at 578 nm. Data calculation and analysis were performed on a Olivetti M24 PC.[38]

Hormone-independent MDA-MB 231 human mammary tumor cells were also obtained from ATCC. Cells were grown in McCOY 5a medium supplemented with 10% NCS and gentamycin (40 μg/mL). Cytostatic activity was determined in a microplate assay as described for MCF-7 cells with one exception: the incubation period was reduced to 2 d.

Mice Uterine Weight Tests. Immature female mice (20 days old, of the NMRI strain) from Charles River, Wiga, Sulzfeld, FRG, were randomly divided into groups of six to ten animals. To determine estrogenic activity, compounds were dissolved in olive oil (100 μL/animal) and injected subcutaneously on three consecutive days. Control animals received the vehicle alone. Twenty-four hours after the last injection, the animals were killed by cervical dislocation and weighed. Uteri were dissected free of fat and fixed in Bouin solution (saturated aqueous picric acid/40% formaldehyde/glacial acetic acid 15:5:1 by vol) for 2 h. Uteri were freed from connective tissue, washed with ethanol, dried at 100 °C for 18 h, and weighed. The relative uterus weight was calculated by the formula: uterine dry weight (mg)/body weight (g), multiplied by 100. To determine the antiestrogenic activity, injections contained a standard dose (0.4 μg) of estrone and increasing doses of the compounds. The inhibition (%) of the estrone-stimulated uterine growth was estimated by the formula: $100 - [(W_{S,T} - W_V)/(W_S - W_V) \times 100]$ ($W_{S,T}$ = rel uterus weight of animals treated with estrone standard (0.4 μg) + test compound; W_V = rel uterus weight of control animals; W_S = rel uterus weight of animals treated with estrone standard).

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Supplementary Material Available: ^{1}H NMR data of 2-(methoxyphenyl)-*N*-[2-(3-methoxyphenyl)ethyl]acetamides (**3a**, **3e–j**), 1-benzyl-1,2,3,4-tetrahydroisoquinolines (**4a**, **4e–j**), 5,6-dihydro-dimethoxyindolo[2,1-*a*]isoquinolines (**5a**, **5e–j**), 12-formyl-5,6-dihydro-dimethoxyindolo[2,1-*a*]isoquinolines (**6a–j**), and diacetoxy-12-formyl-5,6-dihydroindolo[2,1-*a*]isoquinolines (**7a–j**) (6 pages). Ordering information is given on any current masthead page.

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6-Substituted Decahydroisoquinoline-3-carboxylic Acids as Potent and Selective Conformationally Constrained NMDA Receptor Antagonists

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We have prepared a series of 6-substituted decahydroisoquinoline-3-carboxylic acids, and structurally similar analogs, as potential *N*-methyl-D-aspartate receptor antagonists. There is a large body of evidence to support the use of such compounds as cerebroprotective agents in a variety of acute and chronic neurodegenerative disorders, where some component of glutamate-mediated excitotoxicity may exist. The compounds prepared were evaluated in vitro in both receptor binding assays ([^{3}H]CGS19755, [^{3}H]AMPA, and [^{3}H]kainic acid) and in a cortical wedge preparation (versus NMDA, AMPA, and kainic acid) to determine affinity, potency, and selectivity. The new amino acids were also evaluated in vivo for their ability to block NMDA-induced lethality in mice. We synthesized many of the possible diastereomers of the decahydroisoquinoline nucleus in order to examine the spatial and steric requirements for affinity at the NMDA receptor and activity as NMDA antagonists. From our structure–activity relationship we identified two potent and selective NMDA receptor antagonists, the phosphonate- and tetrazole-substituted amino acids **31a** and **32a**, respectively, that show good activity in animals following systemic administration. For example, **31a** and **32a** selectively displaced [^{3}H]CGS19755 binding with IC_{50}s of 55 ± 14 and 856 ± 136 nM, respectively, and selectively antagonized responses due to NMDA in a cortical wedge preparation with IC_{50}s of 0.15 ± 0.01 and 1.39 ± 0.29 μM, respectively. And compounds **31a** and **32a** blocked NMDA-induced lethality in mice with minimum effective doses of 1.25 and 2.5 mg/kg (intraperitoneal), respectively. These novel amino acids are among some of the most potent NMDA antagonists described thus far, and are excellent candidates for development as neuroprotective agents for a number of CNS disorders.

Glutamic acid is an important excitatory amino acid (EAA) neurotransmitter in the central nervous system and is involved in a myriad of neuronal functions.[1] There are at least four well-characterized EAA receptor subclasses.[1] The ionotropic NMDA (for *N*-methyl-D-aspartic acid), AMPA (for 2-amino-3-(5-methyl-3-hydroxyisoxazol-4-yl)-propanoic acid) and kainic acid (KA) receptors are all linked to ion channels permeable to sodium and, in the case of NMDA and AMPA receptors, calcium. The metabotropic or 1*S*,3*R*-ACPD-sensitive glutamate receptor (for (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid) receptor is coupled via a G-protein to phosphoinositide hydrolysis.

Under certain circumstances, glutamic acid can be lethal to cells[2] (excitotoxicity). For example, in cerebral ischemia,[3,4] when cellular energy stores are diminished, gluta-

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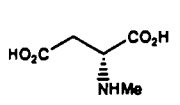
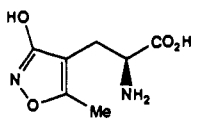
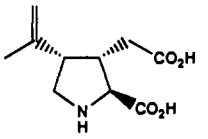


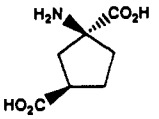
NMDA



AMPA



Kainic Acid



1S,3R-ACPD

mate uptake is compromised and large concentrations of this neurotransmitter are released into the synaptic cleft. This allows for an influx of sodium and calcium ions into cells that leads initially to swelling and subsequently to cell death. When the cell dies, there is a further release of glutamate and the cycle is continued. Blockade of NMDA receptors may prove useful in the treatment of cerebral ischemia and other acute excitotoxic conditions such as spinal cord[5] and head trauma.[6] This pattern of excitotoxicity may also occur in chronic neurodegenerative disorders such as Alzheimer's disease,[7] Parkinson's disease,[8] Huntington's chorea,[9] amyotrophic lateral sclerosis,[10] and AIDS-induced dementia,[11] making NMDA antagonists potentially useful in the treatment of these disorders. NMDA receptor antagonists are also anticonvulsant[12] and may be useful as analgesic agents.[13] Thus, there are a large number of therapeutic targets for NMDA receptor antagonists, and the synthesis of novel antagonist structures is a significant goal for medicinal chemists.

The *N*-methyl-D-aspartic acid subtype of excitatory amino acid receptors is actually a macromolecular complex (Figure 1).[14] There are binding sites for glutamic acid and glycine (glycine is believed to play a cotransmitter role with glutamate), and there is a binding site inside the ion channel which is where compounds such as MK-801 and PCP exert there NMDA antagonist effects. There are also sites that bind divalent cations such as zinc and polyamines such as spermine and spermidine. Possible approaches to NMDA antagonists include compounds that act at the glutamate binding site (competitive antagonists, e.g., **1** (CGS 19755)[15-17]), at the glycine binding site (glycine antagonists, e.g., **2** (L-689,560)[18]), at the ion channel binding site (noncompetitive PCP-like antagonists, e.g., **3** (MK-801)[19]), at the zinc binding site (e.g., **4** (imipramine)[20]), and at the polyamine binding site (polyamine antagonists, e.g., **5** (ifenprodil)[21]). While it is difficult to say at this time that one mode of NMDA antagonism is more advantageous than another, there are clearly behavioral differences between competitive and noncompetitive PCP-like NMDA antagonists that may indicate a more favorable side-effect profile for the former class of compounds[22-24]. We have therefore focused our efforts on the synthesis of competitive NMDA antagonists.

Watkins prepared the prototypic NMDA antagonists, **6** (D-AP5)[25] and **7** (D-AP7)[25] (Chart I). These amino acids define the minimum structural requirements for the ac-

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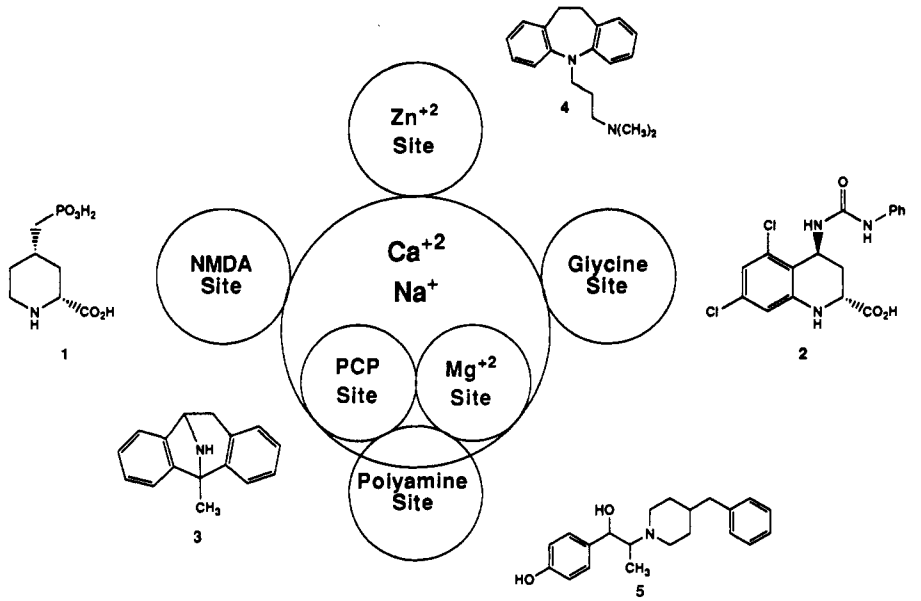
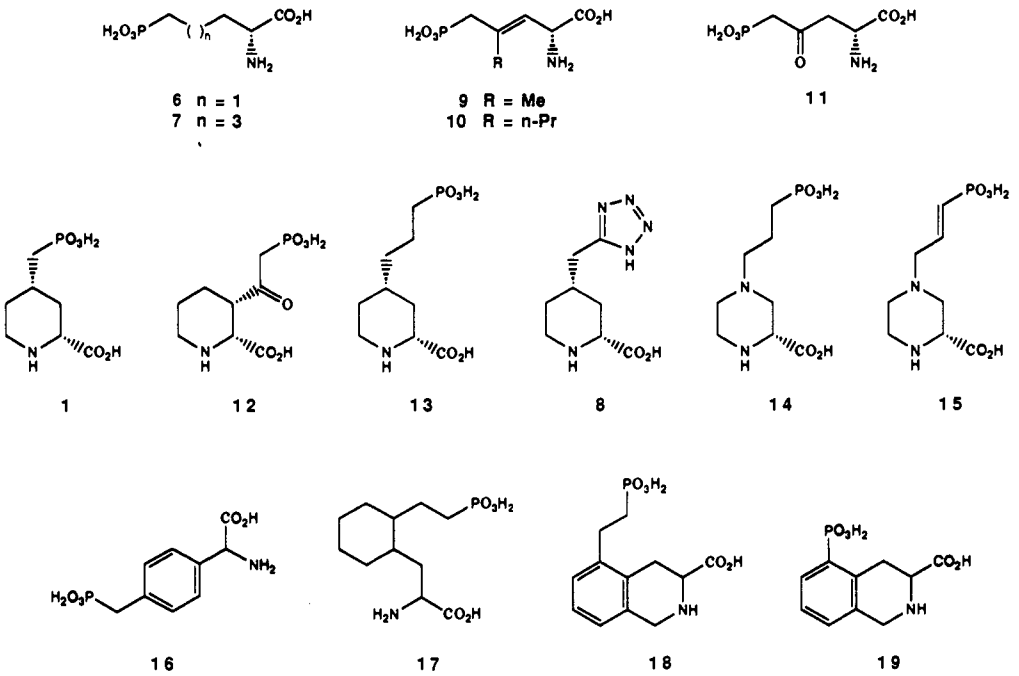


Figure 1. A representation of the NMDA receptor complex, showing the ion channel and associated agonist and antagonist binding sites, along with representative examples of antagonists acting at each of these sites.

Chart I



tivity of competitive antagonists at NMDA receptors, namely an α-amino acid with a distal acid moiety positioned four to six atoms from the proximal acid. The distal acid group can be a carboxylic[26] or phosphonic acid[15-17] or a tetrazole.[27,28] Compounds with a phosphonic acid or tetrazole are usually more potent than those containing a carboxylic acid. One limitation of **6** and **7** was their relatively poor activity in animals following systemic administration.[29] Therefore, the goals of structure–activity studies on NMDA antagonists have been to make changes that would enhance both the in vitro potency and the systemic activity of these compounds and allow for a better understanding of the spatial and steric requirements (tolerances) for activity at NMDA receptors. One approach to solving this problem has been to reduce the conformational mobility of these amino acids. This has been accomplished through incorporation of the requisite acidic amino acid substructure into cyclic systems or the

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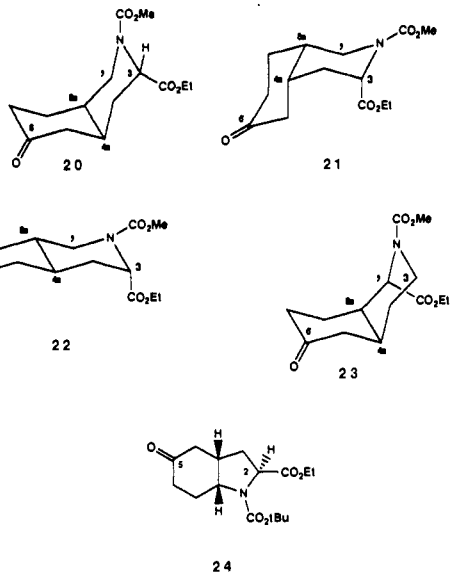
incorporation of constraining elements such as ketones and olefins into acyclic structures. Another approach has been to substitute other acid surrogates, such as the tetrazole, for the polar phosphonic acid group. We have found that by combining these two elements (conformational constraint plus tetrazole) we have been able to develop potent NMDA antagonists, e.g. 8 (LY233053).[27,28]

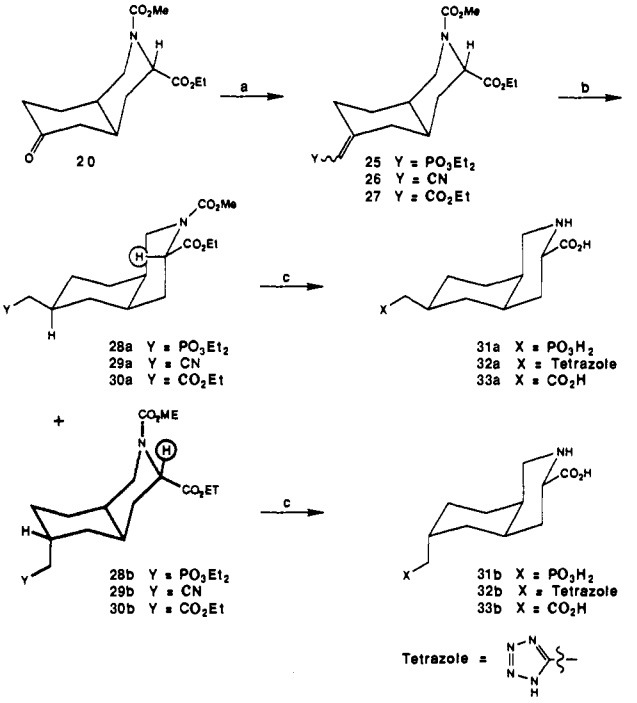
Chart I shows some representative examples of novel competitive NMDA antagonists. For example, incorporation of an olefin or ketone group into 6 afforded the potent antagonists 9 (CGP 37849),[30] 10 (CGP 39653),[31] and 11 (MDL 100,453),[32] respectively. Potent NMDA antagonist activity has been observed for the 3- and 4-substituted piperidine- and piperazine-2-carboxylic acids such as 1,[15-17] 8,[27,28] 12 (MDL 100,925),[33] 13 (LY257883),[16,17] 14 (CPP),[34] and 15 (CPP-ene).[34] Moderately potent NMDA antagonist activity was also described for such compounds as the phenyl glycine derivative 16 (PD 129635),[35] the hexahydrophenylalanine derivative 17 (NPC 12626),[36] and the tetrahydroisoquinolines 18 (SC 48981)[37] and 19 (PD 134705).[38] Thus, a myriad of structural features can be tolerated at the NMDA receptor to allow for enhanced antagonist activity.

We sought to explore the hydroisoquinoline nucleus as a framework for the construction of potent NMDA antagonists. One attractive feature of this system is that with four asymmetric centers, we could prepare a variety of compounds with very distinct three-dimensional structures. We hoped that the relative affinities of these amino acids would provide a better understanding of the optimal stereochemical requirements for binding to the NMDA receptor. We could also explore varying the location of the proximal carboxylic acid moiety between C-1 and C-3 and varying the nature of the distal acid group, e.g., phosphonic acid versus carboxylic acid versus tetrazole. We recently reported that two compounds from this series of decahydroisoquinoline-3-carboxylic acids, 31a (LY274614)[39] and

Chart II



Scheme I



[a] (a) For Y = PO_3Et_2: $(Et_2O_3P)_2CH_2$, NaH, THF, room temperature. For Y = CN: $Et_2O_3PCH_2CN$, NaH, THF, room temperature. For Y = CO_2Et: $Et_2O_3PCH_2CO_2Et$, NaH, THF, room temperature; (b) 60 psi H_2, EtOH, 5% Pd/C, room temperature; (c) For X = PO_3H_2: 6 N HCl, reflux; propylene oxide, H_2O; fractional crystallization from H_2O. For X = tetrazole: n-Bu_3SnN_3, 80 °C; 6 N HCl, reflux; Dowex 50 X-8, 10% aqueous pyridine. For X = CO_2H: 6 N HCl, reflux; Dowex 50 X-8, 10% aqueous pyridine.

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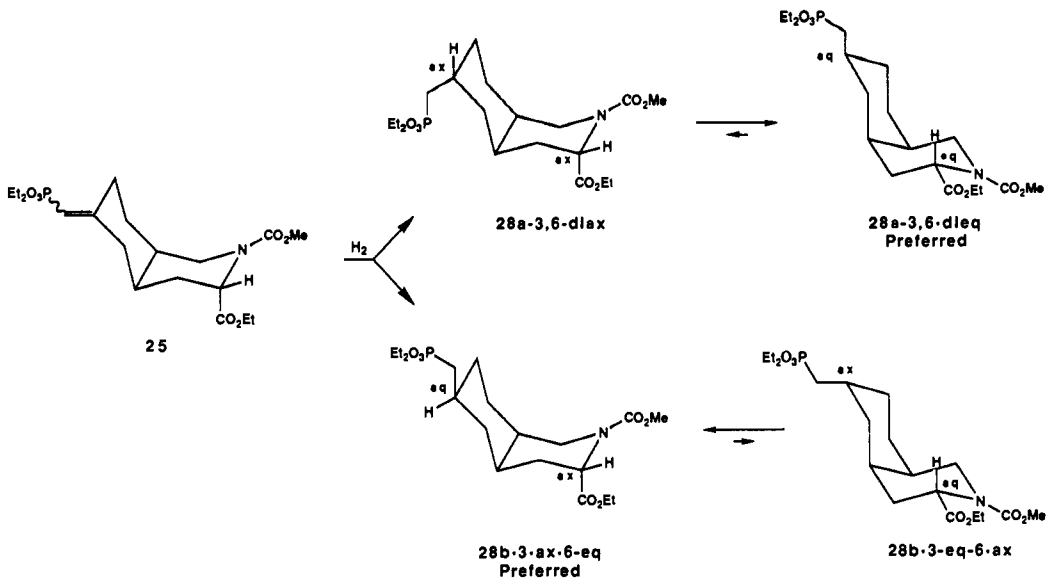


Figure 2. Conformational preferences for the products from hydrogenation of **26**.

32a (LY233536),[40] are potent and selective NMDA antagonists that are active in rats and mice following oral and parenteral administration. Herein we report the synthesis of these and other hydroisoquinoline amino acids, along with two structurally analogous hydroindole-2-carboxylic acids and their characterization as competitive NMDA receptor antagonists.

Chemistry

In order to explore the structure–activity relationships of these hydroisoquinoline amino acids, we sought a flexible approach that would allow for the selective preparation of a variety of stereoisomers, for varying the extent of saturation in the carbocyclic ring, and for attaching the carboxy group at either C-3 or C-1. Key in our synthetic approach to this series of compounds was the obtention of the stereochemically defined ketones **20**,[41] **21**,[41] **22**,[41] **23**, and **24** (Chart II). We felt that the acid-methylene group at C-6 could be introduced through a Horner-Emmons reaction and the amino acid subsequently revealed after exhaustive hydrolysis.

Reaction of **20**[41] (Scheme I) with the sodium salt of either tetraethyl methylenediphosphonate, diethyl phosphonoacetonitrile, or triethyl phosphonoacetate afforded the unsaturated (*E*/*Z* mixture) phosphonate, nitrile, or ester, **25**, **26**, or **27**, respectively. Hydrogenation of **25**, **26**, or **27** afforded a mixture of compounds epimeric at C-6, **28a** and **28b**, **29a** and **29b**, or **30a** and **30b**, respectively. The nitrile epimers **29a** and **29b** were separated by chromatography on silica gel, and each was converted to the tetrazole by treatment with azidotri-*n*-butylstannane neat at 80 °C for 3 days followed by hydrolysis to the amino acids **32a**[42] and **32b**, respectively. The ester epimers **30a** and **30b** were also separated by chromatography on silica gel and each hydrolyzed to the corresponding acids **33a** and **33b**, respectively. The phosphonate epimers **28a** and **28b** were inseparable by chromatography and were hydrolyzed as a mixture, and the amino acids **31a** and **31b** were then separated by fractional crystallization.

We studied a variety of catalyst (5% Pd/C, PtO_2, and Raney nickel) and solvent (ethyl acetate, ethanol, and acetic acid) combinations for the reduction of **25** to the epimeric phosphonates **28a** and **28b**. We could never achieve a highly stereoselective reduction, and in all cases we found that the ratio of **28a**:**28b** would vary at best from about 2:1 to 1:2. Figure 2 shows what we believe might be the conformational preferences for the products of the reduction of **25**. We know that the carbethoxy group at C-3 in ketones **20** and **21** prefers an axial orientation[41] because of $A_{1,3}$ strain between it and the methyl carbamate, and we believe that this conformational preference would also be present in an unsaturated analog such as **25**. Therefore, if reduction occurs from the β-face of the molecule (Figure 2), the product could adopt either the 3,6-diaxial (diax) conformer **28a**-diax, or by ring-flip, the 3,6-diequatorial (dieq) conformer **28a**-dieq. Alternatively,

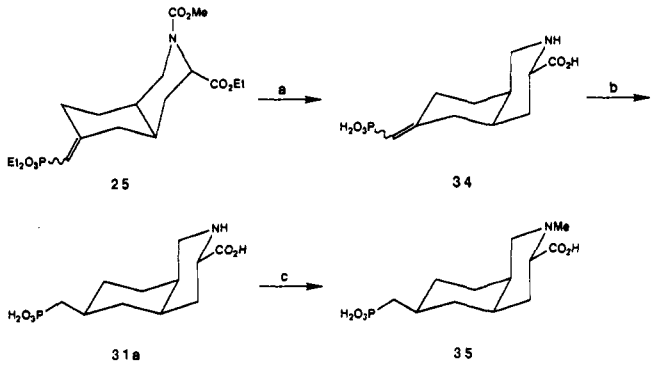
(39) Schoepp, D. D.; Ornstein, P. L.; Salhoff, C. R.; Leander, J. D. Neuroprotectant effects of LY274614, a structurally novel systemically active competitive NMDA antagonist. *J. Neural. Transm.* **1991**, *85*, 131–143.

(40) Ornstein, P. L.; Schoepp, D. D.; Leander, J. D.; Lodge, D. The development of novel competitive N-methyl-D-aspartate antagonists as useful therapeutic agents. Discovery of LY274614 and LY233536. In *Excitatory Amino Acids 1990 Fidia Research Foundation Symposium Series*; Meldrum, B., Moroni, R., Simon, R., Woods, J., Eds.; Raven Press: New York, 1991; Vol. 5, pp 415–423.

(41) Ornstein, P. L.; Arnold, M. B.; Augenstein, N. K.; Paschal, J. W. Syntheses of 6-oxodecahydroisoquinoline-3-carboxylates. Useful intermediates for the preparation of conformationally defined excitatory amino acid antagonists. *J. Org. Chem.* **1991**, *56*, 4388–4392.

(42) The ^1H NMR spectrum of **32a** in DCl/D_2O showed the following: The proton at C-3 was a doublet of doublets at δ 4.07 with coupling constants of 11.8 and 3.3 Hz, and one proton at C-4 was a doublet of triplets at δ 2.00 with coupling constants of 11.8 and 3.3 Hz. The value of the coupling constants for the proton at C-3 indicates that it is axial and coupled to axial and equitorial protons at C-4, and the coupling constants for the aforementioned proton at C-4 (which is presumably axial) indicates that the bridgehead proton at C-4a is equatorial, with respect to the piperidine ring. The protons adjacent to the nitrogen at C-1 were a triplet at δ 3.27 with a coupling constant of 12.9 Hz and a doublet of doublets at δ 3.19 with coupling constants of 12.9 and 4.4 Hz, which indicates that the bridgehead proton at C-8a is axial, with respect to the piperidine ring. The proton at C-6 shows 12.1-Hz couplings to both axial protons at C-5 and C-7, which would indicate that the C-6 proton is axial. On the basis of this analysis and the assumption that both the heterocyclic and carbocyclic rings prefer a chair-like conformation, the C-3 acid and C-6 tetrazolylmethyl groups are in an equatorial orientation and the stereochemistry of **32a** is as shown in Scheme I. With the similarities observed for the chemical shifts and multiplicities of the C-3 protons in **28a** and **29a**, we feel confident that the stereochemistry of **31a** is also as shown in Scheme I.

Scheme II



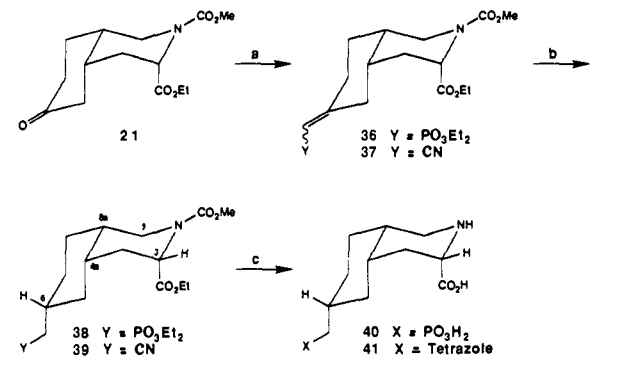
(a) NaOH, H_2O, EtOH, room temperature; TMSI, chloroform, reflux; Dowex 50X-8, 10% aqueous pyridine. (b) 60 psi H_2, H_2O, 5% Pd/C, 40 °C; Dowex 1X-8, 3 N aqueous AcOH. (c) 60 psi H_2, CH_2O, 5% Pd/C, EtOH, H_2O, 50 °C; Dowex 1X-8, 3 N aqueous AcOH.

if reduction occurs from the α-face, the product could adopt either the 3-axial-6-equatorial (axeq) conformer **28b**-axeq, or by ring flip, the 3-equatorial-6-axial (eqax) conformer **28b**-eqax. We believe that the diequatorial conformation would be preferred for **28a** and that the 3-axial-6-equatorial conformation would be preferred for **28b**. In the proton NMR spectra of **28a** and **28b**, we observed that the proton at C-3 is downfield in **28b** relative to **28a** and that the C-3 proton is a triplet in **28a** and two doublets in **28b** (because of the existence of two amide rotamers). If the C-3 proton of **28b** is equatorial, then it would be expected to be downfield relative to the axial proton of **28a**. If the piperidine ring of **28a** is somewhat distorted from a chair-like conformation, this would alleviate the $A_{1,3}$ strain between the ester and carbamate, while at the same time making the angles between the C-3 and C-4 protons are more equivalent, hence the observation of a triplet for the C-3 proton. These same ^1H NMR phenomena were observed for the C-6 epimers of **29** and **30**.

If $A_{1,3}$ strain between the ester and carbamate groups in **25** was the reason for the lack of a highly stereoselective reduction, we felt that removal of the carbamate prior to reduction would solve this problem. We therefore switched the order of steps so that hydrogenation of the double bond took place after deprotection. An alternative synthesis of **31a** is shown in Scheme II. Hydrolysis of the ethyl ester of **25** with aqueous sodium hydroxide in ethanol was followed by removal of the carbamate and phosphonate esters with excess iodotrimethylsilane. The unsaturated amino acid **34** was then isolated after removal of the hydroiodic acid, formed upon aqueous workup, with anion-exchange chromatography. Subsequent hydrogenation of **34** gave only the desired amino acid (>95% by ^1H NMR) **31a**,[42] thus supporting the idea that $A_{1,3}$ strain between the ester and carbamate groups was the source of the lack of selectivity in the hydrogenation of **25**. Treatment of **31a** with formaldehyde, 5% palladium on carbon, and hydrogen in aqueous ethanol afforded the *N*-methyl analog **35** (Scheme II).

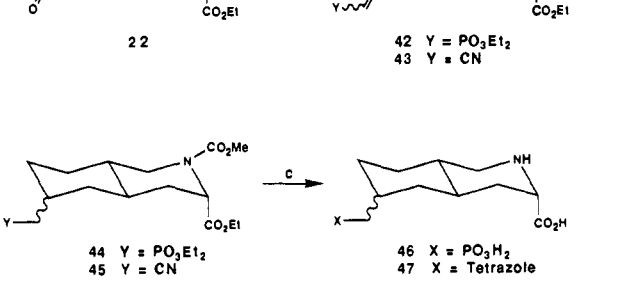
Scheme III describes the conversion of the ketone **21**,[41] epimeric at C-3 relative to ketone **20**,[41] to the amino acids **40** and **41** via the intermediate olefins **36** and **37** and then the saturated compounds **38** and **39**. By ^1H and ^{13}C NMR analysis, the amino acids **40** and **41** appear to be a single diastereomer. The C-3 proton in both amino acid precursors **38** and **39** appears as two doublets, similar to what we observed for **31b**, **32b**, and **33b**. We therefore believe that reduction of the olefin occurs to give the equatorial

Scheme III



(a) For Y = PO_3Et_2: $(Et_2O_3P)_2CH_2$, NaH, THF, room temperature. For Y = CN: $Et_2O_3PCH_2CN$, NaH, THF, room temperature. (b) 60 psi H_2, EtOH, 5% Pd/C, room temperature. (c) For X = PO_3H_2: 6 N HCl, reflux; propylene oxide, H_2O. For X = tetrazole: *n*-Bu_3SnN_3, 80 °C; 6 N HCl, reflux; Dowex 50 X-*, 10% aqueous pyridine.

Scheme IV



(a) For Y = PO_3Et_2: $(Et_2O_3P)_2CH_2$, NaH, THF, room temperature. For Y = CN: $Et_2O_3PCH_2CN$, NaH, THF, room temperature. (b) 60 psi H_2, EtOH, 5% Pd/C, room temperature. (c) For X = PO_3H_2: 6 N HCl, reflux; propylene oxide, H_2O. For X = tetrazole: *n*-Bu_3SnN_3, 80 °C; 6 N HCl, reflux; Dowex 50 X-8, 10% aqueous pyridine.

phosphonomethyl substituent, and hence the relative stereochemistry shown in Scheme III. If epimerization at C-3 had occurred during hydrolysis, we would obtain **31a** as the product, and this is not observed by NMR. Scheme IV describes the conversion of the trans-ring juncture ketone **22**[41] to the amino acids **46** and **47** (obtained as mixtures of C-6 epimers as determined by NMR) again via olefins **42** and **43** and the saturated analogs **44** and **45**.

Scheme V shows the preparation of the tetrahydroisoquinoline amino acids **55** and **56**. The known 6-hydroxytetrahydroisoquinoline **48**[41] was converted to the triflate **49** and then coupled with vinyltri-*n*-butylstannane to yield the styryl compound **50**. We found this olefin to be somewhat unstable and so it was rapidly subjected to a two-step oxidative cleavage with osmium tetroxide followed by sodium metaperiodate, and the intermediate aldehyde was reduced with sodium borohydride to yield the alcohol **51**. Bromination of **51** afforded **52** which was converted to the phosphonate and nitrile, **53** and **54**, respectively, and then to the amino acids **55** and **56**.

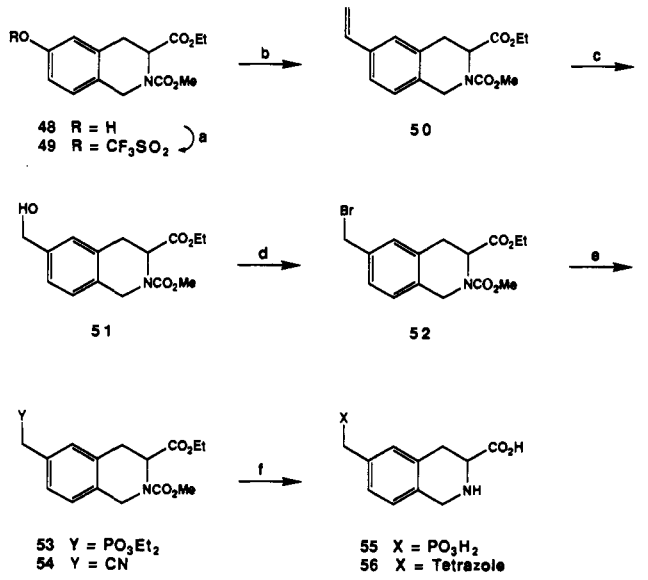
Preparation of the compounds with the carboxy group at C-1 of the decahydroisoquinoline (Scheme VI) began with reaction of 2-(3-hydroxyphenyl)ethylamine with glyoxylic acid to yield an amino acid, which was esterified and then protected as the *tert*-butyl carbamate (BOC) to afford **57**. We found that compound **57** was very resistant to hydrogenation using the same conditions as for the 3-carbethoxy compounds (5% Ru/Al_2O_3, 180 °C, 2000 psi

Table I. In Vitro and in Vivo Data for Acidic Amino Acids

compd[a]	IC_{50} (nM) vs [^{3}H]CGS 19755 binding[b]	IC_{50} (μM) vs NMDA-induced depolarizations in a cortical slice[c]	MED[d] (mg/kg, ip) vs NMDA-induced lethality in mice[e]
1	54 ± 13	1.6 ± 0.13	1.25
8	107 ± 7	4.2 ± 0.4	5
31a	55 ± 14	0.15 ± 0.01	1.25
32a	856 ± 136	1.39 ± 0.29	2.5
33a	4298 ± 589	7.8 ± 1.6	10
31b	815 ± 205	1.7 ± 0.3	2.5
32b	3380 ± 286	4.8 ± 0.8	40
33b	>10000	>100	>160
35	>10000	–	>160
40	3557 ± 366	8.4 ± 1.6	80
41	>10000	>100	>160
46[f]	>10000	>100	>160
47[f]	>10000	>100	>160
55	13810 ± 2170	34.4 ± 1.7	>160
56	26400 ± 1946	36.6 ± 3.6	>160
64[f]	4870 ± 1000	12.7 ± 3.3	>160
65[f]	2480 ± 260	7.8 ± 1.9	160
71[g]	>10000	>100	>160
72[g]	>10000	>100	>160

[a] All compounds are racemic. [b] See ref 44. [c] See ref 47. [d] MED = minimum effective dose. This is the dose at which at least three of the five animals tested survived. [e] See ref 48. [f] Isolated as a mixture of diastereomers at C-6. [g] Isolated as a mixture of diastereomers at C-5.

Scheme V



(a) $PhNTf_2$, *i*-Pr_2NEt, CH_2Cl_2, room temperature. (b) Vinyl tri-*n*-butylstannane, $Pd(Ph_3P)_4$, LiCl, dioxane, 98 °C. (c) OsO_4, $NaIO_4$, toluene, dioxane, room temperature; $NaBH_4$, EtOH, 0 °C. (d) $Ph_3P{\cdot}Br_2$, CH_2Cl_2, pyridine, room temperature. (e) For Y = PO_3Et_2: $(EtO)_3P$, toluene, reflux. For Y = CN: NaCN, DMSO, 70 °C. (f) For X = PO_3H_2: 6 N HCl, reflux; propylene oxide, H_2O. For X = tetrazole: *n*-Bu_3SnN_3, 80 °C; 6 N HCl, reflux; Dowex 50 X-*, 10% aqueous pyridine.

H_2)[41] and that removal of the BOC group with trifluoroacetic acid in dichloromethane followed by treatment with aqueous hydrochloric acid to yield the hydrochloride salt **58** was necessary prior to hydrogenation. Hydrogenation of **58** was sluggish and after BOC protection of the nitrogen and oxidation, a low yield of the desired ketone **59**[43] was realized. This ketone was equilibrated with sodium ethoxide in ethanol to give exclusively the ketone **23**.[43] The homogeneity of ketones **59** and **23** was confirmed by ^{1}H and ^{13}C NMR and GC analysis. Ketone **23** was converted to the amino acids **64** and **65** via the olefins **60** and **61** and the saturated analogs **62** and **63**.

Scheme VII shows the preparation of the 5-substituted octahydroindole-2-carboxylic acids. 5-Hydroxyindole-2-carboxylic acid was hydrogenated and then esterified, BOC protected, and oxidized to afford a separate mixture of the desired ketone **24** along with the descarboxy compound **66**. Ketone **24** appeared to be a single diastereomer by GC[43] and ^{1}H and ^{13}C NMR analysis. When subjected to equilibrating conditions (sodium methoxide in methanol at 60 °C), no change was observed by either GC[43] or NMR. We therefore assigned the stereochemistry based on analogy to the perhydroisoquinoline series, with a cis-ring juncture. To obviate the decarboxylation that we believed was occuring during hydrogenation, we prepared methyl 5-hydroxyindole-2-carboxylate prior to reduction. We found, however that reduction of this ester gave a mixture of products that ultimately yielded none of the desired **24**. Ketone **24** was converted to the amino acids **71** and **72** via the unsaturated phosphonate and nitrile, **67** and **68**, and the corresponding saturated analogs **69** and **70**, respectively.

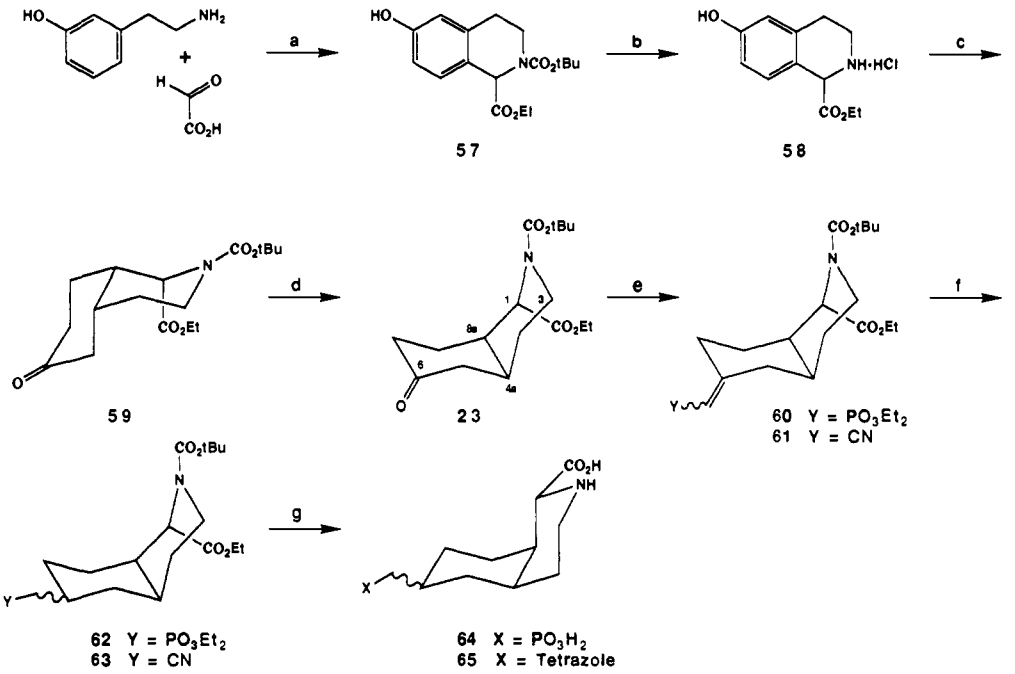
Pharmacology

All of the amino acids that we prepared were evaluated for their ability to displace [^{3}H]CGS 19755 (10 nM) binding,[44] as a measure of their affinity for the glutamate recognition site on the NMDA receptor complex, and the IC_{50} values determined are shown in Table I. Although the data is not shown, each of these compounds was also evaluated for its affinity at AMPA ([^{3}H]AMPA binding[45]) and KA ([^{3}H]KA binding[46]) receptors, and at concentra-

(43) Gas chromatographic analyses were performed on an HP5890 Series II capillary GC with an Ultra 1 crosslinked methyl silicone column, 25 m × 0.32 mm × 0.52 μM, using the following temperature program: Initial temperature of 180 °C for 1 min followed by a 10 °C/min increase to 260 °C, which is then maintained for 5 min. Retention times are as follows: **23**, t_R = 4.71 min; **24**, t_R = 3.72 min; **59**, t_R = 5.13 min.

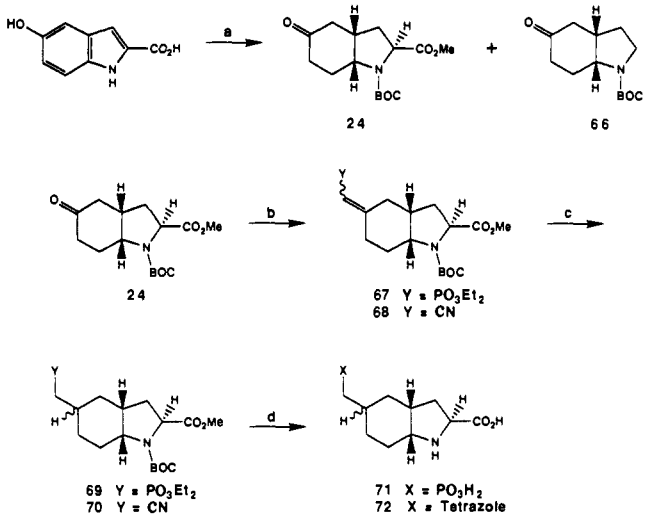
(44) Murphy, D. E.; Hutchinson, A. J.; Hurt, S. D.; Williams, M.; Sills, M. A. Characterization of the binding of [^{3}H]-CGS-19755: a novel N-methyl-D-aspartate antagonist with nanomolar affinity in rat brain. *Br. J. Pharmacol.* **1988**, *95*, 932–938.
(45) Nielsen, E. O.; Madsen, U.; Schaumburg, K.; Krogsgaard-Larsen, P. Studies on receptor-active conformations of excitatory amino acid agonists and antagonists. *Eur. J. Med. Chem. Chim. Ther.* **1986**, *21*, 433–437.
(46) Simon, J. R.; Contrera, J. F.; Kuhar, M. J. Binding of [^{3}H]-kainic acid, an analogue of L-glutamate. *J. Neurochem.* **1976**, *26*, 141–147.

Scheme VI



(a) 5% HCl, 90 °C; EtOH, HCl, reflux; Di-*t*-butyldicarbonate, *i*-Pr_2NEt, CH_2Cl_2, room temperature. (b) TFA, CH_2Cl_2, room temperature; 1 N HCl. (c) 60 psi H_2, 6/1 EtOH/AcOH, 5% Rh/C, 60 °C; Di-*t*-butyldicarbonate, *i*-Pr_2NEt, CH_2Cl_2, room temperature; Pyridinium chlorochromate (PCC), 4Å sieves, CH_2Cl_2, room temperature. (d) NaOEt, EtOH, 80 °C. (e) For Y = PO_3Et_2: $(Et_2O_p)_2CH_2$, NaH, THF, room temperature. For Y = CN: $Et_2O_3PCH_2CN$, NaH, THF, room temperature. (f) 60 psi H_2, EtOH, 5% Pd/C, room temperature. (g) For X = PO_3H_2: 6 N HCl, reflux. For X = tetrazole: *n*-Bu_3SnN_3, 80 °C; 6N HCl, reflux; Dowex 50 X-*, 10% aqueous pyridine.

Scheme VII



(a) 60 psi H_2, H_2O, 5% Rh/Al_2O_3, 60 °C; MeOH, trimethyl orthoformate, HCl, room temperature; di-*tert*-butyl dicarbonate, *i*-Pr_2NEt, CH_2Cl_2, room temperature; PCC, 4Å sieves, CH_2Cl_2, room temperature; (b) For Y = PO_3Et_2: $(Et_2O_3P)_2CH_2$, NaH, THF, room temperature. For Y = CN: $Et_2O_3PCH_2CN$, NaH, THF, room temperature. (c) 60 psi H_2, EtOH, 5% Pd/C, room temperature. (d) For X = PO_3H_2: 6 N HCl, reflux; propylene oxide, H_2O; Dowex 50X-8, H_2O. For X = tetrazole: *n*-Bu_3SnN_3, 80 °C; 6 N HCl, reflux; Dowex 50 X-8, 10% aqueous pyridine.

tions up to 10 000 nM no significant inhibition of ligand binding was observed.

These amino acids were examined in a cortical slice assay[47] for intrinsic agonist activity and ability to antagonize depolarizations induced by 40 μM NMDA, 40 μM quisqualic acid (QUIS) or AMPA, and 10 μM KA. The IC_{50} values for NMDA antagonist activity are shown in Table I. None of these compounds showed any agonist activity when tested alone nor did they significantly inhibit depolarizations due to AMPA (or QUIS) or KA at concentrations up to 100 μM.

As a measure of in vivo NMDA antagonist activity, these amino acids were evaluated for their ability to protect mice from lethality induced by a 200 mg/kg intraperitoneal (ip) injection of NMDA.[48] This dose of NMDA produces lethality in ≥95% of the animals tested, and this assay is very specific in that only NMDA antagonists (e.g., competitive and noncompetitive PCP-like) provide protection. All of the amino acids tested were given ip 30 min prior to administration of NMDA, and the data is reported in Table I as the minimum effective dose (MED) of the compound (i.e. the dose that protects >50% of the animals tested) to provide protection.

Results and Discussion

A series of 6-substituted decahydroisoquinoline-3-carboxylic acids were prepared as potential competitive NMDA receptor antagonists. We synthesized a number of the different possible stereoisomers to understand more about the steric and spatial requirements for activity at the NMDA receptor. We also prepared compounds that would allow us to examine the effect of varying the nature of the distal acidic moiety and the location of the proximal acid group between C-1 and C-3. Except for **31**, **32**, and **33**, no attempt was made to separate the C-6-isomers of the amino acids that we prepared. For comparison, data is also included in Table I for the piperidine phosphonate and tetrazole amino acids, **1** and **8**, respectively.

(47) Harrison, N. L.; Simmonds, M. A. Quantitative studies on some antagonists of N-methyl-D-aspartate in slices of rat cerebral cortex. *Br. J. Pharmacol.* **1985**, *84*, 381–391.

(48) Leander, J. D.; Lawson, R. R.; Ornstein, P. L.; Zimmerman, D. M. N-Methyl-D-aspartic acid-induced lethality in mice: selective antagonism by phenylcyclidine-like drugs. *Brain Res.* **1988**, *448*, 115–120.

Regardless of the distal acid group, the compounds possessing a stereochemical arrangement such as found in **31a**, **32a**, and **33a** showed a greater affinity for the NMDA receptor than the C-6-epimeric compounds **31b**, **32b**, and **33b**. This difference was also observed for NMDA antagonist activity measured in vitro in a cortical slice preparation and in vivo as agents to protect against NMDA-induced lethality in mice. The relative differences in affinity and antagonist potency between the C-6 epimers of the phosphonate and tetrazole-substituted amino acids (about 11–15:1 for **31a**:**31b** and about 3–4:1 for **32a**:**32b**) was about the same in binding and in the cortical wedge assay. However, against NMDA-induced lethality in mice, **31a** was twice as potent at **31b**, while **32a** was 16 times as potent as **32b**. This may represent differences in brain penetration of these amino acids, but no specific data yet exists to explain these observations.

For a series of compounds having the same stereochemical array at all four chiral carbons, such as **31a**, **32a**, and **33a** or **31b**, **32b**, and **33b**, we observed that the phosphonate-substituted amino acids had the highest affinity for the NMDA receptor and were the most potent as antagonists both in vitro and in vivo. The tetrazole-substituted compounds had somewhat lower affinities and antagonist potencies, and the carboxylic acid-substituted compounds showed the lowest affinity and potency. This same trend was also observed for the substituted piperidine-carboxylic acids **1** and **8**. Because the carboxy-substituted amino acids **33a** and **33b** were much less potent than their phosphonic acid and tetrazole counterparts, we focused our subsequent synthetic efforts on the preparation of compounds containing only the latter two acid isosteres.

We prepared the *N*-methyl analog **35** of the potent antagonist **31a**, to evaluate the effects of alkyl substitution at this position. Amino acid **35** showed no affinity for the NMDA receptor and was inactive in vivo as an NMDA antagonist. The same trend was observed for the *N*-methyl analogs of the piperidine amino acids **1**[27] and **8**,[16] which were inactive relative to their unsubstituted counterparts. This may reflect either the need for a proton on the amino acid nitrogen for a potent binding interaction or a lack of steric tolerance at the receptor for a substituent on this atom, even one as small as a methyl group.

The phosphonate-substituted amino acid, **40**, which was epimeric at C-3 relative to **31**, had moderate affinity for the NMDA receptor. This change also resulted in a significant loss of antagonist potency both in vitro and in vivo. The tetrazole-substituted compound **41**, however, was inactive in binding and in the in vitro and in vivo assays for NMDA antagonist activity. Compounds with the trans-ring juncture, **46** and **47**, also showed no affinity for the NMDA receptor and were therefore inactive as antagonists.

We prepared the tetrahydroisoquinoline amino acids **55** and **56** to understand the effect of having a planar carbocyclic ring. These amino acids had little receptor affinity and, although they did demonstrate some weak antagonist activity in the cortical wedge assay, they were inactive in vivo.

The C-1-carboxy-substituted amino acids **64** and **65** did have moderate affinity for the NMDA receptor and were antagonists in the cortical slice assay. But while the tetrazole-substituted compound **65** showed protection against the lethal effects of NMDA in mice, the phosphonate **64** was inactive. This is the only structural variation where the tetrazole-substituted amino acid was more potent than the corresponding phosphonate.

We also prepared the 5-phosphonate- and 5-tetrazole-substituted octahydroindole-2-carboxylic acids, **71** and **72**, respectively. With this very distinctive structural variation, we have excised the C-1 methylene between the nitrogen and the bridgehead carbon of, for example, **31**. In this case, both **71** and **72** had no affinity for the NMDA receptor and showed no antagonist activity both in vitro and in vivo.

We and others have observed that the steric and spatial requirements for affinity at NMDA receptors are very demanding. Very subtle structural changes often lead to significant losses in affinity for the NMDA receptor and/or potency as NMDA antagonists. These trends have also been observed in the series of decahydroisoquinoline amino acids reported herein. The resolution of some of these amino acids and an evaluation of how these amino acids might interact with the NMDA receptor protein relative to other amino acids, such as **1**, **6**, **8**,**9**, **13**, **14**, and **15**, will be reported in a subsequent paper.

Within this series of decahydroisoquinoline-3-carboxylic acids are two very potent and selective NMDA antagonists, **31a** and **32a**, as evidenced by their affinity for the NMDA receptor and their ability to inhibit responses evoked by NMDA in a cortical slice preparation. These two compounds also show excellent activity following systemic administration in mice, rats,[39,40] and pigeons.[49] For example, they block NMDA-induced lethality in mice at minimum effective doses of 1.25 and 2.5 mg/kg, (ip), respectively. This places these compounds amongst some of the most potent NMDA antagonists that we have evaluated. It has also been shown that **31a**[39] and **32a**[40] are systemically active neuroprotective agents in rats, blocking the neuronal degeneration that is observed following focal injection of NMDA into the striatum. The amino acids **31a** and **32a** might be clinically useful agents for the treatment of acute and chronic neurodegenerative diseases, where glutamate-mediated excitotoxicity is playing a role in their pathophysiology.

Experimental Section

All experiments were run under a positive pressure of dry nitrogen. Tetrahydrofuran (THF) was distilled from sodium prior to use. All other solvents and reagents were used as obtained. "Workup" refers to addition to the reaction mixture of a neutral or acidic aqueous solution, separation of the organic layer, and then extraction of the aqueous layer *n* times (×) with the indicated solvent(s). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo, and then purified as indicated. The aqueous solution and organic solvent(s) used are provided parenthetically in the text. "Preparative HPLC" refers to chromatographic separation on a Waters Prep 500 HPLC, using a linear gradient of hexane to the solvent indicated in parentheses in the text. ^1H NMR spectra were obtained on a GE QE-300 spectrometer at 300.15 MHz and a Bruker AM-500 spectrometer at 500 MHz, and ^{13}C NMR spectra were obtained on a GE QE-300 spectrometer at 75.48 MHz with tetramethylsilane as an internal standard. Where indicated, a small amount of 40% aqueous KOD was added to aid solution of NMR samples run in D_2O.

General Procedure for Horner–Emmons Reactions. Ethyl (3*SR*,4a*RS*,8a*RS*)-6-[(Diethylphosphono)methylidene]-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (25). Tetraethyl methylenediphosphonate (75.6 g, 262.2 mmol) was added dropwise to a suspension of sodium hydride (10.49 g, 262.2 mmol, 60% by weight in oil) in 210 mL of THF, and the solution stirred 30 min more

(49) Ornstein, P. L.; Schoepp, D. D.; Fuller, R. W.; Leander, J. D.; Lodge, D. The discovery and development of competitive NMDA antagonists as therapeutic agents. In *Drug Research Related to Neuroactive Amino Acids 1990; Alfred Benzon Symposium No. 32*; Schousboe, A., Diemer, N. H., Kofod, H., Eds.; Munksgaard: Copenhagen, 1992; pp 479–489.

(50) Ammonia in this sample probably derives from residual ammonia on the column which results from ammonium hydroxide used to prepare the column prior to ion exchange.

at room temperature. To the now clear solution was added **20** (53.1 g, 187.3 mmol) in 185 mL of THF, and the mixture was heated to reflux for 2 h. Workup (water/4× ether) and preparative HPLC (ethyl acetate to 10% ethanol/ethyl acetate) afforded 75.7 g (97%) of **25**. ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 5.44 and 5.40 (s, 1 H), 5.37 and 5.30 (s, 1 H), 4.97 and 4.79 (m, 1 H), 3.00–4.30 (m, 12 H), 1.25–2.60 (m, 10 H), 1.25 (m, 9 H). Anal. ($C_{19}H_{32}NO_7P$) C, H, N.

General Procedure for Hydrogenations. Ethyl (3*SR*,4a*RS*,6*SR*,8a*RS*)- and Ethyl (3*SR*,4a*RS*,6*RS*,8a*RS*)-6-[(Diethylphosphono)methyl]-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (28b and 28a). A solution of 88.8 g (212.7 mmol) of **25** in 1850 mL of ethanol was hydrogenated with 50 g of 5% Pd/C at room temperature at 60 psi for 2 h. The mixture was filtered through Celite and concentrated in vacuo, the residue dissolved in ether and again filtered through Celite, and then the filtrate was concentrated in vacuo. Preparative HPLC (ethyl acetate to 10% ethanol/ethyl acetate) afforded 73.1 g (82%) of an inseparable 1.6:1 mixture of **28a**:**28b**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 5.00 and 4.82 (d, J = 6.0 Hz, 1 H), 4.39 (t, J = 4.5 Hz, 1 H), 4.00–4.30 (m, 6 H), 3.00–4.00 (m, 5 H), 1.00–2.30 (m, 22 H). Anal. ($C_{19}H_{34}NO_7P$) C, H, N.

General Procedure for Phosphono Amino Acid Hydrolysis and Isolation. (3*SR*,4a*RS*,6*SR*,8a*RS*)-6-(Phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (31a) and (3*SR*,4a*RS*,6*RS*,8a*RS*)-6-(Phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (31b). An amount of 71.9 g (171.4 mmol) of the mixture of **28a** and **28b** and 1000 mL of 6 N aqueous hydrochloric acid were heated at reflux overnight and then cooled and concentrated in vacuo. Water was added and the mixture concentrated in vacuo twice more. The residue was dissolved in 50 mL of water and treated with 21 mL of propylene oxide (pH 1–2), heated to 50 °C for 30 min, and then concentrated in vacuo. To the residue was added 500 mL of ethanol, and the suspension heated to reflux for 1 hr, then cooled to room temperature, and stirred overnight. The resultant solid was filtered, washed with ethanol, acetone, and ether, suspended in 500 mL of acetone, heated to reflux for 1 h, and then filtered and washed with acetone and ether. This solid was suspended in 200 mL of water, heated to reflux for 2 h, cooled, filtered, and washed with acetone and ether. This procedure was repeated one more time, and the resultant solid was dried in vacuo at 60 °C overnight to afford 12.4 g (26%) of **31**, mp 312–313 °C. The filtrates from the above aqueous suspensions were concentrated in vacuo to about 15 mL, and upon standing a precipitate formed which was filtered and washed with water, acetone, and ether to afford **31b**, mp 254–255 °C. **31a**: ^{1}H NMR (D_2O/KOD) δ 3.23 (d, J = 5.0 Hz, 1 H), 2.72 (d, J = 11.0 Hz, 1 H), 2.56 (m, 1 H), 1.35–2.00 (m, 11 H), 1.10 (m, 1 H), 0.94 (m, 1 H). **31b**: ^{1}H NMR (D_2O/KOD) δ 3.29 (s, 1 H), 2.72 (dd, J = 5.0, 13.0 Hz, 2 H), 1.10–1.80 (m, 13 H). **31a**: Anal. ($C_{11}H_{20}NO_5P$) C, H, N. **31b**: Anal. ($C_{11}H_{20}NO_5P\cdot1.1H_2O$) C, H, N.

(3*SR*,4a*RS*,8a*RS*)-6-(Phosphonomethylidene)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (34). A solution of 45.8 g (109.6 mmol) of **25** and 120 mL of 1 N aqueous sodium hydroxide in 400 mL of ethanol was stirred overnight at room temperature and then concentrated in vacuo. Workup (10% aqueous sodium bisulfate/4× ethyl acetate) was followed by addition of chloroform to the residue and another concentration in vacuo, and then this residue was dissolved in 350 mL of chloroform and 122.4 g (611.7 mmol) of iodotrimethylsilane was added dropwise over 20 min. The solution was heated to reflux for 3 h, cooled, and concentrated in vacuo, and then 300 mL of water was added. The aqueous layer was extracted four times with ether and then concentrated in vacuo. The material was purified by ion-exchange chromatography on Bio-Rad AG1X-8 (hydroxide form, 8 × 12 cm column), eluting with 3 N aqueous acetic acid to afford 27.1 g (94%) of **34**: ^{1}H NMR (D_2O/KOD, doubling due to *E*/*Z* isomers) δ 5.26 and 5.17 (s, 1 H), 3.27 and 2.95 (m, 1 H), 1.20–2.60 (m, 12 H). Anal. ($C_{11}H_{18}NO_5P\cdot0.35H_2O$) C, H, N.

(3*SR*,4a*RS*,6*SR*,8a*RS*)-6-(Phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (31a, LY274614). A solution of 27.1 g (98.5 mmol) of **34** in 460 mL of water was hydrogenated overnight with 13.5 g of 5% Pd/C at 40 °C and 60 psi. The mixture was filtered through Celite and concentrated in vacuo. The catalyst was then refluxed in 400 mL of water and 100 mL of 1 N sodium hydroxide and filtered through Celite while hot, and the filtrate was added to the above material directly isolated from the hydrogenation and then concentrated in vacuo. This residue was dissolved in 50 mL of water at pH 12, filtered through Celite, and purfied by ion-exchange chromatography on Dowex 1×-8 (500 g of resin, 7 × 23 cm column), eluting with 3 N acetic acid and collecting seven 500-mL fractions. Fractions 2–6 were combined and concentrated to about 100 mL. The resultant crystals were filtered and washed with water, acetone, and ether and then dried in vacuo at 60 °C to afford 19.4 g (70%) of **31a**.

(3*SR*,4a*RS*,6*SR*,8a*RS*)-3-Methyl-6-(phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (35). A solution of 0.24 g (0.84 mmol) of **31a** in 100 mL of 1:1 water/ethanol and 5 mL of aqueous formalin was hydrogenated with 0.25 g of 5% Pd/C at 50 °C at 60 psi overnight. The mixture was filtered through Celite and concentrated in vacuo. The residue was dissolved in water at pH 7–8 and purified by ion-exchange chromatography on Dowex 1×-8 (2 × 4 cm column), eluting with 3 N acetic acid to afford 0.15 g (60%) of **35**: mp 301–302 °C; ^{1}H NMR (D_2O/KOD) δ 3.66 (m, 1 H), 3.28 (m, 1 H), 3.17 (m, 1 H), 2.86 (s, 3 H), 1.20–2.30 (m, 8 H), 1.02 (m, 1 H). Anal. ($C_{11}H_{18}NO_5P\cdot0.3H_2O$) C, H, N.

Ethyl (3*SR*,4a*RS*,8a*RS*)-6-(Cyanomethylidene)-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (26). As for **25**, 9.4 g (234.3 mmol) of sodium hydride, 41.5 g (234.3 mmol) of diethyl phosphonoacetonitrile, and 40.0 g (142 mmol) of **20** (one h of reflux) gave 44.7 g (100%) of **26**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 5.18 and 5.04 (s, 1 H), 4.97 and 4.78 (m, 1 H), 3.40–4.30 (m, 7 H), 3.22 and 3.11 (m, 1 H), 2.96 and 2.78 (m, 1 H), 1.40–2.50 (m, 8 H), 1.25 (m, 3 H). Anal. ($C_{16}H_{22}N_2O_4$) C, H, N.

Ethyl (3*SR*,4a*RS*,6*SR*,8a*RS*)-6-(Cyanomethyl)-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (29a) and (3*SR*,4a*RS*,6*RS*,8a*RS*)-6-(Cyanomethyl)-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (29b). A solution of 44.7 g (141.2 mmol) of **26** in 460 mL of ethanol was hydrogenated overnight with 5 g of 5% Pd/C at room temperature at 60 psi. The mixture was filtered through Celite and concentrated in vacuo, the residue dissolved in ether and again filtered through celite, and then the filtrate concentrated in vacuo. Preperative HPLC (hexane to 50% ethyl acetate/hexane) afforded 1.5 g (3.4%) of **29b**, 5.2 g (12%) of a mixture of **29a** and **29b**, and 12.0 g (27%) of **29a**. **29a**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 4.37 (t, J = 6.0 Hz, 1 H), 4.18 (q, J = 7.0 Hz, 2 H), 3.70 (s, 3 H), 3.47 (dd, J = 12.9, 5.2 Hz, 1 H), 3.33 (m, 1 H), 2.29 (d, J = 7.0 Hz, 2 H), 2.20 (m, 1 H), 1.40–2.00 (m, 10 H), 1.26 (t, J = 7.0 Hz, 3 H). **29b**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 5.00 and 4.82 (d, J = 4.4 Hz, 1 H), 4.17 (m, 2 H), 3.93 and 3.79 (d, J = 13.6 Hz, 1 H), 3.70 and 3.68 (s, 3 H), 3.20 and 3.10 (dd, J = 13.6, 2.4 Hz, 1 H), 2.20 (d, J = 7.1 Hz, 2 H), 1.00–2.10 (m, 11 H), 1.26 (t, J = 7.1 Hz, 3 H). **29a**: Anal. ($C_{16}H_{24}N_2O_4$) H, N; C: calcd 62.32; found, 61.75. **29b**: Anal. ($C_{16}H_{24}N_2O_4$) C, H, N.

General Procedure for Tetrazole Amino Acid Synthesis and Isolation. (3*SR*,4a*RS*,6*SR*,8a*RS*)-6-(1*H*-Tetrazol-5-ylmethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (32a). A mixture of 12.0 g (39.0 mmole) of **29a** and 25.9 g (78.0 mmol) of azidotri-*n*-butylstannane was heated to 80 °C for 90 h. A volume of 190 mL of 6 N aqueous hydrochloric acid was added and the mixture heated at 100 °C overnight, then cooled, and concentrated in vacuo. The residue was dissolved in water and extracted 3× with 250 mL of ether, and the aqueous layer was concentrated in vacuo. Ion-exchange chromatography on Dowex 50×-8 (7 × 26 cm column), eluting with 10% pyridine/water afforded 8.23 g (80%) of **32a**: mp 225 °C; ^{1}H NMR (D_2O) δ 3.63 (dd, J = 13.0, 3.0 Hz, 1 H), 3.11 (m, 2 H), 2.90 (d, J = 7.0 Hz, 2 H), 1.30–2.10 (m, 10 H), 1.01 (m, 1 H). Anal. ($C_{12}H_{19}N_5O_2\cdot H_2O$) C, H, N.

(3*SR*,4a*RS*,6*RS*,8a*RS*)-6-(1*H*-Tetrazol-5-ylmethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (32b). As for **32a**, 1.5 g (5.0 mmol) of **29b** and 3.3 g (9.9 mmol) of azidotri-*n*-butylstanne gave 0.54 g (41%) of **32b**: mp 226 °C;

^{1}H NMR (D_2O) δ 3.74 (m, 1 H), 3.12 (m, 4 H), 1.30–2.10 (m, 10 H), 1.03 (m, 1 H). Anal. ($C_{12}H_{19}N_5O_2 \cdot 1.5H_2O$) C, N; H: calcd, 7.58; found, 7.08.

Ethyl (3*SR*,4a*RS*,6*SR*,8a*RS*)-6-[(Ethoxycarbonyl)methyl]-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (30a) and (3*SR*,4a*RS*,6*RS*,8a*RS*)-6-[(Ethoxycarbonyl)methyl]-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (30b). As for **25**, 0.5 g (12.5 mmol) of sodium hydride, 2.5 g (12.5 mmol) of triethyl phosphonoacetate, and 2.8 g (8.7 mmol) of **20** (no reflux, 4 h at room temperature) gave an oil after workup (**27**) which was not purified. The material was directly hydrogenated in 95 mL of ethanol with 1.5 g 5% Pd/C at 40 °C at 60 psi for 4 h at room temperature. Purification by medium pressure LC (Lobar C cloumn, 20% ethyl acetate/hexane, 12 mL/min, 22-mL fractions) gave 1.03 g (33%) of **30b** and 0.94 g (31%) of **30a**. **30a**: ^{1}H NMR ($CDCl_3$) δ 4.39 (t, *J* = 5.0 Hz, 1 H), 4.20 (q, *J* = 7.0 Hz, 2 H), 4.13 (q, *J* = 7.0 Hz, 2 H), 3.72 (s, 3 H), 3.41 (m, 2 H), 2.22 (m, 3 H), 1.90 (m, 3 H), 1.78 (m, 1 H), 1.57 (m, 5 H), 1.25 (m, 6 H), 1.18 (m, 1 H). **30b**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 5.01 and 4.82 (d, *J* = 6.0 Hz, 1 H), 4.17 (m, 2 H), 4.12 (q, *J* = 7.0 Hz, 2 H), 3.92 and 3.77 (d, *J* = 13.0 Hz, 1 H), 3.69–3.71 (s, 3 H), 3.21 and 3.13 (dd, *J* = 13.0, 3.0 Hz, 1 H), 2.14 (d, *J* = 7.0 Hz, 2 H), 2.08 (m, 1 H), 1.30–2.00 (m, 9 H), 1.24 (m, 6 H), 1.00 (m, 1 H). **30a**: Anal. ($C_{18}H_{29}NO_6$) C, H, N. **30b**: Anal. ($C_{18}H_{29}NO_6$) C, H; N: Calcd, 3.94; found, 4.55.

(3*SR*,4a*RS*,6*SR*,8a*RS*)-6-(Carboxymethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (33a). A mixture of 0.90 g (2.5 mmol) of **30a** and 50 mL of 6 N aqueous hydrochloric acid were heated to reflux overnight, then cooled, and concentrated in vacuo. Ion-exchange chromatography on Dowex 50×-8, eluting with 10% pyridine/water afforded 0.39 g (64%) of **33a**: mp >300 °C; ^{1}H NMR (D_2O/KOD) δ 3.36 (m, 1 H), 2.85 (m, 1 H), 2.68 (m, 1 H), 2.12 (d, *J* = 7.5 Hz, 2 H), 1.90 (m, 3 H), 1.66 (m, 5 H), 1.49 (m, 1 H), 1.21 (m, 1 H), 1.06 (m, 1 H). Anal. ($C_{12}H_{19}NO_4$) C, H, N.

(3*SR*,4a*RS*,6*RS*,8a*RS*)-6-(Carboxymethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (33b). As for **33a**, 1.00 g (2.8 mmol) of **30b** gave 0.36 g (52%) of **33b**: mp >300 °C; ^{1}H NMR (D_2O/KOD) δ 3.24 (dd, *J* = 12.5, 2.8 Hz, 1 H), 2.82 (t, *J* = 12.5 Hz, 1 H), 2.58 (dd, *J* = 12.5, 4.0 Hz, 1 H), 2.09 (d, *J* = 7.5 Hz, 2 H), 1.95 (m, 1 H), 1.30–1.85 (m, 9 H), 1.00 (m, 1 H). Anal. ($C_{12}H_{19}NO_4$) C, H, N.

Ethyl (3*SR*,4a*SR*,6*RS*,8a*SR*)-6-[(Diethylphosphono)methyl]-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (38). As for **25**, 0.42 g (10.6 mmol) of sodium hydride, 3.1 g (10.6 mmol) of tetraethyl methylenediphosphonate and 2.0 g (7.1 mmol) of **21** gave an oil after workup (**36**) which was not purified. The material was directly hydrogenated in 100 mL of ethanol with 3.0 g of 5% Pd/C at room temperature at 60 psi for 4 h. Purification by flash chromatography (100 g, silica gel, 5% ethanol/ethyl acetate, 50-mL fractions) gave 2.44 g (82%) of **38**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 4.76 and 4.60 (d, *J* = 7.0 Hz, 1 H), 4.20 (m, 2 H), 4.07 (m, 4 H), 3.75 (m, 1 H), 3.73 and 3.68 (s, 3 H), 3.22 (m, 1 H), 1.50–2.20 (m, 11 H), 1.30 (m, 9 H), 1.04 (m, 2 H). Anal. ($C_{19}H_{34}NO_7P$) C, H, N.

(3*SR*,4a*SR*,6*RS*,8a*SR*)-6-(Phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (40). As for **31a**, 2.32 g of **38** in 50 mL of 6 N aqueous hydrochloric acid gave, after propylene oxide (1.1 mL addition and reflux in ethanol and then in acetone, 1.1 g (72%) of **40**: mp >300 °C; ^{1}H NMR (D_2O) δ 4.01 (d, *J* = 5.0 Hz, 1 H), 3.44 (t, *J* = 13.0 Hz, 1 H), 3.01 (dd, *J* = 13.0, 4.0 Hz, 1 H), 1.90–2.30 (m, 4 H), 1.62 (m, 7 H), 1.09 (m, 2 H); ^{13}C NMR (D_2O) δ 174.10, 58.27, 52.78, 41.43, 35.87, 34.22, 34.12, 34.08, 33.55, 33.49, 33.45, 32.56, 31.68, 31.08, 30.06, 28.80, 28.67, 28.21, 17.63. Anal. ($C_{11}H_{20}NO_5P \cdot 0.5H_2O \cdot 0.4C_3H_6O$) C, H, N.

Ethyl (3*SR*,4a*SR*,8a*SR*)-6-(Cyanomethylidene)-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (37). As for **25**, 0.85 g (21.2 mmol) of sodium hydride, 3.8 g (21.2 mmol) of diethyl phosphonoacetonitrile, and 4.0 g (14.1 mmol) of **21** gave, after flash chromatography (165 g silica gel, 50% ethyl acetate/hexane, 50-mL fractions), 4.0 g (94%) of **37**: ^{1}H NMR ($CDCl_3$) δ 5.09 (bs, 1 H), 4.70 (m, 1 H), 4.21 (m, 2 H), 3.80 (m, 1 H), 3.75 (s, 3 H), 3.36 (m, 1 H), 2.65 (m, 1 H), 1.60–2.30 (m, 9 H), 1.29 (t, *J* = 7.0 Hz, 3 H). Anal. ($C_{16}H_{22}N_2O_4$) C, H, N.

Ethyl (3*SR*,4a*SR*,6*RS*,8a*SR*)-6-(Cyanomethyl)-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (39). As for **29**, hydrogenation of 2.6 g (8.3 mmol) of **37** in 50 mL of ethanol with 1.0 g of 5% Pd/C at room temperature at 60 psi overnight gave, after purification by flash chromatography (80 g silica gel, 50% ethyl acetate/hexane, 25-mL fractions), 1.62 g (63%) of **39**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 4.79 and 4.64 (d, *J* = 7.5 Hz, 1 H), 4.22 (m, 2 H), 3.78 (m, 1 H), 3.75 and 3.70 (s, 3 H), 3.21 (m, 1 H), 1.50–2.40 (m, 11 H), 1.31 (t, *J* = 7.0 Hz, 3 H), 1.14 (m, 2 H). Anal. ($C_{16}H_{24}N_2O_4$) C, H, N.

(3*SR*,4a*SR*,6*RS*,8a*SR*)-6-(1*H*-Tetrazol-5-ylmethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (41). As for **32a**, 1.5 g (4.9 mmol) of **39** and 3.2 g (9.7 mmol) of azidotri-*n*-butylstannane gave 1.1 g (82%) of **41**: mp 282–283 °C; ^{1}H NMR (D_2O/KOD) δ 3.56 (m, 1 H0, 3.28 (m, 1 H), 2.73 (m, 1 H), 2.64 (d, *J* = 7.0 Hz, 2 H), 0.80–2.15 (m, 11 H); ^{13}C NMR (D_2O) δ 177.63, 163.28, 54.19, 41.73, 38.16, 33.15, 32.70, 32.38, 30.93, 28.36, 27.58. Anal. ($C_{12}H_{19}N_5O_2 \cdot 0.3H_2O$) C, H, N.

Ethyl (3*SR*,4a*RS*,8a*SR*)-6-[(Diethylphosphono)methylidene]-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (42). As for **25**, 0.29 g (7.3 mmol) of sodium hydride, 1.9 g (7.3 mmol) of tetraethyl methylenediphosphonate, and 0.9 g (3.0 mmol) of **22** gave, after purification by flash chromatography (70 g silica gel, ethyl acetate, 25-mL fractions), 1.1 g (85%) of **42**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers and/or *E*/*Z* olefins) δ 5.35 and 5.40 (s, 1 H), 4.98 and 4.82 (m, 1 H), 3.90–4.30 (m, 7 H), 3.74 and 3.70 (s, 3 H), 3.41 (m, 1 H), 2.67 (m, 1 H), 2.26 (m, 2 H), 1.40–2.10 (m, 4 H), 1.30 (m, 10 H), 1.15 (m, 2 H). Anal. ($C_{19}H_{32}NO_7P \cdot 0.1CHCl_3$) C, H, N.

Ethyl (3*SR*,4a*RS*,6*SR*,8a*SR*)- and (3*SR*,4a*RS*,6*RS*,8a*SR*)-6-[(Diethylphosphono)methyl]-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (44). As for **28**, hydrogenation of 1.1 g (2.5 mmol) of **42** in 50 mL of ethanol with 0.5 g of 5% Pd/C at room temperature at 60 psi for 4 h gave, after purification by flash chromatography (45 g silica gel, 5% ethanol/ethyl acetate, 25-mL fractions), 0.85 g (80%) of **44**, as a mixture of diastereomers at C_6: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 4.95 and 4.79 (m, 1 H), 3.80–4.30 (m, 8 H), 3.73 and 3.70 (s, 3 H), 2.65 (m, 1 H), 0.90–2.40 (m, 20 H), 0.78 (m, 1 H). Anal. ($C_{19}H_{34}NO_7P$) C, H, N.

(3*SR*,4a*RS*,6*SR*,8a*SR*)- and (3*SR*,4a*RS*,6*RS*,8a*SR*)-6-(Phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (46). As for **31a**, 0.8 g (1.9 mmol) of **44** in 40 mL of 6 N aqueous hydrochloric acid gave, after propylene oxide (0.67 mL) addition and reflux in ethanol and then in acetone, 0.45 g (85%) of **46**, as a mixture of diastereomers at C_6: mp 249–250 °C; ^{1}H NMR (D_2O) δ 4.20 (m, 1 H), 3.19 (m, 1 H), 0.80–2.00 (m, 13 H). Anal. ($C_{11}H_{20}NO_5P \cdot 0.6H_2O \cdot 0.25C_3H_6O$) C, H, N.

Ethyl (3*SR*,4a*RS*,8a*SR*)-6-(Cyanomethylidene)-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (43). As for **25**, 0.18 g (4.5 mmol) of sodium hydride, 0.8 g (4.5 mmol) of diethyl phosphonoacetonitrile, and 0.9 g (3.2 mmol) of **22** gave, after flash chromatography (50 g silica gel, 40% ethyl acetate/hexane, 20-mL fractions), 0.9 g (92%) of **43**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 5.12 (bs, 1 H), 5.00 and 4.81 (m, 1 H), 4.09 (m, 2 H), 3.98 (m, 1 H), 3.73 and 3.70 (s, 3 H), 1.00–3.00 (m, 14 H).

Ethyl (3*SR*,4a*RS*,6*SR*,8a*SR*)- and (3*SR*,4a*RS*,6*RS*,8a*SR*)-6-(Cyanomethyl)-2-(methoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylate (45). As for **29**, hydrogenation of 0.9 g (2.9 mmol) of **43** in 50 mL of ethanol with 0.5 of 5% Pd/C at room temperature at 60 psi overnight gave, after purification by flash chromatography (45 g silica gel, 40% ethyl acetate/hexane, 25-mL fractions), 0.7 g (77%) of **45**, as a mixture of diastereomers at C_6: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 4.99 and 4.80 (m, 1 H), 4.22 (m, 2 H), 4.04 and 3.89 (m, 1 H), 3.74 and 3.70 (s, 3 H), 2.70 (m, 1 H), 2.00–2.45 (m, 4 H), 1.40–2.00 (m, 5 H), 1.27 (m, 3 H), 1.06 (m, 3 H0, 0.85 (m, 1 H). Anal. ($C_{16}H_{24}N_2O_4$) C, H, N.

(3*SR*,4a*RS*,6*SR*,8a*SR*)- and (3*SR*,4a*RS*,6*RS*,8a*SR*)-6-(1*H*-Tetrazol-5-ylmethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic Acid (47). As for **32a**, 0.7 g (2.2 mmol) of **45** and 1.5 g (4.5 mmol) of azidotri-*n*-butylstannane gave 0.5 g (78%) of **47** as a mixture of diastereomers at C_6: mp 228–229 °C; ^{1}H NMR (D_2O) δ 4.02 (m, 1 H), 2.80–3.20 (m, 4 H), 0.70–2.40 (m, 11 H). Anal. ($C_{12}H_{19}N_5O_2$) C, H, N.

Ethyl (3*SR*)-6-[[(Trifluoromethyl)sulfonyl]oxy]-2-(methoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (49). A mixture of 15.6 g (56.0 mmol) of **48**,[39] 25.0 g (70.0 mmol) of *N*-phenyltrifluoromethansulfonimide and 9.7 mL (7.2 g, 56.0 mmol) of *N*,*N*-diisopropyl-*N*-ethylamine in 155 mL of dichloromethane was stirred overnight at room temperature and then concentrated in vacuo. Workup (water/3× ethyl acetate) and preparative HPLC of the residue (hexane to 20% ethyl acetate/hexane) afforded 19.5 g (85%) of **49**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 7.0–7.4 (m, 3 H), 5.19 and 4.98 (m, 1 H), 4.77 (dd, *J* = 14.5, 9.5 Hz, 1 H), 4.58 (dd, *J* = 14.5, 10.5 Hz, 1 H), 4.06 (m, 2 H), 3.80 and 3.76 (s, 3 H), 3.23 (m, 2 H), 1.12 (m, 3 H). Anal. ($C_{15}H_{16}F_3NO_7S$) C, H, N.

Ethyl (3*SR*)-6-Vinyl-2-(methoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (50). A mixture of 10.0 g (24.3 mmol) of **49**, 3.1 g (72.9 mmol) of lithium chloride, 0.57 g (0.49 mmol) of tetrakis(triphenylphosphine)palladium(0) and 7.3 mL (7.9 g, 25.0 mmol) of vinyl tri-*n*-butylstannane in 115 mL of 1,4-dioxane was heated at 98 °C overnight. An additional 0.38 g (0.33 mmol) of tetrakis(triphenylphosphine)palladium(0), 3.6 mL (4.0 g, 12.5 mmol) of vinyltri-*n*-butylstannane, and 1.1 g (26.0 mmol) of lithium chloride were added, and the reaction was again heated at 98 °C overnight. The reaction was cooled to room temperature, 100 mL of ethyl acetate was added, and the mixture was filtered through Celite and concentrated in vacuo. Workup (water/3× dichloromethane; 1× ether) and preparative HPLC afforded 3.8 g (54%) of **50**, which has prone to polymerization. This material was stored under nitrogen at −20 °C and used in the next step as soon as possible ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 7.0–7.3 (m, 3 H), 6.64 (dd, *J* = 17.0, 10.6 Hz, 1 H), 5.71 (d, *J* = 17.0 Hz, 1 H), 5.20 (d, *J* = 10.6 Hz, 1 H), 5.14 and 4.91 (m, 1 H), 4.75 (dd, *J* = 17.5, 7.3 Hz, 1 H), 4.55 (dd, *J* = 17.5, 7.3 Hz, 1 H), 4.07 (m, 2 H), 3.80 and 3.76 (s, 3 H), 3.19 (m, 2 H), 1.14 (m, 3 H).

Ethyl (3*SR*)-6-(Hydroxymethyl)-2-(methoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (51). A mixture of 1.5 g (5.0 mmol) of **50** and 1.5 mL of a 0.1 M solution of osmium tetraoxide in toluene in 17 mL of 1,4-dioxane was stirred 10 min at room temperature, and then 2.1 g (10.0 mmol) of sodium metaperiodate was added in three portions over 20 min. After 30 min more at room temperature, the reaction was worked up (water/3× ether; 1× dichloromethane) to afford 1.4 g (99%) of the aldehyde. This material was dissolved in 15 mL of ethanol, cooled to 0 °C, and treated with 0.19 g (4.9 mmol) of sodium borohydride, and after 20 min at 0 °C, the mixture was concentrated in vacuo. Workup (water/3× dichloromethane; 1× ether) afforded 1.4 g (97%) of **51** and used without further purification: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 7.00–7.35 (m, 3 H), 5.14 and 4.91 (m, 1 H), 4.50–4.80 (m, 3 H), 4.07 (m, 2 H), 3.80 and 3.76 (s, 3 H), 3.56 (m, 1 H), 3.21 (m, 2 H), 1.12 (m, 3 H).

Ethyl (3*SR*)-6-(Bromomethyl)-2-(methoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (52). To a 0 °C suspension of triphenylphosphine dibromide (prepared from 3.0 g (11.4 mmol) of triphenylphosphine and 0.6 mL (1.8 g, 11.4 mmol) of bromine) in 47 mL of dichloromethane was added 2.4 g (8.2 mmol) of **51** and 0.9 mL (0.9 g, 11.4 mmol) of pyridine in 16 mL of dichloromethane. After 35 min at 0 °C, the reaction was worked up (10% aqueous sodium bisulfate/3× dichloromethane; 1× ether). Purification by flash chromatography (150 g silica gel, 30% ethyl acetate/hexane, 50-mL fractions) gave 1.9 g (66%) of **52**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 7.05–7.30 (m, 3 H), 5.16 and 4.93 (m, 1 H), 4.76 (dd, *J* = 16.5, 9.2 Hz, 1 H), 4.56 (dd, *J* = 16.5, 6.0 Hz, 1 H), 4.45 (s, 2 H), 4.08 (m, 2 H), 3.80 and 3.76 (s, 3 H), 3.19 (m, 2 H), 1.12 (m, 3 H). Anal. ($C_{15}H_{18}BrNO_4$) C, H, N.

Ethyl (3*SR*)-6-[(Diethylphosphono)methyl]-2-(methoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (53). A solution of 0.9 g (2.4 mmol) of **52** and 0.9 g (5.4 mmol) of triethyl phosphite was heated to reflux for 5 mL of toluene overnight. The solution was cooled and concentrated in vacuo, and the residue was purified by flash chromatography (50 g silica gel, 75% ethyl acetate/hexane, 30-mL fractions) to afford 0.9 g (85%) of **53**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 7.00–7.20 (m, 3 H), 5.13 and 4.91 (m, 1 H), 4.73 (m, 1 H), 4.54 (m, 1 H), 4.02 (m, 6 H), 3.80 and 3.76 (s, 3 H), 3.16 (m, 2 H), 3.09 (d, $J_{^1H-^{31}P}$ = 21.0 Hz, 2 H), 1.25 (m, 6 H), 1.12 (m, 3 H). Anal. ($C_{19}H_{28}NO_7P$) H, N; C: calcd, 55.20; found, 54.51.

(3*SR*)-6-(Phosphonomethyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (55). As for **31a**, 0.8 g (2.0 mmol) of **53** in 50 mL of 6 N aqueous hydrochloric acid gave, after propylene oxide (0.28 mL) addition and reflux in ethanol and then in acetone, 0.45 g (85%) of **55**: mp >300 °C; ^{1}H NMR (D_2O/KOD) δ 7.00–7.20 (m, 3 H), 3.88 (m, 2 H), 3.38 (dd, *J* = 11.5, 4.0 Hz, 1 H), 2.97 (dd, *J* = 16.0, 4.0 Hz, 1 H), 2.80 (m, 1 H), 2.77 (d, $J_{^1H-^{31}P}$ = 21.0 Hz, 2 H). Anal. ($C_{11}H_{14}NO_5P \cdot 0.25H_2O$) C, N; H: calcd, 5.30; found, 4.82.

Ethyl (3*SR*)-6-(Cyanomethyl)-2-(methoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (54). A mixture of 0.9 g (2.4 mmol) of **52** and 0.3 g (5.3 mmol) of sodium cyanide in 5 mL of dimethyl sulfoxide was heated to 70 °C for 45 min. The reaction was cooled to room temperature and worked up (water and brine/4× dichloromethane; 1× ether). Purification by flash chromatography (40 g silica gel, 40% ethyl acetate/hexane, 30-mL fractions) gave 0.6 g (83%) of **54**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 7.13 (m, 3 H), 5.18 and 4.96 (m, 1 H), 4.77 (dd, *J* = 15.6, 9.2 Hz, 1 H), 4.56 (dd, *J* = 15.6, 6.5 Hz, 1 H), 4.07 (m, 2 H), 3.80 and 3.76 (s, 3 H), 3.71 (s, 2 H), 3.20 (m, 2 H), 1.13 (m, 3 H). Anal. ($C_{16}H_{18}N_2O_4$) C, H, N.

(3*SR*)-6-(1*H*-Tetrazol-5-ylmethyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (56). As for **32a**, 1.4 g (4.6 mmol) of **54** and 3.0 g (9.2 mmol) of azidotri-*n*-butylstannane gave 0.7 g (58%) of **56**: mp 256–258 °C; ^{1}H NMR (D_2O/KOD) δ 6.90 (m, 3 H), 3.94 (s, 2 H), 3.87 (m, 2 H), 3.25 (m, 1 H), 2.84 (m, 1 H), 2.61 (m, 2 H). Anal. ($C_{12}H_{13}N_5O_2 \cdot 0.75H_2O$) C, H, N.

Ethyl (1*SR*)-6-Hydroxy-2-(*tert*-butoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-1-carboxylate (57). A mixture of 36.5 g (0.17 mol) of 2-(3-hydroxyphenyl)-1-aminoethane hybromide and 23.1 g (0.25 mol) to glyoxylic acid monohydrate in 500 mL of 5% aqueous hydrochloric acid was heated to 80 °C for 6.5 h, then cooled, and concentrated in vacuo. The residue was dissolved in 1200 mL of ethanol, hydrochloric acid (g) was bubbled into the solution for 10 min, and the mixture was heated to reflux overnight, then cooled, and concentrated in vacuo. To the resultant orange solid was added 400 mL of dichloromethane, 29 mL (21.6 g, 0.17 mol) of *N*,*N*-diisopropyl-*N*-ethylamine and 30 mL (28.5 g, 0.13 mol) of di-*tert*-butyl dicarbonate in four 7.5-mL portions at room temperature over 1 h. After stirring for another 45 min, 6 mL (4.5 g, 34.4 mmol) of *N*,*N*-diisopropyl-*N*-ethylamine was added to adjust the pH from 5 to 7. After another 30 min, workup (10% aqueous sodium bisulfate/2× dichloromethane; 1× ether) and preparative HPLC gave 33.6 g (62%) of **57**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 7.30 (m, 1 H), 6.71 (m, 1 H), 6.64 (m, 1 H), 6.27 (m, 1 H), 5.48 and 5.33 (s, 1 H), 4.16 (m, 2 H), 3.78 (m, 1 H), 3.69 (m, 1 H), 2.88 (m, 1 H), 2.75 (m, 1 H), 1.09 and 1.07 (s, 9 H), 1.25 (m, 3 H). Anal. ($C_{17}H_{23}NO_5$) C, H, N.

Ethyl (1-*SR*)-6-Hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylate Hydrochloride (58). A solution of 20.0 g (62.2 mmol) of **57** and 90 mL of trifluoroacetic acid in 250 mL of dichloromethane was stirred for 2 h at room temperature and then concentrated in vacuo. The residue was treated with 200 mL of 1 N aqueous hydrochloric acid and then concentrated in vacuo. The resultant solid was filtered, washing three times with ether, once with ethanol, twice with ether, and once with pentane and then dried in vacuo at room temperature to afford 12.0 g of **58**: mp 218.5–219.5 °C; ^{1}H NMR (D_2O) δ 7.39 (d, *J* = 8.8 Hz, 1 H), 6.78 (dd, *J* = 8.8, 2.6 Hz, 1 H), 6.74 (d, *J* = 2.6 Hz, 1 H), 5.33 (s, 1 H), 4.29 (m, 2 H), 3.56 (m, 2 H), 3.02 (m, 2 H), 1.25 (t, *J* = 7.1 Hz, 3 H). Anal. ($C_{12}H_{16}ClNO_3$) C, H, N.

Ethyl (1*SR*,4a*RS*,8a*SR*)-6-Oxo-2-(*tert*-butoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-1-carboxylate (23). A solution of 12.0 g (46.6 mmol) of **58** in 282 mL of ethanol was hydrogenated with 6.0 g of 5% Rh/C at 60 °C at 60 psi for 48 h. The mixture was filtered through Celite

and concentrated in vacuo to an oil. This was dissolved in 200 mL of dichloromethane and treated with 15.0 mL (11.1 g, 86.1 mmol) of *N,N*-diisopropyl-*N*-ethylamine and 10.7 mL (10.2 g, 46.6 mmol) of di-*tert*-butyl dicarbonate for 4 h at room temperature. Workup as for **57** afforded 16.4 g of an oil, used without purification. This material was oxidized as described earlier[39] with 23.7 g (110.0 mmol) of pyridinium chlorochromate (PCC) and 24.3 g of powdered 4-Å molecular sieves in 230 mL of dichloromethane to afford, after preparative HPLC (hexane to 50% ethyl acetate/hexane), 2.1 g (14% from **58**) of a 95:5 mixture of **59**/**23** by GC.[43] This material was dissolved in 50 mL of ethanol, treated with 1.0 mL of a solution of sodium ethoxide in ethanol (from 0.42 g of sodium hydride in 15 mL of ethanol; ca. 0.7 mmol/mL), and equilibrated as described earlier.[39] Purification of the residue by flash chromatography (100 g silica gel, 10–50% ethyl acetate/hexane, 50-mL fractions) gave 2.0 g (13% from **58**) of **23**, one diastereomer by GC.[43] **59**: ^{1}H NMR ($CDCl_3$) δ 4.22 (m, 3 H), 3.77 (m, 1 H), 3.40 (m, 1 H), 2.10–2.60 (m, 7 H), 1.91 (m, 1 H), 1.68 (m, 1 H), 1.54 (m, 1 H), 1.45 (s, 9 H), 1.30 (t, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 210.68, 171.11, 155.09, 80.53, 60.88, 59.05, 45.24, 39.47, 35.68, 35.60, 28.22, 26.69, 23.47, 14.14. **23**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers) δ 4.80 and 4.60 (s, 1 H), 4.21 (m, 2H), 4.11 (m, 1H), 3.98 (m, 1H), 1.80–3.00 (m, 10H), 1.40 (m, 9H), 1.27 (m, 3 H). ^{13}C NMR ($CDCl_3$, doubling due to amide rotamers) δ 210.09 and 209.73, 171.18 and 171.07, 156.07 and 155.48, 80.29, 61.33, 59.41, 58.12, 46.53, 41.57, 40.64 and 40.42, 35.33, 33.91 and 33.68, 28.29, 25.94, 25.33 and 25.13, 14.22. **59**: Anal. ($C_{17}H_{27}NO_5$) C, H, N. **23**: Anal. ($C_{17}H_{27}NO_5$) C, H, N.

Ethyl (1*SR*,4a*RS*,8a*SR*)-6-[(Diethylphosphono)methylidene]-2-(*tert*-butoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-1-carboxylate (60). As for **25**, 9.0 mL (9.0 mmol) of 1.0 M in THF, sodium bis(trimethylsilyl)amide, 2.6 g (9.0 mmol) of tetraethyl methylenediphosphonate, and 2.1 g (6.5 mmol) of **23** gave, after purification by flash chromatography (140 g silica gel, 5% ethanol/ethyl acetate, 50-mL fractions), 2.6 g (87%) of **60**. ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers and/or *E*/*Z* isomers) δ 5.44 and 5.33 (m, 1 H), 4.68 and 4.50 (m, 1 H), 3.90–4.30 (m, 7 H), 3.39 (m, 1 H), 2.92 (m, 1 H), 1.50–2.60 (m, 9 H), 1.46 and 1.44 (s, 9 H), 1.32 (m, 9 H), 1.32 (m, 9 H). Anal. ($C_{22}H_{38}NO_7P$) C, H, N.

Ethyl (1*SR*,4a*RS*,6*SR*,8a*SR*)- and (1*SR*,4a*RS*,6*RS*,8a*SR*)-6-[(Diethylphosphono)methyl]-2-(*tert*-butoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-1-carboxylate (62). As for **28**, hydrogenation of 2.4 g (5.1 mmol) of **60** in 100 mL of ethanol with 0.3 g of 5% Pd/C at room temperature at 60 psi overnight gave 2.1 g (90%) of **62** as a mixture of diastereomers at C_6: ^{1}H NMR ($CDCl_3$) δ 4.00–4.30 (m, 9 H), 3.78 (m, 1 H), 3.15 (m, 1 H), 2.50 (m, 1 H), 2.14 (m, 2 H), 1.50–2.10 (m, 8 H), 1.43 (m, 9 H), 1.30 (m, 9 H). Anal. ($C_{22}H_{40}NO_7P$) C, H, N.

(1*SR*,4a*RS*,6*SR*,8a*SR*)- and (1*SR*,4a*RS*,6*RS*,8a*SR*)-6-(Phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-1-carboxylic Acid Hydrochloride Salt (64). As for **31a**, 1.8 g (3.9 mmol) of **62** in 40 mL of 6 N aqueous hydrochloric acid gave, after washing with acetone and ether, 0.9 g (76%) of **64** as a mixture of diastereomers at C_6: mp 231–232 °C; ^{1}H NMR (D_2O) δ 4.04 (m, 1 H), 3.19 (m, 2 H), 1.10–2.10 (m, 13 H). Anal. ($C_{11}H_{21}ClNO_5P\cdot0.5H_2O$) C, H, N.

Ethyl (1*SR*,4a*RS*,8a*SR*)-6-(Cyanomethylidine)-2-(*tert*-butoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-1-carboxylate (61). As for **25**, 0.37 g (9.1 mmol) of sodium hydride, 1.6 g (9.1 mmol) of diethyl phosphonoacetonitrile, and 2.5 g (7.6 mmol) of **23** gave, after purification by flash chromatography (130 g silica gel, 35% ethyl acetate/hexane, 50-mL fractions), 2.1 g (81%) of **61**: ^{1}H NMR ($CDCl_3$, doubling due to amide rotamers and/or *E*/*Z* olefin isomers) δ 5.18 and 5.07 (s, 1 H), 4.54 and 4.73 (m, 1 H), 4.20 (m, 3 H), 3.98 (m, 1 H), 1.70–3.10 (m, 10 H), 1.45 and 1.43 (s, 9 H), 1.29 (m, 3 H). Anal. ($C_{19}H_{28}N_2O_4$) C, H, N.

Ethyl (1*SR*,4a*RS*,6*SR*,8a*SR*)- and (1*SR*,4a*RS*,6*RS*,8a*SR*)-6-(Cyanomethyl)-2-(*tert*-butoxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-1-carboxylate (63). As for **29**, hydrogenation of 1.9 g (5.5 mmol) of **61** in 100 mL of ethanol with 0.2 g of 5% Pd/C at room temperature at 60 psi overnight gave 1.8 g (93%) of **63** as a mixture of diastereomers at C_6: ^{1}H NMR ($CDCl_3$) δ 4.20 (m, 3 H), 3.77 (m, 1 H), 3.19 (m, 1 H), 2.15–2.40 (m, 3 H), 1.60–1.90 (m, 6 H), 1.45 (m, 12 H), 1.26 (m, 4 H). Anal. ($C_{19}H_{30}N_2O_4\cdot0.08CHCl_3$) C, H, N.

(1*SR*,4a*RS*,6*SR*,8a*SR*)- and (1*SR*,4a*RS*,6*RS*,8a*SR*)-6-(1*H*-Tetrazol-5-ylmethyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-1-carboxylic Acid (65). As for **32a**, 1.6 g (4.6 mmol) of **63** and 3.0 g (9.1 mmol) of azidotri-*n*-butylstannane gave 0.9 g (74%) of **65** as a mixture of diastereomers at C_6: mp 130–131 °C; ^{1}H NMR (D_2O) δ 3.72 (m, 1 H), 3.17 (m, 2 H), 2.82 (m, 2 H), 1.10–2.20 (m, 11 H). Anal. ($C_{12}H_{19}N_5O_2\cdot0.75H_2O\cdot0.1C_3H_6O$) C, H, N.

Methyl 5-Oxo-1-(*tert*-butoxycarbonyl)-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylate (24) and 5-Oxo-1-(*tert*-butoxycarbonyl)-2,3,3a,4,5,6,7,7a-octahydroindole (66). A solution of 25 g (0.14 mol) of 5-hydroxyindole-2-carboxylic acid in 355 mL of water was hydrogenated with 20.0 g of 5% Rh/Al_2O_3 at 60 °C at 60 psi overnight. The reaction was filtered through Celite and concentrated in vacuo. ^{1}H NMR showed the presence of starting material, and so the residue was rehydrogenated in 355 mL of water with 20.0 g of 5% Rh/Al_2O_3 at 60 °C at 60 psi overnight. The reaction was again filtered through Celite and concentrated in vacuo. ^{1}H NMR now showed complete reaction. The residue was dissolved in 1000 mL of methanol, hydrochloric acid (g) was bubbled into the solution for 10 min, followed by the addition of 100 mL of trimethylorthoformate, and then the mixture was stirred 96 h at room temperature and concentrated in vacuo. To the residue was added 500 mL of dichloromethane, 55 mL (41 g, 0.32 mol) of *N,N*-diisopropyl-*N*-ethylamine, and 32 mL (30 g, 0.14 mol) of di-*tert*-butyl dicarbonate in two 16-mL portions at room temperature over 10 min. After another 60 min, the reaction was worked-up (10% aqueous sodium bisulfate/2× dichloromethane; 1× ether). This material was oxidized as described earlier[39] with 50 g (0.23 mol) of PCC and 50 g of powdered 4-Å molecular sieves in 400 mL of dichloromethane to afford, after preparative HPLC, 9.1 g (22%) of **24** and 4.56 g (14%) of **66**. Recrystallization of 9.1 g of **24** from ether/hexane gave 6.6 g of **24**,[43] mp 98–100 °C. **24**: ^{1}H NMR ($CDCl_3$) δ 4.20 (m, 2 H), 3.72 (s, 3 H), 2.75 (m, 1 H), 2.54 (m, 1 H), 2.35 (m, 5 H), 2.08 (m, 1 H), 1.76 (m, 1 H), 1.40 (bs, 9 H); ^{13}C NMR ($CDCl_3$) δ 210.61, 173.37, 154.73, 80.45, 59.40, 55.98, 52.17, 41.43, 36.94, 35.47, 28.29, 26.17. **66**: ^{1}H NMR ($CDCl_3$) δ 4.00 (m, 1 H), 3.40 (m, 2 H), 2.67 (m, 1 H), 2.55 (m, 1 H), 2.28 (m, 3 H), 2.01 (m, 1 H), 1.88 (m, 1 H), 1.64 (m, 2 H), 1.45 (s, 9 H), 1.43 (m, 1 H). **24**: Anal. ($C_{15}H_{23}NO_5$) C, H, N.

Methyl 5-[(Diethylphosphono)methylidene]-1-(*tert*-butoxycarbonyl)-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylate (67). As for **25**, 0.56 g (14.1 mmol) of sodium hydride, 4.1 g (14.1 mmol) of tetraethyl methylenediphosphonate, and 3.0 g (10.1 mmol) of **24** gave, after purification by flash chromatography (160 g silica gel, 5% ethanol/ethyl acetate, 50-mL fractions), 4.1 g (94%) of **67**, as a *E*/*Z* mixture of olefin isomers: ^{1}H NMR ($CDCl_3$) δ 5.49 and 5.42 (d, $J_{^1H-^{31}P}$ = 18.0 Hz, 1 H), 4.22 (m, 1 H), 4.06 (m, 5 H), 3.73 (s, 3 H), 3.33 and 3.22 (m, 1 H), 1.60–2.70 (m, 8 H), 1.43 (m, 9 H), 1.36 (m, 6 H). Anal. ($C_{20}H_{34}NO_7P$) C, H, N.

Methyl 5-[(Diethylphosphono)methyl]-1-(*tert*-butoxycarbonyl)-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylate (69). As for **28**, hydrogenation of 4.0 g (9.3 mmol) of **67** in 95 mL of ethanol with 1.0 g of 5% Pd/C at room temperature at 60 psi for 2 h gave 3.5 g (87%) of **69** as a mixture of diastereomers at C_5: ^{1}H NMR ($CDCl_3$) δ 4.25 (m, 1 H), 4.09 (m, 4 H), 3.78 (m, 1 H), 3.74 (s, 3 H), 1.50–2.40 (m, 9 H), 1.44 (m, 9 H), 1.30 (m, 9 H). Anal. ($C_{20}H_{36}NO_7P$) C, H, N.

5-(Phosphonomethyl)-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic Acid (71). A mixture of 3.4 g (7.8 mmol) of **69** and 100 mL of 6 N aqueous hydrochloric acid were heated to reflux overnight, then cooled, and concentrated in vacuo. After treatment of the residue with 1.6 mL of propylene oxide in 50 mL of water for 1.5 h at 50 °C, the mixture was concentrated in vacuo. Ion-exchange chromatography of the residue on Dowex 50×-8, eluting with water, afforded 1.37 g (67%) of **71** (foam) as a mixture of diastereomers at C_5. ^{1}H NMR (D_2O) δ 4.24 (m, 1 H), 3.69 (m, 1 H), 1.40–2.60 (m, 10 H), 1.17 (m, 1 H), 0.80 (m, 1 H). Anal.[49] ($C_{10}H_{18}NO_5P\cdot0.3H_2O\cdot0.35NH_3$) C, H, N.

Methyl 5-(Cyanomethylidene)-1-(*tert*-butoxycarbonyl)-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylate (68). As for **25**, 0.57 g (14.1 mmol) of sodium hydride, 2.5 g (14.1 mmol) of diethyl phosphonoacetonitrile, and 3.0 g (10.1 mmol) of **24** gave,

after purification by flash chromatography (200 g silica gel, 35% ethyl acetate/hexane, 40-mL fractions), 3.1 g (97%) of **68**. Anal. ($C_{17}H_{24}N_2O_4$) C, H, N.

Methyl 5-(Cyanomethyl)-1-(*tert*-butoxycarbonyl)-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylate (70). As for **29**, hydrogenation of 3.1 g (9.6 mmol) of **68** in 95 mL of ethanol with 1.0 g of 5% Pd/C at room temperature at 60 psi for 1 h gave, after purification by flash chromatography (120 g silica gel, 35% ethyl acetate/hexane, 100-mL fractions), 2.2 g (73%) of **70** as a mixture of diastereomers at C_5. Anal. ($C_{17}H_{26}N_2O_4$) C, H, N.

5-(1*H*-Tetrazol-5-ylmethyl)-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic Acid (72). As for **32a**, 2.1 g (6.6 mmol) of **70** and 4.4 g (13.1 mmol) of azidotri-*n*-butylstannane gave 1.4 g (86%) of **72** as a mixture of diastereomers at C_5: mp 154-157 °C; ^{1}H NMR (D_2O) δ 4.12 (m, 1 H), 3.72 (m, 1 H), 2.82 (m, 2 H), 1.40-2.50 (m, 8 H), 1.12 (m, 1 H), 0.75 (m, 1 H). Anal. ($C_{11}H_{17}N_5O_2 \cdot 0.5H_2O$) C, H, N.

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Carboxamide Group Conformation in the Nicotinamide and Thiazole-4-carboxamide Rings: Implications for Enzyme Binding†

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Ab initio computations (RHF/6-31G*//3-21G*) were performed on the thiazole-4-carboxamide group found in the antitumor drug tiazofurin and its dehydrogenase-binding anabolite thiazole-4-carboxamide adenine dinucleotide (TAD). Results indicate that the carboxamide group is constrained in the conformation in which the amino group is cis-planar to the ring nitrogen. This finding is consistent with carboxamide conformations observed in crystal structures of the thiazole nucleosides. In contrast, ab initio computations on the nicotinamide and dihydronicotinamide rings found in the cofactors NAD^+ and NADH indicate two stable conformations for the carboxamide group. This finding confirms previous computational studies and is consistent with results from a survey of the Cambridge Structural Database. Natural bond orbital analysis indicates that the low-energy carboxamide conformers of all three heterocycles are stabilized by a combination of electrostatic and charge transfer interactions. A survey of the Protein Data Bank indicates that the carboxamide group conformation in TAD is constrained to that favored by dehydrogenase-bound NAD(P)(H).

Introduction

The thiazole nucleoside tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC 286193) has demonstrated clinically effective antitumor activity.[1,2] Recently, attention has also focused on tiazofurin's ability to induce differentiation in neoplastic cells[3-6] and to inhibit G-protein-mediated signaling mechanisms.[6-9] Each of these biological effects appears related to a shutdown of guanine nucleotide synthesis[1-5,10] produced by a dinucleotide anabolite of tiazofurin called tiazofurin adenine dinucleotide (TAD, Figure 1a).[11-13] TAD is a neutral analogue of the cofactor nicotinamide adenine dinucleotide (NAD^+) in which the nicotinamide ring is replaced by the thiazole-4-carboxamide group (Figure 1b).[11-13] TAD acts as an inhibitor of inosine monophosphate dehydrogenase (IMPd), the NAD^+-dependent enzyme catalyzing the rate-limiting step in guanine nucleotide synthesis.[14] Kinetic and modeling studies suggest that TAD inhibits IMPd by mimicking NAD binding at the cofactor site.[15,16]

Although there is no crystal structure of an IMPd-TAD complex, the structural features of free tiazofurin have been studied extensively by X-ray crystallography[17] and quantum chemical computations.[18] X-ray crystallographic studies of a series of thiazole nucleosides[17-21] suggest that the TAD molecule possesses two unique conformational restrictions compared with NAD^+ and NADH. Rotation about both the C-glycosidic bond (C1'-C2) and about the bond to the carboxamide group (C4-C6) is constrained

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† Abbreviations: TAD: thiazole-4-carboxamide adenine dinucleotide; IMPd: inosine monophosphate dehydrogenase; NAD^+: nicotinamide adenine dinucleotide; NADH: dihydronicotinamide adenine dinucleotide; NADP: nicotinamide adenine dinucleotide phosphate; NADPH: dihydronicotinamide adenine dinucleotide phosphate; NAD(P)(H): NAD^+ or NADH or $NADP^+$ or NADPH; NBO: natural bond orbital.

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