Design, Synthesis, SAR, and Biological Evaluation of Highly Potent Benzimidazole-Spaced Phosphono-α-Amino Acid Competitive NMDA Antagonists of the AP-6 Type

Reinhardt B. Baudy,*,† Horace Fletcher III,† John P. Yardley,† Margaret M. Zaleska,‡ Donna R. Bramlett,‡ Rene P. Tasse,‡ Dianne M. Kowal,‡ Alan H. Katz,§ John A. Moyer,‡ and Magid Abou-Gharbia†

Chemical Sciences and Division of Neuroscience, Wyeth-Ayerst Research, CN-8000, Princeton, New Jersey 08543-8000

Received September 5, 2000

A series of 2-amino-(phosphonoalkyl)-1*H*-benzimidazole-2-alkanoic acids was synthesized and evaluated for NMDA receptor affinity using a [^{3}H]CPP binding assay. Functional antagonism of the NMDA receptor complex was evaluated in vitro using a stimulated [^{3}H]TCP binding assay and in vivo by employing an NMDA-induced seizure model. Several compounds of the AP-6 type demonstrated potent and selective NMDA antagonistic activity both in vitro and in vivo. In particular, [*R*(−)]-2-amino-3-(5-chloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic acid (**1**) displayed an IC_{50} value of 7.1 nM in the [^{3}H]CPP binding assay and an ED_{50} value of 0.13 mg/kg (ip) in the NMDA lethality model. Compound **1**, when administered intravenously as a single bolus dose of 3 mg/kg following permanent occlusion of the middle cerebral artery in the rat, reduced the volume of infarcted brain tissue by 45%. These results support a promising therapeutic potential for compound **1** as a neuroprotective agent.

Introduction

While the excitation and depression of mammalian cortical neurons by amino acids were already described in the 1960s by Crawford and Curtis,[1] the concept of excitotoxicity, in particular glutamate excitotoxicity, causing acute neuronal degeneration by excessive stimulation of postsynaptic excitatory amino acid ionotropic receptors was advanced nearly 30 years ago by Olney et al.[2] Such neurotoxic effects of exogenous glutamate in infant mice had already been reported by Lucas and Newhouse in 1957.[3] Later it was shown by Coyle that the effects of exogenous glutamate could be mimicked with *N*-methyl-D-aspartate (NMDA).[4] Evidence supports the primary role of excitotoxic mechanisms in the pathogenesis of neuronal death due to acute brain ischemia[5] and central nervous system (CNS) trauma.[6] Excitatory amino acid (EAA) mediated toxicity contributes to neuronal cell loss in clinical conditions that are both rapid in onset (stroke, head trauma, spinal injury[7−9]) as well as slow in progression (Huntington's disease, amyotropic lateral sclerosis, epilepsy, AIDS-dementia, and Alzheimer disease[10−12]). Acute ischemic stroke, which results in uncontrolled glutamate release and subsequent overactivation of the excitatory amino acid transmitter system, induced in particular by hypoxia and energy depletion, is a leading cause of death and long-term disability worldwide. Antagonists of the voltage-gated calcium channels, and especially antagonists of the NMDA receptor which are among the most extensively studied of this class of receptors, have been shown to offer protection against permanent ischemic damage in animal models even when administered several hours after the ischemic event.[13] A large number of competitive NMDA antagonists have been reported in the literature over the last 20 years (Figure 1), and in the early 1990s a wave of clinical trials testing the efficacy of NMDA antagonists in the treatment of cerebral ischemia was launched.

However, in late 1995, all stroke phase III clinical trials for the competitive NMDA antagonist *cis*-4-(phosphono-methyl)-2-piperidine carboxylic acid (CGS-19755; Selfotel) were terminated. These authors suggest that clinical trials for NMDA antagonists in stroke may have been launched prematurely with 'nonoptimal' compounds. Failure of these early trials have adversely affected the development of potentially superior NMDA antagonists. Competitive antagonists can be displaced by glutamate, necessitating high concentrations of antagonist to overcome the elevated levels of glutamate during ischemia. With the high antagonist concentrations, normal, nonischemic NMDA receptor activity would be blocked, resulting in adverse side effects, as are seen with competitive antagonists such as CGS-19755, NPC-12626, and dCPP-ene.[14] In an effort to discover efficacious NMDA antagonists, our laboratories have identified and reported on several novel NMDA antagonists, including quinoxaline-spaced AP-6 type phosphono α-amino acids[15] and EAA-090,[16] in which the 3,4-diamino-3-cyclobutene-1,2-dione moiety is incorporated as an α-amino acid bioisostere. The earlier discovered quinoxaline-spaced phosphono α-amino acids (Figure 1) led us to the present investigation from which we report on the design and synthesis of 2-amino-(phosphonoalkyl)-1*H*-benzimidazole-2-alkanoic acids, another class of AP-6 type NMDA antagonists. Compound **1** demonstrates one of the most potent competitive NMDA receptor affinities described to date. Its NMDA subtype specificity is currently being evaluated.[17]

* To whom correspondence should be addressed. Phone: (732) 274-4424. Fax: (732) 274-4505. E-mail: Baudyr@war.wyeth.com.
† Chemical Sciences Division.
‡ Neuroscience Division.
§ Biological Chemistry.

10.1021/jm000385w CCC: $20.00
Published on Web 04/12/2001

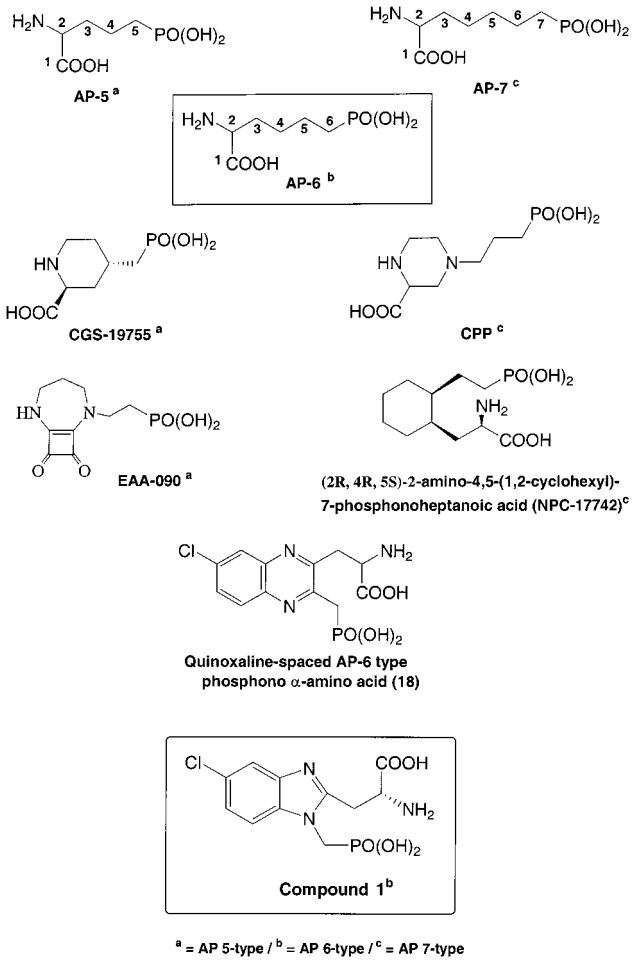


Figure 1. AP-5's and AP-7's versus AP-6's.

Chemistry

The 2-amino-(phosphonoalkyl)-1*H*-benzimidazole-2-alkanoic acids **1**−**17** (Table 1) were synthesized as outlined in Schemes 1−8.

Scheme 1 illustrates the syntheses of a series of chloro-substituted benzimidazoles. Typically, the α-benzyl ester of *N*-Boc-aspartic acid was condensed[18] with an appropriately chloro-substituted 1,2-phenylenediamine **1a**, **2a**, and **5a** via its mixed anhydride, and the resulting malonamic acid derivatives **1b**, **2b**, and **5b** were cyclized in the presence of acetic acid to the benzimidazole derivatives **1c**, **2c**, and **5c**. Alkylation of the benzimidazole nitrogen with trifluoromethanesulfonic acid (dimethoxyphosphinyl) methyl ester in the presence of potassium carbonate produced the desired phosphonate esters **1d**, **2d**, and **5d**, which after hydrogenation over palladium on charcoal afforded the propionic acids **1e**, **2e**, and **5e**. The penultimate intermediates were subsequently hydrolyzed in refluxing 6 N hydrochloric acid to yield the desired chloro-substituted benzimidazole-2-propionic acids **1**, **2**, and **5**.

Scheme 2 depicts the syntheses of compounds **3**, **4**, **10**, and **11** starting from benzimidazole-derived intermediates (**3a**, **4a**, **10a**, and **11a**) which were prepared according to the method of Nestor et al.[19] The compounds were further elaborated to their corresponding phosphonate ethyl esters **3b**, **4b**, **10b**, and **11b** via alkylation with trifluoromethanesulfonic acid (dimethoxyphosphinyl) methyl ester followed by hydrogenation in the presence of palladium to afford the free α-amino acids **3c**, **4c**, **10c**, and **11c**. The remaining phosphonate ester moieties were hydrolyzed to their phosphonic acids **3** and **4** using trimethylsilyl bromide,[20] while **10** and **11** were obtained through hydrolysis in refluxing 6 N hydrochloric acid.

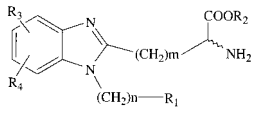
The syntheses of the 4-chloro analogue **6** and the 7-chloro analogue **7** proved challenging. As outlined in Scheme 3, compound **6b** was obtained by treating commercially available 2-chloro-6-fluoroaniline **6a** with fluoboric acid in the presence of sodium nitrite. Regioisomer **7b** was attained by subjecting 2,3-dichloronitrobenzene **7a** to 20% potassium fluoride on calcium fluoride in sulfolane at 150 °C for several days. Treatment of both regioisomers **6b** and **7b** with diethylaminomethyl phosphonate, prepared according to the method of Davidson et al.,[21] afforded the intermediates **6c** and **7c** which, after hydrogenation in the presence of rhodium,[22] produced the aniline derivatives **6d** and **7d**. Coupling[23] with commercial *N*-CBz-D-aspartic acid α-benzyl ester in the presence of bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (BOP·Cl) gave rise to intermediates **6e** and **7e** which, via *p*-toluenesulfonic acid-catalyzed cyclization in refluxing toluene, were transformed to their respective benzimidazole derivatives **6f** and **7f**. Hydrogenation in the presence of palladium and subsequent hydrolyses in refluxing 6 N hydrochloric acid provided the desired 4-chloro- and 7-chloro-regioisomers compounds **6** and **7**, respectively.

Analogues **8** and **9** were prepared (Scheme 4) employing methods similar to those shown in Scheme 2. The use of trifluoromethanesulfonic acid (dimethoxyphosphinyl) methyl ester proved unsuccessful. However, substituting trifluoromethanesulfonic acid [bis-(4-nitrophenylmethoxy) phosphinyl] methyl ester in the alkylation reaction yielded the desired intermediates **8b** and **9b**. Hydrogenation over palladium produced the analogues **8** and **9c**. Treatment of compound **9c** with hydrochloric acid produced the dihydrochloride **9**.

Compound **12**, whose synthesis is shown in Scheme 5, was prepared as a putative prodrug of compound **8**. Coupling the commercially available *N*-CBz-D-aspartic acid α-ethyl ester **12a** to *o*-phenylenediamine via the mixed anhydride followed by acetic acid-catalyzed cyclization afforded the benzimidazole derivative **12b**. Alkylation with trifluoromethanesulfonic acid [bis-(4-nitro phenylmethoxy)phosphinyl] methyl ester in the presence of potassium carbonate gave compound **12c**. Hydrogenation over palladium on carbon yielded the desired ethyl ester **12**.

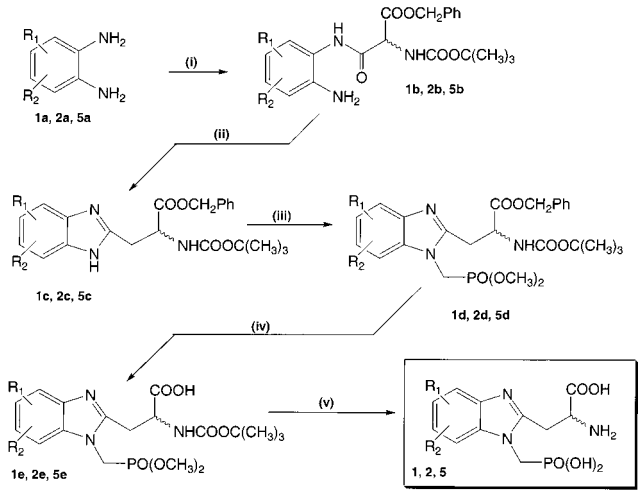
To further assess structure−activity relationships, the phosphonic acid side chain was extended by one carbon as exemplified with the synthesis of compound **13** (Scheme 6). The starting *o*-phenylenediamine **13a** was alkylated with diethyl (2-bromoethyl) phosphonate, giving rise to compound **13b** which, after coupling to *N*-CBz-D-aspartic acid α-benzyl ester, was cyclized in the presence of acetic acid to afford compound **13d**. Removal of the benzyl protecting groups via hydrogenation over palladium yielded the phosphonic acid ethyl ester **13e**. Treatment of **13e** with trimethylsilyl bromide led to the desired compound **13**.

Table 1. α-Amino-1*H*-benzimidazole-2-alkanoic Acid Derivatives



compd	R_1	R_2	R_3	R_4	*n*	*m*	mp, °C	formula
1 (−)-enantiomer	$PO(OH)_2$	H	5-chloro	H	1	1	113−6 (dec)	$C_{11}H_{13}ClN3O_5P{\cdot}HCl$
2 (−)-enantiomer	$PO(OH)_2$	H	H	6-chloro	1	1	205−7 (dec)	$C_{11}H_{13}ClN3O_5P{\cdot}0.8HCl{\cdot}H_2O$
3 (−)-enantiomer	$PO(OH)_2$	H	H	H	1	1	190−200	$C_{11}H_{14}N_3O_5P{\cdot}2HCl{\cdot}H_2O$
4 (−)-enantiomer	$PO(OH)_2$	H	5-chloro	6-chloro	1	1	>220	$C_{11}H_{12}Cl_2N_3O_5P{\cdot}HCl$
5 (+)-enantiomer	$PO(OH)_2$	H	5-chloro	6-chloro	1	1	>220	$C_{11}H_{12}Cl_2N_3O_5P{\cdot}2H_2O$
6 (−)-enantiomer	$PO(OH)_2$	H	4-chloro	H	1	1	>220	$C_{11}H_{13}ClN_3O_5P{\cdot}HCl{\cdot}1.5H_2O$
7 (−)-enantiomer	$PO(OH)_2$	H	H	7-chloro	1	1	148−52	$C_{11}H_{13}ClN_3O_5P{\cdot}1.5H_2O$ 2TRIS
8 (+)-enantiomer	$PO(OH)_2$	H	H	H	1	1	>220	$C_{11}H_{14}N_3O_5P{\cdot}2HCl{\cdot}2H_2O$
9 (−)-enantiomer	$PO(OH)_2$	H	5-methyl	6-methyl	1	1	>220	$C_{13}H_{18}N_3O_5P{\cdot}2HCl$
10 (+)-enantiomer	$PO(OH)_2$	H	H	H	1	2	>220	$C_{12}H_{16}N_3O_5P{\cdot}2HCl{\cdot}MeOH$
11 (−)-enantiomer	$PO(OH)_2$	H	H	H	1	2	>220	$C_{12}H_{16}N_3O_5P{\cdot}2.5H_2O$
12 (−)-enantiomer	$PO(OH)_2$	Et	H	H	1	1	210−2	$C_{13}H_{18}N_3O_5P{\cdot}H_2O$
13 (−)-enantiomer	$PO(OH)_2$	H	H	H	2	1	>220	$C_{12}H_{16}N_3O_5P{\cdot}2HCl{\cdot}0.5$ MeOH
14 (−)-enantiomer	COOH	H	H	H	1	1	86−8	$C_{12}H_{13}N_3O_4{\cdot}2HBr.0.5\ C_6H_{14}O$
15 racemate	$PO(OH)_2$	H	5-trifluoromethyl	H	1	1	>310 (dec)	$C_{12}H_{13}F_3N_3O_5P{\cdot}H_2O$
16 racemate	$PO(OH)_2$	CH_2Ph	5-bromo	H	1	1	203−5	$C_{18}H_{19}BrN_3O_5P{\cdot}HCl{\cdot}0.25H_2O$
17 racemate	$PO(OH)_2$	CH_2Ph	5-chloro	H	1	1	206−8	$C_{18}H_{19}ClN_3O_5P{\cdot}HCl{\cdot}0.5H_2O$

Scheme 1[a]

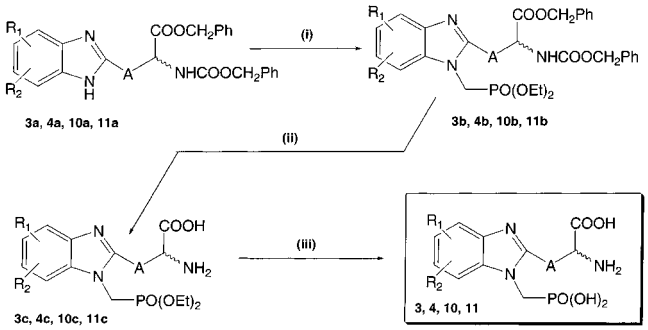


[a] Reagents: (i) *N*-Boc-aspartic acid-α-benzyl ester, ethyl chloroformate, triethylamine; (ii) AcOH; (iii) trifluoromethanesulfonic acid (dimethoxyphoshinyl) methyl ester, potassium carbonate; (iv) hydrogen, Pd/C; (v) HCl. **1a** = **2a**: R_1 = 4-Cl, R_2 = H/**5a**: R_1 = 4-Cl, R_2 = 5-Cl. **1b** = **2b**: 4- and 5-Cl regioisomers/**5b**: R_1 = 4-Cl, R_2 = 5-Cl. **1c** = **2c**: 5- and 6-Cl regioisomers/**5c**: R_1 = 5-Cl, R_2 = 6-Cl. **1d**: R_1 = 5-Cl, R_2 = H/**2d**: R_1 = H, R_2 = 6-Cl/**5d**: R_1 = 5-Cl, R_2 = 6-Cl. **1e**: R_1 = 5-Cl, R_2 = H/**2e**: R_1 = H, R_2 = 6-Cl/**5e**: R_1 = 5-Cl, R_2 = 6- Cl. **1**: R_1 = 5-Cl, R_2 = H (−)-enantiomer. **2**: R_1 = H, R_2 = 6-Cl (−)-enantiomer. **5**: R_1 = 5-Cl, R_2 = 6-Cl (+)-enantiomer.

Scheme 7 displays the synthesis of **14**, a compound in which the phosphonic acid moiety was replaced by the bioisosteric carboxylic acid group. Alkylation of compound **14a**[19] with benzyl 2-bromoacetate led to compound **14b** which was subjected to hydrogenation over palladium to afford the diacid **14c**. Deprotection of the amine in glacial hydrobromic acid[24] led to the desired compound **14** as its dihydrobromide salt.

The final Scheme 8 illustrates the syntheses of two halo-substituted α-amino acid benzyl esters **16** and **17** as well as the preparation of **15**, a trifluoromethyl-substituted benzimidazole phosphono α-amino acid. Alkylation of aminomethyl phosphonic acid diethyl ester with the commercially available starting materials **15a**, **16a**, and **17a** produced the phosphonate esters **15b**, **16b**, and **17b**. Reduction of **15b** with hydrazine over Raney nickel gave the aniline derivative **15c**, while **16b** and **17b** were reduced over rhodium on carbon to afford the analogues **16c** and **17c**, respectively. Coupling of the anilines with *N*-benzyloxycarbonyl-D-aspartic acid-α-benzyl ester followed by cyclization in the presence of propanoic acid yielded compounds **15e**, **16e**, and **17e**. Treatment of **16e** and **17e** with trimethylsilyl iodide[25] afforded the desired benzyl esters **16** and **17**, respectively. Compound **15e** was subjected to hydrogenolysis over palladium on carbon followed by trimethylsilyl bromide deprotection to yield compound **15**.

Scheme 2[a]

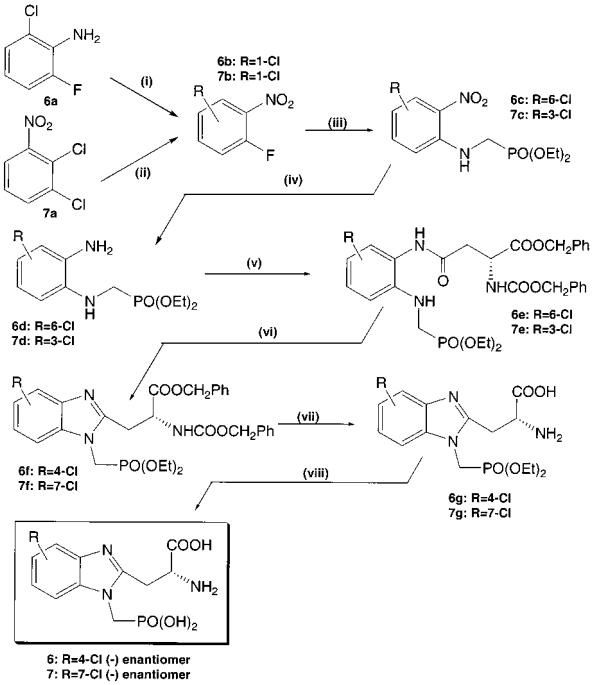


[a] Reagents: (i) trifluoromethanesulfonic acid-(diethoxyphosphinyl)-methyl ester, K_2CO_3; (ii) hydrogen, Pd/C; (iii) HCl or TMS-Br, propylene oxide, HCl. **3a**: R_1 = R_2 = H, A = CH_2; **4a**: R_1 = 5-Cl, R_2 = 6-Cl, A = CH_2; **10a** = **11a**: R_1 = R_2 = H, A = CH_2−CH_2. **3b**: R_1 = R_2 = H, A = CH_2; **4b**: R_1 = 5-Cl, R_2 = 6-Cl, A = CH_2; **10b** = **11b**: R_1 = R_2 = H, A = CH_2−CH_2. **3c**: R_1 = R_2 = H, A = CH_2; **4c**: R_1 = 5-Cl, R_2 = 6-Cl, A = CH_2; **10c** = **11c**: R_1 = R_2 = H, A = CH_2−CH_2. **3**: R_1 = R_2 = H, A = CH_2. **4**: R_1 = 5-Cl, R_2 = 6-Cl, A = CH_2. **10**: R_1 = R_2 = H, A = CH_2−CH_2 (+)-enantiomer. **11**: R_1 = R_2 = H, A = CH_2−CH_2 (−)-enantiomer.

Results and Discussion

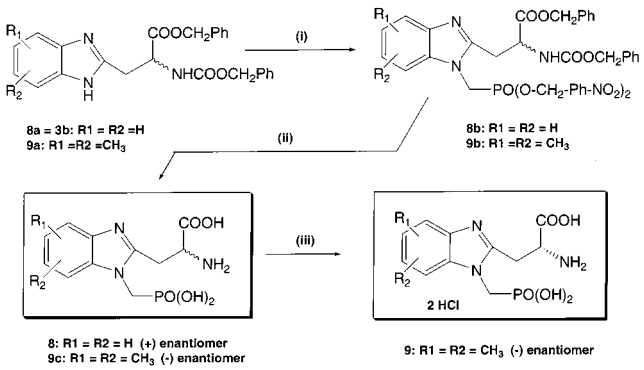
Table 2 lists the biological data for the compounds **1**−**17** along with the results obtained for standard competitive NMDA antagonists CGS-19755, CPP, and AP-7. Affinity for the competitive NMDA receptor site

Scheme 3[a]



[a] Reagents: (i) fluoboric acid, sodium nitrite; (ii) KF, CaF_2; (iii) aminomethyl-phoshonic acid diethyl ester; (iv) hydrogen, Rh/C; (v) *N*-CBz-D-aspartic acid-α-benzyl ester, BOP·Cl, Hunig's base or isobutyl chloroformate; (vi) AcOH or p-TSA; (vii) hydrogen, Pd/C; (viii) HCl.

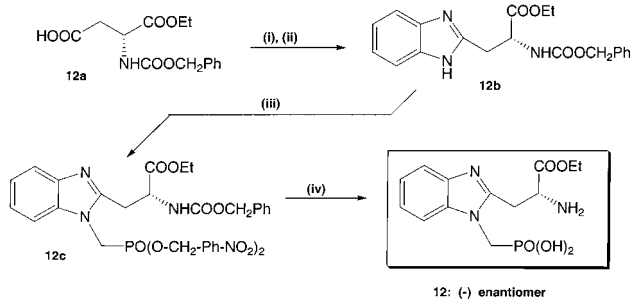
Scheme 4[a]



[a] Reagents: (i) trifluoromethane sulfonic acid [bis-(4-nitrophenylmethoxy)phosphinyl]methyl ester, K_2CO_3; (ii) hydrogen, Pd/C; (iii) HCl.

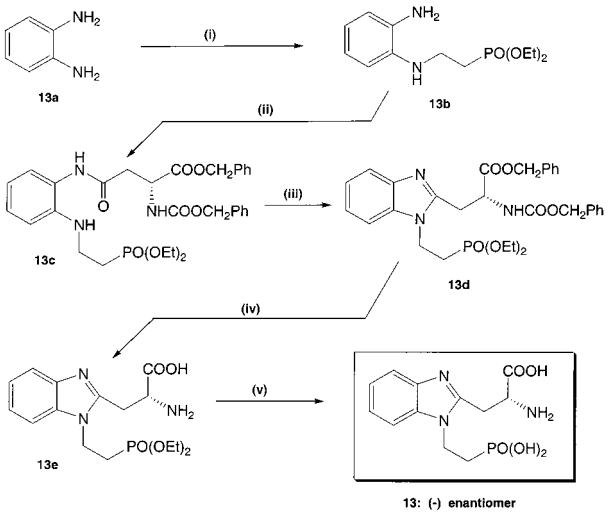
was determined by assessing the ability of the compounds to displace [^{3}H]-CPP from its binding site on rat synaptic membranes. Functional NMDA antagonist activity was determined in vitro using a stimulated [^{3}H]-TCP binding assay.[26] As was discussed in a prior publication,[15] competitive NMDA receptor antagonists decrease the association rate of ligands for the PCP binding site within the ion channel on the NMDA receptor ion channel complex.[26,27] Therefore, competitive antagonists appear to inhibit the stimulated binding of a tritiated PCP analogue, [^{3}H]-TCP, following 1 or 2 h of incubation.[28] Standard competitive NMDA antagonist ligands and compounds **1**−**3**, **16**, and **17** (which exhibited significant NMDA receptor affinity) inhibited stimulated [^{3}H]-TCP binding with a relative potency which paralleled with their affinity for the competitive NMDA receptor. The degree of significance between these two in vitro activities (Figure 5, $\chi^2 = 0.6$, $n = 8$) suggests a

Scheme 5[a]



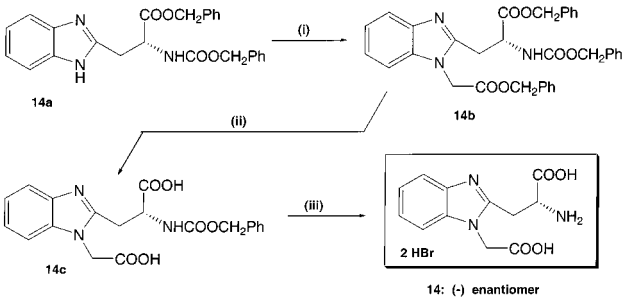
[a] Reagents: 1,2-diaminobenzene, isobutyl chloroformate; (ii) acetic acid; (iii) trifluoromethanesulfonic acid (di-*p*-nitrobenzylphosphinyl) methyl ester, potassium carbonate; (iv) hydrogen, Pd/C.

Scheme 6[a]



[a] Reagents: (i) diethyl 2-bromoethylphosphonate, K_2CO_3; (ii) *N*-CBz-D-aspartic acid-α-benzyl ester, isobutyl chloroformate; (iii) acetic acid; (iv) hydrogen, Pd/C; (v) TMS·Br.

Scheme 7[a]



[a] Reagents: (i) benzyl 2-bromoacetate, K_2CO_3; (ii) hydrogen, Pd/C; (iii) HBr/AcOH.

good correlation. Functional NMDA antagonist activity was confirmed in vivo in mice using an NMDA-induced lethality assay.

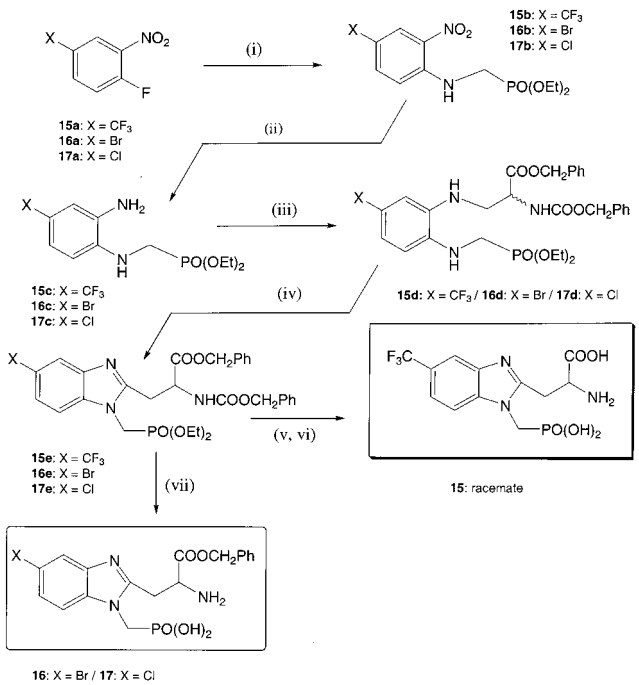
To evaluate compound **1** for in vivo neuroprotective efficacy, a rat model of permanent focal ischemia was used. This model involves an occlusion of the middle cerebral artery (MCA) and is considered to be a relevant animal model of severe ischemic human stroke.[29] As shown in Table 3, administration of compound **1** as a single iv bolus 5 min after the induction of MCA occlusion markedly diminished ischemic damage to the CNS. When evaluated 24 h after MCA occlusion, the

Table 2. Biological Activities of Reference NMDA Antagonists and Compounds **1**−**17**

compd	displacement of [^{3}H]CPP binding IC_{50} (μM)[a,b] [% inhibition at 100 μM]	displacement of [^{3}H]TCP binding IC_{50} (μM)[a, c] [% inhibition at 100 μM]	inhibition of NMDA lethality ED_{50} (mg/kg, ip)[a, d]
CGS-19755	0.028 (22.3−34.2)	22.4 (18.0−27.8)	0.6 (0.42−0.93)
CPP	0.1126 (96.1−132.3)	45.0 (39.1−51.8)	1.0 (0.35−2.89)
AP-7	0.388 (277−511)	302.0 (217−389)	37.6 (31.4−45.1)
1	0.0071 (0.0053−0.0095)	0.72 (0.53−0.91)	0.13 (0.03−3)
2	0.022 (0.018−0.026)	13.6 (11.4−16.3)	2.56 (0.61−2.8)
3	0.077 (0.059−0.098)	11.1 (9.9−12.4)	2.04 (1.28−3.27)
4	0.018 (0.014−0.024)	NT	2.58 (1.85−3.59)
5	1.0 (0.8−1.2)	NT	NT
6	[81]	NT	NT
7	[73]	[28.1]	NT
8	[30]	[29.1]	7.91 (6.42−9.75)
9	[99.7]	[88.5]	NT
10	5.2 (4.5−6.1)	NT	NT
11	6.8 (5.2−9.0)	NT	NT
12	[61]	[0]	NT
13	[39]	[9.5]	NT
14	[30]	[9.5]	NT
15	0.75 (0.512−1.089)	[40]	NT
16	0.056 (0.0435−0.0691)	15.5 (10.9−20.4)	0.47 (0.25−0.88)
17	0.15 (0.123−0.185)	46.5 (34−58.7)	NT

[a] Confidence limits= 95%/NT = not tested. [b] IC_{50} is the concentration of the test compound that inhibited 50% of the binding of a fixed concentration of the standard NMDA antagonist ligand [^{3}H]CPP. [c] IC_{50} represents the concentration of the test compound that decreases 50% of the binding of [^{3}H]TCP to its recognition site within the ion channel of the NMDA/ion channel complex. [d] ED_{50} is the dose of test compound that inhibited 50% of the lethality resulting from administration of an LD_{50} dose of NMDA.

Scheme 8[a]



[a] Reagents: aminomethyl-phosphonic acid diethyl ester; (ii) Raney nickel, hydrazine [**15**] or Rh/C, hydrogen [**16**, **17**]; (iii) *N*-CBz-D-aspartic acid-α-benzyl ester; (iv) propanoic acid [**15**] or p-TSA [**16**, **17**]; (v) Pd/C, H_2; (vi) TMS-Br; (vii) TMS·I [**16**, **17**].

volume of infarcted brain tissue in animals treated with compound **1** was reduced by 45% as compared to the vehicle-treated control group ($p < 0.05$). These results suggest a promising neuroprotective potential for compound **1**.

The unsubstituted parent molecule **8** displaced only 30% [^{3}H]-CPP at a concentration of 100 μM in the binding assay. In the inhibition of NMDA-induced lethality, **8** showed a rather moderate ED_{50} value of 7.91 mg/kg (ip). Since compound **8** represented an AP-6 configuration, we felt that, unlike an earlier series of

Table 3. Effect of Compound **1** on the Volume of Infarcted Brain Tissue in the Rat Permanent Focal Ischemia Model[a]

treatment group	*n*	volume of infarct (mm^3)	% decrease in infarct volume
vehicle	27	93.4 ± 9.3	
compound **1**	28	51.3 ± 6.3	45

[a] Values are presented as mean ± SE. Compound **1** was dissolved in 0.9% NaCl (vehicle) and was administered 5 min following the MCA occlusion as a single intravenous bolus injection at a dose of 3 mg/kg. The volume of brain infarct was quantified at 24 h post occlusion. $P < 0.05$ as compared to the vehicle group.

quinoxaline-spaced compounds[15] displaying high affinity for the NMDA receptor, the conventional AP-7 configuration is required in this series to obtain increased affinity for the receptor. However, compound **10**, an AP-7 construct, displayed an IC_{50} value of 5.2 μM. In a continued pursuance of increased NMDA receptor affinity, we synthesized compound **3**, the *R*-enantiomer of compound **8**. Gratifyingly, compound **3** displaced [^{3}H]-CPP in the binding assay with an IC_{50} value of 77 nM. Likewise, we synthesized compound **11**, the (*R*)(−)-enantiomer of compound **10**; however, its lack of significant affinity with an IC_{50} value of 6.8 μM in the [^{3}H]-CPP binding assay prompted us to prepare one more AP-7 analogue in this series of compounds to ensure ourselves that AP-7-derived compounds were void of robust affinity for the NMDA receptor. Extension of the phosphonic acid side chain by one carbon while maintaining the alanine moiety of the 2-position of benzimidazole resulted in compound **13**. Despite the earlier shown preferred (*R*)-configuration, **13** displaced only 39% [^{3}H]-CPP at a concentration of 100 μM in the binding assay. The binding data thus far encouraged us to pursue structural modifications while maintaining the AP-6 configuration. Replacing the phosphonic acid moiety on compound **3** with a carboxylic acid group led to compound **14**, which exhibited a dramatic decrease in NMDA receptor binding affinity, showing only 30% inhibition at 100 μM. In another synthetic quest we

probed the binding affinity of α-amino acid ester **12** for the NMDA receptor; the compound showed only 61% inhibition at a concentration of 100 μM. Since symmetrically disubstituted benzimidazoles are synthetically reasonably accessible, we first prepared the 5,6-dichloro analogue **4** [(*R*)(−)-enantiomer]. In the NMDA receptor binding assay, **4** turned out to be potent with an IC_{50} value of 18 nM, and an ED_{50} value in the NMDA-induced lethality assay of 2.58 mg/kg (ip). The corresponding (*S*)(+)-enantiomer **5** was also prepared showing an IC_{50} value of 1 μM in the NMDA binding assay. Little change in binding affinity was observed when the 5,6-dichloro substituents were replaced with methyl groups; the 5,6-dimethyl analogue **9** [(*R*)-enantiomer] accomplished 99.7% inhibition at 100 μM in the [^{3}H]-CPP binding assay.

In view of the potent 5,6-dichloro analogue **4** we embarked on the syntheses of monochloro-substituted analogues. The 4-chloro-substituted derivative **6** as well as the 7-chloro-substituted derivative **7** [both are (*R*)(−)-enantiomers] displayed 81% and 73% inhibition, respectively, at 100 μM in the [^{3}H]-CPP binding assay. For compound **2**, the 6-chloro analogue, an IC_{50} value of 22 nM was obtained in the [^{3}H]-CPP binding assay; in the NMDA-induced lethality assay the compound displayed an ED_{50} value of 2.56 mg/kg (ip). In contrast, compound **1**, the 5-chloro analogue, displayed an ED_{50} value of 0.13 mg/kg (ip) in the NMDA lethality assay while its binding affinity (IC_{50} = 7.1 nM) demonstrated only a 3-fold improvement over that seen for compound **2**. Substituting the chlorine of compound **1** with a trifluoromethyl group, compound **15**, brought about a dramatic drop in NMDA receptor affinity with an IC_{50} value of only 750 nM.

In an attempt to facilitate blood brain barrier penetration, the α-amino acid benzylesters **16** and **17** were prepared. The 5-bromo-substituted analogue **16** displayed an IC_{50} value of 56 nM in the binding assay and an ED_{50} value in the NMDA-induced lethality assay of 0.47 mg/kg (ip). The corresponding 5-chloro analogue **17** showed an IC_{50} value of only 150 nM in the displacement of [^{3}H]-CPP. The potency of putative prodrugs **16** and **17** in the binding assay suggests that the benzyl esters of these analogues can also display respectable competitive NMDA affinity in their own right. One possible explanation for the discrepancies between in vitro and in vivo activity for some of the above-mentioned amino acid-phosphonic acids (**1**, **2**, **3**, and **4**) could be their differing abilities to diffuse across the blood-brain barrier. Additionally, literature precedents[30] suggest that active transport could also be a consideration.

Computer Modeling of Compound 1. An overlap of compound **1** with CGS-19755 is shown in Figure 2. A series of constrained minimizations were carried out in which the more flexible benzimidazole (compound **1**) was forced to adopt conformations that matched various phosphonoalkyl rotomers of the CGS compound. In the resulting overlap, the piperdine ring of CGS-19755 matches the pseudo-ring formed by this intramolecular hydrogen bond. The conformations obtained from the best multifit result were minimized to their local minima and overlapped via a four-point rigid fit to produce Figure 2. This overlap is based on the *R*-

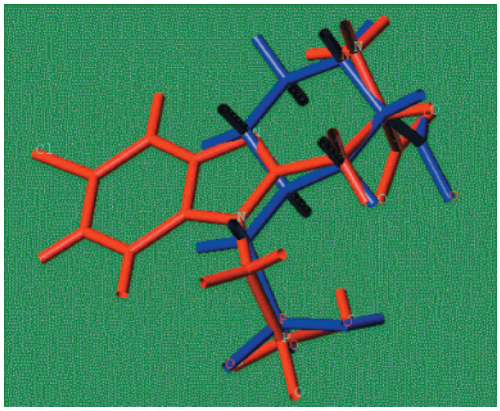


Figure 2. Overlay of the low-energy conformation of compound **1** (red) and CGS-19755 (blue) from the SYBYL program.

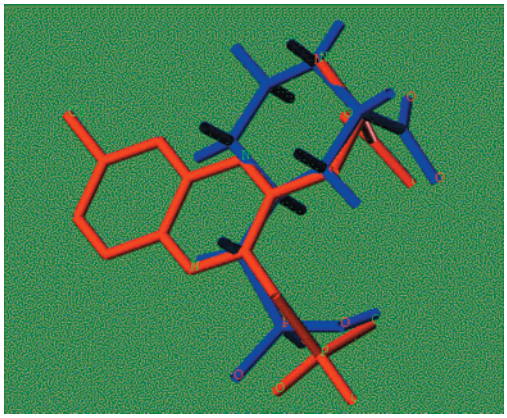


Figure 3. Overlay of the low-energy conformation of the quinoxaline-spaced AP-6 type phosphono α-amino acid (red, **18**) and CGS-19755 (blue) from the SYBYL program.

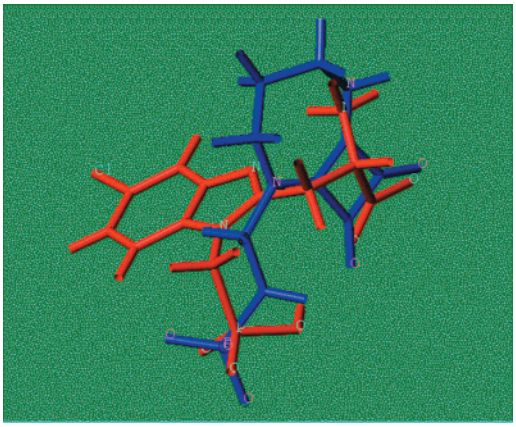


Figure 4. Overlay of the low-energy conformation of compound **1** (red) and EAA-090 (blue) from the SYBYL program.

entantiomer of compound **1**. The *S*-entantiomer cannot simultaneously form the intramolecular hydrogen bond and place the carboxylate group in a similar position in space. This may explain the biological inactivity of the *S*-enantiomer.

A similar overlap of the quinoxaline-spaced AP-6 type phosphono α-amino acid (**18**) and CGS-19755 was created using the same method as above and is shown in Figure 3. Finally, compound **1** was overlapped with EAA-090[16] as shown in Figure 4. Previous work in this laboratory has demonstrated the ability of the amino squarate nucleus to mimic amino acids. The overlap was therefore constructed to allow the squarate oxygens to

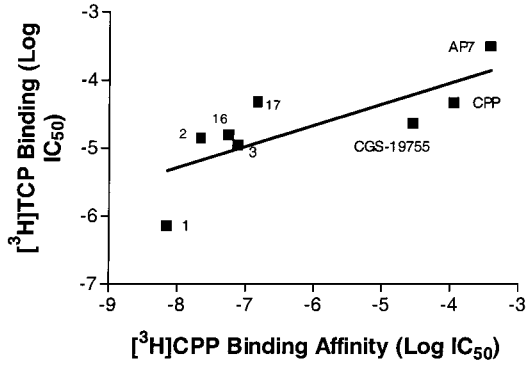


Figure 5. Correlation between affinity for the competitive NMDA receptor binding site (as measured by displacement of [^{3}H]CPP) and for functional antagonism (as measured by inhibition of stimulated [3H]TCP binding).

match the carboxylate oxygens along with the quaternary nitrogens and the phosphorus atoms. This overlap forces the seven-membered ring to lie perpendicular to the plane of compounds **1** and may in part explain its reduced biological activity.

Conclusion

We have demonstrated that 2-amino-(phosphonoalkyl)-1*H*-benzimidazole-2-alkanoic acids are potent AP6-type ligands for the NMDA receptor and are potent systemic inhibitors of NMDA-induced lethality. Compound **1**, [*R*(−)]-2-amino-3-(5-chloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic acid, currently represents one of the most potent known ligands for the NMDA receptor and the most potent inhibitor of NMDA-induced lethality reported to date. A single iv dose of compound **1** resulted in good neuroprotection in a rat focal ischemia model, indicating its potential as a therapeutic agent for the treatment of stroke and other neurodegenerative disorders.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on either a Varian XL-200 or a Bruker AM-400 spectrometer using tetramethylsilane as an internal standard. The chemical shifts are reported in parts per million (δ) downfield from TMS, and coupling constants are reported in hertz (Hz). Mass spectra were recorded on a Hewlett-Packard 5995A spectrometer or a Finnigan 8230 high-resolution instrument. The infrared spectra were recorded on a Perkin-Elmer 784 spectrophotometer. C, H, N combustion analyses were determined on either a Perkin-Elmer 240 or 2400 analyzer, and all analyzed compounds are within ±0.4% of the theoretical value unless otherwise indicated. Organic extracts were dried over magnesium sulfate and were evaporated in vacuo with a rotary evaporator. All products, unless otherwise noted, were purified by flash column chromatography using 230−400 mesh silica gel or by HPLC using a Waters Prep 500 instrument with silica Prep-Pak cartridges. Thin-layer chromatography was performed on silica gel 60 F-254 (0.25 mm thickness) plates. Visualization was accomplished with UV light and/or I_2 vapor.

[*R*(−)]-2-Amino-3-(5-chloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid, 1 Hydrochloride, Scheme 1/Compound 1: 2-*tert*-Butoxycarbonylamino-3-[5-chloro-1-(dimethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic Acid Benzyl Ester (1d). Under dry nitrogen, commercial (*R*)-2-*tert*-butoxycarbonylamino-succinic acid 1-benzyl ester (14.55 g, 45 mmol) was dissolved in dry tetrahydrofuran (350 mL) and cooled to −10 °C. In succession, triethylamine (4.55 g, 45 mmol) and ethyl chloroformate (4.882 g, 45 mmol) were added, and the reaction mixture was stirred for 10 min at −10 °C after which a solution of commercial 4-chloro-benzene-1,2-diamine (**1a**, 6.98 g, 49 mmol) in dry tetrahydrofuran (27 mL) was added slowly. The reaction mixture was allowed to warm slowly to ambient temperature. It was then poured into ice-cold brine (350 mL) and extracted with ethyl acetate (2 × 300 mL). The combined organic layer was washed successively with ice-cold saturated $NaHCO_3$ solution (200 mL) and brine (200 mL), then dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo. The residue was chromatographed on silica gel to afford 15 g of an oil (mixture of regioisomers **2a** and **2b**) that was dissolved in glacial acetic acid (500 mL) and heated to 70−75 °C for 6 h under exclusion of moisture. The reaction mixture was then evaporated in vacuo and the residue flash chromatographed on silica gel (300 g). Elution with 10% ethyl acetate/hexane afforded 9.4 g (mixture of regioisomers **1c** and **2c**) of a dense oil that was treated in acetonitrile (250 mL) with trifluoromethanesulfonic acid-[dimethoxyphosphinyl]-methyl ester (6.528 g, 24 mmol) and anhydrous, powdered K_2CO_3 (8.97 g, 65 mmol) under dry nitrogen and stirring. The reaction mixture was stirred at 25 °C overnight, filtered, and washed with acetonitrile (50 mL). The filtrate was evaporated and the residue partitioned between water and methylene chloride. The separated organic layer was dried and then evaporated in vacuo to dryness. The residue was chromatographed (HPLC); elution with ethyl acetate/hexane provided 2-*tert*-butoxycarbonylamino-3-[5-chloro-1-(dimethoxy-phosphoryl methyl)-1*H*-benzoimidazol-2-yl]-propionic acid benzyl ester (**1d**, 4 g, 16.1% based on (*R*)-2-*tert*-butoxycarbonylamino-succinic acid 1-benzyl ester), which eluted first, and 2-*tert*-butoxycarbonylamino-3-[6-chloro-1-(dimethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic acid benzyl ester (**2d**, 4.4 g, 17.7% based on (*R*)-2-*tert*-butoxycarbonylamino-succinic acid 1-benzyl ester).

^{1}H NMR [**1d**] (DMSO-d_6, 400 MHz): δ 1.16 (s, 9H), 3.31 (m, 2H), 3.57 (s, 3H), 3.59 (s, 3H), 4.75 (m, 1H), 4.92 (m, 2H), 5.12 (s, 2H), 7.27 (dd, J_o = 10.1 Hz, J_m = 2.1 Hz, 1H), 7.29 (m, 6H), 7.61 (d, J = 10.1 Hz, 1H), 7.62 (d, J = 1.9 Hz, 1H).

^{1}H NMR [**2d**] (DMSO-d_6, 400 MHz): δ 1.16 (s, 9H), 3.31 (m, 2H), 3.59 (dd, J_1 = 1.9 Hz, J_2 = 11 Hz, 6H), 4.75 (m, 1H), 4.93 (m, 2H), 5.13 (s, 2H), 7.19 (dd, J_o = 9.5 Hz, J_m = 2 Hz, 1H), 7.28 (m, 6H), 7.56 (d, J = 9.5 Hz, 1H), 7.73 (d, J = 2 Hz, 1H).

2-*tert*-Butoxycarbonylamino-3-[5-chloro-1-(dimethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic Acid (1e). A solution of 2-*tert*-butoxycarbonylamino-3-[5-chloro-1-(dimethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic acid benzyl ester (**1d**, 4 g, 7.25 mmol) in glacial acetic acid (60 mL) was treated with 10% palladium on charcoal (400 mg) and hydrogenated at ambient temperature and atmospheric pressure for 4 h. The mixture was then purged with nitrogen, filtered through Solka-floc, and washed with acetic acid (20 mL), and the filtrate was evaporated to dryness in vacuo. The residue was then stripped with toluene (2 × 20 mL) and finally evaporated in high vacuum to afford the desired intermediate as an oil (3.34 g, 100%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.3 (s, 9H), 3.28 (m, 2H), 3.58 (s, 3H), 3.61 (s, 3H), 4.59 (m, 1H), 4.94 (d, J = 1.2 Hz, 2H), 7.18 (m, 4H), 12.8 (br s, 1H).

[*R*(−)]-2-Amino-3-(5-chloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid, 1 Hydrochloride (1). The above oil (compound **1e**, 3.34 g, 7.2 mmol) was refluxed in 6 N hydrochloric acid (60 mL) for 50 min. The mixture was then evaporated to dryness in vacuo, and the residue was once more evaporated with water (15 mL). The residue was dried in high vacuum and then crystallized from hot water/acetonitrile. The compound was filtered, washed with ether (10 mL), and dried at 1 Torr, 60 °C (over P_2O_5), to yield the title compound (1.4 g, 52%). mp: 113−6 °C (dec.). ^{1}H NMR (DMSO-d_6 +1 drop DCl, 400 MHz): δ 3.86 (t, J = 6.4 Hz, 2H), 4.83 (t, J = 6.8 Hz, 1H), 4.95 (m, 2H), 7.61 (dd, J_o = 8.9 Hz, J_m = 1.9 Hz, 1H), 7.91 (d, J_m = 1.7 Hz, 1H), 7.96 (d, J = 8.9 Hz, 1H). $[\alpha]_D$ = −28.1°, (c = 1.2, ethanolic HCl). MS (−FAB): *m/e* 332

(M − H). Anal. ($C_{11}H_{13}ClN_3O_5P$·1HCl) C, H, N: calcd (found) C, 35.70 (35.87); H, 3.81 (3.94); N, 11.35 (11.26).

[*R*(−)]-2-Amino-3-(6-chloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid, Sesquihydrate, Scheme 1/Compound 2: 2-*tert*-Butoxycarbonylamino-3-[6-chloro-1-(dimethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic Acid (Compound 2e). A solution of 2-*tert*-butoxycarbonylamino-3-[6-chloro-1-(dimethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic acid benzyl ester (**2d**, 4.4 g, 7.98 mmol) in glacial acetic acid (70 mL) was treated with 10% palladium on charcoal (440 mg) and hydrogenated for ∼3 h at 25 °C. The reaction mixture was purged with nitrogen and filtered through Solka-floc, the cake was washed with acetic acid (30 mL), and the filtrate was evaporated to dryness in vacuo. The residue was stripped with toluene (2 × 30 mL) and evaporated in high vacuum to afford 2-*tert*-butoxycarbonylamino-3-[6-chloro-1-(dimethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic acid (**2e**) as an oil (3.77 g, 100%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.3 (s, 9H), 3.41 (m, 2H), 3.59 (dd, J_1 = 1.9 Hz, J_2 = 11 Hz, 6H), 4.55 (m, 1H), 4.92 (m, 2H), 7.08 (d, J = 1.1 Hz, 1H), 7.28 (m, 1 Hz, 1H), 7.6 (m, 2H), 12.8 (br s, 1H).

[*R*(−)]-2-Amino-3-(6-chloro-1-phosphonomethyl-1*h*-benzoimidazol-2-yl)-propionic Acid, Sesquihydrate (2). Compound **2e** (3.7 g, 7.8 mmol) was refluxed in 6 N HCl (70 mL) for 50 min. The reaction mixture was then evaporated to dryness in vacuo, and the residue was stripped with water (2 × 20 mL) and then crystallized from hot water/acetonitrile to afford the title compound (2.9 g, 97.6%). mp: 205−7 °C (dec.). ^{1}H NMR (DMSO-d_6 + 1 drop DCl, 400 MHz): δ 3.87 (dd, J_1 = 5.5 Hz, J_2 = 7.2 Hz, 2H), 4.86 (t, J = 7.2 Hz, 1H), 4.96 (dd, J_1 = 12 Hz, J_2 = 32.7 Hz, 2H), 7.59 (dd, J_o = 8.7 Hz, J_m = 2 Hz, 1H), 7.83 (d, J_o = 8.8 Hz, 1H), 8.13 (d, J_m = 1.9 Hz, 1H). $[\alpha]_D$ = −101.1° (c = 1.08, ethanolic HCl). Anal. ($C_{11}H_{13}ClN_3O_5P$·$1H_2O$·0.8HCl) C, H, N: calcd (found) C, 34.68 (34.80); H, 4.18 (4.29); N, 11.03 (10.65).

[*R*(−)]-2-Amino-3-(1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propanoic Acid Dihydrochloride Dihydrate, Scheme 2/Compound 3. 3-(1*H*-Benzoimidazol-2-yl)-2-benzyloxycarbonylamino-propionic acid benzyl ester (**3a**, 30 g, 70 mmol, prepared according to the method of Nestor et al.[19]), trifluoromethanesulfonic acid-(diethoxyphosphinyl)-methyl ester (23.04 g, 76 mmol), and powdered potassium carbonate (37 g, 268 mmol) were stirred in acetonitrile (500 mL) at ambient temperature for 20 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in dichloromethane (300 mL) and washed with water (500 mL), 5% aqueous sodium bicarbonate (2 × 300 mL), and water (300 mL). The separated organic layer was dried over magnesium sulfate and filtered, and the filtrate was evaporated to dryness in vacuo. The resulting gum (45 g) was subjected to flash column chromatography on silica gel. Elution with a solvent gradient (methylene chloride to ethyl acetate) afforded 10.6 g of the desired intermediate (**3b**) which was dissolved in acetic acid (300 mL) and shaken with 10% palladium on carbon (1 g) under one atmosphere of hydrogen at ambient temperature until the hydrogen uptake ceased. The mixture was filtered through solka floc, and the filtrate was evaporated in vacuo. The residue was once more evaporated with dioxane, resulting in 8 g of the debenzylated material (**3c**) of which 7.5 g was refluxed under dry nitrogen with trimethylsilyl bromide (15 g, 100 mmol) for 1.5 h. The reaction mixture was evaporated in vacuo and the residue partitioned between water (50 mL) and ether (50 mL). The two-layer system was filtered, the solid retained, and the filtrate separated. The aqueous layer was diluted with ethanol (50 mL) and treated with excess propylene oxide under stirring for 0.5 h. The solution was evaporated in vacuo and the solid residue combined with the earlier retained compound. The material was dissolved in 2 N HCl (100 mL) and filtered, and the filtrate was evaporated to dryness in vacuo, affording the title compound (2.27 g, 7%). mp: 190−200 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.8 (d, 2H); 4.8 (m, 3H), 7.5 (m, 2H), 7.7 (d, 1 H), 7.9 (d, 1H), 9.2−8.2 (m, 2H). $[\alpha]_D^{25}$ = −41.6° (c = 1.0, 1 N HCl). HPLC analysis for enantiomeric purity: *R*:*S* = 99:1. Anal. ($C_{11}H_{14}N_3O_5P$·2HCl·$2H_2O$) C, H, N: calcd (found) C, 32.37 (32.57); H, 4.93 (4.87); N, 10.29 (10.53).

[*R*(−)]-2-Amino-3-(5,6-dichloro-1-(phosphonomethyl)-1*H*-benzoimidazole-2-yl)-propionic Acid Dihydrochloride, Scheme 2/Compound 4. The compound was prepared in a manner similar to compound **3**, but using 2-benzyloxycarbonylamino-3-(5,6-dichloro-1*H*-benzoimidazol-2-yl)-propionic acid benzyl ester (**4a**) prepared according to the method of Nestor et al.[19] The title compound was obtained in 15.3% yield mp: >310 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.78 (d, 2H), 4.72 (t, 1H), 4.86 (m, 2H), 8.06 (s, 1H), 8.23 (s, 1H), 8.55 (m, 3H). MS (−FAB): *m*/*e* 366 (M − H). $[\alpha]_D^{25}$ = −50° (c = 1.01, 1 N HCl). HPLC analysis (free amino acid)for enantiomeric purity: *R*:*S* = 97.4:2.6. Anal. ($C_{11}H_{12}Cl_2N_3O_5P$·2HCl) C, H, N: calcd (found) C, 29.96 (29.78); H, 3.20 (3.31); N, 9.53 (9.65).

[*S*(+)]-2-Amino-3-(5,6-dichloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Dihydrate, Scheme 1/Compound 5. The compound was prepared in a manner similar to compound **2**, but using (*S*)-2-*tert*-butoxycarbonylamino-succinic acid 1-benzyl ester. The title compound was obtained in 18% yield. mp: 230 °C (dec.). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.79 (d, 2H), 4.7 (t, 1H), 4.86 (m, 2H), 8.1 (s, 1H), 8.25 (s, 1H), 8.53 (m, 3H). MS (−FAB): *m*/*e* 366 (M − H). $[\alpha]_D^{25}$ = +58.8° (c = 1.0, 1 N HCl). Anal. ($C_{11}H_{12}Cl_2N_3O_5P$·$2H_2O$) C, H, N: calcd (found) C, 32.69 (32.66); H, 3.99 (4.13); N, 10.39 (10.34).

[*R*(−)]-2-Amino-3-(4-chloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Hydrochloride Sesquihydrate, Scheme 3/Compound 6: 1-Chloro-5-fluoro-nitrobenzene (6b). Commercial 2-chloro-6-fluorophenylamine (**6a**, 7.34 g, 50.2 mmol) was mixed with 48% fluoboric acid (50 mL) and cooled to 0 °C after which an ice-cold solution of sodium nitrite (3.45 g, 50 mmol) in water (7 mL) is added dropwise. The reaction mixture was stirred for another 30 min at 0 °C and filtered, and the cake was washed once with cold fluoboric acid (7 mL) followed by a wash with ethanol (2 × 50 mL) and finally ether (3 × 50 mL). The obtained material was suspended in water (40 mL) and added portionwise over the period of 1 h at ambient temperature to a stirred mixture of sodium nitrite (40 g, 580 mmol) dissolved in water (80 mL) to which copper powder (8 g) was added. Thereafter, the reaction mixture was stirred another hour at ambient temperature, and the obtained suspension was filtered and washed with water (100 mL). The cake was mixed with silica gel (30 g), slurried in ethyl acetate (100 mL), filtered, and washed with ethyl acetate (∼200 mL) until there was no more compound detected by UV. The filtrate was evaporated and the residue flash chromatographed on silica gel (250 g). Elution with chloroform/hexane afforded the title compound as an orange yellow foam (3 g, 44%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.86 (m, 3H). MS (EI): *m*/*e* 175 (M+).

[(3-Chloro-2-nitro-phenylamino)-methyl]-phosphonic Acid Diethyl Ester (6c). Aminomethyl-phosphonic acid diethyl ester[21] (2.345 g, 14 mmol) was added to a solution of 1-chloro-3-fluoro-2-nitro-benzene (**6b**, 1.225 g, 7 mmol) in toluene (80 mL). The reaction mixture was refluxed for 8 h and evaporated to dryness in vacuo, and the residue was flash chromatographed on silica gel (120 g). Elution with chloroform/2−10% methanol in chloroform afforded the desired intermediate (1 g, 45%). mp: 62−3 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.2 (t, J = 5.5 Hz, 6H), 3.72 (dd, J_1 = 10.1 Hz, J_2 = 7.6 Hz, 2H), 4.05 (quin, J = 5.5 Hz, 4H), 6.43 (m, 1H), 6.87 (d, J = 8.6 Hz, 1H), 7.08 (d, J = 8.6 Hz, 1H), 7.37 (t, J = 8.6 Hz, 1H).

[(3-Chloro-2-amino-phenylamino)-methyl]-phosphonic Acid Diethyl Ester (6d). Rhodium on charcoal (5%, 60 mg) was added to a solution of [(3-chloro-2-nitro-phenylamino)-methyl]-phosphonic acid diethyl ester (**6c**, 0.4 g, 1.2 mmol) in ethanol (24 mL) and stirred under an atmospheric pressure of hydrogen for 24 h. The mixture was then filtered through Solka-floc and washed with ethanol (10 mL), and the filtrate was evaporated in vacuo to give the desired intermediate (310

mg, 89%). mp: 73−77 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.22 (t, J = 4.8 Hz, 6H), 3.55 (dd, J_1 = 7.6 Hz, J_2 = 5.0 Hz, 2H), 4.03 (quin, J = 4.8 Hz, 4H), 5.0 (m, 1H), 6.5 (t, J = 5.6 Hz, 1H), 6.62 (m, 2 H).

[*R*(−)]-2-Benzyloxycarbonylamino-*N*-{2-chloro-6-[(diethoxy-phosphorylmethyl)-amino]-phenyl}-succinamic Acid Benzyl Ester (6e). Diisopropylethylamine (0.69 g, 6.1 mmol) and commercial (*R*)-2-benzyloxycarbonylamino-succinic acid 1-benzyl ester (1.07 g, 3.4 mmol) were added to a solution of [(3-chloro-2-amino-phenylamino)-methyl]-phosphonic acid diethyl ester (**6d**, 0.8 g, 3.1 mmol) in methylene chloride (44 mL). The mixture was cooled to 0 °C, bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (0.77 g, 3.4 mmol) was added at once, and the reaction mixture was stirred under dry nitrogen at 0 °C overnight. Additional diisopropylamine (0.23 g, 2 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.257 g, 1 mmol) was added to the mixture, and stirring continued at 0 °C for another 5 h, after which the reaction mixture was evaporated and the residue flash chromatographed on silica gel (180 g). Elution with ethyl acetate/hexane afforded the title compound (1.2 g, 61%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.18 (t, J = 7.6 Hz, 6H), 2.7−3.0 (m, 2H), 3.55 (m, 2H), 3.95 (quin, J = 7.6 Hz, 4H), 4.55 (m, 1H), 5.05 (s, 2H), 5.15 (s, 2H), 5.35 (m, 1H), 6.7 (d, J = 9.1 Hz, 1H), 6.75 (d, J = 9.1 Hz, 1H), 7.08 (t, J = 9.1 Hz, 1H), 7.35 (m, 10H), 7.8 (d, J = 8.1 Hz, 1H), 9.35 (s, 1H).

[*R*(−)]-2-(Benzyloxycarbonylamino)-3-[1-(4-chloro-1-diethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic Acid Benzyl Ester (6f). *p*-Toluenesulfonic acid monohydrate (0.36 g, 1.9 mmol) was added at once to a solution of [*R*(−)]-2-benzyloxycarbonylamino-*N*-{2-chloro-6-[(diethoxy-phosphoryl methyl)-amino]-phenyl}-succinamic acid benzyl ester (**6e**, 1.2 g, 1.9 mmol) in toluene (100 mL). The reaction mixture was refluxed with a Dean−Stark trap for 2 h and evaporated in vacuo, and the residue was flash chromatographed on silica (100 g). Elution with 5% methanol/chloroform yielded the title compound (600 mg, 51%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.05 (t, J = 3.1 Hz, 3H), 1.1 (t, J = 3.1 Hz, 3H), 3.4 (m, 2H), 3.95 (m, 5H), 4.86 (d, J = 6.5 Hz, 2H), 4.86 (m, 1H), 5.0 (s, 2H), 5.15 (s, 2H), 7.25 (m, 1H), 7.58 (d, J = 4.5 Hz, 1H), 7.93 (d, J = 4.5 Hz, 1H). MS (EI): *m/e* 613 (M+).

[*R*(−)]-2-Amino-3-[4-chloro-1-(diethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic Acid (6g). Palladium on charcoal (10%, 334 mg) was added to a solution of [*R*(−)]-2-(benzyloxycarbonylamino)-3-[1-(4-chloro-1-diethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic acid benzyl ester (**6f**, 1 g, 1.65 mmol) in acetic acid (135 mL) and hydrogenated for 4 h. The mixture was then filtered through Solka-floc and washed with acetic acid (30 mL), and the filtrate was stripped with benzene (2 × 40 mL) and finally evaporated in vacuo to afford the desired intermediate (600 mg, 94%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.11 (t, J = 5.5 Hz, 3H), 1.23 (t, J = 5.5 Hz, 3H), 3.3 (m, 2H), 3.75 (m, 1H), 4.0 (quin, J = 5.5 Hz, 4H), 4.95 (d, J = 8.1 Hz, 2H), 7.25 (m, 2H), 7.60 (d, J = 6.0 Hz, 1H). MS (CI): *m/e* 390 (M+).

[*R*(−)]-2-Amino-3-(4-chloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Hydrochloride Sesquihydrate (6). A solution of [*R*(−)]-2-amino-3-[4-chloro-1-diethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic acid (**6g**, 0.6 g, 1.54 mmol) in 6 N hydrochloric acid (30 mL) was refluxed for 45 min, then evaporated in vacuo, and stripped with benzene (2 × 30 mL). The beige solid residue was dissolved in hot water (24 mL) and triturated with acetonitrile (>120 mL). Upon cooling to 0 °C, the product crystallized. It was filtered, washed with acetonitrile (30 mL), and dried in vacuo to give the desired title compound as a creamy white powder (240 mg, 39%). mp: >200 °C. ^{1}H NMR (DMSO-d_6, 1 drop DCl, 400 MHz): δ 3.81 (d, J = 7.0 Hz, 2H), 4.8 (m, 1H), 4.95 (m, 2H), 7.45 (t, J = 9.1 Hz, 1H), 7.55 (d, J = 9.1 Hz, 1H), 7.82 (d, J = 9.1 Hz, 1H). MS (−FAB): *m/e* 332 (M − H). Anal. ($C_{11}H_{13}ClN_3O_5P\cdot1HCl\cdot1.5H_2O$) C, H, N: calcd (found) C, 33.27 (33.35); H, 4.31 (4.46); N, 10.58 (10.35).

[*R*(−)]-2-Amino-3-(7-chloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Bis(tromethamine)salt Sesquihydrate, Scheme 3/Compound 7: 1-Chloro-2-fluoro-3-nitro-benzene (7b). Commercial 1,2-dichloro-3-nitro-benzene (**7a**, 35 g, 200 mmol) and 20% potassium fluoride on calcium fluoride (116 g) were heated in sulfolane (100 mL) at 150 °C for 4 days under dry nitrogen. After cooling, the reaction mixture was diluted with water (350 mL), and the obtained solution was extracted with ether (3 × 200 mL). The combined ether extract was washed with water and brine, dried over magnesium sulfate, and filtered, and the filtrate eluted through Grade III silica. The eluent was concentrated in vacuo and the residue distilled under reduced pressure to afford 17 g of 3-chloro-2-fluoronitrobenzene (48.5%/bp 135−140 °C at 12−15 Torr). ^{1}H NMR ($CDCl_3$): δ 7.25 (m, 1H), 7.68 (m, 1H), 7.94 (m, 1H).

[(2-Chloro-6-nitrophenylamino)methyl]phosphonic Acid Diethyl Ester (7c). A mixture of 1-chloro-2-fluoro-3-nitro-benzene (**7b**, 16.5 g, 940 mmol) and diethyl aminomethyl phosphonate[21] (16.7 g, 1 mol) was heated at 50 °C for 20 h, cooled to ambient temperature, and partitioned between water (200 mL) and methylene chloride (300 mL). The organic layer was separated, filtered, dried over magnesium sulfate, filtered again, and the residue was chromatographed on silica gel. Elution with hexanes/ethyl acetate (4:1) gave the desired [(2-chloro-6-nitrophenylamino) methyl]phosphonic acid diethyl ester (14 g, 46%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.79 (m, 2H), 3.9 (m, 4H), 6.59 (m, 1H), 7.03 (t, 1H), 7.74 (dd, 1H), 7.93 (dd, 1H).

[(2-Amino-6-chlorophenylamino)methyl]phosphonic Acid Diethyl Ester (7d). Rhodium on carbon catalyst (5%, 1.4 g) was added to a solution of [(2-chloro-6-nitrophenylamino)-methyl]phosphonic acid diethyl ester (**7c**, 14 g, 43.4 mmol) in ethanol (200 mL) and hydrogenated at ambient temperature overnight in a Parr apparatus at an initial pressure of 40 psi. The catalyst was filtered through solka floc, and the filtrate was evaporated in vacuo to yield the desired aniline (12.5 g, 98%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.23 (m, 6H), 3.89 (m, 1H), 4.01 (m, 6H), 5.46 (m, 2H), 6.54 (m, 1H), 6.61 (m, 2H), 6.73 (t, 1H).

(*R*)-2-Benzyloxycarbonylamino-*n*-{2-chloro-6-[(Diethoxy-phosphorylmethyl)-amino]-phenyl}-succinamic Acid Benzyl Ester (7e). A solution of (*R*)-2-benzyloxycarbonylamino-succinic acid 1-benzyl ester (3.03 g, 10 mmol), *N*-methylmorpholine (1.11 g, 11 mmol), and isobutyl chloroformate (1.37 g, 10 mmol) were dissolved in methylene chloride (150 mL), cooled to −20 °C, and stirred for 15 min. [(2-Amino-6-chlorophenylamino)methyl]phosphonic acid diethyl ester (**7d**, 3.57 g, 10 mmol) was added, and the reaction mixture was stirred for 3 h at ambient temperature after which the solution was washed with water (100 mL) and saturated sodium bicarbonate (80 mL) and dried over magnesium sulfate. The drying agent was removed by filtration, and the filtrate was evaporated in vacuo. The residue was chromatographed on silica gel. Elution with hexanes/ethyl acetate (1:1) gave the desired compound (1.72 g, 27%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.15 (m, 6H), 2.79 (m, 1H), 3.33 (s, 1H), 3.91 (m, 4H), 4.48 (m, 1H), 4.61 (m, 1H), 5.05 (s, 2H), 5.15 (s, 2H), 7.33 (m, 10 H), 7.50 (d, 1H), 7.87 (d, 1H), 9.59 (s, 1H). MS (+FAB): *m/e* 631 (M + H).

[*R*(−)]-2-Amino-3-(7-chloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Bis(tromethamine)-salt Sesquihydrate (Compound 7). Palladium on carbon (10%, 400 mg) was added to a solution of the starting (*R*)-2-Benzyloxycarbonylamino-*N*-{2-chloro-6-[(diethoxy-phosphorylmethyl)-amino]-phenyl}-succinamic acid benzyl ester (**7e**, 2.5 g, 4 mmol) in acetic acid (100 mL) and hydrogenated in a Parr apparatus for 72 h at an initial pressure of 35 psi. The catalyst was filtered off, and the filtrate was evaporated in vacuo. The residue was refluxed in 6 N HCl (100 mL) for 1 h, and the solution was evaporated in vacuo. The residue was triturated in ether and redissolved in water (10 mL), and the pH was adjusted to 2.5 using ammonium hydroxide (10% aqueous). Some suspended material was removed by filtration and the filtrate diluted with ethanol (45 mL), resulting in a suspension which was filtered and washed with 50% aqueous ethanol,

absolute ethanol, and ether. The crude material obtained (1.02 g, 3 mmol) was treated with tromethamine (727 mg, 6 mmol) in warm water (15 mL). Ethanol (80 mL) was added, and the resulting suspension was cooled to 0 °C for 2 h, filtered, washed with ethanol and ether, and dried in vacuo to afford the title compound (1.4 g, 38%). mp: 148−52 °C. MS (−FAB): *m/e* 332 (M − H). Anal. ($C_{19}H_{39}ClN_5O_{13}P$) C, H, N: calcd (found) C, 37.28 (37.64); H, 6.43 (6.35); N, 11.44 (11.37).

[*S*(+)]-2-Amino-3-(1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Dihydrochloride Hydrate, Scheme 4/Compound 8. 3-(1*H*-Benzoimidazol-2-yl)-2-benzyloxycarbonylamino-propionic acid benzyl ester (**8a**, 2.85 g, 6.3 mmol, prepared according to the method of Nestor et al.[19]), trifluoromethanesulfonic acid [bis-(4-nitrophenylmethoxy)-phosphinyl] methyl ester (3.67 g, 8.9 mmol), and powdered potassium carbonate (4 g, 28.9 mmol) were stirred in acetonitrile (100 mL) at ambient temperature for 20 h. Thereafter the procedure of compound **3** was continued, and the obtained tetrabenzyl derivative (4.3 g) was purified on a Waters prep 500 HPLC using a gradient elution of hexanes (100%) to ethyl acetate (100%). The purified tetrabenzyl derivative (**8b**, 1.0 g) was dissolved in acetic acid, treated with palladium on carbon (10%, 150 mg), and hydrogenated at 1 atm. The catalyst was removed by filtration, and the filtrate was evaporated to dryness in vacuo. The residue was twice evaporated with dioxane and then slurried in water (10 mL). The product was filtered and dried to yield the title compound (350 mg, 17.6%); the NMR was identical to compound **3**. HPLC analysis for enantiomeric purity: *R*:*S* = 1.8:98.2. MS (+FAB): *m/e* 300 (M + H). Anal. ($C_{11}H_{14}N_3O_5P\cdot 2H_2O\cdot 2HCl$) C, H, N: calcd (found) C, 41.65 (41.60); H, 5.08 (5.04); N, 13.25 (13.09).

[*R*(−)]-2-Amino-3-(5,6-dimethyl-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Dihydrochloride, Scheme 4/Compound 9. 2-Benzyloxycarbonylamino-3-(5,6-dimethyl-1*H*-benzoimidazol-2-yl)-propionic acid benzyl ester (**9a**, 3.7 g, 8 mmol, prepared according to the method of Nestor et al.[19]), trifluoromethanesulfonic acid [bis-(4-nitrophenyl-methoxy) phosphinyl]methyl ester (5.5 g, 10 mmol), and powdered potassium carbonate (5.5 g, 40 mmol) were stirred in acetonitrile (100 mL) at ambient temperature under dry nitrogen for 20 h. Thereafter the procedure of compound **3** was continued, and the obtained tetrabenzyl derivative (**9b**) was dissolved in acetic acid (100 mL), treated with palladium on carbon (10%, 1 g), and hydrogenated in a Parr apparatus at initially 38 psi for 3 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness in vacuo. The residue (**9c**) was twice evaporated with dioxane and then diluted with water (50 mL). The mixture was adjusted to pH 3 with 6 N HCl and chilled, and the product was filtered. The obtained air-dried solid (2.8 g) was dissolved in water (50 mL) containing 1 N HCl (1 mL) and reprecipitated by adding 1 N NaOH. The product was filtered and washed with water, ethanol, and ether. Thereafter the material was dissolved in 1 N HCl (20 mL), treated with activated carbon, and filtered, and the filtrate was chilled. The product was filtered, washed with ice-cold water, and dried in vacuo to afford the title compound (1.23 g, 37.4%). HPLC analysis for enantiomeric purity: *R*:*S* = 99:1. MS (−FAB): *m/e* 326 (M − H). $[\alpha]_D^{25}$ = −54.4° (*c* = 1.01, 1 N HCl). Anal. ($C_{13}H_{18}N_3O_5P\cdot 2HCl$) C, H, N: calcd (found) C, 39.02 (38.62); H, 5.04 (5.27); N, 10.50 (10.32). ^{1}H NMR (400 MHz, DMSO-d_6 + 2 drops 20% DCl/D_2O): δ 2.35 (s, 3H), 3.85 (m, 1H), 4.87 (m, 3H), 5.7 (s, 1H), 7.75 (s, 1H).

[*S*(+)]- 2-Amino-4-(1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-butyric Acid Dihydrochloride Methanolate, Scheme 2/Compound 10: (*S*)-2-Benzyloxycarbonylamino-4-[1-(diethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-butyric Acid Benzyl Ester (10b). (*S*)-4-(1*H*-Benzoimidazol-2-yl)-2-benzyloxycarbonylamino-butyric acid benzyl ester (**10a**, 4.43 g, 10 mmol, prepared according to the method of Nestor et al.[19]), trifluoromethanesulfonic acid-(diethoxyphosphinyl)-methyl ester (3.3 g, 11 mmol), and powdered potassium carbonate (5.5 g, 40 mmol) were stirred in acetonitrile (75 mL) at ambient temperature under dry nitrogen for 3 days. The reaction mixture was filtered, and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in dichloromethane (100 mL) and washed with water, dried over magnesium sulfate, and filtered, and the filtrate was evaporated to dryness in vacuo. The resulting gum was subjected to flash column chromatography on silica gel. Elution with ethyl acetate gave the desired intermediate (3.1 g, 52%). MS (+FAB): *m/e* 594 (M + H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09−1.31 (m, 6H), 2.40−2.45 (m, 1H), 2.55−2.59 (m, 1H), 2.98−3.02 (t, 2H, *J* = 7.4 Hz), 3.85−4.01 (m, 4H), 4.33−4.36 (d, 2H, *J* = 10.8 Hz), 4.49−4.54 (m, 1H), 5.07 (s, 2H), 5.17 (d, 2H, *J* = 3.95 Hz), 6.46 (d, 1H, *J* = 7.7 Hz), 7.21−7.37 (m, 14 H), 7.67−7.69 (m, 1H).

(*S*)-2-Amino-4-[1-(diethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-butyric Acid (10c). A solution of (*S*)-2-Benzyloxycarbonylamino-4-[1-(diethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-butyric acid benzyl ester (**10b**, 7.6 g, 12.8 mmol) in acetic acid (650 mL) was treated with palladium on carbon (10%, 1 g) and hydrogenated in a Parr apparatus at 40 psi for 3 days. The catalyst was removed by filtration through solka floc, and the filtrate was evaporated in vacuo. The residue was three times evaporated with dioxane and then dried in vacuo to yield the desired intermediate (5 g, 100%). MS (+FAB) 370 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.04−1.22 (m, 6H), 2.17−2.31 (m, 1H), 3.01−3.15 (m, 1H), 3.87−4.08 (m, 4H), 4.82−4.90 (m, 2H), 7.12−7.20 (m, 2H), 7.52−7.56 (t, 2H, *J* = 6.8 Hz).

[*S*(+)]- 2-Amino-4-(1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-butyric Acid Dihydro Chloride Methanolate (10). A solution of (*S*)-2-Amino-4-[1-(diethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-butyric acid (**10c**, 5 g, 13.5 mmol) in 6 N HCl (60 mL) was refluxed overnight and evaporated in vacuo, and the residue was treated with activated carbon in warm water (50 mL). The charcoal was filtered, and the filtrate was evaporated in vacuo. The residue was crystallized from methanol/ether to afford the title compound (2.1 g, 37.2%). mp: 230−40 °C. MS (−FAB): *m/e* 312 (M − H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.38−2.48 (m, 1H), 2.52−2.59 (m, 1H), 3.39−3.59 (m, 5H), 4.08−4.12 (t, 1H, *J* = 6 Hz), 4.78−4.81 (d, 2H, *J* = 6.7 Hz), 7.40−7.75 (m, 2H), 7.77−7.80 (m, 1H), 7.88−7.90 (m, 1H). $[\alpha]_D^{25}$ = +16.4° (*c* = 0.635, MeOH). Anal. ($C_{12}H_{16}N_3O_5P\cdot 2HCl\cdot CH_3OH$) C, H, N: calcd (found) C, 37.32 (37.81); H, 5.31 (5.44); N, 10.04 (9.81).

[*R*(−)] -2-Amino-4-(1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-butyric Acid Dihydrochloride 2.5 Hydrate, Scheme 2/Compound 11. Using the methodology for the above-described *S*-enantiomer, (*R*)-4-(1*H*-benzoimidazol-2-yl)-2-benzyloxycarbonylamino-butyric acid benzyl ester (**11a**, 7.89 g, 17.8 mmol) was converted to the title compound in three steps (1.6 g, 24%). MS (+FAB): *m/e* 314 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): identical to the *S*-enantiomer (**10**). $[\alpha]_D^{25}$ = −20.5° (*c* = 1.08, MeOH). Anal. ($C_{12}H_{16}N_3O_5P\cdot 2.5H_2O$) C, H, N: calcd (found) C, 33.38 (33.24); H, 5.33 (5.61); N, 9.75 (9.82).

[*R*(−)]-2-Amino-3-(1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Ethyl Ester Hydrate, Scheme 5/Compound 12: (*R*)-2-Benzyloxycarbonylamino-3-{1-[bis-(4-nitro-benzyloxy)-phosphorylmethyl]-1*H*-benzoimidazol-2-yl}-propionic Acid Ethyl Ester (12c). A solution of (*R*)-2-benzyloxycarbonylamino-succinic acid 1-ethyl ester (**12a**, 11.7 g, 39.6 mmol) in methylene chloride (150 mL) was cooled to −10 °C, isobutyl chloroformate (5.4 g, 39.6 mmol) was added, and the mixture was stirred for 15 min. *o*-Phenylenediamine (4.27 g, 39.6 mmol) was the added, and the mixture was stirred at ambient temperature overnight. The solution was then washed with water (100 mL) and saturated sodium bicarbonate (80 mL), dried over magnesium sulfate, and filtered, and the filtrate was evaporated in vacuo. The residue was heated for 5 h at 70 °C in glacial acetic acid (200 mL) and then evaporated to dryness in vacuo. The residue was dissolved in methylene chloride (200 mL), washed with saturated sodium bicarbonate (80 mL), dried over magnesium sulfate, filtered, and evaporated to dryness in vacuo. This intermediate (**12b**, 11.52 g, 31.4 mmol), anhydrous potassium

carbonate (17.1 g, 123 mmol), and trifluoromethanesulfonic acid [bis-(4-nitro-phenylmethoxy)phosphinyl] methyl ester (16.2 g, 31.4 mmol) were stirred in acetonitrile (350 mL) overnight at ambient temperature. The solvent was then evaporated in vacuo, and the residue was dissolved in methylene chloride (200 mL), washed with water (100 mL), dried over magnesium sulfate, filtered, and evaporated to dryness in vacuo to afford the desired intermediate (13.5 g, 59%). MS (+FAB): *m/e* 732 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.04−1.11 (t, 3H, J = 7.12 Hz), 4.01−4.04 (m, 2H), 4.72−4.79 (m, 1H), 4.94−5.01 (m, 2H), 5.07−5.30 (m, 6H), 7.11−7.16 (m, 2H), 7.24−7.40 (m, 1H), 7.42−7.46 (m, 4H), 7.52−7.65 (m, 2H), 7.76−7.78 (d, 1H, J = 8.1 Hz), 8.06−8.19 (m, 1H).

[R(−)]-2-Amino-3-(1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Ethyl Ester Hydrate (12). A solution of (*R*)-2-benzyloxycarbonylamino-3-{1-[bis-(4-nitrobenzyloxy)-phosphoryl methyl]-1*H*-benzoimidazol-2-yl}-propionic acid ethyl ester (**12c**, 6.75 g, 9.2 mmol) in acetic acid (100 mL) was treated at once with palladium on carbon (10%, 1 g) and hydrogenated in a Parr apparatus at an initial pressure of 40 psi for 1.5 h. The catalyst was removed by filtration through solka floc, and the filtrate was evaporated in vacuo. The residue was triturated in dioxane, and the obtained solid was filtered, crystallized from water, and dried in vacuo to yield the title compound (180 mg, 6%). mp: 210−212 °C. MS (+FAB): *m/e* 328 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.21−1.27 (t, 3H, J = 7.07 Hz), 3.45−3.61 (m, 4H), 4.14−4.28 (m, 4H), 4.42−4.45 (t, 1H, J = 5.7 Hz), 7.11−7.20 (m, 2H), 7.47−7.59 (m, 2H). Anal. ($C_{13}H_{16}N_3O_4P\cdot H_2O$) C, H, N: calcd (found) C, 45.20 (45.69); H, 5.79 (5.82); N, 12.18 (12.38).

[*R*(−)]-2-Amino-3-[1-(2-phosphono-ethyl)-1*H*-benzoimidazol-2-yl]-propionic Acid Dihydrochloride Hemiethanolate, Scheme 6/Compound 13. [2-(2-Amino-phenylamino)-ethyl]-phosphonic Acid Diethyl Ester (13b). A mixture of benzene-1,2-diamine (**13a**, 4.38 g, 40 mmol), powdered potassium carbonate (5.52 g, 40 mmol), and diethyl bromo ethyl phosphonate (9.95 g, 7.8 mL, 40 mmol) was refluxed under dry nitrogen in acetonitrile (150 mL) for 24 h. The reaction mixture was filtered, the filtrate was evaporated in vacuo, and the residue was dissolved in dichloromethane (150 mL) and washed with water (100 mL) followed by sodium bicarbonate (100 mL). The separated organic layer was dried over magnesium sulfate and filtered, and the filtrate was evaporated in vacuo. The residue was flash chromatographed on silica gel. Elution with ethyl acetate/2% methanol gave the desired intermediate (5.2 g, 52%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.19−1.24 (m, 6H), 2.00−2.08 (m, 2H), 3.16−3.24 (m, 2H), 3.92−4.44 (m, 4H), 6.05−6.23 (m, 1H), 6.38−6.56 (m, 3H).

(*R*)-2-Benzyloxycarbonylamino-*N*-{2-[2-(diethoxy-phosphoryl)-ethylamino]-phenyl}-succinamic Acid Benzyl Ester (13c). (*R*)-[2-(2-Amino-phenylamino)-ethyl]-phosphonic acid diethyl ester (**13b**, 7.5 g, 21 mmol), *N*-methylmorpholine (2.13 g, 2.3 mL, 21 mmol), and isobutyl chloroformate (2.86 g, 2.7 mL, 21 mmol) were stirred in dichloromethane (150 mL) at −10 to −15 °C for 15 min under dry nitrogen. A solution of [2-(2-amino-phenylamino)-ethyl]-phosphonic acid diethyl ester (5.7 g, 21 mmol) in dichloromethane (50 mL) was added dropwise, and the reaction mixture was stirred at ambient temperature for 3 h. The obtained solution was washed with sodium bicarbonate (5%, 100 mL), dried over magnesium sulfate, and filtered, and the filtrate was evaporated to dryness in vacuo to yield the title compound as a dark colored gum (12 g, 96%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.13−1.24 (m, 6H), 1.96−2.05 (m, 1H), 2.71−2.77 (m, 1H), 2.88−2.92 (m, 1H), 3.22−3.26 (m, 2H), 3.90−4.01 (m, 4H), 4.58−4.60 (m, 1H), 4.99−5.14 (m, 4H), 6.08−6.20 (m, 1H), 6.56−6.62 (m, 1H), 7.03−7.07 (m, 1H), 7.26−7.37 (m, 10H), 7.84−7.87 (m, 1H), 9.17 (s, 1H).

(*R*)-2-Benzyloxycarbonylamino-3-{1-[2-(diethoxy-phosphoryl)-ethyl]-1*H*-benzoimidazol-2-yl}-propionic Acid Benzyl Ester (13d). A solution of (*R*)-2-benzyloxycarbonylamino-*N*-{2-[2-(diethoxy-phosphoryl)-ethylamino]-phenyl}-succinamic acid benzyl ester (**13c**, 12 g, 19.6 mmol) in glacial acetic acid (500 mL) was heated at 70 °C for 5 h. Thereafter, the solution was cooled to room temperature and evaporated in vacuo, and the residue was dissolved in dichloromethane (150 mL), washed with sodium bicarbonate (10%, 100 mL), and dried over magnesium sulfate. The drying agent was removed by filtration, and the filtrate was evaporated in vacuo to dryness. The residue was dissolved in glacial acetic acid (150 mL), palladium on carbon (10%, 1.5 g) was added, and the mixture was hydrogenated on a Parr shaker at an initial pressure of 42 psi for 5 h. The catalyst was removed by filtration through Solka Floc, and the filtrate was evaporated and re-evaporated three times with dioxane to afford the desired intermediate (5.3 g, 73%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.11−1.22 (m, 6H), 1.68−1.71 (m, 1H), 2.24−2.41 (m, 2H), 3.19−3.38 (m, 1H), 3.44−3.49 (dd, J = 3.74 Hz, 1H), 3.83−3.89 (m, 1H), 3.91−3.97 (m, 4H), 4.32−4.39 (m, 1H), 7.35−7.39 (m, 2H), 7.47−7.49 (d, J = 7.6 Hz, 1H), 7.56−7.58 (d, J = 7.4 Hz, 1H).

[*R*(−)]-2-Amino-3-[1-(2-phosphono-ethyl)-1*H*-benzoimidazol-2-yl]-propionic Acid Dihydro Chloride Hemiethanolate (13). A mixture of (*R*)-2-benzyloxycarbonylamino-3-{1-[2-(diethoxy-phosphoryl)-ethyl]-1*H*-benzoimidazol-2-yl}-propionic acid benzyl ester (**13d**, 5.3 g, 14 mmol) and trimethylsilyl bromide (13.92 g, 90 mmol) was refluxed in 1,2-dichloroethane (200 mL) under dry nitrogen for 2 h. The solvent was evaporated in vacuo, and the residue was partitioned between water (100 mL) and ether (100 mL). The aqueous layer was separated and diluted with ethanol (100 mL), propylene oxide (5 mL) was added, and the mixture was stirred for 2 h at ambient temperature. The ethanol was evaporated in vacuo, and the aqueous solution was washed with ether. The separated aqueous layer was evaporated to dryness in vacuo, and the residue was dissolved in ethanol containing HCl. Ether was added to precipitate the hydrochloride salt, which was filtered and reprecipitated from methanol with ether to afford the title compound (2 g, 35%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.06−1.09 (m, 1H), 2.11−2.20 (m, 2H), 3.74−3.80 (d, J = 7 Hz, 1H), 4.55−4.64 (m, 2H), 4.76−4.80 (m, 1H), 7.32−7.41 (m, 1H), 7.43−7.52 (m, 2H), 7.74−7.76 (m, 1H), 7.82−7.85 (d, J = 3.7 Hz, 1H), 8.34−8.47 (m, 2H). HPLC analysis for enantiomeric purity: *R*:*S* = 94:6. $[\alpha]_D^{25} = -38.11°$ (c = 1.03, 1 N HCl). Anal. ($C_{12}H_{16}N_3O_5P\cdot 2HCl\cdot 0.5C_2H_5OH$) C, H, N: calcd (found) C, 38.15 (38.05); H, 5.17 (5.46); N, 10.26 (10.08).

[*R*(−)]-2-Amino-3-(1-carboxymethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Dihydrobromide Hemi(Diisopropyl Ether), Scheme 7/Compound 14: (*R*)-2-(Benzyloxycarbonylamino(-3-[1-(benzyloxycarbonylmethyl)-1*H*-benzimidazol-2-yl]propanoic Acid Benzyl Ester (14b). (*R*)-2-Benzyloxycarbonylamino-3-(1-benzyloxycarbonylmethyl-1*H*-benzo imidazol-2-yl)-propionic acid benzyl ester (**14a**, 6.3 g, 15 mmol), benzyl bromoacetate (3.44 g, 15 mmol), anhydrous potassium carbonate (8.5 g, 60 mmol), and 10 drops of triethylamine were stirred in acetonitrile (100 mL) for 20 h at ambient temperature. The solids were filtered, the filtrate was evaporated in vacuo, and the residue was dissolved in methylene chloride (100 mL). The solution was washed with saturated sodium bicarbonate (80 mL) and water (100 mL), dried over magnesium sulfate, filtered, and evaporated in vacuo. The obtained crude product was dissolved in methylene chloride ether (3:1, 50 mL) and passed through a dry chromatography column (150 mL silica gel). The eluate was evaporated in vacuo to give the title compound as a yellow gum (7.7 g, 89.5%). MS (+FAB): *m/e* 578 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.84−4.97 (m, 1H), 5.00−5.02 (d, 2H, J = 5.8 Hz), 5.12−5.14 (d, 2H, J = 3.5 Hz), 5.17 (s, 2H), 5.28 (s, 2H), 7.19−7.25 (m, 2H), 7.29−7.37 (m, 15H), 7.49−7.51 (m, 1H), 7.58−7.60 (m, 1H), 7.83−7.85 (d, 1H, J = 8.3 Hz).

[*R*(−)]-2-Amino-3-(1-carboxymethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Dihydro Bromide Hemi(diisopropyl ether) (14). A solution of (*R*)-2-(benzyloxycarbonylamino(-3-[1-(benzyloxycarbonylmethyl)-1*H*-benzimidazol-2-yl]propanoic acid benzyl ester (**14b**, 7.8 g, 13.4 mmol) in glacial acetic acid (150 mL) was treated with palladium on carbon (10%, 1.5 g) and hydrogenated in a Parr apparatus for 72 h at 40 psi. The catalyst was removed by filtration, and the filtrate

was evaporated. The residue was stirred in hydrobromic acid in acetic acid (32%, 50 mL) for 1 h. The dihydrobromide salt was precipitated with the addition of ether, and the material recrystallized from 2-propanol-isopropyl ether to yield the title compound (1.7 g, 27%). mp: 86−8 °C. MS (−FAB): *m/e* 262 (M − H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.55−3.77 (m, 2H), 4.55−4.59 (m, 1H), 5.44−5.46 (m, 2H), 7.20−7.49 (m, 2H), 7.78−7.80 (m, 1H), 7.84−7.86 (m, 1H). $[\alpha]_D^{25} = -12.75°$ (c = 0.92, EtOH). HPLC analysis for enantiomeric purity: *R*:*S* = 92:8. Anal. ($C_{12}H_{13}N_3O_4 \cdot 2HBr \cdot 0.5C_6H_{14}O$) C, H, N: calcd (found) C, 37.83 (37.92); H, 4.65 (4.83); N, 8.82 (8.45).

2-Amino-3-(1-phosphonomethyl-5-trifluoromethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Hydrate, Scheme 8/Compound 15: [(2-Nitro-4-trifluoromethyl-phenylamino)-methyl]-phosphonic Acid Diethyl Ester (15b). A neat mixture of diethyl aminomethyl phosphonate (13.1 g, 78.4 mmol) and 1-fluoro-2-nitro-4-trifluoromethyl-benzene (**15a**, 5.5 g, 39.2 mmol) was stirred under dry nitrogen. The temperature rose exothermically to 80 °C; cooling was applied to maintain the temperature at 40 °C for 1 h. The reaction mixture was partitioned between methylene chloride (200 mL) and water (200 mL). The two-layer system was filtered, and the organic layer was separated and washed successively with aqueous citric acid (10%, 80 mL), water (100 mL), saturated sodium bicarbonate (80 mL), and then dried over magnesium sulfate. The drying agent was removed by filtration, and the filtrate was evaporated in vacuo to yield the title compound (13.6 g, 97.5%). mp: 55−7 °C (recrystallized from hexane). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.19−1.23 (t, 6H, J = 7 Hz), 4.01−4.10 (m, 6H), 7.38−7.40 (d, 1H, J = 9.9 Hz), 7.81−7.84 (m, 1H), 8.3 (s, 1H), 8.48−8.52 (m, 1H). Anal. ($C_{12}H_{16}F_3N_2O_5P$) C, H, N: calcd (found) C, 40.46 (40.74); H, 4.53 (4.56); N, 7.86 (7.80).

[(2-Amino-4-trifluoromethyl-phenylamino)-methyl]-phosphonic Acid Diethyl Ester (15c). Raney nickel (2 g) was added at once to a solution of [(2-nitro-4-trifluoromethyl-phenylamino)-methyl]-phosphonic acid diethyl ester (**15b**, 10.68 g, 30 mmol) and anhydrous hydrazine (3 mL, 90 mmol) in warm ethanol (150 mL). The mixture was stirred at 50 °C for 1 h, then refluxed for another hour. The catalyst was removed by filtration, and the filtrate was diluted with ether (∼500 mL). A small amount of solids was filtered off, and the filtrate was washed with water (250 mL) and 1 N sodium hydroxide (100 mL) and dried over magnesium sulfate. After removal of the drying agent, the filtrate was evaporated in vacuo to afford the title compound (5.8 g, 57.2%). mp: 75−77 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.18−1.22 (t, 6H, J = 7 Hz), 3.58−3.62 (m, 2H), 3.98−4.05 (m, 4H), 4.97 (s, 2H), 5.18−5.21 (m, 1H), 6.697−6.69 (d, 1H, J = 8.1 Hz), 6.78−6.81 (m, 2H). Anal. ($C_{12}H_{18}F_3N_2O_3P$) C, H, N: calcd (found) C, 44.18 (44.36); H, 5.56 (5.57); N, 8.59 (8.85).

(*R*)-2-Benzyloxycarbonylamino-3-{2-[(diethoxy-phosphorylmethyl)-amino]-5-trifluoromethyl-phenylamino}-propionic Acid Benzyl Ester (15d). A solution of (*R*)-2-benzyloxycarbonylamino-succinic acid 1-benzyl ester (6.02 g, 16.9 mmol) and 4-methylmorpholine (1.7 g, 16.9 mmol) in methylene chloride (100 mL) was treated at−10 °C with a solution of isobutyl chloroformate (2.3 g, 16.9 mmol) in methylene chloride (20 mL) and stirred for 15 min at −10 °C, after which [(2-amino-4-trifluoromethyl-phenylamino)-methyl]-phosphonic acid diethyl ester (**15c**, 5 g, 15.3 mmol) was added. The reaction mixture was stirred at ambient temperature for 2 h, washed with water (100 mL) and saturated sodium bicarbonate (80 mL), dried over magnesium sulfate, filtered, and evaporated to dryness in vacuo. The resulting dark brown residue was crystallized from ethyl acetate/hexanes to afford the title compound (8.5 g, 83%). mp: 110−112 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.16−1.20 (m, 6H), 2.74−2.80 (m, 1H), 2.92−2.97 (m, 1H), 3.61−3.65 (m, 1H), 3.97−4.05 (m, 4H), 4.55−4.60 (m, 1H), 5.04−5.05 (d, 2H, J = 5.4 Hz), 5.14 (s, 1H), 5.78−5.79 (m, 1H), 6.93−6.95 (d, 1H, J = 8.5 Hz), 7.27−7.36 (m, 11H), 7.43 (s, 1H), 7.85−7.87 (d, 1H, J = 7.9 Hz). Anal. ($C_{31}H_{35}F_3N_3O_8P$) C, H, N: calcd (found) C, 55.94 (55.83); H, 5.30 (5.16); N, 6.31 (6.25).

2-Benzyloxycarbonylamino-3-[1-(diethoxy-phosphorylmethyl)-5-trifluoromethyl-1*H*-benzoimidazol-2-yl]-propionic Acid Benzyl Ester (15e). (*R*)-2-Benzyloxycarbonylamino-3-{2-[(diethoxy-phosphorylmethyl)-amino]-5-trifluoromethyl-phenylamino}-propionic acid benzyl ester (**15d**, 8.3 g, 12.4 mmol) was refluxed in propanoic acid (250 mL) for 4 h, then concentrated in vacuo, dissolved in methylene chloride (100 mL), washed three times with saturated sodium bicarbonate (100 mL each), dried over magnesium sulfate, filtered, and evaporated in vacuo to yield the title compound (4.5 g, 56%). The compound racemized during reflux in propanoic acid. A sample was crystallized from hexanes. mp: 75−78 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.00−1.20 (m, 6H), 2.78−2.82 (m, 1H), 3.24−3.50 (m, 1H), 3.70−4.06 (m, 4H), 4.94−5.37 (m, 4H), 7.08−7.45 (m, 10H), 7.46−7.66 (m, 1H), 7.75−7.94 (m, 2H). MS (+FAB): *m/e* 648 (M + H).

2-Amino-3-(1-phosphonomethyl-5-trifluoromethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Hydrate (15). A solution of 2-benzyloxycarbonylamino-3-[1-(diethoxy-phosphorylmethyl)-5-trifluoro methyl-1*H*-benzoimidazol-2-yl]-propionic acid benzyl ester (**15e**, 4 g, 6.18 mmol) in acetic acid (100 mL) was treated with palladium on carbon (10%, 1 g) and hydrogenated in a Parr apparatus at an initial pressure of 40 psi for 3 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The residue (2.5 g) was dissolved in 1,2-dichloroethane (100 mL) and treated with trimethylsilyl bromide (10 mL), refluxed for 2 h, and evaporated in vacuo, and the residue was partitioned under stirring between water (80 mL) and ethyl acetate (80 mL) for 1 h. The aqueous phase was separated, its pH was adjusted to 2.8 with 1 N lithium hydroxide, the mixture was cooled, and the product was filtered and dried to give the title compound (900 mg, 39%). Optical rotation as well as chiral HPLC indicated complete racemization of the material: mp: >310 °C (dec.). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.8−5.0 (m, 5H), 7.0−8.0 (m, 3H). MS (−FAB): *m/e* 366 (M − H). Anal. ($C_{12}H_{13}F_3N_3O_5P \cdot H_2O$) C, H, N: calcd (found) C, 37.40 (37.52); H, 3.93 (3.94); N, 10.91 (10.41).

2-Amino-3-(5-bromo-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Benzyl Ester Hydrochloride Quarter Hydrate, Scheme 8/Compound 16: [(4-Bromo-2-nitro-phenylamino)-methyl]-phosphonic Acid Diethyl Ester (16b). A solution of 4-bromo-1-fluoro-2-nitro-benzene[31] (**16a**, 2.1 g, 9.5 mmol) and aminomethyl-phosphonic acid diethyl ester (3.18 g, 19 mmol) in toluene (200 mL) was refluxed for 1.5 h. After cooling the mixture to room temperature, it was filtered, the cake was washed with toluene, and the filtrate was evaporated in vacuo. The residue was flash chromatographed on silica gel. Elution with ethyl acetate afforded the desired product as an orange solid (3.4 g, 97.5%). MS (PBCI): *m/e* 367 (M + H).

[(2-Amino-4-bromo-phenylamino)-methyl]-phosphonic Acid Diethyl Ester (16c). A solution of [(4-bromo-2-nitro-phenylamino)-methyl]-phosphonic acid diethyl ester (**16b**, 2.6 g, 7 mmol) in ethanol (150 mL) was treated at once with rhodium on carbon (5%, 350 mg) and hydrogenated at atmospheric pressure at ambient temperature for 6 h. The catalyst was removed by filtration through solka floc, the filtrate was evaporated in vacuo, and the residue was flash chromatographed on silica gel. Elution with 2% methanol/ethyl acetate gave the title compound as an amber oil (1.9 g, 81%). MS (EI): *m/e* 336 (M+).

(*R*)-2-Benzyloxycarbonylamino-3-{5-bromo-2-[(diethoxy-phosphorylmethyl)-amino]-phenylamino}-propionic Acid Benzyl Ester (16d). A solution of [(2-amino-4-bromo-phenylamino)-methyl]-phosphonic acid diethyl ester (**16c**, 1.85 g, 5.4 mmol) in methylene chloride (80 mL) was treated at 0 °C with diisopropylethylamine (1.486 g, 11.5 mmol), followed by (*R*)-2-benzyloxycarbonylamino-succinic acid 1-benzyl ester (2.251 g, 6.3 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (1.603 g, 6.3 mmol). The reaction mixture was stirred at 0 °C for 20 h and evaporated in vacuo, and the residue was flash chromatographed on silica gel. Elution with 2% methanol/ethyl acetate gave the desired

material as a colorless foam (2.8 g, 76%). MS (+FAB): *m/e* 676 (M + H).

2-Benzyloxycarbonylamino-3-[5-bromo-1-(diethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic Acid Benzyl Ester (16e). *p*-Toluenesulfonic acid (760 mg, 4 mmol) was added to a solution of (*R*)-2-benzyloxycarbonyl-amino-3-{5-bromo-2-[(diethoxy-phosphorylmethyl)-amino]-phenyl-amino}-propionic acid benzyl ester (**16d**, 2.6 g, 3.8 mmol) in toluene (200 mL) and refluxed for 30 min using a Dean−Stark trap. The mixture was cooled and evaporated in vacuo, and the residue was partitioned between chloroform (80 mL) and saturated sodium bicarbonate (80 mL). The organic layer was separated, dried over magnesium sulfate, filtered, and evaporated in vacuo. The residue was flash chromatographed on silica gel. Elution with ethyl acetate/hexanes (8:1) gave the racemic title compound as a colorless foam (1.6 g, 60%). MS (+FAB): *m/e* 658 (M + H).

2-Amino-3-(5-bromo-1-phosphonomethyl-1*H*-benzoimid-azol-2-yl)-propionic Acid Benzyl Ester Hydrochloride Quarter Hydrate (16). Trimethylsilyl iodide (32 mg, 0.67 mL, 4.66 mmol) was added at once under dry nitrogen to a solution of 2-benzyloxycarbonylamino-3-[5-bromo-1-(diethoxy-phosphorylmethyl)-1*H*-benzoimidazol-2-yl]-propionic acid benzyl ester (**16e**, 440 mg, 0.66 mmol) in acetonitrile (10 mL). The reaction mixture was stirred at 40 °C for 30 min followed at ambient temperature for 3 h after which the mixture was evaporated in vacuo and kept at 0.5 Torr vacuum overnight. The residue was partitioned between water (20 mL) and ether (20 mL) under vigorous stirring for 1 h; the separated organic layer was washed with hydrochloric acid (2%), and the phases were separated. The aqueous layer was evaporated in vacuo and stripped twice with benzene, and the final residue was crystallized from water/acetonitrile. The material was filtered and dried in vacuo (0.5 Torr) overnight to afford the title compound as white micro crystals (150 mg, 48%). mp: 203−5 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.93 (d, J = 8.1 Hz, 2H), 4.85 (d, J = 12.2 Hz, 2H), 5.02 (t, J = 8.1 Hz, 1H), 5.15 (s, 2H), 7.21 (m, 5H), 7.58 (d, J = 8.9 Hz, 1H), 7.78 (s, 1H), 7.91 (d, J = 8.9 Hz, 1H). MS (+FAB): *m/e* 468 (M + H). Anal. ($C_{18}H_{19}BrN_3O_5P\cdot1HCl\cdot0.25H_2O$) C, H, N: calcd (found) C, 42.48 (42.16); H, 4.01 (4.08); N, 8.26 (8.57). **2-Amino-3-(5-chloro-1-phosphonomethyl-1*H*-benzoimidazol-2-yl)-propionic Acid Benzyl Ester Hydrochloride Hemihydrate, Scheme 8/Compound 17.** Compound **17** was prepared in five steps with an overall yield of 21% using the same methodology as for compound **16** but starting from 4-chloro-1-fluoro-2-nitrobenzene (**17a**) prepared according to the procedure of Finger et al.[31] mp: 206−8 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.92 (d, J = 8.0 Hz, 2H), 4.83 (d, J = 11.9 Hz, 2H), 5.03 (t, J = 8.0 Hz, 1H), 5.14 (s, 2H), 7.22 (m, 5H), 7.68 (d, J = 8.7 Hz, 1H), 7.82 (d, J = 8.7 Hz, 1H), 7.91 (s, 1H). MS (+FAB): *m/e* 424 (M + H). Anal. ($C_{18}H_{19}ClN_3O_5P\cdot1HCl\cdot0.5H_2O$) C, H, N: calcd (found) C, 46.11 (45.87); H, 4.41 (4.49); N, 8.96 (9.34).

Computer Modeling of Compound 1. The multifit option in Sybyl was used with the carboxylate carbon, the quaternary nitrogens and the phosphorus atoms were used as constraining atoms. The only acceptable solutions placed the quaternary nitrogen of the benzimidazole in a position to form an intramolecular hydrogen bond with the unsubstituted benzimidazole nitrogen of compound **1**.

In Vitro Pharmacology. Tissue Preparation: Crude synaptic membrane pellets were prepared according to a modification of the method described by Murphy, D. E., et al.[32] from rat whole brain and used in [^{3}H]-CPP and stimulated [^{3}H]-TCP binding assays. Rats were decapitated and their brains were immediately removed, weighed, and placed in ice-cold 10% sucrose. Each brain was homogenized using a Potter−Elvenhjem tissue grinder equipped with a Teflon pestle (12 strokes at approximately 800 rpms). The homogenate was then centrifuged at 1000*g* for 10 min, and the resulting supernatant was centrifuged at 20000*g* for 20 min. The crude mitochondrial pellet was resuspended in ice-cold water and dispersed using a Brinkman Polytron (PT-10, setting of 6 for 30 s), and the suspension was centrifuged at 8000*g* for 20 min. The supernatant and buffy coat were centrifuged at 48000*g* for 20 min. The resulting pellet and buffy coat were then resuspended in 15 volumes of 50 mM TRIS HCl (pH 7.6) buffer containing 0.04% Triton X-100 and incubated at 37 °C for 15 min. The suspension was then centrifuged at 20000*g* for 20 min, after which the pellet was washed twice in ice-cold buffer and finally frozen at −70 °C for subsequent use in binding assays.

[^{3}H]-CPP Binding: The method described by Murphy, D. E., et al.[32] was used. Membrane pellets were thawed, and resuspended in 15 volumes of ice-cold 50 mM TRIS HCl (pH 7.6) buffer. In triplicate, 1 mL of the membrane preparation containing between 0.2 and 0.5 mg protein were incubated at 23 °C for 15 min together with 8 nM [^{3}H]-CPP (specific activity 30−40 Ci/mmol[33]), one of the various test solutions, and an appropriate volume of buffer for a final incubation volume of 2.0 mL using plastic minivials.[34] The reaction was initiated by the addition of the protein to the incubation medium. TRIS buffer and a 1.0 mM NMDA solution were substituted for the test solution in separate triplicates to define "total" and "nonspecific" binding, respectively. The samples were then centrifuged at 48000*g* for 20 min, and the pellets were digested with 0.5 mL/sample of tissue solubilizer (NCS, Amersham) for 1 h. Hydrochloric acid (4 N, 0.1 mL) was added to each sample to reduce chemiluminescence during subsequent counting. Scintillation cocktail (3.2 mL/sample; Aquasol, DuPont) was added, and the samples were prepared for counting using conventional liquid spectroscopy. The compounds were tested at 5−10 concentrations for IC_{50} and 95% confidence limits values.

[^{3}H]-TCP Binding: A modification of the methods described by Kloog, Y., et al.[26] was used. Membrane pellets were thawed and resuspended in 15 volumes of ice-cold 5.0 mM TRIS HCl (pH 7.4) buffer. The homogenate was then centrifuged at 48000*g* for 20 min, and the pellet was resuspended in ice-cold buffer for use in the binding assay. In triplicate, 1 mL samples of the membrane preparation were incubated for 1 h at 25 °C in the presence of 2.5 nM [^{3}H]-TCP (specific activity 45−50 Ci/mmol[35]), 3 μM L-glutamate, 1 μM glycine, one of various test solutions, and an appropriate volume of buffer for a final incubation volume of 2 mL using glass disposable culture tubes. The reaction was started by the addition of membrane protein to the incubation medium. TRIS buffer and a 100 μM solution of MK-801 were substituted for the test solution in separate triplicates to define "total" and "nonspecific" binding, respectively. The samples were then filtered under vacuum using polyethyleneimine (0.05% in buffer) presoaked glass fiber filters (Whatman GF/B) and rinsed with three 2 mL volumes of ice-cold buffer. The filters were placed into individual 20 mL glass scintillation vials and prepared for counting using conventional liquid spectroscopy. The compounds were tested at 5−10 concentrations for determination of IC_{50} and 95% confidence limits values.

In Vivo Pharmacology. NMDA Lethality Model. Antagonism of NMDA-induced lethality was determined using a modification of the NMDA convulsion model described by Hutchison, A. J., et al.[36] Male, Swiss-albino mice (CD-1, 18−22 g[37]) were acclimated for 30 min to an observation chamber and then injected (10 mL/kg) with an intraperitoneal dose of a test solution or vehicle (control). Thirty minutes thereafter, the mice received an intraperitoneal injection of 195 mg/kg NMDA (a lethal dose in 90% of naive mice), and the number of responding (surviving) mice was determined 30 min following the injection of NMDA. Animals were tested in groups of 10 mice/dose level. Data were analyzed using the probit analysis program PS NONLIN (Natural Response Rate Version) for ED_{50} and 95% confidence limits determinations.

Focal Brain Ischemia Model in the Rat. Male Fisher-344 rats (390−310 g) were subjected to a permanent occlusion of the distal middle cerebral artery (MCA) and ipsilateral common carotid arteries according to the procedure described by Brint et al.[38] In brief, animals were anesthetized with 2−4% isoflurane followed by the occlusion of the right common carotid artery. The right MCA was then exposed by crani-

ectomy and electrocoagulated using bipolar electrocautery forceps. Body temperature was maintained at 37 ± 1 °C during the surgical procedure. Test compound or vehicle was administered as a single intravenous bolus injection (1 mL/kg via the tail vein) 5 min following the MCA occlusion. Animals were sacrificed 24 h later; frozen brain sections (20 μm) were cut at 400 μm intervals throughout the forebrain and stained with cresyl violet to visualize the infarcted tissue. The infarct area of each section was quantified by image analysis and the total infarct volume (mm^3) calculated from the sum of all sectional areas multiplied by the interval distance. The results are presented as means ± SE. A one-way ANOVA followed by the least significant difference analysis was used to determine statistical significance of the means between groups.

Acknowledgment. We express our appreciation to Mr. J. Cantone, Ms. S. Ellis, Mr. B. Hofmann, Mr. J. Kulishoff, Mr. J. Mattes, Ms. R. Moore, and Ms. K. Santilli for microanalytical and spectral data.

Supporting Information Available: Tables of elemental analyses for compounds **1**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Crawford, J. M.; Curtis, D. R. The excitation and depression of mammalian cortical neurones by amino acids. *Br. J. Pharmacol.* **1964**, 23313−29.
(2) Olney, J. W.; Ho, O. L.; Rhee, V. Cytotoxic effect of acidic and sulphur-containing amino acids on the infant mouse central nervous system. *Exp. Brain Res.* **1971**, *14*, 61−7.
(3) Lucas, D. R.; NewHouse, J. P. The Toxic Effect of Sodium L-Glutamate on the Inner Layers of the Retina. *AMA Arch. Opthalmol.* **1957**, *58*, 193−201.
(4) Coyle, J. T. Neurotoxic Action of Kainic Acid. *J. Neurochem.* **1981**, *41*, 1−11.
(5) Benveniste, H. The Excitatoxic Hypothesis in Relation to Cerebral Ischemia. *Cerebrovasc. Brain Metab. Rev.* **1991**, *3*, 213−45.
(6) Faden, A. I.; Demediuk, P.; Panter, S. S.; Vink, R. The Role of Excitatory Amino Acids and NMDA Receptors in Traumatic Brain Injury. *Science* **1989**, *244*, 789−800.
(7) Choi, D. W.; Cerebral Ischemia: Some New Approaches and Unanswered Questions. *J. Neurosci.* **1990**, *10*, 2493−2501.
(8) Smith, D. H.; Casey, K.; McIntosh, T. K. Head Injury Treatment in Evolution. Pharmacologic Therapy for Traumatic Brain Injury. Experimental Approaches. *New Horizons* **1995**, *3*, 562−572.
(9) Bullock, R. Introducing NMDA Antagonists into Clinical Practice; Why Head Injury Trials? *Br. J. Clin. Pharmacol.* **1992**, *34*, 396−401.
(10) Lipton, S. A.; Rosenberg, P. A. Mechanisms of Disease. Excitatory Amino Acids as a Final Common Pathway for Neurologic Disorders. *N. Eng. J. Med.* **1994**, *330*, 613−622.
(11) Lees, G. J. Contributory Mechanisms in the Causation of Neurodegenerative Disorders. *Neuroscience* **1993**, *8*, 261−277.
(12) Danysz, W.; Parsons, C. G.; Bresink, I.; Quack, G. A revived Target for Drug Development? Glutamate in CNS Disorders. *Drug News Perspect.* **1995**, *8*, 261−277.
(13) Iversen, L. L.; Woodruff, G. N.; Gill, R. Glutamate Antagonists as Novel Neuroprotective Agents. *J. Cereb. Blood Flow Metab.* **1989**, *9* (suppl), S 743.
(14) Muir, K. W.; Lees, K. R. Clinical Experience with Excitatory Amino Acid Antagonist Drugs. *Stroke* **1995**, *26*, 503−13.
(15) Baudy, R. B.; Greenblatt, L. P.; Jirkovsky, I. L.; Conklin M.; Russo, R. J.; Bramlett, D. R.; Emrey, T. A.; Simmonds, J. T.; Kowal, D. M.; Stein, R. P.; Tasse, R. R. Potent Quinoxaline-Spaced Phosphono-α-Amino Acids of the AP-6 type as Competitive NMDA Antagonists: Synthesis and Biological Evaluation. *J. Med. Chem.* **1993**, *36* (3), 331−342.
(16) Kinney, W. A.; Abou-Gharbia, M.; Garrison, D. T.; Schmid, J.; Kowal, D. M.; Bramlett, D. R.; Miller, T. L.; Tasse, R. P.; Zaleska, M. M.; Moyer, J. A.Design and Synthesis of [2-(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non1(7)-en-2-yl)ethyl] phosphonic Acid (EAA-090), a Potent N-Methyl-D-aspartate Antagonist, via the Use of 3-Cyclobutene-1,2-dione as an Achiral α-Amino Acid Bioisostere. *J. Med. Chem.* **1998**, *41* (2), 236−246.
(17) Manuscript in preparation.
(18) Albertson, N. F. Synthesis of Peptides with Mixed Anhydrides. *Org. React.* **1962**, *12*, 157−355.
(19) Nestor, J. J.; Horner, B. L.; Ho, T. L.; Jones, G. H.; McRae, G. I.; Vickery, B. H. Synthesis of a Novel Class of Heteroaromatic Amino Acids and Their Use in the Preparation of Analogues of Luteinizing Hormone-Releasing Hormone. *J. Med. Chem.* **1984**, *27*, 320−25.
(20) Friedrich, E. C.; DeLucca, G. Interhalogen-Catalyzed Cleavages of Ethers and Esters with Trimethylsilyl Bromide or Chloride. *J. Org. Chem.* **1983**, *48*, (10), 1678−82.
(21) Davidsen, S. K.; Phillips, G. W.; Martin, Stephen F. Geminal Acylation-Alkylation at a Carbonyl Center using Diethyl-N-Benzylideneaminomethyl phosphonate: Preparation of 2-Methyl-2-Phenyl-4-Pentenal. *Org. Synth.* **1987**, *65*, 119−34.
(22) Sokol'skii, D. V.; Omarkulov, T. O.; Nurgozhaev, K. Single-step Conversion Reactions of Nitro Aromatic Compounds to Amino Compounds. *Zh. Org. Khim.* **1978**, *14* (2), 334−7.
(23) Diago-Meseguer, J.; Palomo-Coll, A. L. A New Reagent for Activating Carboxyl Groups: Preparation and Reactions of N,N-Bis[2-oxo-3-ox-azolidinyl]phosphoro diamidic Chloride. *Synthesis* **1980**, 547−51.
(24) Ben-Ishai, D.; Berger, A. Cleavage of N-carbobenzoxy Groups by dry Hydrogen Bromide and Hydrogen Chloride. *J. Org. Chem.* **1952**, *17*, 1564−70.
(25) Lott, R. S.; Virander, C. S.; Stammer, C. H. Trimethylsilyl Iodide as a Peptide Deblocking Agent. *J. Chem. Soc., Chem. Commun.* **1979**, 495−6.
(26) Kloog, Y.; Haring, R.; Sokolovsky, M. Kinetic Characterization of the Phencyclidine-N-Methyl-D-Aspartate Receptor Interaction: Evidence for a Steric Blockade of the Channel. *Biochemistry* **1988**, *27*, 843−48.
(27) Young, A. B.; Fagg, G. E. Excitatory Amino Acid Receptors in the Brain; Membrane Binding and Receptor Autoradiographic Approaches. *Trends Pharmacol. Sci.* **1990**, *11*, 126−133.
(28) Carter, C.; Benavides, J.; Legendre, P.; Vincent, J. D.; Noel, F.; Thuret, F.; Lloyd, K.; Arbilla, S.; Zivkovic, B.; MacKenzie, E. T.; Scatton, B.; Langer, S. Z. Ifenprodil and SL82.0715 as Cerebral Anti-Ischemic Agents. II. Evidence for N-Methyl-D-Aspartate Receptor Antagonist Properties. *J. Pharmacol. Exp. Ther.* **1988**, *247*, 1222−32.
(29) Karpiak, S.; Tagliavia, A,; Wakade, C. G. Animal Models for the Study of Drugs in Ischemic Stroke. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 403−14.
(30) Hansen, D. K. Analytical Methods for the Investigation of the Transport of Natural and Synthetic Glycine Site NMDA Receptor Antagonists Across the Blood-Brain Barrier. *Diss. Abstr. Int.* **1998**, *60* (2), 617.
(31) Finger, G. C.; Gortatowski, M. J.; Shiley, R. H.; White, R. H. Aromatic Fluorine Compounds, VIII. Plant Growth Regulators and Intermediates. *J. Am. Chem. Soc.* **1959**, *81*, 94−101.
(32) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, M. Binding of [3H] 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid to Rat Brain Membranes: A Selective, High-Affinity Ligand for N-Methyl-D-Aspartate Receptors. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 778−84.
(33) Dupont, NEN, Boston, MA.
(34) Skatron Inc., Sterling, VA.
(35) Waters Inc., Milford, MA.
(36) Hutchison, A. J.; Williams, M.; Angst, C.; de Jesus, R.; Blanchard, L.; Jackson, R. H.; Wilusz, E. J.; Murphy, D. E.; Bernard, P. S.; Schneider, J.; Campbell, T.; Guida, W.; Sills, M. A. 4-(Phosphonoalkyl)- and 4-(phosphonoalkenyl)-2-piperidinecarboxylic acids: Synthesis, Activity at N-Methyl-D-Aspartic Acid Receptors, and Anticonvulsant Activity. *J. Med. Chem.* **1989**, *32*, 2171−78.
(37) Charles River, Wilmington, DE.
(38) Brint, S.; Jacewicz, M.; Kiessling, M.; Tanabe, J.; Pulsinelli, W. Focal Brain Ischemia in the Rat: Method for Reproducible Neocortical Infarction using Tandem Occlusion of the Distal Middle Cerebral Artery and Ipsilateral Common Carotid Arteries. *J. Cereb. Blood Flow Metab.* **1988**, *8*, 474−85.

JM000385W