of rat brain tissue (0.2–0.3 mg protein/mL) in the presence of various concentrations of test compounds. Binding was measured by liquid-scintillation counting after standard vacuum filtration and separation of bound tissue from free radioactivity.[27]

Effects of Subacute Treatments on Receptor Binding. Two concentrations of [^{3}H]dihydroalprenolol (DHA) and [^{3}H]-spiperone were employed to estimate β-adrenergic and serotonergic (5-HT_2) receptor binding.

Three groups of 10 rats each were administered either 0.9% saline (1 mL/kg) or 4·HCl (10 mg/kg). All injections were given ip twice a day for 14 days; 24 h after the last injection, rats were decapitated and the frontal cortex (for 5-HT_2) and the remainder of the cortex (for β-adrenergic binding) were dissected out and frozen at −70 °C for subsequent assay in the appropriate in vitro receptor binding assay.

Synaptosomal Uptake. Rat brain cortical synaptosomes were prepared by modification of the sucrose density gradient/vertical rotor centrifugation method of Wood and Wyllie.[19,28] Uptake of [^{3}H]NE and [^{14}C]-5-HT was measured by incubating synaptosomes with both labeled substrates in the presence of varying concentrations of the test compound. After incubation, the mixtures were filtered through 0.45-μM cellulose acetate filters, and residual radioactivity was determined by liquid-scintillation counting. Uptake in the presence of test compound was expressed as a percent of basal uptake (without test compound) over 4 min at 37 °C. An IC_{50} was calculated by determining the concentration of named test compound which resulted in 50% of basal uptake.

Antagonism of Reserpine-Induced Hypothermia. Test compounds, suspended or dissolved in 0.25% Tween 80 in water, were administered ip or po at several dose levels (1, 3, 10, 30, 100, mg/kg) to male mice which had been pretreated (18 h) with 5.0 mg/kg reserpine subcutaneously. Rectal temperatures were recorded at intervals of 1, 2, and 3 h after test-drug administration. Two-way analysis of variance with subsequent Dunnett's comparison to control tests were used to determine the minimally effective dose (MED) for antagonism of hypothermia.

Antagonism of Histamine-Induced ACTH Release. Male Sprague–Dawley rats (200–300 g, Charles River) were used for this assay, an adaptation of the procedure of Reilly and Sigg.[15] Test compounds (10 mg/kg) dissolved in saline, were administered ip 1 h before the injection of either saline or histamine (5 mg/kg, ip). Ten minutes following the second injection, the animals were decapitated and trunk blood samples were collected. Serum samples were prepared through two 2-min centrifugations at 4 °C, divided into 300 μM aliquots, and frozen at −60 °C until radioimmunoassay for ACTH concentrations. These were determined with a commercially available kit (Immuno Nuclear Corp., Stillwater, MN). Results were expressed as pg ACTH/mL serum and subjected to a two-way analysis of variance with Dunnett's comparison to control with Student–Neuman–Keuls multiple comparison tests.

Induction of Noradrenergic Subsensitivity in the Rat Pineal Gland. Male Sprague–Dawley rats were injected twice daily with either saline or a test compound (10 or 30 mg/kg, ip) for 5 days (total of nine injections) and maintained in continuous light throughout the experiment. Another group of rats received saline injections twice daily for 5 days, followed by a single injection of test compound (10 or 30 mg/kg, i.p.) on the 5th day. One hour following the final injection of the test compound or saline, animals were administered either 0.1% ascorbic acid (controls) or isoproterenol (2 μmol/kg in 0.1% ascorbic acid). Rats were decapitated 2.5 min later and the pineal glands were removed, homogenized in perchloric acid, and centrifuged. The cAMP content of the neutralized extract was measured by radioimmunoassay[29] with ^{125}I-labeled antigen and antiserum (New England Nuclear Corp., Boston, MA). Results were calculated as pmol cAMP/pineal and expressed as a percent change from the control isoproterenol-stimulated values. Analysis of variance with Student–Neuman–Keuls tests was used to determine significant differences from control values.

Acknowledgment. We thank Dr. P. Swaminathan for helpful discussions.

Supplementary Material Available: Tables of final atomic positional parameters, atomic thermal parameters, and bond distances and angles for (+)-4·HBr (3 pages). Ordering information is given on any current masthead page.

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Synthesis and Activity of a Potent *N*-Methyl-D-aspartic Acid Agonist, *trans*-1-Aminocyclobutane-1,3-dicarboxylic Acid, and Related Phosphonic and Carboxylic Acids

Robin D. Allan,*[†] Jane R. Hanrahan,[†] Trevor W. Hambley,[‡] Graham A. R. Johnston,[†] Kenneth N. Mewett,[†] and Ann D. Mitrovic[†]

Department of Pharmacology and School of Chemistry, The University of Sydney, New South Wales 2006, Australia.
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We report the synthesis of a series of 3-carboxy-, 3-(carboxymethyl)-, 3-(ω-phosphonoalkenyl)-, and 3-(ω-phosphonoalkyl)-1-aminocyclobutane-1-carboxylic acids for evaluation as agonists or antagonists of neurotransmission at excitatory amino acid receptors, particularly *N*-methyl-D-aspartic acid (NMDA) receptors. The compounds were evaluated as agonists on their ability to depolarize the rat brain cortical wedge preparation or as antagonists of the actions of the selective agonists NMDA, quisqualic acid, and kainic acid. The chain-elongated glutamate derivatives with potential antagonist activity proved to be weak and frequently nonselective antagonists in this assay. The most noteworthy result was that trans isomer **7b** was a very potent agonist, approximately 20 times more active than NMDA at NMDA receptors, while the cis isomer was $^1/_3$ as potent as NMDA.

Of the ligands responsible for neurotransmission at excitatory amino acid receptors in the mammalian central nervous system (CNS), the excitatory amino acids L-glutamic acid and L-aspartic acid appear to be the most important.[1-3] They are, however, rather poor tools for the understanding of this phenomenon since they have other

[†] Department of Pharmacology.
[‡] School of Chemistry.

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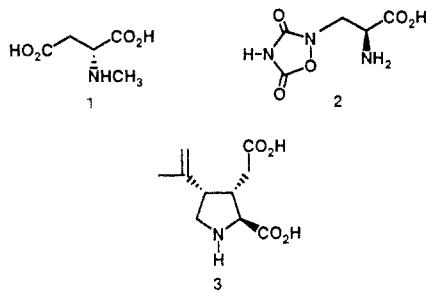


Figure 1. Selective receptor agonists.

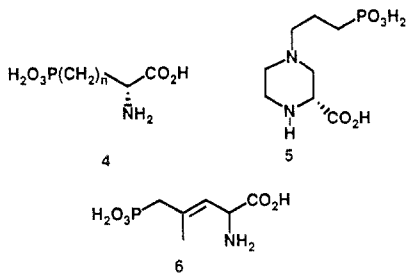


Figure 2. Selective NMDA antagonists.

biochemical roles and their actions are relatively nonselective at receptor subtypes. The development and introduction of selective agonists and antagonists have permitted the classification of excitatory amino acid receptors into at least three subtypes[4-6] as defined by the biochemical[7,8] and electrophysiological[9,10] actions of the protypic agonists *N*-methyl-D-aspartic acid (**1**), L-quisqualic acid (**2**), and kainic acid (**3**) (Figure 1). The agonists **2** and **3** are conveniently combined for broad classification as being "non-NMDA" agonists since no really selective antagonists have yet been developed that unequivocally differentiate between the actions of these agonists which may possibly act at the same receptor.[6] A fourth subytpe, the AP4 receptor, described by the electrophysiological inhibition of certain synaptic responses[5] by L-(−)-2-amino-4-phosphonobutanoic acid (AP4, L isomer of **4**, n = 2), is the least well-characterized or understood of the excitatory amino acid receptors.[5,6]

A major interest in excitatory amino acids is currently centered on the role of endogenous agonists in the etiology of neurodegenerative disorders such as Alzheimer's[11,12] and Hungtington's[13] disease and also in the possible therapeutic application of antagonists as neuroprotective agents minimizing neuronal damage resulting from ischemia.[14,15] Both competitive and noncompetitive NMDA antagonists

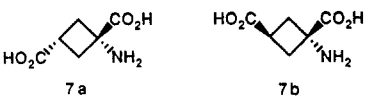
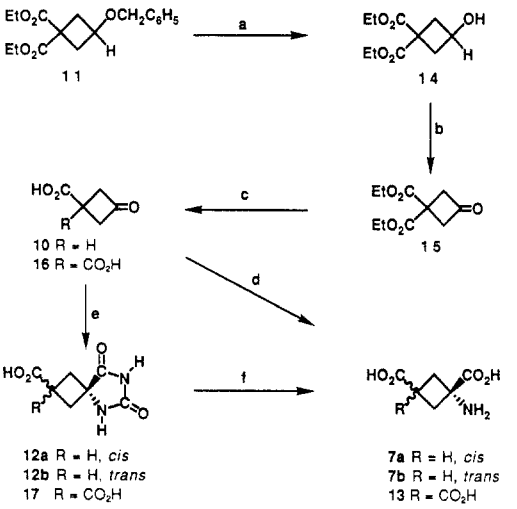


Figure 3. *cis*- and *trans*-1-aminocyclobutane-1,3-dicarboxylic acids.

Scheme I[a]



[a] (a) H_2, 10% Pd/C, EtOH; (b) RuO_4, $NaIO_4$, H_2O, CCl_4; (c) (i) KOH, H_2O, EtOH, (ii) 6 M HCl; (d) (i) NH_4OH, KCN, NH_4Cl, H_2O, (ii) 6 M HCl; (e) (i) NH_4OH, "ammonium carbonate", KCN, H_2O, MeOH, (ii) 6 M HCl; (f) (i) 0.5 M NaOH, reflux, (ii) 6 M HCl.

are also under investigation as antileptic[16] and anticonvulsant agents.[17,18] A considerable number of potent and selective NMDA antagonists have become available over recent years whereas few selective quisqualic acid or kainic acid antagonists have been developed.

Structure–activity studies by Watkins et al.[19,20] showed that the homologue of glutamic acid, D-α-aminoadipic acid, was a selective but weak NMDA antagonist. From expansion of this work, features necessary for NMDA antagonists appear to be (i) an α-amino acid with a second acidic function, preferably a phosphonic acid group, separated from the carboxylic acid by either four or six atoms, (ii) either an unsubstituted α-amino group or an amino group incorporated into a ring system, and (iii) the D configuration of the amino acid. On the basis of these findings, Watkins and colleagues have developed the competitive acylic ω-phosphono-α-amino acids D-2-amino-5-phosphonopentanoic acid[1,21] (D-AP5; **4**, n = 3, Figure 2) and D-2-amino-7-phosphonoheptanoic acid[1] (D-AP7; **4**, n = 5, Figure 2) and the considerably more potent cyclic analogue 4-(3-phosphonoprop-1-yl)piperazine-2-carboxylic acid[22,23] (CPP; **5**, Figure 2), the unsaturated

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analogue of which is more active.[23] A very recent advance has been the report that the simpler unsaturated structure (*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849; **6**, Figure 2) is a very potent NMDA antagonist which is reported to have a long duration of anticonvulsant activity after oral dosing.[24]

Our interest in the synthesis and pharmacological evaluation of cyclobutane analogues of glutamic acid, AP5 and AP7 (**4**, n = 3 and 5 respectively), was stimulated by a literature report[25] that the legume *Ateleia herbert smithii* Pittier (leguminosae), a native of Costa Rica, produces seeds which apparently contain "antifeed" ingredients since the seeds are ignored by most insect seed predators. One of the constituents isolated from the seeds was identified as the non-protein amino acid *cis*-1-aminocyclobutane-1,3-dicarboxylic acid (**7a**, Figure 3). No biological action was ascribed to this component and NMDA receptors may not be involved since the existence of NMDA-sensitive receptors in invertebrates has yet to be established.[6] We report here the synthesis of the cis (**7a**) and trans (**7b**, Figure 3)[26] isomers as well as phosphonic acids including **8a** and **8b** (Scheme III) and **9a** and **9b** (Scheme IV), which are cyclic analogues of AP5 and AP7 and which can also be considered as cyclic analogues of **6**.

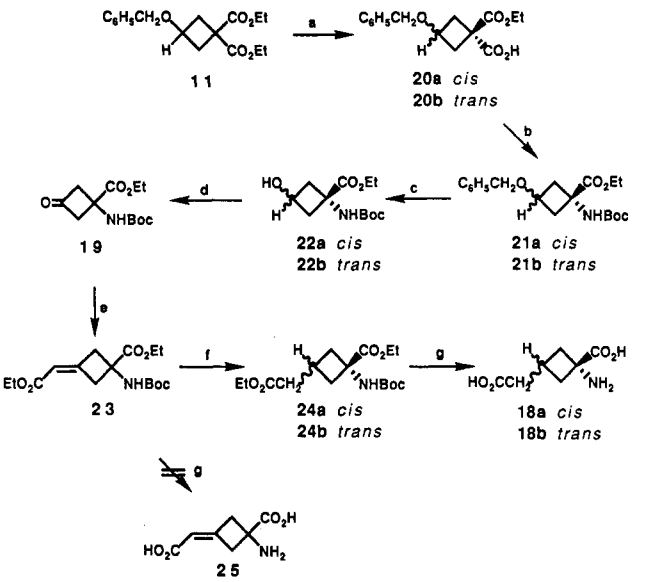
Chemistry

All cyclobutane amino acids were prepared by elaboration of C-1 and C-3 substituted compounds of which 3-oxocyclobutane-1-carboxylic acid[27] (**10**) and diethyl 3-(benzyloxy)cyclobutane-1,1-dicarboxylate[28,29] (**11**) proved to be the most useful. The preparation of glutamic acid analogues with distal carboxylic functions attached directly to the cyclobutane ring is described in Scheme I. Thus the cis and trans isomers **7a** and **7b**, respectively, were prepared in low to moderate yield via a Strecker reaction on **10**, though this procedure was advantageous in that both isomers were readily obtained in a 3:2 cis/trans ratio. An alternative preparation of **7a** and **7b** from **10** by a Bucherer–Bergs reaction via hydantoin **12** gave higher overall yields but a considerably diminished yield of the more active trans isomer. Repetition with a modified Bucherer–Bergs reaction (ammonium chloride addition, hydantoin isolation) was of no practical advantage. This result contrasts with the cyclopentane analogues, where enhanced yields and product ratios comparable to those obtained by the Strecker reaction have been reported.[30]

3-Aminocyclobutane-1,1,3-tricarboxylic acid (**13**) was prepared by a similar procedure. Hydrogenolysis of **11** to **14**, followed by a facile oxidation of the hydroxy intermediate with ruthenium tetroxide and sodium metaperiodate, afforded **15** and, after mild basic hydrolysis, 3-oxocyclobutane-1,1-dicarboxylic acid (**16**). A Bucherer–Bergs synthesis via hydantoin **17** converted **16** to **13**.

Scheme II describes the synthesis of the *cis*- and *trans*-amino acids **18a** and **18b** respectively, in which a methylene group has been inserted between the ring and the carboxylic acid functionality and which, in analogy with 2-aminoadipate,[19,20] might be expected to display antagonist activity. The protected oxo amino acid (**19**) proved to be a crucial intermediate in the synthesis of these compounds as well as proving a valuable synthon for the preparation of various phosphonates. Selective hydrolysis of one of the ester groups of **11** afforded a nonseparable mixture of cis (**20a**) and trans (**20b**) isomers of the monocarboxylic acid. The Curtius reaction of this mixture with diphenyl phosphorazidate and triethylamine,[31] followed by reaction of the unisolated isocyanate intermediates with *tert*-butyl alcohol afforded fair yields of an isomeric mixture of **21a** and **21b**, which was converted by previously described methodology (a and b, Scheme I) to **19** via the intermediate hydroxy compound **22**. The Wittig–Horner (Wadsworth–Emmons) reaction of **19** with triethyl phosphonoacetate and sodium hydride afforded a reasonable yield of **23** which on hydrogenation gave the anticipated mixture of **24a** and **24b**. Separation of these fully protected isomers by chromatography to a "major" and a "minor" isomer could be achieved. The configuration of the major isomer was shown to be trans (**24b**) since the structure of the derived amino acid **18** was subsequently

Scheme II[a]



[a] (a) (i) KOH, H_2O, EtOH, (ii) 6 M HCl; (b) (i) diphenyl phosphorazidate, triethylamine, toluene, 80–110 °C; (c) H_2, 10% Pd/C, EtOH; (d) RuO_4, $NaIO_4$, H_2O, CCl_4; (e) triethyl phosphonoacetate, NaH, dioxane; (f) H_2, 10% Pd/C, EtOH; (g) 6 M HCl, reflux.

(24) Schmutz, M.; Klebs, K.; Olpe, H.-R.; Allgeier, H.; Heckendorn, R.; Angst, C.; Brundish, D.; Dingwall, J. G.; Fagg, G. E. *Br. J. Pharmacol.* **1989**, *97*, 581P.

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(26) During the course of this work, a report describing a synthesis of **7a** and **7b** appeared in the literature: Gaoni, Y. *Tetrahedron Lett.* **1988**, *29*, 1591. Unlike this author, we have defined cis and trans isomers in accordance with the nomenclature described by Chemical Abstracts. Since submission of this paper, Dr. T. Lanthorn has kindly forwarded to us a copy of a manuscript which attributes similar activity and potency to the trans isomer **7b** (described as *cis*-2,4-methanoglutamate): Lanthorn, T. H.; Hood, W. F.; Watson, G. B.; Compton, R. P.; Rader, R. K.; Gaoni, Y.; Monahan, J. B. *Eur. J. Pharmacol.* **1990**, *182*, 397. Correspondence with Dr. Lanthorn and with other investigators in the field of excitatory amino acids has alerted us to potential confusion arising from the current non-IUPAC usage of cis and trans nomenclature (especially when incorporated as part of an acronym) to describe some pharmacologically useful amino acids. This problem requires urgent consideration and it is the recommendation of the authors that the correct IUPAC nomenclature be applied, at least initially, in the case of compounds not yet in widespread use and, if acronyms are used, that they be derived from IUPAC nomenclature.

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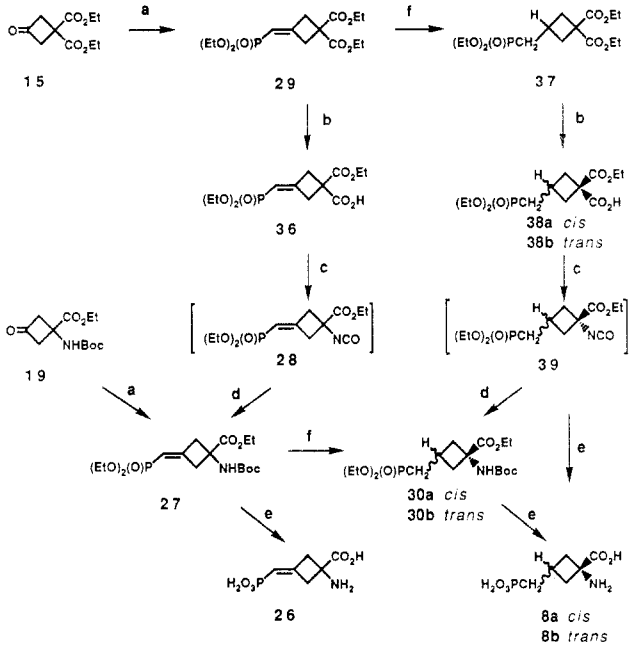
Scheme III[a]



[a] (a) Tetraethyl methylenebisphosphonate, NaH, THF; (b) (i) KOH, H_2O, EtOH, reflux, (ii) 6 M HCl, 0–5 °C; (c) diphenyl phosphorazidate, triethylamine, toluene, 80–110 °C; (d) tBuOH, 110 °C; (e) 6 M HCl, reflux; (f) H_2, 10% Pd/C, EtOH.

determined by X-ray analysis to be **18b** (see later). These protected compounds were hydrolyzed in refluxing 6 M HCl. This procedure was also adopted as standard for the hydrolysis of subsequent protected phosphonates; reflux times of 18–24 h were found to be adequate. However, the hydrolysis of **23** did not afford amino acid **25** but rather a mixture of products shown by ^1H NMR spectroscopy to be devoid of unsaturation. This ready Michael addition of water to strained cyclobutylideneacetic acid esters was not without precedent.[32]

Phosphonate analogues **26**, **8a**, and **8b** (Scheme III) were prepared by utilization and adaptation of the methodology already described for the synthesis of **18a** and **18b** in an earlier preparation (Scheme II). Thus **15** was transformed through a series of steps to **27** (Scheme III). A more efficient route to **27**, developed later, used synthon **19**. Hydrolysis of either **27** or the in situ hydrolysis of the isocyanate intermediate **28** of the Curtius reaction afforded an impure sample with the unsaturated phosphono amino acid **26** as the major component. The lower propensity for the conjugated phosphonic acid **26** or ester **27** to undergo nucleophilic addition presumably accounts for the observation of this species.

Scheme III also outlines the preparation of a mixture of the AP5 analogues **8a** and **8b**, initially from **29** through a multistep synthesis, and later through a two-step synthesis from **27**. Initially, no separation of either the isomeric mixtures of the fully protected precursors or of the free amino acids could be accomplished and therefore the mixture of cis- and trans-phosphonic acids 8 was examined for pharmacological activity. Subsequently, after extensive fractional crystallization, a sample of a crystalline isomer of the precursor **30** was obtained sufficiently pure to permit characterization by X-ray analysis (see later) as *cis* isomer **30a**. Hydrolysis of a slightly less isomerically pure sample of **30a** afforded a specimen of **8a** (>95% pure

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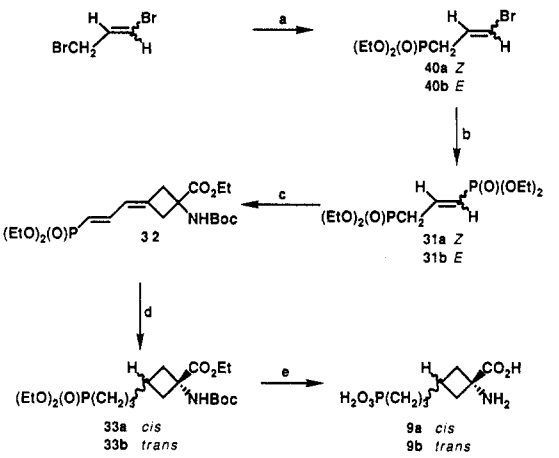
Scheme IV[a]



[a] (a) Triethyl phosphite; (b) triethyl phosphite, $NiCl_2$; (c) NaH, compound 15, dioxane; (d) H_2, 10% Pd/C, EtOH; (e) 6 M HCl, reflux.

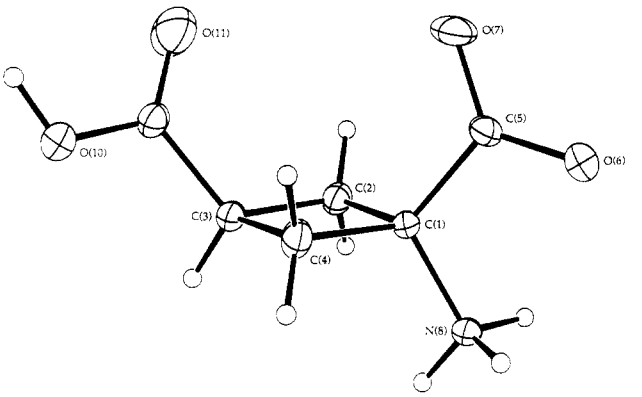


Figure 4. ORTEP plot of amino acid **7b**.

by ^1H NMR) which was tested for pharmacological activity.

Scheme IV outlines the preparation of the phosphono amino acids **9a** and **9b** in which the side chain has been extended by a further two methylene units to make conformationally restricted analogues of AP7. The preparation of the bis-phosphonate[33] (a mixture of *E* (**31a**) and *Z* (**31b**) isomers; 19:1 by ^1H NMR assignment of the larger HC=CH coupling constant value of 18 Hz[34] to the *E* isomer and the lower value of ca. 13.5 Hz to the *Z* isomer) was best accomplished in two consecutive Michaelis–Arbuzov reactions from an isomeric mixture of 1,3-dibromoprop-1-ene. Attempts to purify the mixture of **31a** and **31b** by distillation invariably led to an alteration in the ratio of isomers to favor the formation of the initially least abundant *Z* isomer; consequently the isomers were separated by column chromatography and the *E* isomer was used in the subsequent step. Reaction of **31a** with **19** was followed by immediate reduction of the intermediate **32** (an *E* assignment of stereochemistry was made on the basis of a coupling constant value of 17 Hz) to prevent polymerization. Once again no ready separation of the fully protected isomers **33a** and **33b** or of the free amino acids **9a** and **9b** could be accomplished and the amino acid mixture was tested for potential antagonist activity.

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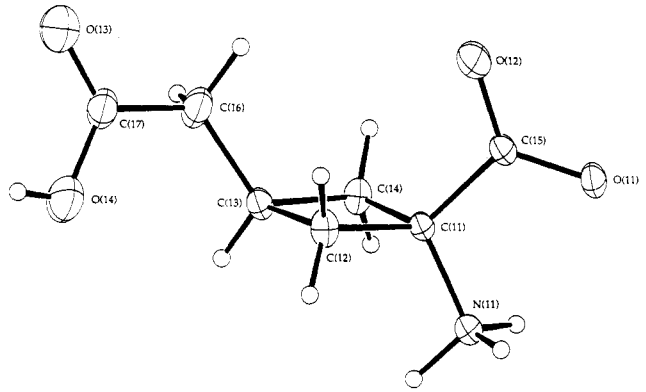


Figure 5. ORTEP plot of amino acid **18b**.

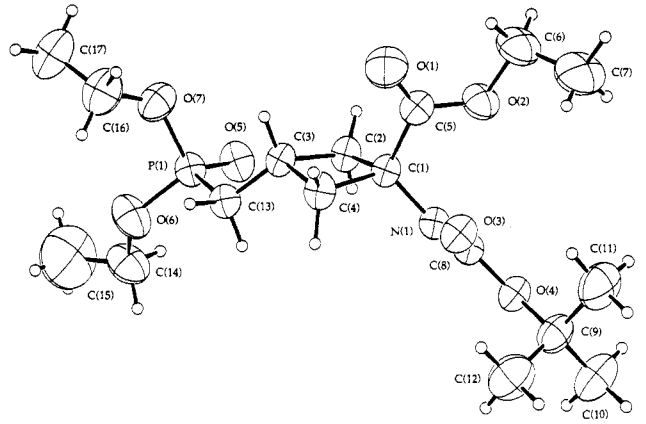


Figure 6. ORTEP plot of protected amino acid **30a**.

X-ray Structures and Ring Puckering

Computer generated ORTEP[35] plots of compounds **7b** and **18b** and the protected amino acid **30a** are shown in Figures 4, 5, and 6, respectively. The structural analyses show that **7b** and **18b** crystallize as zwitterions and that **18b** crystallizes with two independent molecules and a single water molecule in the asymmetric unit. In all three structures there are intermolecular hydrogen bonds which, in some cases, are very strong. The cyclobutane rings show varying degrees of nonplanarity: fold angles, defined as the angle between the planes through C(1), C(2), and C(4) and through C(2), C(3), and C(4), are 3.5° (**7b**), 4.4° and 9.9° (**18b**), and 16.1° (**30a**). It appears that this folding is significantly affected by solid-state effects since the immediate environment of the cyclobutane ring in each structure is similar and there is a difference in the values for the two independent molecules of **18b**. Thus, there must be a relatively low barrier to ring puckering and hence some freedom in the disposition of the functional groups. Bond lengths and angles associated with the cyclobutane group are significantly strained, as is typical of four-membered rings. The crystallographic data are summarized in Table I.

Pharmacology

Cortical Wedge. A modified and simpler version[36] of the cortical wedge preparation described by Harrison and Simmonds[37] was used to evaluate pharmacological effects of the cyclobutane amino acids in excitatory amino acid receptors. NMDA (10 μM), quisqualic acid (5 μM) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (5 μM), and kainic acid (2 μM) were used as standard agonists while (±)-AP5 and (±)-CPP were used as NMDA receptor antagonists. Because of time constraints and difficulties associated with recovery at or near saturation, dose–response curves of NMDA and potent agonists were not taken to saturation. In preference to an ED_{50} value, the relative potencies of these new agonists are described in terms of the concentration producing the equivalent depolarizing response to that observed with 10 μM NMDA.

Dose–response curves for NMDA, **7a**, **7b**, and **13** are shown in Figure 7. Amino acid **7b** proved to be the most potent agonist, being some 20 times more active than NMDA (0.4 μM equivalent to 10 μM NMDA), and we believe it to be the most potent NMDA agonist reported to date. Cis isomer **7a** and **13** were considerably weaker as agonists; **7a** being approximately $^1/_3$ as potent (30 μM) as NMDA while **13** was less than $^1/_{10}$ as potent (140 μM). The depolarizing responses induced by **7a** and **7b** were as sensitive to the NMDA antagonist (±)-AP5 (10 μM) as those of NMDA, and the responses to all three agonists were completely abolished by the more potent antagonist (±)-CPP (10 μM).

Table II summarizes the results from the pharmacological testing of the carboxylic acid homologues **18a** and **18b**, as well as all the phosphonic acid homologues. Only the carboxylic acids displayed some agonist activity at high concentrations. As antagonists, **18a** was inactive while the others were weak and frequently nonspecific.

In Vivo Studies. When injected ip into male mice (15–25 g) at 180 mg/kg, **7b** caused convulsions and death for six out of 10 mice but was nonlethal to mice at 60 mg/kg. In a comparative study, ip injection of NMDA at

Table I. Crystal Data

	7b	18b	30a
space group	$P2_1/n$	$P2_1/n$	$P\bar{1}$
a, Å	12.235 (2)	11.587 (2)	7.909 (3)
b, Å	5.8784 (9)	9.149 (2)	9.626 (4)
c, Å	13.360 (2)	16.854 (3)	15.375 (7)
α, deg			90.34 (3)
β, deg	97.61 (1)	104.06 (2)	98.19 (3)
γ, deg			103.04 (3)
V, Å^3	796.7 (3)	1733.2 (8)	1127.8 (9)
formula weight	177.16	364.36	393.42
D_{calcd}, g cm^{-3}	1.477	1.396	1.158
empirical formula	$C_6H_{11}NO_5$	$C_{14}H_{24}N_2O_9$	$C_{17}H_{32}NO_7P$
Z	4	4	2
absorption coeff, cm^{-1}	0.85	0.76	1.23
temperature, °C	21	21	21
λ, Å	0.71069	0.71069	0.71069
R, (F_o)	0.038	0.049	0.045
R_w	0.047	0.057	0.051

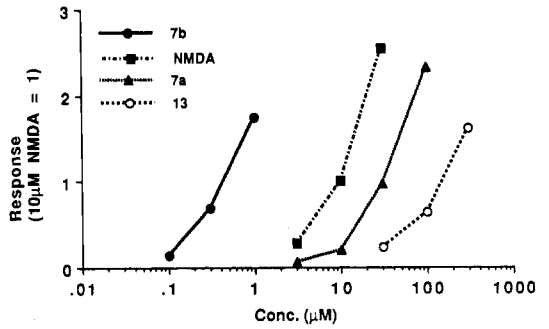


Figure 7. Dose–response curves of depolarizations induced by 1-min application of NMDA and cyclobutane agonists. Data are mean values for a minimum of three experiments.

(35) Figures were drawn with ORTEP (Johnson, C. K. *ORTEP, A Thermal Ellipsoid Plotting Program*; Oak Ridge National Labs.: Oak Ridge, TN, 1965.

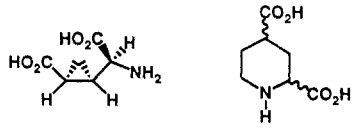
(36) Allan, R. D.; Drew, C. A.; Ong, J.; Tran, H. W. *Neurosci. Lett.* **1990**, *112*, 223.

(37) Harrison, N. L.; Simmonds, M. A. *Br. J. Pharmacol.* **1985**, *84*, 381.

Table II. Depression of Depolarizing Responses of Agonists by Cyclobutane Analogues of 2-Aminoadipic Acid, AP5 and AP7

no.	concn, μM	agonist activity response[a]	% antagonist activity[b] NMDA (10 μM)	quis/AMPA (5 μM)	kain (2 μM)
18a	500	v weak (0.65)	ns[c]	ns	ns
18b	500	v weak (0.25)			
	200		~50	ns	ns
26	500	inactive			
	100		30–40	25–30	~25
8a/8b (mix.)	500	inactive			
	200		~40	~25	~25
8b	200	inactive	25–40	ns	ns
9a/9b (mix.)	500	inactive	40–50	ns	ns

[a] Approximate response vs 10 μM NMDA (=1). [b] Approximate depression of response of standard agonist concentration. [c] Not significant.



34 35

Figure 8. Cyclic glutamic acid analogues for comparison with 7.

180 mg/kg caused convulsions and death for four out of nine mice, while **7a** did not cause convulsions at 180 mg/kg.

Discussion

It has long been recognized that discrete conformational arrangements of agonists or antagonists are required for the activation or blockake of excitatory amino acid receptors. Consequently, considerable effort has been directed toward structure–activity relationships using conformationally restricted analogues in order to develop antagonists of increased potency. In the case of the NMDA receptor, many strategies have been employed in order to restrict the conformational flexibility of potential antagonists. These include the incorporation of aromatic[38] and heterocyclic rings[34,39] in addition to unsaturation in acyclic compounds.[24] Another successful approach in limiting conformational flexibility has been in the use of cyclopropane rings. Shinozaki and co-workers[40] have recently described the actions of the four diastereoisomers of L-α-(carboxycyclopropyl)glycine (L-CCG). Of these, the 2*S*,3*R*,4*S* isomer (L-CCG-IV; **34**, Figure 8) was about 5 times more potent than NMDA in depolarizing NMDA receptors and its action was blocked by NMDA antagonists. Shinozaki described the conformation of this isomer as analogous to a folded conformation of glutamic acid and concluded that a preferred conformation of glutamic acid for activating the NMDA receptor is a folded form. From other studies on phenyl-spaced 2-amino-(5–9)-phosphonoalkanoic acids, Bigge and co-workers[38] have also concluded that the NMDA receptor prefers a "folded" rather than an "extended" conformation of glutamic acid.

It was also our contention that conformational restriction imposed by small rings such as cyclopropane and cyclobutane could lead to potentially potent agonists and antagonists. In the case of glutamate analogues incorporating a cyclobutane ring, the conformation is considerably restricted, but considerable flexibility remains because of the mobility of the cyclobutane which can "flip" from one conformation to another through low-energy barriers (on the order of 1.4 kcal mol^{-1} for the parent unsubstituted cyclobutane;[41,42] undoubtedly higher with increasing substitution). This flexibility may permit access and binding to receptor sites. 1,3-Substituted cyclobutanes were of particular interest because of the lead afforded by the isolated natural product, the existence of suitable synthetic intermediates, and because the plane of symmetry in the molecule means that optical isomers are not a complicating factor. Furthermore, the restrictions imposed by the cyclobutane ring allow 1,3-substituents that are cis to one another to get into close proximity and mimic conformations expected for a "folded" conformation.

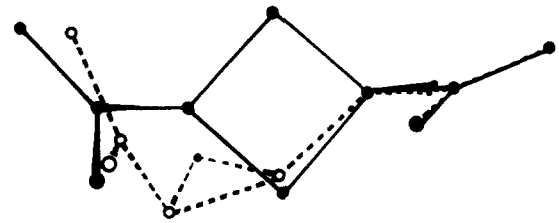


Figure 9. Possible conformations of the potent NMDA agonists **7b** and **34** in which the polar groups occupy similar regions in space. The figure shows the carboxyl groups projecting upward from the plane of the cyclobutane ring. Hydrogen atoms have been omitted.

The most important result from this work is the extremely high agonist potency of trans compound **7b** (in which the α- and ω-carboxylic acid groups are actually held cis to one another). Two recent papers[40,43] describe structure–activity studies on analogues whose conformations can be related to these cyclobutanes. The activity of a series of piperidinedicarboxylic acids (PDAs) tested on the rat cortical wedge preparation by Madsen et al.[43] showed that a trans arrangement of the carboxylic acid groups in 2,3-PDA and 2,4-PDA gave quite potent agonists, while the corresponding cis isomers are a partial agonist and an antagonist, respectively. Superficially this is consistent with our results in that **7a** (with a trans arrangement of carboxylic acid groups) has a potency similar to *trans*-2,3-PDA and *trans*-2,4-PDA (**35**, Figure 8). Compound **7b**, however, has exceptionally high activity and a rationale may be that incorporation of the nitrogen into a piperidine ring may decrease the potential agonist activity and that the primary amino group of **7b** is better placed for receptor interaction. From the closely related series of analogues of L-glutamic acid containing a cyclopropane ring investigated by Shinozaki et al.,[40] **34**, with a primary amino group, as well as the two carboxylic acid groups in a cis arrangement about the ring, is also more potent than NMDA. Figure 9 shows one of the possible ways in which the conformation of the cyclopropyl analogue **34** can match the structure of **7b** which is drawn in the X-ray conformation. Such conformations of **34** can be produced by overlapping the α-amino acid groups and then adjusting the polar ω-amino acid group of **34**. However, these conformations do not have good overlap of the "carbon backbone" of the glutamate analogues as can be seen from Figure 9. More detailed computer-aided mo-

(38) Bigge, C. F.; Drummond, J. T.; Johnson, G.; Malone, T.; Probert, A. W., Jr.; Marcoux, F. W.; Coughenour, L. L.; Brahce, L. J. *J. Med. Chem.* **1989**, *32*, 1580.
(39) Ornstein, P. L.; Schaus, J. M.; Chambers, J. W.; Huser, D. L.; Leander, J. D.; Wong, D. T.; Paschal, J. W.; Jones, N. D.; Deeter, J. B. *J. Med. Chem.* **1989**, *32*, 827.
(40) Shinozaki, H.; Ishida, M.; Shimamoto, K.; Ohfune, Y. *Brain Res.* **1989**, *480*, 355.
(41) Cremer, D. *J. Am. Chem. Soc.* **1977**, *99*, 1307.
(42) Tang Kai, A. H.; Combs, L. L. *Int. J. Quantum Chem., Quantum Chem. Symp.* **1979**, *13*, 175.
(43) Madsen, U.; Brehm, L.; Schaumburg, K.; Jørgensen, F. S.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1990**, *33*, 374.

lecular modeling studies are currently being undertaken and will be reported at a later date. It is possible that a certain amount of conformational flexibility, with relative motion between the polar end groups, may be important for potent receptor activation. If this flexibility can be shown to be a significant factor, then **7b**, with considerable conformational restraint but extremely high activity, will be an important NMDA agonist for structure–activity relationships.

As far as antagonist activity is concerned, the results were disappointing, and this may be explained if a different conformation is required for antagonist–receptor interaction. Our cyclobutane analogues are more than 2 orders of magnitude less potent than the piperazine CPP (**5**) and the acyclic antagonist **6**. This would suggest a major conformational mismatch between the active antagonist conformations and those available to our potential antagonists. Another consideration that may be relevant to the lack of potency of the cyclobutane antagonists is that all phosphonic acids reported to be potent competitive antagonists at NMDA receptors are α-amino acids without further substitution at the α carbon.

Conclusion

We have demonstrated that restricting the conformational mobility of glutamic acid by the incorporation of a cyclobutane ring into the structure leads to a highly potent NMDA agonist, **7b**, which appears to be the most potent NMDA agonist reported to date. However, application of this strategy to phosphonic acids AP5 and AP7 has proved unsuitable for preparing antagonists of worthwhile potency.

Experimental Section

Melting points were determined on a Reichert hot-stage apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded at 89.6 and 22.5 MHz, respectively, on a JEOL FX90Q NMR spectrometer and were consistent with the proposed structures. The peaks (^1H) of compounds measured in $CDCl_3$ and D_2O are described in ppm downfield from TMS (tetramethylsilane) (internal standard) and DSS (2,2-dimethyl-2-silapentane-5-sulfonate) (internal standard), respectively; peaks (^{13}C) of compounds measured in $CDCl_3$ are described in ppm downfield from TMS (internal standard) while internal dioxane (δ 67.7 downfield from external TMS) was used as internal standard for peaks (^{13}C) of compounds recorded in D_2O/NaOD. Coupling constants reported for ^{13}C NMR refer to ^{13}C–^{31}P couplings. Mass spectral data refer to chemical ionization using methane as reagent gas on a TSQ46 Finnigan/MAT spectrometer. Where analyses are indicated by the symbol of the elements, the results are within ±0.4% of the theoretical value. Analyses were performed by the Australian Microanalytical Service. TLC was carried out with 0.25 mm silica gel F254 (E. Merck) aluminium-backed plates. Chromatographic separations were performed with short-column vacuum chromatography[44] on Merck silica gel H (TLC grade) unless stated otherwise. Petroleum refers to the fraction of bp 65–70 °C.

Rat Brain Cortical Wedge Preparation. The rat brain cortical wedge preparation has been described elsewhere.[36] Briefly, cerebral wedges of rat brain were prepared and placed between layers of absorbent fiber ("nappy liner") supported on an inclined block at room temperature, the corpus callosum side being raised onto a nonpolarizable wick electrode (and enclosed in petroleum jelly). DC potentials between the cingulate cortex and corpus callosum were monitored by Ag/AgCl electrodes via agar/saline bridges and a high input impedance DC amplifier and displayed on a chart recorder. The preparations were continuously superfused with magnesium-free Krebs medium to generate repetative spontaneous discharges.

***cis*-(12a) and *trans*-3-Carboxycyclobutane-1-spiro-5′-hydantoin (12b).** Concentrated NH_4OH was added to a solution of ketonic acid (**10**; 2.28 g, 20 mmol, sublimed before use), prepared according to the method of Pigou and Schiesser,[27] in a mixture of H_2O (20 mL) and MeOH (20 mL) until the solution was neutral (pH ~6–7). To this neutralized solution was added KCN (1.42 g, 21.8 mmol), ammonium carbonate (NH_4HCO_3·$NH_2CO_2NH_4$) (6.32 g, ca. 40 mmol), and NH_4Cl (1.07 g, 20 mmol) and the solution was stirred and heated at 65 °C overnight. The mixture was cooled, acidified (pH 1.5–2) by the dropwise addition of 6 M HCl, cooled further, filtered and the filtrate washed with cold H_2O and air-dried to give a mixture of the isomeric hydantoins **12a** and **12b** as a white solid (2.90 g, 79%) which was used without further purification in a subsequent step.

***cis*-(7a) and *trans*-1-Aminocyclobutane-1,3-dicarboxylic Acid (7b). A: Strecker Synthesis.** The pH of a solution of **10** (2.63 g, 23 mmol, sublimed before use) in H_2O (20 mL) was adjusted to ca. pH 8 by the dropwise addition of concentrated NH_4OH. KCN (1.54 g, 23.6 mmol) and NH_4Cl (1.27 g, 23.7 mmol) were added, the solution stirred until all the solids had dissolved and then it was allowed to stand at room temperature for 7 days. Concentrated HCl (20 mL of 32% w/v) was added, the solution was stirred at room temperature for 2.5 h and then refluxed overnight (ca. 18 h). Most HCl and other volatiles were removed initially by concentration under reduced pressure. Removal of final traces of HCl was accomplished by repetitive addition of H_2O (ca. 20 mL) and concentration under reduced pressure. The final residue was dissolved in a minimum volume of H_2O and partly purified (no separation of isomers was attempted at this stage) by ion-exchange chromatography (Dowex AG 50W, H^+ form, 120 mL of resin) with H_2O as eluent. Fractions giving a positive ninhydrin test were combined and concentrated to a residue of brownish solid which was recrystallized from H_2O to give exclusively the cis isomer monohydrate **7a** as off-white needles (975 mg, 24%). An analytical sample of **7a** was prepared by recrystallization from H_2O: mp 250–252 °C dec (darkening from 225 °C); ^1H NMR (D_2O) δ 3.6–3.2 [1 H, m (approximating quintet with δ 3.36, J = 9.7 Hz)], 2.9–2.4 (m, 4 H); ^{13}C NMR (D_2O/2 M NaOD) δ 185.9 (CO_2^-), 184.6 (CO_2^-), 56.1 (C-1), 40.1 (C-2 and C-4), 33.9 (C-3); MS m/z 160 (MH^+, 100), 142 (43), 114 (20). Anal. ($C_6H_9NO_4$·H_2O) C, H, N.

The filtrate from above was rechromatographed more carefully by ion-exchange chromatography (AG 50W, H^+ form; H_2O); 500-mL fractions were collected, each fraction was concentrated to a suitable volume (ca. 10 mL), and the contents were examined by TLC (3:1:1 nBuOH/AcOH/H_2O, visualized by spraying with ninhydrin). Initial elution gave fractions containing additional cis isomer which on recrystallization afforded pure *cis*-monohydrate **7a** as a white, crystalline solid (170 mg, 4%; total 1.145 g, 28%). Further elution using H_2O yielded trans isomer hydrate **7b** as a slightly off-white, crystalline solid (800 mg, 20%). An analytical sample of **7b** was prepared by recrystallization from H_2O: mp 257–259 °C dec (darkening from 237 °C); ^1H NMR (D_2O) δ 3.68–3.28 (m, 1 H), 3.11–2.50 (m, 4 H); ^{13}C NMR (D_2O/2 M NaOD) δ 186.0 (CO_2^-), 185.3 (CO_2^-), 56.5 (C-1), 37.4 (C-2 and C-4), 34.6 (C-3); MS m/z 160 (MH^+, 94), 142 (77), 114 (100). Anal. ($C_6H_9NO_4$·H_2O) C, H, N.

B: Modified Bucherer–Bergs Synthesis. The mixed hydantoins **12a** and **12b** (2.9 g, 15.7 mmol) were hydrolyzed with 0.5 M NaOH (160 mL) over 24 h. The hydrolysis solution was concentrated to ca. 25 mL, the pH was adjusted to ca. 7.0 by the addition of 6 M HCl, and the small quantity of precipitated solid was filtered off and discarded. The pH of the filtrate was further adjusted to ca. pH 3.5, the solution/precipitate cooled, and the precipitate was filtered off and washed with ice-cold H_2O. The solid was recrystallized from H_2O and the product air-dried to yield **7a** as a white, crystalline solid (1.16 g). The filtrates were combined and chromatographed by ion-exchange chromatography as described previously to afford further **7a** (830 mg; total 1.99 g, 71%, 56% from **10**) followed by pure **7b** which was obtained as a white, crystalline solid (370 mg, 13%, 10% from **10**).

C: Normal Bucherer–Bergs Synthesis. An alternative synthesis of the amino acids from **10** (2.28 g, 20 mmol) using "normal" Bucherer–Bergs reaction conditions (i.e. the absence of NH_4Cl in the preparation of **12**) and the in situ hydrolysis of the hydantoin (in preference to isolation and hydrolysis) followed by ion-exchange chromatography afforded a comparable yield of both **7a** (1.96 g, 55%) and **7b** (250 mg, 7%).

(44) Ravi, B. N.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 129.

Diethyl 3-Oxocyclobutane-1,1-dicarboxylate (15). Hydrogenolysis of **11**[28,29] over 10% Pd/C at 1 atm according to a modified literature procedure[28] and subsequent chromatography (4:1 petroleum/EtOAc) afforded pure diethyl 3-hydroxycyclobutane-1,1-dicarboxylate (**14**) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ ca. 4.6–4.2 (m, 1 H), 4.21 (q, 4 H, J = 7 Hz), 3.00–2.78 [m, 3 H (includes hydroxyl proton)], 2.56–2.34 (m, 2 H), 1.26 (t, 6 H, J = 7 Hz).

The ruthenium tetroxide (RuO_4) catalyst was prepared as a CCl_4 solution by stirring ruthenium trichloride (65 mg, 0.3 mmol) in CCl_4 (35 mL) with a solution of sodium metaperiodate (900 mg) in H_2O (35 mL) overnight. The lower layer was separated and the aqueous layer was extracted with CCl_4 (2 × 10 mL). The (combined) bright yellow organic layer was used in the subsequent oxidation.

Hydroxy compound **14** (17.85 g, 82.6 mmol) was added to the solution of the catalyst from above, a solution of sodium metaperiodate (35.3 g, 165 mmol) in H_2O (ca. 250 mL) was added, and the two-phase mixture was stirred vigorously until a yellow color had been restored in the organic layer (ca. 24 h). The lower layer was run off and the aqueous layer was extracted with $CHCl_3$ (2 × 50 mL). The combined organic extracts were washed in turn with H_2O (2 × 50 mL) and saturated NaCl solution (2 × 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure to afford crude **15** as a pale yellow oil containing a trace of finely dispersed black solid (probably RuO_2). Distillation (Kugelrohr) under reduced pressure gave pure **15** as a colorless oil (16.3 g, 92%): bp 150–155 °C/0.1 mm (lit.[28] 112–113 °C/3 mm); ^{1}H NMR ($CDCl_3$) δ 4.28 (q, 4 H, J = 7 Hz), 3.63 (s, 4 H), 1.30 (t, 6 H, J = 7 Hz).

3-Aminocyclobutane-1,1,3-tricarboxylic Acid (13). The normal Bucherer–Bergs reaction of a solution of 3-oxocyclobutane-1,1-dicarboxylic acid (**16**; 2.0 g, 12.6 mmol), prepared by the alkaline hydrolysis of diester **15**, KCN (2.05 g, 31.5 mmol), and ammonium carbonate (5.07 g, ca. 63 mmol) in a mixture of MeOH (20 mL) and H_2O (20 mL) (refluxed for 3 h) followed by basic hydrolysis of crude hydantoin **17** and purification of the final product by ion-exchange chromatography (Dowex AG 50 W, H^+ form; H_2O followed by 1 M pyridine as eluents) afforded crude **13** as an off-white, crystalline solid (600 mg, 23%). A sample for pharmacological testing was obtained by recrystallization from H_2O: mp >300 °C; ^{1}H NMR (D_2O) δ 3.21 and 2.87 (AA'BB' quartet with some fine structure, $J \cong 13.5$ Hz); MS m/z 204 (MH^+, 30%).

***cis*-(20a) and *trans*-3-(Benzyloxy)-1-carbethoxycyclobutane-1-carboxylic Acid (20b).** A solution of diester **11** (7.65 g, 25 mmol) and KOH (1.5 g of 85%) in a mixture of EtOH (50 mL) and H_2O (12.5 mL) was refluxed for 8 h. The mixture was concentrated under reduced pressure, the residue was dissolved in H_2O (ca. 40 mL), and this solution was extracted with Et_2O (3 × 25 mL) to remove unhydrolyzed diester. The aqueous layer was cooled in ice, Et_2O (25–30 mL) was added, and the rapidly stirred biphasic mixture was treated dropwise with 6 M HCl until the pH of the aqueous phase was ≤pH 2. The Et_2O layer was removed and the aqueous layer was extracted with Et_2O (1 × 25, 2 × 10 mL). The combined Et_2O extracts were washed successively with H_2O (25 mL) and saturated NaCl (2 × 25 mL), dried over Na_2SO_4, and concentrated under reduced pressure to yield a crude mixture of the cis (**20a**) and trans (**20b**) isomers as a viscous pale yellow oil (5.0 g, 72%) which was not purified further: ^{1}H NMR ($CDCl_3$) δ 7.32 (br s, 5 H), 4.43 (s, 2 H), ca. 4.4–4.0 [superimposed m and q ($J \cong 7$ Hz) from two isomers, total 3 H], 2.95–2.46 (m from two isomers, total 4 H), 1.27 (coincidental t from two isomers, total 3 H, J = 7 Hz).

***cis*-(21a) and *trans*-Ethyl 3-(Benzyloxy)-1-[[(*tert*-butyloxy)carbonyl]amino]cyclobutane-1-carboxylate (21b).** In a typical experiment, a solution of mixed isomeric half esters **20a** and **20b** (5.42 g, 19.5 mmol), diphenyl phosphorazidate (5.57 g, 20.2 mmol), and triethylamine (2.25 g, 22.2 mmol) in dry toluene (20 mL) was heated with stirring under nitrogen at 80 °C for 1 h then at 110 °C for an additional 1 h. tBuOH (1.84 mL, 19.5 mmol) was added in one portion and the mixture was heated at 110 °C under nitrogen for ca. 48 h. Solvents and other volatiles were removed under reduced pressure to afford a viscous dark-brown oil. The residue was taken up into a mixture of Et_2O (30 mL) and cold H_2O (30 mL), the Et_2O layer was separated, and the aqueous layer was extracted with further Et_2O (4 × 10 mL). The combined Et_2O extracts were washed in turn with H_2O (20 mL) and saturated NaCl (2 × 20 mL), dried over Na_2SO_4, and concentrated under reduced pressure to a viscous dark brown oil. Initial chromatography (1:1 petroleum/EtOAc) afforded inter alia an unseparated mixture of the cis (**21a**) and trans (**21b**) isomers which also contained traces of minor contaminants as an off-white to yellow viscous oil which partly crystallized on standing. Trituration of this material with petroleum (which removed the more readily soluble noncrystalline isomer) and filtration afforded a pure sample of one isomer (the major isomer in the original mixture) as a sparingly hexane-soluble white solid (1.16 g, 17%), recrystallized from petroleum: mp 85–87 °C; ^{1}H NMR ($CDCl_3$) δ 7.33 (s, 5 H), 4.92 (br s, 1 H), 4.44 (s, 2 H), ca. 4.4–4.1 [m (possibly quintet), 1 H], 4.19 (q, 2 H, J = 7 Hz), 2.76–2.33 (m, 4 H), 1.43 (s, 9 H), 1.27 (t, 3 H, J = 7 Hz).

The filtrate from above was rechromatographed (17:3 petroleum/EtOAc) to yield a mixture of the cis (**21a**) and trans (**21b**) isomers free from contaminants as a viscous oil (2.77 g, 41%) (total 3.93 g, 58%): ^{1}H NMR ($CDCl_3$) δ 5.10 and 4.93, respectively (N*H* peaks in approximate ratio 3:2, respectively); remaining peaks are not sufficiently well resolved for assignment to individual isomers.

***cis*-(22a) and *trans*-Ethyl 1-[[(*tert*-Butyloxy)carbonyl]amino]-3-hydroxycyclobutane-1-carboxylate (22b).** A solution of the unseparated *cis*- and *trans*-benzyl ethers **21a** and **21b** (in approximately equal amounts after the separation of a quantity of one of the pure isomers and free of detectable contaminants) (5.46 g, 15.6 mmol) in EtOH (40 mL) was hydrogenolyzed over 10% Pd/C (500 mg) at 1 atm for 4 days. Additional catalyst (500 mg) was then added and hydrogenolysis at 1 atm continued until hydrogen uptake ceased. The suspension was filtered through Celite to remove catalyst and the filter cake was washed with EtOH. The filtrate was concentrated under reduced pressure to give a quantitative yield of a cis (**22a**) and trans (**22b**) mixture of the title compound as an off-white oil which crystallized on cooling to an off-white to buff solid (4.05 g). This material was used in the subsequent oxidation step without further purification: ^{1}H NMR ($CDCl_3$) δ 5.64 and 5.02 (N*H* peaks of major and minor isomeric products, respectively, in approximate ratio 3:2; remaining peaks are not sufficiently well resolved for assignment to individual isomers).

The isomerically pure benzyl ether (unknown assignment, either **21a** or **21b**) (2.62 g, 7.5 mmol) in EtOH (20 mL) was hydrogenolyzed in an analogous manner to afford a quantitative yield of an isomerically pure hydroxy compound (unknown assignment, either **22a** or **22b**) as an off-white solid: mp 112–114 °C; ^{1}H NMR ($CDCl_3$) δ 5.02 (br s, 1 H), ca. 4.6–4.3 (m, 1 H), 4.22 (q, 2 H, J = 7 Hz), ca. 2.8–2.4 (m, 5 H), 1.43 (s, 9 H), 1.29 (t, 3 H, J = 7 Hz).

Ethyl 1-[[(*tert*-Butyloxy)carbonyl]amino]-3-oxocyclobutane-1-carboxylate (19). The ruthenium tetroxide (RuO_4) catalyst in CCl_4 (15–17.5 mL) was prepared as described previously from ruthenium trichloride (21 mg, 0.1 mmol) and sodium metaperiodate (300 mg). A crude mixture of isomers **22a** and **22b** (2.59 g, 10 mmol), from the previous preparation, was treated with the solution of catalyst from above and a solution of sodium metaperiodate (4.3 g, 20 mmol) in H_2O (ca. 40–50 mL) and the product was worked up as described previously to afford crude **19** as essentially a pale yellow oil (contaminated with a trace of black suspension of ruthenium tetroxide) which slowly crystallized. Chromatography (1:1 petroleum/EtOAc) yielded pure title compound as a colorless solid (2.05 g, 80%) which was recrystallized from petroleum: mp 81–82 °C; ^{1}H NMR ($CDCl_3$) δ 5.45 (br s, 1 H), 4.28 (q, 2 H, J = 7.1 Hz), 3.59 (br s, 4 H), 1.50 (s, 9 H), 1.31 (t, 3 H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 201.7 (*C*=O), 172.8 (CO_2Et), 155.2 (HN*C*=O), 80.7 [O*C*(CH_3)$_3$], 62.3 (O*C*H_2CH$_3$), 57.8 (C-2 and C-4), 49.8 (C-1), 28.2 [C(*C*H_3)$_3$], 14.1 (OCH$_2$*C*H_3). Anal. ($C_{12}H_{19}O_5N$) C, H, N.

Ethyl 1-[[(*tert*-Butyloxy)carbonyl]amino]-3-(carbethoxymethylidene)cyclobutane-1-carboxylate (23). A solution of triethyl phosphonoacetate (785 mg, 3.5 mmol) in anhydrous dioxane (2–3 mL) was added dropwise with stirring, over ca. 3–5 min, to a suspension of NaH [84 mg, 3.5 mmol; obtained by washing an 80% emulsion of NaH in oil (105 mg) with dry pentane] in dry dioxane (5 mL) under nitrogen. The resulting clear solution was cooled to the stage where the dioxane was on the

verge of crystallizing and, while stirring was continued, a solution of **19** (900 mg, 3.5 mmol) in anhydrous dioxane (2.5 mL) was added over 5 min while the temperature was maintained at ca. 10 °C. The solvent was removed under reduced pressure and the residue was taken up into a mixture of Et_2O (20 mL) and cold H_2O (20 mL). The Et_2O layer was separated and the aqueous phase was extracted further with Et_2O (3 × 5 mL). The combined Et_2O extracts were washed in turn with H_2O (10 mL) and saturated NaCl (2 × 10 mL), dried over Na_2SO_4, and concentrated to a yellow/tan oil (990 mg). Purification by chromatography (4:1 petroleum/EtOAc) gave **23** as a viscous, very pale yellow oil (800 mg, 70%) which did not crystallize: ^1H NMR ($CDCl_3$) δ 5.75 [quintet, 1 H, J (cis and trans) = 2.2–2.4 Hz], 5.33 (br s, 1 H), 4.23 (q, 2 H, J = 7 Hz), 4.15 (q, 2 H, J = 7 Hz), 3.8–3.2 (m, 4 H), 1.44 (s, 9 H), 1.28 (t, 3 H, J = 7 Hz), 1.27 (t, 3 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 173.0 (CO_2R), 166.1 (=$CHCO_2R$), 157.4* (C-3), 154.9* (NHC=O), 114.8 (HC=), 80.2 [$OC(CH_3)_3$], 61.8 and 59.9 (OCH_2CH_3), 55.7 (C-1), 44.1 and 42.3 (C-2 and C-4, unassigned), 28.3 [$C(CH_3)_3$], 14.4 and 14.1 (OCH_2CH_3) (*these assignments may be reversed).

***cis*-(24a) and *trans*-Ethyl 1-[[(*tert*-Butyloxy)carbonyl]amino]-3-(carbethoxymethyl)cyclobutane-1-carboxylate (24b).** Compound **23** (870 mg, 2.66 mmol) in absolute EtOH (ca. 10–15 mL) was hydrogenated over 10% Pd/C (75 mg) at 1 atm. The reaction mixture was filtered through Celite to remove catalyst and the filtrate, after concentration under reduced pressure, afforded a pale yellow viscous oil. The isomeric products were separated by chromatography (92.5:7.5 petroleum/EtOAc); initial elution afforded the minor isomer (cis, **24a**) as a viscous straw-colored oil (290 mg, 33%) which would not crystallize: ^1H NMR ($CDCl_3$) δ 5.2 (br s, 1 H), 4.23 (q, 2 H, J = 7 Hz), 4.11 (q, 2 H, J = 7 Hz), 2.8–2.1 (m, 7 H), 1.43 (s, 9 H), 1.29 (t, 3 H, J = 7 Hz), 1.24 (t, 3 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 174.1 (CO_2R), 172.2 (CO_2R), 154.7 (NHC=O), 79.8 [$OC(CH_3)_3$], 61.4 and 60.3 (OCH_2CH_3), 55.1 (C-1), 41.2 (C-2 and C-4), 37.6 (CH_2CO_2R), 28.3 [$C(CH_3)_3$], 24.8 (C-3), 14.2 (OCH_2CH_3).

Continued elution with the same solvent system yielded the major isomer (trans, **24b**) as a viscous straw-colored oil (525 mg, 60%) which also would not crystallize: ^1H NMR ($CDCl_3$) δ 5.05 (br s, 1 H), 4.19 (q, 2 H, J = 7 Hz), 4.11 (q, 2 H, J = 7 Hz), 3.1–2.7 (m, 1 H), 2.6–2.3 (m, 6 H), 1.43 (s, 9 H), 1.28 (t, 3 H, J = 7 Hz), 1.24 (t, 3 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 173.1 (CO_2R), 172.2 (CO_2R), 155.1 (NHC=O), 80.0 [$OC(CH_3)_3$], 61.3 and 60.3 (OCH_2CH_3), 56.0 (C-1), 40.2 (C-2 and C-4), 36.6 (CH_2CO_2R), 28.2 [$C(CH_3)_3$], 25.4 (C-3), 14.2 (OCH_2CH_3).

***cis*-1-Amino-3-(carbethoxymethyl)cyclobutane-1-carboxylic Acid Hemihydrate (18a).** An initial suspension of the minor cis isomer from above (**24a**; 275 mg, 0.83 mmol) was hydrolyzed by refluxing with 6 M HCl for ca. 24 h. The reaction mixture was concentrated under reduced pressure, and final traces of HCl were removed by the repeated addition of H_2O and concentration under reduced pressure. The residue of hydrochloride thus obtained was dissolved in a minimum of H_2O and purified by ion-exchange chromatography (Dowex AG 50 W, H^+ form) using H_2O only as the eluent to yield free amino acid **18** as a white, crystalline solid (125 mg, 82%). An analytical sample was prepared by recrystallization from H_2O: mp 245–248 °C dec (darkening from 235 °C); ^1H NMR (D_2O) δ 3.02–2.54 (m, 5 H), 2.26–2.01 (m, 2 H); MS m/z 174 (MH^+, 100), 156 (19), 128 (22). Anal. ($C_7H_{11}NO_4 \cdot 0.5H_2O$) C, H, N.

***trans*-1-Amino-3-(carbethoxymethyl)cyclobutane-1-carboxylic Acid Hemihydrate (18b).** In a similar manner the major (trans) isomer from above (**24b**; 250 mg, 0.76 mmol) was hydrolyzed and chromatographed to afford the free amino acid **18b** as a white, crystalline solid (85 mg, 61%) which was recrystallized from H_2O: mp 249–251 °C dec (softening from 225 °C); ^1H NMR (D_2O) 3.01–2.41 (overlapping m); MS m/z 174 (MH^+, 100), 156 (40), 128 (51). Anal. ($C_7H_{11}NO_4 \cdot 0.5H_2O$) C, H, N.

Diethyl 3-[(Diethoxyphosphinyl)methylene]cyclobutane-1,1-dicarboxylate (29). A solution of tetraethyl methylenebisphosphonate (7.2 g, 25 mmol) in anhydrous THF (10 mL) was added dropwise with stirring at room temperature, over 5–10 min, to a suspension of NaH [600 mg, 25 mmol; obtained by washing an 80% emulsion in oil (750 mg) with pentane] in anhydrous THF (30 mL) under nitrogen. A slight increase in temperature was observed. The solution was cooled to room temperature, a solution of **15** (5.35 g, 25 mmol) in THF (10 mL) was added dropwise over ca. 5–10 min and the resulting solution was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure and worked up by the general procedure outlined for the synthesis of **23** to afford crude **29** as a yellow oil (7.75 g). Chromatography (gradient; 2:1 petroleum/EtOAc rising to 1:2 petroleum/EtOAc) yielded initially unchanged ketone **15** (520 mg, 10% recovery) followed by phosphonate **29** as a very pale yellow oil (6.10 g, 70%): ^1H NMR ($CDCl_3$) δ 5.51 (d quin, 1 H, J = 16.6 Hz, J = 2.4 Hz), 4.23 (q, 4 H, J = 7.1 Hz), 4.07 (dq, 4 H, J = 7.8, 7.0 Hz), 3.58 [m (approximate dd with J = 3.7, 3.1 Hz), 2 H], 3.36 [m (approximate dd with J = 5.0, 2.4 Hz), 2 H], 1.33 (dt, 6 H, J = 7.0, 0.5 Hz), 1.27 (t, 6 H, J = 7 Hz).

1-(Carbethoxy)-3-[(diethoxyphosphinyl)methylene]cyclobutane-1-carboxylic Acid (36). Compound **29** (1.74 g, 5 mmol) was refluxed in a solution of KOH (ca. 300 mg of 85%) in a mixture of H_2O (5 mL) and EtOH (20 mL) for 8 h and worked up as described in previous examples to yield, together with unreacted **29** (280 mg, 16% recovery), the title monoacid **36** as a viscous, virtually colorless oil (1.15 g, 72% or 86% based on consumed **29**): ^1H NMR ($CDCl_3$) δ 8.8 (variable depending on concentration and traces of H_2O, br s, 1 H), 5.50 (d of apparent quintet, 1 H, J = 17, 2.2 Hz), 4.24 (q, 2 H, J = 7 Hz), ca. 4.10 and 4.09 (both dq, total 4 H, J = 7.9, 7.0 Hz) (arising from two magnetically nonequivalent OCH_2CH_3 groups attached to P=O), 3.7–3.5 (m, 4 H), 1.32 (t, 6 H, J = 7 Hz), 1.28 (t, 3 H, J = 7 Hz); MS m/z 321 (MH^+, 100), 305 (4), 277 (16), 276 (3), 249 (5), 233 (3).

Ethyl 1-[[(*tert*-Butyloxy)carbonyl]amino]-3-[(diethoxyphosphinyl)methylene]cyclobutane-1-carboxylate (27). Method A. A solution of monoester **36** (800 mg, 2.5 mmol), diphenyl phosphorazidate (725 mg, 2.63 mmol), and triethylamine (275 mg, 2.72 mmol) in toluene (5 mL) was reacted as described previously. tBuOH (0.25 mL, 2.65 mmol) was added in one portion and the mixture was heated at 110 °C for ca. 40 h. Solvent and other volatiles were removed under reduced pressure to afford a viscous, dark brown oil. This residue was taken up into a mixture of Et_2O (10 mL) and H_2O (10 mL), the Et_2O layer was separated, and the aqueous layer was extracted further with Et_2O (4 × 5 mL). The combined Et_2O extracts were washed in turn with H_2O (10 mL) and saturated NaCl (2 × 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure to yield crude **27** as a viscous, dark brown oil (550 mg). Chromatography (gradient; 3:2 petroleum/EtOAc rising to 1:2 petroleum/EtOAc) afforded a fraction containing **27** which was isolated as a straw-colored to colorless, viscous oil (400 mg, 41%): ^1H NMR ($CDCl_3$) δ 5.54 (d of apparent quin, 1 H, J = 17, 2.2 Hz), 5.37 (variable) (br s, 1 H), 4.22 (q, 2 H, J = 7 Hz), 4.07 (dq, 2 H, J = ~7.7, 7 Hz), 4.06 (dq, 2 H, J = ~7.7, 7 Hz), 3.7–2.9 (m, 4 H), 1.44 (s, 9 H), 1.32 (t, 6 H, J = 7 Hz), 1.28 (t, 3 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 173.0 (CO_2R), 160.1 (C-3, d, J = 3.4 Hz), 154.9 (NHC=O), 109.9 (PCH=, d, J = 189 Hz), 80.2 [$OC(CH_3)_3$], 61.7 (OCH_2CH_3), 61.5 ($OPOCH_2CH_3$, d, J = 6.1 Hz), 55.0 (C-1), 44.4, 44.0, 43.6, 43.3 (two superimposed or proximal doublets; δ and J values unassigned[45]) (C-2 and C-4, unassigned), 28.3 [$C(CH_3)_3$], 16.4 ($OPOCH_2CH_3$, d, J = 6.1 Hz), 14.1 ($OCOCH_2CH_3$).

Method B. A solution of tetraethyl methylenebisphosphonate (576 mg, 2 mmol) in anhydrous dioxane (2 mL) was added dropwise with stirring over ca. 3–5 min to a suspension of NaH [48 mg, 2 mmol; obtained by washing an 80% emulsion of NaH in oil (60 mg) with dry pentane] in dry dioxane under nitrogen. The resulting clear solution was treated with a solution of **19** (514 mg, 2 mmol) in dry dioxane (2 mL) as described for the synthesis of **23** and the mixture was stirred at room temperature overnight. The product was worked up by the procedure already described for the synthesis of **23** to give crude **27** as a brown oil. Chromatography as before afforded **27** as a straw-colored oil (200 mg, 26%).

(45) Four distinct peaks (two pairs of doublets) were observed at 90 MHz; at both 200 and 400 MHz, the signal collapsed into a broad singlet and no additional details of chemical shift and coupling constants could be determined.

1-Amino-3-(phosphonomethylene)cyclobutane-1-carboxylic Acid (26). Method A. The intermediate isocyanate **28** was generated from a solution of half-ester **36** (320 mg, 1 mmol), diphenyl phosphorazidate (290 mg, 1.05 mmol), and triethylamine (110 mg, 1.09 mmol) in anhydrous toluene (ca. 5 mL) by heating under nitrogen for 1 h at 80 °C followed by 2 h at 110 °C. Toluene and other volatiles were removed under reduced pressure to afford a dark brown oil which was suspended in 6 M HCl (5 mL), and the mixture was refluxed for ca. 48 h. The solution was concentrated under reduced pressure, and the final traces of HCl were removed in the normal manner. Ion-exchange chromatography (Dowex AG 1, acetate form; gradient, H_2O rising to 3 M AcOH) afforded a fraction (mostly eluted with 1 M AcOH) which on concentration and removal of the last traces of AcOH by the repeated addition and evaporation of H_2O yielded an impure sample of the title compound **26** as white, glassy crystals (95 mg, 42% calcd as hydrate) which was extremely hygroscopic/deliquescent on exposure to air. Impurities of higher molecular weight could be detected in a mass spectrum of this product: ^{1}H NMR (D_2O) δ 5.78 (d of apparent quintet, 1 H, J = ~15, 2.5 Hz), 3.78–2.87 (m, 4 H), impurity peaks at δ 2.58, 2.39, 2.29 and 2.08 (2–5% assuming similar structure); MS m/z 208 (MH^+).

Method B. An (initial) suspension of **27** (330 mg, 0.84 mmol) was hydrolyzed with 6 M HCl (10 mL) for ca. 40 h and the resulting solution was worked up in the normal manner to afford a residue of crude hydrochloride. An alternative ion-exchange chromatographic separation (Dowex AG 50, H^+ form) using only H_2O as the eluent afforded initially a viscous oil which on complete drying gave an impure sample of **26** as a colorless, glass solid (140 mg, 74% based on monohydrate).

Diethyl 3-[(Diethoxyphosphinyl)methyl]cyclobutane-1,1-dicarboxylate (37). Hydrogenation of **29** (3.53 g, 10.1 mmol) in EtOH (25 mL) over 10% Pd/C gave crude **37** which was purified by chromatography (1:1 petroleum/EtOAc) and isolated as a very pale yellow oil (quantitative yield). Distillation (Kugelrohr) afforded pure **37** as a colorless oil: bp 205 °C/0.05 mm; ^{1}H NMR ($CDCl_3$) δ 4.23 (q, 2 H, J = 7 Hz), 4.19 (q, 2 H, J = 7 Hz), 4.08 (dq, 4 H, J = ~7.7, 7 Hz), 2.8–2.3 (m, 5 H), 1.91 (dd, 2 H, $J \cong$ 18, 6.5 Hz), 1.31 (t, 6 H, J = 7 Hz), 1.27 (t, 3 H, J = 7 Hz), 1.25 (t, 3 H, J = 7 Hz).

***cis*-(38a) and *trans*-1-(Carbethoxy)-3-[(diethoxyphosphinyl)methyl]cyclobutane-1-carboxylic Acid (38b).** Hydrolysis of **37** (5.57 g, 16 mmol) with KOH (1.02 g of 85%) in a mixture of EtOH (57.5 mL) and H_2O (17.5 mL) and subsequent acidification as described previously afforded a mixture of the cis and trans title compound (**38a** and **38b**, unknown assignment; predominantly one isomer (>70%) by comparison of ^{1}H NMR signals of ester methyl protons) as a colorless, crystalline solid (3.89 g, 76%): ^{1}H NMR ($CDCl_3$) δ 8.66 (variable, br s, 1 H), 1.28 and 1.26 (t, J = 7 Hz and t, J = 7 Hz respectively, total 3 H; from major and minor isomeric products, respectively); the remaining spectrum was insufficiently well resolved for assignment of individual peaks.

All attempts to achieve separation of the isomers by fractional crystallization proved unsuccessful and the unseparated mixture of isomers was used in a subsequent step.

***cis*- and *trans*-Ethyl 1-[[(*tert*-Butyloxy)carbonyl]amino]-3-[(diethoxyphosphinyl)methyl]cyclobutane-1-carboxylate (30a and 30b, Unknown Assignment).** Chromatography of the crude isomeric mixture of the title compound, obtained as a viscous brown oil from reaction of the mixture of half-esters **38a** and **38b** (2.42 g, 7.5 mmol), diphenyl phosphorazidate (2.15 g, 7.8 mmol), triethylamine (0.85 g, 8.4 mmol), and tBuOH (0.75 mL, 8.0 mmol) by a previously described procedure, yielded inter alia a fraction affording a mixture of the cis and trans isomers (unknown assignment, one major and one minor isomeric components) as a straw-colored oil (580 mg) which slowly crystallized and which was free of impurities: ^{1}H NMR ($CDCl_3$) δ 5.21 and 5.02 (N*H* peaks in approximate ratio 1:1 with the intensity of the peak at 5.21 being marginally greater).

A subsequent fraction from the column consisted of a slightly impure mixture of the isomers as a pale yellow oil (770 mg, 26%) (total 1.35 g, 46%) which partly crystallized on standing. These fractions were combined and repeatedly (>20 times) recrystallized from pentane to afford a sample of the major isomer [shown to be reasonably free of the minor (noncrystalline) isomer by ^{1}H NMR analysis] as colorless needles (ca. 125 mg). Further recrystallization (more than five times) from pentane eventually afforded a small sample (<25 mg) of the major cis isomer (**30a**) in which contamination by the other isomer could barely be detected by ^{1}H NMR: mp 82.5–85 °C; ^{1}H NMR ($CDCl_3$) δ 5.21 (br s, 1 H), 4.23 (q, 2 H, J = 7 Hz), 4.08 (dq, 4 H, J = ~7.7, 7 Hz), 2.9–2.2 (m, 4 H), 1.98 (dd, 2 H, J = 18, ~7.2 Hz), 1.43 (s, 9 H), 1.31 (t, 6 H, J = 7 Hz), 1.30 (t, 3 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 173.9 (CO_2R), 154.6 (NH*C*=O), 79.8 [O*C*(CH_3)$_3$], 61.4 (O*C*H_2CH$_3$), 61.4 (O=POCH_2CH$_3$, d, J = ca. 6.5 Hz), 55.1 (C-1), 38.8 (C-2 and C-4, d, J = 12.2 Hz), 33.0 (O=P*C*H_2, d, J = 138 Hz), 28.3 [C(*C*H_3)$_3$], 22.9 (C-3, d, J = 6.1 Hz), 16.5 (O=POCH_2*C*H_3, d, J = 6.1 Hz), 14.2 (O=COCH_2*C*H_3); MS m/z 394 (MH^+, 63), 378 (4), 366 (10), 338 (26), 320 (15), 294 (100). Anal. ($C_{17}H_{32}NO_7P$) C, H, N.

A quantity (100 mg) of less isomerically pure (≥95%) sample of the major cis isomer (**30a**) was recovered from the latter filtrates and used in a subsequent hydrolysis.

***cis*- and *trans*-1-Amino-3-(phosphonomethyl)cyclobutane-1-carboxylic Acid (Mixture of 8a and 8b, Unassigned).** Hydrolysis of the mixture of isocyanate intermediates **39** prepared from a mixture (322 mg, 1 mmol) of the isomeric half-esters **38a** and **38b**, diphenyl phosphorazidate (288 mg, 1.05 mmol) and triethylamine (108 mg, 1.05 mmol) and purification of the crude product by chromatography [as described for the preparation (method A) of the unsaturated analogue **26**] afforded a mixture of the cis (**8a**) and trans (**8b**) isomers (unknown ratio) as an off-white, hygroscopic glass (75 mg, 33%).

***cis*-1-Amino-3-(phosphonomethyl)cyclobutane-1-carboxylic Acid (8a).** Hydrolysis of the sample of **30a** from above (100 mg, 0.25 mmol) and purification by ion-exchange chromatography (Dowex AG50 W, H^+ form; eluent H_2O only) afforded **8a** as a colorless, crystalline solid (ca. 55 mg, almost quantitative yield): ^{1}H NMR (D_2O) δ 3.05–2.53 (m, 5 H), 1.95 (dd, 2 H, J = ~17, 7 Hz).

***(E)*- (31a) and *(Z)*-Tetraethyl 2-Propenyl-1,3-bisphosphonate (31b).** A mixture of (*E*)- and (*Z*)-diethyl 3-bromoprop-2-enylphosphonate (**40a** and **40b**, unassigned) was obtained as a colorless to pale yellow oil (72.9 g, 74%) of bp 79–85 °C/0.1 mm from the Michaelis–Arbuzov reaction of a mixture of *E* and *Z* isomers of 1,3-dibromopropene[46] with triethyl phosphite according to a modification of the procedure described for the preparation of the chloro analogue(s).[46]

In a large-scale experiment,[47] triethyl phosphite (18.0 mL) was added dropwise with stirring, over ca. 30 min, to a mixture of *E* and *Z* isomers from above (25.6 g, 0.1 mol) and anhydrous powdered $NiCl_2$ (1.0 g) which was maintained at ca. 150 °C under nitrogen (evolved ethyl bromide was collected in a Dean–Stark trap). The reaction mixture was heated for an additional 12 h while two further additions of both $NiCl_2$ (each of 500 mg) and triethyl phosphite (each of 2 mL) were made after 4 and 8 h. Excess triethyl phosphite was distilled off under reduced pressure (ca. 5–10 mm, pot temperature <180 °C) [an ^{1}H NMR spectrum of the crude mixture at this stage indicated complete conversion to predominantly one isomer (>95% of *E* isomer (**31a**); for assignments see below)]. The residue was distilled under reduced pressure to afford two fractions: (a) a mixture of isomers [**31a** and **31b**, now containing ca. 10–20% of the minor *Z* isomer (**31b**)] as a colorless to pale yellow oil (14.35 g, 46%; bp 150–155 °C/0.05–0.1 mm) and (b) a similar mixture of isomers as a colorless to pale yellow oil [5.1 g, 16% (total 19.45 g, 62%); bp 155–158 °C/0.05–0.1 mm] with **31b** making a smaller but still significant (ca. 10%) contribution.

These fractions were recombined and a portion (10 g) was chromatographed on silica gel (gradient; 100% EtOAc rising to 1:19 EtOH/EtOAc) to afford initially **31b** as a colorless oil (300 mg) [^{1}H NMR ($CDCl_3$) δ 7.03–6.63 and 6.46–6.06 (dm, 1 H, $J \cong$ 50 Hz), 5.94–5.52 (sym m, 1 H), 4.14 (dq, 4 H, J = ~7.8, 7 Hz), 4.08 (dq, 4 H, J = ~7.8, 7 Hz), 3.32 (dddd, 2 H, J = ~22, 7, ~4,

(46) Lavielle, G.; Sturtz, G.; Normant, H. *Bull. Soc. Chim. Fr.* **1967**, 4186.

(47) When performed at half-scale, the addition of extra anhydrous nickel(II) chloride and triethyl phosphite was found to be unnecessary while the reaction had gone to completion essentially within 4 h.

~2 Hz), 1.33 (t, 12 H, J = 7 Hz)], followed by a small quantity of mixed isomers and finally reasonably isomerically pure *E* isomer **31a** as a colorless oil (9.4 g): ^{1}H NMR ($CDCl_3$) δ 7.03–6.36 (m, 1 H), 5.83 (dddt, J = ~18, ~18, 4.5, ~1.5 Hz), 4.12 (dq, 4 H, J = ~7.8, 7 Hz), 4.09 (dq, 4 H, J = ~7.8 7 Hz), 2.78 (ddt, 2 H, J = 22.5, 7.4, ~1.5 Hz), 1.33 (t, 12 H, J = 7 Hz). This latter fraction was used in subsequent reactions.

Ethyl 1-[[(*tert*-Butyloxy)carbonyl]amino]-3-[(*E*)-3-(diethoxyphosphiny)prop-2-enylidene]cyclobutane-1-carboxylate (33). Compound **19** (900 mg, 3.5 mmol) in anhydrous dioxane was treated with a solution of the anion generated from the reaction of **31a** (1.10 g, 3.5 mmol) with NaH (84 mg, 3.5 mmol) in anhydrous dioxane (7 mL) under nitrogen at ca. 10 °C, and the reaction mixture was worked up as described for the preparation of **23** to afford a crude sample of **32** as a viscous straw-colored oil (ca. 1.5 g) free of unreacted ketone **19**. Chromatography (1:1 petroleum/EtOAc) yielded **32** as a viscous pale yellow oil (1.05 g, 72%) which was hydrogenated immediately upon isolation (see below): ^{1}H NMR ($CDCl_3$) δ 7.03 (ddd, 1 H, J = 21, 17, 11 Hz), 6.06 (dm, 1 H, J = 11 Hz), 5.55 (dd, 1 H, J = 19.5, 17 Hz), 5.3 (br s, 1 H), 4.23 (q, 2 H, J = 7 Hz), 4.08 (dq, 4 H, J = ca 7.5, 7 Hz), 3.55–3.02 (m, 4 H), 1.44 (s, 9 H), 1.32 (t, 6 H, J = 7 Hz), 1.28 (t, 3 H, J = 7 Hz).

Mixture of *cis*-(33a) and *trans*-Ethyl 1-[[(*tert*-Butyloxy)carbonyl]amino]-3-[3-(diethoxyphosphinyl)propyl]cyclobutane-1-carboxylate (33b). Compound **33** (1.0 g, 2.4 mmol) in absolute EtOH (25 mL) was reduced over 10% Pd/C at 1 atm. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure to a pale yellow, viscous oil (980 mg). Chromatography (1:1 petroleum/EtOAc) gave a virtually colorless oil (770 mg, 76%) shown by ^{1}H NMR to be a mixture of cis and trans isomers (**33a** and **33b**, respectively) (ca. 3:1 unassigned major/minor isomer): ^{1}H NMR ($CDCl_3$) δ 5.12 and 5.03 (br s of minor and major isomers, respectively, total 1 H), 4.24 and 4.19 (q of minor and major isomer, respectively, total 2 H, J = 7 Hz) 4.11 (two superimposed dq, not resolved, total 4 H), 2.83–1.55 (two superimposed m, total 11 H), 1.46 (s, 9 H), 1.34 (t, 6 H, J = 7 Hz), 1.29 and 1.26 (t of minor and major isomers, respectively, total 3 H, J = 7 Hz).

Mixture of *cis*-(9a) and *trans*-1-Amino-3-(3-phosphonopropyl)cyclobutane-1-carboxylic Acid (9b). A mixture of **33a** and **33b** (250 mg, 0.6 mmol) was hydrolyzed by refluxing in 6 M HCl (ca. 10 mL) for 24 h. The mixture was concentrated under reduced pressure and the last trace of HCl was removed by the repeated addition of H_2O and concentration under reduced pressure. The residue of mixed amino acid hydrochlorides was dissolved in a minimum volume of H_2O and purified by ion-exchange chromatography (Dowex AG 50 W, H^+ form) using H_2O as the eluent. Two fractions containing mixtures of the cis and trans isomers were collected. Concentration of the first fraction gave a mixture of **9a** and **9b** as a white, crystalline solid (55 mg) shown by ^{13}C NMR to consist of a mixture of one major and one minor geometrical isomer: ^{13}C NMR (D_2O/2 M NaOD) δ 186.3 and 185.9 (CO_2^- of major and minor isomers, respectively) and 57.5 and 56.9 ($H_2NCCO_2^-$ of major and minor isomers, respectively, unassigned configurations). Concentration of the second fraction also afforded a mixture of isomers as a white, crystalline solid (65 mg; total 120 mg, 79% calcd as monohydrate) shown by ^{13}C NMR to consist of a mixture of approximately equal proportions of **9a** and **9b**.

Crystallography. Cell constants were determined by least-squares fits to the setting values of 25 independent reflections, measured and refined on an Enraf-Nonius CAD4-F diffractometer with a graphite monochromator. The crystallographic data are summarized in Table I. Data were reduced and Lorentz, polarization, and decomposition corrections were applied by using the Enraf-Nonius Structure Determination Package.[48] The structures were solved by direct methods and refined by full-matrix least-squares techniques by using SHELX-76.[49] The three ethyl groups of **30a** were disordered over two sites. All non-hydrogen atoms with the exception of minor contributors to disordered atoms were refined anisotropically, hydrogen atoms were refined with isotropic thermal parameters in all structures except that of **30a** in which the hydrogen atoms of the groups other than the cyclobutane ring were included at calculated sites (C–H 0.97 Å) with group isotropic thermal parameters. Scattering factors used were those supplied in SHELX-76.[49] The atomic nomenclature is defined in Figures 5–7.[35] Listing of all atom coordinates, thermal parameters, details of least-squares planes calculations, and observed and calculated structure factor amplitudes have been deposited.

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Supplementary Material Available: Tables listing X-ray crystallographic data including atomic coordinates, thermal parameters, and least-squares planes calculations for **7b**, **18b**, and **30a** (18 pages); tables listing observed and calculated structure factor amplitudes (36 pages). Ordering information is given on any current masthead page.

(48) *Enraf-Nonius Structure Determination Package (SDP)*; Enraf Nonius: Delft, Holland, 1985.

(49) Sheldrick, G. M. *SHELX-76, A Program for X-Ray Crystal Structure Determination*; University of Cambridge: Cambridge, England, 1976.