Potent Quinoxaline-Spaced Phosphono α-Amino Acids of the AP-6 Type as Competitive NMDA Antagonists: Synthesis and Biological Evaluation

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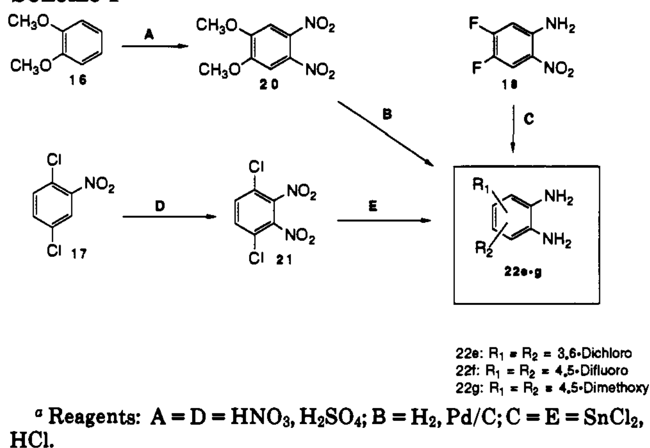
A series of α-amino-3-(phosphonoalkyl)-2-quinoxalinepropanoic acids was synthesized and evaluated for NMDA receptor affinity using a [^{3}H]CPP binding assay. Functional antagonism of the NMDA receptor complex was evaluated in vitro using a stimulated [^{3}H]TCP binding assay and in vivo by employing an NMDA-induced seizure model. Some analogues also were evaluated in the [^{3}H]-glycine binding assay. Several compounds of the AP-6 type show potent and selective NMDA antagonistic activity both in vitro and in vivo. In particular α-amino-7-chloro-3-(phosphonomethyl)-2-quinoxalinepropanoic acid (1) displayed an ED_{50} of 1.1 mg/kg ip in the NMDA lethality model. Noteworthy is α-amino-6,7-dichloro-3-(phosphonomethyl)-2-quinoxalinepropanoic acid (3) with a unique dual activity, displaying in the NMDA receptor binding assay an IC_{50} of 3.4 nM and in the glycine binding assay an IC_{50} of 0.61 μM.

Many diverse pathological processes cause ischaemic cerebrovascular diseases, of which, focal cerebral ischaemia (stroke) is prominent in man. Considerable research efforts are ongoing to elucidate the fundamental mechanisms of ischaemia-induced neurodegeneration. We set out to develop an effective therapeutic strategy to intervene early on in the cascade-like events which, after an ischaemic insult, lead to neuronal death. Overactivation of excitatory amino acid transmitter systems, induced by excess glutamate, aspartate, and perhaps other related amino acids, are believed to play the primary role in the sequence of biochemical events which result in neuronal injury and death.[1-4] The excitatory amino acids mediate their action via at least four major subtypes of receptors, namely, the NMDA (N-methyl-D-aspartatic acid), the kainate, the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), and the metabotropic receptor.[5] NMDA receptors play a crucial role in ischaemia-induced neurotoxicity by opening calcium-permeable ion channels.[6-8] NMDA antagonists offer protection against permanent ischaemic damage even when administered several hours after the ischaemic event, thus suggesting that they may find utility in the acute therapy of cerebral ischaemia.[9] Therefore we pursued the synthesis and biological evaluation of diverse, novel NMDA antagonists, (Figure 1).

Numerous competitive NMDA antagonists have been reported in the literature since 1980. With few exceptions, the most potent analogues incorporate α-amino acids tethered to a phosphonic acid moiety (Figure 1) via an alkyl chain consisting of either three-carbon (such as 2-amino-5-phosphonovaleric acid, AP-5[10] or five-carbon (2-amino-7-phosphonoheptanoic acid, AP-7[11]). Interestingly, AP-6 analogues have demonstrated much reduced efficacy. Enhanced affinity for the NMDA receptor has been obtained by conformationally restraining the phosphono α-amino acid unit, but the AP-5/AP-7 rule still applies to these unsaturated and/or cyclic analogues like (E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849[12]), 4-(3-phosphonopropyl)-2-piperazinecarboxylic acid (CPP[13,14]), α-[4-(phosphonomethyl)phenyl]glycine (SC 46643[15]), 4-(phosphonomethyl)-2-piperidinecarboxylic acid (CGS 19755[16-18]), and 2-amino-4,5-(1,4-butanediyl)-7-phosphonoheptanoic acid (NPC 12626[19]). Using the quinoxaline ring system as a template, we have synthesized various phosphono α-amino acids. Somewhat surprisingly, 1, an AP-6 type of competitive NMDA antagonist, turned out to be the most active compound of the present series.

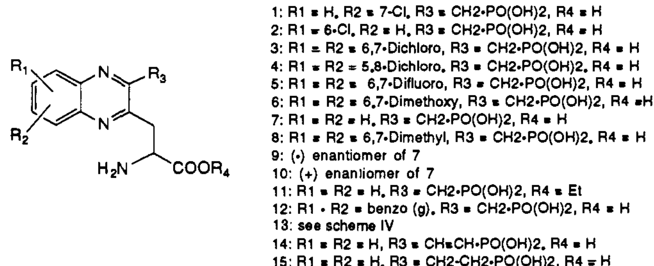
Scheme I [a]



[a] Reagents: A = D = HNO_3, H_2SO_4; B = H_2, Pd/C; C = E = $SnCl_2$, HCl.

Chemistry

The α-amino-3-(phosphonoalkyl)-2-quinoxalinepropanoic acids 1–15 were synthesized as outlined in Schemes II–V. Scheme I depicts the syntheses of some substituted



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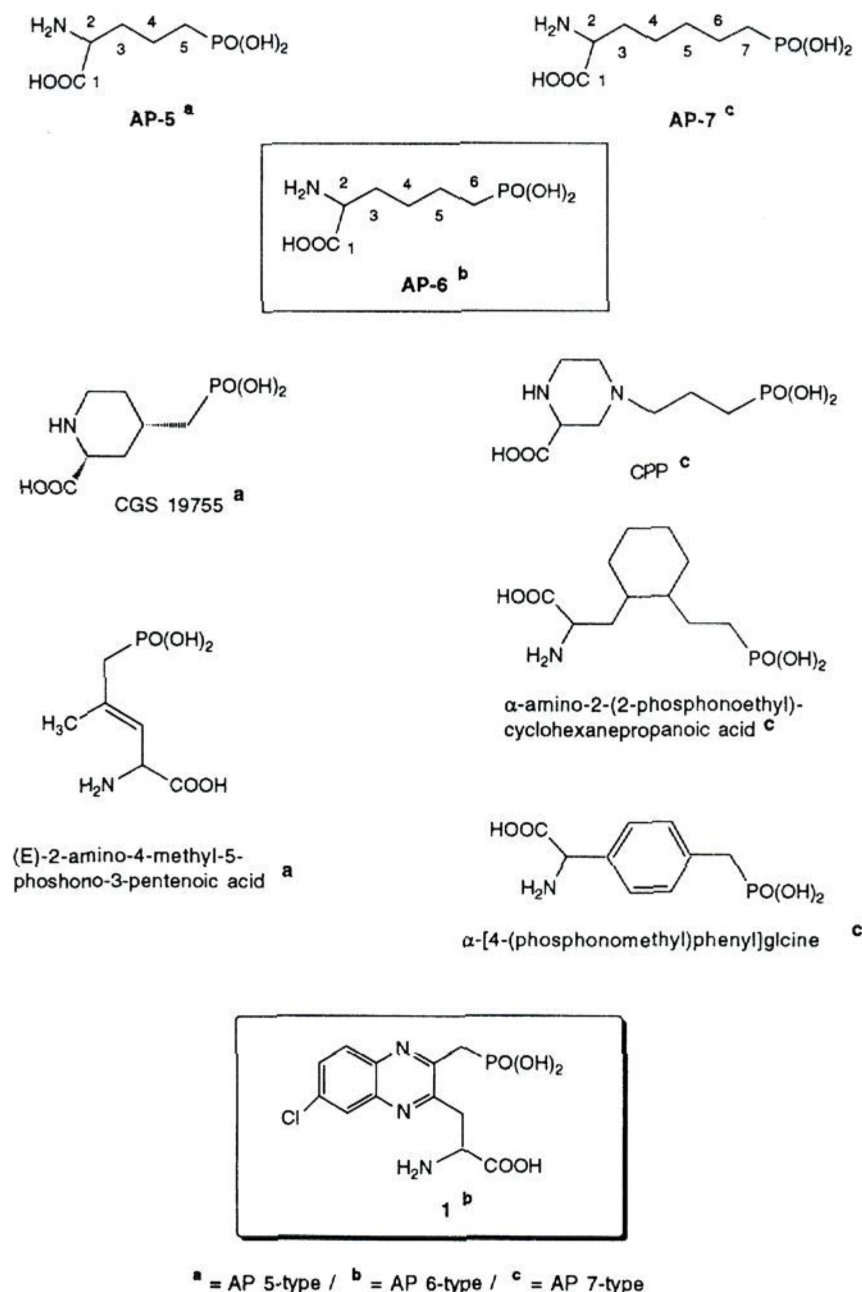


Figure 1. AP-5's and AP-7's versus AP-6's.

1,2-phenylenediamine starting materials which were not commercially available.

Typically (Scheme II), the appropriately substituted 1,2-phenylenediamines **22a–h** were condensed with 1,4-dibromo-2,3-butanedione to afford the 2,3-bis(bromomethyl)quinoxalines **23a–h** in high yields. An Arbuzov reaction using trimethyl phosphite and the bis(bromomethyl)quinoxaline precursor in equimolar amounts led to the corresponding phosphonate esters **24a–h** with yields in the range of 35–50%. Increasing the amount of trimethyl phosphite led to the formation of undesired bisphosphonate esters. The regioisomeric 6- and 7-monochloroquinoxalines **24b** and **24c** were only accessible through HPLC separation. Despite a distinct difference in the H-NMR between these two regioisomers, conclusive assignment of the position of the chlorine atom was not possible. Therefore compound **24b** was subjected to X-ray crystallography, confirming the chlorine to be in position 6, as illustrated in Figure 3. Compounds **25b–g** were obtained by alkylating the sodium salt of diethyl acetamidomalonate in ethanol with the corresponding bromomethylquinoxalines **24b–g**. Hydrolysis and decarboxylation in refluxing 6 N HCl followed by treatment with propylene oxide in ethanol gave the desired phosphono amino acids **1–6**. The unsubstituted compounds **26i** and **26j**, as well as **26k** were obtained by alkylating the potassium salt of the appropriate *N*-benzylideneglycine ester in THF with **24a** and **24h**. Acidic hydrolysis and subsequent treatment with propylene oxide in ethanol led to compounds **7** and **8**. Treatment of **26j** with bromotrimethylsilane led to compound **11**. The dibenzoyl-L-tartrate of **26i** was prepared and repeatedly crystallized from acetonitrile to yield a pure diastereomeric salt, which after treatment with sodium bicarbonate followed by hydrolysis with 6 N HCl and treatment with propylene oxide gave compound **10**. Compound **9** was obtained similarly starting with **26i** and dibenzoyl-D-tartaric acid.

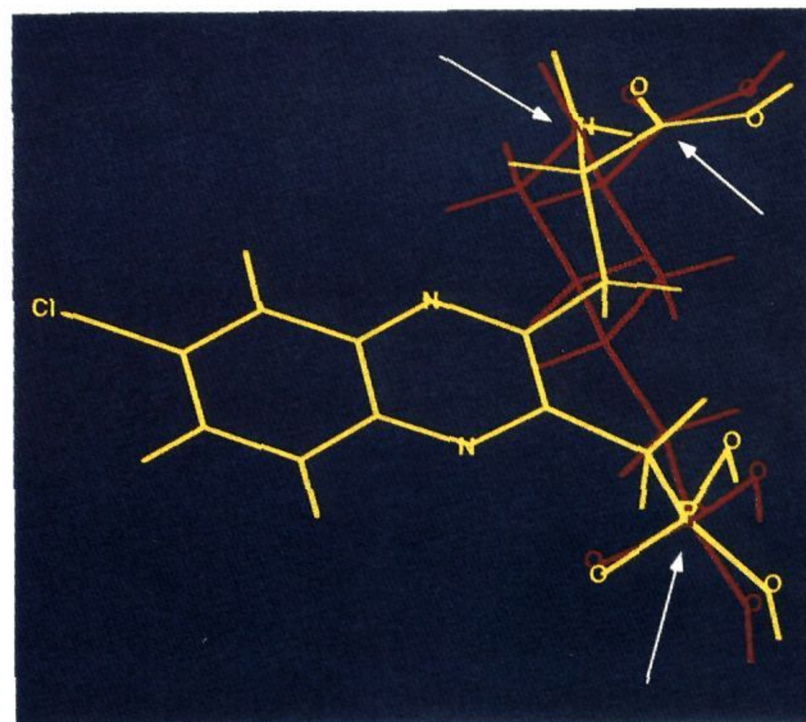


Figure 2. An overlay of the low-energy conformations of compound 1 (yellow) and the (–)-gauche form of CGS-19755 (red) from the SYBYL program.

As outlined in Scheme III the ammonium salt of α-amino-3-(phosphonomethyl)benzo[*g*]quinoxaline-2-propanoic acid (**12**) was obtained by condensing 2,3-diamononaphthalene (**19**) with 1,4-dibromo-2,3-butanedione to yield **27**. An Arbuzov reaction led to **28** which after alkylating the potassium salt of *N*-benzylideneglycine benzyl ester gave compound **29**. Treatment of the phosphonate ester with bromotrimethylsilane furnished **30**, which, after hydrogenation and subsequent treatment with ammonia, yielded compound **12**.

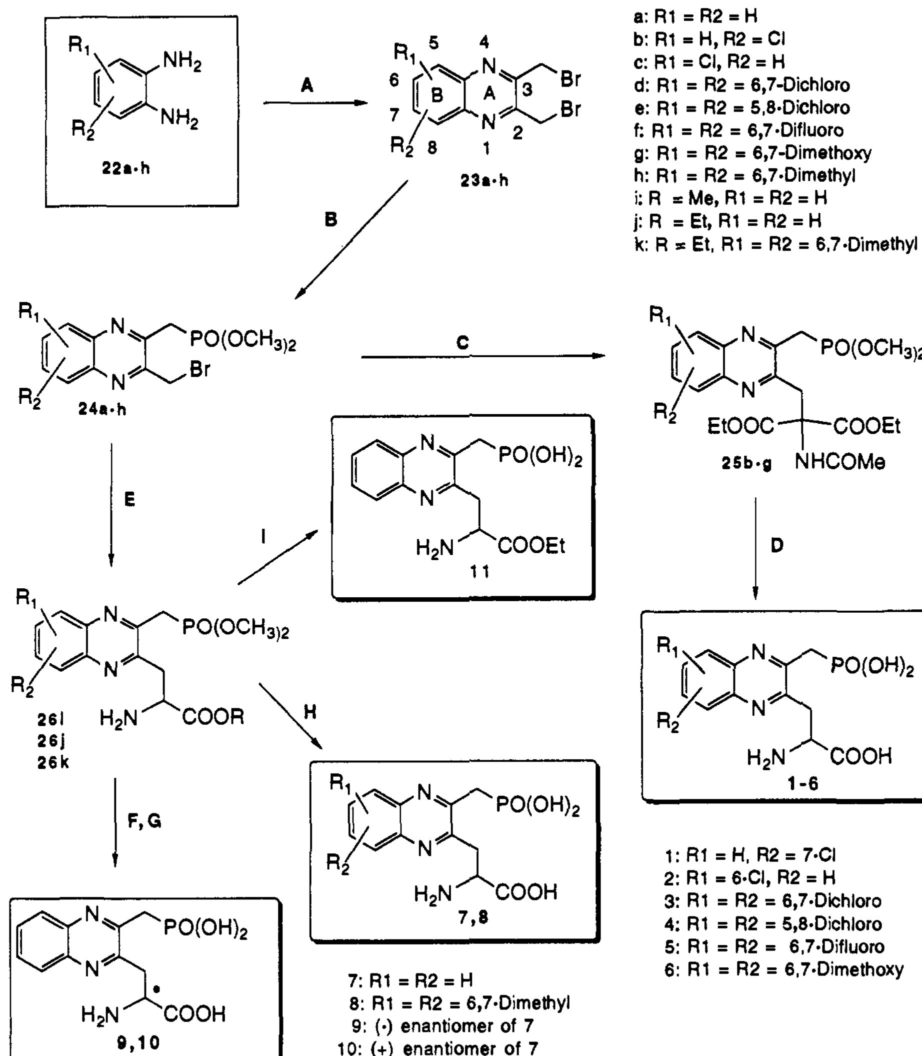
Treating 4,5-dimethyl-1,2-phenylenediamine (**22h**) with glyoxal gave compound **31** (Scheme IV). Bromination of **31** with *N*-bromosuccinimide led to 6,7-bis(bromomethyl)-quinoxaline (**32**) which after an Arbuzov reaction gave compound **33**. Alkylation of the potassium salt of *N*-benzylglycine methyl ester with **33** led to **34**, which after HCl hydrolysis and treatment with propylene oxide gave the desired α-amino-7-(phosphonomethyl)-6-quinoxalinepropanoic acid (**13**).

In order to extend the phosphonic acid side chain by one carbon, compound **23a** was converted to the 2-carboxaldehyde derivative **35** by interaction with the sodium salt of 2-nitropropane (Scheme V). In a Wittig-type reaction with sodium dimethyl methanephosphonate, **35** was transformed to the vinyl phosphonate ester **36**. Only the trans isomer was obtained. Treatment of the resulting vinyl phosphonate with the sodium salt of diethyl acetamidomalonate provided compound **37**. Acidic hydrolysis of **37** followed by the removal of HCl with propylene oxide yielded the desired vinyl phosphonic acid **14**. Hydrogenation of **37** in the presence of Pd/C gave **38** which, after the usual hydrolysis and HCl removal, gave the desired α-amino-3-(2-phosphonoethyl)-2-quinoxalinepropanoic acid **15**.

Results and Discussion

The biological data for the compounds **1–15** are listed in Table I along with test results on some standards AP-7,

Scheme II [a]



[a] Reagents: A = 1,4-dibromo-2,3-butanedione; B = $P(OMe)_3$; C = NaOEt, diethyl acetamidomalonate; D, G, H = (1) HCl, (2) propylene oxide; E = *t*-BuOK, PhCH=NCH_2COOR; F = dibenzoyltartaric acid (L or D); I = (1) TMSBr, (2) propylene oxide.

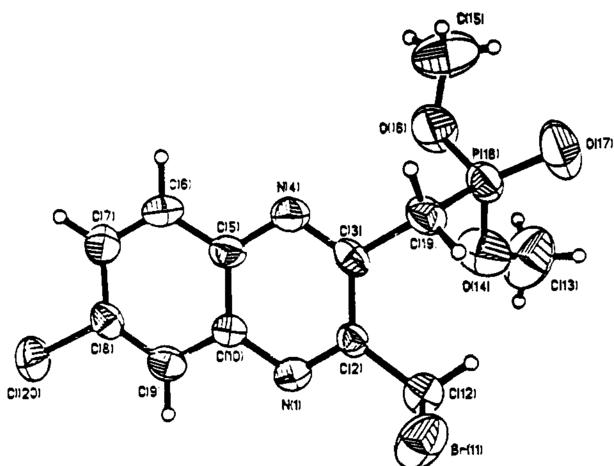
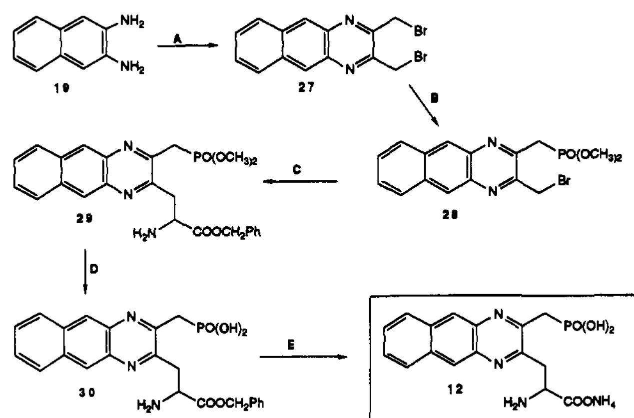


Figure 3. X-ray structure of **24b**.

Scheme III [a]

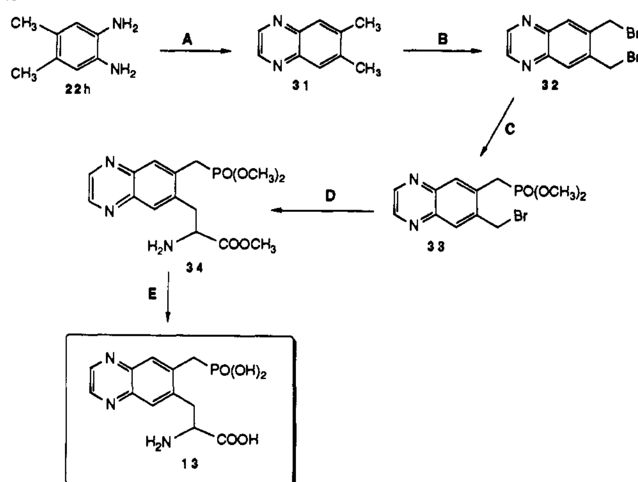


[a] Reagents: A = 1,4-Dibromo-2,3-butanedione; B = $P(OCH_3)_3$; C = *t*-BuOK, PhCH=N-CH_2COOCH_2Ph; D = TMSBr; E = (1) H_2, Pd–C(2) NH_4OH.

CGS-19755, and CPP. Affinity for the competitive NMDA receptor site was determined by assessing the ability of the compounds to displace [^{3}H]CPP, a known ligand for this binding site, from rat synaptic membranes. In vivo NMDA antagonist activity was determined using an NMDA-induced lethality assay, in which a compound's ability to prevent the effects of a lethal dose of NMDA, was assessed.

Competitive and noncompetitive NMDA receptor antagonists, in contrast to agonists, decrease the association rate of tritiated ligands for the PCP receptor[20,21] and, thereby, appear to inhibit stimulated [^{3}H]TCP binding following 1 or 2 h of incubation.[22] All standard NMDA antagonists inhibited the stimulated binding of [^{3}H]TCP with a relative potency compared to CGS-19,755 which

Scheme IV [a]



[a] Reagents: A = $(CHO)_2$; B = NBS; C = $P(OCH_3)_3$; D = $PhCH{=}NCH_2COOCH_3$; E = (1) HCl, (2) propylene oxide.

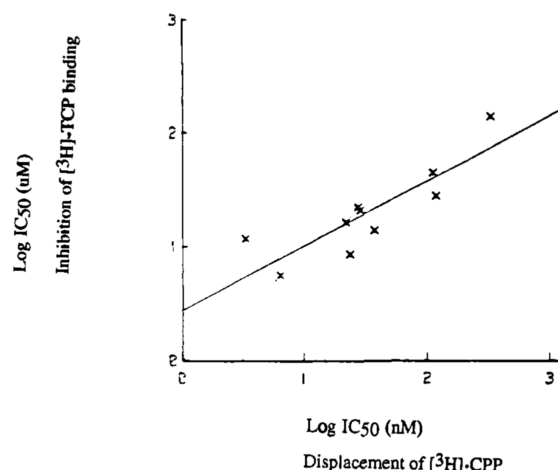


Figure 5. Correlation between affinity for the competitive NMDA receptor binding site (as measured by displacement of [^{3}H]CPP) and for functional antagonism (as measured by inhibition of stimulated [^{3}H]TCP binding).

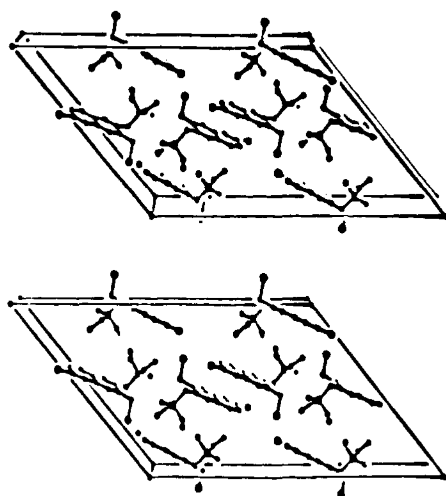


Figure 4. Packing diagram of **24b**.

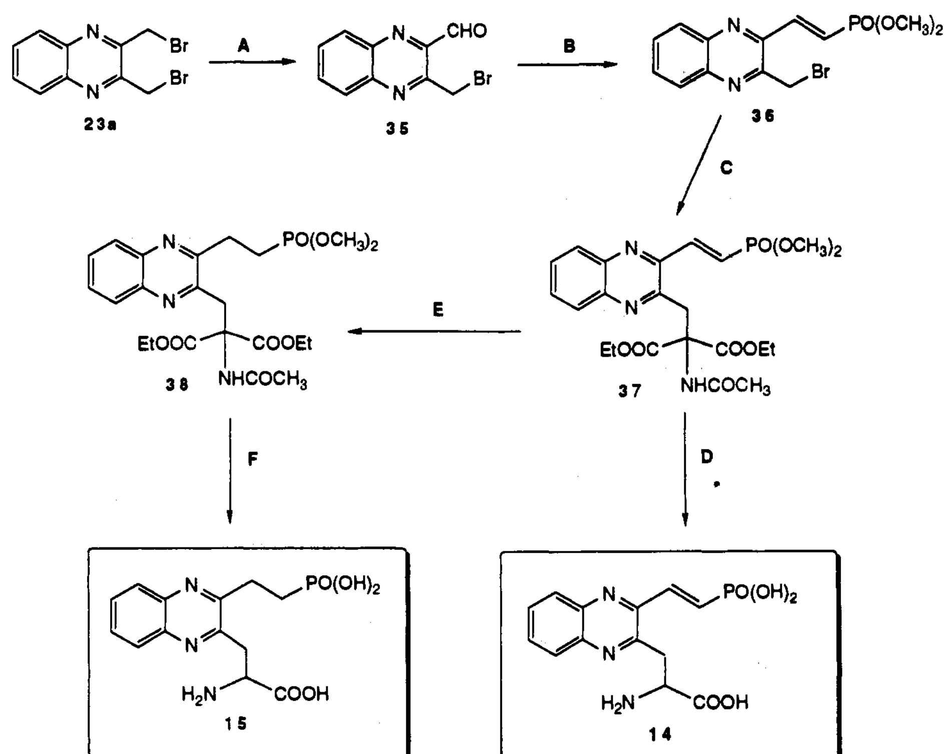
correlated with the relative affinities determined in the NMDA receptor binding assay. Compounds **1–3**, **7–9**, and **12**, which exhibited significant NMDA receptor affinity, also inhibited stimulated [^{3}H]TCP binding, suggesting that these compounds possessed antagonist activity at the NMDA receptor. The correlation between the NMDA binding assay and the stimulated [^{3}H]TCP binding assay was obtained for both the standard compounds and the quinoxaline analogues (**1–3**, **7–9**, **12**). The high degree of significance obtained from the comparisons of these two in vitro activities (Figure 5, Chi^2 = 0.949, n = 10) suggests a sound correlation.

The first compound synthesized, **7**, had an IC_{50} value of 119 nM in the NMDA receptor binding assay and an ED_{50} value in the NMDA-induced lethality test of 3.7 mg/kg (ip). Thus while about equipotent in binding with CPP, compound **7** was somewhat less potent in vivo.

The in vivo potency seen with compound **7** was gratifying, but the relatively high affinity for the NMDA receptor was quite surprising. Recent structure–activity relationship (SAR) and molecular modeling studies suggest that the generally accepted AP-5/AP-7 pharmacophore is required for potent binding to the competitive NMDA receptor site.[23-25] Contrary to this hypothesis, we have found that AP-6-type molecules such as compound **7** can display high NMDA receptor affinity. The reasons for this apparent paradox were not exhaustively explored. However, molecular modeling studies revealed that the quinoxaline-derived AP6 pharmacophore (exemplified by compound **1**) can easily overlap with the minimum energy conformation of the potent AP-5 analogue CGS-19755 (Figure 2). This result encouraged us to elucidate the structure–activity relationships among quinoxaline phosphono α-amino acids. We set out (a) to synthesize various substituted quinoxaline phosphono α-amino acids maintaining the AP-6 configuration (compounds **1–8**, **12**), (b) to resolve compound **7** into its enantiomers (compounds **9** and **10**), (c) to determine if an α-amino acid ester maintains activity (compound **11**), (d) to examine the importance of the nitrogen loci in the quinoxaline ring system with respect to activity (compound **13**), and (e) to prepare AP-7 analogues (compounds **14** and **15**).

Since symmetrically disubstituted quinoxalines are synthetically easily accessible, we first prepared the 6,7-dichloro analogue **3**. In the NMDA receptor binding assay **3** turned out to be more potent than any ligand yet reported for the NMDA receptor (IC_{50} = 3.4 nM). In the inhibition of NMDA-induced lethality **3** showed a marked improvement over **7** (ED_{50} = 1.6 mg/kg, ip). In contrast, compound **4**, the 5,8-dichloro-substituted quinoxaline, did not significantly inhibit NMDA-induced lethality (ED_{50} = >10 mg/kg) and showed rather moderate displacement in the NMDA binding assay (IC_{50} = 245 nM). While some of the other disubstituted analogues displayed some NMDA binding affinity (compound **8**: IC_{50} = 22.3 nM; compound **5**: IC_{50} = 166 nM), their in vivo potency left much to be desired. However, benzo[*g*]quinoxaline analogue **12** scored a respectable IC_{50} = 6.5 nM in the NMDA binding assay and displayed an ED_{50} value of 5.3 mg/kg (ip) in the NMDA-induced lethality model. Resolution of compound **7** revealed, that the eutomer (compound **9**) is 4 times more potent than the racemate in the [^{3}H]CPP binding assay and the distomer (compound **10**) is less than maximally effective (85% displacement) at the 10 μM concentration. When an ethyl ester moiety was substituted for the carboxylic acid group (compound **11**), both in vitro and in vivo activity was diminished. Placing the nitrogen atoms of the quinoxaline ring system further away from the phosphonic and α-amino acid moieties (compound **13**) resulted in reduced activity (both, in vitro and in vivo). From the limited number of examples it was impossible to deduce the reasons for the lower activity seen when the

Scheme V [a]



[a] Reagents: A = NaOEt, 2-nitropropane; B = NaH, $CH_2[PO(OCH_3)_2]_2$; C = NaOEt, diethyl acetamidomalonate; D = (1) HCl, (2) propylene oxide; E = H_2, Pd–C; F = (1) HCl (2) propylene oxide.

Table I. Biological Activities of Reference EAA Antagonists and Compounds 1–15

compound	displacement of [^{3}H]CPP binding: IC_{50} (nM)[a]	displacement of [^{3}H]TCP binding: IC_{50} (μM)[a]	displacement of [^{3}H]glycine binding: IC_{50} (μM)[a]	inhibition of NMDA lethality: ED_{50} (mg/kg, ip)[a]
CGS-19,755	28.0 (22.3–34.2)	22.4 (18.0–27.8)	>100	0.6 (0.42–0.93)
CPP	112.6 (96.1–132.3)	45.0 (39.1–51.8)	>100	1.0 (0.35–2.89)
AP-7	388.0 (277.0–511.0)	302.0 (217.0–389.0)	NT	37.6 (31.4–45.1)
glycine	NT[b]	NT	0.086 (0.073–0.10)	NT
1	24.0 (17.2–32.6)	8.6 (5.8–11.7)	>100	1.1 (0.63–2.12)
2	38.0 (28.5–52.3)	13.9 (13.0–14.8)	1.80 (1.17–2.76)	>10
3	3.4 (2.8–4.1)	11.7 (10.0–13.6)	0.61 (0.32–1.17)	1.6 (0.82–3.03)
4	245.0 (197.1–304.7)	NT	NT	>10
5	166.0 (131.9–210.2)	NT	NT	>10
6	832.0 (686.1–1010)	NT	NT	>10
7	119.0 (87.4–163.4)	28.0 (24.6–31.9)	4.7 (3.35–6.55)	3.7 (2.68–5.15)
8	22.3 (17.7–30.2)	16.4 (15.3–17.7)	NT	~10
9	29.3 (23.5–38.8)	21.0 (16.8–26.2)	NT	1.5 (1.06–2.02)
10	>1000	NT	NT	>10
11	>1000	NT	NT	NT
12	6.5 (9.0–11.4)	5.6 (4.2–7.6)	NT	5.3 (3.99–7.14)
13	>1000	NT	NT	>10
14	>1000	NT	NT	>10
15	>1000	NT	NT	>10

[a] 95% confidence limits. [b] NT = not tested.

nitrogen atoms were moved to the B-ring. However, this interesting finding suggests that the electronic makeup of the A-ring is very important for binding to the NMDA receptor to take place. The AP-7 type compounds **14** and **15** displayed reduced activity in the [^{3}H]CPP binding assay.

An important remaining task was to synthesize a monosubstituted analogue. In view of the biological results obtained for compound **3**, a chloro-substituted derivative was chosen. While the 7-chloro analogue **1** turned out to be the most potent compound in vivo (ED_{50} = 1.1 mg/kg ip) in the quinoxaline series, its affinity for the NMDA receptor was 7 times less (IC_{50} = 24 nM) than that seen with the 6,7-dichloro analogue (compound **3**). Surprisingly, compound **2**, the 6-chloro analogue, was inactive in vivo, yet possessed a respectable affinity for the NMDA receptor (IC_{50} = 38 nM). While all compounds have negligible interaction at the AMPA and kainate receptor sites up to 10 μM, some compounds displayed some rather intriguing affinity at the glycine site. In particular compound **3** possessed a noteworthy IC_{50} of 609 nM (half as potent as 7-chlorokynureic acid) in the glycine receptor assay representing an 8-fold increase in glycine affinity over the unsubstituted analogue **7** (IC_{50} = 4.7 μM). Compounds **1** and **2** displayed an inverse binding profile for the glycine site. While compound **1** turned out to be a potent ligand for the NMDA receptor, it was practically void of any affinity for the glycine site. Yet, compound **2**, which displayed little affinity for the NMDA binding site, scored rather significantly in the inhibition of glycine

binding (IC_{50} of 1.8 μM). It has been shown that competitive NMDA antagonists can indirectly inhibit the binding of glycine to its coagonist site.[26-28] However, the paradoxical binding profile of compounds **1** and **2** suggests that at least part of the apparent glycine affinity displayed by the chloro analogues **2** and **3** results from a direct action on the glycine binding site. Compounds **1-3** are racemic and their resolution will be described in a forthcoming paper.

The discrepancies between in vitro and in vivo activity for the abovementioned compounds (**1-3**, **7-9**, and **12**) may be explained by considering differing abilities to cross the blood-brain barrier. All the compounds described are very polar and may enter the brain via some active transport mechanism, which does not necessarily coincide in its recognition properties with those of the NMDA receptor complex. Furthermore, dissimilar bioavailabilities due to differences in metabolism and/or distribution may play a significant role.

Conclusion

We have demonstrated that α-amino-3-(phosphonomethyl)-2-quinoxalinepropanoic acids are potent and selective AP6-type ligands for the NMDA receptor and are potent systemic inhibitors of NMDA-induced lethality. Compound **3** (α-amino-6,7-dichloro-3-(phosphonomethyl)-2-quinoxalinepropanoic acid) currently represents the most potent known ligand for the NMDA receptor and is, to date, the only NMDA antagonist reported which appears to interact with both the glycine and NMDA sites.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^1H 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. The enantiomers of compound **7** were separated by HPLC using a copper(II) complexation method. A Novopak phenyl column (150 × 3.9 mm)[29] was used with an eluent consisting of aqueous 0.005 M $CuSO_4$/0.005 M $NH_4H_2PO_4$/0.01 M L-proline at pH 4.6. Detection was at UV 320 nm. Run times were 9 and 11 min for the L and D forms, respectively. To establish the elution order of the racemate, the separation was also performed on a Crownpak CR-(−) column[30] using an eluent of 97/3 (v/v) aqueous $HClO_4$, pH 2.0/2-propanol. Compound **7** was compared to the enantiomers **9** and **10** and it was found indeed, that compound **9** is the (−) form. The unique chemistry of the Crownpak CR(−) stationary phase has been shown to elute the (+) form of α-amino acids usually first, followed by the (−) form.

Compounds **16-19**, **22a,d,h** were purchased from Aldrich Chemical Co., Inc. Compounds **20**, **21**, and **22e-g** were prepared according to literature procedures.[31-35]

4,5-Dimethoxy-1,2-dinitrobenzene (20): light-yellow needles, mp 122-4 °C; yield 94%; ^1H NMR ($CDCl_3$, 200 MHz) δ 4.00 (s, 6 H, 2 × OCH_3), 7.32 (s, 2 H, ArH).

4,5-Dimethoxy-1,2-phenylenediamine (22g): dark red-brown solid, mp 151-4 °C; yield 95%; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.2 (br s, 4 H, 2 × NH_2), 3.77 (s, 6 H, 2 × OCH_3), 6.36 (s, 2 H, ArH).

3,6-Dichloro-1,2-dinitrobenzene (21): creamy solid, mp 98-100 °C; yield 11%; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.66 (s, 2 H, ArH).

3,6-Dichloro-1,2-phenylenediamine (22e): beige solid, mp 88-89 °C; yield >90%; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.82 (br s, 4 H, 2 × NH_2), 6.75 (s, 2 H, ArH).

4,5-Difluoro-1,2-phenylenediamine (22f): pink solid, mp 131-2 °C; yield 100%; ^1H NMR (DMSO-d_6, 400 MHz) δ 4.55 (s, 4 H, 2 × NH_2), 6.42 (t, J = 10.5 Hz, 2 H, ArH).

2,3-Bis(bromomethyl)quinoxaline (23a) was prepared by the reported literature[36] procedure: mp 152-6 °C: ^1H NMR ($CDCl_3$, 200 MHz) δ 4.9 (s, 4 H, 2 × CH_2Br), 7.78 (m, 2 H, H-6, H-7), 8.05 (m, 2 H, H-5, H-8).

2,3-Bis(bromomethyl)-6,7-dichloroquinoxaline (23d): a mixture of **22d** (28 mmol, 6.9 g) and 1,4-dibromo-2,3-butanedione (28 mmol, 6.9 g) was refluxed in benzene (100 mL) with a Dean-Stark trap for 1 h. The reaction mixture was then evaporated to dryness and the residue flash chromatographed on silica gel. Elution with hexane/$CHCl_3$ afforded 9.9 g (92%) of **23d** as a yellow solid: mp 154-6 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 4.86 (s, 4 H, 2 CH_2Br), 8.18 (s, 2 H, ArH).

Compounds **23b,e-h** were prepared according to the procedure described for compound **23d**.

2,3-Bis(bromomethyl)-6-chloroquinoxaline (23b): mp 149-50 °C; yield 89%; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.89 (s, 2 H, CH_2), 4.9 (s, 2 H, CH_2), 7.74 (dd, J_1 = 2.3 Hz, J_2 = 8.9 Hz, 1 H, H-7), 8.01 (d, J = 8.8 Hz, 1 H, H-8), 8.07 (d, J = 2.2 Hz, 1 H, H-5).

2,3-Bis(bromomethyl)-5,8-dichloroquinoxaline (23e): mp 155-8 °C; yield 100%; ^1H NMR ($CDCl_3$, 200 MHz) δ 5.02 (s, 4 H, 2 × CH_2Br), 7.82 (s, 2 H, ArH).

2,3-Bis(bromomethyl)-6,7-difluoroquinoxaline (23f): mp 144-5 °C; yield 100%; ^1H NMR (DMSO-d_6, 400 MHz) δ 5.01 (s, 4 H, 2 × CH_2Br), 8.20 (t, J = 9.7 Hz, 2 H, Ar-H).

2,3-Bis(bromomethyl)-6,7-dimethoxyquinoxaline (23g): mp 182-3 °C; yield 93%; ^1H NMR ($CDCl_3$, 200 MHz) δ 4.06 (s, 6 H, 2 × OCH_3), 4.88 (s, 4 H, 2 × CH_2Br), 7.33 (s, 2 H, ArH).

2,3-Bis(bromomethyl)-6,7-dimethylquinoxaline (23h): mp 152-4 °C; yield 92%; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.46 (s, 6 H, 2 × CH_3), 4.99 (s, 4 H, 2 × CH_2Br), 7.84 (s, 2 H, H-5, H-8).

[[3-(Bromomethyl)-2-quinoxalinyl]methyl]phosphonic acid dimethyl ester (24a): a mixture of **23a** (15.8 mmol, 5 g) and trimethyl phosphite (15.8 mmol, 1.96 g) was refluxed in toluene (200 mL) for 6 h. The reaction mixture was then evaporated in vacuo to dryness and the residue flash chromatographed on silica gel. Elution with EtOAc gave 2.36 g (43%) of **24a** as an off-white solid: mp 99-102 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.73 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3), 3.85 (d, J = 21.7 Hz, 2 H, CH_2P), 4.98 (s, 2 H, CH_2Br), 7.74 (m, 2 H, ArH), 8.02 (m, 2 H, ArH).

Compounds **24b-h** were prepared according to the procedure described for compound **24a**.

[[3-(Bromomethyl)-6-chloro-2-quinoxalinyl]methyl]phosphonic acid dimethyl ester (24b): mp 159-60 °C; yield 27%; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.77 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 3.84 (d, J = 22.4 Hz, 2 H, CH_2P), 4.95 (s, 2 H, CH_2Br), 7.69 (dd, J_o = 8.9 Hz, J_m = 2.3 Hz, 1 H, H-7), 7.98 (dd, J_o = 8.9 Hz, J_p = 0.5 Hz, 1 H, H-8), 8.42 (dd, J_m = 2.3 Hz, J_p = 0.5 Hz, 1 H, H-5).

[[3-(Bromomethyl)-7-chloro-2-quinoxalinyl]methyl]phosphonic acid dimethyl ester (24c): mp 129-30 °C; yield 31.5%; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.78 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 3.85 (d, J = 21.6 Hz, 2 H, CH_2P), 4.96 (s, 2 H, CH_2Br), 7.70 (m, J_o = 8.7 Hz, J_m = 2.1 Hz, J_{H-P} = 0.9 Hz, 1 H, H-6), 7.99 (dd, J_o = 8.7 Hz, J_p = 0.3 Hz, 1 H, H-5), 8.05 (dt, J_m = 2.1 Hz, J_p = 0.3 Hz, J_{H-P} = 0.4 Hz, 1 H, H-8).

[[3-(Bromomethyl)-6,7-dichloro-2-quinoxalinyl]methyl]phosphonic acid dimethyl ester (24d): mp 128-30 °C; yield 41%; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.74 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 3.83 (d, J = 15 Hz, 2 H, CH_2P), 4.93 (s, 2 H, CH_2Br), 8.17 (s, 2 H, ArH).

[[3-(Bromomethyl)-5,8-dichloro-2-quinoxalinyl]methyl]phosphonic acid dimethyl ester (24e): mp 140-4 °C; yield

43%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.8 (s, 3 H, OCH_3), 3.9 (s, 3 H, OCH_3), 3.97 (d, J = 22.7 Hz, 2 H, CH_2P), 5.02 (s, 2 H, CH_2Br), 7.7 (s, 2 H, H-6, H-7).

[[3-(Bromomethyl)-6,7-difluoro-2-quinoxalinyl]methyl]-phosphonic acid dimethyl ester (24f): colorless oil; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.69 (s, 3 H, OCH_3), 3.69 (s, 3 H, OCH_3), 3.87 (d, J = 22.2 Hz, 2 H, CH_2P), 5.04 (s, 2 H, CH_2Br), 8.17 (t, J = 10.1 Hz, 2 H, ArH).

[[3-(Bromomethyl)-6,7-dimethoxy-2-quinoxalinyl]methyl]phosphonic acid dimethyl ester (24g): mp 119–22 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.82 (m, 8 H, 2 × OCH_3 + CH_2P), 4.07 (s, 6 H, 2 × OCH_3), 4.96 (s, 2 H, CH_2Br), 7.34 (s, 2 H, ArH).

[[3-(Bromomethyl)-6,7-dimethyl-2-quinoxalinyl]methyl]-phosphonic acid dimethyl ester (24h): mp 111–2 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.45 (s, 3 H, CH_3), 2.46 (s, 3 H, CH_3), 3.67 s, 3 H, OCH_3), 3.70 (s, 3 H, OCH_3), 3.82 (d, J = 21.9 Hz, 2 H, CH_2P), 5.01 (s, 2 H, CH_2Br), 7.82 (s, 2 H, ArH).

(Acetylamino)[[7-chloro-3-[(dimethoxyphosphinyl)methyl]-2-quinoxalinyl]methyl]propanedioic acid diethyl ester (25b): a solution of diethyl acetamidomalonate (5.2 mmol, 1.145 g) in ethanol (70 mL) was treated dropwise with a solution of sodium (5.2 mmol, 122 mg) in ethanol (15 mL) under dry nitrogen at ambient temperature. The reaction mixture was stirred until all the sodium was dissolved after which a solution of **24b** (52 mmol, 2 g) was added at once. The resulting solution was stirred overnight and evaporated in vacuo, and the residue was partitioned between 5% $NaHCO_3$ (50 mL) and EtOAc (100 mL). The organic layer was separated, washed with brine (70 mL), dried, and evaporated to dryness in vacuo. The residue was flash chromatographed on silica gel. Elution with 2% MeOH in $CHCl_3$ afforded 1.9 g (70%) of **25b** as a colorless gum: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.15 (t, J = 3.5 Hz, 6 H, 2 × OCH_2CH_3), 1.81 (s, 3 H, $COCH_3$), 3.64 (s, 3 H, OCH_3), 3.67 (s, 3 H, OCH_3), 3.70 (d, J = 10.4 Hz, 2 H, CH_2P), 4.02 (br s, 2 H, CH_2C), 4.20 (m, 4 H, 2 × OCH_2CH_3), 7.84 (dd, J_o = 8.9 Hz, J_m = 2.2 Hz, 1 H, H-6), 7.96 (d, J_m = 2.2 Hz, 1 H, H-8), 8.06 (d, J_o = 8.9 Hz, 1 H, H-5).

Compounds **25c–g** were prepared according to the procedure described for compound **25b**.

(Acetylamino)[[6-chloro-3-[(dimethoxyphosphinyl)methyl]-2-quinoxalinyl]methyl]propanedioic acid diethyl ester (25c): white foam; yield 81%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.15 (t, J = 3.9 Hz, 6 H, 2 × OCH_2CH_3), 1.81 (s, 3 H, $COCH_3$), 3.64 (s, 3 H, OCH_3), 3.67 (s, 3 H, OCH_3), 3.71 (d, J = 10.8 Hz, 2 H, CH_2P), 3.99 (s, 2 H, CH_2C), 4.1 (m, 4 H, 2 × OCH_2CH_3), 7.84 (dd, J_o = 8.9 Hz, J_m = 2.3 Hz, 1 H, H-7), 7.92 (d, J_o = 8.9 Hz, 1 H, H-8), 8.10 (d, J_m = 2.3 Hz, 1 H, H-5).

(Acetylamino)[[6,7-dichloro-3-[(dimethoxyphosphinyl)-methyl]-2-quinoxalinyl]methyl]propanedioic acid diethyl ester (25d): beige solid, mp 130–2 °C; yield 40%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.23 (t, J = 8.6 Hz, 6 H, 2 × OCH_2CH_3), 1.88 (s, 3 H, COCH), 3.64 (d, J = 23.3 Hz, 2 H, CH_2P), 3.78 (s, 3 H, OCH_3), 3.83 (s, 3 H, OCH_3), 4.2–4.35 (m, 6 H, 2 × OCH_2CH_3, CH_2C), 6.97 (s, 1 H, NH), 7.98 (s, 1 H, H-8), 8.13 (s, 1 H, H-5).

(Acetylamino)[[5,8-dichloro-3-[(dimethoxyphosphinyl)-methyl]-2-quinoxalinyl]methyl]propanedioic acid diethyl ester (25e): waxy yellow solid, mp 74–8 °C; yield 66%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.2 (t, 6 H, J = 7.6 Hz, 2 × OCH_2CH_3), 1.89 (s, 3 H, $COCH_3$), 3.68 (d, J = 22.8 Hz, 2 H, CH_2P), 3.80 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 4.2–4.35 (m, 6 H, 2 × OCH_2CH_3, CH_2C), 7.24 (s, 2 H, H-6, H-7).

(Acetylamino)[[6,7-difluoro-3-[(dimethoxyphosphinyl)-methyl]-2-quinoxalinyl]methyl]propanedioic acid diethyl ester (25f): green-yellow oil, yield 100%; ^{1}H NMR (DMSO-d_6, 400 MHz), δ 1.15 (t, J = 7.1 Hz, 6 H, 2 × CH_2CH_3), 1.81 (s, 3 H, $COCH_3$), 3.64 (s, 3 H, OCH_3), 3.67 (s, 3 H, OCH_3), 3.66 (d, J = 22.4 Hz, 2 H, CH_2P), 3.98 (s, 2 H, CH_2C), 4.2 (m, 4 H, 2 × CH_2CH_3), 7.91 (t, J = 8.5 Hz, 1 H, ArH), 8.12 (t, J = 8.5 Hz, 1 H, ArH).

(Acetylamino)[[6,7-dimethoxy-3-[(dimethoxyphosphinyl)methyl]-2-quinoxalinyl]methyl]propanedioic acid diethyl ester (25g): off-white crystals, mp 136–9 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.08 (t, J = 5.5 Hz, 6 H, 2 × CH_2CH_3), 1.81 (s, 3 H, $COCH_3$), 3.56 (d, J = 17.5 Hz, 2 H, CH_2P), 3.61 (s, 3 H, $POCH_3$), 3.63 (s, 3 H, $POCH_3$), 3.9 (m, 8 H, CH_2C, 2 × OCH_3), 4.2 (q, J = 5.5 Hz, 4 H, 2 × CH_2CH_3), 7.12 (s, 1 H, Ar-H), 7.31 (s, 1 H, ArH).

α-Amino-7-chloro-3-(phosphonomethyl)-2-quinoxalinepropanoic acid (1): **25b** (106 mmol, 5.5 g) was refluxed in 6 N HCl (100 mL) for 3 h and evaporated to dryness in vacuo, and the residue taken up in ethanol (50 mL). The resulting solution was treated at once with propylene oxide (5 mL), affording almost immediately a suspension which was stirred for 2 h, filtered, and washed with ethanol (20 mL) and ether (20 mL). The material was dried at 80 °C at 1 Torr over P_2O_5 to give 3.4 g (93%) of **1** as a greenish-yellow powder: mp 181–92 °C; ^{1}H NMR (DMSO-d_6 + 1 drop DCl, 400 MHz) δ 3.56 (m, 2 H, CH_2P), 3.81 (d, J = 5.6 Hz, 2 H, $CHCH_2$), 4.54 (t, J = 5.9 Hz, 1 H, $CHCH_2$), 7.82 (dd, J_o = 8.9 Hz, J_m = 2.4 Hz, 1 H, H-6), 8.03 (d, J_o = 8.9 Hz, 1 H, H-5), 8.07 (d, J_m = 2.3 Hz, 1 H, H-8). Anal. ($C_{12}H_{13}ClN_3O_5P$·$0.25H_2O$) C, H, N.

Compounds **2–6** were prepared according to the procedure described for compound **1**.

α-Amino-6-chloro-3-(phosphonomethyl)-2-quinoxalinepropanoic acid (2): yellow powder, mp 176–7 °C dec; yield 82%; ^{1}H NMR (DMSO-d_6 + 1 drop DCl, 400 MHz) δ 3.54 (dd, J_1 = 16.6 Hz, J_2 = 22.1 Hz, 2 H, partially exchanged with DCl, CH_2P), 3.80 (d, J = 5.7 Hz, 2 H, $CHCH_2$), 4.55 (t, J = 5.7 Hz, 1 H, $CHCH_2$), 7.82 (dd, J_o = 9 Hz, J_m = 2.2 Hz, 1 H, H-7), 8.01 (d, J_o = 9 Hz, 1 H, H-8), 8.07 (d, J_m = 2.2 Hz, 1 H, H-5). Anal. ($C_{12}H_{13}ClN_3O_5P$·$0.25H_2O$) H, N, C: calcd, 39.63; found, 40.74.

α-Amino-6,7-dichloro-3-(phosphonomethyl)-2-quinoxalinepropanoic acid hydrochloride (3): pinkish-beige microcrystals, mp 184 °C dec; yield 60%; ^{1}H NMR (DMSO-d_6 + 1 drop DCl, 400 MHz) δ 3.82 (d, J = 5.6 Hz, 2 H, $CHCH_2$), 4.54 (t, J = 5.6 Hz, 1 H, $CHCH_2$), 8.29 (s, 1 H, H-8), 8.32 (s, 1 H, H-5); MS (+FAB) m/e 380 (M + H). Anal. ($C_{12}H_{12}Cl_2N_3O_5P$) C, H, N.

α-Amino-5,8-dichloro-3-(phosphonomethyl)-2-quinoxalinepropanoic acid hydrochloride (4): light-beige microcrystals, mp 193 °C dec; yield 62%; ^{1}H NMR (DMSO-d_6 + 1 drop DCl, 400 MHz) δ 3.68 (dd, J_1 = 4 Hz, J_2 = 22 Hz, 2 H, CH_2P), 3.88 (d, J = 5.8 Hz, 2 H, $CHCH_2$), 4.62 (t, J = 5.9 Hz, 1 H, $CHCH_2$), 7.97 (d, J = 1.4 Hz, 2 H, H-6, H-7); MS (−FAB) m/e 378 (M − 2). Anal. ($C_{12}H_{12}Cl_2N_3O_5P$·$0.5H_2O$) C, H, N.

α-Amino-6,7-difluoro-3-(phosphonomethyl)-2-quinoxalinepropanoic acid hydrochloride (5): light-ochre microcrystals, mp 208 °C dec; yield 70%; ^{1}H NMR (DMSO-d_6 + 1 drop DCl) δ 3.55 (m, 2 H, CH_2P), 3.77 (s, 2 H, CH_2CH), 4.52 (s, 1 H, $CHCH_2$), 8.05 (m, 2 H, ArH); MS (−FAB) m/e 346 (M − H). Anal. ($C_{12}H_{12}F_2N_3O_5P$·H_2O) C, H, N.

α-Amino-6,7-dimethoxy-3-(phosphonomethyl)-2-quinoxalinepropanoic acid hydrochloride (6): light-yellow powder, mp 220 °C dec, yield 87%; ^{1}H NMR (DMSO-d_6 + 1 drop DCl, 400 MHz) δ 3.55 (m, 2 H, CH_2P), 3.69 (d, J = 5.7 Hz, 2 H, CH_2-CH), 3.92 (s, 6 H, 2 × OCH_3), 4.51 (t, J = 5.9 Hz, 1 H, $CHCH_2$), 7.37 (s, 1 H, ArH), 7.39 (s, 1 H, ArH); MS (−FAB) m/e 370 (M − H). Anal. ($C_{14}H_{18}N_3O_7P$·H_2O) C, H, N.

α-Amino-3-[(dimethoxyphosphinyl)methyl]-2-quinoxalinepropanoic acid methyl ester (26i): a solution of N-benzylideneglycine methyl ester (29 mmol, 5.13 g) in THF (40 mL) was added dropwise at −78 °C under dry nitrogen to a solution of t-BuOK (29 mmol, 3.25 g) in dry THF (100 mL) over 3 min. The resulting dark-yellow solution was then treated at once with a solution of **24a** (0.029 mol, 10 g) in dry THF (150 mL) at −78 °C. The reaction mixture was stirred for 2 h at −78 °C and then allowed to reach −40 °C at which point it was poured into ice-cold 10% $NaHCO_3$ (200 mL). The product was extracted with EtOAc (3 × 100 mL), and the combined organic layer washed with brine, dried, and evaporated. The residue was flash chromatographed on silica gel. Elution with a gradient of 2–6% MeOH in $CHCl_3$ gave 10 g (98%) of **26i** as an amber oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.98 (br s, 2 H, NH_2), 3.55 (m, 2 H, CH_2CH), 3.68 (d, J = 21.8 Hz, 2 H, CH_2P), 3.73 (s, 3 H, OCH_3), 3.76 (s, 3 H, OCH_3), 3.80 (s, 3 H, CH_3), 4.41 (m, 1 H, $CHCH_2$), 7.7 (m, 2 H, ArH), 7.98 (m, 2 H, ArH).

α-Amino-3-[(dimethoxyphosphinyl)methyl]-2-quinoxalinepropanoic acid ethyl ester (26j): this compound was prepared like **26i** using N-benzylideneglycine ethyl ester; amber oil; yield 85%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.22 t, J = 7.8 Hz, 3 H, CH_2CH_3), 3.55 (m, 2 H, CH_2CH), 3.67 (d, J = 20.3 Hz, 2 H, CH_2P), 3.75 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 4.15 (m, 3 H, CH_2CH_3, $CHCH_2$), 7.7 (m, 2 H, ArH), 7.98 (m, 2 H, ArH).

α-Amino-6,7-dimethyl-3-[(dimethoxyphosphinyl)methyl]-2-quinoxalinepropanoic acid ethyl ester (26k) was prepared like **23i** but starting from **24g**: amber oil; yield 85%; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.22 (t, J = 7.8 Hz, 3 H, OCH_2CH_3), 3.55 (m, 2 H, OCH_2CH), 3.70 (d, J = 20.3 Hz, 2 H, CH_2P), 3.75 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 4.15 (m, 3 H, OCH_2CH_3 and $CHCH_2$), 7.7 (m, 2 H, ArH), 7.98 (m, 2 H, ArH).

α-Amino-3-(phosphonomethyl)-2-quinoxalinepropanoic acid ethyl ester (11): bromotrimethylsilane (15 mmol, 2 mL) was added to a solution of **26j** (19 mmol, 880 mg) in dry methylene chloride (20 mL). The reaction mixture was refluxed for 4 h then evaporated to dryness. The residue was triturated with water (20 mL) and washed with ether (2 × 10 mL), and the aqueous layer was evaporated to dryness. The resulting gum was stripped with benzene (2 × 10 mL) and dissolved in ethanol (10 mL). Propylene oxide (4 mL) was added, the mixture stirred for 1 h and then evaporated, and the residue triturated in ethanol, acetonitrile, and ether after which the compound precipitated. It was filtered, washed with acetonitrile/ether, and air-dried. The crude compound was recrystallized from ethanol/ether to afford 450 mg (70%) of **11** as an off-white powder: mp 169–72 °C dec; ^{1}H NMR (D_2O, 400 MHz) δ 0.96 (t, J = 7.1 Hz, 3 H, CH_2CH_3), 3.28–3.41 (m, 2 H, CH_2P), 3.86 (dd, J_1 = 4.2 Hz, J_2 = 13.7 Hz, 2 H, $CHCH_2$), 4.12 (q, J = 7 Hz, 2 H, CH_2CH_3), 7.67 (m, 2 H, H-6, H-7), 7.86 (d, J = 8 Hz, 2 H, H-5, H-8); MS (-FAB) *m/e* 338 (M – H). Anal. ($C_{14}H_{18}N_3O_5P$·0.3EtOH) C, H: calcd, 5.65; found, 5.21; N: calcd, 11.90; found, 11.32.

(+)-α-Amino-3-(phosphonomethyl)-2-quinoxalinepropanoic acid (10): a solution of **26i** (21 mmol, 7.43 g) in acetonitrile (30 mL) was treated at once with a hot solution of dibenzoyl-L-tartaric acid monohydrate (21 mmol, 8 g) and left standing for 3 days. The obtained crystals were filtered, washed with acetonitrile (10 mL), and twice recrystallized from acetonitrile to afford 2.6 g (mp 121–4 °C) of α-amino-3-[(dimethoxyphosphinyl)methyl]-2-quinoxalinepropanoic acid methyl ester dibenzoyl-L-tartrate, of which 1.8 g were treated with ice-cold 10% $NaHCO_3$ (30 mL). The liberated amino ester was extracted with methylene chloride (3 × 30 mL), and the combined organic layers were dried and evaporated to dryness. The resulting residue (660 mg) was refluxed in 6 N HCl (10 mL) for 45 min, hot-charcoaled, and filtered through Celite. The filtrate was evaporated to dryness and the residue stripped with water (2 × 30 mL) and then with toluene (2 × 20 mL). The resulting gummy material was dissolved in ethanol (10 mL) and treated at room temperature with propylene oxide (0.75 mL). The resulting suspension was stirred for 15 min, filtered, and the filtercake washed successively with ethanol, hot methanol, and ether. The light-yellow microcrystals were dried at 1 Torr over P_2O_5 at 80 °C to afford 310 mg (40%) of **10**: mp 182 °C dec; $[\alpha]^{25}_D$ = +2.27°; ^{1}H NMR (DMSO-d_6 + 1 drop DCl, 400 MHz) δ 3.55 (m, 2 H, partially exchanged with DCl, CH_2P), 3.79 (d, J = 5.6 Hz, 2 H, CH_2CH), 4.55 (t, J = 5.8 Hz, 1 H, $CHCH_2$), 7.8 (m, 2 H, ArH), 8.0 (m, 2 H, ArH); MS (-FAB) *m/e* 310 (M – H). Anal. ($C_{12}H_{14}N_3O_5P$·0.75H_2O) C, H, N.

(–)-α-Amino-3-(phosphonomethyl)-2-quinoxalinepropanoic acid (9): the filtrate obtained from the above dibenzoyl-L-tartrate salt of α-amino-3-[(dimethoxyphosphinyl)-methyl]-2-quinoxalinepropanoic acid methyl ester (10) was evaporated to dryness and the residue partitioned between ice-cold 10% $NaHCO_3$ (50 mL) and methylene chloride (3 × 50 mL). The combined organic layers were dried and evaporated to give 2.2 g of a residue which was filtered through a plug of silica gel using 5% MeOH in $CHCl_3$. The free amino ester (6 mmol, 2.14 g) was dissolved in hot acetonitrile (10 mL) and treated with a hot solution of dibenzoyl-D-tartaric acid (6 mmol, 2.15 g) in acetonitrile (10 mL). Ether (10 mL) was added to the clear solution, which after 48 h standing at ambient temperature resulted in the crystal formation of the diastereomer. The salt was filtered and repeatedly (5×) recrystallized from acetonitrile until a constant melting point (130–5 °C) was obtained. It was then converted to the free amino ester by partitioning it between ice-cold 10% $NaHCO_3$ (20 mL) and methylene chloride (3 × 20 mL). The combined organic layers were dried and evaporated in vacuo to dryness. The obtained residue (660 mg) was refluxed in 6 N HCl (10 mL) for 40 min, hot-charcoaled, and filtered through Celite. The clear yellow filtrate was washed with ether (2 × 20 mL) and then evaporated to dryness. The residue was dissolved in ethanol (10 mL) and treated with propylene oxide (0.65 mL) at ambient temperature. The resulting precipitate was filtered and washed successively with ethanol, hot methanol, and ether. The light yellow powder was dried at 1 Torr at 25 °C over P_2O_5 to give 370 mg of **9** (3.2% overall yield starting from the racemic amino ester): mp 176 °C dec; $[\alpha]^{25}_D$ = –3.10°; ^{1}H NMR (DMSO-d_6 + 1 drop DCl, 400 MHz) δ 3.58 (m, 2 H, partially exchanged with DCl, CH_2P), 3.80 (d, J = 6 Hz, 2 H, CH_2CH), 4.56 (t, J = 6.1 Hz, 1 H, $CHCH_2$), 7.79 (m, 2 H, ArH), 7.99 (m, 2 H, ArH); MS (-FAB) 310 (M – H). Anal. ($C_{12}H_{14}N_3O_5P$·0.25EtOH·0.5H_2O) C, H, N.

α-Amino-3-(phosphonomethyl)-2-quinoxalinepropanoic acid (7): **26j** (11.7 mmol, 4.3 g) was refluxed in 6 N HCl (25 mL) for 3 h. The resulting dark green solution was treated with charcoal in the heat and filtered through Celite and the clear yellow filtrate washed with ether (30 mL). After evaporating the solution to dryness in vacuo, the residue was stripped with water (50 mL) and benzene (3 × 50 mL). The obtained solids were dissolved in ethanol (75 mL) and treated at once with propylene oxide (8.4 mL), and the reaction mixture was kept stirring at ambient temperature for 12 h. The resulting suspension was filtered, washed with ether (2 × 10 mL) and dried at 1 Torr at 80 °C to afford 2.4 g (59%) of compound **7** as an off-white powder: mp 178 °C dec; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.56 (m, 2 H, CH_2P), 3.80 (d, J = 6.7 Hz, 2 H, CH_2CH), 4.57 (t, J = 6.7 Hz, 1 H, $CHCH_2$), 7.79 (m, 2 H, ArH), 8 (m, 2 H, ArH); MS (–FAB) *m/e* 310 (M – H). Anal. ($C_{12}H_{14}N_3O_5P$·HCl) C, H; N: calcd, 12.08, found, 11.62.

α-Amino-6,7-dimethyl-3-(phosphonomethyl)-2-quinoxalinepropanoic acid (8): the compound was prepared from **26h** according to the procedure given for **7**: mp 198–200 °C; yield 79%; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.43 (s, 6 H, 2 × CH_3), 3.37 (m, 2 H, CH_2P), 3.75 (m, 2 H, CH_2CH), 4.52 (t, J = 5.7 Hz, 1 H, CH_2CH), 7.76 (s, 1 H, ArH), 7.77 (s, 1 H, ArH). Anal. ($C_{14}H_{18}N_3O_5P$·H_2O) C, H; N: calcd, 47.05; found, 47.54.

2,3-Bis(bromomethyl)benzo[*g*]quinoxaline (27): a mixture of **19** (20 mmol, 3.16 g) and 1,4-dibromo-2,3-butanedione (20 mmol, 4.88 g) was refluxed in benzene (120 mL) with a Dean-Stark trap for 2 h. The reaction mixture was then cooled to room temperature, and the precipitate filtered, washed with hexane (30 mL), and air-dried to yield 6.8 g (93%) of **27** as green crystals: mp 190–1 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.07 (s, 4 H, 2 × CH_2Br), 7.68 (dd, J_1 = 3.2 Hz, J_2 = 6.8 Hz, 2 H, H-7, H-8), 8.26 (dd, J_1 = 3.3 Hz, J_2 = 6.4 Hz, 2 H, H-6, H-9), 8.78 (s, 2 H, H-5, H-10).

[[3-(Bromomethyl)benzo[*g*]quinoxalin-2-yl]methyl]phosphonic acid dimethyl ester (28): **27** (10 mmol, 3.66 g) and trimethyl phosphite (12 mmol, 1.488 g) were refluxed in toluene (300 mL) for 6 h. The mixture was evaporated in vacuo and the residue flash chromatographed on silica gel. Elution with ethyl acetate/hexane (4:1) furnished 1.5 g (38%) of **28** as an orange wax: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.73 (s, 3 H, OCH_3), 3.76 (s, 3 H, OCH_3), 3.94 (d, J = 22 Hz, 2 H, CH_2P), 5.10 (s, 2 H, CH_2Br), 7.66 (dd, J_1 = 2 Hz, J_2 = 3.4 Hz, 2 H, H-7, H-8), 8.24 (dd, J_1 = 1.9 Hz, J_2 = 4.8 Hz, 2 H, H-6, H-9), 8.74 (s, 2 H, H-5, H-10).

α-Amino-3-[(dimethoxyphosphinyl)methyl]benzo[*g*]quinoxaline-2-propanoic acid phenylmethyl ester (29): a solution of *N*-benzylideneglycine benzyl ester (4.5 mmol, 1.159 g) in dry THF (20 mL) was added dropwise at –78 °C under dry nitrogen to a solution of *t*-BuOK (4.6 mmol, 515 mg) in dry THF (10 mL). The resulting bright yellow solution was stirred for 5 min at –78 °C after which a solution of **28** (4.5 mmol, 1.8 g) in dry THF (30 mL) was added in a dropwise fashion. The obtained red-brown reaction mixture was stirred at 25 °C overnight, poured into water (100 mL), and extracted with ether (3 × 50 mL). The combined organic layers were dried and evaporated to dryness. The residue was flash chromatographed on silica gel. Elution with 2% methanol in chloroform afforded 1.3 g (60%) of **29** as an amber oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.48 (br s, 2 H, NH_2), 3.51 (d, J = 6 Hz, 2 H, CH_2CH), 3.67 (s, 3 H, OCH_3), 3.70 (s, 3 H, OCH_3), 3.78–3.96 (m, 2 H, CH_2P), 4.10 (t, J = 6.2 Hz, 1 H, $CHCH_2$), 5.13 (d, J = 5.5 Hz, 2 H, CH_2Ph), 7.21 (m, 3 H, H-3′,

H-4′, H-5′), 7.27 (m, 2 H, H-2′, H-6′), 7.60–7.63 (m, 2 H, H-6, H-9), 8.16–8.22 (m, 2 H, H-7, H-8), 8.48 (s, 1 H, Ar-H), 8.66 (s, 1 H, ArH).

α-Amino-3-(phosphonomethyl)benzo[g]quinoxaline-2-propanoic acid phenylmethyl ester (30): bromotrimethylsilane (13.5 mmol, 2.065 g) was added to a stirred solution of **29** (2.7 mmol, 1.3 g) in methylene chloride (50 mL). The solution was refluxed under exclusion of moisture for 4 h and evaporated to dryness in vacuo and the residue triturated with water (40 mL) for 10 min and then washed with ether (50 mL). The aqueous layer was separated and evaporated to dryness in vacuo and the residue dissolved in ethanol (40 mL). Propylene oxide (2 mL) was added to the solution and the resulting fine suspension kept at 0 °C overnight. A waxy green material was isolated by filtration, washed with ether (20 mL), and dried at 1 Torr at ambient temperature to yield 940 mg (77%) of **30**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.4 (m, 2 H, CH_2P), 3.8 (m, 2 H, CH_2CH), 4.75 (s, 1 H, $CHCH_2$), 5.2 (s, 2 H, CH_2Ph), 7.1 (d, J = 7 Hz, 3 H, H-3′, H-4′, H-5′), 7.2 (d, J = 7 Hz, 2 H, H-2′, H-6′), 7.6 (m, 2 H, H-6, H-9), 8.11–8.21 (2 m, 2 H, H-7, H-8), 8.39 (s, 1 H, ArH), 8.62 (s, 1 H, ArH).

α-Amino-3-(phosphonomethyl)benzo[g]quinoxaline-2-propanoic acid ammonium salt (12): palladium (10%) on charcoal (100 mg) was added to the solution of **30** (2.3 mmol, 910 mg) in acetic acid (30 mL). The mixture was hydrogenated for 14 h at normal pressure, then diluted with water (40 mL), and filtered through Celite. The filtrate was evaporated and the residue dissolved in ammonia (5%, 30 mL). The resulting solution was hot-charcoaled and filtered through Celite and the filtrate concentrated in vacuo. Acetone was added to the filtrate to induce crystallization. The resulting suspension was kept at 0 °C for 2 h and the product filtered, washed with acetone, and air-dried to yield 600 mg (68%) of **12** as a yellow powder: mp 215 °C dec; ^{1}H NMR (DMSO-d_6 + 1 drop DCl, 400 MHz) δ 3.82–3.88 (m, 2 H, $CHCH_2$), 4.6 (m, 1 H, $CHCH_2$), 7.6 (dd, J_1 = 3.3 Hz, J_2 = 6.5 Hz, 2 H, H-7, H-8), 8.2 (dd, J_1 = 6.3 Hz, J_2 = 9.5 Hz, 2 H, H-6, H-9), CH_2 exchanged with DCl, 8.62 (s, 1 H, ArH), 8.66 (s, 1 H, ArH); MS (–FAB) m/e 362 (M + 1). Anal. ($C_{16}H_{19}N_4O_5P \cdot 0.25H_2O$) C, H, N.

6,7-Dimethylquinoxaline (31): a solution of 40% aqueous glyoxal (50 mmol, 7.25 g) and sodium hydrogen sulfite (10.4 g, mixture of sodium bisulfate and sodium metabisulfite) in water (80 mL) was heated to 70 °C and then poured into a suspension of **22h** (0.050 mol, 6.8 g) in water (100 mL). The reaction mixture was then allowed to reach room temperature at which point it was basified to pH 7.5 with solid sodium carbonate. The organic materials were extracted with chloroform (3 × 100 mL) and the combined organic extracts dried and evaporated to dryness. The residue was flash chromatographed on silica gel. Elution with 2% methanol in chloroform gave 6 g (76%) of **31** as a brown solid: mp 99–100 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 2.44 (s, 6 H, 2 × CH_3), 7.83 (s, 2 H, ArH), 8.80 (s, 2 H, H-2, H-3).

6,7-Bis(bromomethyl)quinoxaline (32): a solution of **31** (37.9 mmol, 6 g) in carbon tetrachloride (400 mL) was treated at once with benzoyl peroxide (200 mg), followed by NBS (76 mmol, 13.53 g). The reaction mixture was refluxed for 4 h and cooled to 0 °C and the precipitated succinimide removed by filtration. The filtrate was evaporated and the residue chromatographed on silica gel. Elution with 2% methanol in chloroform furnished 6 g (50%) of **32** as an amber oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.10 (s, 4 H, 2 × CH_2Br), 8.28 (s, 2 H, H-5, H-8), 9.00 (s, 2 H, H-2, H-3).

[[7-(Bromomethyl)quinoxalin-6-yl]methyl]phosphonic acid dimethyl ester (33): a mixture of **32** (18.9 mmol, 6 g) and trimethyl phosphite (20 mmol, 2.36 mL) was refluxed in toluene (250 mL) for 6 h. The solvent was evaporated and the residue flash chromatographed on silica gel. Elution with 2% methanol in $CHCl_3$ gave 1.9 g (30.6%) of **33** as an amber oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.62 (s, 3 H, OCH_3), 3.65 (s, 3 H, OCH_3), 3.70 (d, J = 22.1 Hz, 2 H, CH_2P), 5.09 (s, 2 H, CH_2Br), 8.08 (d, J = 3.6 Hz, 1 H, H-5), 8.25 (s, 1 H, H-8), 8.93 (s, 2 H, H-2, H-3).

α-Amino-7-[(dimethoxyphosphinyl)methyl]-6-quinoxalinepropanoic acid methyl ester (34): under stirring and dry nitrogen a solution of N-benzylideneglycine methyl ester (5.5 mmol, 980 mg) in dry THF (15 mL) was added dropwise at –78 °C to a solution of t-BuOK (5.5 mmol, 620 mg) in dry THF (15 mL). The reaction mixture was stirred for 2 min at –78 °C after which a solution of **33** (5 mmol, 1.9 g) in dry THF (60 mL) was added. Stirring was continued for 1 h at –78 °C and then at ambient temperature overnight. The mixture then was poured into ice-cold saturated brine (100 mL) and extracted with ethyl acetate (2 × 80 mL). The combined organic extracts were dried, filtered, and evaporated to dryness in vacuo. The residue was flash chromatographed on silica gel. Elution with 5% methanol in chloroform gave 450 mg (24%) of **34** as a greenish-yellow oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.18 (m, 2 H, CH_2CH), 3.58 (s, 3 H, CH_3), 3.59 (s, 3 H, OCH_3), 3.62 (s, 3 H, OCH_3), 3.65 (m, 3 H, CH_2P, CH_2CH), 7.90 (s, 1 H, H-5), 7.99 (d, J = 3.3 Hz, 1 H, H-8), 8.87 (s, 2 H, H-2, H-3).

α-Amino-7-(phosphonomethyl)-6-quinoxalinepropanoic acid (13): **34** (12.7 mmol, 450 mg) was refluxed in 6 N HCl (10 mL) for 1 h, hot-charcoaled, filtered through Celite, and evaporated to dryness. The residue was dissolved in ethanol (15 mL) and treated with propylene oxide (1.5 mL). The precipitate was filtered, titurated in hot methanol, and dried in vacuo at 80 °C to yield 280 mg (68%) of **13**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.3–3.6 (m, 5 H, CH_2P, CH_2CH), 7.99 (d, J = 3.5 Hz, 1 H, H-8), 8.03 (s, 1 H, H-5), 8.89 (d, J = 2 Hz, 2 H, H-2, H-3); MS (–FAB) m/e 310 (M – H). Anal. ($C_{12}H_{14}N_3O_5P \cdot 0.3EtOH$) C, H, N.

3-(Bromomethyl)quinoxaline-2-carboxaldehyde (35): a solution of sodium (760 mg) in ethanol (90 mL) was treated with 2-nitropropane (35 mmol, 3.115 g) under dry nitrogen at ambient temperature. At 0 °C a solution of **23a** (31 mmol, 9.8 g) in THF (200 mL) and ethanol (200 mL) was added by cannula. The reaction mixture was stirred at 25 °C overnight and evaporated to dryness and the residue partitioned between water (100 mL) and chloroform (3 × 100 mL). The combined organic layers were dried and evaporated to dryness. The residue was chromatographed on silica gel. Elution with chloroform afforded 1.8 g (34%) of **35** as a white powder: mp 164–5 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 5.18 (s, 2 H, CH_2Br), 8.0–8.35 (m, 4 H, ArH), 10.2 (s, 1 H, CHO).

[[3-(Bromomethyl)-2-quinoxalinyl]ethenyl]phosphonic acid dimethyl ester (36): a mixture of tetramethyl methylenediphosphonate (7.6 mmol, 1.76 g) and sodium hydride (0.0075 mol, 300 mg, 60%) was stirred in toluene (50 mL) for 30 min at 25 °C and then added dropwise to a solution of **35** (7.17 mol, 1.8 g) in toluene (200 mL) at 0 °C. The mixture was allowed to reach ambient temperature over 1 h and then washed with water (100 mL). The organic layer was separated, dried, and evaporated to dryness to yield 2.5 g (97%) of **36** as a white waxy solid: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.75 (s, 3 H, OMe), 3.80 (s, 3 H, OMe), 4.78 (s, 2 H, CH_2Br), 7.1–7.35 (m, 2 H, PCH=CH), 7.7 (m, 2 H, H-6, H-7), 8.0 (m, 2 H, H-5, H-8).

(Acetylamino)[[3-[(dimethoxyphosphinyl)ethenyl]-2-quinoxalinyl]methyl]propanedioic acid diethyl ester (37): a solution of sodium (97 mg) in ethanol (10 mL) was added dropwise to a solution of diethyl acetamidomalonate (4.2 mol, 911 mg) in ethanol (20 mL) under dry nitrogen. After stirring at 25 °C for 30 min, a solution of **36** (3.92 mol, 1.4 g) in ethanol (30 mL) was added at once. Stirring was continued for 3 h. The solvent was removed in vacuo and the residue partitioned between sodium bicarbonate (10% aqueous) and ethyl acetate. The organic layer was dried and evaporated to afford 1.85 g (96%) of **37** as an amber oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.19 (t, J = 3.1 Hz, 6 H, 2 × OCH_2CH_3), 1.79 (s, 3 H, $COCH_3$), 3.71 (s, 3 H, OMe), 3.73 (s, 3 H, OMe), 3.99 (s, 2 H, CCH_2), 4.20 (m, 4 H, 2 × OCH_2CH_3), 7.10 (dd, J_1 = 16.6 Hz, J_2 = 20.2 Hz, 1 H, CCH=CH), 7.63 (dd, J_1 = 16.7 Hz, J_2 = 21.1 Hz, 1 H, CCH=CHP), 7.86 (m, 2 H, ArH), 7.92 (m, 1 H, ArH), 8.08 (m, 1 H, ArH), 8.15 (s, 1 H, NH).

(Acetylamino)[[3-[(dimethoxyphosphinyl)ethyl]-2-quinoxalinyl]methyl]propanedioic acid diethyl ester (38): a solution of **37** (1.85 mol, 900 mg) in methanol (20 mL) was treated with 10% Pd/C (100 mg). The mixture was hydrogenated at ambient temperature and atmospheric pressure for 4 h. The catalyst was removed by filtration and the filtrate was evaporated. The residue was chromatographed on silica gel. Elution with 2% methanol in chloroform gave 620 mg (69%) of **38** as an faintly yellow oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.18 (t, J = 4.3 Hz, 6 H, 2 × OCH_2CH_3), 1.82 (s, 3 H, $COCH_3$), 2.32 (m, 2 H, CH_2P), 3.11 (m, 2 H, CH_2CH_2P), 3.64 (s, 3 H, OMe), 3.66 (s, 3 H, OMe),

3.92 (s, 2 H, CCH_2), 4.2 (m, 4 H, 2 × OCH_2CH_3), 7.78 (m, 2 H, H-6, H-7), 7.89 (m, 1 H, ArH), 8.00 (m, 1 H, ArH), 8.22 (s, 1 H, NH); MS (+FAB) *m/e* 496 (M + H).

α-Amino-3-(2-phosphonoethenyl)-2-quinoxalinepropanoic acid (14): 37 (1.7 mmol, 850 mg) was refluxed in 6 N HCl (40 mL) for 2.5 h, hot-charcoaled, and filtered through Celite. The filtrate was evaporated to dryness and the residue dissolved in ethanol (30 mL). Propylene oxide (2 mL) was added and the precipitated compound removed by filtration and crystallized from water/ethanol to yield 200 mg (35%) of 14 as off-white microcrystals: mp 207-10 °C; ^{1}H NMR (DMSO-d_6 plus 1 drop DCl, 400 MHz) δ 3.8 (m, 2 H, CH_2CH), 4.6 (m, 1 H, CH_2CH), 7.14 (dd, J_1 = 16.7 Hz, J_2 = 18.3 Hz, 1 H, CH=CH), 7.59 (dd, J_1 = 16.7 Hz, J_2 = 20.5 Hz, 1 H, CH=CH), 7.84 (m, 2 H, H-6, H-7), 8.02 (m, 2 H, H-5, H-8); MS (-FAB) *m/e* 322 (M - H). Anal. ($C_{13}H_{14}N_3O_5P{\cdot}H_2O$) C, H, N.

α-Amino-3-(2-phosphonoethyl)-2-quinoxalinepropanoic acid (15) was prepared like 14: yield 71%; white microcrystals, mp 185-7 °C dec; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.02 (m, 2 H, CH_2P), 3.5 (m, 2 H, CH_2CH_2P), 3.62 (m, 2 H, $CHCH_2$), 4.31 (m, 1 H, $CHCH_2$), 7.72 (br s, 2 H, H-6, H-7), 7.96 (m, 2 H, H-5, H-8); MS (-FAB) *m/e* 324 (M - H). Anal. ($C_{13}H_{16}N_3O_5P{\cdot}0.75H_2O$) C, H, N.

Computer Modeling of Compound 1. A superimposition at the three pharmacophoric sites of compound 1 on those of the low-energy (-)-gauche form of CGS 19755[18] is shown in relative 3-D orientation in Figure 2. These structures were obtained by a flexible fit optimization of closely related low-energy conformations using the MAXIMIN MULTIFIT procedure in the molecular modeling software program SYBYL.[37a] The partial atomic charges for the molecules were calculated using the MOPAC semiempirical method.[37b] Five pharmacophoric atom pair points (P=O, C=O, and N) were chosen and the MULTIFIT procedure was run using the Tripos force field with electrostatic terms included.[37a] The resulting root mean square distance (RMS) between the atom pairs was 0.08 Å with a resulting sacrifice in energy for CGS 19755 of 0.2 kcal/mol above the global minimum and a sacrifice of 0.7 kcal/mol above the local minimum for compound 1.

Single Crystal X-Ray Analysis of 24b. Needle-shaped crystals were obtained from ether/hexane. Intensity data were collected at 296 K using graphite monochromated Mo Kα (λ = 0.71073 Å) radiation on a Siemens R3m diffractometer, employing $\omega/2\theta$ scans with variable speeds and a range of 0.80°. Three standard reflections (measured once every 97 reflections) used to monitor data collection did not reveal significant intensity decay during the period of data collection. A total of 2824 reflections were collected having $-25 \leq h \leq 25$, $-2 \leq k \leq 8$, $-14 \leq l \leq 24$. Of these, 2526 were unique and 1482 ($F > 4.0\sigma(F)$) observed. Unit cell constants were determined by a least squares fit of the 2θ values of 20 reflections having $25° \leq 2\theta \leq 30°$. The X-ray intensities were corrected for scan speed, background, and Lorentz and polarization factors. Absorption corrections were not applied.

The structure was solved by direct methods and refined using the SHELTXL Plus package of programs.[38] All hydrogen atoms were located on a difference Fourier map. Final refinement was performed using the full matrix least square method. The non-hydrogen atoms were refined with anisotropic thermal parameters, and all hydrogen atoms were allowed to ride the carbon to which they are attached (C-H = 0.96 Å, X-C-H = 109° or 120°). The analytical scattering factors for the neutral atoms were used,[39] and all non-hydrogen scattering factors were corrected for both the real and imaginary anomalous dispersion.[40] The largest residual peaks were in the vicinity of the bromine atom. Crystal data and selected details of the refinement calculations are listed in Table II. The X-ray structure of 24b is depicted in Figure 3 and the packing diagram is presented in Figure 4.

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.[41] from rat whole brain and used in [^{3}H]CPP, [^{3}H]glycine, 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-Elvehjem tissue grinder

Table II. Crystal Data and Selected Details of the Refinement Calculation for **24b**

formula	$C_{12}H_{13}BrClN_2O_3P$
formula weight	379.6
crystal size, mm	0.20 × 0.24 × 0.62
crystal system	monoclinic
space group	*C2/c*
a, Å	27/571 (7)
b, Å	6.963 (2)
c, Å	20.410 (4)
β, deg	128.29 (2)
volume, Å^3	3066.9 (13)
Z	8
density (calcd), mg/mm^3	1.644
absorption coefficient, mm^{-1}	2.938
range, deg	$3 < 2\theta < 50$
$R = \sum\lvert F_o - F_c\rvert/\sum F_o$	0.068
$R_w = \sum w^{1/2}\lvert F_o - F_c\rvert/\sum w^{1/2}F_o$	0.072

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.[41] was used. Membrane pellets were thawed and resuspended in 15 volumes of ice-cold 50 mM Tris HCl (pH 7.6) buffer. In triplicate, 1000 μL of the membrane homogenate containing between 0.2 and 0.5 mg of protein were incubated at 23 °C for 15 min together with 8 nM [^{3}H]CPP (specific activity 30-40 Ci/mmol[42]), one of the various test solutions, and an appropriate volume of buffer for a final incubation volume of 2.0 mL using plastic minivials.[43] The reaction was initiated by the addition of the homogenate 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. A 4 N hydrochloric acid solution (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]Glycine Binding. The method of Kemp, J. A. et al.[44] was adopted for these assays. Membrane pellets were thawed and washed with 50 mM Tris citrate and resuspended in 15 volumes of Tris citrate buffer. In triplicate, samples (1000 μL) were incubated at 4 °C for 20 min in a final incubation volume of 2 mL containing 20 nM [^{3}H] glycine (specific activity 45-50 μCi/mL; Aquasol, DuPont) and one of several test solutions. Separate triplicates also contained vehicle and 1 mM glycine to define "total" and "nonspecific" [^{3}H]glycine binding. The reaction was initiated by the addition of homogenate to the incubation medium. The samples were then centrifuged at 48000*g* for 20 min, and the resulting pellets were digested using tissue solubilizer (0.5 mL/sample; NCS, Amersham). A 4 N hydrochloric acid solution (0.1 mL) was added to each sample to reduce potential chemiluminescence during subsequent counting. Scintillation cocktail (3.2 mL/sample; Aquasol, DuPont) was then added to each sample, and the samples were counted for radioactivity using conventional liquid spectroscopy. The compounds were tested at 5-10 concentrations for determination of IC_{50} and 95% confidence limits values.

[^{3}H]TCP Binding. A modification of the methods described by Kloog, Y. et al.[20] 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 48000g for 20 min and the pellet resuspended in ice-cold buffer for use in the binding assay. In triplicate, 1000-μL samples of the homogenate were incubated for 1 h at 25 °C in the presence of 2.5 nM [^{3}H]TCP (specific activity 45–50 Ci/mmol[29]), 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 homogenate 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. Antagonism of NMDA-induced lethality was determined using a modification of the NMDA convulsion model described by Hutchison, A. J. et al.[18] Male, Swiss-albino mice (CD-1, 18–22 g[45]) were acclimated for 30 min to an observation chamber and then injected with one of several intraperitoneal doses 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.

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Supplementary Material Available: A listing of atomic coordinates, bond lengths and angles, isotropic and anisotropic thermal parameters, and H-atom coordinates for compound **24b** is available (41 pages). Ordering information is given on any current masthead page.

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