The supernatant was clarified, and the virus particles were then pelleted at 40000 rpm for 30 min by using a rotor (70.1 Ti; Beckman Instruments, Inc., Fullerton, Calif.) and suspended in virus-disrupting buffer. The RT assay was performed by a modification of the method of Spira et al.[34] in 96-well microdilution plates using (rA)n·$(dT)_{12-18}$ as template-primer. The RT results were expressed in dpm/milliliter of originally clarified supernatant. The drugs were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells and also in African green Monkey kidney (Vero) cells that were obtained from the American Type Culture Collection, Rockville, MD. The Vero cells were maintained in minimum essential medium supplemented with 2% heat-inactivated fetal calf serum, penicillin (100 units/mL), and streptomycin (100 μg/mL), and the toxicity assay was performed as described previously.[34] The PBM and Vero cells were cultured with and without drug for 6 and 4 days, respectively, at which time they were counted for cell proliferation and viability by using the trypan blue exclusion method.[30,34] Only the effects on cell growth are reported since these correlated well with cell viability. The median effective concentration (EC_{50}) and inhibitory concentration (IC_{50}) values were derived from the computer-generated median-effect plot of the dose-effect data as described previously.[35]

Acknowledgment. The technical assistance of V. Saalmann and D. L. Cannon is gratefully acknowledged. This work was supported by Public Health Service Grants AI 26055 and AI 25899 from the National Institutes of Health and the Veterans Administration.

Registry No. 1, 69530-93-4; **2**, 126502-03-2; **3**, 126502-04-3; 4, 126541-12-6; **5**, 126541-13-7; **6**, 117723-57-3; **7a**, 85326-07-4; **7b**, 120503-35-7; **7c**, 120503-63-1; **7d**, 120503-30-2; **8a**, 126637-92-1; **8b**, 126638-02-6; **8c**, 126638-03-7; **9**, 126637-93-2; **10**, 126502-05-4; 11, 126637-94-3; **12a**, 126502-06-5; **12b**, 126502-18-9; **12c**, 126502-19-0; **12d**, 126502-20-3; **13a**, 126637-95-4; **13b**, 126637-99-8; **13c**, 126638-00-4; **13d**, 126638-01-5; 14, 126502-07-6; **15**, 126637-96-5; **16**, 126502-08-7; **17**, 126637-97-6; **18**, 120503-34-6; **19**, 126637-98-7; **20**, 126502-09-8; **21**, 126502-10-1; **22**, 107550-76-5; **23**, 443-72-1; **24**, 97614-44-3; **25a**, 126502-11-2; **26**, 126502-12-3; **27**, 126502-13-4; **28**, 126502-14-5; **30**, 126502-15-6; **31**, 126502-16-7; **32**, 126502-17-8; CS_2, 75-15-0; chlorotrimethylsilane, 75-77-4; *tert*-butyldimethylsilyl chloride, 18162-48-6; *N,N*-thiocarbonyldiimidazole, 6160-65-2; 6-chloropurine, 87-42-3.

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Synthesis and Bioactivity of a New Class of Rigid Glutamate Analogues. Modulators of the *N*-Methyl-D-aspartate Receptor

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A variety of derivatives of azetidine-2,4-dicarboxylic acid were synthesized and examined for their ability to stimulate $^{45}Ca^{2+}$ uptake in cultures of cerebellar granule cells. Of the compounds tested, the *cis*-azetidine-2,4-dicarboxylic acid (**10f**) was found to be the most potent agent in potentiating glutamate, aspartate, or *N*-methyl-D-aspartate (NMDA) stimulated $^{45}Ca^{2+}$ uptake at the NMDA receptor. The mechanism of action of **10f** was further investigated in [^{3}H]MK-801 binding assays and [^{3}H]GABA release from cultured embryonic rat forebrain neurons. All of the results from the functional studies of azetidine **10f** are consistent with a selectivity of action at the NMDA receptor. Moreover, azetidine **10f** appears to exhibit a dual type of action, behaving as a glutamate-like agonist at higher concentrations and as a positive modulator at concentrations below 50 μM.

Currently four L-glutamate receptor subtypes have been identified on the basis of the ligand structural features essential for receptor binding, as well as, in part, the coupling of these recognition sites to specific signal transduction systems.[1] The *N*-methyl-D-aspartate (NMDA) receptors are activated by glutamate, aspartate, and NMDA, and are competitively antagonized by D-2-amino-5-phosphonovaleric acid (D-APV) or noncompetitively by phencyclidine (PCP) and MK-801. NMDA receptors are coupled to a Na^+/Ca^{2+} permeable ion channel which exhibits a voltage dependent Mg^{2+} blockade. The NMDA receptor operated ion channel is noncompetitively blocked by Zn^{2+}, which appears to act at a site distinct from that for Mg^{2+}.[2] Quisqualate receptors are activated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and are antagonized by agents such as γ-glutamyl aminomethanesulfonate (GAMS) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX).[3] One subtype of quisqualate receptor is linked to a fast response mediated by Na^+ channels and a second type to a slower response involving the activation of a phospholipase C with the production of inositol 1,4,5-trisphosphate. Kainate receptors are also antagonized by CNQX and their response mediated by Na^+ ion channels, much like that of one of the quisqualate receptor subtypes.[4]

Of these four glutamate receptor subtypes, the NMDA receptor in particular has captured the attention of many neurobiologists, for it has been shown to play a key role

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§ Fidia-Georgetown Institute.

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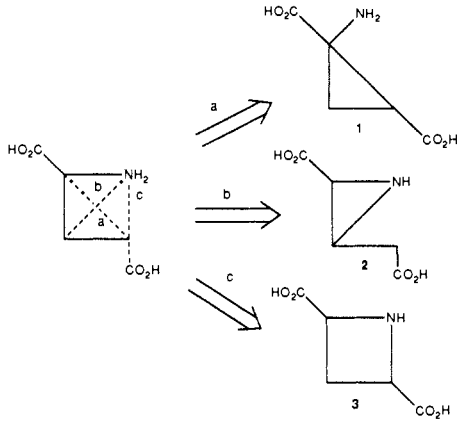
Scheme I. Rigid Glutamate Analogues

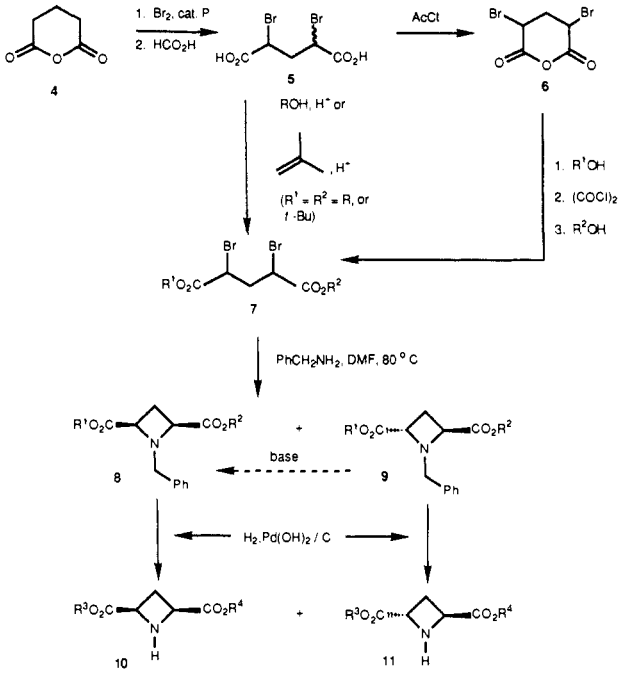


in both the induction of LTP (long term potentiation, a current model of memory)[5] and in the neuronal degeneration resulting from the ischemic conditions accompanying a stroke or heart attack.[6] The latter state is presumably reached when excessive amounts of the excitatory amino acids, glutamate or aspartate, are released, leading to an over-stimulation of NMDA receptors with consequent alterations in the calcium fluxes. Additionally, xanthine oxidase generated superoxide radicals may play an important role in this degenerative process.[7]

Voluminous data can now be found regarding the ability of agents like PCP[8] to inhibit NMDA receptor function.[9] Memory deficits have in fact been shown to be associated with PCP abuse in man, an expected consequence of the inhibition of calcium fluxes through the NMDA receptor channel.[10] Consequently, the design of safe but efficacious negative modulators of this receptor which are able to penetrate the blood–brain barrier would appear likely to hold promise as neuroprotective agents in disease states involving excessive neurotransmitter release.[11]

Of equal interest would be the development of agents which potentiate NMDA receptor function, for one might suppose that such modulators may serve as cognition enhancing agents, facilitating possibly both the acquisition of memory and the process of learning. Recently, through electrophysiological experiments the simplest of amino acids, glycine, has been shown to function as a positive allosteric modulator of the NMDA receptor. Glycine binds to an allosteric regulatory site on the NMDA receptor complex and is able to increase both the duration of channel open time and, most markedly, the frequency of

Scheme II. Synthesis of Azetidine-2,4-dicarboxylic Acid Derivatives



	R^1	R^2	R^3	R^4
a	Me	Me	Me	Me
b	Me	*t*-Bu	Me	*t*-Bu
c	*t*-Bu	*t*-Bu	*t*-Bu	*t*-Bu
d	Me	Ch_2Ph	Me	H
e	*t*-Bu	Ch_2Ph	*t*-Bu	H
f	Ch_2Ph	Ch_2Ph	H	H

opening of the NMDA receptor associated ion channel.[12]

In an effort to learn more about the possible modulation of NMDA receptor function through the action of small organic molecules, we set out to synthesize a new class of conformationally rigidified glutamate molecules.[13] The methods for the synthesis of these structures, and the effect of these compounds in various biochemical assays of glutamate receptor function are detailed below.

Chemistry

The new class of rigid glutamate analogues we selected for synthesis was arrived at in a very simple way by examining the glutamate molecule itself and considering the possible ways of rigidifying its structure. Three possibilities for accomplishing this which do not involve lactam formation or the introduction of additional atoms are drawn in Scheme I. One of these compounds, the aziridine **2** was deemed to be potentially neurotoxic, because of the likelihood of ring-opening processes involving its strained three-membered ring.[14]

The azetidinedicarboxylic acid **3** appeared particularly intriguing, especially in light of the fact that the compound had apparently never been reported in the literature, let

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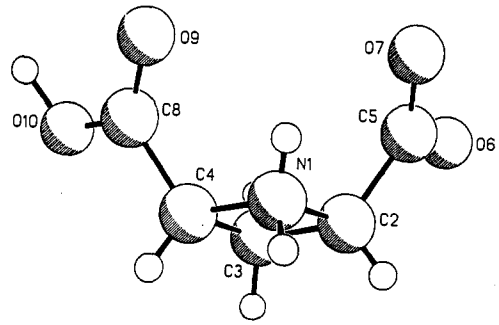
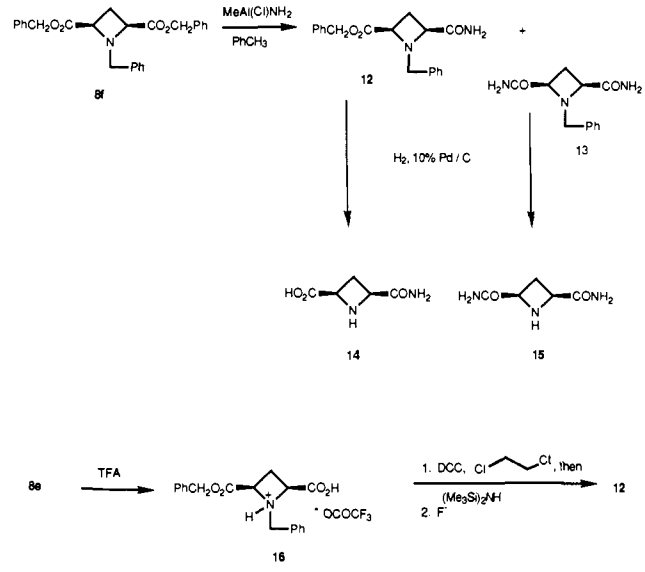


Figure 1. An X-ray structure of *cis*-azetidine-2,4-dicarboxylic acid (**10f**).

alone examined for its action as an excitatory amino acid analogue. Methods for the preparation of the diester derivatives of azetidine-2,4-dicarboxylic acid have, however, been reported. Baldwin has recently described a synthesis of *tert*-butyl methyl azetidine-2,4-dicarboxylate by reaction of a dibromoglutarate with benzylamine.[15]

Use of Baldwin's adaptation of Cromwell's original azetidine synthesis[16] was therefore made in the present work. As outlined in Scheme II, glutaric anhydride (4) was converted to the 2,4-dibromide **5** and then to 2,4-dibromoglutaric acid dibenzyl ester (**7f**) by reaction with benzyl alcohol and *p*-toluenesulfonic acid. This dibromide was next heated with benzylamine in DMF at 80 °C to yield a ~1:1 mixture of *cis*- and *trans*-dibenzyl 1-benzylazetidine-2,4-dicarboxylate (**8f** and **9f**). This mixture could easily be separated by silica gel chromatography. The pure isomers were then converted to their crystalline amino diacids **10f** and **11f** by hydrogenolysis over $Pd(OH)_2/C$. This hydrogenolysis procedure greatly facilitated isolation of the very polar, water-soluble products by avoiding the use of an aqueous saponification procedure. Both the cis and the trans diacid were obtained as crystalline solids. The ^1H NMR spectrum of the cis diacid exhibited an A_2XY pattern, that of the trans diacid an AA'XX' pattern. The cis diacid was further characterized by X-ray analysis.[17] The computer-generated drawing of **10f** is shown in Figure 1. It is of some importance to mention that the trans diester **9f** can be converted in modest yield to the cis diester **8f** by treatment with sodium benzyl oxide in a mixture of toluene and DMF. This result is in line with the expected higher ground state energy of the trans isomer

Scheme III. Synthesis of Amide Derivatives



for which two equilibrating conformations can be drawn, both of which exhibit a pseudoaxial-benzyl ester/*N*-benzyl interaction.[18]

Since biological studies revealed compound **10f** to potentiate the action of glutamate or aspartate at the NMDA receptor (see the following section), a variety of diester, acid–ester, acid–amide, and diamide analogues were synthesized in order to probe the structural features essential to activity. Some of these compounds were also of interest for study as prodrug forms of **10f**, which because of their greater lipophilicity were expected to exhibit a greater propensity to penetrate the blood–brain barrier.

Synthesis of the di-*tert*-butyl ester **10c** was accomplished by treating 2,4-dibromoglutaric acid with isobutylene and sulfuric acid. Reaction of the resulting diester with benzylamine in DMF at 80 °C for 2 h provided a mixture of the *cis*- and *trans*-azetidine diesters **8c** and **9c**, which were separable by column chromatography. The purified *cis*-1-benzylazetidine diester **8c** was hydrogenated over $Pd(OH)_2/C$ to yield the cis diester **10c** as a low-melting solid. The *cis*-azetidine-2,4-dicarboxylic acid dimethyl ester (**10a**) was prepared in a manner identical with that described for the dibenzyl ester. The unsymmetrical azetidine-2,4-dicarboxylic acid *tert*-butyl methyl ester (**10b**) was prepared according to Baldwin's procedure[11] which entails reaction of 2,4-dibromoglutaric anhydride with methanol to afford the monomethyl ester of 2,4-dibromoglutaric acid. This intermediate was converted to its acid chloride, and thence to the methyl *tert*-butyl ester by reaction with *tert*-butyl alcohol. Lastly, reaction of the 2,4-dibromoglutaric acid diester and benzylamine furnished a ~1:1 mixture of the cis- and trans-disubstituted azetidines **8b** and **9b**. These compounds were separated by column chromatography and the pure cis isomer hydrogenated as before to furnish azetidine **10b**.

Two racemic monoester derivatives **10d** and **10e** were also prepared. Compound **10d** was synthesized via the anhydride **6** by reaction first with methanol to provide the monoester, then with oxalyl chloride to give the acid chloride ester, and then with benzyl alcohol to provide the mixed diester. Benzylamine treatment, chromatography, and hydrogenolysis as before yielded the *cis*-azetidine-2,4-dicarboxylic acid monomethyl ester as a yellowish glass.

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(17) Rigaku AFC5 diffractometer with rotating anode X-ray generator, Ni-filtered Cu Kα X-radiation, 18 °C. Monoclinic, space group P_n, a = 5.3949 (4) Å, b = 5.5322 (5) Å, c = 10.4713 (6) Å, β = 97.019 (6)°, J = 2, density (calcd) = 1.56 g cm^{-3}. 515 reflections with $I > 2.5\sigma(I)$. Positions of all hydrogen atoms were initially determined from difference Fourier maps. Their positional coordinates and isotropic temperature factors were refined independently. Final R = 0.034 and 0.032 (weighted). The molecule exists as a zwitterion. Selected bond lengths and angles: N(1)–C(2), 1.506 (4); N(1)–C(4), 1.494 (4); C(2)–C(3), 1.546 (4); C(2)–C(5), 1.524 (5); C(3)–C(4), 1.546 (5); C(4)–C(8), 1.501 (5); C(5)–O(6), 1.267 (4); C(5)–O(7), 1.231 (5); C(8)–O(9), 1.200 (5); C(8)–O(10), 1.306 (4) Å. N(1)–C(2)–C(3), 90.6 (2); N(1)–C(2)–C(5), 111.0 (3); N(1)–C(4)–C(3), 91.1 (2); N(1)–C(4)–C(8), 111.1 (2); C(2)–C(3)–C(4), 87.4 (2); C(2)–N(1)–C(4), 90.9 (2); C(2)–C(5)–O(6), 115.1 (3); C(2)–C(5)–O(7), 119.2 (3); C(3)–C(2)–C(5), 115.8 (3); C(3)–C(4)–C(8), 116.2 (3); C(4)–C(8)–O(9), 122.6 (3); C(4)–C(8)–O(10), 112.1 (3); O(6)–C(5)–O(7), 125.7 (3); O(9)–C(8)–O(10), 125.2 (4)°.

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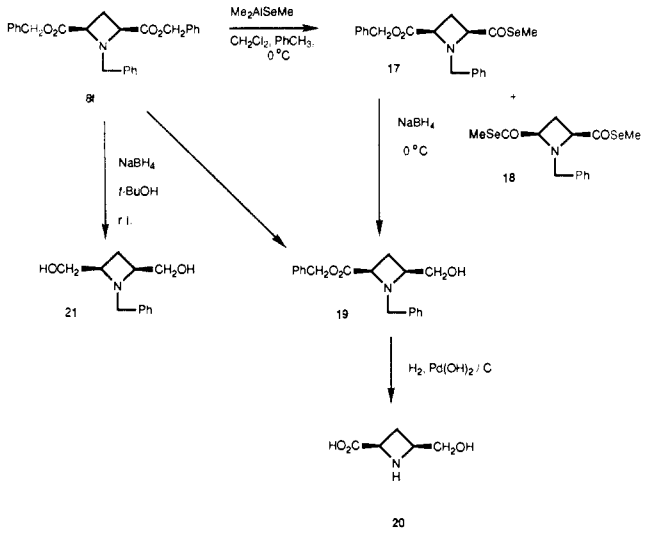
Scheme IV. Synthesis of 4-(Hydroxymethyl)azetidine-2-carboxylic Acid



The mono-*tert*-butyl ester was prepared in a very similar fashion; however, this time the anhydride was treated with benzyl alcohol first. The pure cis mono-*tert*-butyl ester **10e** was obtained as a crystalline solid (mp 157–159 °C).

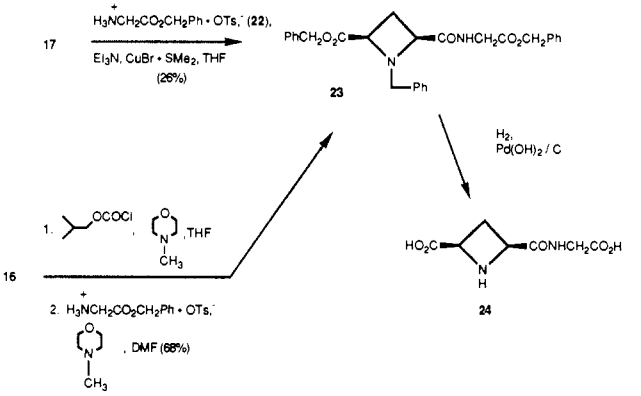
The mono- and diamide derivatives of **10f** (Scheme III) could be prepared from the diester **8f** as well. This was accomplished by exposing **8f** to the aluminum reagent prepared from finely powdered ammonium chloride and trimethylaluminum.[19] The diamide precipitated during the workup of this reaction while the monoamide was obtained in pure form by column chromatography. Hydrogenolysis of the *N*-benzyl group from **12** and **13** then provided the desired monoamide **14** and diamide **15**. Since the yield of **14** obtained by this process was low (~11%), an alternative method of preparation was developed. The monobenzyl ester **16**, prepared from **8e** by trifluoroacetic acid treatment, was reacted with DCC and hexamethyldisilazane (HMDS) in the presence of DMAP. Workup and chromatography provided the pure amide ester **12** in 50% yield which was converted to **14** by hydrogenolysis. To the best of our knowledge, the use of HMDS as an ammonia equivalent in the DCC-mediated amidation of carboxylic acids has not been described previously,[20] and we believe that it holds considerable promise as a mild and convenient way to effect this transformation.

The preparation of 4-(hydroxymethyl)azetidine-2-carboxylic acid (**20**) was accomplished in one of two ways (Scheme IV). First, by treating the diester **8f** with dimethylaluminum methylselenolate,[21] a mixture of the mono- and bis-selenol esters **17** and **18** was generated which could be separated by silica gel chromatography. The benzyl methaneselenol ester **17** was then stirred with sodium borohydride in a mixture of methylene chloride and ethanol to provide the desired alcohol **19** in 72% yield. Removal of the *N*-benzyl group was again brought about by hydrogenolysis over $Pd(OH)_2/C$ to yield **20**.

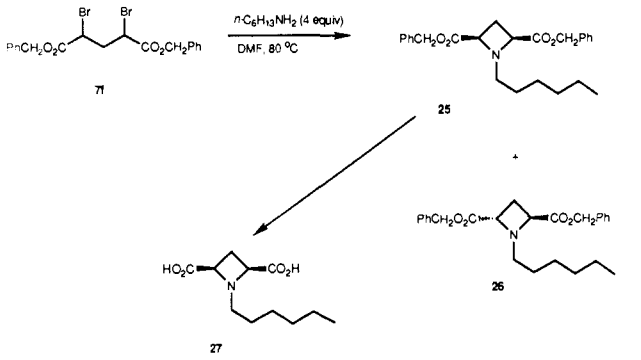
(19) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989.

(20) Acid chlorides yield amides on treatment with HMDS, followed by acidic hydrolysis: Bower, J. R.; Williams, P. J.; Kurz, K. *J. Org. Chem.* **1983**, *48*, 4111. Pellegate, R.; Halia, A.; Villa, M.; Paluisano, G.; Lesina, G. *Synthesis* **1985**, 517. A mixture of trimethylsilyl esters and *N*-(trimethylsilyl)amides was obtained from carboxylic acid anhydrides and HMDS: Koetzsch, H. J.; Vahlensieck, H. J. Ger. Offen. DE 3,443,960 (1986); *Chem. Abstr.* **1986**, *105*, 191384x.

Scheme V. Synthesis of the Dipeptide **24**



Scheme VI. Synthesis of *cis*-*N*-Hexylazetidine-2,4-dicarboxylic Acid



Since this first route to alcohol **20** suffered from a lack of specificity in formation of the monoselenol ester, we also investigated the direct reduction of the diester **8f** to the alcohol **19**. While sodium borohydride reduction in ethanol led to ester exchange as well as reduction, the use of *tert*-butyl alcohol as the solvent prevented the former process and provided **19** in 44% isolated yield together with some of the bis(hydroxymethyl)azetidine **21** (16%).

The selenol ester **17** was also used in the preparation of a dipeptide derivative **24** of azetidine-2,4-dicarboxylic acid (Scheme V). Compound **17** was thus reacted with the *p*-toluenesulfonic acid salt **22** of glycine benzyl ester[22] in the presence of triethylamine and cuprous bromide–dimethyl sulfide complex in THF as solvent.[21] The protected dipeptide derivative **23** was isolated in 26% yield. Alternatively, preparation of a mixed anhydride from the monobenzyl ester **16** and isobutyl chloroformate, followed by coupling with glycine benzyl ester,[23] provided **23** in a more acceptable yield of 68%. Hydrogenolysis of **23** under the standard conditions then provided the dipeptide **24** in which the azetidine diacid has been joined with glycine.

To examine the effect of an *N*-alkyl or *N*-aryl substituent on the biological activity of the azetidinedicarboxylic acid, the dibromide **7f** was reacted with an excess of *n*-hexylamine in DMF at 80 °C to furnish a 1:2 cis/trans mixture of the azetidines **25** and **26**. Separation of these materials could be accomplished only by HPLC. The pure cis isomer was then hydrogenated over $Pd(OH)_2/C$ to provide the desired *cis*-1-hexylazetidine-2,4-dicarboxylic acid **27** (Scheme VI). The *N*-benzyl derivative **28** could, on the other hand, be procured from **8f** by partial hydrogenolysis (see Experimental Section).

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(22) Hershenson, F. M. *J. Org. Chem.* **1975**, *40*, 1260.

(23) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012.

Table I. Azetidine Diacid **10f** Potentiates Calcium Influx Stimulated by Agonists of NMDA Receptors[a]

addition	$^{45}Ca^{2+}$ influx, nmol/mg of protein: control	$^{45}Ca^{2+}$ influx, nmol/mg of protein: azetidine **10f**	stimulation, %
basal	16 ± 1.5	23 ± 1.9[b]	144
+ NMDA 50 μM	91 ± 6.5	136 ± 8.4[b]	151
+ glutamate 1 μM	39 ± 3.8	85 ± 7.6[b]	268
+ aspartate 10 μM	43 ± 2.9	75 ± 4.1[b]	193
CPP 10 μM	5.4 ± 0.3	4.9 ± 0.2	91
+ kainate 100 μM	56 ± 3.7	52 ± 2.3	93
+ quisqualate 100 μM	15 ± 1.2	15 ± 0.8	100

[a] Granule cells were preincubated for 15 min in control conditions or with 50 μM azetidine **10f** followed by 10 min of incubation without (basal) or with the indicated concentrations of agonists. Incubations with kainate and quisqualate were performed in the presence of 10 μM CPP to block the NMDA receptors. The percent of stimulation refers to the stimulation caused by azetidine **10f** over the action of agonist alone (100%) after subtracting the respective basal values. Data are means ± SEM from four experiments. [b] Significantly ($p < 0.05$) different from the stimulation produced by glutamate alone, by Student's t test.

Lastly, to probe the contribution of the basic nitrogen atom of the azetidines to bioactivity, the *N*-acetyl derivative **29** was prepared from **10f** by reaction with acetic anhydride in pyridine and water.

Biological Evaluation of the Azetidines

The azetidine derivatives were first tested by using primary cultures of cerebellar granule cells prepared from 8-day-old rats as previously described.[24] These cultures are characterized by the presence of ionotropic NMDA and kainate receptors which upon stimulation induce the influx of extracellular Ca^{2+} ions.[8] They also possess the metabolotropic quisqualate receptor coupled to phospholipase C.[8]

cis-Azetidine-2,4-dicarboxylic acid (**10f**), the first compound prepared in this series, was examined for its ability to induce $^{45}Ca^{2+}$ uptake in the cerebellar granule cells.[25] As can be seen from data presented in Table I, a moderate concentration (50 μM) of the compound when tested alone produced a low enhancement of the basal Ca^{2+} influx. However, **10f** was capable of potentiating the action of NMDA, glutamate and aspartate, the agonists of the NMDA receptor. The activity of these agonists and the potentiation induced by azetidine **10f** were inhibited by 3-(2-carboxypiperazin-4-yl)propane-1-phosphonic acid (CPP), a competitive NMDA receptor antagonist (data not shown). In contrast, azetidine **10f** failed to affect the activity of kainate and quisqualate, when these agonists were used in the presence of CPP in order to inhibit a simultaneous activation of NMDA receptors (Table I). This finding indicates the lack of azetidine action at non-NMDA ionotropic receptors. Similarly, azetidine **10f** had no effect on the stimulation of phosphoinositide hydrolysis induced by metabolotropic quisqualate receptors (data not shown). The above results show that the action of azetidine **10f** is selective for the ionotropic NMDA receptors.

Table II reveals that the potentiation of agonist action at the NMDA receptors is dependent upon azetidine stereochemistry. Both the cis diacid **10f** and its mono-*tert*-butyl ester **10e** are capable of potentiating glutamate function, the monoester being somewhat less active. In contrast, the trans diacid **11f** and its mono-*tert*-butyl ester **11e** are inactive. In the case of the *cis*- and *trans*-azetidine-2,4-dicarboxylic acid *tert*-butyl methyl ester **10b** and **11b**, neither compound was found active.

Table II. Stereoselectivity of Action of the Azetidines on Glutamate-Stimulated Calcium Influx in Cerebellar Granule Cells[a]

azetidine (50 μM)	$^{45}Ca^{2+}$ influx, nmol/mg of protein: basal	$^{45}Ca^{2+}$ influx, nmol/mg of protein: glutamate	stimulation, %
none	15 ± 0.8	51 ± 1.3	100
10b	14 ± 0.7	54 ± 1.9	111
11b	15 ± 0.6	52 ± 1.2	105
10e	15 ± 1.6	67 ± 1.4[b]	144
11e	16 ± 0.8	51 ± 1.8	97
10f	25 ± 3.5	92 ± 5.7[b]	186
11f	14 ± 1.4	52 ± 2.3	106

[a] Granule cells were preincubated for 15 min with 50 μM azetidine followed by 10 min of incubation without (basal) or with 10 μM glutamate. The percent of stimulation refers to the stimulation caused by glutamate (100%) after subtracting the respective basal values. Data are means ± SEM from four experiments. [b] Significantly ($p < 0.005$) different from the stimulation produced by glutamate alone, by Student's t test.

Table III. Action of *cis*-Azetidines on Basal and Aspartate-Stimulated Calcium Influx in Cerebellar Granule Cells[a]

azetidine (50 μM)	$^{45}Ca^{2+}$ influx, nmol/mg of protein: basal	$^{45}Ca^{2+}$ influx, nmol/mg of protein: aspartate	stimulation, %
none	16 ± 2.5	43 ± 2.9	100
10a	19 ± 2.0	46 ± 2.8	99
10b	14 ± 1.9	43 ± 3.7	107
10d	18 ± 4.5	53 ± 4.1[b]	130
10e	15 ± 2.0	52 ± 3.9[b]	136
10f	21 ± 1.2	68 ± 2.2[b]	175
14	20 ± 1.6	55 ± 2.5[b]	132
15	15 ± 2.3	44 ± 3.4	107
20	19 ± 1.0	58 ± 3.3[b]	147
27	16 ± 2.7	49 ± 3.7	122
28	16 ± 0.1	46 ± 4.3	113
29	15 ± 2.1	47 ± 2.2[b]	119
(*S*)-(−)-azetidine-2-carboxylic acid	16 ± 2.1	44.5 ± 6.2	105

[a] Granule cells were preincubated for 15 min with 50 μM azetidine followed by 10 min of incubation without (basal) or with 10 μM aspartate. The percent of stimulation refers to the stimulation caused by aspartate (100%) after subtracting the respective basal values. Data are means ± SEM from four experiments. [b] Significantly ($p = 0.005$) different from the stimulation produced by glutamate alone, by Student's t test.

As a consequence of the lack of action of the *trans*-azetidine-2,4-dicarboxylic acid, the effect of the remaining azetidine derivatives on glutamate- or aspartate-stimulated calcium uptake was studied with use of only the cis isomers. As is apparent from Table III, the diacid shows the greatest potentiating effect upon aspartate action. Those derivatives which contain one carboxyl function and an ester or amide group retain some activity, while the diesters possess no action. The presence of an *N*-hexyl, *N*-benzyl, or *N*-acetyl substituent (**27**, **28**, and **29**, respectively) leads to lowered activity or to the complete loss of activity. The commercially available (*S*)-(−)-azetidine-2-carboxylic acid was found inactive in these experiments.

Since the diacid **10f** was found to be the most active compound in the azetidine series, it was used in further experiments. Employing the $^{45}Ca^{2+}$ influx assay in cerebellar granule cells, dose–response experiments were performed in the absence and in the presence of NMDA. Figure 2 shows that in the absence of agonist azetidine **10f**

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(25) Wroblewski, J. T.; Nicoletti, F.; Costa, E. *Neuropharmacology* **1985**, *24*, 919. Wroblewski, J. T.; Fadda, E.; Mazzetta, J.; Lazarewicz, J. W.; Costa, E. *Neuropharmacology* **1989**, *28*, 447.

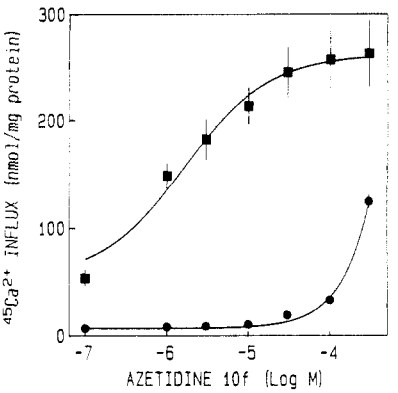

Figure 2. Dose-dependent effects of azetidine **10f** on $^{45}Ca^{2+}$ influx in cerebellar granule cells. The cells were preincubated for 15 min with the indicated concentrations of azetidine **10f** followed by 10 min of incubation in the absence (circles) or in the presence of 25 μM NMDA (squares). The points represent means from three experiments with bars indicating SEM.

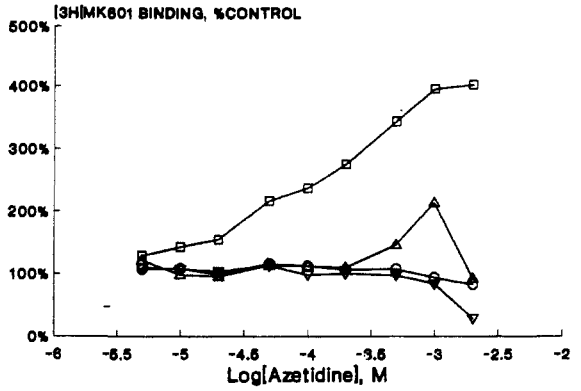

Figure 3. Azetidine **10f** increases [^{3}H]MK-801 binding to well-washed rat-brain membranes. [^{3}H]MK-801 binding was measured in the presence of azetidine **10f** (squares and circles) in the absence and presence of 100 μM glutamate and 30 μM glycine, respectively, at the concentrations shown. Similarly, binding was measured using azetidine **11f** (upright and inverted triangles) in the absence and presence of glutamate and glycine. The results represent a typical experiment that was repeated two further times with similar results.

shows a stimulatory action which becomes prominent at concentrations above 100 μM. However, when NMDA was included in the assay, the potentiating effect of azetidine **10f** was already visible at 1 μM concentration. These data suggest a dual action for azetidine **10f** at the NMDA receptor, a direct agonist action at higher concentrations and a possible modulatory action at lower concentrations.

In subsequent experiments the mechanism of action of azetidine **10f** on the NMDA receptor was investigated by employing the [^{3}H]MK-801 binding assay as previously described.[26] Azetidine **10f** increased [^{3}H]MK-801 binding to well-washed rat-brain membranes with an EC_{50} value of 168 ± 77 μM (mean ± SEM, n = 3). The trans isomer, azetidine **11f** was significantly less effective (Figure 3). As shown in Figure 3, the enhancement of [^{3}H]MK-801 binding was not seen when 100 μM glutamate and 30 μM glycine were included in the assay, implying an action of azetidine **10f** at either the glutamate or glycine site. To determine which of these sites was responsible for the action of azetidine **10f**, dose–response curves to 7-chlorokynurenate and CGS 19755 were performed in the absence and presence of 0.5 mM azetidine **10f**. As shown in Figure

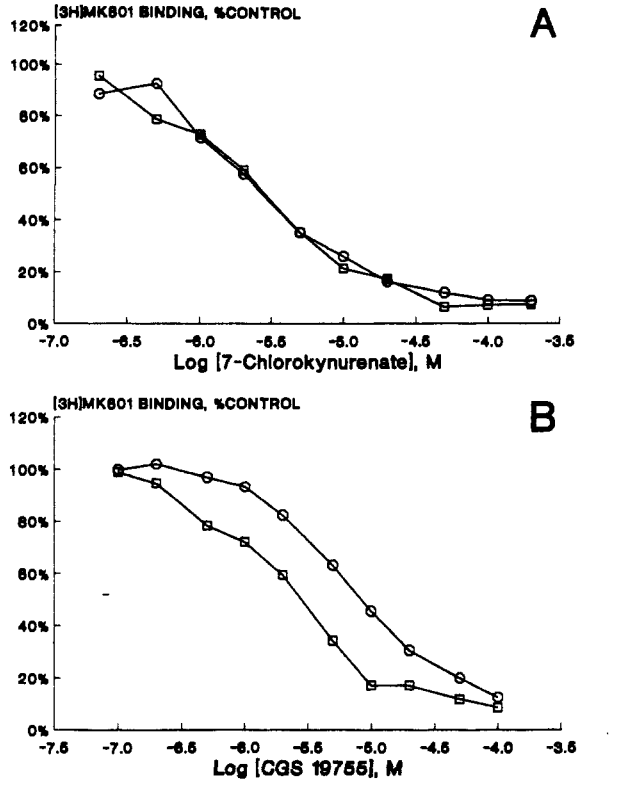

Figure 4. Azetidine **10f** reduces the apparent potency of CGS 19755 but not 7-chlorokynurenate against [^{3}H]MK-801 binding to well washed rat brain membranes. Dose response curves to (A) 7-chlorokynurenate and (B) CGS 19755 in the absence (squares) and presence (circles) of 0.5 mM azetidine **10f**. The results represent a typical experiment that was repeated two further times with similar results.

Table IV. Effects of Azetidine **10f** on [^{3}H]GABA Release from Rat Forebrain Neurons in Culture[a]

agonist	fractional [^{3}H]GABA release, % above basal: control	+ 100 μM CGS 19755
azetidine **10f** (1 mM)	1.04 ± 0.11	−0.08 ± 0.06
NMDA (100 μM)	0.73 ± 0.09	0.09 ± 0.38
kainate (100 μM)	2.65 ± 0.33	2.63 ± 0.24

[a] Results represent the mean ± SEM of 4–6 wells of cells. [^{3}H]-GABA release reflects agonist-evoked tritium efflux with basal efflux subtracted and is expressed as a proportion of the remaining tritium contained in the cells. The competitive NMDA antagonist CGS 19755, when used, was added for 5 min prior to the addition of agonists.

4, inhibition of [^{3}H]MK-801 binding by 7-chlorokynurenate, an NMDA receptor antagonist which is selective for the glycine binding site,[27] was not altered by the presence of the azetidine (IC_{50} values of 3.00 ± 2.3 μM and 2.20 ± 1.64 μM in the absence and presence of azetidine **10f**, respectively). In contrast, the addition of the azetidine to dose–response curves with CGS 19755 causes a decrease in the apparent potency of this selective antagonist at the glutamate site (IC_{50} values of 1.90 ± 1.30 μM and 17.5 ± 13.3 μM in the absence and presence of 0.5 mM azetidine **10f**, respectively). The results clearly indicate that azetidine **10f** at high concentrations acts as an NMDA-like agonist at the NMDA receptor and has little or no activity at the glycine recognition site.

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(27) Kemp, J. A.; Foster, A. C.; Leeson, P. D.; Priestly, T.; Tridgett, R.; Iversen, L. L.; Woodruff, G. N. *Proc. Natl. Acad. Sci., U.S.A.* **1988**, *85*, 6547.

The effects of azetidine **10f** on the release of [^{3}H]GABA from cultured embryonic rat forebrain neurons was also examined (Table IV). Azetidine **10f** increased [^{3}H]GABA release between 0.01 and 1 mM, which is consistent with the results from the [^{3}H]MK-801 binding assay. The effects of 1 mM azetidine **10f** were completely abolished by 100 μM CGS 19755, as were the effects of 100 μM NMDA (Table IV). This concentration of CGS 19755 did not alter the response to kainate (100 μM). These results support the concept that azetidine **10f** may act as an NMDA-like agonist, and, as the effects are completely reversed by a selective NMDA receptor antagonist, these results imply a selective action at NMDA-preferring glutamate receptors.

Conclusions

This article details the synthesis and biological activity of a new class of conformationally constrained analogues of glutamate. All of the results from the functional evaluations are consistent with the selective action of azetidine **10f** at the NMDA subtype of excitatory amino acid receptors, but not at kainate or quisqualate receptors. The activity of the compounds in the azetidine series is restricted to the cis stereoisomers possessing at least one carboxyl function. Azetidine **10f** shows a dual type of action, behaving as a glutamate-like agonist at higher concentrations, but also displaying positive modulatory properties at concentrations below 50 μM. However, as shown, this modulation is not related to interaction with the glycine-modulatory site present within the NMDA receptor domain.

Experimental Section

Column chromatography was performed on Merck No. 7734 Kieselgel 60 (0.063–0.200 mm). HPLC separations were performed on a Waters Millipore 7.8 mm × 30 cm μPorasil column (cat. no. 84175) by using two Waters 501 HPLC pumps with automated gradient controller and a model 441 absorbance detector operating at 254 nm. The flow rate was 5 mL/min. Merck precoated silica gel 60F-254 plates were used for analytical TLC. NMR spectra were recorded on a Bruker AF 300 instrument at 300 MHz with TMS or sodium 3-(trimethylsilyl)propanesulfonate as standards. Coupling constants were evaluated by first-order rules with an estimated accuracy of 0.5 Hz. IR spectra were measured on an IBM IR/32 FTIR spectrophotometer; mass spectra, on Varian CH 5 and VG 70-G instruments. Elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, Tennessee. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected.

2,4-Dibromoglutaric Acid Di-*tert*-butyl Ester (7c). To 1.45 g (5 mmol) of **5** in 1.5 mL of ether was added at −78 °C 1.5 mL of liquefied isobutene, followed by 3 drops of concentrated sulfuric acid. The solution was stirred at room temperature in a screw-capped vial (slight pressure formation; use adequate shielding) for 22 h, cooled, and poured into a mixture of saturated aqueous $NaHCO_3$ solution and ether. The organic phase was dried over $MgSO_4$ and chromatographed on SiO_2(EtOAc/hexanes 1:25) to yield 0.97 g (48%) of **7c** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 18 H); *dl* isomer δ 4.38 (t, 2 H, J = 7 Hz) 2.56 (t, 2 H, J = 7 Hz); meso isomer δ 4.28 (t, 2 H, J = 7.5 Hz), 2.77 (dt, 1 H, J = 7 (t), 15 Hz (d)), 2.53 (dt, 1 H, J = 7.5 (t), 14.5 Hz (d)); *dl*/meso = 1:2; IR (neat) 2980, 2934, 1733, 1458, 1395, 1370, 1302, 1260, 1146, 843, 761 cm^{-1}; MS (EI) m/e 292, 290, 288 (1, 2.5, 1, M^+ − 2 C_4H_6), 57 (100); calcd for $C_5H_6O_4{}^{79}Br^{81}Br$ 289.8612, found 289.8612.

2,4-Dibromoglutaric Acid Benzyl Methyl Ester (7d). **6** (2.18 g, 8 mmol), 2 mL of methylene chloride, and 0.33 mL (8.2 mmol) of methanol were stirred in a closed vial for 3 h and evaporated. Benzene (20 mL) and 40 μL of DMF were added, the mixture was cooled in an ice bath, and 0.77 mL (8.8 mmol) of oxalyl chloride in 10 mL of benzene was added dropwise during 30 min. Stirring was continued at 0 °C for 80 min and then at room temperature for 50 min. After evaporation, 10 mL of methylene chloride was added, and the resulting solution was added dropwise with ice cooling over a period of 30 min to a solution of 1.02 mL (10 mmol) of benzyl alcohol, 1.4 mL (10 mmol) of triethylamine, and 45 mg (0.4 mmol) of DMAP in 10 mL of CH_2Cl_2. Stirring at 0 °C and room temperature was continued for 20 min each. The solution was washed with 10% aqueous $KHSO_4$ solution, dried over $MgSO_4$, evaporated, and filtered over silica gel (EtOAc/hexanes 1:10) to afford 2.65 g (84%) of the product as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.38 (br s, 5 H), 5.22 (narrow AB q, 2 H), 3.80 (s, 3 H); *dl* isomer δ 4.55 (t, 1 H, J = 7.5 Hz), 4.52 (t, 1 H, J = 7.5 Hz), 2.68 (t, 2 H, J = 7 Hz); meso isomer δ 4.44 (t, 1 H, J = 7.5 Hz), 4.39 (t, 1 H, J = 7.5 Hz), 2.90 (dt, 1 H, J = 7 (t), 15 Hz (d)), 2.65 (dt, 1 H, J = 7.5 (t), 15 Hz (d)); *dl*/meso = 2.5:1; IR (neat) 3034, 2953, 1742, 1437, 1275, 1156, 735, 698 cm^{-1}; MS (EI) m/z 315, 313 (3, 3, M^+ − Br), 297, 295 (9, 8), 209, 207 (18, 20), 91 (100); calcd for $C_{13}H_{14}O_4{}^{79}Br$ 313.0075, found 313.0075.

2,4-Dibromoglutaric Acid Benzyl *tert*-Butyl Ester (7e). **6** (2.18 g, 8 mmol), 0.83 mL (8 mmol) of benzyl alcohol, and 2 mL of chloroform were stirred for 1 h at room temperature and for 1 h under reflux. Benzene (20 mL) and 0.1 mL of DMF were added, the solution was cooled in an ice bath, and 1.05 mL (12 mmol) of oxalyl chloride in 5 mL of benzene was added dropwise. Stirring was continued for 40 min at 0 °C and 70 min at room temperature, after which time gas evolution had virtually ceased. Volatiles were evaporated in vacuo, and the residue was taken up in 5 mL of CH_2Cl_2. This solution was added dropwise with ice cooling to a solution of 1.4 mL (15 mmol) of *tert*-butyl alcohol, 1.4 mL (10 mmol) of triethylamine, and 19.5 mg (0.16 mmol) of 4-(dimethylamino)pyridine in 15 mL of CH_2Cl_2. After stirring at 0 °C and room temperature for 20 min each, the mixture was washed with aqueous $KHSO_4$ solution, dried over $MgSO_4$, evaporated, and filtered over silica gel to afford 2.65 g (76%) of a yellowish oil: ^{1}H NMR ($CDCl_3$) δ 7.37 (br, s, 5 H), 5.22 (2 narrow AB q, 2 H), 1.48 (s, 9 H); *dl* isomer δ 4.54 (t, 1 H, J = 7 Hz), 4.39 (t, 1 H, J = 7 Hz), 2.63 (t, 2 H, J = 7 Hz); meso isomer δ 4.44 (t, 1 H, J = 7.5 Hz), 4.26 (t, 1 H, J = 7.5 Hz), 2.83 (dt, 1 H, J = 7 (t), 15 Hz (d)), 2.59 (dt, 1 H, J = 7.5 Hz (t), 15 Hz (d)); *dl*/meso = 1:2; IR (neat) 2980, 1736, 1456, 1370, 1266, 1148, 843, 752, 696 cm^{-1}; MS (EI) m/z 382, 380, 378, (0.4, 0.7, 0.4, M^+ − C_4H_6), 301, 299 (7, 7), 107 (59), 91 (94), 57 (100); calcd for $C_{12}H_{12}O_4{}^{79}Br^{81}Br$ 379.9082, found 379.9082. Anal. ($C_{13}H_{14}O_4Br_2$) C, H, Br.

2,4-Dibromoglutaric Acid Dibenzyl Ester (7f). **5** (4.63 g, 16 mmol), 4.1 mL (40 mmol) of benzyl alcohol, 100 mg of $TsOH{\cdot}H_2O$, and 20 mL of benzene were refluxed under a Dean–Stark trap for 8 h. Evaporation and filtration over silica gel (EtOAc/hexanes 1:15) afforded 6.60–6.95 g (88–92%) of the product as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.37 (br s, 10 H), 5.23, 5.19 (AB q, 4 H, J = 12.5 Hz); *dl* isomer δ 4.55 (dd, 2 H, J = 6.5, 7.5 Hz), 2.69 (dd, 2 H, J = 6.5, 7.5 Hz); meso isomer δ 4.42 (t, 2 H, J = 7.5 Hz), 2.90 (dt, 1 H, J = 7 (t), 15 Hz (d)), 2.66 (dt, 1 H, J = 7.5 (t), 15 Hz (d)); *dl*/meso = 6:1; IR (neat) 3034, 1740, 1499, 1456, 1385, 1273, 1154, 750, 696 cm^{-1}; MS (EI) m/z 381, 379, 377 (0.2, 0.4, 0.2, M^+ − C_7H_7), 107 (100), 91 (94); calcd for $C_{12}H_{11}O_4{}^{79}Br^{81}Br$ 378.9004, found 378.9004. Anal. ($C_{19}H_{18}O_4Br_2$) C, H, N.

Other 1-Benzylazetidine-2,4-dicarboxylates. General Procedure. Dibromoglutarates **7** (1–35 mmol), 3 molar equiv of benzylamine, and enough DMF to prepare a solution ca. 0.12 M in **7** were stirred at 80 °C for 2–4 h. The solvent was distilled into a cold trap at 0.2–0.3 Torr and 40 °C bath temperature, the residue was taken up in CH_2Cl_2, and the solution was washed with saturated aqueous $NaHCO_3$ solution. After the solution was dried over $MgSO_4$ and evaporated, the residue was chromatographed on silica gel with EtOAc/hexanes to yield, after a forerun, first the trans and then the cis isomer as light yellow to amber, viscous oils, some of which solidify to waxy masses on prolonged standing.

1-Benzylazetidine-2,4-dicarboxylic Acid Dimethyl Ester (Eluent: EtOAc/hexanes 1:6, and then 1:3 after the trans isomer was completely eluted). **A. Trans isomer 9a**: yield 36%; ^{1}H NMR ($CDCl_3$) δ 7.3–7.2 (m, 5 H), 4.22 (t, 2 H, J = 7 Hz), 3.89, 3.84 (AB q, 2 H, J = 12.5 Hz), 3.65 (s, 6 H), 2.51 (t, 2 H, J = 7 Hz); IR (neat) 2953, 1736, 1437, 1354, 1200, 1030, 749, 698 cm^{-1}. **B. Cis isomer 8a**: yield 32%; ^{1}H NMR ($CDCl_3$) δ 7.37–7.22 (m, 5 H), 3.88 (s, 2 H), 3.64 (s, 6 H), 3.63 (t, 2 H, J = 8.5 Hz), 2.50 (dt, 1 H, J = 8.5 Hz (t), 10.5 Hz (d)), 2.34 (dt, 1 H, J = 8 Hz (t), 10.5 Hz (d)); IR (neat) 3029, 2953, 1744, 1437, 1225, 1202, 1042,

700 cm^{-1}; MS (EI) m/z 263 (4, M$^+$), 204 (87), 177 (36), 117 (28), 100 (22), 91 (100); calcd for $C_{14}H_{17}NO_4$ 263.1158, found 263.1159.

1-Benzylazetidine-2,4-dicarboxylic Acid Di-*tert*-butyl Ester (Eluent: EtOAc/hexanes 1:15, then 1:8). **A. Trans isomer 9c**: yield 36%; mp 46–48 °C (from hexanes); ^{1}H NMR ($CDCl_3$) δ 7.34–7.18 (m, 5 H), 4.07 (t, 2 H, J = 7 Hz), 3.95, 3.88 (AB q, 2 H, J = 13 Hz), 2.41 (t, 2 H, J = 7 Hz), 1.41 (s, 18 H); IR (neat) 2978, 2933, 1726, 1455, 1393, 1368, 1239, 1154, 1021, 840, 735, 700 cm^{-1}. **B. Cis isomer 8c**: yield 38%; mp 53–56 °C (from hexanes); ^{1}H NMR ($CDCl_3$) δ 7.34–7.19 (m, 5 H), 3.84 (s, 2 H), 3.44 (t, 2 H, J = 8 Hz), 2.39 (dt, 1 H, J = 8.5 Hz (t), 11 Hz (d)), 2.23 (dt, 1 H, J = 8 Hz (t), 10.5 Hz (d)), 1.37 (s, 18 H); IR (neat) 2978, 2932, 1735, 1455, 1393, 1368, 1233, 1156, 845, 737, 705 cm^{-1}; MS (EI) m/z 290 (1, M$^+$ – C_4H_9), 246 (34), 190 (88), 91 (100), 57 (25); calcd for $C_{16}H_{20}NO_4$ 290.1932, found 290.1932.

1-Benzylazetidine-2,4-dicarboxylic Acid Benzyl Methyl Ester (Eluent: EtOAc/hexanes 1:8, then 1:5). **A. Trans isomer 9d**: yield 31%; ^{1}H NMR ($CDCl_3$) δ 7.38–7.18 (m, 10 H), 5.10 (narrow AB q, 2 H), 4.24 (t, 1 H, J = 6.5 Hz), 4.23 (t, 1 H, J = 6.5 Hz), 3.89, 3.85 (AB q, 2 H, J = 13 Hz), 3.64 (s, 3 H), 2.51 (t, 2 H, J = 6.5 Hz); IR (neat) 3031, 2950, 2851, 1734, 1495, 1455, 1347, 1173, 1028, 911, 737, 698 cm^{-1}; MS (EI) m/z 339 (0.2, M$^+$), 280 (11), 204 (38), 91 (100); calcd for $C_{20}H_{21}NO_4$ 339.1471, found 339.1471. **B. Cis isomer 8d**: yield 28%; ^{1}H NMR ($CDCl_3$) δ 7.38–7.2 (m, 10 H), 5.12, 5.02 (AB q, 2 H, J = 12.5 Hz), 3.89 (narrow AB q, 2 H), 3.66 (t, 1 H, J = 8.5 Hz), 3.62 (t, 1 H, overlapping), 3.62 (s, 3 H), 2.53 (dt, 1 H, J = 8.5 (t), 10.5 Hz (d)), 2.34 (dt, 1 H, J = 8 (t), 10.5 Hz (d)); IR (neat) 3031, 2952, 1744, 1497, 1455, 1223, 1175, 1028, 911, 739, 698 cm^{-1}.

1-Benzylazetidine-2,4-dicarboxylic Acid Benzyl *tert*-Butyl Ester (Eluent: EtOAc/hexanes 1:12, then 1:8). **A. Trans isomer 9e**: yield 30%; ^{1}H NMR ($CDCl_3$) δ 7.40–7.17 (m, 10 H), 5.09 (narrow AB q, 2 H), 4.20 (t, 1 H, J = 7 Hz), 4.10 (t, 1 H, J = 7 Hz), 3.90 (narrow AB q, 2 H), 2.47 (t, 2 H, J = 7 Hz), 1.42 (s, 9 H); IR (neat) 3031, 2977, 1728, 1497, 1455, 1368, 1156, 1026, 911, 845, 737, 698 cm^{-1}. **B. Cis isomer 8e**: yield 33%; ^{1}H NMR ($CDCl_3$) δ 7.38–7.18 (m, 10 H), 5.10, 5.03 (AB q, 2 H, J = 12.5 Hz), 3.89, 3.84 (AB q, 2 H, J = 13 Hz), 3.61 (t, 1 H, J = 8 Hz), 3.49 (t, 1 H, J = 8 Hz), 2.48 (dt, 1 H, J = 8.5 (t), 10.5 Hz (d)), 2.28 (dt, 1 H, J = 8 (t), 10.5 Hz (d)), 1.36 (s, 9 H); IR (neat) 3031, 2979, 2932, 1738, 1497, 1455, 1368, 1229, 1159, 1028, 845, 745, 698 cm^{-1}; MS (EI) m/z 324 (0.3, M$^+$ – C_4H_9), 280 (13), 246 (6), 218 (27), 190 (19), 91 (100), 57 (60); calcd for $C_{19}H_{18}NO_4$ 324.1236, found 324.1236.

1-Benzylazetidine-2,4-dicarboxylic Acid Dibenzyl Ester (Eluent: EtOAc/hexanes 1:8, then 1:4). **A. Trans isomer 9f**: yield 32%; ^{1}H NMR ($CDCl_3$) δ 7.4–7.15 (m, 15 H), 5.09 (narrow AB q, 4 H), 4.25 (t, 2 H, J = 7 Hz), 3.90, 3.85 (AB q, 2 H, J = 13 Hz), 2.52 (t, 2 H, J = 7 Hz); IR (neat) 3033, 2953, 1732, 1497, 1455, 1345, 1171, 1028, 909, 737, 698 cm^{-1}. Anal. ($C_{26}H_{25}NO_4$) C, H, N. **B. Cis isomer 8f**: yield 34%; ^{1}H NMR ($CDCl_3$) δ 7.4–7.2 (m, 15 H), 5.12, 5.04 (AB q, 4 H, J = 12.5 Hz), 3.91 (s, 2 H), 3.68 (t, 2 H, J = 8 Hz), 2.58 (dt, 1 H, J = 8.5 (t), 10.5 Hz (d)), 2.38 (dt, 1 H, J = 8 (t), 10.5 Hz (d)); IR (neat) 3033, 1744, 1497, 1455, 1173, 1028, 911, 735, 696 cm^{-1}; MS (EI) m/z 415 (0.2, M$^+$), 324 (2), 280 (51), 91 (100). Anal. ($C_{26}H_{25}NO_4$) C, H, N.

Epimerization of 9f. NaH (0.20 g, 5 mmol, 60% in oil) was washed with toluene. Toluene (5 mL) was added, followed by 0.78 mL (7.5 mmol) of benzyl alcohol. After the solution was stirred at room temperature for 30 min, 8.31 g (20 mmol) of **9f** in 60 mL of DMF was added, and the mixture was stirred at 80 °C for 20 h. After cooling, 0.34 mL (6 mmol) of acetic acid and 5 mL of diphenyl ether were added, and the DMF was pumped off as described above. Distillation was then continued until the boiling point reached 63 °C (0.15 Torr) and subsequently fell. This procedure removed all of the benzyl alcohol (the remainder of which might interfere with the subsequent chromatography since it follows the product rather closely) and most of the diphenyl ether (which was then easily removed in the forerun). SiO_2 (20 g) was added, the solvent was evaporated, and the residue was applied on a silica gel column, from which EtOAc/hexanes 1:8 eluted 0.69 g (8%) of unchanged **9f**; EtOAc/hexanes 1:6 subsequently eluted 3.41 g (41%) of the cis isomer **8f**.

Hydrogenolysis of 1-Benzylazetidine-2,4-dicarboxylic Acid Esters. General Procedure. Substrate (0.3–15 mmol) as a 25–100 mmolar methanolic solution was hydrogenated in a Parr shaker under 3–4 bar of H_2 over 50–100 mg/mmol of 20% Pd-$(OH)_2$/C (Aldrich, containing 31% H_2O) for a period of 4–8 h. In some cases, palladium on carbon also gave good results. The catalyst was filtered off through a double paper filter and repeatedly washed with methanol. Evaporation yielded the crude products, which were purified according to individual procedures. The free diacids were obtained according to a modified procedure described below.

***cis*-Azetidine-2,4-dicarboxylic Acid Dimethyl Ester (10a).** 8a (3.96 g, 15 mmol), after filtration over SiO_2 with EtOAc and bulb-to-bulb distillation (oven temperature 65–70 °C (0.15 Torr)) yielded 1.94 g (70%) of the product as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 4.27 (dd, 2 H, J = 7, 8.5 Hz), 3.76 (s, 6 H), 2.95 (dt, 1 H, J = 8.5 (t), 12 Hz (d)), 2.60 (dt, 1 H, J = 6.5 (t), 12 Hz (d)); IR (neat) 3345, 2955, 1740, 1437, 1231, 1042 cm^{-1}; MS (EI) m/z 173 (7, M$^+$), 114 (100), 82 (44), 54 (98); calcd for $C_7H_{11}NO_4$ 173.0688, found 173.0688.

Azetidine-2,4-dicarboxylic Acid *tert*-Butyl Methyl Ester. A. Trans isomer 10b: yield 59%; ^{1}H NMR ($CDCl_3$) δ 4.34 (dd, 1 H, J = 6.5, 9 Hz), 4.11 (dd, 1 H, J = 5.5, 9 Hz), 3.77 (s, 3 H), 2.91 (br s, 1 H), 2.73 (ddd, 1 H, J = 6.5, 9, 11.5 Hz), 2.60 (ddd, 1 H, J = 5.5, 9, 11.5 Hz), 1.49 (s, 9 H); IR (neat) 3328, 2979, 1732, 1370, 1237, 1159, 1059, 845 cm^{-1}; MS (EI) m/z 215 (2, M$^+$), 174 (2), 156 (10), 114 (100), 100 (40), 82 (42), 59 (37), 57 (60), 54 (77). **B. Cis isomer 11b**: yield 48%; mp 45.5–47 °C; ^{1}H NMR ($CDCl_3$) δ 4.21 (dd, 1 H, J = 6, 9 Hz), 4.10 (dd, 1 H, J = 6, 9 Hz), 3.76 (s, 3 H), 3.12 (br s, 1 H), 2.96 (dt, 1 H, J = 9 (t), 12 Hz (d)), 2.49 (dt, 1 H, J = 6 (t), 12 Hz (d)), 1.48 (s, 9 H); IR (neat) 3276, 2975, 1728, 1370, 1269, 1159, 1100, 1088, 1026, 833, 776, 764 cm^{-1}.

Azetidine-2,4-dicarboxylic Acid Di-*tert*-butyl Ester. A. Cis isomer 10c: The crude product from 2.08 g (6 mmol) of **8c**, after filtration over SiO_2 with EtOAc/hexanes 1:1 and crystallization from hexanes, afforded 1.32–1.35 g (85–87%) of colorless crystals: mp 49.5–50.5 °C; ^{1}H NMR ($CDCl_3$) δ 4.04 (dd, 2 H, J = 5.5, 9 Hz), 3.2 (br, 1 H), 2.97 (dt, 1 H, J = 9 (t), 12 Hz (d)), 2.38 (dt, 1 H, J = 5.5 (t), 12 Hz (d)), 1.48 (s, 18 H); IR (neat) 3328, 2979, 2934, 1731, 1480, 1458, 1394, 1369, 1280, 1257, 1156, 1048, 1031, 846, 734 cm^{-1}; MS (EI) m/z 257 (0.2, M$^+$), 201 (0.5), 156 (29), 100 (100), 182 (14), 57 (23); calcd for $C_{13}H_{23}NO_4$ 257.1627, found 257.1626. **B. Trans isomer 11c**: **9c** (347 mg, 1 mmol) afforded, in the same way, 191 mg (74%) of **11c**: mp 64–65 °C; ^{1}H NMR ($CDCl_3$) δ 4.13 (t, 2 H, J = 7.5 Hz), 2.61 (t, 2 H, J = 7.5 Hz), ca. 1.6 (very broad, 1 H), 1.48 (s, 18 H); IR (neat) 3335, 2975, 2932, 1730, 1368, 1354, 1239, 1215, 1152, 1059, 1019, 847, 737, 695 cm^{-1}.

***cis*-Azetidine-2,4-dicarboxylic Acid Monomethyl Ester (10d).** The crude product from 238 mg (0.7 mmol) of starting material **8d** was taken up in water, washed with three portions of ether to remove nonpolar material, evaporated, and dried in vacuo to yield 93 mg (85%) of a yellowish glass: ^{1}H NMR (D_2O) δ 5.13 (dd, 1 H, J = 8.5, 9.5 Hz), 4.80 (1 H, overlapping with HDO), 3.86 (s, 3 H), 3.14 (dt, 1 H, J = 10 (t), 12.5 Hz (d)), 2.68 (dt, 1 H, J = 8 (t), 12.5 Hz (d)); IR (Nujol) 3393, 3135, 2629, 1750, 1617, 1408, 1315, 1250, 1080, 1065, 1038, 982, 936, 882, 828, 764 cm^{-1}; MS (EI) m/z 159 (1, M$^+$), 134 (1), 114 (37), 100 (100), 82 (39), 54 (73); calcd for $C_6H_9NO_4$ 159.0532, found 159.0531.

Azetidine-2,4-dicarboxylic Acid Mono-*tert*-butyl Ester. A. Trans isomer 11e: **9e** (538 mg, 1.41 mmol), after evaporation and washing of the solid residue with ether (which removes small amounts of yellowish impurities), yielded 236 mg (83%) of crude product. This material was dissolved in 6 mL of warm methanol, precipitated by addition of 12 mL of ether, filtered, and dried at 95 °C (0.1 Torr) for 20 h to recover 180 mg (63%) of pure material: mp 175–177 °C dec; ^{1}H NMR (D_2O) δ 4.91 (dd, 1 H, J = 7, 10 Hz), 4.71 (dd, 1 H, J = 7.5, 10 Hz), 2.93, 2.83 (AB q, 2 H, J = 12.5 Hz, each part split into dd, J = 7, 10.5 Hz), 1.52 (s, 9 H); IR (KBr) 3438, 2982, 1740, 1607, 1632, 1570, 1389, 1364, 1281, 1248, 1175, 1080, 855, 779, 515, 469 cm^{-1}. Anal. ($C_9H_{15}NO_4$) C, H, N. **B. Cis isomer 10e**: **8e** (846 mg, 2.22 mmol) afforded, in the same way, 380 mg (85%) of crude product. Recrystallization (10 mL of methanol, 20 mL of ether) and drying at 55 °C (0.1 Torr) for 5 h yielded 273 mg (61%) of pure material. Another 52 mg (12%) was recovered from the mother liquid by chromatography on SiO_2 (2-propanol/H_2O/concentrated NH_3 17:2:1): mp 157–159 °C dec; ^{1}H NMR (D_2O) δ 4.98 (dd, 1 H, J = 8, 10 Hz), 4.75 (dd, 1 H, J = 8, 10 Hz), 3.15 (dt, 1 H, J = 10 Hz (t),

12.5 Hz (d)), 2.57 (dt, 1 H, J = 8 (t), 12.5 Hz (d)), 1.51 (s, 9 H); IR (KBr) 3434, 2998, 1738, 1617, 1563, 1385, 1368, 1320, 1248, 1161, 885, 843, 777, 764 cm^{-1}; MS (EI) m/z 100 (100, $M^+ - COOC_4H_9$), 82 (33), 57 (81), 54 (37). Anal. ($C_9H_{15}NO_4$) C, H, N.

Azetidine-2,4-dicarboxylic Acid. A. Trans isomer 11f: 9f (1.25 g, 3 mmol) in 90 mL of methanol was hydrogenated in a Parr shaker under 4 atm of hydrogen over 180 mg of 20% $Pd(OH)_2/C$ for 4 h. Hot (65 °C) water (135 mL) was added to dissolve the partially precipitated product and, in particular, its N-benzyl derivative, which would otherwise escape from further reaction and contaminate the final product. Hydrogenation was continued for another 5 h. The catalyst was filtered off and washed with water, and the solution concentrated to a small volume, whereon the product precipitated in form of colorless, fine crystals which were of high purity. THF was added to complete precipitation. The crystals were filtered off, washed with aqueous THF, and dried in vacuo at room temperature. Yield: 346 mg (79%). The compound has no defined mp; it decomposes from ca. 200 °C on: ^1H NMR (D_2O) δ 4.88 (t, 2 H, J = 8.5 Hz), 2.93 (t, 2 H, J = 8.5 Hz); IR (Nujol) 2639, 2473, 1746, 1601, 1580, 1250, 1217, 812, 776 cm^{-1}; MS (FAB) m/z 101 ($M + H^+ - COOH$). Anal. ($C_5H_7NO_4$) C, H, N. **B. Cis isomer 10f**: Prepared in the same way from **8f**: yield 81–89%; mp 223–230 °C dec; ^1H NMR (D_2O) δ 4.87 (dd, 2 H, J = 8, 10 Hz), 3.16 (dt, 1 H, J = 10 Hz (t), 12 Hz (d)), 2.60 (dt, 1 H, J = 8 Hz (t), 12 Hz (d)); IR (Nujol) 3158, 2624, 2408, 1732, 1657, 1563, 1246, 1161, 1044, 1033, 978, 868, 843, 779, 752, 727, 604 cm^{-1}. Anal. ($C_5H_7NO_4$) C, H, N.

***cis*-1-Benzylazetidine-2,4-dicarboxylic Acid (29).** This compound was formed, together with **10f**, when the second stage of the above hydrogenolysis was omitted. It could be easily isolated in pure form as a consequence of its low solubility. More conveniently, 521 mg (1.5 mmol) of **8c** was stirred at room temperature for 12 h in a mixture of each 4.5 mL of MeOH, THF, and 1 M aqueous NaOH. Aqueous HCl (4.5 mL, 1 M) was added and the solution partially evaporated. The precipitated crystals were sucked off and washed with water. Concentration of the mother liquid yielded a second fraction; together 324 mg (92%) after drying in vacuo: mp 225–228 °C dec after sintering from ca. 210 °C; ^1H NMR (D_2O) δ 7.50 (br s, 5 H), 4.77 (t, 2 H, J = 9.5 Hz), 4.50 (s, 2 H), 3.05 (dt, 1 H, J = 10 Hz (t), 11.5 Hz (d)), 2.45 (dt, 1 H, J = 9 (t), 12 Hz (d)); IR (Nujol) 3108, 1746, 1239, 1211, 1022, 772, 725 cm^{-1}; MS (EI) m/z 235 (0.2, M^+), 234 (0.2), 190 (29), 163 (7), 100 (6), 91 (100); calcd for $C_{12}H_{13}NO_4$ 235.0845, found 235.0844.

***cis*-1-Benzyl-4-carbamoylazetidine-2-carboxylic Acid Benzyl Ester (12) and *cis*-1-Benzylazetidine-2,4-dicarboxamide (13) from 8f.** To 108 mg (2 mmol) of finely powdered ammonium chloride was added under a dry argon atmosphere via syringe at 0 °C 1 mL of trimethylaluminum (2 M in toluene). After the mixture was stirred at 0 °C for 20 min and at room temperature for 1 h, a solution of 623 mg (1.5 mmol) of **8f** in 1 mL of toluene was added via syringe, and the mixture was stirred at 50 °C for 2.5 h. The mixture was then cooled in an ice bath, diluted with 10 mL of methylene chloride, and quenched by addition of 0.84 g (20 mmol) of NaF, followed by *cautious* dropwise addition of 0.36 mL (20 mmol) of water. After gas evolution had ceased, stirring was continued at room temperature for 20 min, and the fluoroaluminates were removed by suction filtration through a medium porosity sintered glass funnel and thoroughly washed with methylene chloride. Evaporation to a small volume and dilution with ethyl acetate/hexanes (1:5) caused precipitation of **13**, which was collected on a sintered glass funnel and washed with the same solvent. The mother liquid and washings were combined, evaporated, and chromatographed on silica gel to yield, in the sequence of elution, unreacted starting material (with ethyl acetate/hexanes 1:3, 266 mg, 43%), benzyl alcohol, and **12** (with ethyl acetate).

12: yield 56 mg (11.5%); 2-fold crystallization from CH_2Cl_2/hexanes furnished colorless needles; mp 104–106.5 °C; ^1H NMR ($CDCl_3$) δ 7.4–7.2 (m, 10 H), 6.95 (br, 1 H), 5.58 (br, 1 H), 5.07 (s, 2 H), 3.82, 3.71 (AB q, 2 H, 13 Hz), 3.80 (t, 1 H, J = 8.5 Hz), 3.61 (t, 1 H, J = 8.5 Hz), 2.70 (dt, 1 H, J = 8.5 (t), 11 Hz (d)), 2.25 (dt, 1 H, J = 8 (t), 11 Hz (d)); IR (neat) 3438, 3063, 3031, 2961, 2925, 2892, 1742, 1686, 1580, 1497, 1455, 1175, 1061, 1022, 747, 698 cm^{-1}; MS (EI) m/z 280 (24%, $M^+ - CONH_2$), 253 (3), 189 (5), 162 (4), 91 (100), (CI, isobutane) m/z 325 (100%, M + H^+), 280 (24), 253 (6), 189 (8), 162 (6), 91 (62). Anal. ($C_{19}H_{20}N_2O_3$) C, H, N.

13: yield 80 mg (23%); colorless solid; mp 209–212 °C dec after filtration over SiO_2 (ethyl acetate/methanol 4:1); ^1H NMR ($CDCl_3$) δ 7.4–7.25 (m, 5 H), 6.29 (br, 2 H), 5.21 (br, 2 H), 3.76 (s, 2 H), 3.72 (t, 2 H, J = 8.5 Hz), 2.89 (dt, 1 H, J = 9 Hz (t), 11.5 Hz (d)), 2.17 (dt, 1 H, J = 8.5 (t), 11.5 Hz (d)); IR (Nujol) 3474, 3409, 3318, 3156, 1673, 1647, 1570, 1418, 1323, 1291, 1148, 1080, 762, 708 cm^{-1}; MS (EI) m/z 234 (0.2%, M + H^+), 189 (37), 162 (8), 117 (6), 91 (100); (CI, isobutane) m/z 234 (100), 189 (30), 162 (6), 91 (25); calcd for $C_{11}H_{13}N_2O$ 189.1028, found 189.1027.

12 from 8e. **8e** (280 mg, 0.73 mmol) was treated with 2.8 mL of trifluoroacetic acid as described below. To the crude product dissolved in 3 mL of 1,2-dichloroethane were added sequentially 180 mg (1.47 mmol) of DMAP, 303 mg (1.47 mmol) of DCC, and 0.46 mL (2.2 mmol) of hexamethyldisilazane. After the mixture was stirred at room temperature for 14 h, the dicyclohexylurea was filtered off and washed with 1,2-dichloroethane, and the solution was evaporated. The residue was taken up in 5 mL of THF, and 1.5 mL of Bu_4NF (1.5M in THF) was added. After stirring for 20 min at room temperature, the solution was evaporated, and the residue directly chromatographed on SiO_2 with ethyl acetate (R_f ca. 0.38) to yield 145 mg (61%) of **12** as a slightly colored solid. (Omission of the desilylation step resulted in incomplete desilylation even on repeated chromatography, giving **12** in a substantially reduced yield and the less polar N-monosilyl derivative as a byproduct.)

***cis*-4-Carbamoylazetidine-2-carboxylic Acid (14).** **12** (186 mg, 572 μmol) in 25 mL of methanol was hydrogenated at atmospheric pressure over 73 mg of 20% $Pd(OH)_2/C$ for 1.5 h. Warm water (10 mL) was added, and the hydrogenation continued for 17.5 h. The mixture was filtered through a fluted filter, and the catalyst was washed with several portions of methanol/water. After evaporation, the residue was chromatographed on SiO_2 with 2-propanol/water/concentrated NH_3 12:7:1 to obtain 70 mg of impure material, which crystallized partially on standing. It was dissolved in hot methanol/water (2:1) and kept in the freezer for several days, yielding 30.5 mg (37%) of pure **14**. The compound has no defined mp, but decomposes with darkening and shrinking from ca. 195 °C on: ^1H NMR (D_2O) δ 5.00 (t, 1 H, J = 9 Hz), 4.75 (t, 1 H, J = 9 Hz), 3.14 (dt, 1 H, J = 10 (t), 12 Hz (d)), 2.52 (dt, 1 H, J = 8 (t), 12 Hz (d)); IR (Nujol) 3399, 3141, 3019, 2537, 2409, 1688, 1615, 1563, 1391, 1294, 1217, 1165, 1115, 1048, 968, 887, 810, 762, 696, 637 cm^{-1}; MS (EI) m/z 142 (40, $M^+ - 2$ H), 100 (100), 82 (27), 68 (28), 54 (50), 44 (95); (CI, isobutane) m/z 145 (100, M + H^+), 100 (24), 84 (15), 72 (22). Anal. ($C_5H_8N_2O_3$) C, H, N.

***cis*-Azetidine-2,4-dicarboxamide (15).** **13** (45.8 mg, 196 μmol) in 10 mL of glacial acetic acid (no reaction occurred in methanol) was hydrogenated in a Parr shaker over 11 mg of 10% Pd/C under a hydrogen pressure of 4 atm for 7 h. The mixture was filtered through a fluted filter and the catalyst washed with several portions of methanol. Evaporation and drying in vacuo yielded a colorless film which crystallized slowly on addition of a few drops of methanol. After standing in a freezer overnight, the crystals were isolated, washed with a small volume of ethyl acetate/methanol (5:1), and dried in vacuo; yield 16.7 mg (60%): mp 215–218 °C dec after strong shrinking and darkening from ca. 200 °C on (measured with oil bath preheated to 190 °C); ^1H NMR (D_2O) δ 4.29 (t, 2 H, J = 8.5 Hz), 2.98 (dt, 1 H, J = 9 (t), 11.5 Hz (d)), 2.23 (dt, 1 H, J = 8 (t), 11.5 Hz (d)); IR (Nujol) 3391, 3335, 3208, 3073, 1683, 1669, 1281, 1211, 1109, 1030, 843, 749, 722, 677 cm^{-1}; MS (CI, isobutane) m/z 144 (100, M + H^+), 99 (56).

***cis*-1-Benzylazetidine-2,4-dicarboxylic Acid Monobenzyl Ester Trifluoroacetate (16).** **8e** (973 mg, 2.55 mmol) in 10 mL of trifluoroacetic acid was kept at room temperature for 21 h. Evaporation was followed by addition of ether, in which the resulting syrup dissolved. Crystallization set in rapidly. Filtration, washing with ether, and drying in vacuo afforded 817 mg (73%) of **16** as colorless fine crystals: mp 141–143 °C; ^1H NMR ($CDCl_3$) δ 7.4–7.22 (m, 10 H), 7.18 (br, 2 H), 5.11 (s, 2 H), 4.28 (t, 2 H, J = 8.5 Hz), 4.16, 4.10 (AB q, 2 H, J = 13 Hz), 2.81 (m, 1 H), 2.53 (m, 1 H); IR (Nujol) 1742, 1690, 1254, 1210, 1127, 1038, 756, 723, 700 cm^{-1}; MS (EI) m/z 280 (0.3, $M^+_{base} - COOH$), 234 (0.2), 190 (4), 114 (2), 91 (13), 69 (64), 51 (37), 45 (100); (CI, isobutane) m/z 326 (37, $M_{base} + H^+$), 190 (15), 108 (13), 91 (100); calcd for C_{18}-

$H_{18}NO_2$ 280.1338, found 280.1337.

1-Benzyl-4-(hydroxymethyl)azetidine-2-carboxylic Acid Benzyl Ester (19). To 2.08 g (5 mmol) of **8f** in 50 mL of *tert*-butyl alcohol was added all at once 0.95 g (25 mmol) of $NaBH_4$. After the mixture was stirred at room temperature for 45 min, 5.7 mL (100 mmol) of acetic acid was added dropwise, and stirring at room temperature was continued for another 15 min, after which period hydrogen evolution had virtually ceased. The solution was evaporated (*foaming*!), 80 mL of saturated aqueous $NaHCO_3$ was added (*foaming*!), and the mixture was extracted with 4 × 20 mL of methylene chloride. After drying over $MgSO_4$ and evaporation, the residue was chromatographed on SiO_2. EtOAc/hexanes (1:3) eluted the byproduct, benzyl alcohol; EtOAc/hexanes (1:1) then eluted the title compound. Evaporation and drying in vacuo afforded 678 mg (44%) of a colorless oil with the following spectral characteristics: mp 25–30 °C; ^1H NMR ($CDCl_3$) δ 7.37–7.25 (m, 10 H), 5.09 (s, 2 H), 3.86, 3.65 (AB q, 2 H, J = 12.5 Hz), 3.73 (t, 1 H, J = 8.5 Hz), 3.39 (tt, 1 H, J = 2.5, 8 Hz), 3.24–3.13 (m, 2 H), 2.58 (br dd, J = 2, 11.5 Hz), 2.35, 2.27 (AB q, 2 H, J = 11 Hz, both parts split into d with J = 8 Hz; IR (neat) 3401, 3065, 3031, 2924, 2872, 1742, 1613, 1497, 1455, 1395, 1216, 1177, 1094, 1028, 749, 698 cm^{-1}; MS (EI) m/z 311 (M^+, 0.1), 309 (0.2), 294 (0.3), 292 (0.2), 280 (33), 176 (26), 91 (100); calcd for $C_{19}H_{20}NO_2$ (M^+ – OH) 294.1494, found 294.1495.

In order to recover the byproduct **21**, the above column was stripped with methanol. The crude material obtained on evaporation was chromatographed again (SiO_2, EtOAc/MeOH 4:1) and bulb-to-bulb distilled (oven 140 °C (0.06 Torr)) to obtain 164 mg (16%) of a clear, yellowish syrup which solidified in the freezer and then had the following: mp 72–74 °C; ^1H NMR ($CDCl_3$) δ 7.33–7.23 (m, 5 H), 3.71 (s, 2 H), 3.35–3.25 (m, 4 H), 3.07 (s, 2 H), 2.10–1.96 (m, 2 H); (D_2O) δ 7.39 (s, 5 H), 3.78 (s, 2 H), 3.45–3.27 (m, 4 H), 2.30 (m, 1 H), 1.61 (m, 1 H); IR (neat) 3366, 3063, 3029, 2924, 2869, 1655, 1495, 1455, 1090, 1030, 957, 737, 702 cm^{-1}; MS (EI) m/z 207 (0.2, M^+), 176 (40), 91 (100); calcd for $C_{11}H_{14}NO$ 176.1075, found 176.1076.

4-(Hydroxymethyl)azetidine-2-carboxylic Acid (20). **19** (179 mg, 575 μmol) and 60 mg of 20% $Pd(OH)_2/C$ (containing 31% of water) in 30 mL of methanol were hydrogenated in a Parr shaker under 4 atm of hydrogen for 3.5 h. The catalyst was filtered off through a double paper filter and washed with methanol, the solution was evaporated, and the residue was chromatographed on silica gel (2-propanol/water/concentrated NH_3 14:5:1) to yield, after evaporation and drying in vacuo, 53.6 mg (71%) of **20** as a colorless solid: mp 196–202 °C dec; ^1H NMR (D_2O) δ 4.71 (t, 1 H, J = 9 Hz), 4.56 (m, 1 H), 3.87–3.74 (m, 2 H), 2.81 (dt, 1 H, J = 9.5 (t), 12 Hz (d)), 2.43 (dt, 1 H, J = 8.5 (t), 12.5 Hz (d)); IR (Nujol) 3239, 3146, 2589, 2438, 1620, 1565, 1410, 1327, 1302, 1188, 1090, 1038, 980, 885, 828, 758, 725 cm^{-1}; MS (EI) m/z 100 (100), 86 (24), 82 (31), 54 (42); (CI, isobutane) m/z 132 (100, M + H^+), 114 (28), 100 (15), 86 (22), 68 (23); calcd for $C_4H_6NO_2$ 100.0399, found 100.0399.

***N*-[[*cis*-1-Benzyl-4-[(benzyloxy)carbonyl]azetidin-2-yl]carbonyl]glycine Benzyl Ester (23). (a) From 17.** To 80.5 mg (0.2 mmol) of **17** and 74.2 mg (0.22 mmol) of glycine benzyl ester tosylate in 1 mL of THF was added 84 μL (0.6 mmol) of triethylamine, followed by 45.2 mg (0.22 mmol) of cuprous bromide/dimethyl sulfide complex. After stirring for 2 h at room temperature, the suspension was diluted with methylene chloride, 2 g of SiO_2 was added, and the whole was evaporated and chromatographed on SiO_2. EtOAc/hexanes (1:6) eluted 37.4 mg (46%) of unreacted **17**, followed by 1.2 mg of **8f**. An unidentified impurity was eluted with EtOAc/hexanes (1:2) and finally the title compound with EtOAc/hexanes (2:1). Evaporation and drying in vacuo yielded 25.0 mg (26%) of **23** as a colorless syrup.

(b) **From 16.** **8e** (49.6 mg, 0.13 mmol) was treated with trifluoroacetic acid as described above. The crude material was dissolved in 1 mL of THF and the solution cooled to –20 °C. *N*-Methylmorpholine (35 μL, 0.32 mmol) was added, followed by 17 μL (0.13 mmol) of isobutyl chloroformate. After 15 min at –20 °C, a solution of 44 mg (0.13 mmol) of glycine benzyl ester tosylate and 14 μL (0.13 mmol) of *N*-methylmorpholine in 0.2 mL of DMF was added, and after another 10 min at –20 °C the mixture was warmed to room temperature and poured into water. Extraction with CH_2Cl_2, drying over $MgSO_4$, evaporation, and column chromatography (SiO_2, EtOAc/hexanes 1:1) yielded 42 mg (68%) of the product: ^1H NMR ($CDCl_3$) δ 7.72 (br t, 1 H, J = 5.5 Hz), 7.4–7.2 (m, 15 H), 5.17 (narrow AB q, 2 H), 5.04 (narrow AB q, 2 H), 3.97, 3.84 (AB q, 2 H, J = 18 Hz, both parts split into d with J = 5.5 Hz), 3.80 (t, 1 H, overlapping), 3.78, 3.73 (AB q, 2 H, overlapping), 3.67 (t, 1 H, J = 8.5 Hz), 2.67 (dt, 1 H, J = 8.5 (t), 11 Hz (d)), 2.23 (dt, 1 H, J = 8 (t), 11 Hz (d)); IR (neat) 3371, 3064, 3032, 2923, 2851, 1746, 1679, 1520, 1498, 1456, 1357, 1188, 1029, 741, 698 cm^{-1}; MS (EI) m/z 472 (0.2, M^+), 381 (2), 337 (5), 310 (3), 280 (40), 253 (8), 91 (100); calcd for $C_{28}H_{28}N_2O_5$ 472.1998, found 472.1998.

***N*-[(*cis*-4-Carboxyazetidin-2-yl)carbonyl]glycine (24).** **23** (18.5 mg, 39 μmol) in 10 mL of methanol was hydrogenated in a Parr shaker over 34.5 mg of 20% $Pd(OH)_2/C$ (containing 31% of water) under 4 atm of hydrogen for 4 h. Water (5 mL) was added, the catalyst was removed by filtration through a double paper filter and washed thoroughly with methanol/water (2:1), and the solution was evaporated. Filtration over SiO_2 (2-propanol/water/concentrated NH_3 14:5:1), evaporation and drying in a vacuum desiccator over P_2O_5 and KOH afforded 6.7 mg (85%) of a colorless glass: ^1H NMR (D_2O) δ 4.98 (t, 1 H, J = 8.5 Hz), 4.73 (t, 1 H, overlapping), 3.86, 3.74 (AB q, 2 H, J = 17.5 Hz), 3.08 (dt, 1 H, J = 10 (t), 11.5 Hz (d)), 2.55 (dt, 1 H, J = 8.5 (t), 12 Hz (d)); MS (EI) m/z 143 (6), 100 (97), 84 (97), 57 (100); (DCI, NH_3) m/z 220 (6, M + NH_4^+), 202 (19); calcd for $C_5H_7N_2O_3$ 143.0457, found 143.0457.

1-Hexylazetidine-2,4-dicarboxylic Acid Dibenzyl Ester (25). **7f** (470 mg, 1 mmol) and 0.53 mL (4 mmol) of hexylamine in 10 mL of DMF were stirred at 80 °C for 12 h. After workup as for the 1-benzyl compounds (see general procedure), the crude product was chromatographed on SiO_2 with EtOAc/hexanes 1:7. Both isomers appeared on TLC as one spot (R_f ca. 0.43 with EtOAc/hexanes 1:5), preceded by and followed by several unidentified impurities. In this way, 178 mg (43%) of an ca. 2:1 trans/cis mixture was obtained as an amber oil which could be separated only by HPLC. This was done in ca. 5-mg batches with EtOAc/hexanes 1:9 as eluent. In contrast to all 1-benzyl derivatives described above, the cis isomer eluted first. Both isomers were oils. **A. Cis isomer 25:** t_R 7.5 min; ^1H NMR ($CDCl_3$) δ 7.34 (br s, 10 H), 5.19, 5.14 (AB q, 2 H, J = 12.5 Hz), 3.54 (t, 2 H, J = 8.5 Hz), 2.66 (t, 2 H, J = 7.5 Hz, NCH_2), 2.56, 2.46 (AB q, 2 H, J = 10.5 Hz, both parts split into triplets with J = 9 and 8.5 Hz, respectively), 1.38 (m, 2 H), 1.30–1.15 (m, 6 H), 0.85 (t, 3 H, J = 7 Hz); IR (neat) 3065, 3034, 2962, 2930, 2857, 1746, 1499, 1456, 1379, 1362, 1339, 1271, 1175, 1026, 909, 749, 734, 696 cm^{-1}; MS (EI) m/z 409 (1, M^+), 338 (8), 318 (2), 274 (100), 91 (78). Calcd for $C_{25}H_{31}NO$ 409.2253, found 409.2252. **B. Trans isomer 26:** t_R 8.8 min; ^1H NMR ($CDCl_3$) δ 7.35 (br s, 10 H), 5.18 (narrow AB q, 2 H), 4.21 (t, 2 H, J = 6.5 Hz), 2.58 (t, 2 H, J = 7 Hz, NCH_2), 2.50 (t, 2 H, 6.5 Hz), 1.33–1.10 (m, 8 H), 0.85 (t, 3 H, J = 7 Hz); IR (neat) 3067, 3034, 2958, 2930, 2857, 1732, 1499, 1456, 1379, 1343, 1167, 1026, 909, 735, 698 cm^{-1}.

***cis*-1-Hexylazetidine-2,4-dicarboxylic Acid (27).** **25** (25.5 mg, 62 mmol) in 5 mL of ethyl acetate and 5 mL of methanol was stirred under 1 bar of hydrogen (balloon) over 39 mg of 20% $Pd(OH)_2/C$ for 1.5 h. The catalyst was filtered off and washed with methanol and ethyl acetate, and the solution was evaporated and dried in vacuo to leave 12.6 mg (88%) of an off-white, amorphous solid: mp 150–162 °C dec; ^1H NMR ($CD_3OD/CDCl_3$ 2:1) δ 4.64 (t, 2 H, J = 9 Hz), 3.28 (t, 2 H, J = 7.5 Hz, NCH_2), 3.04 (m, 1 H), 2.50 (m, 1 H), 1.65 (m, 2 H), 1.47–1.26 (m, 6 H), 0.91 (t, 3 H), MS (EI) m/z 229 (3, M^+), 184 (93), 158 (100), 140 (24), 114 (34), 100 (47); calcd for $C_{11}H_{19}NO_4$ 229.1314, found 229.1315.

***cis*-1-Acetylazetidine-2,4-dicarboxylic Acid (28).** To 29.2 mg (0.2 mmol) of **10f** and 65 μL (0.8 mmol) of pyridine in 0.5 mL of water was added with stirring in several portions of 150 μL (1.6 mmol) of acetic anhydride within 1.5 h. After 3.5 h at room temperature, the mixture was filtered over a short column of Dowex 50W-X8 resin (H^+ form, washed with 10% HCl and deionized water before use) to remove pyridine (elution with water). The crude material obtained after evaporation was chromatographed on SiO_2 (2-propanol/water/acetic acid 14:5:1) and evaporated. The product held acetic acid tenaciously; after drying at 100 °C (0.1 Torr, overnight), 35.5 mg of an amber glass was obtained which still contained 4% of acetic acid (corrected yield: 91%): ^1H NMR (D_2O) δ 5.06 (dd, 2 H, J = 5.5, 10 Hz),

3.07 (dt, 1 H, J = 10 (t), 11.5 Hz (d)), 2.43 (dt, 1 H, J = 5.5 (t), 12 Hz (d)), 1.96 (s, 3 H); MS (EI) m/z 143 (3%, $M^+ - CO_2$), 100 (21), 43 (100); (DCI, NH_3) m/z 205 (38, $M + NH_4^+$), 188 (100, $M + H^+$), 144 (25); calcd for $C_6H_9NO_3$ 143.0582, found 143.0582.

Calcium Uptake. The uptake of $^{45}Ca^{2+}$ was studied in cerebellar granule cells cultured for 8–9 days.[25] Culture dishes were washed twice and preincubated for 15 min at 37 °C with 1 mL of medium containing 154 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 1.3 mM $CaCl_2$, 5.6 mM glucose, and 10 mM Hepes buffer, pH 7.35. Then 1 μCi of $^{45}CaCl_2$ (New England Nuclear, specific activity 10 Ci/g) was added, and 10 min was allowed for the equilibration of the radioactive tracer in the various compartments. At the end of this preincubation period the azetidine derivative (50 μM) was added followed after 15 min by the indicated concentrations of agonists. In experiments with quisqualate and kainate the medium contained 1 mM $MgCl_2$ and 10 μM CPP, which were used in order to block the NMDA receptors. The incubation was then continued for 10 min. The incubations were terminated by rapidly washing the cultures with a solution containing 154 mM choline chloride, 2 mM EGTA, and 10 mM Tris–HCl, pH 7.35. The cells were dried and dissolved in 0.5 M NaOH, and aliquots were used for the measurement of both incorporated radioactivity and protein concentration.

[^{3}H]MK-801 Binding. [^{3}H]MK-801 binding was measured in well-washed rat-brain membranes.[26] Assays contained 1–200 μg membrane protein, 0.5 nM [^{3}H]MK-801 (New England Nuclear, specific activity 15 Ci/mmol), and drugs as appropriate. Incubations were performed in 10 mM HEPES–NaOH at room temperature for 2 h, were terminated by vacuum filtration over glass fiber filters (Schleicher and Schuell #32), and radioactivity was determined. Typically, specific [^{3}H]MK-801 was 0.1–0.2 pmol/mg of protein when the assay mixture was supplemented with glutamate and glycine. Lower levels of binding were obtained without this addition. When [^{3}H]MK-801 binding was maximally enhanced nonspecific binding was 6% of total binding.

[^{3}H]GABA Release. The release of [^{3}H]GABA from embryonic rat forebrain neurons, cultured for 8–9 days was studied.[28] Cells were loaded with 1 μCi/mL [^{3}H]GABA (New England Nuclear, specific activity 25 Ci/mmol) for 60–90 min at 37 °C. Cells were then washed at 5 min intervals with eight exchanges of HEPES-buffered physiological saline devoid of added Mg^{2+}. Release of [^{3}H]GABA was triggered by the addition of NMDA, azetidine **10f**, or kainate. The final two washes and the stimulus fractions were collected for determination of radioactivity. Release of [^{3}H]GABA was expressed as a fraction of the total [^{3}H]GABA remaining in the cells at the appropriate time. Typically, basal release represented 4–5% of the total remaining [^{3}H]GABA. Residual cell-associated [^{3}H]GABA was determined by solubilizing cells with 0.2% SDS at the end of the experiment.

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