Potent, Orally Active, Competitive *N*-Methyl-D-aspartate (NMDA) Receptor Antagonists Are Substrates for a Neutral Amino Acid Uptake System in Chinese Hamster Ovary Cells†,1

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Received January 31, 1995®

A series of enantiomerically pure (phosphonomethyl)-substituted phenylalanine derivatives related to SDZ EAB 515 (**1**) were prepared as competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists. Unlike most known competitive NMDA antagonists, analogs in this series with the *S*-configuration are potent NMDA antagonists whereas analogs with the unnatural *R*-configuration are weak NMDA antagonists, as determined by receptor binding experiments and their anticonvulsant action in mice. Examination in a previously reported competitive NMDA pharmacophore model revealed that receptor affinity can be explained partially by a cavity that accommodates the biphenyl ring of **1**, while the biphenyl ring of the *R*-enantiomer **2** extends into a disallowed steric region. We proposed that analogs with the natural *S*-configuration and a large hydrophobic moiety would have an advantage *in vivo* over analogs with an *R*-configuration by being able to use a neutral amino acid uptake system to enhance both peripheral adsorption and transport into the brain. Examination in a system L neutral amino acid transport carrier assay shows that **1** competes with L-Phe for transport in an apparent competitive and stereospecific manner (estimated $K_i = 50\ \mu M$). The 1- and 2-naphthyl derivatives **3a**,**3b** were found to be among the most potent, competitive NMDA antagonists yet discovered, being ca. 15-fold more potent than **1** *in vitro* and *in vivo*, with a long duration of action. The title compound **3a** had potent oral activity in MES ($ED_{50} = 5.0$ mg/kg). **3a** also retains its ability to compete, albeit more weakly than **1** (estimated $K_i = 200\ \mu M$), for L-Phe uptake to CHO cells. In this series, analogs with the *R*-configuration are not substrates for the system L neutral amino acid transport carrier. These results provide evidence that central nervous system active agents can be designed as substrates of a neutral amino acid transporter as a means to enhance penetration of the blood–brain barrier.

Introduction

N-Methyl-D-aspartate (NMDA) receptors are members of a family of ionotropic glutamate receptors that function as a major excitatory neurotransmission pathway in the brain. The NMDA receptor is a multiprotein complex that forms a ligand-gated cation channel and requires glutamate and glycine as coagonists for activation.[2] It contains a number of other sites that can modulate receptor activity. Glutamate receptors in general, and NMDA receptors in particular, have been implicated in a phenomenon known as excitotoxicity which may accompany acute ischemic events such as stroke or cerebral trauma[3] and may be a factor in several other neurological disorders including convulsive disorders, neuropathic pain, and anxiety.[4] NMDA receptor antagonists may also be useful in preventing tolerance to opiate analgesia and helping to control withdrawal symptoms from addictive drugs.[5] Additionally, NMDA receptors may be a significant causal factor in chronic neurodegenerative disorders such as Alzheimer's disease,[6] Huntington's disease,[7] Parkinson's disease,[8] human immunodeficiency virus (HIV)-related neuronal injury, and amyotrophic lateral sclerosis (ALS).[9] Antagonists of NMDA receptor function are expected to be useful in the treatment of some of these neurological disorders, if adverse behavioral effects can be limited.

Competitive NMDA receptor antagonists have demonstrated neuroprotective activity in animal models of focal cerebral ischemia and cerebral trauma.[10] A recent review describes most of the recent structural types of NMDA receptor antagonists.[11] It is evident from the literature that most potent competitive NMDA receptor antagonists have an unnatural *R*-configuration of the common α-amino acid moiety. However, there are exceptions to this including the biphenyl derivative (*S*)-α-amino-5-(phosphonomethyl)[1,1′-biphenyl]-3-propanoic acid (SDZ EAB 515, **1**)[12a] and the decahydroisoquinoline derivatives (−)-(*S*)-6-(phosphonomethyl)- and (−)-(*S*)-6-(tetrazolylmethyl)decahydroisoquinoline-3-carboxylic acids (LY235959 and LY202157).[12b] The decahydroisoquinoline derivatives are atypical amino acids and were not used for comparison in this study. Examination of **1** in an NMDA competitive antagonist pharmacophore model revealed that the biphenyl moiety with the *S*-configuration might fit into a region of steric tolerance near the glutamate recognition site (Figure 1), whereas in the *R*-configuration, the biphenyl system encroached into a region of steric intolerance.[2,13]

One of the primary amino acid transporters through the blood–brain barrier is the large neutral amino acid

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® Abstract published in *Advance ACS Abstracts*, May 1, 1995.

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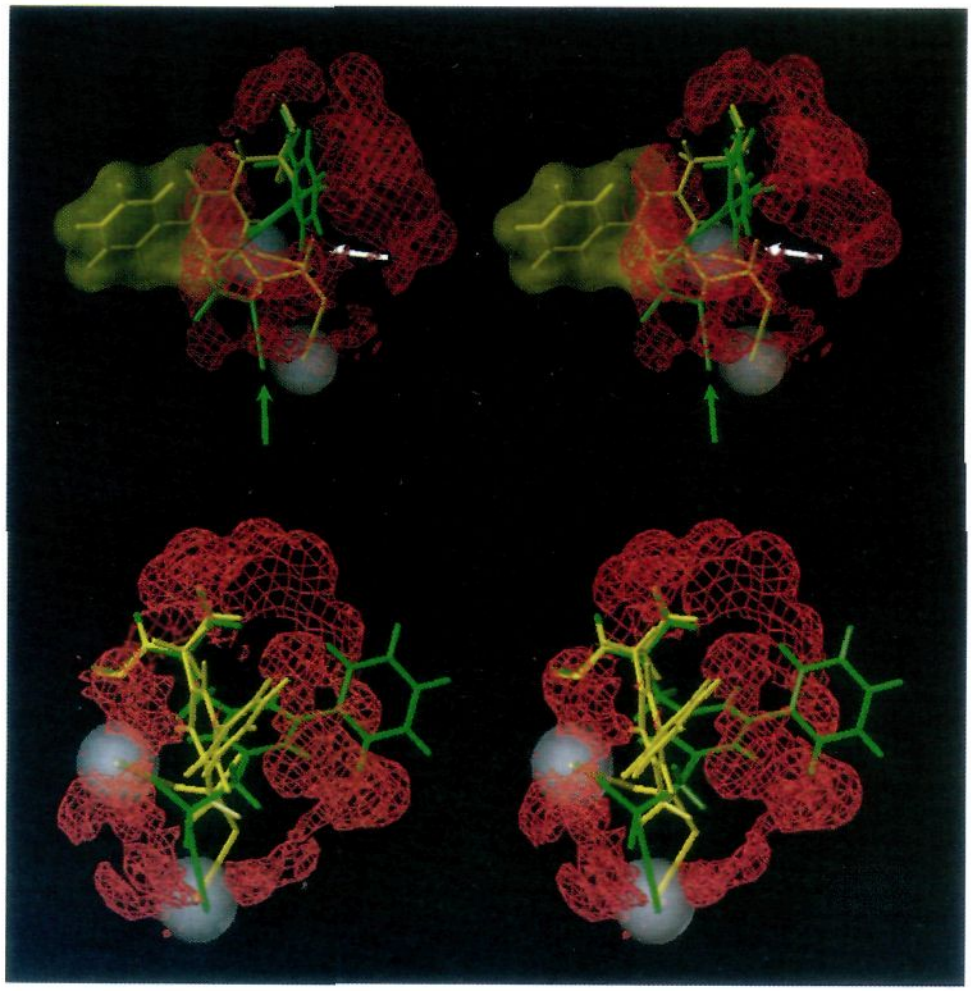
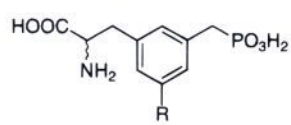


Figure 1. Top: stereoview of *S*-**1** (yellow) and the less potent *R*-**2** (green) enantiomer of SDZ EAB 515 docked in the competitive NMDA receptor pharmacophore model.[13] In red is shown the previously defined receptor-excluded volume. The two spheres in white represent receptor binding sites for the terminal phosphonic acid moiety. The second phenyl ring of the *R*-**2** enantiomer encountered disallowed space in the area denoted by the white arrow. The reduced potency of *R*-**2** may be due to this disallowed steric interaction, as well as a poor interaction with one of the receptor binding sites (green arrow), whereas *S*-**1** shows good fit to the pharmacophore model and the second phenyl ring (yellow translucent volume) projects through a cavity of the volume map. Bottom: orthogonal stereoview of the top. The second phenyl ring of *S*-**1** slips neatly through a cavity in the volume map of the receptor with the volume coresponding to the phenyl ring (in top picture) removed for clarity.

carrier system L, which is located in brain capillaries.[14] System L is known to facilitate the transport of branched chain and aromatic amino acids. Previous work has established that competitive inhibitors of L-Phe transport are generally substrates for the transporters.[15b] Because **1** is structurally related to L-Phe, a known substrate for this transporter, we hypothesized that it might be capable of utilizing this transporter to enhance uptake into the brain. To test this theory, the ability of **1**, its enantiomer **2**, and several structurally related compounds to be transported was evaluated with a competitive inhibition of [^{3}H]-L-Phe transport assay in Chinese hamster ovary cells. Compounds with the *S*-configuration described herein were designed specifically to act as competitive NMDA receptor antagonists and potentially to utilize a neutral amino acid transport system to enhance their access into the central nervous system (CNS).

Molecular Modeling and Chemistry

A previous article from our laboratory described compounds such as 3-(phosphonomethyl)phenylalanine



1 (*S*) R = Ph
2 (*R*) R = Ph
3a (*S*) R = 1-naphthyl
3b (*S*) R = 2-naphthyl
4 (*R,S*) R = H

(**4**; $IC_{50} = 3.3\ \mu M$ in [^{3}H]CPP binding assay), which had modest antagonist activity at NMDA receptors.[15] The related compound **1**, which contains a phenyl substituent *meta* to both the phosphonomethyl and α-amino acid substituents, was found to have much greater affinity at the recognition site.[12] In the present study, replacement of the phenyl ring with a naphthylene ring system resulted in even greater increase in affinity for the NMDA recognition site (Table 1). When examined in an NMDA competitive antagonist pharmacophore model,[13] the biphenyl ring of **1** projected neatly through a cavity in the volume map of the receptor, whereas the biphenyl ring of the *R*-enantiomer **2** encountered disal-

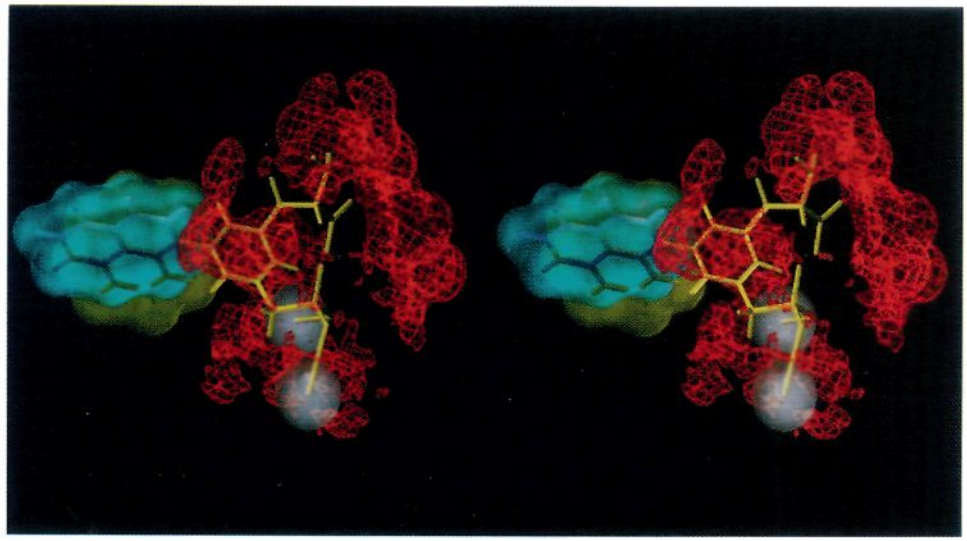
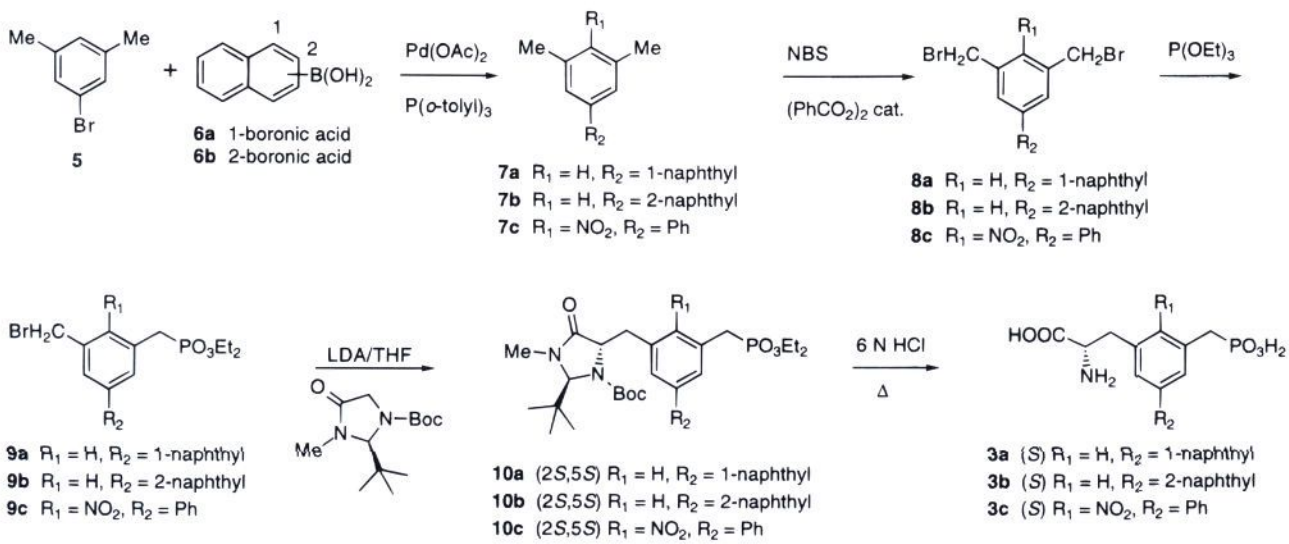


Figure 2. Stereoview of fit of potent *S*-**3a** (yellow) and *S*-**3b** (blue) onto the competitive NMDA receptor pharmacophore model, in a low-energy conformation as determined by a conformational search analysis using SYBYL as previously described.[13] In red is shown the previously defined receptor-excluded volume, and in yellow and blue is shown the volume occupation of the 1- and 2-naphthalene groups, respectively. For clarity, only the blue naphthalene ring from **3b** is shown. The two spheres in white represent receptor binding sites for the terminal phosphonic acid moiety.

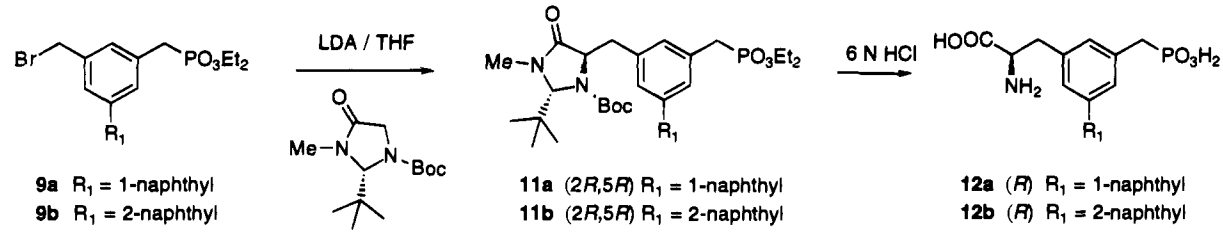
Scheme 1



lowed space (Figure 1). In addition, a low-energy conformer of **2** could not be made to reach one of the phosphonic acid interaction sites (the secondary interaction site). Thus, the reduced affinity of this analog may be due to a disallowed steric interaction combined with a suboptimal interaction with one of the phosphonate receptor interaction sites in the pharmacophore model. The biphenyl ring system of **1** must provide either additional hydrophobic or electronic interactions via the π-cloud of the second phenyl ring at the receptor surface that stabilize binding as demonstrated by the 40-fold increase in affinity compared with **4**. The novel naphthylene derivatives **3a,b** support these assumptions. With the binding to the primary receptor interaction sites of the pharmacophore intact, the naphthylene ring system extends into the same pocket as the phenyl ring of **1** and increases the volume of tolerated space available. We suggest that this additional volume may be near or on the receptor surface. However, the increased affinity of naphthyl > phenyl > H suggests that a specific hydrophobic interaction with the extended aromatic π-cloud may exist. The increased steric occupation by the naphthyl ring of **3a,b** when these compounds are fit to the pharmacophore model is shown in Figure 2. Fitting molecules with the *R*-configuration into the primary interaction sites defined by the pharmacophore model clearly shows that the aromatic ring protrudes into a region of steric intolerance. When loosely fit, with concomitant loss in binding affinity, the *R*-enantiomers can find the cavity.

The target *S*-α-amino acid phosphonates **3a**–**c** were prepared according to Scheme 1. 2-Naphthylboronic acid was prepared in 62% yield by treatment of the Grignard reagent of 2-bromonaphthalene with a solution of trimethyl borate followed by acid hydrolysis of the resulting borate ester with aqueous hydrochloric acid. The unsymmetrical biaryl compounds 1-(3,5-dimethylphenyl)naphthalene (**7a**) and 2-(3,5-dimethylphenyl)naphthalene (**7b**) were obtained in yields of 73% and 69%, respectively, by palladium(II)-catalyzed

Scheme 2

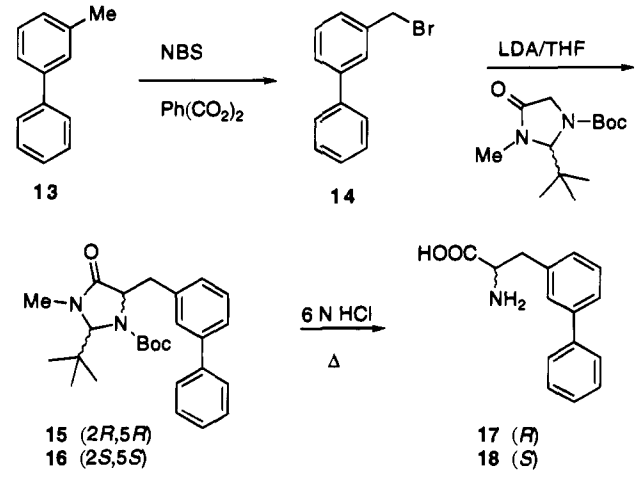


Suzuki cross-coupling[16] of 1,3-dimethyl-5-bromobenzene and **6a** or **6b**. Benzylic bromination of the methyl groups of **7a–c** with 2.1 equiv of *N*-bromosuccinimide and a catalytic amount of benzoyl peroxide under a tungsten light afforded the bis-bromomethyl derivatives **8a–c** in yields of 70–80%. Alternatively, the monobromomethyl derivatives could also be obtained in good yield when only 1.1 equiv of *N*-bromosuccinimide was used under similar conditions without irradiation with the tungsten lamp. The Michaelis–Arbuzov reaction of **8a–c** with 1.1 equiv of triethyl phosphite in xylene gave the monophosphonates **9a–c** in reasonable yields (48–70%). Seebach's (*S*)-(−)-imidazolidinone chiral auxiliary was used to effect the displacement of the benzylic bromide derivatives **9a–c** by treating with the lithium enolate of the (*S*)-(−)-imidazolidinone to give the coupled adducts **10a–c** in yields of 53–81%. Hydrolysis of the imidazolidinone diethyl phosphonates with 6 N HCl produced directly the enantiomerically pure products **3a–c** in good to excellent yields (80–92%). The *R*-enantiomers **12a,b** were obtained by an identical procedure by coupling Seebach's (*R*)-(+)-imidazolidinone chiral auxiliary with **9a,b** followed by hydrolysis according to Scheme 2.

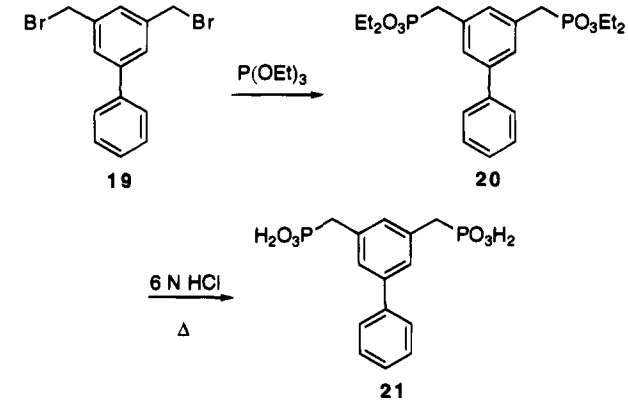
Both ^{13}C- and ^{1}H-NMR indicated that each of **10a–c** and **11a,b** were single diastereomers and illustrate the stereospecificity and efficiency of the Seebach reagent. The absolute configurations of **10a–c** and **11a,b** were deduced according to the reported chiral auxiliary reactions[17] and further confirmed by ^{1}H-NMR experiments by observing the positive NOE of the C-5 methine proton upon saturation of the 2-(*tert*-butyl) protons. Each pair of *R*- and *S*-enantiomers (**3a** and **12a, 3b** and **12b**) was distinguished on TLC Chiralplate; each final product showed a single spot on the chiral TLC plate indicating that they were enantiomerically pure.

Two other types of molecules, the *R*- and *S*-3-phenyl Phe derivatives **17** and **18** and the bis-phosphonic acid **21**, were prepared as references for the system L transport studies. Using a straightforward sequence outlined in Scheme 3, 3-phenyltoluene was transformed into 3-(bromomethyl)-1,1′-biphenyl (**14**), which was displaced in turn by the lithium enolates of *R*- and *S*-Seebach chiral auxiliaries to give the imidazolidinone derivatives **15** and **16**. Acidic hydrolysis provided both the *R*- and *S*-3-phenyl Phe derivatives **17** and **18**. Enantiomeric purity of each compound was confirmed by thin layer chromatography on Chiralplate adsorbent plates. The [1,1′-biphenyl]-3,5-bis(methanephosphonic acid) **21** was obtained from a Michaelis–Arbuzov reaction of 3,5-bis(bromomethyl)-1,1′-biphenyl and triethyl phosphite (92%), followed by acidic hydrolysis of [1,1′-biphenyl]-3,5-bis(diethyl methanephosphonate) **20** according to Scheme 4.

Scheme 3



Scheme 4



Results

Compounds were tested in rat synaptosomal membrane preparations for their ability to displace [^{3}H]-glutamate, [^{3}H]glycine, and [^{3}H]CGP 39653 by previously reported procedures.[18–20] As seen in Table 1, assays employing glutamate and CGP 39653 give similar results. All compounds were essentially inactive in glycine binding. Consistent with other competitive NMDA receptor antagonists, both the α-amino acid and phosphonic acid moieties were found to be essential for high-affinity binding to the glutamate recognition site of the NMDA receptor. This is most easily demonstrated by the low binding affinity of **17** and **18**, which lack the phosphonomethyl side chain moiety, and **21**, which does not have the α-amino acid side chain. We resynthesized both *S*-**1** and *R*-**2** as reference agents and found comparable binding affinity to that reported in the literature,[12] with **1** being approximately 40-fold more potent than **2** in CGP 39653 binding. **1** is only 3-fold more potent than racemic CPP, suggesting that the active *R*-enantiomer of CPP has similar affinity. As

Table 1. Inhibition of Receptor Binding Affinity in Rat Brain Synaptosomal Membrane

compd	IC_{50} (μM)[a] [^{3}H]-Glu[b]	[^{3}H]CGP 39653[c]	[^{3}H]-Gly[d]	LDH release[e]
CPP	0.837	0.148	>200	
1 (*S*)-SDZ EAB 515	0.13	0.088	224	1.0
2 (*R*)-SDZ EAB 515	13.5	5.21	>200	
3a (PD 158473)	0.015	0.006	65.4	0.05
3b (PD 159913)	0.014	0.012		
3c	2.3	1.08	11.5	
12a	0.32	0.25		
12b	0.65	0.59		
17 (*R*)	90%[f]	100%[f]	81%[f]	
18 (*S*)	58%[f]	52%[f]	52%[f]	
21	80%[f]	86%[f]	100%[f]	

[a] The IC_{50} values in the table are mean values for a minimum of two experiments run in triplicate. The accuracy of each assay is reflected by SEM of reference agents as shown in footnotes b–d. [b] SEM for CPP in the [^{3}H]Glu binding assay ($n = 4$) is ± 0.19 μM. [c] SEM for CPP in [^{3}H]CGP 39653 binding assay ($n = 5$) is 0.015 μM. [d] SEM for 5,7-dichlorokynurenic acid in [^{3}H]Gly binding assay ($n = 11$) is 0.011 μM; $IC_{50} = 0.11$ μM. [e] Concentration of compound needed to prevent cell death induced by a 5 min application of 100 μM NMDA.[21] [f] Reported as percent of control at 100 μM concentration of test compound.

Table 2. Maximal Electroshock Results in Mice after iv Administration

compd	dose[a]	time	protected[b]	ataxic[c]	ED_{50}	TI[d]
CPP					2.35	2.21
1 (*S*)	3	15 min	1/5	0/5		
		1 h	0/5	0/5		
		2 h	1/5	0/5		
	10	15 min	5/5	0/5		
		1 h	4/5	1/5		
		2 h	3/5	0/5		
2 (*R*)	10	15 min	0/5	0/5		
		1 h	0/5	0/5		
		2 h	0/5	0/5		
3a (*S*)-PD 158473					0.53	2.28
3b (*S*)-PD 159913	0.3	15 min	1/5	0/5		
		1 h	0/5	0/5		
		2 h	2/5	0/5		
	1	15 min	1/5	0/5		
		1 h	5/5	1/5		
		2 h	5/5	0/5		
12a	10	15 min	0/5	0/5		
		1 h	0/5	0/5		
		2 h	1/5	0/5		
	30	15 min	3/5	1/5		
		1 h	3/5	0/5		
		2 h	5/5	0/5		

[a] Dose = mg/kg iv. [b] Number of animals protected/number of animals tested at each dose or time point. [c] Number of animals with ataxia/number of animals tested. [d] TI (therapeutic index) = ataxia ED_{50}/MES ED_{50}.

mentioned above, the unsubstituted derivative **4** is also 40-fold weaker than **1**, indicating that the phenyl moiety provides a specific molecular interaction with the receptor. The *S*-1- and -2-naphthyl derivatives **3a,b** have as high affinity at the NMDA receptor recognition site as any previously reported in the literature. They are ca. 10-fold more potent than **1** and 400-fold more potent than the parent compound **4**.[15a] In contrast, the *R*-naphthyl derivatives **12a,b** are 30- and 70-fold less potent than their enantiomers, similar to the difference seen between **1** and **2**. The 2-nitro derivative, **3c**, of **1** had substantially reduced receptor affinity. It is unclear at this time if this is due to a steric interaction or an electronic effect. Reduction of the nitro group of **3c** resulted in lactam formation with the carboxylic acid functionality and greatly decreased receptor binding. Of some interest, **3c** shows limited affinity at the glycine site, but functional assays have not been done to confirm this activity.

There is a significant correlation between binding at the NMDA receptor and functional activity, as demonstrated by the ability of antagonists to inhibit glutamate-induced influx of Ca^{2+} ions into cultured cortical neurons or the prevention of NMDA-induced lethality in mice.[15b] As a measure of their functional NMDA antagonist activity, **1** and **3a** were compared in an excitotoxicity cell culture assay. This assay measures the release of lactate dehydrogenase related to neuronal cell death induced by the application of NMDA.[21] As before, we found **3a** to be 20-fold more potent with respect to its ability to be neuroprotective *in vitro* than **1** with IC_{50}'s = 0.05 and 1.0 μM, respectively. Other functional assays of NMDA antagonist activity confirm that **3a** is 20-fold more potent than **1** and more than 1000-fold more potent than **2**.[22]

Anticonvulsant activity was determined in a model of maximal electroshock induced seizures (MES). Adult male CF-1 strain mice (18–24 g body wt) were administered drug intravenously. Maximal electroshock was performed according to conventional methods, with a 60 Hz sinusoidal current (50 mA, base to peak, 0.2 s) applied by corneal electrodes,[23] and ataxia was quantified by an inverted screen procedure.[24] In this study the reference agents **1**, **2**, and CPP were compared with **3a,b** and **12a** (see Table 2). For **1**, maximal protection from MES was seen at 15 min, with anticonvulsant activity extending past 2 h. The median effective dose was between 3 and 10 mg/kg. The *R*-enantiomer **2** was not active at any time point tested at a dose of 30 mg/kg. **3a**, the *S*-1-naphthyl derivative, had an $ED_{50} = 0.53$ mg/kg or 5-fold greater potency than CPP in the MES model. The *R*-enantiomer **12a** had an ED_{50} between 10 and 30 mg/kg, comparable to the differences in their affinity for the NMDA receptor. Similar in potency to **3a**, the *S*-2-naphthyl derivative **3b** had an ED_{50} between 0.3 and 1 mg/kg. In addition, **3a** and CPP exhibited a similar therapeutic index, when their anticonvulsant activity was compared with their ability to cause ataxia. **3a** also had potent oral activity in MES ($ED_{50} = 5.0$ mg/kg) and a duration of anticonvulsant action lasting between 8 and 16 h, with its peak activity at 6 h.

We have not conducted kinetic experiments comparing the receptor binding of 1, **3a,b**, and **4**, but one might predict that, upon the basis of their *in vivo* activity in MES, the larger molecules **3a,b** will have a slower k_{on} and a much slower k_{off} that would be consistent with their high receptor affinity and long duration of action. The extended duration of action and the separation of protection and ataxia at longer time points suggest that these derivatives may have potential therapeutic value.

Transport Studies. The transport capacity of **1**, **2**, **3a**, **17**, **18**, and **21** was determined using an assay that measured their ability to compete with L-Phe for uptake. Earlier studies have established that approximately 70% of L-Phe transport in CHO cells is attributable to the sodium-independent system L transporter for neutral amino acids. The remainder of the transport activity is attributable to system ASC, a sodium-dependent carrier that shows a strong preference for alanine, serine, and cysteine.[25a] The k_m of transport for substrates of the system L transporter, such as L-phenylalanine, has been estimated to be approximately

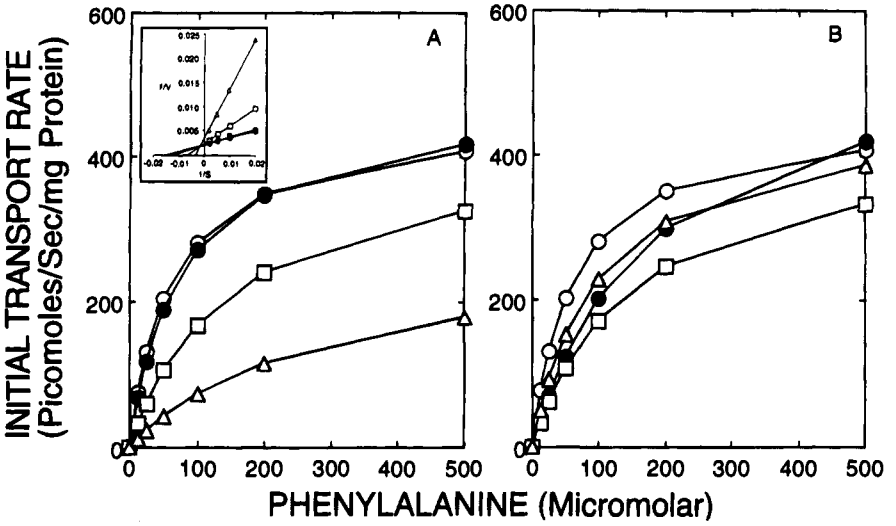


Figure 3. Initial transport rates of L-Phe in CHO cells in the presence of enantiomers of SDZ EAB 515. Transport was evaluated at 37 °C for a 30 s interval in the presence of varying concentrations of *S*-1 (A) and *R*-**2** (B). Analysis of transport inhibition in the presence of 1 by a Lineweaver–Burk plot is shown in the inset. Concentrations of each of the enantiomers are indicated by symbols (closed circle, 0 μM; open circle, 2 μM; open square, 20 μM; open triangle, 200 μM).

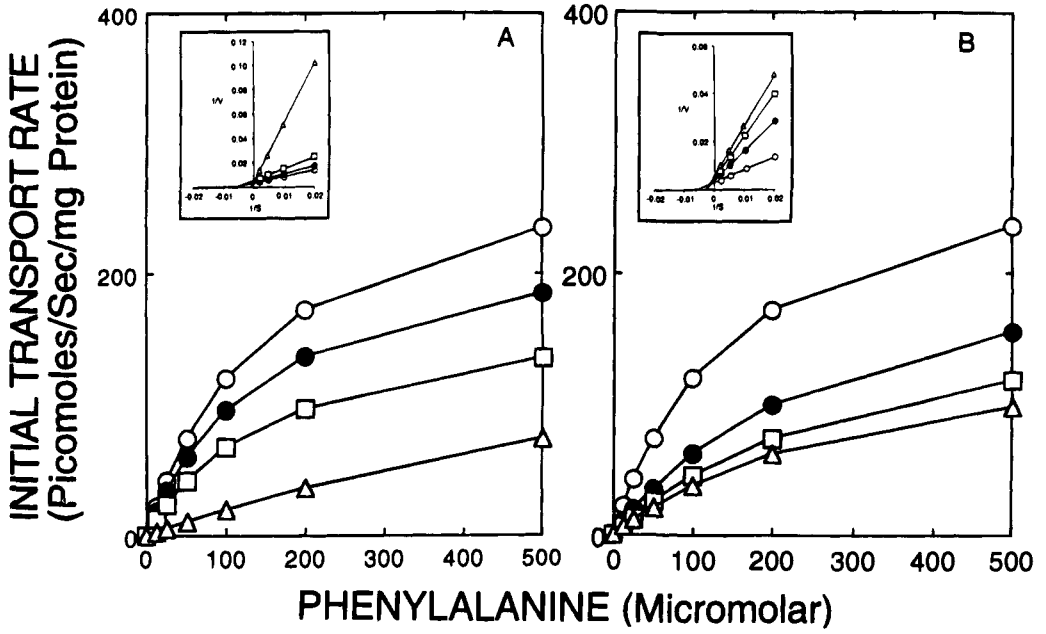


Figure 4. Inhibition of initial transport rates of L-Phe in CHO cells in the presence of **18** and **3a**. Analyses of transport inhibition by a Lineweaver–Burk plot are shown in the inset. Symbols are as described in Figure 3.

30–50 μM in Chinese hamster ovary cells.[25b] As shown in Figure 3A, initial rates of L-Phe transport were inhibited in a concentration-dependent manner in the presence of 1 at concentrations ranging from 2 to 200 μM. No detectable inhibition of the initial rates of transport was observed when assays were performed in the presence of the *R*-enantiomer **2** (Figure 3B), **17**, or **21** (data not shown), which supports the stereospecific nature of the transporter inhibition and a role for the carboxyl moiety in the transport process. Comparable results for **12a** are assumed, owing to its structural resemblance to **2** and **17**. Compound **1** competitively inhibited L-Phe uptake, as shown in a Lineweaver–Burk plot (Figure 3, inset).

Since α-amino acids are zwitterionic under physiological conditions, it is assumed that this is the ionic state required for substrates of the system L neutral amino acid carrier. The additional anionic character of the phosphonic acid moiety of **1** was expected to have a detrimental effect upon its ability to compete for L-Phe uptake. Initial rates of L-Phe uptake were determined in the presence of **18**, a derivative of **1** that lacks the phosphonic acid moiety. As shown in Figure 4A, initial rates of L-Phe transport were inhibited in a concentration-dependent manner in the presence of **18**. A Lineweaver–Burk plot indicated competitive inhibition of Phe uptake (Figure 4B). Estimates of the inhibition constants determined from Dixon plots (Figure 5) show that **1** and **18** displayed estimated K_i values of 50 and 25 μM, respectively. Therefore, these observations indicate that the phosphonic acid moiety does not substantially reduce the overall capacity of this class of compounds to serve as substrates for the neutral amino acid carrier.

The transport capacity of the 1-naphthyl derivative **3a**, which displayed increased potency as a competitive NMDA receptor antagonist relative to **1**, was also examined. **3a** displayed a similar concentration-de-

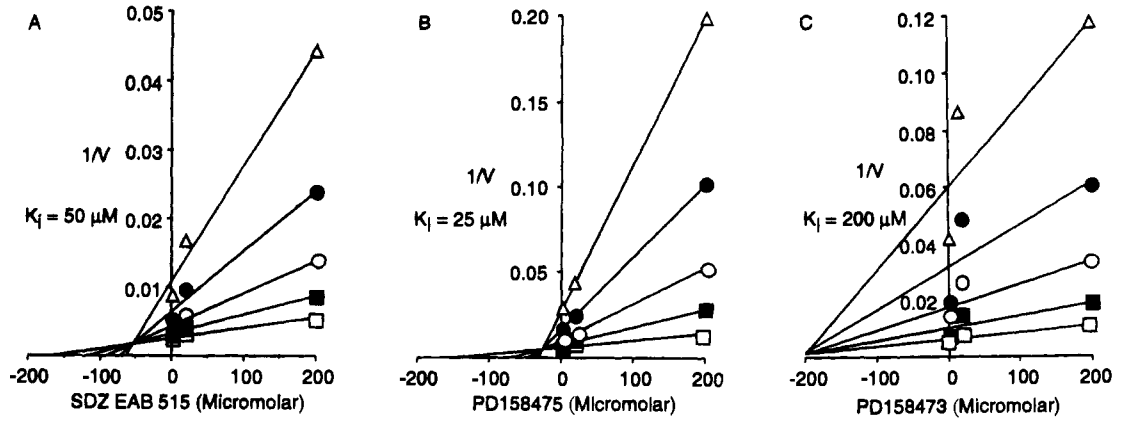


Figure 5. Dixon plot (1/initial rate versus inhibitor concentration) estimations of inhibition constants for 1, 18, and **3a**. The concentrations of L-Phe are indicated by symbols (open square, 500 μM; closed square, 200 μM; open circle, 100 μM; closed circle, 50 μM; open triangle, 25 μM).

pendent competitive inhibition of Phe uptake as was observed for **1** (Figure 4, insets). However, the estimated inhibition constant was significantly higher (K_i = 200 μM), indicating a reduced capacity for transport compared to **1**. The higher K_i may indicate a steric limitation of the transporter or reflect a reduced tolerance for side chain modifications since side chain characteristics of amino acids are a determinant in substrate recognition by transporters.

These results provide evidence that the NMDA antagonist **1** and its derivatives are able to utilize a neutral amino acid carrier and may be substrates for the large neutral amino acid carrier of the blood–brain barrier. Further experiments are needed to fully characterize their action. The neutral amino acid carrier has been shown to be capable of facilitating the uptake of a number of amino acid drugs such as L-DOPA, α-methyl-DOPA, azaserine, melphalan, 6-diazo-5-oxo-L-norleucine, and acivicin.[26]

Conclusions

The 1- and 2-naphthyl derivatives **3a,b** are among the most potent, competitive NMDA receptor antagonists yet described, with low nanomolar affinity. This high receptor affinity translates into powerful *in vivo* anticonvulsant activity as demonstrated by their activity in the mouse MES assay. This is expected to be predictive of neuroprotective activity in models of focal cerebral ischemia and cerebral trauma. Because these derivatives have a natural *S*-configuration of the α-amino acid moiety, they may use the L system neutral amino acid transporter to enhance oral adsorption and access into the central nervous system. Although they are not subtype-selective NMDA receptor antagonists[22] and do not have greater separation between anticonvulsant activity and adverse behavioral deficits than previously reported competitive NMDA antagonists (Table 2), the fact that they are potent and orally active may represent an opportunity for therapeutic use at submaximal neuroprotective doses in conditions such as stroke or head trauma, or, for example, as an adjuvant therapy in the treatment of pain or Parkinson's disease.

Experimental Section

Transport Studies. Cell Culture and Culture Method. All transport studies were performed using the CHO-K1 cell line, a proline auxotroph of the Chinese hamster ovary cell line. The cells were grown in monolayers to confluency, maintained at 37 °C in F-12 nutrient mixture (HAM) medium containing L-glutamine (GIBCO-BRL, Grand Island, NY) and supplemented with 5% (v/v) fetal calf serum, 120 units/mL penicillin, and 120 units/mL streptomycin.

Preparation of Compounds. Stock solutions for each of the test compounds were prepared by suspension in phosphate-buffered saline [37 mM NaCl, 2.7 mM KCl, 10.6 mM Na_2PO_4 (pH 7.4)] containing 2% (v/v) NH_4OH and stored at −20 °C until use.

Transport Assays. Cells suspended in F-12 nutrient mixture medium supplemented with 5% calf serum were plated into 12-well culture dishes (Costar, Cambridge, MA) and allowed to recover for 16–24 h at 37 °C before measurements of transport activity were performed. The cells were washed once in phosphate-buffered saline containing 5.6 mM D-glucose, 0.49 mM $MgCl_2$, and 0.68 mM $CaCl_2$ and incubated at 37 °C for 30 min in the same buffer (2 mL/well) to allow depletion of internal amino acid pools. Following the 30 min incubation period, the phosphate-buffered saline solution was removed from the wells by inverting and shaking the dish. To each well, a 225 μL aliquot of phosphate-buffered saline containing 25 μM–1 mM [^{3}H]-L-phenylalanine (0.02 μCi/μL) (Amersham Corp., Arlington Heights, IL) and the test compound, at final concentrations of 2–200 μM, were added. [^{3}H]-L-Phenylalanine transport activity was measured at 30 s intervals in the presence of each of the test compounds. At the conclusion of the uptake period, the medium was rapidly removed by aspiration and the transport terminated by two sequential 2 mL washes/well with ice-cold phosphate-buffered saline. After removal of the residual buffer, 220 μL of 10% (w/v) trichloroacetic acid was added and each well allowed to incubate for 1 h at room temperature. A 200 μL aliquot was removed, and radioactivity was quantified in a liquid scintillation counter. Cellular protein was determined using a modification of the method of Zak and Cohen[27] with bovine serum albumin as a standard.

Kinetic Analysis. Initial rates of uptake were determined for each of the indicated concentrations of L-Phe in the presence of the indicated test compound. Analyses of the kinetic constants were performed by nonlinear curve fit of the data to the expression: $v = (V_{max}[S]/k_m + [S]) + P[S]$ where P is the first-order term describing nonsaturable uptake.

Pharmacology Experimentals. Receptor binding experiments were run by measuring displacement of radioligands in rat cortex synaptosomal membranes according to literature methods.[18–20]

A standard model of electroshock-induced convulsions in mouse was used to determine anticonvulsant activity.[23] In this test mice received the test substance in a dosage of 0.1–100 mg/kg iv. At a given time (30 s–60 min) following the drug administration, a 50 mA, 200 ms long shock was applied with corneal electrodes smeared with electrolyte jelly. This suprathreshold shock produces tonic extensor convulsions of all extremities. Inhibition of the hindlimb extension is taken as

a protective action. After investigation of several dose levels and times, an ED_{50} was estimated.

Ataxia in mice was assessed by the inverted screen procedure in which mice were individually placed on a 4.0-in. square of wire mesh that was subsequently inverted. Any mouse that fell from the wire mesh during a 60 s test period was rated ataxic.[24]

Molecular Modeling. The structures of 1, **2**, **3a,b**, and **12a** were built using version 6.04 of the SYBYL software,[29] minimized with MAXIMIN, and oriented with the basic amine at the origin, the α-methylene of the amino acid along the x axis, and the carbon of the CO_2H in the $x-y$ plane.[28] To evaluate their fit to the existing pharmacophore model,[13] each analog was subjected to the following analysis. A 2 Å tensor was defined that was perpendicular to the plane of the proximal CO_2H, piercing through the hydroxyl oxygen. Using MULTIFIT,[29] the structures were then constrained to superimpose the end points of this tensor, the basic nitrogen, its lone pair, and two phosphonate receptor interaction points with analogous points on a reference compound (CGS 19755) taken from the previous pharmacophore model. The geometry of the reference compound was not allowed to change.

Intramolecular spring constants of 20 kcal/mol Å^2 were used on all fitting atoms, except for one of the phosphonic acid interaction points. The spring force constant for this atom was reduced to 10 kcal/mole Å^2 to reflect the somewhat reduced importance of tight interaction with this "secondary" site for maintenance of high affinity to the NMDA receptor. Charges were not included in the calculations. After running MULTIFIT, the structures were allowed to relax by rerunning MAXIMIN on the multifit geometry without any constraints. To check that low-energy conformations resulted from these fitting experiments, conformational searches (no charges employed, 30° increment on all rotatable bonds) were run to determine the minimum energy conformations. See ref 13 for details. In all cases, the fit conformations were within 2 kcal/mol of the minimum energy conformations.

Chemistry. General. The NMR spectra were recorded at 93.94 kG (400 MHz for ^{1}H, 100 MHz for ^{13}C), 70.45 kG (300 MHz for ^{1}H, 75 MHz for ^{13}C), and 46.97 kG (200 MHz for ^{1}H, 50 MHz for ^{13}C) in $CDCl_3$, unless otherwise stated. Residual $CHCl_3$ (δ 7.24) and the center line of the $CDCl_3$ triplet (δ 77.0) were used as internal references for ^{1}H- and ^{13}C-NMR, respectively. All OH and NH proton assignments were confirmed by D_2O exchange. D_2O was the solvent of choice for target amino acid derivatives, and the chemical shift values are reported relative to sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TSP) as internal standard. All flash chromatography was run using standard flash silica gel 60 (240–400 mesh) as adsorbent. Chiral thin layer chromatography was done on Chiralplate (Cat. No. 811056, Machery-Nagel). Melting points were observed on Mel-Temp II (Lab Devices) and are uncorrected. Optical rotations were obtained on a Perkin-Elmer-241 polarimeter, cell length 10 cm. All starting materials, unless otherwise stated, were commercially available and used without purification. Anhydrous tetrahydrofuran (THF) and anhydrous dimethylformamide (DMF) were purchased from Aldrich and used directly. Diisopropylamine was distilled from calcium hydride.

General Procedure A: Preparation of 1- and 2-(3,5-Dimethylphenyl)naphthalene (7a,b) via Palladium-Catalyzed Cross-Coupling of 3,5-Dimethyl-1-bromobenzene with 1- or 2-Naphthaleneboronic Acid. A mixture of 1,3-dimethyl-5-bromobenzene (1.85 g, 10 mmol), 1- or 2-naphthaleneboronic acid (1.89 g, 11 mmol), triethylamine (2.8 mL, 20 mmol), tri-2-tolylphosphine (0.125 g, 0.4 mmol), palladium(II) diacetate (0.045 g, 0.4 mmol), and anhydrous dimethylformamide (25 mL) with 5 Å molecular sieves (0.5 g) was stirred at 100 °C under a nitrogen atmosphere for 4 h. The solvent was evaporated in vacuo, and the residue was treated with 5% aqueous ammonia solution, extracted with ethyl acetate, and washed with saturated aqueous sodium chloride. After filtration to remove a black precipitate, the organic layer was separated and dried over magnesium sulfate, filtered, and evaporated. The residue was chromatographed on silica gel.

General Procedure B: Preparation of 1- and 2-[3,5-Bis(bromomethyl)phenyl]naphthalene (8a,b), 3,5-Bis-(bromomethyl)-4-nitrobiphenyl (8c), and 3-(Bromomethyl)biphenyl (14). A solution of the biaryl product of **7a–c** or **13** in carbon tetrachloride was treated with a catalytic amount of benzoyl peroxide and then *N*-bromosuccinimide (2.1 equiv for **8a–c** or 1.1 equiv for monophosphonylation product **14**). The mixture was refluxed and irradiated under a tungsten lamp for 1.0–3.0 h and then cooled to 0 °C. The precipitated succinimide was removed by filtration. The filtrate was evaporated, and the yellow residue was chromatographed on silica gel.

General Procedure C: Preparation of [3-(Bromomethyl)-5-naphthalen-1-ylbenzyl]phosphonic Acid Diethyl Ester (9a), [3-(Bromomethyl)-5-naphthalen-2-ylbenzyl]phosphonic Acid Diethyl Ester (9b), [[5-(Bromomethyl)-4-nitrobiphenyl-3-yl]methyl]phosphonic Acid Diethyl Ester (9c), and [[5-[(Diethoxyphosphoryl)methyl]biphenyl-3-ylmethyl]phosphonic Acid Diethyl Ester (20) via Michaelis–Arbuzov Phosphonylation. A solution of the bromomethyl adduct of **8a–c** in xylene was treated with triethyl phosphite and heated at reflux (140 °C) for 1.5–4.0 h as indicated in each experiment. The solvent was evaporated in vacuo, and the residue was purified by silica gel chromatography.

General Procedure D: Preparation of 10a–c, 11a,b, 15, and 16 via Stereospecific Substitution with (*S*)- or (*R*)-1-(*tert*-Butoxycarbonyl)-2-*tert*-butyl-3-methyl-4-imidazolidinone. To a solution of diisopropylamine (0.32 mL, 2.29 mmol) in 10 mL of anhydrous tetrahydrofuran was added *n*-butyllithium (1.43 mL, 1.6 M in hexane, 2.29 mmol) at −20 °C over 10 min. After stirring for 20 min at 0 °C, the LDA solution was cooled to −78 °C, and a solution of (*S*)-(−)- or (*R*)-(+)-1-(*tert*-butoxycarbonyl)-2-*tert*-butyl-3-methyl-4-imidazolidinone (0.586 g, 2.29 mmol) in anhydrous tetrahydrofuran (5 mL) was added over 20 min. The reaction mixture temperature was raised to −20 °C for 30 min (light yellow color), the mixture was recooled to −78 °C, and then a solution of **9a**, **9b**, **9c**, or **14** (2.08 mmol) in tetrahydrofuran (5 mL) was added to the reaction mixture over 20 min (orange color). After stirring for 3 h, the volume of the mixture was reduced to about 5 mL and then the mixture poured onto 30 mL of saturated ammonium chloride and extracted with diethyl ether. The organic layer was washed with saturated sodium chloride, dried over magnesium sulfate, filtered, and evaporated. The oily residue was purified by silica gel chromatography. All chromatography fractions were carefully checked; no trace amount of the corresponding undesired diastereoisomer was detected for any one of the reactions according to ^{1}H- and ^{13}C-NMR spectroscopy.

General Procedure E: Preparation of (*S* and *R*)-2-Amino-3-[3-naphthalen-1-yl-5-(phosphonomethyl)phenyl]-propionic Acid (3a and 12a), (*S* and *R*)-2-Amino-3-[3-naphthalen-2-yl-5-(phosphonomethyl)phenyl]propionic Acid (3b and 12b), (*S*)-2-Amino-3-[4-nitro-5-(phosphonomethyl)biphenyl-3-yl]propionic Acid (3c), and (*R* and *S*)-2-Amino-3-biphenyl-3-ylpropionic Acid (18 and 19). A solution of one of the imidazolidinone adducts (**10a,–c**, **11a,b**, **15**, **or 16**) (3.0 mmol) in 6 N HCl (20 mL), unless otherwise stated, was refluxed for 24 h. After cooling, Dowex 50W × 4 resin (2.0 g) was added to the reaction mixture and the solvent evaporated. The residue/resin was washed in a Dowex 50W × 4 resin column sequentially with water (50 mL), ethanol (50 mL), water (50 mL), 5% ammonium hydroxide (100 mL), and 30% ammonium hydroxide (50 mL). The product was identified by C18 reverse phase silica gel TLC plates (water:acetonitrile, 1:8) using ninhydrin as a stain. The fractions containing product were combined and evaporated in vacuo (foaming). Some of the products were further purified by preparative TLC (Alltech; precoated silica gel RP 18, "hybrid plate").

2-Naphthaleneboronic acid (6b): using an adaptation of a previously reported procedure.[30] To magnesium (12.16 g, 500 mmol) in anhydrous THF (500 mL) was added 10 mL of 2-bromonaphthalene (103.5 g, 500 mmol) in THF (150 mL). The mixture was heated to reflux until the boiling became

spontaneous, and the remaining solution of the bromide compound was added at a rate that maintained constant reflux. After the spontaneous reflux subsided, the dark solution was heated at reflux for an additional 20 min. A solution of trimethyl borate (54.08 g, 0.52 mmol) in THF (600 mL) was added dropwise to the Grignard reagent at −5 °C equipped with a mechanical stirrer. A white sludge separated from the solution during addition. After stirring for 15 min at room temperature, 10% HCl (500 mL) was added to the suspension. Partition of the acidic solution with ether (500 mL), drying of the organic extract with magnesium sulfate, and removal of the solvent gave a white solid in 62% yield: mp 263−264 °C (lit.[31] mp 263−266 °C).

1-(3,5-Dimethylphenyl)naphthalene (7a): prepared from 3,5-dimethyl-1-bromobenzene and 1-naphthaleneboronic acid according to general procedure A. Purification by flash chromatography (methylene chloride:petroleum ether, 1:2) gave a colorless oil (1.7 g) in 73% yield. The oil crystallized upon standing: mp 68−70 °C; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 8.01−7.84 (m, 3H), 7.58−7.40 (m, 6H), 7.10 (s, 1H), 2.42 (s, 6H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 140.6, 140.5, 137.7, 133.7, 132.5, 131.7, 128.8, 128.2, 127.9, 127.4, 126.7, 126.2, 125.8, 125.7, 125.6, 125.3, 21.4.

2-(3,5-Dimethylphenyl)naphthalene (7b): prepared from 3,5-dimethyl-1-bromobenzene and 2-naphthaleneboronic acid (**6b**) according to general procedure A. Purification by flash chromatography (methylene chloride:hexane, 1:10) gave a white solid (1.28 g) in 69% yield: mp 72−74 °C; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 8.05−7.35 (m, 10H), 2.45 (s, 6H).

1-[3,5-Bis(bromomethyl)phenyl]naphthalene (8a): prepared from **7a** (6.0 g, 25.9 mmol), benzoyl peroxide (0.193 g, 0.8 mmol), and *N*-bromosuccinimide (9.68 g, 54.4 mmol) in carbon tetrachloride (100 mL) according to general procedure B. The mixture was refluxed for 1.5 h. Purification by flash chromatography (methylene chloride:hexane, 1:2) gave a colorless oil (8.1 g) in 80% yield: ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.95−7.80 (m, 3H), 7.50−7.39 (m, 7H), 4.56 (s, 4H).

2-[3,5-Bis(bromomethyl)phenyl]naphthalene (8b): prepared from 2-(3,5-dimethylphenyl)naphthalene, **7b** (4.0 g, 17.2 mmol), benzoyl peroxide (0.121 g, 0.5 mmol), and *N*-bromosuccinimide (6.4 g, 36.1 mmol) in carbon tetrachloride (100 mL) according to general procedure B. The mixture was refluxed for 3 h. Purification by flash chromatography (methylene chloride:hexane, 1:5) gave white needle crystals (5.03 g) in 75% yield: mp 160−162 °C; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 8.05−7.45 (m, 10H), 4.57 (s, 4H); MS (CI) *m/e* 388 (M^+). Anal. ($C_{18}H_{14}Br_2$) C, H.

3,5-Bis(bromomethyl)-4-nitrobiphenyl (8c): prepared from 2,6-dimethyl-4-phenylnitrobenzene, **7c** (2.27 g, 10.0 mmol), benzoyl peroxide (0.072 g, 0.3 mmol), and *N*-bromosuccinimide (3.74 g, 21.0 mmol) in carbon tetrachloride (100 mL) according to general procedure B. The mixture was refluxed for 4 h. Purification by flash chromatography (methylene chloride:hexane, 1:3) gave white needle crystals (2.69 g) in 70% yield: mp 133−135 °C; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.67 (s, 2H), 7.56−7.45 (m, 5H), 4.57 (s, 4H).

[3-(Bromomethyl)-5-naphthalen-1-ylbenzyl]phosphonic acid diethyl ester (9a): prepared from **8a** (4.6 g, 14.8 mmol) and triethyl phosphite (2.7 g, 16.2 mmol) in xylene (50 mL) according to general procedure C. The mixture was refluxed for 2.0 h. Purification by flash chromatography (methylene chloride:ethyl acetate, 4:1) gave the diethyl phosphonate as a colorless oil (3.8 g) in 70% yield: ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.99−7.80 (m, 3H), 7. 58−7.30 (m, 7H), 4.54 (s, 2H), 4.08 (q, J = 7.1 Hz, 2H), 4.04 (q, J = 7.1 Hz, 2H), 3.22 (d, J = 21.7 Hz, 2H), 1.27 (t, t, J = 7.1, 7.1 Hz, 6H).

[3-(Bromomethyl)-5-naphthalen-2-ylbenzyl]phosphonic acid diethyl ester (9b): prepared from **8b** (2.07 g, 5.33 mmol) and triethyl phosphite (1.22 g, 7.33 mmol) in xylene (40 mL) according to general procedure C. The mixture was refluxed for 1.5 h. Purification by flash chromatography (methylene chloride:ethyl acetate, 10:1, as eluant) gave the diethyl phosphonate as a colorless oil (1.5 g) in 63% yield: ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 8.03−7.35 (m, 10H), 4.58 (s, 2H), 4.10 (q, J = 7.1 Hz, 2H), 4.08 (q, J = 7.1 Hz, 2H), 3.25 (d, J = 21.7 Hz, 2H); 1.29 (t, t, J = 7.1, 7.1 Hz, 6H).

[[5-(Bromomethyl)-4-nitrobiphenyl-3-yl]methyl]phosphonic acid diethyl ester (9c): prepared from **8c** (0.9 g, 2.33 mmol) and triethyl phosphite (0.426 g, 2.56 mmol) in xylene (30 mL) according to general procedure C. The mixture was refluxed for 2.5 h. Purification by flash chromatography (methylene chloride: ethyl acetate 1:1) gave the diethyl phosphonate as a colorless oil (0.505 g) in 48% yield: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.80−7.45 (m, 7H), 4.56 (s, 2H), 4.04 (q, J = 7.0 Hz, 2H), 4.03 (q, J = 7.0 Hz, 2H), 3.65 (d, J = 22.2 Hz, 2H), 1.24 (t, J = 7.0 Hz, 6H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 148.9 (d, J = 7.4 Hz), 144.0 (d, J = 2.9 Hz), 138.0, 131.4 (d, J = 2.9 Hz), 131.1 (d, J = 5.3 Hz), 129.1 (2C), 128.9, 128.8, 127.2, 126.5 (d, J = 8.8 Hz), 62.6, 62.4, 29.6 (d, J = 139.0 Hz), 27.5, 16.3, 16.2.

(2*S*)-*trans*-2-*tert*-Butyl-5-[3-[(diethoxyphosphoryl)methyl]-5-naphthalen-1-ylbenzyl]-3-methyl-4-oxoimidazolidine-1-carboxylic acid *tert*-butyl ester (10a): prepared from **9a** (0.93 g, 2.08 mmol) according to general procedure D using (*S*)-(−)-1-(*tert*-butoxycarbonyl)-2-*tert*-butyl-3-methyl-4-imidazolidinone chiral auxiliary. The crude product was purified by silica gel chromatography (ethyl acetate:methanol, 5:1, as eluant) to give the adduct as a colorless oil (0.685 g) in 53% yield: $[\alpha]^{20}_D$ +22° (c = 1.6, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.90−7.72 (m, 3H), 7.50−7.32 (m, 4H), 7.15−7.05 (m, 3H), 4.70 (s, 1H), 4.30 (br s, 1H), 4.13−3.92 (m, 4H), 3.82 (d, J = 14.0 Hz, 1H), 3.26 (d, J = 14.0 Hz, 1H), 3.12 (d, J = 22 Hz, 2H), 2.72 (s, 3H), 1.35−1.12 (m, 12H), 0.89 (s, 9H).

(2*R*)-*trans*-2-*tert*-Butyl-5-[3-[(diethoxyphosphoryl)methyl]-5-naphthalen-1-ylbenzyl]-3-methyl-4-oxoimidazolidine-1-carboxylic acid *tert*-butyl ester (11a): prepared from **9a** (0.90 g, 2.0 mmol) according to general procedure D using (*R*)-(−)-1-(*tert*-butoxycarbonyl)-2-*tert*-butyl-3-methyl-4-imidazolidinone chiral auxiliary; 0.94 g (72%) of **11a** was obtained as a colorless oil: $[\alpha]^{20}_D$ −19° (c = 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 200 MHz) as for **10a**.

(2*S*)-*trans*-2-*tert*-Butyl-5-[3-[(diethoxyphosphoryl)methyl]-5-naphthalen-2-ylbenzyl]-3-methyl-4-oxoimidazolidine-1-carboxylic acid *tert*-butyl ester (10b): prepared from **9b** (0.93 g, 2.08 mmol) according to general procedure D using (*S*)-(−)-1-(*tert*-butoxycarbonyl)-2-*tert*-butyl-3-methyl-4-imidazolidinone chiral auxiliary. The crude product was purified by silica gel chromatography (ethyl acetate as eluant) to give the adduct as a colorless oil (1.1 g) in 85% yield: $[\alpha]^{20}_D$ +38° (c = 1.6, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 8.10 (s, 1H) 7.90−7.68 (m, 4H), 7.55−7.36 (m, 4H), 7.06 (s, 1H), 4.67 (s, 1H), 4.34 (br s, 1H), 4.06 (q, J = 7.1 Hz, 2H), 4.04 (q, J = 7.1 Hz, 2H), 3.82 (d, J = 14.0 Hz, 1H), 3.28 (d, J = 14.0 Hz), 3.16 (d, J = 21.6 Hz, 2H), 2.81 (s, 3H), 1.38 (s, 9H), 1.27 (t, J = 7.1 Hz, 3H), 1.26 (t, J = 7.1 Hz, 3H), 0.92 (s, 9H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 171.5, 152.1, 140.7 (d, J = 3.0 Hz), 138.0, 136.8 (d, J = 2.7 Hz), 133.6, 132.6, 131.7 (d, J = 2.9 Hz), 130.6 (d, J = 6.8 Hz), 128.3, 128.2, 127.7, 127.6, 127.1 (d, J = 26 Hz), 126.2, 125.9, 125.7, 125.5, 81.6 (2C), 62.2 (d, $J_{p\text{-}c}$ = 4.2 Hz), 62.1 (d, $J_{p\text{-}c}$ = 4.2 Hz), 60.6, 41.0, 34.0, 33.8 (d, $J_{p\text{-}c}$ = 137.8 Hz), 31.9, 28.3 (3C), 26.6 (3C), 16.5, 16.4.

(2*R*)-*trans*-2-*tert*-Butyl-5-[3-[(diethoxyphosphoryl)methyl]-5-naphthalen-2-ylbenzyl]-3-methyl-4-oxoimidazolidine-1-carboxylic acid *tert*-butyl ester (11b): prepared from **9b** (0.90 g, 2.0 mmol) according to general procedure D using (*R*)-(−)-1-(*tert*-butoxycarbonyl)-2-*tert*-butyl-3-methyl-4-imidazolidinone chiral auxiliary; 1.07 g (82%) of **11b** was obtained as a colorless oil: $[\alpha]^{20}_D$ −36° (c = 1.6, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 200 MHz) as for **10b**.

(2*S*)-*trans*-2-*tert*-Butyl-5-[[5-[(diethoxyphosphoryl)methyl]-4-nitrobiphenyl-3-yl]methyl]-3-methyl-4-oxoimidazolidine-1-carboxylic acid *tert*-butyl ester (10c): prepared from **9c** (0.48 g, 1.7 mmol) according to general procedure D using (*S*)-(−)-1-(*tert*-butoxycarbonyl)-2-*tert*-butyl-3-methyl-4-imidazolidinone chiral auxiliary. The crude product was purified by silica gel chromatography (ethyl acetate: methylene chloride, 1:5, as eluant) to give the adduct as a colorless oil (0.893 g) in 85% yield: $[\alpha]^{20}_D$ −19° (c = 1.6, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.56−7.35 (m, 6H), 7.22 (s, 1H), 5.01 (s, 1H), 4.34 (dd, J = 6.1, 3.0 Hz, 1H), 4.12−3.90 (m, 4H), 3.65 (dd, J = 16.6, 3.0 Hz, 1H), 3.45 (dd, J = 16.6, 6.1 Hz, 1H), 3.33 (dd, $J_{p\text{-}H}$ = 22.2 Hz, J = 8.2 Hz, 2H), 2.95 (s,

3H), 1.31 (s, 9H), 1.26 (m, 6H), 0.98 (m, 9H); ^{13}C-NMR ($CDCl_3$, 75 MHz) 152.7, 150.3, (d, J_{p-c} = 7.7 Hz), 142.8 (d, J_{p-c} = 3.1 Hz), 139.1, 131.1, 129.0 (2C), 128.7 (d, J_{p-c} = 5.4 Hz), 127.2 (2C), 126.6 (d, J_{p-c} = 3.3 Hz), 125.3 (d, J_{p-c} = 9.0 Hz), 81.6, 81.0, 62.5, 62.4, 58.3, 40.7, 32.1, 29.5 (d, J_{p-c} = 130.0 Hz), 27.9 (3C), 26.4 (3C), 16.4, 16.2.

(*S*)-2-Amino-3-[3-naphthalen-1-yl-5-(phosphonomethyl)phenyl]propionic acid (3a): prepared from **10a** (1.96 g, 3.0 mmol) according to general procedure E. A white solid (0.959 g) was obtained in 83% yield after lyophilization: mp >262 °C dec; $[\alpha]^{20}_D$ −12°, $[\alpha]^{20}_{365}$ −31° (*c* = 1.0, 10% NH_4OH in H_2O); TLC Chiralplate (CH_3CN:H_2O, 1:2) R_f 0.50, pure; ^{1}H-NMR (D_2O, 300 MHz) δ 7.79 (m, 1H), 7.68−7.56 (m, 2H), 7.36−7.24 (m, 4H), 7.20 (s, 1H), 7.15 (s, 1H), 7.01 (s, 1H), 3.90 (dd, *J* = 9.1, 4.4 Hz, 1H), 3.24 (dd, *J* = 14.3, 4.4 Hz, 1H), 2.91 (d, *J* = 19.8 Hz, 2H), 2.85 (dd, *J* =14.3, 9.1 Hz, 1H); ^{13}C-NMR (D_2O, 75 MHz) δ 176.7, 143.5, 142.1, 139.9, 139.8, 138.1, 136.2, 133.6, 133.1 (d, J_{p-c} = 6.1 Hz), 131.9 (d, J_{p-c} = 5.2 Hz), 131.0, 130.9, 130.5, 129.8, 129.1, 128.8, 128.4, 58.8, 39.5 (d, J_{p-c} = 126 Hz), 38.2; LRMS (ES, 50/50 MeOH/H_2O + 0.1% NH_4OH) *m*/*e* 384 (M^- − 1); HRMS calcd for $C_{20}H_{20}NO_5P$ 386.1157, found 386.1152. Anal. ($C_{20}H_{20}NO_5P$) C, H, N.

(*R*)-2-Amino-3-[3-naphthalen-1-yl-5-(phosphonomethyl)phenyl]propionic acid (12a): prepared from 2*R*,5*R*- **11a** (1.96 g, 3.0 mmol) according to general procedure E. A white solid (0.924 g) was obtained in 80% yield after lyophilization: mp > 265 °C dec; $[\alpha]^{20}_D$ +11°, $[\alpha]^{20}_{365}$ +31° (*c* = 1.0, 10% NH_4OH in H_2O); TLC Chiralplate (CH_3CN:H_2O, 1:2) R_f 0.42, pure; ^{1}H-NMR (D_2O, 300 MHz), ^{13}C-NMR (D_2O, 75 MHz), and LRMS (ES, 50/50 MeOH/H_2O + 0.1% NH_4OH) as for **3a** (*S*); HRMS calcd for $C_{20}H_{20}NO_5P$ 386.1157, found 386.1157. Anal. ($C_{20}H_{20}NO_5P$) C, H, N.

(*S*)-2-Amino-3-[3-naphthalen-2-yl-5-(phosphonomethyl)phenyl]propionic acid (3b): prepared from **10b** (1.96 g, 3.0 mmol) according to general procedure E. The crude product was further purified by preparative TLC plate (CH_3CN:H_2O, 1:5). A white solid (0.75 g) was obtained in 65% yield after lyophilization: TLC Chiralplate (CH_3CN:H_2O, 1:2) R_f 0.50, pure; mp >270 °C dec; $[\alpha]^{20}_D$ −7.5° (*c* = 1.0, 10% NH_4OH in H_2O); ^{1}H-NMR (2% NaOD in D_2O, 300 MHz) δ 8.19 (s, 1H), 7.99−7.86 (m, 4H), 7.63−7.52 (m, 3H), 7.44 (s, 1H), 7.26 (s, 1H), 3.60 (dd, *J* = 8.0, 4.1 Hz, 1H), 3.13 (dd, *J* = 13.3, 4.1 Hz, 1H), 2.98 (d, J_{p-H} = 19.7 Hz, 2H), 2.98 (dd, *J* = 13.3, 8.0 Hz, 1H); ^{13}C-NMR (2% NaOD in D_2O, 75 MHz) δ 182.1, 139.6, 139.2, 139.1, 138.5, 137.8, 133.0, 131.9, 129.5 (d, J_{p-c} = 4.4 Hz), 128.1, 127.8, 127.2, 126.2, 125.8, 125.2, 125.0, 124.3, 57.1, 40.7, 36.9 (d, J_{p-c} = 122 Hz); LRMS (ES, 50/50 MeOH/H_2O + 0.1% NH_4OH) *m*/*e* 384 (M^- − 1); HRMS calcd for $C_{20}H_{20}NO_5P$ 386.1157, found 386.1162. Anal. ($C_{20}H_{20}NO_5P$) C, H, N.

(*R*)-2-Amino-3-[3-naphthalen-2-yl-5-(phosphonomethyl)phenyl]propionic acid (12b): prepared from **11b** (0.652 g, 1.0 mmol) according to general procedure E. The crude product was further purified by preparative TLC plate (CH_3CN:H_2O, 1:5). A white solid (0.25 g) was obtained in 65% yield after lyophilization: mp >270 °C dec; $[\alpha]^{20}_D$ +7° (*c* = 1.0, 10% NH_4OH in H_2O); TLC Chiralplate (CH_3CN:H_2O, 1:2) R_f 0.40, pure; ^{1}H-NMR (D_2O, 300 MHz), ^{13}C-NMR (D_2O, 75 MHz), and LRMS (ES, 50/50 MeOH/H_2O + 0.1% NH_4OH) as for **3b** (*S*); HRMS calcd for $C_{20}H_{20}NO_5P$ 386.1157, found 386.1162. Anal. ($C_{20}H_{20}NO_5P$) C, H, N.

(*S*)-2-Amino-3-[4-nitro-5-(phosphonomethyl)biphenyl-3-yl]propionic acid (3c): prepared from **10c** (1.02 g, 1.6 mmol) according to general procedure E. The crude product was further purified by preparative TLC plate (CH_3CN:H_2O:TFA, 25:85:0.1). A white solid (0.38 g) was obtained in 62% yield after lyophilization: TLC Chiralplate (CH_3CN:H_2O, 1:2) R_f 0.7, pure; mp >250 °C dec; $[\alpha]^{20}_D$ −10°, $[\alpha]^{20}_{365}$ −71° (*c* = 1.0, 10% NH_4OH in H_2O); ^{1}H-NMR (2% TFA-d_1 in D_2O, 200 MHz) δ 7.39−7.17 (m, 7H), 4.13 (dd, *J* = 6.7, 6.7 Hz, 1H), 3.12 (d, J_{p-H} = 21.9 Hz, 2H), 3.10 (dd, *J* = 8.0, 6.1 Hz, 1H), 2.93 (dd, *J* = 8.0, 6.7 Hz, 1H); LRMS (ES, 50/50 MeOH/H_2O + 0.1% NH_4OH) *m*/*e* 379 (M^- − 1); HRMS calcd for $C_{16}H_{17}N_2O_7P$ 381.0852, found 381.0830.

3-(Bromomethyl)biphenyl (14): prepared from 3-phenyltoluene (4.88 g, 29.0 mmol), benzoyl peroxide (0.193 g, 0.8 mmol), and *N*-bromosuccinimide (5.68 g, 31.9 mmol) in carbon tetrachloride (80 mL) according to general procedure B without application of tungsten lamp. The mixture was refluxed for 1.5 h. Purification by flash chromatography (methylene chloride:hexane, 1:8) gave white needles (4.44 g) in 62% yield: mp 55−56 °C; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.70−7.39 (m, 9H), 4.59 (s, 2H).

1,1-Dimethylethyl (2*R*)-*trans*-5-([1,1'-biphenyl]-3-ylmethyl)-2-(1,1-dimethylethyl)-3-methyl-4-oxo-1-imidazolidinecarboxylate (15): prepared from **14** (0.75 g, 3.04 mmol) according to general procedure D. The crude product was purified by silica gel chromatography (CH_2Cl_2:EtOAc, 10:1) to give the adduct as a colorless oil (0.92 g) in 72% yield: $[\alpha]^{20}_D$ −55° (*c* = 0.15, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.58−7.14 (m, 9H), 4.61 (s, 1H), 4.35 (s, 1H), 3.85 (d, *J* = 13.9 Hz, 1H), 3.25 (d, *J* = 13.9 Hz, 1H), 2.75 (s, 3H), 1.42 (s, 9H), 0.93 (s, 9H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 171.3, 150.3, 140.9, 140.7, 136.2, 128.9, 128.7, 128.5 (2C), 128.2, 127.0 (2C), 125.3, 80.9 (2C), 60.6, 40.8, 34.0, 31.7, 28.1 (3C), 26.5 (3C).

1,1-Dimethylethyl (2*S*)-*trans*-5-([1,1'-biphenyl]-3-ylmethyl)-2-(1,1-dimethylethyl)-3-methyl-4-oxo-1-imidazolidinecarboxylate (16): prepared from **14** (0.75 g, 3.04 mmol) according to general procedure D. The crude product was purified by silica gel chromatography (CH_2Cl_2:EtOAc, 10:1) to give the adduct as a colorless oil (1.13 g) in 88% yield: $[\alpha]^{20}_D$ +52° (*c* = 0.15, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 200 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz) as for **3a** (*S*).

(*R*)-2-Amino-3-biphenyl-3-ylpropionic acid (17): prepared from **15** (0.9 g, 2.13 mmol) according to general procedure E, but refluxed for 3 h. A white solid (0.38 g) was obtained in 74% yield after concentration of the 2% NH_4OH/H_2O elution from the resin column: TLC Chiralplate (CH_3CN:CH_3OH:H_2O, 1:1:2) R_f 0.10, pure; mp 225 °C dec; $[\alpha]^{20}_{365}$ −20° (*c* = 1.0, 10% NH_4OH in H_2O); ^{1}H-NMR ($CDCl_3$, 200 MHz, 2% NaOD in D_2O) δ 6.77−6.51 (m, 9H), 2.81 (dd, *J* = 8.3, 3.4 Hz, 1H), 2.45 (dd, *J* = 12.9, 3.4 Hz, 1H), 2.05 (dd, *J* = 12.9, 8.3 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 50 MHz, 2% NaOD in D_2O) δ 184.5, 143.2, 143.1, 141.8, 131.8, 131.5 (2C), 131.0, 130.4, 130.0, 129.6 (2C), 127.6, 60.1, 44.0; LRMS (ES, 50/50 MeOH/H_2O + 0.1% NH_4OH) *m*/*e* 242 (M^- + 1); HRMS calcd for $C_{15}H_{15}NO_2$ 242.1181, found 242.1180. Anal. ($C_{15}H_{15}NO_2$) C, H, N.

(*S*)-2-Amino-3-biphenyl-3-ylpropionic acid (18): prepared from **16** (0.5 g, 1.18 mmol) according to general procedure E but refluxed for 2 h in 2 N HCl. A white solid (0.1 g) was obtained in 35% yield after concentration of the 2% NH_4OH/H_2O elution from the resin column: TLC Chiralplate (CH_3CN:CH_3OH:H_2O, 1:1:2) R_f 0.18, pure; mp 227 °C dec; $[\alpha]^{20}_{365}$ +20° (*c* = 1.0, 10% NH_4OH in H_2O); ^{1}H-NMR ($CDCl_3$, 200 MHz, 2% NaOD in D_2O), ^{13}C-NMR ($CDCl_3$, 50 MHz, 2% NaOD in D_2O), and LRMS (ES, 50/50 MeOH/H_2O + 0.1% NH_4OH) *m*/*e* 242 (M^- + 1) as for **17**; HRMS calcd for $C_{15}H_{15}NO_2$ 242.1181, found 242.1177. Anal. ($C_{15}H_{15}NO_2$) C, H, N.

[[5-[(Diethoxyphosphoryl)methyl]biphenyl-3-yl]methyl]phosphonic acid diethyl ester (20): prepared from 1,3-bis(bromomethyl)-5-phenylbenzene (**19**)[12] (1.02 g, 3.0 mmol) and triethyl phosphite (2.7 g, 10.0 mmol) in xylene (20 mL) according to general procedure C. The mixture was refluxed for 4 h. Purification by flash chromatography (ethyl acetate) gave the product (1.25 g) as a colorless oil in 92% yield: ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 7.60−7.15 (m, 8H), 4.04 (q, *J* = 7.0 Hz, 4H), 4.00 (q, *J* = 7.0 Hz, 4H), 3.16 (d, *J* = 22 Hz, 4H), 1.22 (t, *J* = 7.0 Hz, 12H).

[[5-(Phosphonomethyl)biphenyl-3-yl]methyl]phosphonic acid (21): prepared from **20** (0.97 g, 2.14 mmol) by refluxing in 6 N HCl (15 mL) for 48 h. The solution was reduced to one-half its volume (7 mL) and cooled at 0 °C. The resulting precipitates were filtered and collected. The solid residue was purified by crystallization (MeOH:H_2O, 1:5) to give a white solid (0.68 g) in 93% yield: mp 265−267 °C dec; ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.60−7.18 (m, 8H), 3.17 (d, *J* = 21.9 Hz, 4H); MS (ES, 50/50 MeOH in H_2O + 1% NH_4OH) *m*/*e* 342.7. Anal. ($C_{14}H_{16}O_6P_2$) C, H.

Acknowledgment. We wish to acknowledge the intellectual contributions of Peter A. Boxer for discus-

sions related to these potent NMDA antagonists and supporting experiments conducted by Richard M. Woodward (Acea Pharmaceuticals, Inc.), Lillian Robichaud (cortical wedge), Gregory Campbell (LDH release), and Linda Coughenour (TCP binding) that confirm the competitive NMDA antagonist activity of **1**, and **3a,b**. We would also like to thank the Analytical Chemistry group for C,H,N analyses and high-resolution mass spectroscopy.

Supplementary Material Available: Coordinates of the fit conformation of **3b** in SYBYL Mol2 file format (2 pages). Ordering information may be found on any current masthead page.

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JM950061L