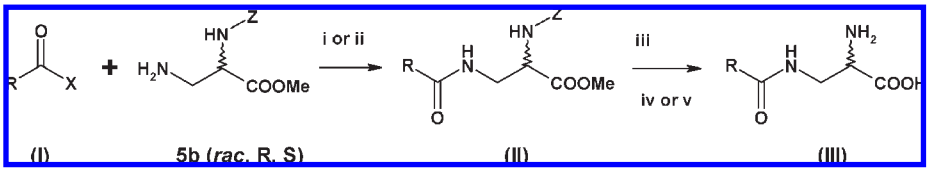
The 2-ethylphenyl-substituted naphthalenecarboxylic acid **28a** (for target compound **28**) and most of the 4-aryl substituted benzothiophene-2- (for **27**, **31**) and indole-2-carboxylic acids (for **26**, **40**−**42**, **44**, **45**) were prepared from their bromo counterparts by Pd-catalyzed coupling with the corresponding arylboronic acids (Scheme 4).

R-26 was prepared in good yields as described in Scheme 5. Benzyl ester **R-5c** was synthesized from **R-5a**[26] (Chart 2) and condensed with the carboxylic acid **26a** to the Z-protected aminoester **26b**, enabling deprotection of the product in one step by hydrogenolysis under mild conditions.

For target compound **39**, the intermediate **22a** was used as coupling partner for phenylboronic acid in the Pd-catalyzed reaction (Scheme 6), generating in one step carboxylic acid **39a** that was subsequently transformed into **39**. On the other hand, for **43** the stannylated indole ester **43a** was coupled with 1-benzyl-2-bromobenzene to **43b** (Scheme 7). Saponification to **43c** and further reaction according to Scheme 1 delivered **43**.

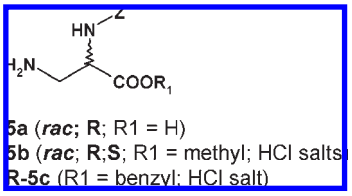
Several functional group transformations in positions 3 and 4 of the indole and benzothiophene scaffolds were accomplished

Scheme 1[a]



[a] (i) For X = Cl: CH_2Cl_2−pyridine 1:1, −10 °C. (ii) For X = OH: 1,1′-carbonyldiimidazole, pyridine, room temp. (iii) NaOH aq, THF or dioxane, room temp. (iv) H_2/Pd−C, THF−H_2O 1:1, room temp. (v) $ISiMe_3$, CH_2Cl_2, 0 °C.

Chart 2



delivering the desired substituted acids for the condensation reaction with ***rac*-5b**. These reactions are summarized in Scheme 8. 4-Bromo-1*H*-indole-3-carboxylic acid **23b**, used for target compound **23**, was prepared from 4-bromo-1*H*-indole by trichloroacetylation to **23a** and hydrolysis of the trichloromethyl group under mild conditions to avoid decarboxylation.[29] 4-Cyanobenzothiophene-2-carboxylic acid **29c**, the precursor for target compound **29**, was obtained by cyanation of the bromo ester **29a** with cuprous cyanide at high temperature, followed by selective hydrolysis of the ester group of **29b**. Target compound **49**, carrying a nitro group in position 3 of the indole nucleus, required the indole acid **49a** that was obtained from nitration of 4-bromo-1*H*-indole-2-carboxylic acid.

To obtain the N-methylated indole derivatives **52** and **53**, 4-bromo-1*H*-indole-2-carboxylic acid methyl ester was N-methylated to **52a**. Pd-catalyzed coupling of **52a** with 2-ethylphenylboronic acid gave **53a**. Saponification of the methyl esters and condensation with ***rac*-5b** followed by deprotection provided the two target compounds (Scheme 9).

Results

Some prototype 3-acylamino-2-aminopropionic acid derivatives with various acyl substituents were tested in radioligand binding assays for the strychnine-insensitive glycine site at the NMDA receptor complex ([^{3}H]glycine) and, in most cases, also the glutamate recognition site ([^{3}H]CGP39653). The resulting pK_i values are given in Table 1. Whereas the carbamate **6** and the urea **7** were virtually inactive, amino acids with aroyl or heteroaroyl groups at the β-nitrogen atom displayed micromolar affinities for the glycine site. The activity of compounds with single aromatic ring substitution in this position (**8**−**11**) was moderate, whereas bicyclic aromatic rings attached to the carbonyl group conferred affinities in the low micromolar range to compounds **13**, **R-14**, **15**, **17**−**21**. A methylene spacer between the aromatic ring and the carbonyl group (**16**) or the attachment of the ring system at a saturated C-atom (compare the inactive indanyl derivative **12** with the active indenyl compound **13**) were not tolerated.

Compounds with attachments of various substituents at bicyclic aromatic ring systems are listed in Tables 2 and 3. A most promising ring system turned out to be indole: A bromine atom in position 4 of the indole nucleus significantly enhanced the binding affinity for the glycine site, as shown by a comparison of the racemic compound ***rac*-22** (pK_i = 6.8) and its *R*-enantiomer **R-22** (pK_i = 7.0) with the unsubstituted compound **R-14** (pK_i = 5.3, Table 1). Other ring systems were much less affected by such a bromo substituent (cf. benzothiophenes **17**/**24**; 2-naphthyl derivatives **20**/**25**). In the case of the 3-indolyl compound **15**, bromo substitution in position 4 of the indole ring resulted in a strong loss of affinity (**23**). The compound with the highest affinity at the glycine site prepared in this work was amino acid **26** with a 2-ethylphenyl group attached in position 4 of the indole nucleus, with a pK_i value of 7.6 for the *R*-enantiomer. Similar to the bromine atom in this position, the 2-ethylphenyl group did not induce or only slightly induced an increase in affinity when it was attached to other ring systems, e.g., as illustrated by the pairwise comparison of the benzothiophene derivatives **17** (pK_i = 5.9) and **27** (pK_i = 6.2) or the 2-naphthyl derivatives **20** (pK_i = 5.9) and **28** (pK_i = 5.7).

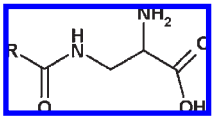
Therefore, besides a few more benzothiophenes (**29**, **30**, **31**), the derivatization of molecules with the carbonyldiaminopropionic acid side chain attached in position 2 of an indole ring was further pursued. The results from several single enantiomers suggested that the *R*-form has the strongest activity at the glycine site (cf. Table 1, **R-14/S-14**; Table 2, **R-22**/***rac*-22**, **R-26**/***rac*-26**), indicating that the binding mode of the amino acid part of the 3-acylamino-2-aminopropionic acids discussed here resembles that of the parent compound D-serine. For simplicity, however, in most cases only the racemic compounds were prepared (compounds **9** and **10** only as *R*-enantiomers). Of course, this needs to be considered when comparisons of their affinities with those of *R*-enantiomers are made.

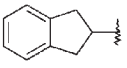
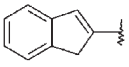
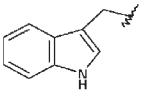
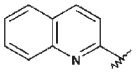
To determine if the aforementioned position 4 of the indole ring is the most promising one for further derivatization, the derivatives with chloro at the four different positions of the benzene ring were compared: whereas chloro substitution in positions 5 and 6 of the indole ring (R3, R4, Table 3) did not enhance the affinities of the compounds for the glycine site (**34**, **35**), substitution at position 7 (R5, **36**, pK_i = 5.5) resulted in a little improvement and in position 4 (R2, **37**, pK_i = 6.0) in even more improvement over the unsubstituted **R-14** (pK_i = 5.3). Substitution with other groups in position 4, such as methoxy (**38**), was also beneficial.

Position 3 of the indole ring was varied by attaching alkyl side chains (R1, Table 3). Whereas methyl (**32**) increased the affinity only a little, a significant enhancement of glycine site affinity was obtained with propyl (**33**) by a factor of 10, compared with **R-14**. Methylation of the indolyl N-atom (R6, Table 3) had no effect on binding affinity (**46**, pK_i = 4.9) when compared to the unsubstituted indolyl compound **R-14**.

Of particular interest was the observed increase of affinity with the 4-phenyl substituent, leading to the question of whether substitution of the phenyl ring in **39** was a means to further strengthen binding affinity. This was indeed the case,

Table 1. Affinities of 3-Acylamino-2-aminopropionic Acid Derivatives for the Glycine and Glutamate Recognition Sites of the NMDA Receptor: Unsubstituted Chains and Ring Systems as R Groups

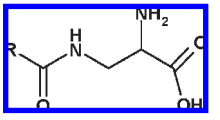


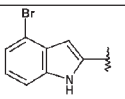
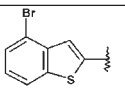
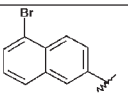
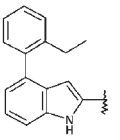
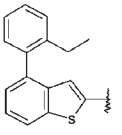
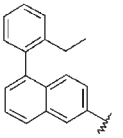
No	R	glycine[a] pKi	glutamate[a] pKi
6	MeO	4.1 ± 0.18	n.t.
7	EtNH	< 4	n.t.
8	2-pyridyl	4.4 ± 0.08	n.t.
9	2-pyrrolyl	4.9 ± 0.18	n.t.
10	2-furyl	4.9 ± 0.26	n.t.
11	2-thienyl	5.1 ± 0.16	n.t.
12		< 4	n.t.
13		5.5 ± 0.28	< 4
R-14	2-indolyl	5.3 ± 0.17	3.8 ± 0.02
S-14	2-indolyl	< 4	n.t.
15	3-indolyl	5.4 ± 0.11	n.t.
16		< 4	n.t.
17	benzo[b]thiophen-2-yl	5.9 ± 0.08	< 4
18	benzofuran-2-yl	5.2 ± 0.12	< 4
19		5.6 ± 0.10	< 4
20	2-naphthyl	5.9 ± 0.07	< 4
21	1-naphthyl	5.9 ± 0.07	< 4

[a] The affinities of the compounds shown were determined in [^{3}H]glycine and [^{3}H]CGP39653 binding assay as described in the Experimental Section. The data shown are expressed as pK_i values and represent mean values ± SEM from at least three independent experiments. n.t., not tested.

as alkyl substitution in position 2 of the phenyl ring produced derivatives with high affinity for the glycine site: benzyl (**43**), methyl (**40**), ethyl (***rac*-26**), propyl (**41**), and even the sterically demanding isopropyl group (**42**) gave a 3- to 100-fold increase of affinity compared to **39**, the optimum being found with ethyl in the already mentioned compounds ***rac*-26** and **R-26**. No increase in glycine site affinity (compared to **39**) was obtained with more polar substituents in the ortho-position of the phenyl ring, such as in **44** and **45**.

Table 2. Effect on Affinities of Bromine- and 2-Ethylphenyl Substitution at Indolyl, Benzothienyl, and Naphthyl in the Acyl Group of the 3-Acylamino-2-aminopropionic Acid Derivatives

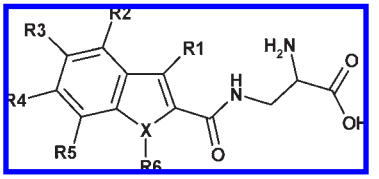


R	No	glycine[a] pKi	glutamate[a] pKi
	**R-22**	7.0 ± 0.05	4.6 ± 0.01
	***rac*-22**	6.8 ± 0.13	4.7 ± 0.02
	**23**	4.3 ± 0.08	< 4
	**24**	6.4 ± 0.09	< 4
	**25**	6.6 ± 0.06	4.3 ± 0.05
	**R-26**	7.6 ± 0.07	5.4 ± 0.27
	***rac*-26**	7.5 ± 0.1	4.9 ± 0.1
	**27**	6.2 ± 0.14	3.9 ± 0.15
	**28**	5.7 ± 0.12	4.5 ± 0.10

[a] The affinities of the compounds for the glycine and glutamate recognition sites are expressed as pK_i values and were determined in [^{3}H]glycine and [^{3}H]CGP39653 binding assays as described in the Experimental Section. The data shown are mean values ± SEM from at least three independent experiments for [^{3}H]glycine binding (at least two for [^{3}H]CGP39653 binding).

The binding affinities of ***rac*-22** and ***rac*-26** for the glycine site could not be improved by various disubstitution patterns at the indole ring (Table 4). For example, a propyl substituent in position 3 (R1), which by itself had a positive effect (such as in **33**) compared to **R-14**, showed no synergistic effect when combined with a bromo- or 2-ethylphenyl group in position 4 but on the contrary lowered glycine site affinity (compare **47** with ***rac*-22**, or **48** with ***rac*-26**). The introduction of an electron-withdrawing group in position 3, such as nitro, which presumably enhances the NH-donor function of the indolyl N-atom, reduced the affinity for the glycine site (compare **49** with ***rac*-22**). It is also noteworthy that a chloro atom in position 7 (R5, Table 3), which by itself only slightly improved the affinity for the glycine site (**36** vs **R-14**), was rather detrimental when combined with an 2-ethylphenyl substituent in position 4 (**50** vs ***rac*-26**). Similarly, whereas a methyl group

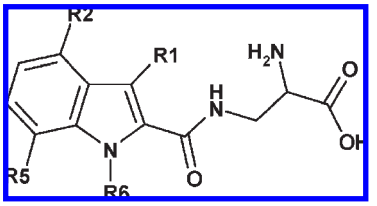
Table 3. Affinities of 3-Acylamino-2-aminopropionic Acid Derivatives for the Glycine and Glutamate Recognition Sites of the NMDA Receptor: Monosubstituted Bicyclic Ring Systems in the Acyl Group



compd	X	R1	R2	R3	R4	R5	R6	glycine[a] pK_i	glutamate[a] pK_i
29	S	H	CN	H	H	H		5.9 ± 0.04	< 4
30	S	H	Cl	H	H	H		6.5 ± 0.03	< 4
31	S	H	2,4-dichlorophenyl	H	H	H		4.7 ± 0.12	< 4.5
32	N	methyl	H	H	H	H	H	5.5 ± 0.15	n.t.
33	N	propyl	H	H	H	H	H	6.3 ± 0.10	3.8 ± 0.16
34	N	H	H	Cl	H	H	H	4.7 ± 0.09	n.t.
35	N	H	H	H	Cl	H	H	4.9 ± 0.17	n.t.
36	N	H	H	H	H	Cl	H	5.5 ± 0.05	n.t.
37	N	H	Cl	H	H	H	H	6.0 ± 0.19	4.1 ± 0.08
38	N	H	OCH_3	H	H	H	H	5.9 ± 0.07	4.1 ± 0.05
39	N	H	phenyl	H	H	H	H	5.6 ± 0.08	4.7 ± 0.03
40	N	H	*o*-tolyl	H	H	H	H	6.5 ± 0.11	5.2 ± 0.13
41	N	H	2-propylphenyl	H	H	H	H	7.3 ± 0.11	5.4 ± 0.05
42	N	H	2-isopropylphenyl	H	H	H	H	7.3 ± 0.07	5.6 ± 0.10
43	N	H	2-benzylphenyl	H	H	H	H	6.0 ± 0.11	< 5.5
44	N	H	2-dimethylamino-phenyl	H	H	H	H	5.8 ± 0.04	4.1 ± 0.01
45	N	H	2-methoxyphenyl	H	H	H	H	5.6 ± 0.04	4.3 ± 0.12
46	N	H	H	H	H	H	methyl	4.9 ± 0.09	n.t.

[a] The affinities of the compounds for the glycine and glutamate recognition sites are expressed as pK_i values and were determined in [^{3}H]glycine ($N \geq 3$) and [^{3}H]CGP39653 ($N \geq 2$) binding assays as described in the Experimental Section. The data shown are mean values ± SEM. n.t.: not tested.

Table 4. Affinities of 3-Acylamino-2-aminopropionic Acid Derivatives for the Glycine and Glutamate Recognition Sites of the NMDA Receptor: Disubstituted Bicyclic Ring Systems in the Acyl Group



compd	R1	R2	R5	R6	glycine[a] pK_i	glutamate[a] pK_i
47	propyl	Br	H	H	6.5 ± 0.02	4.2 ± 0.25
48	propyl	2-ethylphenyl	H	H	6.8 ± 0.07	5.3 ± 0.04
49	nitro	Br	H	H	6.1 ± 0.05	4.9 ± 0.12
50	H	2-ethylphenyl	Cl	H	6.9 ± 0.06	< 4.5
51	H	Cl	Cl	H	6.8 ± 0.15	4.0 ± 0.11
52	H	Br	H	methyl	5.0 ± 0.04	n.t.
53	H	2-ethylphenyl	H	methyl	3.9 ± 0.05	n.t.

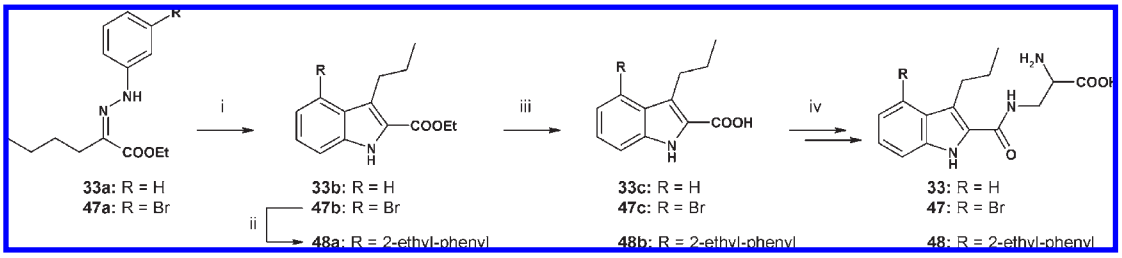
[a] The affinities for the glycine and glutamate recognition sites of the compounds listed in the table are expressed as pK_i values and were determined in [^{3}H]glycine ($N \geq 3$) and [^{3}H]CGP39653 ($N \geq 2$) binding assays as described in the Experimental Section. The data shown are mean values ± SEM. n.t.: not tested.

at the indolyl N-atom had no effect in the otherwise unsubstituted molecule **46**, it dramatically decreased the affinities of the compounds bearing a bromo- or 2-ethylphenyl group in position 4 (**52**, **53**). In other cases, however, appropriate disubstitution could well increase glycine affinity when compared to the corresponding monosubstitutions. For example, the 4,7-dichloro compound **51** had a higher affinity than the two monochloro derivatives **36** and **37**.

The results in Tables 1−4 show that many of the compounds described in this study bind not only to the strychnine-insensitive glycine coagonist site but also, albeit with much weaker affinities, to the glutamate recognition site, labeled by [^{3}H]CGP39653. Their activity at this site was not due to indirect (allosteric) inhibition of [^{3}H]CGP39653 binding via agonism at the glycine site,[30,31] since the glycine antagonist 5,7-dichlorokynurenic acid was added to the assay mixture to block such an interaction. On the other hand, a limited selection of 3-acylamino-2-aminopropionic acid derivatives (**9**, **10**, **17**, **R-14**, **15**, **20**, **R-22**, **24**, **R-26**, **27**, **29**, **31**, **33**) was tested in assays for the ion channel site ([^{3}H]MK-801 binding measured under equilibrium conditions) or the polyamine site associated with the NMDA receptor ([^{3}H]spermidine binding). None of them showed any activity (data not shown).

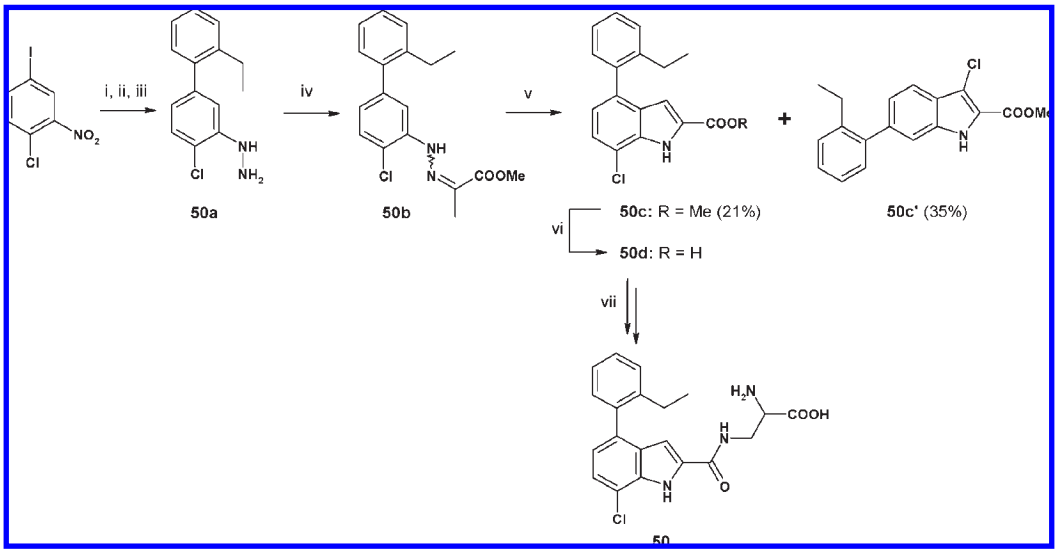
When measured under nonequilibrium conditions, the stimulation of the binding of [^{3}H]MK-801 can be used as a functional assay for compounds acting at the NMDA receptor complex.[32,33] The rate of association of the noncompetitive NMDA receptor antagonist [^{3}H]MK-801 to its binding site inside the receptor-operated ion channel depends on the degree of channel opening, which is, in turn, a function of the activation of the glutamate and glycine sites on the receptor. In our experiments, stimulation of the glycine coagonist site was monitored under conditions where the transmitter recognition site was maximally activated by a saturating concentration of glutamate. In this setup glycine, ACC, and both enantiomers of serine stimulated the binding of [^{3}H]MK-801 to the same maximal level, with the expected potencies and enantioselectivity and with D-serine being about a 100-fold more potent than L-serine (data not shown in detail). The well established partial agonist D-cycloserine,[34] on its own, stimulated the binding of [^{3}H]MK-801 maximally by about 50% of the effect obtained with a saturating concentration of

Scheme 2[a]



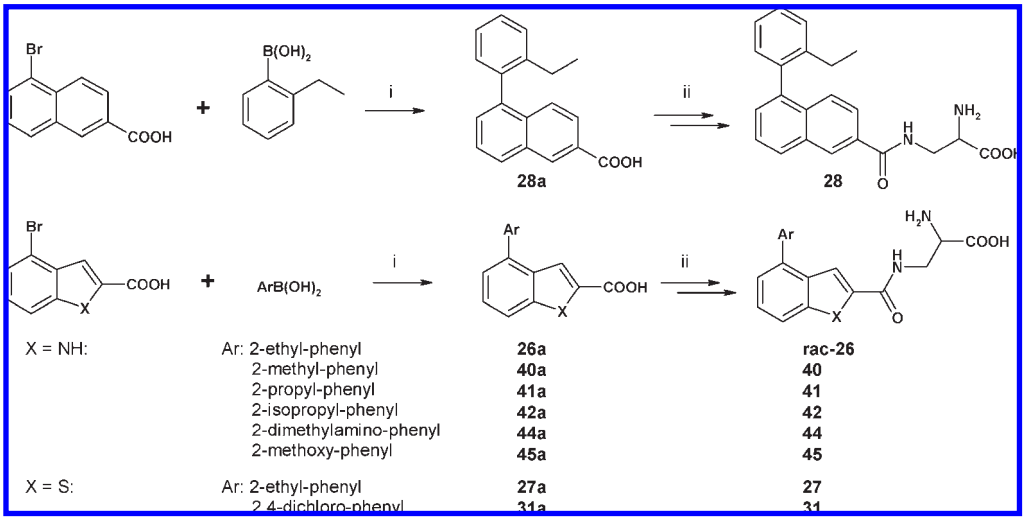
[a] (i) H_2SO_4, ethanol, reflux; (ii) 2-ethylphenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, ethanol, toluene, reflux; (iii) aqueous base, methanol or ethanol, reflux; (iv) according to Scheme 1.

Scheme 3[a]



[a] (i) 2-Ethylphenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, ethanol, toluene, reflux; (ii) Fe, NH_4Cl, H_2O, methanol, reflux; (iii) $NaNO_2$, HCl aq, 0 °C, 5 h, then $SnCl_2$, HCl conc, 30 min; (iv) 2-oxopropionic acid methyl ester, H_2SO_4 conc, H_2O, ethanol, 10 °C; (v) polyphosphoric acid, 140 °C; (vi) 1 M NaOH, methanol, reflux; (vii) according to Scheme 1.

Scheme 4[a]

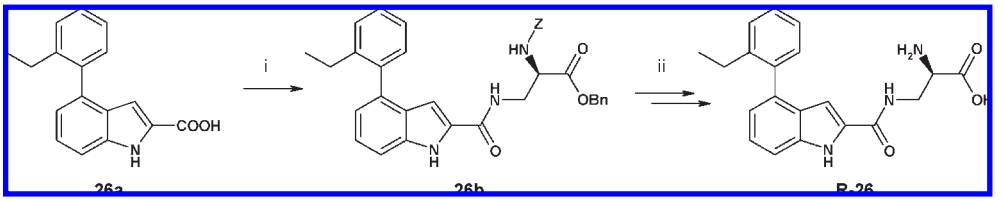


[a] (i) $Pd(PPh_3)_4$ cat., 2 M Na_2CO_3, ethanol, toluene, reflux; (ii) according to Scheme 1.

glycine (Figure 2). In the presence of a maximally active glycine concentration, however, D-cycloserine inhibited [^{3}H]MK-801 binding down to a level similar to the maximal activation that it produced when added alone. The same typically partial agonistic behavior was found with compound **R-26** (Figure 2).

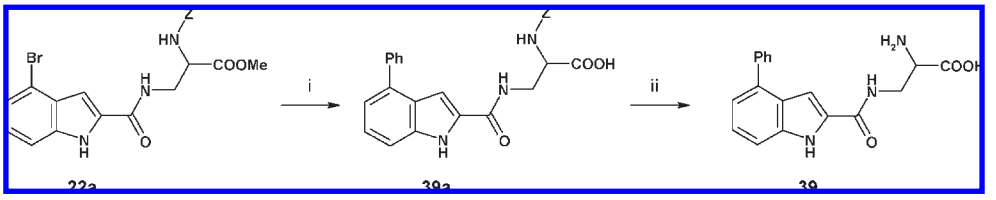
However, the determination of the maximal degree of agonistic effect ("efficacy") of a compound in this assay is complicated by the fact that under basal conditions (i.e., without addition of an agonist), the glycine site is already activated to some extent by endogenous glycine presumably

Scheme 5[a]



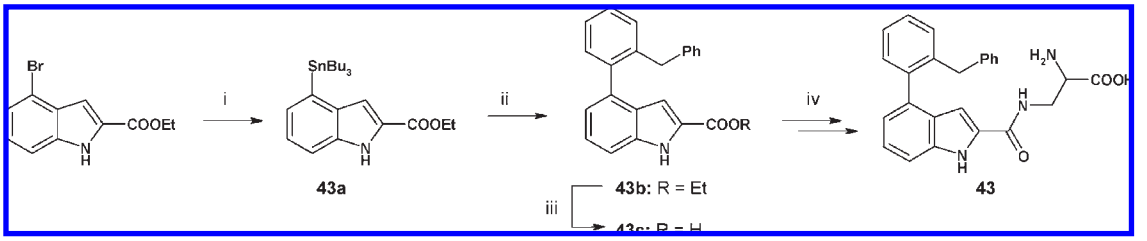
[a] (i) **R-5c**, 1,1′-carbonyldiimidazole, pyridine, room temp (81%); (ii) H_2, Pd−C, AcOH, room temp (89%).

Scheme 6[a]



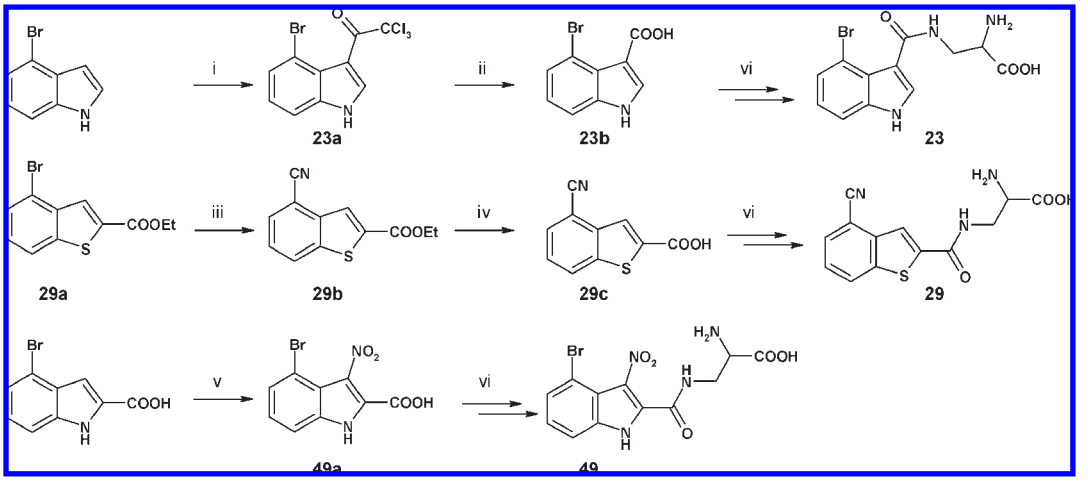
[a] (i) Phenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, ethanol, toluene, reflux; (ii) $ISiMe_3$, CH_2Cl_2, 0 °C.

Scheme 7[a]



[a] (i) $(SnBu_3)_2$, $Pd(PPh_3)_4$, 2 M Na_2CO_3, ethanol, toluene, reflux; (ii) 1-benzyl-2-bromobenzene, $PdCl_2(PPh_3)_2$, DMF, 120 °C; (iii) 1 M NaOH, methanol, reflux; (iv) according to Scheme 1.

Scheme 8[a]

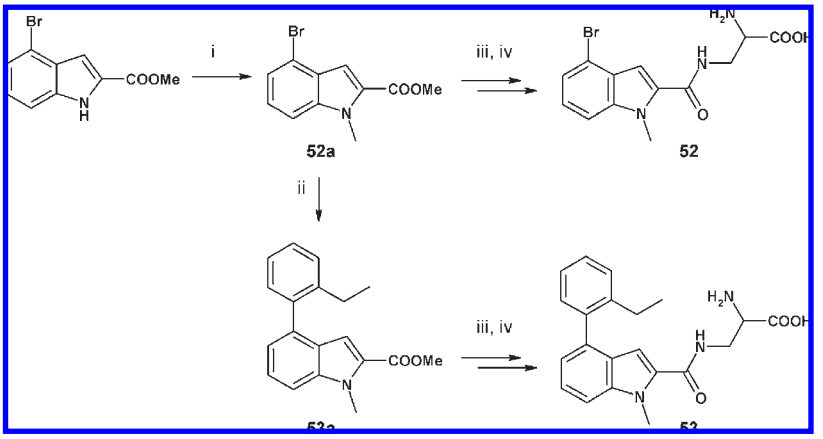


[a] (i) Cl_3CCOCl, pyridine, dioxane, reflux; (ii) 1 M KOH, ethanol, room temp; (iii) CuCN, *N*-methylpyrrolidone, reflux; (iv) 1 equiv of Na_2CO_3, H_2O, methanol, dioxane, reflux; (v) HNO_3, acetic anhydride, 0 °C; (vi) according to Scheme 1.

present in the membrane preparation.[35] This phenomenon is illustrated by the observation that [^{3}H]MK-801 binding under basal conditions is inhibited by the glycine site antagonist 5,7-dichlorokynurenic acid (Figure 3).[36] For this reason, we calculated the efficacies of compounds with reference to a "zero level" which was determined in the presence of a maximally active concentration (30 μM) of 5,7-dichlorokynurenic acid (Figure 3). According to this procedure, a compound like *cis*-methyl-HA966 (L-687414[37]), which also inhibits [^{3}H]MK-801 binding under basal conditions but not to the same extent as the pure antagonist 5,7-dichlorokynurenic acid, is recognized as a partial agonist with an efficacy of 14%, which is less than the degree of basal receptor activation by endogenous glycine (Figure 3).

Table 5 gives an overview of the potencies (pEC_{50} or pA_2 values) and efficacies of a selection of 3-acylamino-2-aminopropionic acid derivatives in this assay. Their potencies showed an excellent correlation with the affinities found in

Scheme 9[a]



[a] (i) NaH, MeI, DMF, room temp; (ii) 2-ethylphenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, ethanol, toluene, reflux; (iii) 1 M NaOH, methanol, reflux; (iv) according to Scheme 1.

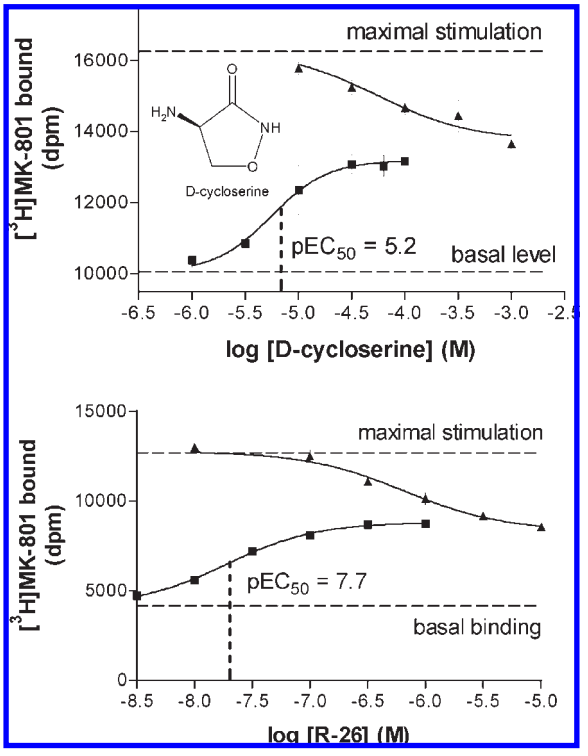


Figure 2. Partial agonism at the glycine site by D-cycloserine (top) and compound **R-26** (bottom). The potentiation of [^{3}H]MK-801 binding was measured under nonequilibrium conditions in the presence of a maximally active concentration of glutamate, as described in the methods section. The compounds were tested either alone (squares) or in combination with 1 μM glycine (triangles). Basal levels were determined in the absence of any glycine ligand. Maximal stimulation was measured in the presence of 3 μM glycine, and 10 μM glutamate was present in all samples.

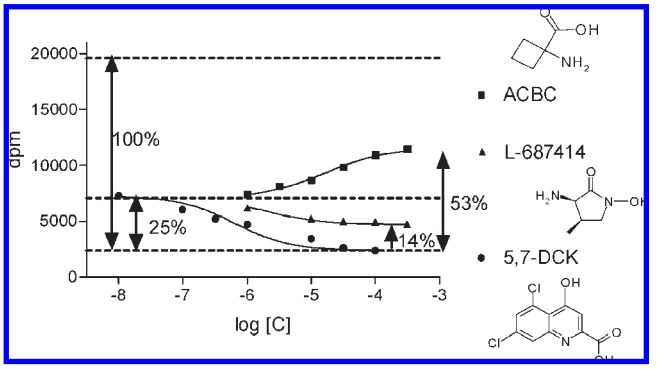


Figure 3. Method of determining the efficacy of partial agonists acting at the glycine site. The nonequilibrium binding of [^{3}H]MK-801 under basal conditions (no agonist added, middle dashed line) is already activated to about 25% relative to the maximally possible stimulation (with a saturating concentration of glycine, upper dashed line) because of the endogenous glycine present. This is demonstrated by an inhibition of [^{3}H]MK-801 binding under basal conditions by the glycine site antagonist 5,7-dichlorokynurenic acid (5,7-DCK, ●). The efficacies (maximal stimulation obtained, relative to maximal effect of glycine) of partial agonists were therefore determined relative to a zero level (lower dashed line) measured in the presence of a saturating concentration of 5,7-DCK. This principle is illustrated with ACBC, having an efficacy of 53%. A low-efficacy partial agonist such as L-687414 (▲) having an efficacy that is lower than the basal activity because of endogenous glycine will result in a partial inhibition of this basal activity.

the [^{3}H]glycine binding assay (slope = 1.0, $r^2 = 0.82$, $p < 0.01$). On the other hand, their intrinsic efficacies (maximal degrees of stimulation) did not go in parallel with their binding affinities. For example, the compound with the highest efficacy (about 80% of the maximal effect of glycine, Table 5) found in the series was the furane analogue **10**, and the corresponding pyrrolo and thiophene analogues **9** and **11** also stimulated [^{3}H]MK-801 binding to a relatively high level (about 60% of the maximal effect of glycine). All three compounds had only relatively weak binding affinities for the glycine site (Table 1). On the other hand, the compound with the highest binding affinity, **R-26**, had somewhat less intrinsic efficacy (about 50%). Interestingly, some compounds with very low efficacies (e.g., **29** around 20%) or even fully antagonistic properties (**31**), were also found (Table 5).

In the search of a pharmacophore model accounting for the affinities of the 3-acylamino-2-aminopropionic acids at the strychnine-insensitive glycine site, we assessed conformational preferences of **R-26**, **27**, and **53**. The *N*-methylamides **54**−**56** were used as respective model systems for molecular modeling (Table 6). On the basis of ab initio energy differences, the s-cis conformation of the indoles **54** and **55** is favored by 4.7 and 5.0 kcal/mol, respectively, whereas no significant energy difference between the s-cis and s-trans conformations results with the benzothiophene **56**. Therefore, we assume that the

s-cis conformation corresponds to the active one which is, according to our calculations, also accessible to the benzothiophene **56**.

Discussion

Partial agonism at the glycine coagonist site of the NMDA receptor complex might be an attractive therapeutic principle because it would make it possible to stabilize the receptor efficacy at a certain desired level without completely closing down normal synaptic signal transmission. To date, only a small number of partial glycine site agonists are known, such as D-cycloserine,[34,39] HA-966,[40,41] or some cyclic homologues of glycine.[23] Retaining agonistic activity in glycine-derived molecules has proven to be difficult because of severe space restrictions for substituents at or in the immediate vicinity of the amino acid group. However, for the reasons outlined in the introduction, we felt that mimicking the hydroxy group of D-serine in a way that preserves its potential for hydrogen bond formation, i.e., by an amido group, might allow substitution and thereby exploration of the space adjacent to this part of the molecule.

Table 5. Potencies and Efficacies of 3-Acylamino-2-aminopropionic Acid Derivatives in a Functional Assay (Stimulation of [^{3}H]MK-801 Binding under Nonequilibrium Conditions) for the Glycine Recognition Site of the NMDA Receptor

compd	pEC_{50} or pA_2	efficacy (%)[a]	*N*
10	5.1 ± 0.10	81 ± 5	8
41	7.6 ± 0.16	64 ± 1	5
9	5.0 ± 0.15	63 ± 7	4
11	5.6 ± 0.10	63 ± 3	5
40	6.9 ± 0.14	62 ± 4	4
44	6.5 ± 0.05	59 ± 2	3
R-26	7.7 ± 0.09	55 ± 2	4
43	6.5 ± 0.10	55 ± 10	2
R-22	6.6 ± 0.09	45 ± 4	4
R-14	5.3 ± 0.10	42 ± 1	3
47	6.0 ± 0.03[b]	38 ± 2.5	3
51	6.2 ± 0.18[b]	32 ± 2	3
33	5.7 ± 0.12[b]	28 ± 1	3
29	5.5 ± 0.23[b]	22 ± 2	3
31	5.1 ± 0.20[b]	none (antagonist)	3

[a] The compounds listed were tested in a functional NMDA receptor assay (potentiation of [^{3}H]MK-801 binding under nonequilibrium conditions) as described in the Experimental Section. The efficacies are expressed relative to the maximal effect of glycine (=100%) and are based on a zero-level defined in the presence of 30 μM 5,7-dichlorokynurenic acid (Figure 3). [b] For the compounds with a low intrinsic efficacy (similar to the basal level determined by the presence of endogenous glycine or less), potencies are expressed as pA_2 values and were determined from their apparent antagonism against glycine (Schild plots[38]). The data shown are mean values ± SEM from *N* independent determinations.

It quickly became clear that the resulting 3-acylamino-2-aminopropionic acids indeed bear a considerable potential as ligands at the strychnine-insensitive glycine coagonist site associated with the NMDA receptor complex. Compounds with bicyclic aromatic ring systems (**13**, **R-14**, **15**, **17**−**21**), were more potent than monocyclic analogues (**8**−**11**), provided the conjugation between the ring system and the carbonyl group of the side chain was maintained (see the inactive compounds **12** and **16**, Table 1). Attachments of substituents to the bicyclic ring systems reveal that the 2-indolyl scaffold was the most promising of those tried for optimization of binding affinity. For example, the attachment of a bromo- or 2-ethylphenyl substituent in position 4 of the indole ring resulted in massive increases in glycine site binding affinity, while similar substitutions at the 3-indolyl, 2-benzothiophene, or the 2-naphthyl scaffolds lead to less or no improvement of affinities (Table 2).

Structure−activity relationships in the indole-2-carbonyl-diaminopropionic acid series showed that introducing substituents in the indole position 3 (R1) and, even more importantly, position 4 (R2) was the most successful measure to further increase the affinities of these types of compounds. The increase of affinities (compared to the unsubstituted **R-14**) obtained by different lipophilic substituents in position 4 of these compounds is a sign that the groups attached reach into a specifically shaped lipophilic area of the glycine site. Thus, groups such as methoxy (**38**), chloro (**37**), bromo (***rac*-22**), or phenyl (**39**) give rise to significant increases of affinity. The highest increase was obtained with 2-ethylphenyl (***rac*-26**) in position 4. A comparison between phenyl and 2-ethylphenyl indicates a special role for the ethyl group: as calculated by quantum chemical geometry optimization of achiral model systems (not shown), the torsion angle between the indolyl system and the 4-phenyl ring (as in **39**) as well as between the indolyl system and the 4-(2-ethylphenyl) ring (as in ***rac*-26**) corresponds for both at their minimum energy to a gauche conformation; in other words, the ethyl group of ***rac*-26** has no critical influence on the preferred orientation of the phenyl ring. From this we conclude that the 2-ethyl substituent improves the binding affinity likely by interacting with an additional part of the glycine site and not by mere stabilization of the active conformation.

While methylation of the indolyl N-atom has no effect on the affinity of the unsubstituted compound **R-14** (compare with **46**), it dramatically reduces the one of the 2-ethylphenyl

Table 6. Energy Differences from ab Initio Geometry Optimization of Model Systems **54**−**56**[a]

	"s-cis"		"s-trans"		
X	*t* (deg)	energy (hartree)	*t* (deg)	energy (hartree)	*E*(s-cis) − *E*(s-trans) (kcal/mol)
NH (**54**)	0.0	−571.445 418	159.1	−571.437 954	−4.7
N-Me (**55**)	20.4	−610.721 292	143.4	−610.713 387	−5.0
S (**56**)	17.9	−914.237 384	179.7	−914.238 429	0.7

[a] Structures **54**−**56** are taken as model systems of respective compounds **26**, **27**, and **53**. Constrained geometry optimizations were carried out with the Turbomole 5.5 program on the BP86 level of theory, taking advantage of the RI-DFT method using the SV(P) basis set. The torsion angle *t* was constrained from 180° to 0° in steps of 20°. *t*: torsion angle X−C−C=O.

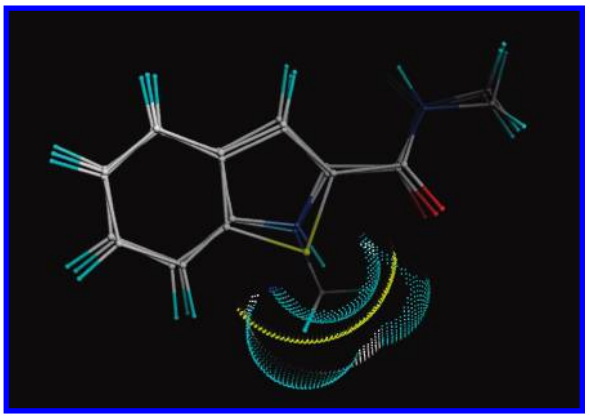


Figure 4. Display of the least-squares superimposition of S-cis models of **55** and **56** on **54** with respect to their common substructures of non-hydrogen atoms. The z-clipped van der Waals surfaces of the NH (H in cyan), S (in yellow), and NCH_3 (C in white, H in cyan) groups illustrate the significant change in volume requirements of **54**, **56**, **55**. The unclipped surface areas vary from 14 $Å^2$ (**54**) and 26 $Å^2$ (**56**) to 37 $Å^2$ (**55**).

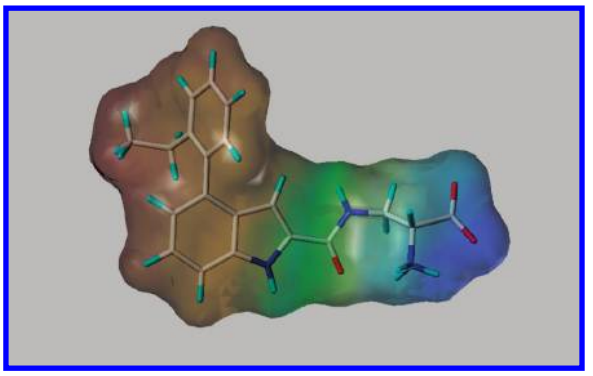


Figure 5. Hypothetical active s-cis conformation of **R-26** displayed with solvent-accessible surface colored by lipophilicity. Highly polar 3-acylamino-2-aminopropionic acid substructure is colored from blue to green, and highly lipophilic biphenyl substructure is colored in brown. Model and lipophilic surface representation was generated with MOLCAD within Sybyl8.0 (Tripos International, 1699 South Hanley Road, St. Louis, MO 63144) using default parameters.

derivative ***rac*-26** (compare with **53**). It seems likely that the decrease of affinity produced by indolyl-N-methylation comes from a steric conflict evoked by the 4-phenyl substituent, which would be moved into a position unfavorable for receptor binding. The fact that the affinity of the 2-ethylphenylbenzothiophene analogue **27** (pK_i = 6.2) is between those of the NH- and NCH_3-indolyl derivatives (***rac*-26**, pK_i = 7.5; **53**, pK_i = 3.9) supports this contention, since the space occupied by a sulfur atom is intermediate between that of an NH and an N methyl groups, respectively (Figure 4). The same rank order of affinities is found in the 4-bromo-substituted series **52** (4-bromoindolyl-*N*-methyl, pK_i = 5.0), **24** (4-bromobenzothiophene, pK_i = 6.4), and ***rac*-22** (4-bromoindolyl-NH, pK_i = 6.8), again illustrating the increase of affinity with decreasing steric bulk at the heteroatom of the bicyclic ring system. The differences are less pronounced, however, which is in line with the smaller size of the bromo atom compared with the 2-ethylphenyl group. With an even smaller chloro atom in position 4, no steric hindrance is produced anymore by replacing the indolyl by a benzothiophene ring system; in fact, compound **30** (pK_i = 6.5) is somewhat more potent than **37** (pK_i = 6.0), suggesting that the slightly increased bulk of the S-atom moves the 4-chloro atom into a more favorable position for binding. A similar steric influence is exerted by a chloro-atom in position 7 of the indole nucleus. While it has no effect on glycine site affinity on its own (compare **36** with **R-14**), it results in increased affinity of the 4,7-dichloro substituted compound **51** in comparison with **37**, again suggesting that the slightly bulky 7-chloro group moves the 4-chloro substituent into a favorable position for binding. In conjunction with the much more space-filling 2-ethylphenyl substituent, however, the 7-chloro atom has a detrimental effect on glycine site affinity (**50** vs ***rac*-26**), although much less than indolyl-N-methylation.

One hypothetical active conformation of **R-26**, as derived in part by conformational analysis, is shown in Figure 5. Since the phenyl ring on its own did not increase glycine site affinity (compare **R-14** and **39**), it seems conceivable that the essential part of the phenylethyl substituent is the ethyl group, reaching into a separate attachment site, and that the phenyl ring merely acts as a "scaffold" to support this ethyl group. The solvent-accessible surface shown in Figure 5 may be considered as an approximation of the contour of the ligand-induced receptor conformation.

In order to assess our pharmacophore hypothesis, we attempted to model the binding of **R-26** to the ligand binding domain of NR1 using the conformation observed in the X-ray structure of the NR1/cycloleucine complex.[42] We chose the cycloleucine complex for modeling because the binding cavities revealed in the complexes with glycine, D-serine, DCS, ACC, and ACBC were too small for accommodation of **R-26**. Several different binding modes were modeled whereby each mode involved the salt bridges with Arg523 and Asp732 also reported for the complex of cycloleucine and related partial agonists. Different regions of the NR1 glycine binding domain were found to be accessible to the *o*-ethylphenyl moiety of **R-26**, but in contrast to the observed hydrogen bonding of D-serine, any hydrogen bond of its amide proton was absent. However, given the significant ligand-induced conformational changes of the NR1 constructs, the validity of our models is too uncertain to support any pharmacophore hypothesis.

3-Acylamino-2-aminopropionic acid analogues also bind to the glutamate recognition site at the NMDA receptor, labeled by [^{3}H]CGP39653 (Tables 1−4). However, the increases in glycine site affinities obtained by the derivatizations discussed above do not lead to similar increases in affinities for the glutamate site; as a result, more than a 100-fold selectivity for the glycine over the glutamate site were found for a number of compounds, for example, **R-22** and many others; see Tables 1−4.

The structure−activity relationships that govern the affinities of these types of compounds do not predict their intrinsic efficacies at the glycine coagonist site of the NMDA receptor. In the series of compounds presented here, the one with the highest efficacy to stimulate [^{3}H]MK-801 binding under nonequilibrium conditions was the furane derivative **10**, which had only a moderate binding affinity for the glycine site (cf. Tables 1 and 5). At the other end of the scale, compound **31**, which had a similar affinity, was found to be a "silent" glycine antagonist without any intrinsic efficacy at all. At present, it seems that no pattern of structure−activity relationships can be derived from our data to explain the rules governing the intrinsic efficacies of 3-acylamino-2-aminopropionic acids at the glycine site.

In conclusion, the compounds of the 3-acylamino-2-aminopropionic acid type presented here are a novel class of ligands acting at the strychnine-insensitive glycine coagonist site associated with the NMDA receptor. They provide the proof that it is possible to retain high affinity and different degrees of intrinsic efficacy in molecules derived from D-serine. Extension of an amido analogue of D-serine by monocyclic or bicyclic (preferably indolyl) aromatic ring systems maintains moderate to high affinity for the glycine site. Occupation of an additional lipophilic pocket by suitable substituents in position 4 of the indolyl ring system confers nanomolar affinity to this type of compound but leaves little steric tolerance for further substitution. The resulting compounds have various degrees of intrinsic activity, ranging from pure antagonism to high efficacy (80%) partial agonism.

Experimental Section

Binding Assays for Various Recognition Sites on the NMDA Receptor Complex. The rat brain cortex membrane preparation used was the same for all NMDA receptor related binding assays and was based on procedures described earlier.[43] The binding of [^{3}H]glycine to the strychnine-insensitive coagonist site was measured as described previously.[43] [^{3}H]CGP39653[44] was used as a ligand to label the NMDA recognition site. The binding assay was performed as described previously,[45] with the following modification. Because complex interactions between the glutamate and glycine recognition sites are known to occur in radioligand binding studies,[30,31] 30 μM 5,7-dichlorokynurenic acid was added to the assay to occlude an allosteric inhibition of [^{3}H]CGP39653 binding produced by agonism of the test compounds at the glycine site. To determine the affinities of compounds for the MK-801 site within the NMDA receptor-operated ion channel, [^{3}H]MK-801 binding was measured under equilibrium conditions, also as described earlier.[45] The affinities of compounds for the polyamine site associated with the NMDA receptor complex were assessed in binding experiments using [^{3}H]spermidine as a radioligand.[46] The assay mixture (final volume of 1 mL) in 50 mM Tris-HCl buffer (pH 7.0) consisted of an aliquot of membranes prepared as described above (corresponding to approximately 10 mg of original tissue weight), 20 nM [^{3}H]spermidine, and the test compounds at the appropriate concentrations. Nonspecific binding was measured in the presence of 10 mM unlabeled spermidine. The samples were incubated for 30 min at 0 °C and were then filtered through Whatman GF/B filters which had been soaked in a 0.3% polyethyleneimine solution for at least 1 h prior to use. The filters were then washed with ice-cold incubation buffer and further processed as described above.

In all receptor binding assays, pIC_{50} values were derived from the curves describing the inhibition of specific [^{3}H]ligand binding by the test compounds and converted into pK_i values using the Cheng−Prusoff relationship. Prism 3.0 software (GraphPad Software, San Diego, CA) was used for these calculations.

Functional NMDA Receptor Test. MK-801 Potentiation Assay. The final pellet of the rat brain membrane preparation[43] was resuspended in ice-cold Tris-HCl buffer (10 mM, pH 7.4) to give a final concentration of 10−15 mg of original tissue weight (about 50−200 mg of protein) per sample. In the binding experiment, this membrane suspension was preincubated for 15 min at 25 °C in the presence of a saturating concentration (10 μM) of glutamate and varying concentrations of compounds acting at the glycine site as indicated in Results. [^{3}H]-MK-801 (15−30 Ci/mMol, DuPont NEN) was then added to give a concentration of 3 nM in a final assay volume of 2 mL. After further incubation at 25 °C for 15 min (nonequilibrium conditions), bound and free radioligand were separated by filtration through Whatman GF/B filters, using a Brandel M24R cell harvester. The radioactivity on the filters was then determined by liquid scintillation counting.

Data Analysis. The binding in the presence of 10 μM glutamate, but in the absence of any added glycine agonist, was named "basal binding". However, it was found that a glycine antagonist like 5,7-dichlorokynurenic acid inhibited this basal binding to a considerable extent, suggesting that the glycine site was already activated by about 25% by endogenous glycine under basal conditions (Figure 3). A "zero level" was therefore determined in the presence of 30 μM 5,7-dichlorokynurenic acid, and the intrinsic efficacies of partial agonists were calculated with reference to this zero level. Figure 3 illustrates this principle.

Molecular Modeling. Modeling was performed using our in-house software WitNotP [Widmer, A. *WITNOTP: A Computer Program for Molecular Modelling*; Novartis Pharma AG: Basel, Switzerland, 1997] using the Tripos force field (TAFF) of Sybyl 6.4 [Tripos, Inc., St. Louis, MO] and Turbomole5.5 on a HPxw8200 workstation under Linux.[47] Geometries of molecules were obtained by building molecular models from fragments, followed by TAFF energy minimization. The vdW surfaces were calculated with WitNotP.

Chemistry. Chromatographic separations in preparative scale were performed either by flash chromatography[48] or by medium pressure liquid chromatography (MPLC) using a UV detector and a pump. In all cases 40−60 μm silica gel (Merck) was used. If not otherwise specified, ^{1}H NMR spectra for intermediates were taken on a Gemini 200 MHz spectrometer. The target compounds were uniformly obtained as white powders and characterized by ^{1}H NMR spectra (Bruker 360 MHz spectrometer). Chemical shifts for ^{1}H are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard. Combustion analyses were performed by the Analytical Department of Novartis AG, and values are within 0.4% of theoretical values unless otherwise specified.

Method A: General Procedure for the Acylation of *rac*-5b with a Carboxylic Acid Chloride. To a 10% solution of ***rac*-5b** in a 1:1 mixture of dry pyridine and dichloromethane at −10 °C a solution of 1.3 equiv of the acid chloride in dichloromethane was slowly added. After being stirred for 45 min at −10 °C, the mixture was poured onto ice−water and extracted with ethyl acetate. The combined organic phases were washed with aqueous citric acid, dried over Na_2SO_4, and evaporated. The residue was purified by MPLC with *tert*-butyl methyl ether as eluent.

Method B: General Procedure for the Condensation of Carboxylic Acids with *rac*-5b. To a suspension of the carboxylic acid in pyridine (1−2%) an amount of 1.1 equiv of carbonyldiimidazole was added. The mixture turned to a yellow solution and was stirred overnight. After addition of 1 equiv of ***rac*-5b** stirring was continued for 2 days, the mixture evaporated to dryness, and the residue crystallized from hot ethanol.

Method C: General Procedure for the Saponification of *rac*-3-Acylamino-2-benzyloxycarbonylaminopropionic Esters. To a 3% solution of the substrate in THF 1 equiv of 1 M aqueous NaOH solution was added. The mixture was stirred at room temperature until the starting material had disappeared (TLC). After dilution with H_2O, the THF was evaporated, the aqueous phase was acidified with 1 M aqueous HCl solution and extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated to dryness.

Method D: General Procedure for Removal of the Z-Group by Hydrogenation. A 5% solution of the Z-protected amino acid in 1:1 THF/H_2O was treated with about 20% (w/w) of Pd on carbon (5%) and hydrogenated until no hydrogen was taken up. After filtration the catalyst was washed with 1:1 THF/H_2O and the filtrate evaporated to dryness. The residue was crystallized from the specified solvent.

Method E: General Procedure for Removal of the Z-Group by Iodotrimethylsilane. A 5% solution of the substrate in dichloromethane was treated with 2.2 equiv of iodotrimethylsilane at 0 °C, and the mixture was stirred for 1 h. The reaction mixture was concentrated and the residue distributed between water and

ethyl acetate. The pH of the water phase was adjusted to 6 with 0.1 M NaOH solution, and the precipitate was collected and recrystallized from water/isopropanol.

Method F: General Procedure for the Pd-Catalyzed Coupling Reaction between Arylboronic Acids and Aryl Halogenides. An amount of 1 equiv of the aryl halogenide, 1.1−1.7 equiv of the arylboronic acid, and 3 mol % of tetrakistriphenylphosphine-palladium were refluxed in a 10:3:2 mixture of toluene, ethanol, and 2 M Na_2CO_3 solution until the aryl halogenide disappeared (TLC). After filtration the phases were separated and the aqueous phase was extracted with diethyl ether and acidified with 6 M HCl solution. Extraction of the acidic water phase with ethyl acetate, drying of the organic phase over Na_2SO_4, and evaporation yielded the coupling product, in most cases sufficiently pure for the next step.

***rac*-3-Amino-2-benzyloxycarbonylaminopropionic Acid *rac*-5a.** ***rac*-5a** was prepared (69%) using the literature procedure for **R-5a**[26] but starting from *rac*-Z-asparagin. Mp 235−239 °C (dec). ^{1}H NMR (D_2O + DCl): 7.40 br s, 5H; 5.15 s, 2H; 4.60−4.45 m, 1H; 3.60−3.45 m, 1H; 3.40−3.20 m, 1H.

***rac*-3-Amino-2-benzyloxycarbonylaminopropionic Acid Methyl Ester Hydrochloride *rac*-5b.** ***rac*-5b** was prepared (71%) using the literature procedure for **R-5b**[26] but starting from ***rac*-5a**. Mp 136−138 °C. ^{1}H NMR (CD_3OD): 7.40−7.25 m, 5H; 5.10 s, 2H; 4.55−4.45 m, 1H; 3.75 s, 3H; 3.50−3.40 m, 1H; 3.30−3.15 m, 1H.

(*R*)-2-Amino-3-[(1*H*-indole-2-carbonyl)amino]propionic Acid R-14. Acylation of 4.33 g of **R-5b** with indole-2-carbonyl chloride according to method A gave 5.71 g (96%) of (*R*)-2-benzyloxycarbonylamino-3-[(1*H*-indole-2-carbonyl)amino]-propionic acid methyl ester **R-14a**, mp 178 °C. ^{1}H NMR ($CDCl_3$): 9.50 s, 1H; 7.60 d, J = 9 Hz, 1H; 7.40 d, J = 9 Hz, 1H; 7.35−7.20 m, 6H; 7.15−7.05 m, 1H; 7.05−6.95 m, 1H; 6.85 s, 1H; 6.5 d, J = 7 Hz, 1H; 5.10 s, 2H; 4.65−4.50 m, 1H; 3.95−3.80 m, 2H; 3.75 s, 3H. A solution of 3.95 g (10 mmol) of **R-14a** in 50 mL of THF was added to a slurry of 11.3 mL of Dowex OH^- (capacity, 0.974 mmol OH^-/mL, prepared by elution of Dowex Cl^- with 2 M NaOH solution until the eluent gave no precipitation with diluted $AgNO_3$ solution) in 30 mL of H_2O. The mixture was stirred for 20 h at room temperature, during which time small portions of Dowex OH^- were added until no starting material was detected. The Dowex OH^- was filtered and, after washing with 300 mL of 1:1 THF/H_2O, filled into a glass column and eluted with 2 M HCl solution/THF, 1:1. The product fractions were combined and concentrated and the residue was dissolved in hot acetone, resulting in a deeply dark solution that was decolorized by addition of carbon. After filtration, the product was precipitated by addition of H_2O, yielding 2.20 g (57.6%) of (*R*)-2-benzyloxycarbonylamino-3-[(1*H*-indole-2-carbonyl)amino]propionic acid **R-14b** as a colorless powder, mp 196−206 °C. $[\alpha]^{20}_D$ 6.90 (*c* 0.990, acetone). ^{1}H NMR ([(CD_3)$_2$SO]): 11.60 s, 1H; 8.55 br t, 1H; 7.70−7.55 m, 2H; 7.40 d, J = 9 Hz, 1H; 7.35−7.25 m, 5H; 7.15 t, J = 9 Hz, 1H; 7.10−6.95 m, 3H; 5.00 s, 2H; 4.35−4.20 m, 1H; 3.75−3.50 m, 2H. Anal. ($C_{20}H_{19}N_3O_5$) C, H, N, O. Deprotection of **R-14b** according to method D yielded (95%) **R-14** as a white powder, mp 242 °C (dec; H_2O/THF, 1:1). Thin layer chromatography on Chiralplate (Machery-Nagel)[49] with $CH_3CN/CH_3OH/H_2O$, 4/1/1, showed a single spot, R_f = 0.46 (ninhydrine). No contamination with the enantiomer **S-14** was observed. $[\alpha]^{20}_D$ 2.5° (*c* 1.00, 1 M NaOH). ^{1}H NMR (D_2O + DCl): 7.75 d, J = 7 Hz, 1H; 7.58 d, J = 8 Hz, 1H; 7.38 t × d, J = 7 and 1 Hz, 1H; 7.20 t, J = 7 Hz, 1H; 7.16 s, 1H; 4.40−4.35 m, 1H; 4.06−3.90 m, 2H. Anal. ($C_{12}H_{13}N_3O_3 \cdot 0.3H_2O$) C, H, N, O.

(*S*)-2-Amino-3-[(1*H*-indole-2-carbonyl)amino]propionic Acid S-14. Acylation of **S-5b**[27] with indole-2-carbonyl chloride (method A, 87%) and removal of the protecting groups (method C, 86%; method D, 79%) gave **S-14**, mp 225−250 °C (dec; H_2O/THF, 1:1). Thin layer chromatography on Chiralplate (Machery-Nagel)[49] with $CH_3CN/CH_3OH/H_2O$, 4:1:1, showed a single spot, R_f = 0.52 (ninhydrine). No contamination with the enantiomer **R-14** was observed. $[\alpha]^{20}_D$ −1.8° (*c* 1.00, 1 M NaOH). ^{1}H NMR (D_2O + DCl): 7.75 d, J = 7 Hz, 1H; 7.58 d, J = 8 Hz, 1H; 7.38 t × d, J = 7 and 1 Hz, 1H; 7.22 t × d, J = 7 and 1 Hz, 1H; 7.16 s, 1H; 4.40−4.35 m, 1H; 4.10−3.90 m, 2H. Anal. ($C_{12}H_{13}N_3O_3 \cdot 0.3H_2O$) C, H, N, O.

***rac*-2-Amino-3-[(benzo[b]thiophene-2-carbonyl)amino]propionic Acid 17.** Condensation of benzo[*b*]thiophene-2-carboxylic acid with ***rac*-5b** (method B, 88%) and removal of the protecting groups (method C, 86%; method E, 74%) gave **17**, mp 230 °C (dec). ^{1}H NMR (D_2O + DCl): 7.7−7.6 m, 2H; 7.53 s, 1H; 7.25−7.10 m, 2H; 4.15−4.05 m, 1H; 3.75−3.50 m, 2H. Anal. ($C_{12}H_{12}N_2O_3S$) C, H, N, O, S.

***rac*-2-Amino-3-[(naphthalene-2-carbonyl)amino]propionic Acid 20.** Condensation of naphthalene-2-carboxylic acid with ***rac*-5b** (method B, 87%) and removal of the protecting groups (method C, 95%; method E, 63%) gave **20**, mp 220 °C (dec). ^{1}H NMR ([(CD_3)$_2$SO]): 8.9 br s, 1H; 8.45 s, 1H; 8.1−7.9 m, 4H; 7.65−7.55 m, 2H; 3.8−3.6 m, 2H; 3.5−3.4 m, 1H. Anal. ($C_{14}H_{14}N_2O_3$) C, H, N, O.

***rac*-2-Amino-3-[(4-bromo-1*H*-indole-2-carbonyl)amino]propionic Acid *rac*-22.** Condensation of 2.00 g of 4-bromo-1*H*-indole-2-carboxylic acid with ***rac*-5b** according to method B gave 3.15 g (79%) of 2-benzyloxycarbonylamino-3-[(4-bromo-1*H*-indole-2-carbonyl)amino]propionic acid methyl ester **22a** as a slightly yellow solid, mp 187−193 °C. ^{1}H NMR ([(CD_3)$_2$SO]): 12.05 s, 1H; 8.75 t, J = 6 Hz, 1H; 7.75 d, J = 6 Hz, 1H; 7.45, d, J = 6 Hz, 1H; 7.35 br s, 5H; 7.25 d, J = 6 Hz, 1H; 7.18−7.10 m, 2H; 5.05 s, 2H; 4.35 q, J = 6 Hz, 1H; 3.70−3.55 m, 5H. Deprotection of **22a** (method C, 97%; method E, 60%) gave ***rac*-22**, mp 252 °C (dec). ^{1}H NMR (D_2O + DCl): 7.38 d, J = 8.4 Hz, 1H; 7.25 d, J = 6 Hz, 1H; 7.12−7.08 m, 1H; 6.98 s, 1H; 4.32−4.30 m, 1H; 4.0−3.8 m, 2H. Anal. ($C_{12}H_{12}BrN_3O_3$) C, H, Br, N, O.

(*R*)-2-Amino-3-[(4-bromo-1*H*-indole-2-carbonyl)amino]propionic Acid R-22. Condensation of 4-bromo-1*H*-indole-2-carboxylic acid with **R-5b** (method B, 66%) and removal of the protecting groups (method C, 96.4%; method E, 73%) gave **R-22**, mp 248 °C (dec). Thin layer chromatography on Chiralplate (Machery-Nagel)[49] with $CH_3CN/CH_3OH/H_2O$, 4:1:2, showed a single spot, R_f = 0.39 (ninhydrine). As a control, the racemate ***rac*-22** showed two spots with R_f = 0.39 and 0.48. $[\alpha]^{20}_D$ −0.5° (*c* 1.00, 1 M NaOH). ^{1}H NMR ([(CD_3)$_2$SO] + DCl): 7.52 d, J = 7 Hz, 1H; 7.32 d, J = 7 Hz, 1H; 7.22 s, 1H; 7.18 t, J = 7 Hz, 1H; 4.10−4.05 m, 1H; 3.85−3.80 m, 2H. Anal. ($C_{12}H_{12}BrN_3O_3$) C, H, Br, N, O.

***rac*-2-Amino-3-[(4-bromobenzo[*b*]thiophene-2-carbonyl)amino]-propionic Acid 24.** Condensation of 4-bromobenzo[*b*]thiophene-2-carboxylic acid with ***rac*-5b** (method B, 42.9%) and removal of the protecting groups (method C, 100%; method E, 69.5%) gave **24**, mp 198 °C (dec). ^{1}H NMR (D_2O + NaOD): 7.75 d, J = 7.2 Hz, 1H; 7.00 s, 1H; 7.55 d, J = 7.2 Hz, 1H; 7.3−7.2 m, 1H; 3.8−3.6 m, 1H; 3.6−3.4 m, 2H. Anal. ($C_{12}H_{11}BrN_2O_3S \cdot 0.5H_2O$) C, H, Br, N, O, S.

***rac*-2-Amino-3-[(5-bromonaphthalene-2-carbonyl)amino]propionic Acid 25.** Condensation of 5-bromonaphthalene-2-carboxylic acid with ***rac*-5b** (method B, 88%) and removal of the protecting groups (method C, 99%; method E, 63%) gave **25**, mp 207 °C (dec). ^{1}H NMR ([(CD_3)$_2$SO]): 9.1 br s, 1H; 8.55 s, 1H; 8.17 d, J = 9 Hz, 1H; 8.12 d × d, J = 9 and 2 Hz, 1H; 8.08 d, J = 7.2 Hz; 7.95 d, J = 7.2 Hz, 1H; 7.50 d × d, J = 7.8 and 7.8 Hz, 1H; 3.9−3.7 m, 1H; 3.7−3.5 m, 2H. Anal. ($C_{14}H_{13}BrN_2O_3 \cdot 0.4H_2O$) C, H, Br, N, O.

***rac*-2-Amino-3-{[4-(2-ethylphenyl)-1*H*-indole-2-carbonyl]amino}-propionic Acid *rac*-26.** An amount of 7.65 g (31.9 mmol) of 4-bromo-1*H*-indole-2-carboxylic acid and 8.20 g (54.7 mmol) of 2-ethylphenylboronic acid were coupled according to method F, yielding 6.11 g (72%) of 4-(2-ethylphenyl)-1*H*-indole-2-carboxylic acid **26a** as a beige powder, mp 216 °C (dec). ^{1}H NMR ([(CD_3)$_2$SO]): 12.9 br s, 1H; 11.9 s, 1H; 7.50−7.20 m, 5H; 6.95 d, J = 7 Hz, 1H; 6.60 d, J = 0.2 Hz, 1H; 2.50 q, J = 7 Hz, 2H; 0.90 t, J = 7 Hz, 3H. Condensation of 4-(2-ethyl-phenyl)-1*H*-indole-2-carboxylic acid **26a** with ***rac*-5b** (method B, 45%) and removal of

the protecting groups (method C, 100%; method D, 83.5%) gave ***rac*-26**, mp 258 °C (dec). ^{1}H NMR ([(CD$_3$)$_2$SO]): 7.45 d, J = 8 Hz, 1H; 7.40−7.35 m, 2H; 7.30−7.25 m, 2H; 7.20 d, J = 8 Hz, 1H; 6.90 d, J = 7 Hz, 1H; 6.80 s, 1H; 4.1−4.0 m, 1H; 3.8−3.6 m, 2H; 2.5−2.4 m, 2H; 0.95 t, J = 6 Hz, 3H. Anal. ($C_{20}H_{21}N_3O_3$) C, H, N, O.

(*R*)-2-Amino-3-{[4-(2-ethylphenyl)-1*H*-indole-2-carbonyl]amino}-propionic Acid R-26. Procedure I. Condensation of 4-(2-ethylphenyl)-1*H*-indole-2-carboxylic acid **26a** with **R-5b** (method B, 96%) and removal of the protecting groups (method C, 82%; method E, 15.4%) gave **R-26**, mp 240 °C (dec). Thin layer chromatography on Chiralplate (Machery-Nagel)[49] with CH$_3$CN/MeOH/H$_2$O, 4:1:1) showed one single spot with R_f = 0.31 (ninhydrine). A racemic sample gave two spots with R_f = 0.31 and 0.43, respectively. $[\alpha]^{20}_D$ −6.5° (c 1.00, DMSO). ^{1}H NMR ([(CD$_3$)$_2$SO]): 11.90 s, 1H; 8.62 t, J = 6 Hz, 1H; 7.9−7.4 br, ∼3H; 7.45 d, J = 7 Hz, 1H; 7.40−7.32 m, 2H; 7.30−7.18 m, 3H; 6.90 d, J = 7 Hz, 1H; 6.72 s, 1H; 3.78−3.70 m, 1H; 3.52−3.38 m, 2H; 2.55−2.40 m, 2H; 0.95 t, J = 7 Hz, 3H. Anal. ($C_{20}H_{21}N_3O_3$) C, H, N, O.

Procedure II. : An analytically pure sample with the same optical rotation and ^{1}H NMR data was obtained in the following way. Condensation of 5.64 g (21.26 mmol) of 4-(2-ethylphenyl)-1*H*-indole-2-carboxylic acid **26a** with 7.76 g (21.26 mmol) of **R-5c** according to method B gave 9.99 g (81.6%) of (*R*)-2-benzyloxycarbonylamino-3-{[4-(2-ethyl-phenyl)-1*H*-indole-2-carbonyl]-amino}propionic acid benzyl ester **26b** as a yellow foam. An amount of 5.00 g of this foam was dissolved in 87 mL of glacial acetic acid, an amount of 500 mg of 5% Pd on carbon was added, and the mixture was hydrogenated at normal pressure for 3 h. After filtration from the catalyst and evaporation of the solvent, the residue was triturated with water, filtered, and recrystallized from isopropanol/water, yielding 2.73 g (89.4%) of **R-26**, identical in all properties with the sample from procedure (I).

***rac*-2-Amino-3-{[4-(2-ethylphenyl)benzo[*b*]thiophene-2-carbonyl]amino}propionic Acid 27.** An amount of 5.00 g (19.5 mmol) of 4-bromobenzo[*b*]thiophene-2-carboxylic acid and 5.00 g (33.1 mmol) of 2-ethylphenylboronic acid was coupled according to method F, yielding 4.87 g (88%) of 4-(2-ethylphenyl)-benzo[*b*]thiophene-2-carboxylic acid **27a** as beige powder, mp 207−210 °C (H$_2$O/isopropanol). ^{1}H NMR ([(CD$_3$)$_2$SO]): 13.5 s, 1H; 8.07 d, J = 9 Hz, 1H; 7,62−7.58 m, 1H; 7.45−7.42 m, 2H; 7.40 s, 1H; 7.35−7.30 m, 2H; 7.20 d, J = 6 Hz, 1H; 2.50−2.25 m, 2H; 1.40 t, J = 6 Hz, 3H. Condensation of **27a** with ***rac*-5b** (method B, 97%) and removal of the protecting groups (method C, 81%; method E, 33.1%) gave **27**, mp 212 °C (dec). ^{1}H NMR ([(CD$_3$)$_2$SO]): 9.0−8.9 br m, 1H; 8.05 d, J = 7.4 Hz, 1H; 7.60 s, 1H; 7.50 t, J = 6 Hz, 1H; 7.45−7.50 m, 2H; 7.35−7.3 m, 1H; 7.25 d, J = 6 Hz, 1H; 7.20 d, J = 6 Hz, 1H; 3.7−3.6 m, 1H; 3.4−3.3 m, 2H; 2.50−2.25 m, 2H; 0.95 t, J = 6 Hz, 3H. Anal. ($C_{20}H_{20}N_2O_3S\cdot 0.6H_2O$) C, H, N, O, S.

***rac*-2-Amino-3-{[5-(2-ethylphenyl)naphthalene-2-carbonyl]amino}-propionic Acid 28.** Amounts of 1.60 g (6.37 mmol) of 5-bromonaphthalene-2-carboxylic acid and 1.05 g (7.0 mmol) of 2-ethylphenylboronic acid were coupled according to method F, yielding 1.53 g (87%) of 5-(2-ethylphenyl)naphthalene-2-carboxylic acid **28a** as a beige powder, mp 154−168 °C. ^{1}H NMR ([(CD$_3$)$_2$SO]): 13.1 s, 1H; 8.70 s, 1H; 8.20 d, J = 7 Hz, 1H; 7.95 d, J = 7 Hz, 1H; 7.70 t, J = 7 Hz, 1H; 7.55−7.25 m, 5H; 7.20 d, J = 7 Hz, 1H; 2.40−2.10 m, 2H; 0.90 t, J = 7 Hz, 3H. Condensation of 5-(2-ethylphenyl)naphthalene-2-carboxylic acid **28a** with ***rac*-5b** (method B, 80%) and removal of the protecting groups (method C, 96%; method D 54%) gave **28**, mp 178−189 °C (dec; THF/H$_2$O, 2:1). ^{1}H NMR ([(CD$_3$)$_2$SO]): 9.0 br t, 1H; 8.56 s, 1H; 8.05 d, J = 9 Hz, 1H; 7.75 d × d, J = 9 and 1 Hz, 1H; 7.65−7.60 m, 1H; 7.45−7.40 m, 3H; 7.40−7.10 m, 2H; 7.18 d, J = 7.8 Hz, 1H; 3.85−3.70 m, 1H; 3.65−3.45 m, 2H; 2.40−2.10 m, 2H; 0.90 t, J = 6 Hz, 3H. Anal. ($C_{22}H_{22}N_2O_3\cdot 0.4H_2O$) C, H, N, O.

***rac*-2-Amino-3-[(3-propyl-1*H*-indole-2-carbonyl)amino]propionic Acid 33.** A solution of 5.64 g (35.65 mmol) of 2-oxohexanoic acid ethyl ester and 55 mL of ethanol was added at room temperature to a mixture of 3.55 mL (36.08 mmol) of phenylhydrazine, 41 mL of concentrated H$_2$SO$_4$, 69 mL of H$_2$O, and 103 mL of ethanol. After the mixture was stirred for 1 h, H$_2$O was added and the mixture extracted with *tert*-butyl methyl ether. The organic phase was washed with brine, dried over Na$_2$SO$_4$, and evaporated to yield 5.54 g (63%) of 2-(phenylhydrazono)hexanoic acid ethyl ester **33a** as a red oil. ^{1}H NMR (CDCl$_3$): 12.0 br s, 1H (NH of *E*-isomer); 7.90 br s, 1H (NH of *Z*-isomer); 7.30−7.20 m, 2H; 7.20−7.10 m, 2H; 7.00−6.85 m, 1H; 4.30 q, J = 8 Hz, 2H; 2.50 pent, J = 8 Hz, 2H; 1.60−1.30 m, 7H; 0.90 t, J = 8 Hz, 3H. A mixture of 5.54 g of **33a**, 5.6 mL of concentrated H$_2$SO$_4$, and 56 mL of dry ethanol was heated at 100 °C for 90 min. After cooling and dilution with water, the reaction mixture was extracted with toluene and the organic phase washed with 1 M NaHCO$_3$ solution and brine and evaporated to dryness, leaving 5.52 g of a yellow oil. From another reaction with 6.95 g of the starting hydrazone, 6.94 g of this oil was obtained. Both oils were combined and purified by MPLC on silica gel with toluene/cyclohexane, 4:1, yielding 6.50 g (56%) of 3-propyl-1*H*-indole-2-carboxylic acid ethyl ester **33b** as a slightly yellow solid. ^{1}H NMR (CDCl$_3$): 8.65 br s, 1H; 7.70 d, J = 8 Hz, 1H; 7.40−7.25 m, 2H; 7.13 t × d, J = 8 and 2 Hz, 1H; 4.40 q, J = 7 Hz, 2H; 3.05 t, J = 7 Hz, 2H; 1.70 hex, J = 7 Hz, 2H; 1.40 t, J = 7 Hz, 3H; 0.95 t, J = 7 Hz, 3H. A mixture of 6.5 g (28.12 mmol) of **33b**, 32.4 mL of 1 M NaOH solution, and 140 mL of ethanol was heated at 70 °C for 2 h. After evaporation of the ethanol the residual aqueous solution was diluted with H$_2$O, washed with diethyl ether, acidified with 2 M HCl solution, and extracted with diethyl ether. Drying of the organic phase over Na$_2$SO$_4$ and evaporation yielded 5.27 g (92%) of 3-propyl-1*H*-indole-2-carboxylic acid **33c** as slightly yellow crystals, mp 114−119 °C. ^{1}H NMR (CDCl$_3$): 8.77 s, 1H; 7.70 d, J = 8 Hz, 1H; 7.45−7.30 m, 2H; 7.20−7.10 m, 1H; 6.5−5.5 br, 1H; 3.10 t, J = 8 Hz, 2H; 1.75, hex, J = 8 Hz, 2H; 1.00 t, J = 8, 3H. Acylation of ***rac*-5b** with 3-propyl-1*H*-indole-2-carbonyl chloride (prepared in 90% yield by refluxing **33c** with 10 equiv of thionyl chloride in dichloromethane) (method A, 96%) and removal of the protecting groups (method C, 94%; method E, 82%) gave **33**, mp 225 °C (dec). ^{1}H NMR ([(CD$_3$)$_2$SO]): 11.9 br s, 1H; 8.6 br t, 1H; 8.0−7.8 br, ∼3H; 7.58 d, J = 7.2 Hz, 1H; 7.45 d, J = 7.2 Hz, 1H; 7.15 t, J = 7.2 Hz, 1H; 7.00 t, J = 7.2 Hz, 1H; 3.80−3.65 m, 2H; 3.6−3.5 m, 1H; 3.1−3.0 t, J = 9 Hz, 2H; 1.60 hex, J = 9 Hz, 2H; 0.90 t, J = 9 Hz, 3H. Anal. ($C_{15}H_{19}N_3O_3\cdot 0.1H_2O$) C, H, N, O.

***rac*-2-Amino-3-[(4-phenyl-1*H*-indole-2-carbonyl)amino]propionic Acid 39.** Amounts of 4.74 g (10 mmol) of *rac* 2-benzyloxycarbonylamino-3-[(4-bromo-1*H*-indole-2-carbonyl)amino]propionic acid methyl ester **22a** and 1.34 g (11 mmol) of phenylboronic acid were coupled according to method F, yielding 3.14 g (68.6%) of 2-benzyloxycarbonylamino-3-[(4-phenyl-1*H*-indole-2-carbonyl)amino]propionic acid **39a** as a brownish, highly viscous oil. ^{1}H NMR ([(CD$_3$)$_2$SO]): 12.5 br, 2H; 11.90 s, 1H; 8.65 br t, 1H; 7.70−7.40 m, 7H; 7.38−7.20 m, 7H; 7.10 d, J = 7 Hz, 1H; 5.10−4.95 m, 2H; 4.25−4.20 m, 1H; 3.75−3.50 m, 2H. Removal of the Z-group of **39a** (method E, 44%) gave **39**, mp 231 °C (dec). ^{1}H NMR ([(CD$_3$)$_2$SO]): 12.0 br s, 1H; 8.82 br t, 1H; 8.1−7.5 br, ∼3H; 7.65 d, J = 7 Hz, 2H; 7.52 t, J = 8 Hz, 2H; 7.47 d, J = 8 Hz, 1H; 7.39 t, J = 7 Hz, 1H; 7.28 s, 1H; 7.27 t, J = 8 Hz, 1H; 7.12 d, J = 7 Hz, 1H; 3.85−3.75 m, 1H; 3.60−3.45 m, 2H. Anal. ($C_{18}H_{17}N_3O_3\cdot 0.2H_2O$) C, H, N, O.

***rac*-2-Amino-3-[(4-bromo-1-methyl-1*H*-indole-2-carbonyl)amino]-propionic Acid 52.** To a suspension of 1.41 g of NaH (55% in oil, washed with pentane; 32.3 mmol) in 79 mL of DMF, an amount of 7.4 g (29.3 mmol) of 4-bromo-1*H*-indole-2-carboxylic acid methyl ester was added, and the mixture was stirred for 20 min. This was followed by the addition of 4.2 g (29.6 mmol) of methyl iodide in 10 mL of DMF and stirring for 3 h at room temperature. The reaction mixture was diluted with 250 mL of H$_2$O, and the formed precipitate was filtered and separated by MPLC on silica gel with toluene/ethyl acetate, 4:1, yielding 5.73 g (73%) of 4-bromo-1-methyl-1*H*-indole-2-carboxylic acid methyl ester **52a** as white powder, mp 83−84 °C. ^{1}H NMR ([(CD$_3$)$_2$SO]): 7.65 d, J = 11 Hz, 1H; 7.40 d, J = 11 Hz, 1H; 7.30 t, J = 11 Hz, 1H; 7.10 s, 1H; 4.05 s, 3H;

3.90 s, 3H. Saponification was accomplished by heating 1.93 g of **52a** in a mixture of 15 mL of 1 M NaOH solution and 30 mL of methanol for 30 min. After acidification with 2 M HCl solution and filtration, an amount of 1.74 g (95%) of 4-bromo-1-methyl-1*H*-indole-2-carboxylic acid **52b** was obtained as a white powder, mp 240−245 °C. ^{1}H NMR ([$(CD_3)_2SO$]): 13.5−12.9 br s, 1H; 7.65 d, J = 11 Hz, 1H; 7.40 d, J = 11 Hz, 1H; 7.25 t, J = 11 Hz, 1H; 7.10 s, 1H; 4.10 s, 3H. Condensation of **52b** with ***rac*-5b** (method B, 95%) and removal of the protecting groups (method C, 93%; method E 78%) gave **52**, mp 212 °C (dec). ^{1}H NMR ([$(CD_3)_2SO$]): 8.80 br t, 1H; 8.0−7.4 br, ∼3H; 7.58 d, J = 9 Hz, 1H; 7.32 d, J = 7.8 Hz, 1H; 7.20 t, J = 7.2 Hz, 1H; 7.12 s, 1H; 4.00 s, 3H; 3.75−3.65 m, 1H; 3.60−3.40 m, 2H. Anal. ($C_{13}H_{14}BrN_3O_3 \cdot 0.4H_2O$) C, H, Br, N, O.

***rac*-2-Amino-3-{[4-(2-ethylphenyl)-1-methyl-1*H*-indole-2-carbonyl]amino}propionic Acid 53.** Amounts of 3.5 g (13.05 mmol) of 4-bromo-1-methyl-1*H*-indole-2-carboxylic acid methyl ester **52a** and 2.15 g (14.3 mmol) of 2-ethylphenylboronic acid were coupled according to method F, yielding a dark residue which was purified by MPLC on silica gel. Elution with hexane/*tert*-butyl methyl ether, 9:1, afforded 4.2 g (96%) of 4-(2-ethylphenyl)-1-methyl-1*H*-indole-2-carboxylic acid methyl ester **53a** as a colorless oil. ^{1}H NMR ($CDCl_3$): 7.45−7.30 m, 6H; 7.08−7.02 m, 1H; 7.00 s, 1H; 4.13 s, 3H; 3.85 s, 3H; 2.60−2.40 m, 2H; 1.00 t, J = 8 Hz, 3H. A mixture of 4 g of **53a**, 27 mL of 1 M NaOH solution, and 50 mL of methanol was refluxed for 30 min. After dilution with ice the reaction mixture was extracted with diethyl ether and the aqueous phase acidified with 2 M HCl solution. The precipitate was filtered and dried yielding 3.56 g (93%) of 4-(2-ethylphenyl)-1-methyl-1*H*-indole-2-carboxylic acid **53b** as a white powder, mp 210−212 °C. ^{1}H NMR ([$(CD_3)_2SO$]): 12.9 br s, 1H; 7.60 d, J = 7 Hz, 1H; 7.50−7.25 m, 4H; 7.20 d, J = 7 Hz, 1H; 7.00 d, J = 7 Hz, 1H; 4.10 s, 3H; 2.6−2.4 m, 2H; 0.95 t, J = 8 Hz, 3H. Condensation of **53b** with ***rac*-5b** (method B, 88%) and removal of the protecting groups (method C, 100%; method D, 76%) gave **53**, mp 220 °C (dec; AcOH/THF/H_2O, 2:2:1). ^{1}H NMR ([$(CD_3)_2SO$]): 8.52 br t, 1H; 7.52 d, J = 8.4 Hz, 1H; 7.40−7.30 m, 3H; 7.25 d × t, J = 7 and 1 Hz, 1H; 7.20 d, J = 7 Hz, 1H; 6.95 d, J = 6 Hz, 1H; 6.75 s, 1H; 4.02 s, 3H; 3.68−3.58 m, 1H; 3.50−3.40 m, 2H; 2.60−2.40 m, 2H; 0.95 t, J = 7.2 Hz, 3H. Anal. ($C_{21}H_{23}N_3O_3 \cdot 0.1H_2O$) C, H, N, O.

Supporting Information Available: Syntheses and spectral data of the target compounds and intermediates not described in the Experimental Section; elemental analysis results of all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kemp, J. A.; McKernan, R. M. NMDA receptor pathways as drug targets. *Nat. Neurosci*. **2002**, *5*, 1039–1042.
(2) Johnson, J. W.; Ascher, P. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* **1987**, *325*, 529–531.
(3) Thomson, A. M. Glycine is a coagonist at the NMDA receptor/channel complex. *Prog. Neurobiol*. **1990**, *35*, 53–74.
(4) Leeson, P. D.; Iversen, L. L. The glycine site on the NMDA receptor: structure−activity relationships and therapeutic potential. *J. Med. Chem*. **1994**, *37*, 4053–4067.
(5) Danysz, W.; Parsons, C. G. Glycine and *N*-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. *Pharmacol. Rev*. **1998**, *50*, 597–664.
(6) Hood, W. F.; Gray, N. M.; Dappen, M. S.; Watson, G. B.; Compton, R. P.; Cordi, A. A.; Lanthorn, T. H.; Monahan, J. B. Characterization of indole-2-carboxylate derivatives as antagonists of *N*-methyl-D-aspartate receptor activity at the associated glycine recognition site. *J. Pharmacol. Exp. Ther*. **1992**, *262*, 654–660.
(7) Carling, R. W.; Leeson, P. D.; Moore, K. W.; Moyes, C. R.; Duncton, M.; Hudson, M. L.; Baker, R.; Foster, A. C.; Grimwood, S.; Kemp, J. A.; Marshall, G. R.; Tricklebank, M. D.; Saywell, K. L. 4-Substituted-3-phenylquinolin-2(1*H*)-ones: acidic and nonacidic glycine site *N*-methyl-D-aspartate antagonists with in vivo activity. *J. Med. Chem*. **1997**, *40*, 754–765.
(8) Balsamini, C.; Bedini, A.; Diamantini, G.; Spadoni, G.; Tontini, A.; Tarzia, G.; Di Fabio, R.; Feriani, A.; Reggiani, A.; Tedesco, G.; Valigi, R. (*E*)-3-(2-(*N*-Phenylcarbamoyl)vinyl)pyrrole-2-carboxylic acid derivatives. A novel class of glycine site antagonists. *J. Med. Chem*. **1998**, *41*, 808–820.
(9) Donati, D.; Di Fabio, R. Synthesis and pharmacological properties of novel glycine antagonists. *Pharm. Acta Helv*. **2000**, *74*, 239–245.
(10) Poschenrieder, H.; Hofner, G.; Stachel, H. D. Grevellin analogs with affinity to the *N*-methyl-D-aspartate (glycine site) receptor, a novel lead structure. *Arch. Pharm. (Weinheim, Ger.)* **2000**, *333*, 211–216.
(11) Snell, L. D.; Claffey, D. J.; Ruth, J. A.; Valenzuela, C. F.; Cardoso, R.; Wang, Z. J.; Levinson, S. R.; Sather, W. A.; Williamson, A. V.; Ingersoll, N. C.; Ovchinnikova, L.; Bhave, S. V.; Hoffman, P. L.; Tabakoff, B. Novel structure having antagonist actions at both the glycine site of the *N*-methyl-D-aspartate receptor and neuronal voltage-sensitive sodium channels: biochemical, electrophysiological, and behavioral characterization. *J. Pharmacol. Exp. Ther*. **2000**, *292*, 215–227.
(12) Jansen, M.; Dannhardt, G. Antagonists and agonists at the glycine site of the NMDA receptor for therapeutic interventions. *Eur. J. Med. Chem*. **2003**, *38*, 661–670.
(13) Buchstaller, H.-P.; Siebert, C. D.; Steinmetz, R.; Frank, I.; Berger, M. L.; Gottschlich, R.; Leibrock, J.; Krug, M.; Steinhilber, D.; Noe, C. R. Synthesis of thieno[2,3-*b*]pyridinones acting as cytoprotectants and as inhibitors of [^{3}H]glycine binding to the *N*-methyl-D-aspartate (NMDA) receptor. *J. Med. Chem*. **2006**, *49*, 864–871.
(14) Cai, S. X. Glycine/NMDA receptor antagonists as potential CNS therapeutic agents: ACEA-1021 and related compounds. *Curr. Top. Med. Chem*. **2006**, *6*, 651–662.
(15) Nagata, R.; Katayama, S.; Ohtani, K.; Tanaka, H.; Shimago, K. Tricyclic quinoxalinediones, aza-kynurenic acids, and indole-2 carboxylic acids as in vivo active NMDA-glycine antagonists. *Curr. Top. Med. Chem*. **2006**, *6*, 733–745.
(16) Catarzi, D.; Colotta, V.; Varano, F. Competitive Gly/NMDA receptor antagonists. *Curr. Top. Med. Chem*. **2006**, *6*, 809–821.
(17) Bare, T. M.; Brown, D. G.; Horchler, C. L.; Murphy, M.; Urbanek, R. A.; Alford, V.; Barlaam, C.; Dyroff, M. C.; Empfield, J. B.; Forst, J. M.; Herzog, K. J.; Keith, R. A.; Kirschner, A. S.; Lee, C. M. C.; Lewis, J.; McLaren, F. M.; Neilson, K. L.; Steelman, G. B.; Trivedi, S.; Vacek, E. P.; Xiao, W. Pyridazinoquinolinetriones as NMDA glycine-site antagonists with oral antinociceptive activity in a model of neuropathic pain. *J. Med. Chem*. **2007**, *50*, 3113–3131.
(18) Newcomer, J. W.; Krystal, J. H. NMDA receptor regulation of memory and behavior in humans. *Hippocampus* **2001**, *11*, 529–542.
(19) Goff, D. C.; Coyle, J. T. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am. J. Psychiatry* **2001**, *158*, 1367–1377.
(20) Shim, S. S.; Hammonds, M. D.; Kee, B. S. Potentiation of the NMDA receptor in the treatment of schizophrenia: focused on the glycine site. *Eur. Arch. Psychiatry Clin. Neurosci*. **2008**, *258*, 16–27.
(21) Snell, L. D.; Morter, R. S.; Johnson, K. M. Structural requirements for activation of the glycine receptor that modulates the *N*-methyl-D-aspartate operated ion channel. *Eur. J. Pharmacol*. **1988**, *156*, 105–110.
(22) McBain, C. J.; Kleckner, N. W.; Wyrick, S.; Dingledine, R. Structural requirements for activation of the glycine coagonist site of *N*-methyl-D-aspartate receptors expressed in Xenopus oocytes. *Mol. Pharmacol*. **1989**, *36*, 556–565.
(23) Watson, G. B.; Lanthorn, T. H. Pharmacological characteristics of cyclic homologues of glycine at the *N*-methyl-D-aspartate receptor-associated glycine site. *Neuropharmacology* **1990**, *29*, 727–730.
(24) Monahan, J. B.; Hood, W. F.; Compton, R. P.; Cordi, A. A.; Williams, R. M. Identification of a novel structural class of positive modulators of the *N*-methyl-D-aspartate receptor, with actions mediated through the glycine recognition site. *Eur. J. Pharmacol., Mol. Pharmacol. Sect*. **1990**, *189*, 373–379.
(25) Furukawa, H.; Gouaux, E. Mechanisms of activation, inhibition and specificity: crystal structures of the NMDA receptor NR1 ligand-binding core. *EMBO J*. **2003**, *22*, 2873–2885.
(26) Aebischer, B.; Frey, P.; Haerter, H.; Herrling, P. L.; Mueller, W. Synthesis and NMDA antagonistic properties of the enantiomers of 4-(3-phosphonopropyl)piperazine-2-carboxylic acid (CPP) and for the unsaturated analogue (*E*)-4-(3-phosphonoprop-2-enyl)-piperazine-2-carboxylic acid (CPP-ene). *Helv. Chim. Acta* **1989**, *72*, 1043–1051.
(27) Moore, S.; Patel, R. P.; Atherton, E.; Kondo, M.; Meienhofer, J.; Blau, L.; Bittman, R.; Johnson, R. K. Synthesis of actinomycin and analogs. *J. Med. Chem*. **1976**, *19*, 766–772.
(28) Ishii, H.; Murakami, Y.; Ishikawa, T. Abnormal Fischer indolization and its related compounds. XXII. Fischer indolization of ethyl

pyruvate 2-(2-chloro- and 2,6-dichlorophenyl)methylhydrazones. *Chem. Pharm. Bull*. **1990**, *38*, 597–604.
(29) Bergmann, J. Reaction of *N*-acylpyridinium salts with indole. *J. Heterocycl. Chem*. **1970**, *7*, 1071–1076.
(30) Grimwood, S.; Wilde, G. J. C.; Foster, A. C. Interactions between the glutamate and glycine recognition sites of the *N*-methyl-D-aspartate receptor from rat brain, as revealed from radioligand binding studies. *J. Neurochem*. **1993**, *60*, 1729–1738.
(31) Mugnaini, M.; Giberti, A.; Ratti, E.; Van Amsterdam, F. T. M. Allosteric modulation of [^{3}H]CGP39653 binding by glycine in rat brain. *J. Neurochem*. **1993**, *61*, 1492–1497.
(32) Bonhaus, D. W.; Yeh, G. C.; Skaryak, L.; McNamara, J. O. Glycine regulation of the *N*-methyl-D-aspartate receptor-gated ion channel in hippocampal membranes. *Mol. Pharmacol*. **1989**, *36*, 273–279.
(33) Kloog, Y.; Nadler, V.; Sokolovsky, M. Mode of binding of [^{3}H]dibenzocycloalkenimine [MK-801] to the *N*-methyl-D-aspartate (NMDA) receptor and its therapeutic implication. *FEBS Lett*. **1988**, *230*, 167–170.
(34) Hood, W. F.; Compton, R. P.; Monahan, J. B. D-Cycloserine, a ligand for the *N*-methyl-D-aspartate coupled glycine receptor has partial agonist characteristics. *Neurosci. Lett*. **1989**, *98*, 91–95.
(35) Berger, M. L. On the true affinity of glycine for its binding site at the NMDA receptor complex. *J. Pharmacol. Toxicol. Methods* **1995**, *34*, 79–88.
(36) Foster, A. C.; Kemp, J. A.; Tricklebank, M. D.; Leeson, P. D.; Carling, R. W.; Williams, B. J.; Grimwood, S.; Donald, A. E.; Priestley, T.; Marshall, G. R.; Saywell, K.; Gill, R. Selective antagonists for the glycine site of the *N*-methyl-D-aspartate receptor. *Mol. Neuropharmacol*. **1992**, *2*, 97–102.
(37) Priestley, T.; Marshall, G. R.; Hill, R. G.; Kemp, J. A. L-687,414, a low efficacy NMDA receptor glycine site partial agonist in vitro, does not prevent hippocampal LTP in vivo at plasma levels known to be neuroprotective. *Br. J. Pharmacol*. **1998**, *124*, 1767–1773.
(38) Arunlakshana, O.; Schild, H. O. Some quantitative uses of drug antagonists. *Br. J. Pharmacol*. **1959**, *14*, 48–58.
(39) Watson, G. B.; Bolanowski, M. A.; Baganoff, M. P.; Deppeler, C. L.; Lanthorn, T. H. D-Cycloserine acts as a partial agonist at the glycine modulatory site of the NMDA receptor expressed in Xenopus oocytes. *Brain Res*. **1990**, *510*, 158–160.
(40) Foster, A. C.; Kemp, J. A. HA-966 antagonizes *N*-methyl-D-aspartate receptors through a selective interaction with the glycine modulatory site. *J. Neurosci*. **1989**, *9*, 2191–2196.
(41) Singh, L.; Donald, A. E.; Foster, A. C.; Hutson, P. H.; Iversen, L. L.; Iversen, S. D.; Kemp, J. A.; Leeson, P. D.; Marshall, G. R.; Oles, R. J.; Priestley, T.; Thorn, L.; Tricklebank, M. D.; Vass, C. A.; Williams, B. J. Enantiomers of HA-966 (3-amino-1-hydroxypyrrolid-2-one) exhibit distinct central nervous system effects: (+)-HA-966 is a selective glycine/*N*-methyl-D-aspartate receptor antagonist, but (−)HA-966 is a potent γ-butyrolactone-like sedative. *Proc. Natl. Acad. Sci. U.S.A*. **1990**, *87*, 347–351.
(42) Inanobe, A.; Furukawa, H.; Gouaux, E. Mechanism of partial agonist action at the NR1 subunit of NMDA receptors. *Neuron* **2005**, *47*, 71–84.
(43) Schneider, C. I.; Urwyler, S. Biochemical and thermodynamic aspects of the binding of [^{3}H]glycine to its strychnine-insensitive recognition site associated with the *N*-methyl-D-aspartate receptor complex. *Biochem. Pharmacol*. **1992**, *43*, 1693–1699.
(44) Sills, M. A.; Fagg, G.; Pozza, M.; Angst, C.; Brundish, D. E.; Hurt, S. D.; Wilusz, E. J.; Williams, M. [^{3}H]CGP39653: a new *N*-methyl-D-aspartate antagonist radioligand with low nanomolar affinity in rat brain. *Eur. J. Pharmacol*. **1991**, *192*, 19–24.
(45) Urwyler, S.; Laurie, D.; Lowe, D. A.; Meier, C. L.; Muller, W. Biphenyl-derivatives of 2-amino-7-phosphonoheptanoic acid, a novel class of potent competitive *N*-methyl-D-aspartate receptor antagonists. 1. Pharmacological characterization in vitro. *Neuropharmacology* **1996**, *35*, 643–654.
(46) Yoneda, Y.; Ogita, K.; Enomoto, R.; Suzuki, T.; Kito, S. Identification and characterization of specific binding sites of [^{3}H]spermidine in synaptic membranes of rat brain. *Brain Res*. **1991**, *563*, 17–27.
(47) Ahlrichs, R.; Bar, M.; Haser, M.; Horn, H.; Kolmel, C. Electronic structure calculations on workstation computers: the program system turbomole. *Chem. Phys. Lett*. **1989**, *162*, 165–169.
(48) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem*. **1978**, *43*, 2923–2925.
(49) Gunther, K. Thin-layer chromatographic enantiomeric resolution via ligand exchange. *J. Chromatogr*. **1988**, *448*, 11–30.