α-Spirocyclopentyl- and α-Spirocyclopropyl-γ-butyrolactones: Conformationally Constrained Derivatives of Anticonvulsant and Convulsant α,α-Disubstituted γ-Butyrolactones

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To further study the putative γ-butyrolactone site of the $GABA_A$/chloride channel complex, constrained derivatives of convulsant and anticonvulsant α,α-disubstituted γ-butyrolactones (α-spirocyclopropyl- and α-spirocyclopentyl-γ-butyrolactones) were synthesized and evaluated biologically. Most of the spirocyclopropyl agents were anticonvulsants when tested against pentylenetetrazole-induced seizures in mice. These agents effectively displaced ^{35}[S]-*tert*-butylbicyclophosphorothionate (^{35}[S]-TBPS), a ligand for the picrotoxin binding site of the $GABA_A$/chloride channel, from rat neuronal membranes and affected the GABA-mediated current in hippocampal neurons. The monomethyl-substituted spirocyclopropyl agent with a methyl group *cis* to the carbonyl (**15**) potentiates GABA-induced current whereas the *trans* derivative (**16**) blocks the current. The only anticonvulsant in the spirocyclopentyl series was the unsubstituted spirocyclopentyl compound **2**. All the other substituted spirocyclopentyl targets were inactive *in vivo* at the highest dose tested except for convulsant **9**, which has a *trans* 2,5-dimethyl-substituted cyclopentyl ring. All the spirocyclopentyl derivatives displaced ^{35}[S]-TBPS from rat neuronal membranes very effectively, and they also all potentiated GABA-induced chloride current except for convulsant **9** which blocked the current. From the data obtained in this investigation, it appears that when the volume occupied above and below the lactone ring is as large as that occupied by spirocyclopentyl agent **9**, convulsant activity is observed. Groups with less volume in these areas either are inactive in the behavioral test or have anticonvulsant activity. When bound to the $GABA_A$/chloride channel, the larger molecules may stabilize the closed state of the channel whereas the smaller molecules may stabilize the open state.

γ-Butyrolactones (GBL's) have been shown to affect neuronal activity. When these compounds are substituted with small alkyl groups in the α position, the resulting agents prevent seizures induced by pentylenetetrazole in mice.[1,2] The corresponding β-substituted GBL's, on the other hand, cause seizures in mice.[3] The site of action of these GBL's is associated with the picrotoxin receptor of the $GABA_A$/chloride channel complex in neurons.[4] The $GABA_A$/chloride channel opens when GABA binds to its receptor site. However, when picrotoxin, a convulsant, is also present and binds to the picrotoxin site, the frequency of channel opening is diminished.[5] This action of picrotoxin increases neuronal excitability and the probability of seizure propagation. Binding studies using ^{35}[S]-*tert*-butylbicyclophosphorothionate (^{35}[S]-TBPS), a ligand specific for the picrotoxin receptor, indicate that some GBL's act at a site allosterically linked to the picrotoxin receptor.[6] In addition, electrophysiological studies indicate that the convulsant β-GBL's, like picrotoxin, block GABA-induced chloride currents in neurons. The α-GBL's, on the other hand, either have no effect on the chloride current or potentiate it in the presence of GABA. The α-GBL's can also antagonize the current blockage induced by either the β-GBL's or picrotoxin.[7] Because of the actions described above, the α-GBL's have potential as anticonvulsant agents.

A variety of alkyl-substituted α-GBL's have been synthesized and evaluated. Structure–activity relationships indicate that α-alkyl groups containing more than five carbons (e.g., α,α-diisopropyl-GBL) are convulsants, whereas groups containing three or four carbons are anticonvulsants.[8] In an attempt to explore the GBL receptor site more closely, conformationally constrained derivatives of α,α-disubstituted GBL's have been synthesized and evaluated for (1) anticonvulsant activity in mice, (2) the ability to displace ^{35}[S]-TBPS from the picrotoxin receptor, and (3) the ability to potentiate or inhibit chloride currents in cultured hippocampal neurons. The constrained analogs, α-spirocyclopentyl- and α-spirocyclopropyl-GBL's, are illustrated in Charts 1 and 2.

Chemistry

The synthesis of the spirocyclopentyl derivatives (**2**, **4**, **7**, **9**, and **10**) involved the formation of the appropriate cyclopentanecarboxylic acid ester, alkylation with allyl bromide, ozonolysis, reduction with sodium borohydride, and cyclization in aqueous acid to form the lactone (Scheme 1). Commercially available methyl cyclopentanecarboxylate (**19**) readily underwent this procedure and provided the lead compound **2** in the cyclopentyl series. The other cyclopentanecarboxylic acid esters were synthesized by three methods (Scheme 2). Esters **21a** and **23** were produced from the basic hydrolysis and esterification of the corresponding cyclopentanenitriles **28** and **30**. The cyclopentanenitriles were made by reaction of the cyclopentanones **27** and **29** with tosylmethyl isocyanide (TOSMIC).[9] Ester **25** could not be readily made by this method since the 2,5-dimethylcyclopentanone was not commercially available. Therefore, compound **25** was constructed using the procedure of Jacobs and Florheim,

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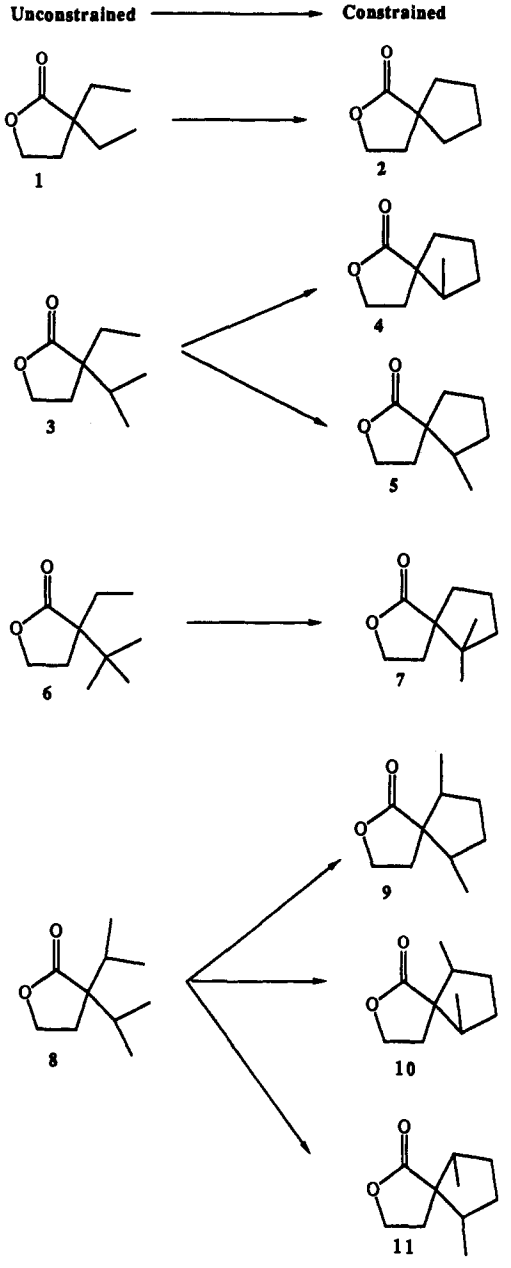
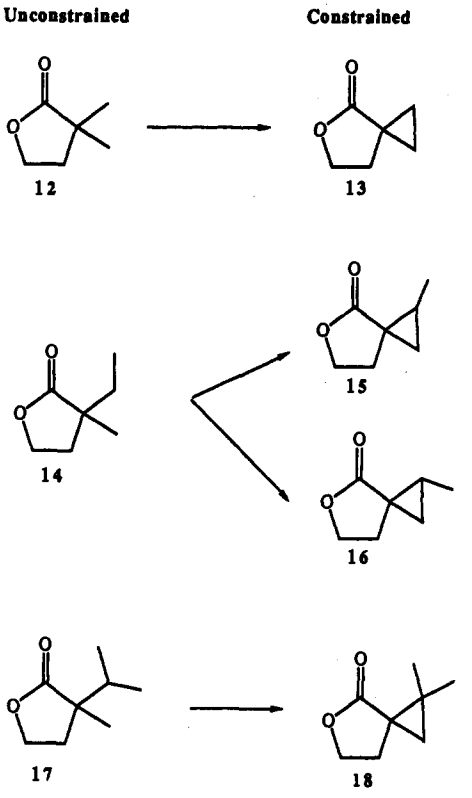
Chart 1. Unconstrained and Constrained α-GBL's

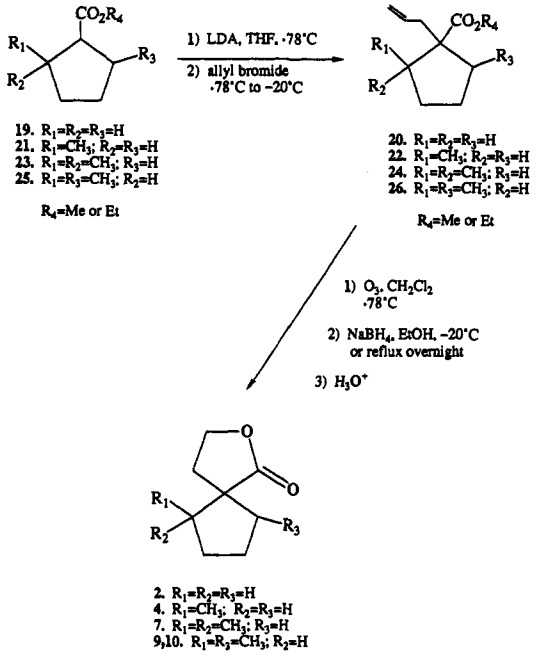


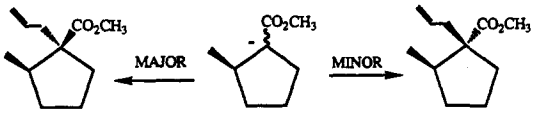
by cyclization of 2,5-dibromohexane (**33**) with diethyl malonate to form compound **34**[10] followed by decarboxylation with LiCl/H_2O in DMSO.[11] Alternative syntheses of compounds **21a** and **23** also were examined since it was somewhat difficult to scale up the TOSMIC reaction and the yields were quite low. The ethyl ester analog **21b** of intermediate **21a** could be synthesized by the cyclization procedure used to make ester **25**. Starting with 1,4-dibromopentane (**31**), ethyl ester **21b** was constructed. Finally, intermediate **23** was synthesized from cyclopentenyl triflate **35** by palladium-catalyzed carbonylation[12] followed by hydrogenation of the double bond. For the reaction sequence illustrated in Scheme 1, the diastereomers of the cyclopentanecarboxylic acid esters and the allyl intermediates were used as mixtures; however, the diastereomeric lactones were separated chromatographically. The target lactones, in many cases, were composed of a mixture of enantiomers, and this mixture was used in the pharmacological assays. Due to the preferred backside attack of allyl bromide in the LDA reaction, the lactone isomers where one or two methyl groups on the spirocyclopentyl ring are *trans* to the carbonyl (**5** and **11**) were not obtained in sufficient yield for *in vivo* testing (see below).

Chart 2. Unconstrained and Constrained α-GBL's



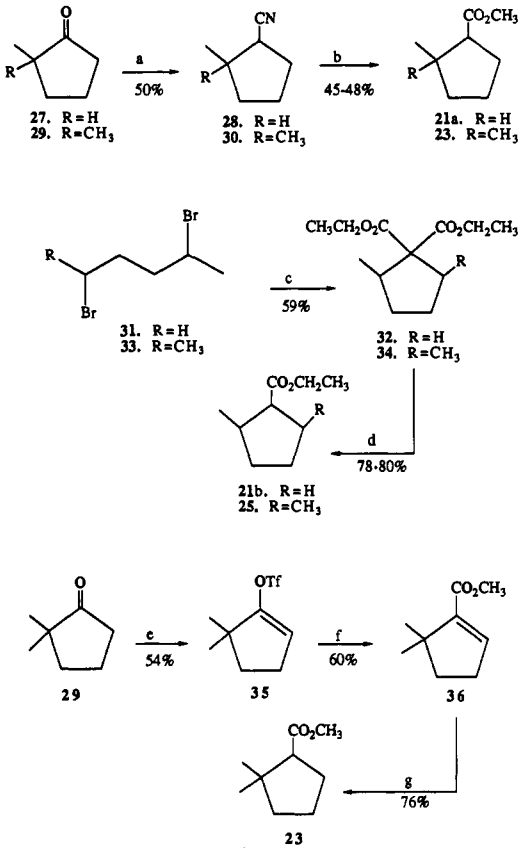
Scheme 1





The spirocyclopropyl targets (**13**, **15**, **16**, and **18**) were synthesized from their α ene precursors by three different methods (Scheme 3). α-Methylene-GBL (**37**) is commercially available. α-Isopropylidene-GBL (not shown) and α-ethylidine-GBLs **39** and **40** were synthesized from α-phosphonate-GBL **38**[13] using the method of Minami et

Scheme 2[a]



[a] (a) TOSMIC, *t*-BuOK, MeOH, DMSO, ~20 °C, overnight; (b) i: KOH, H_2O, diethylene glycol, reflux 1–2 days; ii: MeOH, HCl; (c) i: NaOEt, EtOH; ii: $H_2C(CO_2Et)_2$, reflux overnight; (d) LiCl, H_2O, DMSO, 190 °C, overnight; (e) i: 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2; ii: $(CF_3SO_2)_2O$; (f) $Pd(OAc)_2$, Et_3N, triphenylphosphine, MeOH, DMF, CO_2; (g) H_2, PtO_2, EtOAc.

al.[14] Compound **18** was formed using the procedure of Minami et al.[15] from α-isopropylidine-GBL by reaction with dimethylsulfoxonium methylide (reaction not shown). Unfortunately, when this sulfur ylide chemistry was applied to α ene intermediates **37**, **39**, and **40**, only unidentifiable aqueous-soluble products were obtained. The target compound **13** was formed by reacting α-methylene-γ-butyrolactone (**37**) with palladium acetate and diazomethane;[16] however, when compounds **39** and **40** were treated with these conditions, no reaction occurred and the starting material was recovered. Therefore, target molecules **15** and **16** were made from α enes **39** and **40** via pyrazolines.[17] Intermediates **39** and **40** were reacted with diazomethane to form pyrazolines **41** and **42**, respectively. Photolysis of the pyrazolines provided target compounds **15** and **16** and a small amount of a side product, α-(2-isopropenyl)-γ-butyrolactone (not shown). Again, enantiomeric mixtures of the spirocyclopropyl targets were used in the pharmacological assays.

Most of the unconstrained agents illustrated in Charts 1 and 2 were synthesized as described previously.[8] α-Ethyl-α-*tert*-butyl-GBL (**6**) was synthesized by reacting α-*tert*-butyl-GBL with ethyl iodide using lithium diisopropylamide as the base.

Pharmacological Results

The α-spiro-substituted lactones and their unconstrained counterparts[8] were evaluated for (a) anticonvulsant activity against pentylenetetrazole-induced seizures in mice, (b) toxicity, determined by the rotorod test, to mice, and (c) effects on chloride current in hippocampal neurons (see Tables 1 and 2). The constrained agents were also investigated for their effects on the binding of

Scheme 3

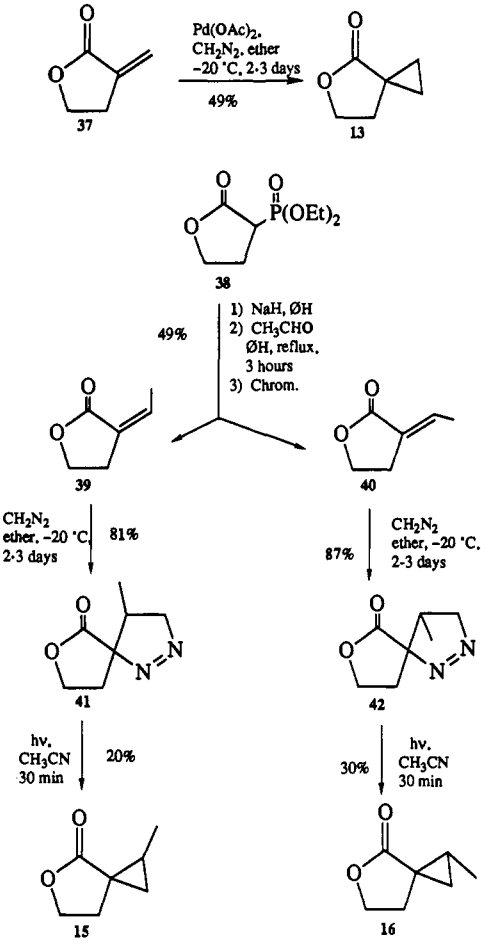


Table 1. Behavioral Activity, Neurotoxicity, and Electrophysiological Activity of α,α-Disubstituted γ-Butyrolactones and Congeneric α-Spirocyclopropyl-γ-butyrolactones

compd	ED_{50}[a] (mg/kg)	CD_{50}[b] (mg/kg)	TD_{50}[c] (mg/kg)	percent response relative to current produced by GABA[d]
12[e]	>500 [90][f]		>500 [155]	41 ± 5 [6]
13[g]	NA[h] [11]	NA [11]	>500 [11]	62 ± 4 [24]
14[e]	259 [45] (230–291)[i]		621 [80] (481–1215)	170 ± 12 [9]
15	210 [20] (185–255)		>300[j] [20]	149 ± 9 [10]
16	259 [20] (171–398)		>500[k] [20]	48 ± 6 [10]
17[e]	198 [50] (171–232)		278 [90] (239–329)	116 ± 5 [13]
18	221 [20] (175–264)		254 [20] (205–379)	247 ± 42 [10]

[a] Dose at which 50% of the mice were protected from clonic seizures induced by pentylenetetrazole (85 mg/kg). [b] Dose at which 50% of the mice had clonic convulsions. [c] Dose at which 50% of the mice failed the rotorod neurotoxicity test. [d] Percent response relative to current produced by 1 μM GABA. GABA response is 100%. Compounds were tested at 10 mM. Data reported as the mean ± SEM. [e] Behavioral and neurotoxicity data are from ref 8. [f] Numbers in brackets are the total number of mice tested for behavioral effects or the total number of cells tested for electrophysiological effects. [g] The highest dose tested was 500 mg/kg. All five mice given this dose passed the rotorod test. [h] NA = not active. [i] Numbers in parentheses are the 95% fiducial limits. [j] At 300 mg/kg, 1/5 of the mice failed the rotorod test. [k] At 500 mg/kg, 1/5 of the mice failed the rotorod test.

Table 2. Behavioral Activity, Neurotoxicity, and Electrophysiological Activity of α,α-Disubstituted γ-Butyrolactones and Congeneric α-Spirocyclopentyl-γ-butyrolactones

compd	ED_{50}[a] (mg/kg)	CD_{50}[b] (mg/kg)	TD_{50}[c] (mg/kg)	percent response relative to current produced by GABA[d]
1[e]	191 [55][f] (172–211)[g]		416 [100] (372–492)	301 ± 61 [9]
2	385 [20] (308–492)		*h*	220 ± 40 [5]
3[e]	169 [20] (103–325)		>300[i] [20]	222 ± 33 [15]
4[j]	NA[k] [5]	NA [5]	>500 [5]	252 ± 26 [10]
6[e]		133 [22] (92–206)		47 ± 2 [5]
7[l]	NA [10]	NA [10]	*m* [10]	327 ± 64 [10]
8[e]		137 [20] (108–184)		328 ± 72 [7]
9		198 [20] (112–254)		80 ± 13 [6]
10[n]	NA [10]	NA [10]	>500 [10]	198 ± 39 [8]

[a] Dose at which 50% of the mice were protected from clonic seizures induced by pentylenetetrazole (85 mg/kg). [b] Dose at which 50% of the mice had clonic convulsions. [c] Dose at which 50% of the mice failed the rotorod neurotoxicity test. [d] Percent response relative to current produced by 1 μM GABA. GABA response is 100%. Compounds 1–4, 7, 8, and 10 were tested at 10 mM. Compounds 6 and 9 were tested at 1 mM. Data reported as the mean ± SEM. [e] Behavioral and neurotoxicity data are from ref 8. [f] Numbers in brackets are the total number of animals tested for behavioral effects or the total number of cells tested for electrophysiological effects. [g] Numbers in parentheses are the 95% fiducial limits. [h] All animals were toxic at the only dose evaluated in the rotorod test (562 mg/kg). [i] At 300 mg/kg, 3/5 of the mice passed the rotorod test. [j] All mice were evaluated at a dose of 500 mg/kg. All mice passed the rotorod test. [k] NA = not active. [l] The highest dose tested was 500 mg/kg. [m] At 300 mg/kg, 2/5 of the mice failed the rotorod test, and at 500 mg/kg, 3/5 of the mice failed this test. [n] The highest dose tested was 500 mg/kg. All mice passed the rotorod test.

Table 3. Effects of α,α-Disubstituted, α-Spirocyclopentyl-, and α-Spirocyclopropyl-γ-butyrolactones on ^{35}[S]-TBPS Binding

compd	^{35}[S]-TBPS displacement[a] IC_{50} (mM)	Scatchard analysis of ^{35}[S]-TBPS Binding[a] K_d (nM)	Scatchard analysis of ^{35}[S]-TBPS Binding[a] B_{max} (pmol/mg of protein)
1	0.75 ± 0.05[b]	37.2 ± 1.3*[c]	1.51 ± 0.08
		(28.0 ± 1.1	1.53 ± 0.11)[d,e]
2	0.81 ± 0.03	48.8 ± 6.33*	1.04 ± 0.19
		(21.6 ± 3.28	1.05 ± 0.21)
3	0.24 ± 0.01	35.2 ± 2.4*	1.50 ± 0.1
4	0.68 ± 0.08	39.5 ± 1.27*	0.93 ± 0.08
		(19.6 ± 1.30	1.05 ± 0.21)[f]
6	0.31 ± 0.04	46.8 ± 1.2*	1.54 ± 0.12
7	0.36 ± 0.03	41.8 ± 3.00*	1.00 ± 0.12
8	0.22 ± 0.02[b]	33.4 ± 1.8*	1.49 ± 0.11
9	0.20 ± 0.04	35.6 ± 2.20*	1.04 ± 0.14
10	0.78 ± 0.20	38.5 ± 2.10*	0.54 ± 0.11*
12	9.2 ± 0.7[b]		
13	>10[g]		
14[h]	2.28 ± 0.17	80.7 ± 8.6*	1.27 ± 0.02
		(40.7 ± 3.3	1.35 ± 0.3)
15	7.17 ± 0.83	44.1 ± 7.79*	0.83 ± 0.16
		(24.6 ± 5.54	1.20 ± 0.33)[i]
16	3.41 ± 0.61	40.4 ± 10.9*	1.14 ± 0.24
17	0.74 ± 0.11	47.0 ± 0.11*	0.99 ± 0.15
		(22.5 ± 1.52	1.10 ± 0.08)
18	1.14 ± 0.11	35.4 ± 1.75*	0.86 ± 0.08
		(20.7 ± 1.00	1.01 ± 0.12)

[a] Binding data are presented as the mean ± SEM of two or three experiments performed in triplicate. Scatchard analysis was carried out with ~IC_{50} concentrations of compounds. [b] The IC_{50} is from the literature.[8] [c] *$P < 0.05$ as compared with control, t test with Bonferroni correction. [d] Numbers in parentheses are comparison control values for ^{35}[S]-TBPS binding alone. [e] These are also the control values for Scatchard analysis of compounds 3, 6, and 8. [f] These are also the control values for Scatchard analysis of compounds 7, 9, and 10. [g] Partial displacement (24%) by 10 mM compound 13 was observed. [h] All binding data are from the literature.[4a] [i] These are also the control values for Scatchard analysis of compound 16.

^{35}[S]-TBPS to the picrotoxin receptor (Table 3). The cyclopropyl derivatives had activities similar to those of the unconstrained analogs. The unsubstituted derivative 13 had no anticonvulsant activity and weakly displaced ^{35}[S]-TBPS at a concentration as high as 10 mM. This agent is similar in structure to analog 12, a compound devoid of anticonvulsant activity and toxicity at low doses and a weak displacer of ^{35}[S]-TBPS. At high concentrations, lactones 12 and 13 both block chloride current in hippocampal neurons. Since agents 12 and 13 were only very weak displacers of ^{35}[S]-TBPS, Scatchard analyses were not performed on these compounds. The target molecule 18 has activity similar to that of the α-isopropyl-α-methyl-substituted compound 17. Both agents are anticonvulsants, potentiate chloride currents, and are somewhat toxic. The unconstrained lactone 17 is similar to the constrained derivative 18 as a displacer of ^{35}[S]-TBPS. The monomethyl agents 15 and 16 were both similar in anticonvulsant activity to the unconstrained congener 14. However, agent 16 appeared to displace ^{35}[S]-TBPS at a concentration similar to compound 14, and it was a much more effective displacer than analog 15. On the other hand, agent 15, like compound 14, potentiates chloride current in hippocampal neurons whereas analog 16 blocks the current. In the Scatchard analysis of spirocyclopropyl targets 15, 16, and 18, changes in the K_d, but not the B_{max}, of ^{35}[S]-TBPS binding were observed (Table 3). Although these data suggest that the lactones affect ^{35}[S]-TBPS binding in a competitive manner, the dissociation rate of ^{35}[S]-TBPS from its receptor in the presence of these agents must be studied to determine if they are truly competitive. Previous studies on compound 17 indicated that although this agent affected the K_d but not the B_{max} of ^{35}[S]-TBPS binding in the Scatchard analysis, the dissociation rate of ^{35}[S]-TBPS was also affected by this molecule and therefore the inhibition of ^{35}[S]-TBPS binding by compound 17 cannot be considered competitive.[6]

The spirocyclopentyl targets, unlike most of their unconstrained analogs, are devoid of observable anticonvulsant activity when the cyclopentyl ring is substituted with methyl groups. The unsubstituted compound 2, is the only agent exhibiting anticonvulsant activity. However, agent 2 is less potent than the unconstrained analog 1 in preventing seizures. Agent 9 is a convulsant and thus appears to mimic the *in vivo* conformation of its unconstrained analog, convulsant 8. All the α-spirocyclopentyl-substituted compounds displaced ^{35}[S]-TBPS very effectively, although their unconstrained counterparts displaced the ligand slightly better. As observed with the spirocyclopropyl targets, all the spirocyclopentyl compounds except agent 10 affected the K_d but not the B_{max} of ^{35}[S]-TBPS binding. Again, to determine whether agents 2, 4, 7, and 9 are competitive inhibitors of ^{35}[S]-TBPS binding, the dissociation rates of ^{35}[S]-TBPS in the presence and absence of these agents must be determined. Compound 10 affects both the K_d and the B_{max} of ^{35}[S]-TBPS binding and therefore is not a competitive ligand at this site. All of these compounds potentiate GABA-mediated chloride currents in cultured hippocampal neurons except for convulsant 9, which blocks currents. Interestingly, compound 6, the unconstrained derivative of potentiator 7, blocks GABA currents, and compound

8, the unconstrained derivative of the current blocker and convulsant 9, potentiates current.

Discussion

The goal of the present study was to further explore the putative γ-butyrolactone receptor site on the $GABA_A$/chloride channel complex. Previous work in this area has indicated that the β-substituted and large alkyl-α-substituted γ-butyrolactones are convulsants. When the substituents on the α position consist of three or four carbons, the compounds are anticonvulsants when tested against pentylenetetrazole-induced seizures in mice.[8] Here, we have investigated constrained derivatives of both the convulsant and anticonvulsant α,α-disubstituted γ-butyrolactones. The smaller α-alkyl substituents have been constrained with an α-spirocyclopropyl ring. This produces very rigid structures (13, 15, 16, and 18), and the methyl substituents on the cyclopropyl ring are essentially locked into one position. The larger α-alkyl substituents have been constrained with an α-spirocyclopentyl ring. These are less rigid structures (2, 4, 7, 9, and 10); however, the position of the methyl substituents is greatly restricted. By investigating the biological activities of these rigid and semirigid compounds, we can gain insight into the preferred conformation of these agents at their $GABA_A$/chloride channel receptor site.

From the data obtained in this study, it appears that constraining the substituents on the α-carbon of γ-butyrolactones does not enhance anticonvulsant activity. In the case of the spirocyclopropyl derivatives, some of the binding affinity is lost; however, the anticonvulsant efficacy and the relative toxicities are maintained with these agents. The most interesting result from this series comes from the electrophysiological result in which anticonvulsants 15 and 16, analogs of anticonvulsant 14, exhibit opposite electrophysiological effects despite similar *in vivo* activities. Agent 15, which has the methyl group locked into a position pointing in the same direction as the lactone carbonyl, has an effect similar to compound 14 and potentiates chloride current; thus, 15 probably resembles the active conformation of lactone 14 *in vivo*. Agent 16, however, has the methyl group pointing in a direction opposite that of the carbonyl and blocks the GABA-induced chloride current. Analog 16 may be acting at a receptor other than the $GABA_A$ receptor when it is producing its anticonvulsant effects *in vivo*, and the activity at this other site may overcome the current blocking effects of 16 at the $GABA_A$/chloride channel. Alternatively, since some lactones have been shown recently to have activities that differ depending on the subunit composition of the $GABA_A$ receptor,[18] analogs 15 and 16 may have actions that depend on $GABA_A$ receptor subunit composition. The $GABA_A$ receptors found in cultured hippocampal neurons may not have the subunit composition of those *in vivo* $GABA_A$ receptors involved in mediation of the behavioral effects of compounds 15 and 16. Additional studies which are beyond the scope of the investigation reported here are needed to address these possible explanations.

The spirocyclopropyl analog 13, like its previously studied unconstrained counterpart 12,[8] is inactive *in vivo* against pentylenetetrazole-induced seizures, and it is also a weak displacer of ^{35}[S]-TBPS. This result is the expected result, given the similarity in the size and shape of the α-substituents in both compounds. The result reconfirms the minimum size requirement needed for α-alkyl substituents to confer anticonvulsant activity on the GBL ring structure. We also found that these agents can block currents in hippocampal neurons. Both the mechanism of the current-blocking effects of these compounds and the reason why the compounds are not *in vivo* convulsants are unclear at this time.

The results from the spirocyclopentyl lactones have proven to be more informative than those of the spirocyclopropyl lactones. When the α-substituents are constrained with a spirocyclopentyl ring, the binding affinity is maintained while the *in vivo* effects are greatly attenuated. The only anticonvulsant α-spirocyclopentyl lactone, 2, is a much less potent anticonvulsant than its unconstrained analog 1. Likewise, the constrained derivative of anticonvulsant lactone 3, agent 4, is devoid of observable *in vivo* activity. When groups with more than five carbons are constrained, most of the convulsant activity disappears in the resulting spirolactones. Most notably, when convulsants 6 and 8 are constrained to form spirolactones 7 and 10, respectively, they lose all observable *in vivo* activity. The convulsant activity is, however, maintained in agent 9, another analog of lactone 8; therefore, compound 9 must resemble the receptor-bound conformation of convulsant lactone 8.

The effects of the spirocyclopentyl lactones on chloride current further distinguish these agents from their unconstrained counterparts. Anticonvulsants 1, 2, and 3 and inactive agent 4 all potentiate chloride current. However, while convulsant 6 blocks chloride current, its inactive (*in vivo*) constrained congener 7 potentiates the current. Also, convulsant 8 surprisingly potentiates current while its constrained derivative 10 (inactive, *in vivo*) potentiates and constrained derivative 9 (convulsant) blocks current.

An analysis of the structures of agents 6–10 has revealed that the unconstrained convulsants 6 and 8 occupy more space on both the *top* and the *bottom* of the carbonyl plane of the lactone than do the constrained derivatives 7, 9, and 10. Comparison of the volume maps of the minimized conformations of unconstrained analog 6 and spirocyclopentyl-constrained analog 7 is illustrated in Figure 1.[19] Although we do not know what the receptor-bound conformations of agents 6 and 7 are, it is unlikely that unconstrained derivative 6 will be in a conformation exactly like 7 due to the steric hindrance imposed by the carbons that would mimic the two bridge carbons of compound 7. Figure 1 indicates that the volume unique to compound 6 includes additional space on the *top* and the *bottom* of the carbonyl plane and the volume unique to compound 7 includes the space occupied by the two *bridge* carbons.

When combined with the biological data discussed above, the structural comparisons of 6 and 7 suggest that the volume occupied on the *top* and the *bottom* of the carbonyl plane of the γ-butyrolactones is important for inhibiting GABA-mediated chloride currents in hippocampal neurons and causing convulsions in mice. Enders and Vigelius found that α-spirocyclohexyl-γ-butyrolactone had convulsant activity in mice but α-spirocyclopentyl-γ-butyrolactone (2) did not.[20] We now report that compound 2 actually has anticonvulsant activity against pentylenetetrazole-induced seizures in mice. Therefore, the size limit on both the *top* and the *bottom* of the carbonyl plane of the lactone ring for going from anticonvulsant/inactive to convulsant α-substituted γ-butyrolactones must be between the volume occupied by α-spirocyclopentyl- and α-spirocyclohexyl-γ-butyrolactones in the space above

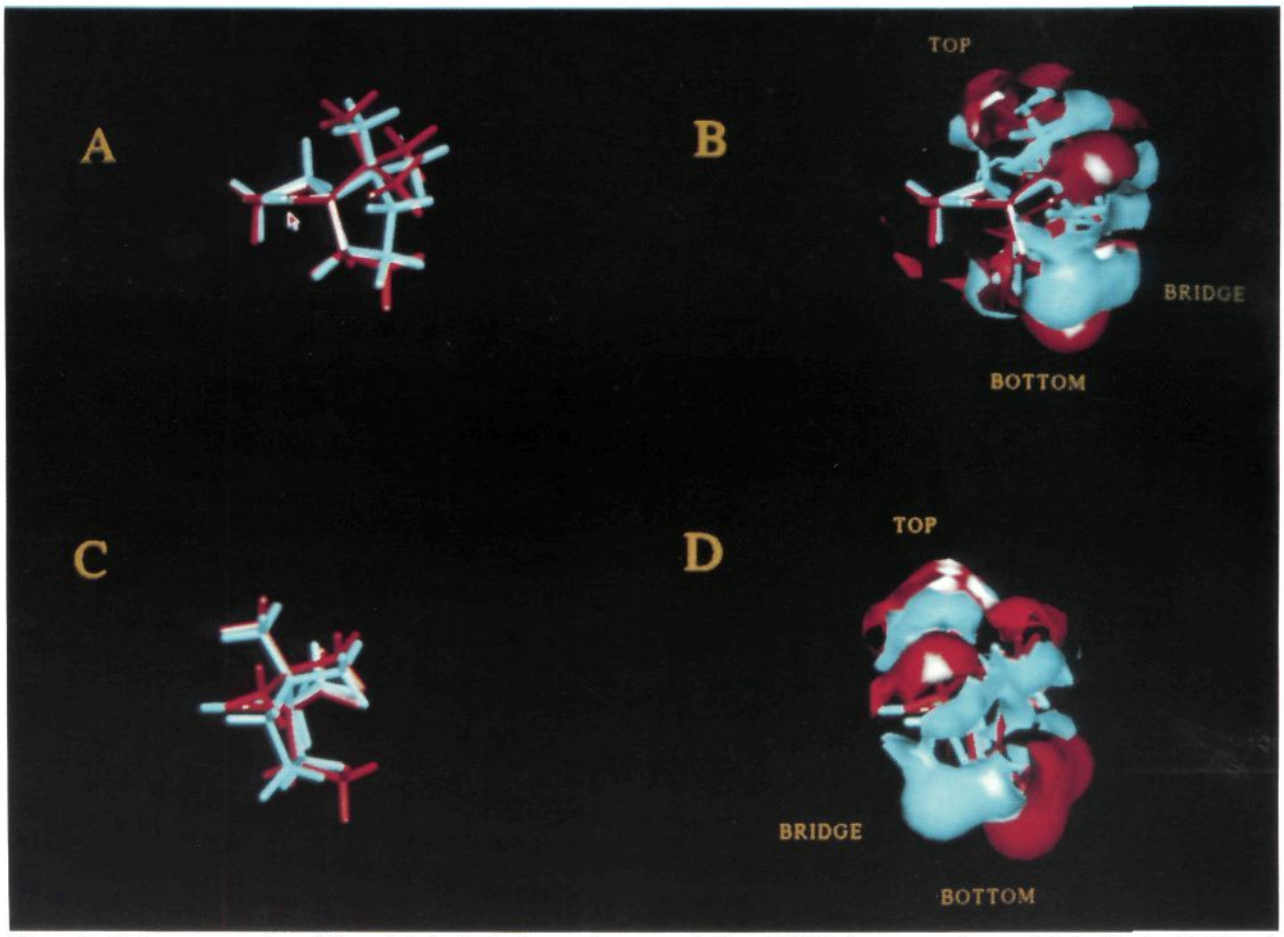


Figure 1. Volume comparison of the minimized structures of compounds **6** and **7**.[19] Each compound was minimized using the Powell method initially and then the conjugant gradient method. The molecules were fit together using the carbon and oxygen of the carbonyl and the lactone ring oxygen. The MVolume feature of Sybyl was used for volume comparisons and contoured on a grid size of 0.5 Å. (A) Superimposed structures of compounds **6** (red) and **7** (blue) with the carbonyl pointing out of the paper approximately on the *z* axis. The carbonyl of the molecules is designated by the red arrow. (B) The volume of **6** minus the volume of **7** (red) and the volume of **7** minus the volume of **6** (blue) where the molecules are in the same orientation as in (A). (C) Same as (A) except the carbonyl plane is approximately in the *xz* plane and the lactone ring is behind the α-substituents. (D) Same as (B) except in the orientation found in (C).

and below the lactone ring. The receptor for the lactones also must accommodate the space occupied by the carbon *bridge* of the spirocyclopentyl compounds since these agents also displace ^{35}[S]-TBPS very effectively.

Ehlert proposed a two-state model for the benzodiazepine receptor.[21] He suggested that the site where benzodiazepines and β-carbolines bind exists in different conformational states depending on whether the chloride channel is open or closed. The β-carbolines prefer binding to the channel when it is closed and stabilize the closed state of the channel. The benzodiazepines prefer binding to the open state and, when bound along with GABA, stabilize the open state of the channel. If we apply this model to the lactone site and the data obtained here, we can say that a certain amount of hydrophobic bulk is necessary above and below the lactone ring to stabilize the channel in a closed state and thus block chloride current. When this bulk is pulled in and repositioned out to the side of the ring in the form of a two-carbon bridge as with the spirocyclopentyl groups, the agent can no longer stabilize the channel in the closed state and, thus, no current blockage or convulsant activity is observed. Since spirocyclopentyl agent **9** both blocks chloride current and has convulsant activity, the hydrophobic bulk on the side of the lactone ring near the β-carbon must also play some role in stabilizing the channel in the closed state. Similarly, current block is also seen with agent **16**, which has bulk in this region, and with the β-substituted γ-butyrolactones. If occupying this space below the α-spiro ring in the region of the β-carbon of the lactone ring is important for stabilizing the channel in the closed state, then one can predict that the two missing spirocyclopentyl compounds in this study, **5** and **11** (see Chart 1), will probably be current blockers.

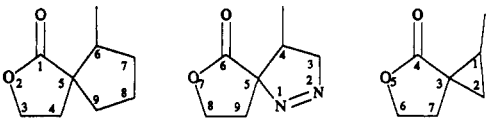
The fact that the inactive spirocyclopentyl compounds (**4**, **7**, and **10**) can potentiate chloride current suggests that these agents would be anticonvulsants at higher doses. (For the present study, agents were considered inactive if no anticonvulsant activity was observed at a 500 mg/kg dose.) The smaller methyl-substituted spirocyclopropyl compounds and their unconstrained counterparts must stabilize the channel in an open state since they potentiate current and have anticonvulsant activity. However, when the two-carbon bridge of the inactive spirocyclopentyl compounds is added, these agents may not stabilize the channel in the open state as well, and therefore, larger doses of these spiro compounds may be necessary to achieve an anticonvulsant effect. Alternatively, the lack of *in vivo* effects of these spirocyclopentyl compounds may be explained by their pharmacokinetic properties (e.g., rates

of absorption and metabolism) instead of their pharmacodynamic properties.

In conclusion, from the results obtained in this study, we have been able to more clearly define the γ-butyrolactone receptor site on the $GABA_A$/chloride channel. The binding studies suggest that the receptor site can accommodate large α-substituents on the lactone ring; however, at least three carbons are necessary at the α position to have an effective hydrophobic binding interaction. The upper size limitation above and below the lactone ring necessary for maintaining anticonvulsant activity lies somewhere between the volume consumed by an unsubstituted α-spirocyclopentyl group (2) and a monomethyl-substituted α-spirocyclopentyl group (4). When the volume consumed above and below the lactone ring is as large as a spirocylohexyl group, convulsant activity is observed.[20] Also, the position of methyl substituents on the spiro rings determines whether agents will block or potentiate current. Agents with methyl groups on the spirocyclopentyl ring pointing in a direction opposite that of the lactone carbonyl may have an increased chance of acting like the β-substituted GBL's and thus stabilize the closed channel, inhibit current, and act as convulsants. Finally, it is now known that $GABA_A$ receptors can be found in a variety of subunit combinations *in vivo*, each of which is often affected differently by ligands such as benzodiazepines, picrotoxinin, or bicuculline.[22] In the binding and electrophysiological evaluations of the compounds presented here, mixtures of these receptor subunit combinations were most likely used since cultured hippocampal neurons are composed of heterogeneous cell populations. A more detailed study of the GBL's using specific subunit combinations will be necessary to further differentiate the effects of these conformationally constrained α-substituted GBLs.

Experimental Section

Chemistry. General. The starting materials were either purchased commercially or synthesized by the literature procedures indicated. Ozonolysis was performed using a Model T-408 Welsbach ozonator (Philadelphia, PA). Catalytic hydrogenations were performed with a Parr hydrogenation apparatus. Silica gel (32–63 μm) for flash chromatography was purchased from Universal Scientific (Atlanta, GA). Preparative HPLC was performed on a Waters Prep LC/System 500A liquid chromatograph using 2 PrepPAK-500/silica cartridges for normal phase separations. A Waters Model M6000A liquid chromatograph equipped with an Alltech Econosil silica (250-mm × 10-mm) column, a Beckman ultrasphere Si (25-cm × 10-mm) column, or an Altex Ultrasphere Si column (25-cm × 10-mm) was used for normal phase separations. An Alltech Econosil C18 column (250-mm × 10-mm) was used for reverse-phase separations. The purifications were monitored by GC and TLC. A Varian Model 3700 gas chromatograph containing a 6-ft glass column (0.25-in. i.d.) packed with 1% SP2401 on 80/100 mesh Supelcoport (Supelco, Inc., Bellefonte, PA) or a Hewlett-Packard 5890A GC equipped with an Ultra 1 capillary column (0.2-mm i.d., 0.11-μm film thickness, 25-m length) was used to follow reactions. Thin-layer chromatography was performed using Analtech (Newark, DE) precoated (250-mm) silica gel GF plates or silica gel GHLF plates. The plates were evaluated by observation under UV light, development taking place in an I_2 chamber and/or by spraying with a 5% sulfuric acid solution and then heating on a hot plate. IR spectra were taken on a Perkin-Elmer 1710 FT-IR spectrometer after applying a neat film of the sample on a silver chloride plate. NMR spectra were recorded at ambient temperature with a 5-mm probe on a Varian Gemini-300 spectrometer operating at either 300 MHz (^1H) or 75 MHz (^{13}C). For ^1H NMR and ^{13}C NMR spectra, the internal references were TMS (δ 0.00) and $CDCl_3$ (δ 77.00), respectively. Peak multiplicity is designated as s (singlet), d (doublet), t (triplet), q (quartet), qu (quintuplet), or m (multiplet). Coupling constants (*J*) are reported in hertz. The numbering system used to identify NMR peaks for the spirolactone molecules is shown below. Elemental analyses were obtained from MHW Laboratories (Phoenix, AZ) or Galbraith Labs Inc. (Knoxville, TN).



General Procedure To Make Cyclopentanenitriles 28 and 30. Potassium *tert*-butoxide powder was added to an ice-cold solution of tosylmethyl isocyanide (TOSMIC) in anhydrous DMSO. After being stirred for 5 min, the cyclopentanone (**27** or **29**) and anhydrous methanol were added. A thick brown reaction mixture formed, and this was stirred at room temperature under N_2 overnight. The mixture was diluted with water, adjusted to pH 3–4 with 6 N HCl, and extracted with petroleum ether. The organic layers were combined, extracted with saturated NaCl solution, and dried ($MgSO_4$). The product was isolated as a light brown liquid identified as pure product **28** or as a brown liquid that was purifed on a silica column to provide pure **30** as a yellow liquid.

2-Methylcyclopentanenitrile (28).[23] Potassium *tert*-butoxide powder (24.0 g, 0.204 mol), TOSMIC (12.5 g, 0.064 mol), anhydrous DMSO (40 mL), methanol (2.5 mL), and 2-methylcyclopentanone (**27**, 4.87 g, 0.050 mol); water (500 mL) and petroleum ether (3 × 200 mL). The organic layers were combined and dried ($MgSO_4$): light brown liquid (2.69 g, 50%); bp ~75 °C (35 mmHg); IR (cm^{-1}) 2963 (s), 2876 (m), 2360 (w), 2236 (m), 1647 (w), 1454 (m), 1382 (w), 1020 (w); ^{13}C NMR ($CDCl_3$) δ 123.1 (CN), 122.0 (CN), 40.7, 36.7, 35.9, 34.7, 33.6, 32.5, 30.4, 30.1, 23.7, 23.1, 18.5, 16.8. Anal. ($C_7H_{11}N$) C,H,N.

2,2-Dimethylcyclopentanenitrile (30). Potassium *tert*-butoxide (35.6 g, 0.318 mol), TOSMIC (20.7 g, 0.106 mol), DMSO (75 mL), 2,2-dimethylcyclopentanone (**29**, 9.96 g, 0.089 mol), and anhydrous methanol (3.8 mL); water (800 mL), petroleum ether (3 × 500 mL), and saturated NaCl solution (200 mL). Upon filtration and evaporation of the filtrate, a brown liquid remained (9.45 g). This was placed on a silica column (265 g of silica), and the column was eluted successively with mixtures of CH_2Cl_2 in petroleum ether (10%, 1 L; 25%, 2 L; 40%, 1 L). Product **30** was isolated as a light yellow liquid (5.44 g, 50%): IR (cm^{-1}) 2964 (s), 2873 (m), 2236 (m), 1461 (m), 1390 (w), 1372 (m), 1316 (w), 1223 (w), 1155 (w), 1010 (w), 952 (w); ^1H NMR ($CDCl_3$) δ 2.43 (t, *J* = 8.8, 1H, HCCN), 2.19–2.09 (m, 1H), 2.04–1.57 (m, 4H), 1.52–1.42 (m, 1H), 1.15 (s, 3H, CH_3), 1.13 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 121.5 (CN), 42.3, 40.5, 39.6, 28.9, 27.4, 23.9, 22.0. Anal. ($C_8H_{13}N$) C,H,N.

General Procedure To Synthesize Cyclopentanecarboxylic Acid Esters 21a and 23 from the Cyclopentanenitriles. The cyclopentanenitrile (**28** or **30**), diethylene glycol, and a solution of potassium hydroxide (KOH) in water were combined and refluxed for 24–48 h. The reaction mixture was acidified and extracted with ether. The organic fractions were combined, extracted with saturated NaCl solution, and dried ($MgSO_4$). The crude acid products were isolated as light brown/yellow liquids. These were combined with anhydrous methanol and a few drops of acetyl chloride and stirred at room temperature overnight. The methanol was evaporated, and the remaining liquid was diluted with ether and extracted with saturated $NaHCO_3$ solution. The aqueous layers were extracted with ether again. The ether portions were combined and dried ($MgSO_4$). The products were isolated as clear/light yellow liquids.

Methyl 2-Methylcyclopentanecarboxylate (21a).[24] 2-Methylcyclopentanenitrile (**28**, 2.69 g, 0.025 mol), KOH (6.0 g, 0.107 mol), diethylene glycol (30 mL), and water (30 mL); ether (3 × 100 mL) and saturated NaCl solution (100 mL). The acid was isolated as a brown liquid (3.0 g). Anhydrous methanol (40 mL); ether (80 mL) and saturated $NaHCO_3$ solution (3 × 20 mL). The dried organic layer was filtered and the filtrate reduced to a brown liquid (2.5 g). This was distilled to provide pure **21a** as a light yellow liquid (1.56 g, 45%): bp 75–85 °C (25 mmHg); IR (cm^{-1}) 2956 (s), 2873 (m), 1737 (s), 1436 (m), 1375 (m), 1265 (m), 1202 (s), 1159 (s), 1036 (w). Anal. ($C_8H_{14}O_2$) C,H.

Methyl 2,2-Dimethylcyclopentanecarboxylate (23).[25] 2,2-Dimethylcyclopentanenitrile (**30**, 4.1 g, 0.033 mol), diethylene glycol (40 mL), KOH (7.5 g, 0.134 mol), and water (40 mL); ether (3 × 150 mL) and saturated NaCl solution (100 mL). The acid was isolated as a light yellow liquid (3.59 g). Anhydrous methanol (40 mL); ether (200 mL) and saturated $NaHCO_3$ solution (3 × 40 mL); ether (100 mL). After filtration and evaporation of the filtrate, a clear liquid was obtained and identified as 90% pure **23** (2.76 g, 48%). A small portion of the product was further chromatographed to give an analytical sample: IR (cm^{-1}) 2957 (s), 2873 (m), 1737 (s), 1456 (m), 1435 (m), 1387 (w), 1369 (m), 1358 (m), 1218 (m), 1192 (s), 1169 (s); ^1H NMR ($CDCl_3$) δ 3.67 (s, 3H, OCH_3), 2.44 (t, J = 8.5, 1H, $HCCO_2Et$), 2.11–2.00 (m, 1H), 1.93–1.46 (m, 5H), 1.15 (s, 3H, CH_3), 0.88 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 175.7 (C=O), 54.3, 51.0, 42.6, 41.5, 29.0, 27.3, 23.7, 22.1. Anal. ($C_8H_{16}O_2$) C,H.

The other 10% of the product mixture was the intermediate amide from the hydrolysis: IR (cm^{-1}) 3383 (s), 3194 (m), 2959 (m), 1650(s), 1455(w), 1366 (w), 1314 (w), 1283 (w); ^1H NMR ($CDCl_3$) δ 5.50–5.20 (br, 2H, NH_2), 2.24 (t, J = 8.5, 1H, CHC=O), 2.15–2.00 (m, 1H), 1.95–1.44 (m, 5H), 1.17 (s, 3H, CH_3), 0.96 (s, 3H, CH_3).

Diethyl 2-Methyl-1,1-cyclopentanedicarboxylate (32).[26] Sodium metal (4.8 g, 0.209 mol) was dissolved in absolute ethanol (100 mL). This solution was cooled in an ice bath and stirred with a mechanical stirrer while diethyl malonate (15.0 g, 0.094 mol) was slowly added. A white precipitate formed, and the reaction mixture became very thick. 1,4-Dibromopentane (**31**, 20.0 g, 0.084 mol) was added and the mixture stirred at 90 °C overnight. The ethanol was evaporated, and the remaining mixture was diluted with water (100 mL) and adjusted to pH 5 with 1 N HCl. The aqueous mixture was then extracted with ether (3 × 100 mL). The ether layers were combined, extracted with saturated NaCl solution (100 mL), and dried ($MgSO_4$). The drying mixture was filtered and the filtrate reduced to a yellow liquid (19.0 g). Distillation gave pure **32** as a clear liquid (11.3 g, 59%): bp 86–90 °C (4 mmHg); IR (cm^{-1}) 3463 (w), 2979 (s), 2876 (m), 1729 (s), 1464 (m), 1367 (m), 1260 (s), 1179 (s), 1097 (m), 1038 (m), 856 (w), 759 (w); ^1H NMR ($CDCl_3$) δ 4.28–4.09 (m, 4H, 2 × OCH_2CH_3), 2.72–2.64 (m, 1H), 2.49–2.39 (m, 1H), 2.06–1.78 (m, 3H), 1.61–1.36 (m, 2H), 1.25 (m, 6H, 2 × OCH_2CH_3), 0.99 (d, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 173.0, 171.8, 63.6, 60.9, 60.8, 40.4, 33.7, 33.3, 22.7, 16.3, 13.9, 13.8. Anal. ($C_{12}H_{20}O_4$) C,H.

Diethyl 2,5-Dimethyl-1,1-cyclopentanedicarboxylate (34).[10,27] The compound was prepared by a literature method[10] and obtained as a colorless liquid: ^1H NMR ($CDCl_3$) δ 4.29–4.09 (m, 4H, 2 × OCH_2CH_3), 2.80–2.70 (m, 1H), 2.49–2.39 (m, 1H), 2.10–1.83 (m, 2H), 1.69–1.58 (m, 1H), 1.32–1.22 (m, 7H, 2 × OCH_2CH_3 and 1 ring H), 1.12 (d, J = 6.8, 3H, CH_3), 0.95 (d, J = 7.0, 3H, CH_3). Anal. ($C_{13}H_{22}O_4$) C,H.

General Procedure To Make Esters 21b and 25. The diethyl 1,1-cyclopentanedicarboxylate (**32** or **34**) was combined with lithium chloride, water, and DMSO, and the mixture was heated to 190 °C overnight. The dark smelly reaction mixture was then cooled and diluted with water. The aqueous mixture was extracted with pentane or petroleum ether. The organic layer was extracted with saturated NaCl solution and dried ($MgSO_4$). The products were isolated as brown liquids which were purified by distillation or on a silica column.

Ethyl 2-Methylcyclopentanecarboxylate (21b).[28] Diethyl 2-methyl-1,1-cyclopentanedicarboxylate (**32**, 10.0 g, 0.044 mol), lithium chloride (2.9 g, 0.068 mol), water (1.25 mL, 0.069 mol), and DMSO (50 mL); water (150 mL), petroleum ether (3 × 100 mL), and saturated NaCl solution (70 mL). The drying mixture was filtered and the filtrate reduced to a light brown liquid (6.14 g). This was purified by short path distillation to provide pure **21b** as a light yellow liquid (5.45 g, 80%): bp 75–85 °C (20 mmHg); IR (cm^{-1}) 2960 (s), 2873 (m), 1733 (s), 1451 (m), 1376 (m), 1303 (m), 1260 (m), 1181 (s), 1157 (s), 1042 (m); ^1H NMR ($CDCl_3$) δ 4.18–4.09 (m, 2H, OCH_2CH_3), 2.78 (m, 0.3H, $CHCO_2Et$), 2.36–1.32 (m, 6.4H), 1.30–1.12 (m, 7.4H), 1.06 (d, J = 6.6, 2H, $CHCH_3$), 0.91 (d, J = 6.8, 1H, $CHCH_3$), 0.93–0.83 (m, 0.3H); ^{13}C NMR ($CDCl_3$) δ 176.5, 175.3, 60.1, 60.0, 52.0, 48.3, 39.4, 37.4, 34.9, 33.8, 30.0, 27.4, 24.4, 23.8, 19.6, 16.2, 14.4, 14.3. Anal. ($C_9H_{16}O_2$) C,H

Ethyl 2,5-Dimethylcyclopentanecarboxylate (25).[10,29] Diethyl 2,5-dimethyl-1,1-cyclopentanedicarboxylate (**34**, 6.89 g, 0.028mol), lithium chloride (2.45 g, 0.058 mol), water (1.1 mL, 0.06 mol), and DMSO (50 mL); water (200 mL) and pentane (3 × 200 mL). The drying mixture was filtered and reduced to a light brown liquid (4.06 g). The liquid was placed on a silica column (192 g of silica), and the column was eluted with mixtures of CH_2Cl_2 in petroleum ether (10%, 1 L; 20%, 1 L; 40%, 1 L). A light yellow liquid was isolated and identified as pure **25** (3.77 g, 78%): ^1H NMR ($CDCl_3$) δ 4.20–4.09 (m, 2H, OCH_2CH_3), 2.68 (t, J = 6.5, 0.3H, $HCCO_2Et$), 2.46–2.15 (m, 2.5H), 1.99–1.58 (m, 2.5H), 1.40–0.85 (m, 6.7H). Anal. ($C_{10}H_{18}O_2$) C,H.

5,5-Dimethyl-1-cyclopentenyl Trifluoromethanesulfonate (35). To an ice-cold solution of 2,2-dimethylcyclopentanone (**29**, 5.03 g, 0.045 mol) and 2,5-di-*tert*-butyl-4-methylpyridine (14.3 g, 0.07 mol) in anhydrous dichloromethane (150 mL) was added triflic anhydride (15 mL, 0.089 mol) over 5 min. The solution was stirred under a drying tube from 0 °C to room temperature overnight during which time a white precipitate formed. The solvent was removed, and the remaining residue was combined with pentane (300 mL). The insoluble precipitate was filtered off, and the filtrate was extracted with cold 1 N HCl (2 × 150 mL) and saturated NaCl solution and then dried (K_2CO_3). The drying mixture was filtered, and the filtrate was reduced to a yellow liquid (9.61 g). This was distilled and provided chromatographically (GC) pure **35** as a clear liquid (5.96 g, 54%): bp 66–68 °C (20 mmHg); IR (cm^{-1}) 2968 (m), 1656 (m), 1470 (m), 1423 (s), 1252 (s), 1219 (s), 1144 (s), 1066 (s), 918 (m), 897 (m), 862 (m), 603 (m); ^1H NMR ($CDCl_3$) δ 5.54 (t, J = 2.6, 1H, $HC=CR_2$), 2.39–2.32 (m, 2H, $C=CCH_2$), 1.87–1.82 (m, 2H, $(CH_3)_2CCH_2$), 1.15 (s, 6H, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 155.8, 121.0, 116.6, 112.7, 42.9, 37.0, 25.4, 25.0. Anal. ($C_8H_{11}O_3SF_3$) C,H,S,F.

Methyl 5,5-Dimethyl-1-cyclopentene-1-carboxylate (36). A mixture of 5,5-dimethyl-1-cyclopentenyl trifluoromethanesulfonate (**35**, 2.08 g, 8.5 mmol), triethylamine (2.3 mL, 16 mmol), palladium acetate (55 mg, 0.24 mmol), triphenylphosphine (142 mg, 0.5 mmol), methanol (15 mL, 0.37 mol), and DMF (32 mL) was purged with carbon monoxide for 8 min and then stirred under a CO balloon at room temperature overnight. The reaction mixture changed color from brown/orange to red. Ether was added to the reaction, and it became dark brown. The ether mixture was extracted with water (3 × 100 mL) and saturated NaCl solution (50 mL) and then dried ($MgSO_4$). The drying mixture was filtered and reduced to a brown liquid (1.42 g). This was placed on a silica column (112 g of silica packed with hexane), and the column was eluted with mixtures of CH_2Cl_2 in hexane (5%, 500 mL; 10%, 1 L; 20%, 1 L). The product **36** was isolated as a clear liquid (796 mg, 60%): IR (cm^{-1}) 2953 (s), 1718 (vs), 1620 (m), 1457 (m), 1436 (s), 1382 (m), 1361 (m), 1343 (m), 1303 (m), 1262 (s), 1224 (m), 1190 (m), 1173 (m), 1067 (s), 764 (m); ^1H NMR ($CDCl_3$) δ 6.70 (t, J = 2.6, 1H, $HC=CR_2$), 3.72 (s, 3H, OCH_3), 2.39 (apparent dt, J = 7.2, J = 2.7, 2H, $C=CCH_2$), 1.82–1.77 (apparent t, J = 7.2, 2H, $(CH_3)_2CCH_2$), 1.23 (s, 6H, $C(CH_3)_2$). Anal. ($C_9H_{15}O_2$) C,H.

Methyl 2,2-Dimethylcyclopentanecarboxylate (23). A mixture of methyl 5,5-dimethyl-1-cyclopentene-1-carboxylate (**36**, 506 mg, 3.26 mmol), platinum(IV) oxide (28 mg, 0.12 mmol), and ethyl acetate (25 mL) was shaken in an H_2 atmosphere (20 lb/in^2) for 45 min. The PtO_2 was filtered off and washed with EtOAc. The filtrate and washings were combined and reduced to give pure **23** (387 mg, 76%) as a clear liquid having spectroscopic properties identical to those reported above when this compound was prepared by hydrolysis of compound **30**.

General Procedure To Make 1-(2-Propenyl)cyclopentanecarboxylic Acid Esters 20,[30] 22, 24, and 26. To a −78 °C solution of lithium diisopropylamide in THF (either purchased or made from diisopropylamine and butyllithium) under N_2 was added the cyclopentanecarboxylic acid ester (**19**, **21**, **23**, or **25**) dissolved in anhydrous THF. The solution was allowed to warm to −50 or 0 °C and then recooled to −78 °C. Allyl bromide was then added and the reaction solution stirred from −78 °C to room temperature overnight. The reaction was quenched with 5–10% HCl and the mixture diluted with ether. The phases were partitioned, and the organic layer was extracted with saturated $NaHCO_3$ solution. The $NaHCO_3$ fractions were extracted again with ether. All the organic layers were combined, extracted with saturated NaCl solution, and dried ($MgSO_4$). The products were isolated as yellow/brown liquids and purified by distillation or column chromatography.

Methyl 1-(2-Propenyl)-2-methylcyclopentanecarboxylate (22). Lithium diisopropylamide/tetrahydrofuran solution (12 mL of a 1.5 M LDA/THF solution, 18 mmol) and methyl 2-methylcyclopentanecarboxylate (**21a**, 1.93 g, 13.6 mmol) in THF (20 mL). The solution was allowed to warm to about −50 °C and then recooled to −78 °C. Allyl bromide (2.4 mL, 27.7 mmol); quenched with 5% HCl solution (30 mL); ether (125 mL) and saturated $NaHCO_3$ solution (3 × 30 mL); ether (50 mL). Upon filtration of the drying mixture and evaporation of the filtrate, a light brown liquid was obtained. This liquid was purified on a silica gel column (98 g), eluting with mixtures of CH_2Cl_2 in hexane (10%, 1 L; 20%, 2 L). Product **22** was obtained as a clear liquid (93% pure, 1.6 g, 60%). A small portion was further chromatographed to give an analytical sample: IR (cm^{-1}) 2957 (s), 2928 (s), 2873 (m), 1729 (s), 1641 (w), 1457 (m), 1200 (m, br), 1152 (m), 915 (w); ^1H NMR ($CDCl_3$) δ 5.80–5.66 (m, 1H, CH=CH_2), 5.09–5.01 (m, 2H, CH=CH_2), 3.66 (s, 3H, OCH_3), 2.68–2.61 (m, 1H, CH_2=$CHCH_2$), 2.07–2.00 (m, 1H, CH_2=$CHCH_2$), 2.19–2.14 (m, 1H), 1.91–1.75 (m, 2H), 1.63–1.55 (m, 2H), 1.44–1.24 (m, 2H), 0.99 (d, J = 6.8, 0.3H, $CHCH_3$), 0.89 (d, J = 6.6, 2.7H, $CHCH_3$). Anal. ($C_{11}H_{18}O_2$) C,H.

Methyl 1-(2-Propenyl)-2,2-dimethylcyclopentanecarboxylate (24). Methyl 2,2-dimethylcyclopentanecarboxylate (**23**, 976 mg, 6.2 mmol) in anhydrous THF (5 mL) and lithium diisopropylamide in cyclohexane (6 mL of a 1.5 M LDA/THF complex in cyclohexane, 9 mmol). The solution was stirred under N_2 at −78 to −10 °C and then recooled to −78 °C. Allyl bromide (1.2 mL, 13.9 mmol); quenched with 5% HCl (10 mL) and then diluted with ether (50 mL); saturated $NaHCO_3$ solution (3 × 20 mL); ether (100 mL). After filtration and evaporation of the filtrate, a light brown liquid remained (1.3 g). This was purified on a silica column (66 g of silica), and the column was eluted with mixtures of CH_2Cl_2 in hexane (10%, 500 mL; 20%, 1 L). The product was isolated as a light brown liquid (848 mg) and purified further by short-path distillation to give pure product **24** (712 mg, 58%): bp 70 °C (11 mmHg); IR (cm^{-1}) 3077 (w), 2962 (s), 2875 (m), 1729 (s), 1640 (w), 1469 (m), 1436 (m), 1387 (w), 1369 (w), 1323 (w), 1200 (s), 1135 (s), 915 (m); ^1H NMR ($CDCl_3$) δ 5.69–5.55 (m, 1H, CH=CH_2), 5.07–5.01 (m, 2H, CH=CH_2), 3.66 (s, 3H, OCH_3), 2.68–2.61 (m, 1H, CH_2=$CHCH_2$), 1.98–1.91 (m, 1H, CH_2=$CHCH_2$), 2.33–2.25 (m, 1H), 1.76–1.51 (m, 5H), 1.07 (s, 3H, CH_3), 0.87 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 176.5, 135.6, 117.7, 59.0, 51.1, 44.6, 39.8, 37.6, 30.1, 26.0, 23.5, 19.6. Anal. ($C_{12}H_{20}O_2$) C,H.

Ethyl 1-(2-Propenyl)-2,5-dimethylcyclopentanecarboxylate (26). Diisopropylamine (14.0 mL, 0.1 mol), anhydrous THF (25 mL), and butyllithium (40 mL of a 2.5 M solution in hexanes, 0.1 mol); ethyl 2,5-dimethylcyclopentanecarboxylate (**25**, 73% pure, 14.3 g, 0.062 mol) in dry THF (5 mL); allyl bromide (9.1 mL, 0.1 mol); 1 N HCl (200 mL); ether (600 mL) and saturated $NaHCO_3$ solution (2 × 110 mL); ether (100 mL). The drying mixture was filtered, and the filtrate was reduced to a dark brown liquid. This was distilled to provide pure **26** (9.49 g, 73%): bp 64–70 °C (5 mmHg); IR (cm^{-1}) 3077 (w), 2959 (s), 2875 (s), 1724 (s), 1639 (w), 1462 (m), 1380 (m), 1225 (s), 1193 (s), 1129 (m), 1037 (m), 913 (m); ^1H NMR ($CDCl_3$) δ 5.98–5.80 (m, 1H, CH=CH_2), 5.10–5.00 (m, 2H, CH=CH_2), 4.19–4.10 (m, 2H, OCH_2CH_3), 2.61–2.55 (m, 1H), 2.43–2.25 (m, 2H), 2.07–1.79 (m, 3H), 1.64–1.58 (m, 1H), 1.32–1.21 (m, 4H, OCH_2CH_3 and CH), 0.97 (d, J = 7.1, 3H, CH_3), 0.91 (d, J = 6.8, 3H, CH_3). Anal. ($C_{13}H_{22}O_2$) C,H.

The isomers were separated on a silica column for an analytical sample and identified by NMR. **Isomer 1**: ^1H NMR ($CDCl_3$) δ 5.85 (m, 1H, CH=CH_2), 5.07 (m, 2H, CH=CH_2), 4.15 (q, J = 7.1, 2H, OCH_2CH_3), 2.41 (d, J = 7.5, 2H, CH_2CH=CH_2), 1.95 (m, 2H), 1.80 (m, 2H), 1.60 (m, 2H), 1.28 (t, 3H, OCH_2CH_3), 0.98 (m, 1H), 0.91 (d, J = 8.0, 6H, 2 × CH_3). **Isomer 2**: ^1H NMR ($CDCl_3$) δ 5.90 (m, 1H, CH=CH_2), 5.02 (m, 2H, CH=CH_2), 4.14 (q, J = 7.1, 2H, OCH_2CH_3), 2.59 (m, 1H), 2.28 (m, 2H, CH_2CH=CH_2), 2.01 (m, 2H), 1.83 (m, 1H), 1.26 (t, J = 7.1, 3H, OCH_2CH_3, overlapping m, 2H), 0.97 (d, J = 7.0, 3H, CH_3), 0.91 (d, J = 7.0, 3H, CH_3).

General Procedure To Make Spirocyclopentanes 2,[31] 4, 7, 9, and 10. A solution of the 1-(2-propenyl)cyclopentanecarboxylate ester (**20**, **22**, **24**, or **26**) in $CHCl_3$ was cooled in a dry ice/CCl_4 bath. Ozone was bubbled in until a blue color was maintained. Oxygen was then bubbled in until the blue color disappeared. A cloudy solution of $NaBH_4$ in absolute ethanol was then dripped into the cool $CHCl_3$ solution and the reaction stirred at −22 °C to room temperature overnight (for compounds **9** and **10**, the $CHCl_3$ was removed and the ethanolic mixture was heated to 75–80 °C for 3 h after stirring overnight at room temperature). The reaction mixture was recooled to −22 °C and the reaction quenched with 10% HCl. The volatile solvents were removed, and the remaining aqueous solution was extracted with ether. The organic portions were combined, extracted with saturated $NaHCO_3$ solution and/or saturated NaCl solution, and dried ($MgSO_4$). Chromatographically (GC) pure products were obtained via distillation or chromatography.

(5α,6α)-6-Methyl-2-oxaspiro[4.4]nonan-1-one (4). Methyl 1-(2-propenyl)-2-methylcyclopentanecarboxylate (**22**, 559 mg, 3.07 mmol) and $NaBH_4$ (210 mg, 5.55 mmol) in absolute ethanol (15 mL); 10% HCl solution (10 mL); ether (3 × 75 mL) and saturated NaCl solution (50 mL). Upon filtration and evaporation of the filtrate, a clear liquid was obtained. This was distilled and provided a mixture of the diastereomers **4** and **5** (259 mg, 55%, 4:5 ratio ≃ 95:5): bp 100–115 °C (5 mmHg); IR (cm^{-1}) 2959 (s), 2874 (m), 1764 (s), 1457 (m), 1373 (m), 1217 (m), 1175 (s), 1125 (m), 1030 (s); ^1H NMR ($CDCl_3$) δ 4.28–4.18 (m, 2H, CH_2, H-3), 2.24–2.14 (m, 3H), 2.04–1.84 (m, 3H), 1.72–1.62 (m, 3H), 1.05 (d, J = 6.9, 2.9H, CH_3), 0.97 (d, J = 7.0, 0.1H, CH_3). Anal. ($C_9H_{14}O_2$) C,H.

HPLC in 10% EtOAc in hexane was used to separate the isomers. The major isomer, **4**, was characterized by NMR: ^1H NMR ($CDCl_3$) δ 4.28–4.21 (m, 2H, CH_2, H-3), 2.24–2.14 (m, 3H), 2.04–1.84 (m, 3H), 1.71–1.59 (m, 3H), 1.05 (d, J = 7.0, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 181.0, 65.4, 51.8, 43.7, 36.5, 36.0, 33.4, 22.9, 15.4.

6,6-Dimethyl-2-oxaspiro[4.4]nonan-1-one (7). Methyl 1-(2-propenyl)-2,2-dimethylcyclopentanecarboxylate (**24**, 1.94 g, 9.2 mmol) in $CHCl_3$ (30 mL); $NaBH_4$ (0.90 g, 24 mmol) in absolute ethanol (60 mL); 10% HCl solution (30 mL) and ether (3 × 125 mL). The dried organic layer was filtered and reduced to a liquid/white solid mixture (2.68 g). The mixture was placed on a silica column (204 g of silica), and the column was eluted with mixtures of EtOAc in hexane (10%, 1 L; 20%, 1 L). The product **7** was isolated as a clear liquid (1.01 g, 98% pure, 64% yield): IR (cm^{-1}) 2963 (s), 2872 (m), 1764 (s), 1465 (m), 1370 (m), 1170 (s), 1111 (m), 1035 (s); ^1H NMR ($CDCl_3$) δ 4.25–4.14 (m, 2H, OCH_2), 2.40–2.30 (m, 1H), 2.20–2.09 (m, 2H), 2.04–1.95 (m, 1H), 1.90–1.72 (m, 3H), 1.54–1.48 (m, 1H), 1.06 (s, 3H, CH_3), 0.96 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 181.0, 65.2, 54.7, 43.6, 38.8, 35.1, 31.2, 25.0, 22.8, 20.1. Anal. ($C_{10}H_{16}O_2$) C,H.

6,9-Dimethyl-2-oxaspiro[4.4]nonan-1-ones 9 and 10. Ethyl 1-(2-propenyl)-2,5-dimethylcyclopentanecarboxylate (**26**, 7.8 g, 0.037 mol) in $CHCl_3$ (120 mL) and $NaBH_4$ (3.9 g, 0.103 mol) in ethanol (240 mL). More $NaBH_4$ (3.5 g, 0.092 mol) in absolute ethanol was added after overnight stirring and the solution stirred at room temperature for 48 more hours. An aldehyde peak was still present in the IR; therefore, the reaction was heated to 75–80 °C for 3 h, and at this time, the aldehyde peak disappeared. The reaction was quenched with water (50 mL) and the mixture refluxed for 1 h. Upon cooling, 1 N HCl (200 mL) was added and the organic solvents were evaporated. The acidic aqueous solution was extracted with CH_2Cl_2 (3 × 200 mL). The organic layers were combined, extracted with saturated $NaHCO_3$ solution (2 × 150 mL) and saturated NaCl (200 mL), and dried ($MgSO_4$). After filtration and evaporation of the drying mixture, a yellow liquid was obtained (5.14 g). Isomers **9** and **10** were separated via normal phase preparative HPLC using 8% EtOAc in hexane. Isomer **10** was isolated as a white solid (544 mg, mp 52–54 °C), and it was purified by sublimation (40 °C, 1.5 mmHg) to provide pure **10** as a white gummy solid (501 mg). Isomer **9** was isolated as a clear liquid (1.44 g), and it was purified by short path distillation (bp 85–90 °C, 2.5 mmHg) to provide authentic **9** (1.38 g). Total yield of pure **9** and **10**: 32%.

Compound 9: IR (cm^{-1}) 2961 (m), 2875 (w), 1764 (s), 1459 (w), 1373 (w), 1219 (w), 1177 (m), 1032 (m); ^1H NMR ($CDCl_3$) δ 4.32–4.16 (m, 2H, OCH_2), 2.48–2.31 (m, 2H, CH_2, H-4), 2.15–1.81 (m, 4H), 1.52–1.39 (m, 1H), 1.30–1.20 (m, 1H), 1.03 (d, J = 7.0, 3H, CH_3), 0.96 (d, J = 7.0, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 180.9, 65.3, 55.0, 42.8, 38.9, 32.6, 32.3, 30.8, 17.2, 16.2. Anal. ($C_{10}H_{16}O_2$) C,H.

Compound 10: IR (cm^{-1}) 2956 (s), 2872 (m), 1756 (s), 1461 (w), 1376 (m), 1190 (m), 1138 (s), 1031 (m); ^1H NMR ($CDCl_3$) δ

4.24 (t, J = 7.7, 2H, OCH_2), 2.24 (t, J = 7.7, 2H, CH_2, H-4), 2.06–2.00 (m, 2H), 1.90–1.70 (m, 4H), 1.05 (d, J = 6.8, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$) δ 65.5, 56.3, 44.6, 31.7, 30.2, 14.7 (no C=O observed). Anal. ($C_{10}H_{16}O_2$) C,H.

5-Oxaspiro[2.4]heptan-4-one (13).[32] α-Methylene-γ-butyrolactone (**37**, 3.1 g, 30.6 mmol), palladium acetate (45 mg, 0.2 mmol), and ether (50 mL) were combined, and the suspension was cooled in an ice bath. A cool solution of diazomethane in ether (260 mL of a 0.32 M solution) was dripped into the cool suspension over 20 min. The resulting brown suspension was stirred at 0 °C to room temperature over 3 h. The excess diazomethane was allowed to evaporate, and then, the suspension was filtered. The filtrate was reduced to a brown liquid (4.01 g). This liquid was placed on a silica column (224 g of silica packed with hexane), and the column was eluted with mixtures of EtOAc in hexane (5%, 1 L; 10%, 1 L; 15%, 1 L; 20%, 1 L; 25%, 1 L). The product **13** was isolated as a clear liquid (1.27 g) and also as a mixture with **37** (607 mg of 70% pure **13**). Total yield: 1.69 g, 49%. **Pure product 13**: bp 75–85 °C (2.5 mmHg); IR (cm^{-1}) 2964 (m), 2917 (m), 1762 (s), 1382 (m), 1296 (m), 1261 (m), 1110 (s), 1021 (s), 800 (m); ^1H NMR ($CDCl_3$) δ 4.45 (t, J = 7.5, 2H, OCH_2), 2.34 (t, J = 7.5, 2H, CH_2, H-7), 1.27 (dd, J = 4.3, J = 7.1, 2H, CH, H-1, CH *cis* to the carbonyl, H-2), 1.00 (dd, J = 4.2, J = 7.2, 2H, CH, H-1, CH *trans* to the carbonyl, H-2); ^{13}C NMR ($CDCl_3$) δ 180.6, 65.6, 29.4, 19.4, 14.8. Anal. ($C_6H_8O_2$) C,H.

(*Z*)- and (*E*)-2-Ethylidene-γ-butyrolactone (39 and 40). To a suspension of sodium hydride (3.37 g, 0.140 mol) in anhydrous benzene (75 mL) was slowly added a solution of α-(diethylphosphono)-γ-butyrolactone (**38**, 25.0 g, 0.113 mol) in anhydrous benzene (175 mL). The resulting solution was heated to 50–60 °C for 3 h, and over time, the clear solution became orange. The solution was cooled to room temperature, and a solution of acetaldehyde (12 mL, 0.215 mol) in anhydrous benzene (50 mL) was added. The solution was refluxed under N_2 overnight. The reaction was cooled, and 1 N HCl (500 mL) was added. The layers were separated, and the aqueous layer was extracted with ether (3 × 200 mL). The benzene and ether layers were combined, extracted with water (200 mL) and saturated $NaHCO_3$ solution (200 mL), and dried ($MgSO_4$). The drying mixture was filtered and reduced to a red liquid (9.8 g). Purification of this material by distillation did not separate the isomers; therefore, the mixture of isomers obtained from the distillation (6.85 g) was placed on a silica column (325 g of silica packed with hexanes). The column was eluted with mixtures of EtOAc in hexane (3%, 1 L; 8%, 1 L; 10%, 2 L; 20%, 2 L; 40%, 1 L; 50%, 1 L). Isomers **39** (2.22 g) and **40** (4.13 g) were isolated as clear liquids. Further purification of the isomers by short path distillation provided pure samples of **39** (2.17 g) and **40** (4.08 g). Total yield of both isomers: 6.25 g, 49%.

Compound 39: bp 85–105 °C (3.5–4 mmHg); IR (cm^{-1}) 2983 (w), 2917 (m), 2859 (w), 1751 (s), 1674 (m), 1486 (w), 1444 (m), 1373 (m), 1350 (w), 1212 (s), 1120 (s), 1026 (s), 958 (m), 860 (m); ^1H NMR ($CDCl_3$) δ 6.34 (m, 1H, R_2C=CHR), 4.32 (t, J = 7.4, 2H, OCH_2), 2.92 (m, 2H), 2.17 (dt, J = 7.2, J = 2.4, 3H, R_2C=CHCH_3); ^{13}C NMR ($CDCl_3$) δ 170.6, 138.9, 124.4, 65.2, 28.8, 13.6. Anal. ($C_6H_8O_2$) C,H.

Compound 40: bp 96–118 °C (3.5 mmHg); IR (cm^{-1}) 2985 (w), 2918 (m), 1757 (s), 1682 (s), 1486 (w), 1440 (m), 1376 (m), 1336 (m), 1217 (s), 1132 (m), 1034 (s), 1010 (s), 987 (m), 714 (m); ^1H NMR ($CDCl_3$) δ 6.77 (m, 1H, R_2C=CHR), 4.37 (t, J = 7.5, 2H, OCH_2), 2.87 (m, 2H), 1.85 (dt, J = 7.1, J = 2.1, 3H, R_2C=CHCH_3); ^{13}C NMR ($CDCl_3$) δ 171.4, 135.7, 126.4, 65.2, 24.5, 15.3. Anal. ($C_6H_8O_2$) C,H.

(4α,5α)-4-Methyl-6-oxo-7-oxa-1,2-diazaspiro[4.4]non-1-ene (41). (*Z*)-2-Ethylidene-γ-butyrolactone (**39**, 487 mg, 4.3 mmol) was dissolved in ether (10 mL) and cooled in an ice bath. Diazomethane in ether (50 mL of a 0.32 M solution) was added, and the flask was sealed, warmed to room temperature, and allowed to stir for 72 h. The flask was again cooled in an ice bath and opened to allow the excess diazomethane to evaporate. The reaction solution was then reduced to a yellow liquid (661 mg). This material was placed on a silica column (40 g of silica packed with hexane), and the column was eluted with mixtures of EtOAc in hexane (10%, 500 mL; 15%, 500 mL; 25%, 500 mL; 40%, 500 mL). The product was isolated as a clear liquid (545 mg, 81%): IR (cm^{-1}) 2969 (m), 2922 (m), 1768 (s), 1548 (w), 1377 (m), 1219 (m), 1179 (s), 1148 (m), 1024 (s), 980 (m); ^1H NMR ($CDCl_3$) δ 4.91 (dd, J = 17.2, J = 8.2, 1H, CH, H-3), 4.17 (dd, J = 17.2, J = 9.6, 1H, CH, H-3), 4.77–4.70 (m, 1H, CH, H-8), 4.51–4.43 (m, 1H, CH, H-8), 3.18–3.09 (m, 1H, CH, H-9), 2.40–2.31 (m, 1H, CH, H-9), 2.19–2.10 (m, 1H, CH, H-4), 1.19 (d, J = 7.0, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 171.3, 94.4, 82.9, 66.3, 36.3, 32.4, 11.6. Anal. ($C_7H_{10}N_2O_2$) C,H,N.

(4α,5β)-4-Methyl-6-oxo-7-oxa-1,2-diazaspiro[4.4]non-1-ene (42). (*E*)-2-Ethylidene-γ-butyrolactone (**40**, 563 mg, 4.9 mmol) was dissolved in ether and cooled in an ice bath. A cool solution of diazomethane in ether (50 mL of a 0.32 M solution) was added, the flask was cooled, and the reaction was allowed to warm to room temperature and stir for 96 h. The excess diazomethane was allowed to evaporate, and the reaction solution was reduced to a light yellow liquid (809 mg). This was placed on a silica column (35 g of silica packed with hexane), and the column was eluted with mixtures of EtOAc in hexane (5%, 500 mL; 10%, 500 mL; 25%, 500 mL; 30%, 500 mL). Product **42** was isolated as a clear liquid (732 mg, 88%): IR (cm^{-1}) 2973 (m), 2923 (m), 2879 (w), 1772 (s), 1554 (w), 1431 (m), 1377 (m), 1221 (s), 1181 (s), 1025 (s), 959 (m); ^1H NMR ($CDCl_3$) δ 4.93–4.78 (m, 2H, CH, H-3 and H-8), 4.66–4.59 (m, 1H, CH, H-8), 4.49 (dd, J = 17.7, J = 3.4, 1H, CH, H-3), 2.59–2.48 (m, 3H, CH, H-9 and H-4), 0.87 (d, J = 7.1, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 173.8, 96.6, 85.8, 67.1, 30.7, 28.0, 15.2. Anal. ($C_7H_{10}N_2O_2$) C,H,N.

(1α,3α)-1-Methyl-5-oxaspiro[2.4]heptan-4-one (15).[33] (4α,5α)-4-Methyl-6-oxo-7-oxa-1,2-diazaspiro[4.4]non-1-ene (**41**, 1.1 g, 7.1 mmol) was dissolved in acetonitrile (900 mL). The solution was irradiated by a Hanovia 450-W lamp for 20 min while the reaction flask was bathed in a cool water bath. The reaction solution was reduced to a yellow liquid (928 mg). This liquid was placed on a silica column (60 g of silica packed with hexane), and the column was eluted with mixtures of EtOAc in hexane (10%, 1 L; 20%, 1 L). The product mixture was isolated as a yellow liquid (608 mg). A small amount of this was purified by normal phase semipreparative HPLC using 10% EtOAc in hexane as the eluent. Four peaks were isolated and characterized by NMR and IR. The product ratio, based on HPLC and GC, is about 40:4:13:4 (for peaks 1:2:3:4). Peak 1 (i.e., first peak eluted) was the desired product **15** (see data below). Peak 2 was α-(2-isopropenyl)-γ-butyrolactone. Peak 3 was compound **16**. Peak 4 was α-ethylene-δ-lactone.

Since peaks 2 and 4 were not desired materials, they were eliminated by ozonolysis as follows: The rest of the yellow liquid (560 mg) was dissolved in dichloromethane (40 mL) and cooled in a dry ice/isopropyl alcohol bath. Ozone was bubbled in until a blue color was maintained. Oxygen was then bubbled in until the solution became clear again. Dimethyl sulfide (2 mL) was added to the clear solution, and after 30 min, the solvents were removed. The remaining clear liquid was placed on a silica column (20 g of silica packed with hexane), and the column was eluted with mixtures of EtOAc in hexane (10%, 1 L; 20%, 500 mL). The product was isolated as a clear liquid which was a mixture of **15** and **16**. This mixture was purified on semipreparative HPLC using 10% EtOAc in hexane as the eluent. The first peak to elute was the desired product **15** and was obtained as a clear liquid (208 mg). This was purified further by short-path distillation to provide pure **15** (178 mg, 20%): bp 60–70 °C (0.3 mmHg); IR (cm^{-1}) 2935 (w), 1759 (s), 1457 (w), 1441 (w), 1378 (m), 1116 (s), 1025 (m); ^1H NMR ($CDCl_3$) δ 4.47–4.31 (m, 2H, OCH_2), 2.49–2.39 (m, 1H, CH, H-7), 2.14–2.06 (m, 1H, CH, H-7), 1.43–1.34 (m, 1H), 1.19–1.10 (m, 2H), 1.26 (d, J = 5.9, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 178.6, 65.6, 31.5, 24.6, 23.9, 20.9, 12.1. Anal. ($C_7H_{10}O_2$) C,H.

The second peak, isomer **16**, was also obtained as a clear liquid (172 mg). This was purified by short-path distillation to furnish pure **16** (159 mg, 18%).

(1α,3β)-1-Methyl-5-oxaspiro[2.4]heptan-4-one (16).[33] (4α,5β)-4-Methyl-6-oxo-7-oxa-1,2-diazaspiro[4.4]non-1-ene (**42**, 1.92 g, 12.5 mmol) was dissolved in acetonitrile (900 mL) and cooled in a cold water bath. The solution was irradiated by a Hanovia 450-W lamp for 30 min. The solvent was evaporated, and a yellow liquid remained (1.58 g). This was placed on a silica column (164 g of silica packed with hexane), and the column was eluted with mixtures of EtOAc in hexane (10%, 1 L; 15%, 1 L; 20%, 1 L; 25% 1 L). The crude product was isolated as a yellow liquid (1.14 g). A portion was purified by semipreparative normal phase HPLC in 10% EtOAc in hexane, and the same four peaks

were observed as with the irradiation of 41 in a ratio of 5:10:75:10 for peaks 1:2:3:4 (see procedure above for spectroscopic data). The rest of the liquid was dissolved in dichloromethane and ozonized as described above. The product of ozonolysis was purified on a silica column and then by HPLC. Product 16 was isolated as a clear liquid (563 mg). This was purified by short-path distillation to furnish pure 16 (478 mg, 30%): bp 65–75 °C (0.3 mmHg); IR (cm^{-1}) 2963 (w), 1765 (s), 1457 (w), 1397 (m), 1379 (m), 1288 (m), 1222 (m), 1130 (s), 1103 (m), 1027 (s); ^1H NMR ($CDCl_3$) δ 4.48–4.40 (m, 2H, OCH_2), 2.40–2.30 (m, 1H, CH, H-7), 2.20–2.11 (m, 1H, CH, H-7), 1.56–1.40 (m, 2H), 1.13 (d, J = 6.0, 3H, CH_3), 0.63–0.59 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 180.9, 65.9, 25.1, 23.8, 22.2, 19.9, 14.3. Anal. ($C_7H_{10}O_2$) C,H.

Isomer 15 was also isolated from HPLC as a clear liquid (36 mg, 2%).

1,1-Dimethyl-4-oxo-5-oxaspiro[2.4]heptane (18). This compound was prepared according to a literature method.[15]

α-*tert*-Butyl-α-ethyl-γ-butyrolactone (6). To a solution of diisopropylamine (5.5 mL, 0.039 mol) in THF (30 mL) cooled in a dry ice/isopropyl alcohol bath was added butyllithium (16 mL of a 2.5 M solution in hexanes, 0.04 mol). After 5 min, a solution of α-*tert*-butyl-γ-butyrolactone[8] (5.0 g, 0.035 mol) in THF (25 mL) was added and the resulting solution stirred for 35 min. Ethyl iodide (3.1 mL, 0.039 mol) was added and the solution stirred from −78 °C to room temperature overnight. The reaction was quenched with 10% HCl (50 mL) and water (25 mL). The layers were separated, and the aqueous layer was extracted with ether (3 × 100 mL). The organic layers were combined, extracted with saturated $NaHCO_3$ solution (100 mL), and dried ($MgSO_4$). The dried organic layer was filtered and the filtrate reduced to a brown liquid. This liquid was dissolved in ether (100 mL) and extracted with saturated sodium thiosulfate solution (2 × 60 mL) and saturated NaCl solution (2 × 50 mL). The organic fraction was reduced to a light brown liquid (5.87 g). This was distilled to provide 93% of the pure product as a gummy white solid [3.45 g, bp 76–77 °C (0.6–0.7 mmHg)]. This solid was further purified on a silica column (168 g of silica packed with hexane) eluting with 0.5–5.0% EtOAc in hexane. Pure 6 was isolated as a white solid (3.23 g, 54%): mp 51 °C; ^1H NMR ($CDCl_3$) δ 4.22–4.14 (m, 2H, OCH_2), 2.41–2.30 (m, 1H), 2.16–2.05 (m, 1H), 1.98–1.87 (m, 1H, CH_2CH_3), 1.56–1.40 (m, 1H, CH_2CH_3), 1.04 (s, 9H, $C(CH_3)_3$), 0.94 (t, J = 7.4, 3H, CH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 180.5, 65.1, 53.0, 36.2, 28.2, 25.7, 9.6. Anal. ($C_{10}H_{18}O_2$) C,H.

Pharmacology. Materials. Picrotoxinin, pentylenetetrazole (PTZ), and polyethyleneglycol-400 (PEG) were purchased from Sigma Chemical Co. (St. Louis, MO); ^{35}[S]-labeled TBPS was purchased from New England Nuclear (Boston, MA) and unlabeled TBPS was purchased from Research Biochemicals (Natick, MA).

Neurological Effects. Female CF-1 strain mice (Harlan, 6–8 weeks old) were maintained on a 12:12 light–dark cycle with food and water available *ad libitum*. Drug screening was accomplished by methods based on those of Swinyard and Woodhead.[34] Test compounds were dissolved in 30% PEG and given by intraperitoneal (ip) injections (5–10 mice/dose) in a volume of 0.01 mL/g of body weight. Following the injection of the test compound, the mice were observed for 30 min and any seizure activity was noted. Compounds that did not induce clonic seizures were tested for anticonvulsant activity by examining their ability to block seizures caused by PTZ. PTZ (85 mg/kg) was administered as a 0.85% solution in 0.9% NaCl, and the mice were observed for 30 min for seizure activity. Protection was defined as the absence of clonic seizures.

Neurotoxic effects were assessed using the rotorod toxicity test.[35] In this test, the mouse was placed on a 1-in. diameter rod rotating at 6 rpm. The chemical was considered toxic if the mouse fell from the rotating rod twice during the 10-min testing period.

The median effective doses (ED_{50}s), median toxic doses (TD_{50}s), and median convulsive doses (CD_{50}s) were determined by $\log_{10}$ probit analysis of the dose–response data.[36] The CD_{50} was determined from the number of animals which had clonic seizures following the administration of the test compound.

^{35}[S]-TBPS Binding. ^{35}[S]-TBPS binding was performed according to previously described methods.[37] The cerebral cortex from female Sprague–Dawley rats (250–300 g) was removed over ice immediately following decapitation. The brains were homogenized in 20 mL of ice-cold 0.32 M sucrose/g of tissue and centrifuged at 1000g for 10 min. The supernatant was carefully decanted and centrifuged at 150 000g for 30 min. The resulting pellet was resuspended in 20 volumes of ice-cold deionized water and centrifuged at 150 000g for 30 min. The pellet was resuspended in 20 volumes of 50 mM Tris–citrate buffer (pH 7.5) and centrifuged at 50 000g for 30 min. The resulting pellet was resuspended in 20 volumes of 50 mM Tris–citrate buffer. The membrane suspensions were stored at −70 °C and thawed and resuspended in 50 mM Tris–citrate buffer immediately prior to use. The protein concentration was determined according to the Lowry method.[38]

For binding assays, 100 μL of rat brain membranes was added to a solution containing 50 μL of ^{35}[S]-TBPS (specific activity 60–85 Ci/mmol) in 1 M NaBr and 50 μL of the test compound dissolved in 50 mM Tris–citrate buffer. For the inhibition experiments, the final ^{35}[S]-TBPS concentration was 2 nM, and for saturation studies, the ^{35}[S]-TBPS concentrations ranged from 1 to 300 nM. The samples were incubated in triplicate for 90 min at 25 °C, diluted with 3 mL of 0.9% NaCl, rapidly filtered through Whatman GF/B filters, and washed twice with 3 mL of 0.9% NaCl. Filter-bound radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined as that observed in the presence of 10 μM unlabeled TBPS or 100 μM unlabeled picrotoxinin.

The determination of the drug concentration that displaced 50% of specifically bound ^{35}[S]-TBPS (IC_{50}) and the curve fitting of sigmoidal concentration–displacement curves were accomplished by logistic analysis using at least five different drug concentrations. Binding constants were determined by Scatchard analysis of the saturation data.

Electrophysiology. Hippocampal cells were cultured from 1-day-old female Sprague–Dawley rat pups by previously described methods.[39] Electrophysiological experiments were carried out on the stage of an inverted microscope (Leitz) using whole-cell patch clamp techniques on cells that had been in culture for 10–21 days. The growth medium was removed, and the cells were placed in an extracellular recording solution which contained 140 mM NaCl, 10 mM NaHEPES (pH 7.3), 3 mM KCl, 4 mM $MgCl_2$, and 5.5 mM glucose. Electrodes (4–10-MΩ resistance) were filled with an intracellular recording solution that contained 130 mM CsCl, 10 mM TEACl, 10 mM NaHEPES (pH 7.3), 2 mM MgATP, 1.1 mM EGTA, and 2mM QX-314. Neurons were voltage-clamped at −30 mV using a standard patch amplifier (DAGAN). Drugs were applied for 2 s at a rate of 30 μL/s from a linear array of six 340-μm i.d. glass tubes positioned within 100 μm of the cell. After the drug applications, the culture dish was perfused at 2 mL/min to prevent accumulation of drug in the extracellular solution. For measurement of modulatory effects of drugs on GABA-mediated currents, control GABA current was obtained followed by GABA + drug and then control GABA. Only data in which control was recovered ±10% were used. The control current amplitude was taken as the mean of the pre- and postdrug responses.

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