N-Substituted 4-Amino-3,3-dipropyl-2(3*H*)-furanones: New Positive Allosteric Modulators of the $GABA_A$ Receptor Sharing Electrophysiological Properties with the Anticonvulsant Loreclezole

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1,4-Addition of benzylamine to 2(5*H*)-furanone followed by dialkylation of the 3-position with allylbromide gave (±)-4-benzyl-3,3-diallyl-2(3*H*)-furanone (**8**), which served as the intermediate for the synthesis of various N-substituted 4-amino-3,3-dipropyl-2(3*H*)-furanones (±)-**9a**−**l**. The compounds were evaluated for their capacity to potentiate or inhibit GABA-evoked currents in *Xenopus laevis* oocytes expressing recombinant $\alpha1\beta2\gamma2$ $GABA_A$ receptors. The benzyl, ethyl, and allyl carbamates ((*R*)-**9a** (100 μM), (±)-**9b** (100 μM), (±)-**9c** (200 μM)) stimulated GABA currents by 279 ± 47%, 426 ± 8%. and 765 ± 61%, respectively, while the phenylcarboxamide (±)-**9f** (200 μM) stimulated currents by 420 ± 33%. Concentration−response studies showed that compound **9c** was approximately twice as potent in stimulating GABA currents as α-EMTBL (**2**), the most potent 3,3-dialkylbutyrolactone known to date. On the other hand, the *N*-sulfonyl analogues were much less active or even inhibited GABA-evoked currents. In vitro radioligand displacement studies on rat brain membranes showed that these compounds did not bind to the benzodiazepine or GABA recognition sites of the $GABA_A$ receptor. However, these compounds generally weakly displaced [^{35}S]-TBPS (approximately 50% displacement at 100 μM), though potencies did not correlate with GABA current potentiation. Results obtained with $\alpha1\beta1$ and mutant $\alpha1\beta2$N265S receptors, which compared to $\alpha1\beta2$ receptors are both much less sensitive to current stimulation produced by the anticonvulsant loreclezole, suggest that at least some of these aminobutyrolactones, (e.g., **9a**, **9c**), and interestingly also α-EMTBL, share stimulatory properties with loreclezole.

Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the central nervous system. GABA interacts with three major receptor classes, the $GABA_A$, $GABA_B$, and $GABA_C$ receptors. Of these three, the first has by far received the most attention, and many compounds interacting with this receptor (e.g., benzodiazepines such as Valium) have found great clinical use. The $GABA_A$ receptor is a pentameric supramolecular complex forming a ligand-gated ion channel controlling neuronal chloride ion flux. The protein subunits comprising the receptor have been classified as α, β, γ, δ, ϵ, π, and θ on the basis of sequence homology.[1−6] Some of these subunits also exist in the form of several isoforms (e.g., $\alpha_1-\alpha_6$, $\beta_1-\beta_3$, etc.), consequently leading to a potentially very large number of $GABA_A$ receptor subtypes. In the mammalian brain, the major subtype has the composition $2\alpha_1 2\beta_2\gamma_2$.[7,8]

Opening and closing of the chloride ion channel, and thus the level of neuroinhibition, can be controlled not only by the interaction of GABA with its particular recognition site (for which specific agonists and antagonists are also known, for example, muscimol and (+)-bicuculline, respectively) but also by compounds interacting at allosteric modulatory sites situated elsewhere on the receptor complex.[9] A number of such sites have been described including that for benzodiazepines, barbiturates, picrotoxin, neurosteroids, and ROD compounds.[10−13] An added feature of some of these binding sites is that, depending on the chemical structure of the ligand, either positive or negative allosteric modulation of GABA-evoked currents (corresponding to stimulation or inhibition of chloride ion flux) can result. Thus, while the clinically useful 1,4-benzodiazepines are positive allosteric modulators of the $GABA_A$ receptor,[14] many compounds belonging to the β-carboline family (e.g., methyl β-carboline-3-carboxylate or β-CCM) negatively modulate the $GABA_A$ receptor by binding to the same benzodiazepine recognition site.[14,15]

The 3,3- and 4,4-dialkylbutyrolactones, thiobutyrolactones, and related compounds, largely developed by Covey and Ferrendelli over the past 2 decades, are another class of compounds that appears to interact with distinct allosteric binding sites on the $GABA_A$ receptor.[16−21] Thus, while the 4,4-dialkyl derivatives (e.g., **1**, Chart 1) were found to generally block GABA-induced chloride currents in neurons and were conse-

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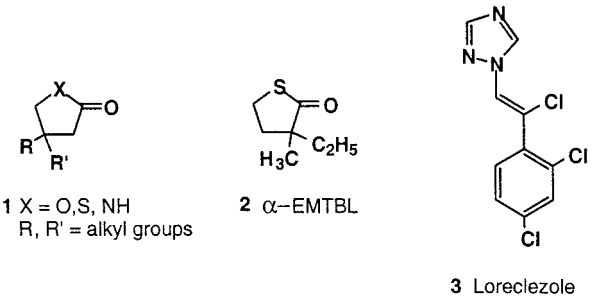
Chart 1



quently convulsant in vivo, the 3,3-dialkyl analogues (best represented by α-EMTBL, **2**)[18,22−24] potentiated currents and were anticonvulsant. Because both classes of compounds displaced [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]-TBPS), a ligand specific for the picrotoxin binding site, in in vitro binding studies, it was at first assumed that the lactones acted via the picrotoxin site.[22] However, subsequent electrophysiological studies, especially with mutant receptors unresponsive to picrotoxin, led to the hypothesis that the positive allosteric modulatory butyrolactones act mainly at a distinct binding site termed the lactone site, while the negative modulatory activity would be predominantly due to interaction with the picrotoxin site.[23,24] Voltage-dependent calcium channels have also been implicated in the convulsant and anticonvulsant properties of these compounds.[25]

Another anticonvulsant agent acting at the $GABA_A$ receptor is loreclezole (**3**) whose activity was also originally hypothesized to be due to an interaction with the benzodiazepine binding site.[26,27] However, the observation that the anticonvulsant action of loreclezole is not inhibited by the benzodiazepine receptor antagonist flumazenil,[28,29] together with various binding and electrophysiological evidence, has pointed to a distinct loreclezole-sensitive allosteric modulatory site. Only β_2 and β_3, but not β_1, containing receptors respond strongly to loreclezole, and a point mutation in β_2 to the amino acid residue present in the homologous position in β_1, that is, β_2N265S, makes the receptor much less responsive to loreclezole.[30−34]

Both the butyrolactone and loreclezole binding sites of the $GABA_A$ receptor thus represent relatively unexplored targets for the development of agents having fewer of the undesired effects of the widely prescribed benzodiazepines (e.g., somnolence, amnesia, dependence, tolerance). In this paper, then, we report that N-substituted 4-amino-3,3-propyl-substituted butyrolactones are potent positive allosteric modulators of the $GABA_A$ receptor that appear to exhibit an action partially similar to that of loreclezole.

Chemistry

The starting material for this study was 4-benzylamino-4,5-dihydro-2(3*H*)-furanone **5**,[35] prepared first in racemic form by conjugate addition of benzylamine to 2(5*H*)-furanone **4** in methanol (Scheme 1).[36,37] Compound (±)-**5** was then transformed into the 3,3-diallyl derivative (±)-**8** by treatment at −78 °C with lithium hexamethyldisilazide in THF followed by addition of excess allyl bromide in HMPA.[37] Minor amounts of the cis and trans monoallyl derivatives ((±)-**6** and (±)-**7**, respectively) were also isolated from the reaction mixture. Hydrogenation of compound (±)-**8** in the presence of palladium catalyst led to simultaneous reduction of the double bonds of the allyl groups and removal of the *N*-benzyl group. The resulting primary amine function was then acylated or sulfonylated using the appropriate acyl or sulfonyl chloride (or Boc anhydride) in the presence of triethylamine and DMAP, affording compounds **9a**−**l** as racemic mixtures (Table 1). Similarly, for comparative purposes, compound (±)-**5** was converted into the *N*-Cbz derivative (±)-**10** using the same hydrogenation−acylation (with benzyl chloroformate) sequence.

The *R* and *S* enantiomers of the *N*-Cbz derivative (±)-**9a** could be separated by HPLC on a chiral OD column. To assign the absolute configuration to the two fractions obtained, (*R*)-**9a** was prepared unambiguously starting from (*R*)-4-*N*-Cbz-aminobutyrolactone (*R*)-**10** (Scheme 2), itself prepared from D-aspartic acid by a published procedure.[38,39] Because C-3 dialkylation of compound (*R*)-**10** was found to be inefficient using the conditions that were successful for the *N*-benzyl analogue (±)-**5**, the *N*-Cbz group of (*R*)-**10** was replaced by an *N*-benzyl group. Thus, hydrogenolytic cleavage of the Cbz group of (*R*)-**10** followed by reductive alkylation of the resulting amine with benzaldehyde provided (*R*)-**5**, which could now be dialkylated at C-3 as before using LiHMDS/allyl bromide to give (*R*)-**8**. Finally, the latter was transformed into the 3,3-dipropyl *N*-Cbz derivative (*R*)-**9a** by hydrogenation and acylation as before. Compound (*R*)-**9a** was found to have a retention time corresponding to that of the faster-moving component isolated by chiral phase HPLC of (±)-**9a**. The slower-moving component could thus be assigned the *S* configuration.

Scheme 1

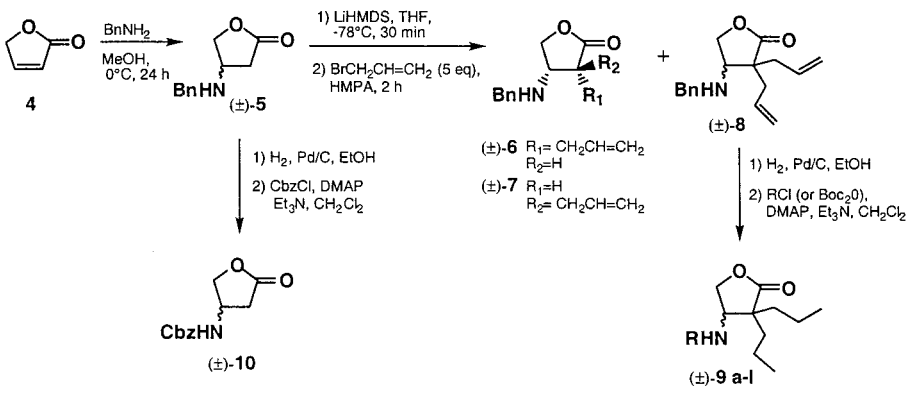
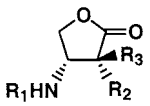


Table 1. Effect of Compounds **5**−**10** on GABA-Elicited Currents in *Xenopus* Oocytes Expressing α1β2γ2 $GABA_A$ Receptors



compd	R_1	R_2	R_3	% stimulation of GABA-induced currents[a] (μM)
2 (α-EMTBL)				+352 ± 23 (200)
(±)-**5**	Bn	H	H	0 ± 1 (200)
(±)-**6**	Bn	allyl	H	+73 ± 18 (200)
(±)-**7**	Bn	H	allyl	−1 ± 3 (200)
(±)-**8**	Bn	allyl	allyl	+115 ± 17 (200)
(±)-**9a**	$-CO_2Bn$	propyl	propyl	+279 ± 94 (100)
(*R*)-**9a**	$-CO_2Bn$	propyl	propyl	+279 ± 47 (100)
(*S*)-**9a**	$-CO_2Bn$	propyl	propyl	+104 ± 4 (100)
(±)-**9b**	$-CO_2Et$	propyl	propyl	+426 ± 8 (200)
(±)-**9c**	$-CO_2$allyl	propyl	propyl	+765 ± 61 (200)
(±)-**9d**	$-CO_2-t$-Bu	propyl	propyl	+46 ± 5 (200)
(±)-**9e**	$-CO_2Ph$	propyl	propyl	+330 ± 35 (200)
(±)-**9f**	−COPh	propyl	propyl	+420 ± 33 (200)
(±)-**9g**	$-SO_2Ph-p$-Cl	propyl	propyl	+23 ± 8 (5)
(±)-**9h**	$-SO_2Ph-p$-OMe	propyl	propyl	−22 ± 10 (200)
(±)-**9i**	$-SO_2Ph-p-CF_3$	propyl	propyl	−44 ± 1 (200)
(±)-**9j**	$-SO_2-2$-naphthyl	propyl	propyl	+4 ± 3 (50)
(±)-**9k**	$-SO_2-2$-thiophene	propyl	propyl	−16 ± 5 (200)
(±)-**9l**	$-SO_2NMe_2$	propyl	propyl	−1 ± 6 (200)
(±)-**10**	$-CO_2Bn$	H	H	+47 ± 5 (200)

[a] Determined electrophysiologically in *X. laevis* oocytes expressing α1β2γ2 $GABA_A$ receptors as previously described.[40,41] Average of three assays ± SEM.

Results and Discussion

No radioligands are available for the proposed butyrolactone binding site of the $GABA_A$ receptor, and furthermore, previous studies of this class of molecules have not convincingly demonstrated any consistent correlation between [^{35}S]-TBPS displacement and GABA-evoked current stimulations by these compounds.[19] For these reasons, it was decided to first perform electrophysiological screening of the 4-aminobutyrolactone derivatives and subsequently to investigate in vitro binding characteristics of the more active compounds. Thus, compounds **5**−**10** (200 μM, 100 μM or less, depending on solubility) were tested for their aptitude to activate, stimulate, or inhibit GABA-evoked currents in *Xenopus laevis* oocytes expressing rat brain recombinant α1β2γ2 $GABA_A$ receptors.[40,41] Results are shown in Table 1. None of the tested compounds acted as an agonist of the channel at the concentrations given in Table 1, but many of them acted as modulators of GABA-induced currents. In the following, the structural requirements for modulation are discussed. The first important observation that can be made is that an amide-type functionality at C-4 of a butyrolactone ring is more effective than an amine functionality in stimulating GABA currents. Thus, the *N*-benzyl derivative (±)-**5** (200 μM) had no effects on GABA currents while the *N*-Cbz analogue (±)-**10** (200 μM) was able to stimulate currents, albeit weakly (47 ± 5%). In both cases, addition of alkyl or dialkyl substituents at C-3 had a beneficial effect on activity. Thus, the *cis*-3-allyl-4-*N*-benzyl derivative and its 3,3-diallyl analogue ((±)-**6** and (±)-**8**, respectively) now had significant, though still relatively weak, stimulatory activity (73 ± 18% and 115 ± 17%, respectively, at 200 μM). Interestingly, the trans isomer (±)-**7** was totally inactive. In the case of the *N*-Cbz analogue, the presence of the dialkyl substituents at C-3 also had a very strong effect on activity, compound (±)-**9a** (100 μM) now stimulating currents by about 280%. Furthermore, this stimulatory activity resides mainly in the *R* enantiomer ((*R*)-**9a**, 279% stimulation at 100 μM), the *S* enantiomer being approximately 2.5 times less active. Other carbamate derivatives prepared (except for the sterically bulky *N*-Boc compound (±)-**9d**) were even more potent (i.e., ethyl carbamate (±)-**9b**, 426 ± 8% stimulation at 200 μM), stimulations being at the same level as those of α-EMTBL (352 ± 23% stimulation at 200 μM), the most active butyrolactone-type compound reported so far. In the case of the allyl carbamate (±)-**9c** (765 ± 61% stimulation at 200 μM), the activity of α-EMTBL was largely surpassed, a result confirmed in the concentration−response study described below. Finally, a carboxamide functionality ((±)-**9f**) was found to display stimulatory activity (420 ± 33% at 200 μM) of the same order as the carbamate analogues. In contrast, and except for the *p*-chlorophenylsulfonamide (±)-**9g** (which showed +23 ± 8% stimulation at 5 μM but could not be assayed at higher concentrations because of insolubility), none of the sulfonamide derivatives synthesized ((±)-**9h**−**l**) showed any significant current stimulation. In fact, in some of these compounds [(±)-**9h**,**i**,**k**], a weak inhibitory effect on currents was observed.

The in vitro binding profile of the more active compounds of Table 1 was investigated with respect to selected binding sites of the $GABA_A$ receptor. Thus, interaction with the GABA, benzodiazepine, and picrotoxin binding sites was evaluated by determination of

Scheme 2

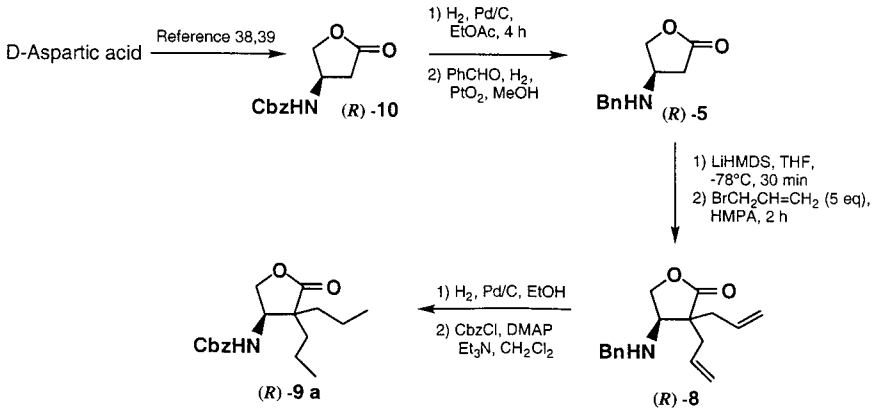


Table 2. Displacement of Radioactive Ligands from Rat Brain Membranes by Selected 4-Aminobutyrolactones

	displacement of radioactive ligand at 100 μM[a]		
compd	[^{3}H]-flunitrazepam	[^{3}H]-muscimol	[^{35}S]-TBPS
2 (α-EMTBL)	0%	0%	25%
(±)-**6**	0%	0%	60%
(±)-**8**	14% stimulation	0%	60%
(±)-**9a**	0%	0%	70%
(±)-**9b**	0%	0%	30%
(±)-**9c**	0% (ce) 15% stimulation (fb)	0%	50%
(±)-**9e**	25% stimulation	25% stimulation (ce) 0% (fb)	75%
(±)-**9f**	15% stimulation	10% stimulation (ce) 0% (fb)	65%
(±)-**9g**	25% stimulation	0%	85%
(±)-**9h**	0% stimulation	0%	80%
(±)-**9i**	20% stimulation	0%	40% (ce) 70% (fb)
(±)-**9j**	0%	0%	75%

[a] Determined in rat brain tissue as previously described.[42,43] Values are given as percent displacement by each substance. Experiments were performed at least three times in triplicate; "ce" refers to rat cerebellar tissue; "fb" refers to rat forebrain tissue (whole brain minus cerebellum). When tissue is not specified, values were identical for fb and ce.

the percent displacement of the appropriate selective radioligand (respectively, [^{3}H]-muscimol, [^{3}H]-flunitrazepam, and [^{35}S]-*tert*-butylbicyclophosphorothionate (([^{35}S]-TBPS)) by 100 μM of the substance.[42,43] As shown in Table 2, none of the compounds tested showed any binding affinity for the GABA recognition site. In fact, a slight increase (10−25%) in [^{3}H]-muscimol binding could be observed in some cases in cerebellar tissue (compounds (±)-**9e**,**f**). Similarly, displacement of [^{3}H]-flunitrazepam was either negligible ((±)-**9b**) or produced a 15−25% stimulation of radioactive ligand binding ((±)-**9e**,**f**). This latter effect did not correlate with effects on GABA current, since, for example, compound (±)-**9i**, which also stimulated [^{3}H]-flunitrazepam binding by 20%, actually inhibited GABA currents by 44 ± 1%.

The 4-amino-3,3-dipropylbutyrolactones were somewhat more active in displacing [^{35}S]-TBPS from its binding site. Thus, compounds (±)-**9a**−**c**,**e**,**f** displaced from 30% to 75% of radioligand binding at 100 μM. Though these values remain relatively modest, they are superior to the displacement by α-EMTBL (25% at 100 μM). Again, there is little correlation between [^{35}S]-TBPS displacement potencies by these compounds and effects on GABA-evoked currents. Thus, compound (±)-**9j**, which had practically no effect on currents, still displaced 75% of [^{35}S]-TBPS binding at 100 μM while compound (±)-**9h**, which actually inhibited currents (−22 ± 10% at 100 μM) displaced 90% of [^{35}S]-TBPS binding at the same concentration. Studies by Covey and co-workers using mutant GABA receptors insensitive to picrotoxin (the postulated site of action of TBPS), showed that α-EMBTL still potentiated GABA currents, leading to the hypothesis that the dialkylbutyrolactones are acting at two different sites, i.e., the picrotoxin/TBPS site (which would account for inhibition of GABA currents by low concentrations of α-EMTBL) and a putative "butyrolactone" site (interaction that produces current stimulations observed at higher concentrations of α-EMTBL).[24] Concentration−response values for compounds (±)-**9c** and (±)-**9f** were also determined and compared to those of α-EMTBL (Table 3). The EC_{50} values could not be determined because of solubility problems that made analysis at higher concentrations impossible. However (±)-**9c** and (±)-**9f** were more potent than α-EMTBL at the same concentration. No evidence for a biphasic effect was observed for the substances investigated.

Table 3. Concentration−Response Values of Compounds **2**, (±)-**9c**, and (±)-**9f** on GABA-Elicited Currents in *Xenopus* Oocytes Expressing α1β2γ2 Receptors

	% stimulation of GABA induced currents[a]		
concn, μM	**2** (α-EMTBL)	(±)-**9c**	(±)-**9f**
2	+10 ± 2	+26 ± 5	+17 ± 3
20	+39 ± 11	+229 ± 14	+207 ± 17
200	+386 ± 25	+765 ± 61	+420 ± 33

[a] Average of three assays ± SEM.

Table 4. Comparison of Current Stimulation of Compounds **2** (α-EMTBL), (*R*)-**9a**, (±)-**9c**, and (±)-**9f** in α1β2 and α1β2N265S Receptors

	% stimulation of GABA-induced currents	
compd[a]	α1β2	α1β2N265S
(*R*)-**9a**	324 ± 9	123 ± 20
2 (α-EMTBL)	192 ± 36	83 ± 6
(±)-**9c**	2132 ± 569	627 ± 95
(±)-**9f**	557 ± 9	648 ± 81

[a] Compound **2** was assayed at 200 μM. The other compounds were assayed at 100 μM.

Compound (*R*)-**9a** was also tested on α1β1 and α1β2 recombinant receptors. At 100 μM, the compound was 3- to 4-fold more active in α1β2 receptors than in α1β1 receptors (not shown). Interestingly, this profile resembles that of the anticonvulsant loreclezole, which similarly enhances the activity in β2 (as well as β3) but not in β1 subunit containing $GABA_A$ receptors.[33] To test this hypothesis, the effect of (*R*)-**9a** on GABA currents in the presence of the point mutant $\alpha_1\beta$2N265S receptor, which shows a diminished response to loreclezole,[44,45] was studied. As shown in Table 4, current stimulations by (*R*)-**9a** were also greatly decreased by about 65% in this case, again suggesting similarity in action with loreclezole. Interestingly, the current stimulation by α-EMTBL was also reduced in mutant α1β2N265S receptors compared to wild-type α1β2 receptors by more than 50% (Table 4).

In oocytes expressing rat recombinant α1β2N265S receptors, stimulation by (±)-**9c** (100 μM) was also reduced to about 30% of the current potentiation observed in the wild-type receptor (Table 4). However, stimulation by (±)-**9f** (100 μM) was not significantly affected by the point mutation β2N265S. Thus, at least some (**9a**, **9c**) but not all (**9f**) of the butyrolactones analyzed may share similarity in their action to the anticonvulsant loreclezole.

In conclusion, the addition of an *N*-carboxamide or carbamate (but not *N*-sulfonyl) group at C-4 of a 3,3-dialkylbutyrolactone has the effect of significantly increasing the GABA-evoked current potentiation produced by this class of compounds possibly by directing them to the loreclezole allosteric recognition site of the $GABA_A$ receptor. While the latter point will obviously require further study and the availability of more selective compounds, it is clear that the addition of a substituted amine functionality to the butyrolactone nucleus allows for a great deal of structural variation

and considerable leeway for the optimization of activity. These studies are currently underway.

Experimental Section

General. Melting points were determined on a Büchi apparatus and are uncorrected. IR spectra of samples were obtained as KBr or as films with a Nicolet 205 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR were determined on a Bruker 200, 250, or 300 MHz instrument. Chemical shifts are given as δ values with reference to Me_4Si as internal standard. Electron impact and chemical ionization mass spectra were recorded on AEI MS-50 and AEI MS-9 spectrometers, respectively. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. Thin-layer chromatography was performed on Merck silica gel plates with fluorescent indicator. The plates were visualized with UV light (254 nm) or with a 3.5% solution of phosphomolybdic acid in ethanol. All column chromatography was conducted on a Waters 990 apparatus. All reagents were purchased from the Aldrich Chemical Co. and were used without further purification. α-EMTBL was prepared as described.[46] Elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette.

(*R,S*)-4-Benzylamino-4,5-dihydro-2(3*H*)-furanone ((±)-5).[35] A solution of 2(5*H*)-furanone (2.5 g, 29.7 mmol) in methanol (3 mL) was treated at 0 °C with benzylamine (3.82 g, 35.7 mmol). The reaction mixture was stirred at 0 °C for 24 h, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (ethyl acetate−hexane 1:1), affording compound (±)-**5** as a yellow oil (3.5 g, 60%): CIMS *m/z* 191 (M^+); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.62 (s, 1H, NH), 2.40 (dd, 1H, $J_{3a,4}$ = 5.3 Hz, J_{gem} = 16.0 Hz, H-3a), 2.70 (dd, 1H, $J_{3b,4}$ = 8.0 Hz, J_{gem} = 16.0 Hz, H-3b), 3.68 (m, 1H, H-4), 3.80 (s, 2H, CH_2Ph), 4.13 (dd, 1H, $J_{5a,4}$ = 1.6 Hz, J_{gem} = 10.7 Hz, H-5a), 4.48 (dd, 1H, $J_{5b,4}$ = 5.3 Hz, J_{gem} = 10.7 Hz, H-5b), 7.20−7.50 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.9, 51.9, 54.0, 73.6, 127.7, 128.4, 128.9, 139.6, 176.5. Anal. ($C_{11}H_{13}NO_2$) C, H, N.

***cis*- and *trans*-3-Allyl-4-benzylamino-4,5-dihydro-2(3*H*)-furanone ((±)-6 and (±)-7, Respectively) and (*R,S*)-4-Benzyl-3,3-diallyl-4,5-dihydro-2(3*H*)-furanone ((±)-8).** To a solution of lithium hexamethyldisilazide (5.4 mmol, 2.2 equiv) in anhydrous THF (50 mL) was added at −78 °C under argon a solution of compound (±)-**5** (0.46 g, 2.44 mmol). The solution was stirred at −78 °C for 30 min, and a solution of allyl bromide (1.16 mL, 12.2 mmol) in HMPA (5 mL) was added by cannula. The reaction mixture was stirred for 2 h at −78 °C, saturated aqueous ammonium chloride was added, and the mixture was extracted with dichloromethane (3 × 20 mL). The organic extracts were combined, the solvent was removed under reduced presure, and the residue was purified by chromatography on silica gel (ethyl acetate−hexane 1:1). The first compound to be eluted was the diallyl derivative (±)-**8**, obtained as an oil (340 mg, 52%): IR (film) 1639, 1770, 3338 cm^{-1}; CIMS *m/z* 272 $(MH)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (s, 1H, NH), 2.33 (m, 2H, CH_2CH=CH_2), 2.45 (m, 2H, CH_2-CH=CH_2), 3.54 (dd, 1H, $J_{4,5a}$ = 8.4 Hz, $J_{4,5b}$ = 7.7 Hz, H-4), 3.80 (s, 2H, CH_2N), 3.81 (dd, 1H, $J_{5a,4}$ = 8.4 Hz, J_{gem} = 17.6 Hz, H-5a), 4.28 (dd, 1H, $J_{5b,4}$ = 7.7 Hz, J_{gem} = 17.6 Hz, H-5b), 5.00−5.22 (m, 4H, 2 × CH=CH_2), 5.68 (m, 1H, CH=CH_2), 5.88 (m, 1H, CH=CH_2), 7.31 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 36.0, 39.4, 49.1, 52.4, 59.1, 70.5, 119.3, 119.7, 127.5, 128.0, 128.6, 132.8, 133.1, 139.8, 178.7. Anal. ($C_{17}H_{21}NO_2$) C, H, N.

Further elution of the chromatography column afforded compound (±)-**6** as an oil (50 mg, 9%): IR (film) 1772 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62 (s, 1H, NH), 2.39 (m, 2H, CH_2-CH=CH_2), 2.65 (m, 1H, H-3), 3.59 (m, 1H, H-4), 3.80 (m, 2H, CH_2N), 4.15 (dd, 1H, $J_{4,5a}$ = 4.3 Hz, J_{gem} = 9.7 Hz, H-5a), 4.27 (dd, 1H, $J_{4,5b}$ = 1.6 Hz, J_{gem} = 9.7 Hz, J_{gem} = 9.7 Hz, H-5b), 5.14 (m, 2H, CH=CH_2), 5.86 (m, 1H, CH−CH_2), 7.26−7.36 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.3, 45.9, 51.7, 58.4, 72.2, 118.4, 127.5, 128.1, 128.6, 134.2, 139.5, 179.1. HRCIMS calcd for $C_{14}H_{18}NO_2$ *m/z* 232.1337, found *m/z* 232.1339.

Continued elution of the chromatography column afforded compound (±)-**7** as an oil (100 mg, 18%): IR (film) 1773 cm^{-1}; CIMS *m/z* 232 $(MH)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.69 (s, 1H, NH), 2.37 (m, 1H, H-3), 2.52 (m, 2H, CH_2CH=CH_2), 3.39 (q, 1H, J = 6.6 Hz, H-4), 3.75 (s, 2H, CH_2N), 3.91 (dd, 1H, $J_{4,5a}$ = 6.6 Hz, J_{gem} = 9.1 Hz, H-5a), 4.33 (dd, 1H, $J_{4,5b}$ = 6.6 Hz, J_{gem} = 9.1 Hz, H-5b), 5.12 (m, 2H, CH=CH_2), 5.77 (m, 1H, CH=CH_2), 7.24−7.32 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.2, 46.1, 51.8, 58.5, 72.1, 118.5, 127.5, 128.1, 128.6, 134.1, 139.4, 177.2. Anal. ($C_{14}H_{17}NO_2$) C, H, N.

(*R,S*)-4-Benzyloxycarbonylamino-4,5-dihydro-2(3*H*)-furanone ((±)-10). A solution of compound (±)-**5** (306 mg, 1.6 mmol) in ethanol (10 mL) was hydrogenated at 40 psi for 16 h in the presence of 10% palladium on carbon (100 mg). The reaction mixture was filtered through Celite, the filter pad was washed with ethanol, and the combined filtrates were evaporated under reduced pressure. The residue was dissolved in dichloromethane (3 mL), and to the solution was added at 0 °C triethylamine (0.4 mL, 2.8 mmol), DMAP (45 mg, 0.37 mmol), and benzyl chloroformate (325 mg, 1.9 mmol). The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 12 h. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel (ethyl acetate−hexane 1:1), affording compound (±)-**10** as a white solid (230 mg, 61%): mp 101−102 °C (lit. mp (*S* isomer) 103−104 °C)[47]; CIMS *m/z* 236 $(MH)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.47 (dd, 1H, $J_{3a,4}$ = 3.0 Hz, J_{gem} = 17.7 Hz, H-3a), 2.85 (dd, 1H, $J_{3b,4}$ = 7.6 Hz, J_{gem} = 17.7 Hz, H-3b), 4.23 (m, 1H, H-4), 4.50 (m, 2H, H-5), 5.12 (s, 2H, CH_2O), 7.36 (s, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.8, 48.1, 67.2, 73.6, 128.2, 128.4, 128.6, 135.9, 155.7, 175.1.

(*R,S*)-4-Benzyloxycarbonylamino-4,5-dihydro-3,3-dipropyl-2(3*H*)-furanone ((±)-9a). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.37 mmol) was hydrogenated and then treated with benzyl chloroformate (86 mg, 0.5 mmol) to afford compound (±)-**9a** as a white powder (61 mg, 52%): mp 91−92 °C; IR (KBr) 1557, 1684, 1774 cm^{-1}; CIMS *m/z* 320 $(MH)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, 3H, J = 7.2 Hz, CH_2CH_3), 0.92 (t, 3H, J = 7.0 Hz, CH_2CH_3), 1.23−1.64 (m, 8H, 2 × CH_2CH_2), 3.84 (dd, 1H, $J_{4,5a}$ = 8.6 Hz, $J_{4,5b}$ = 7.4 Hz, H-4), 4.49 (m, 1H, H-5a), 4.57 (m, 1H, H-5b), 5.12 (s, 2H, CH_2O), 7.36 (s, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 14.5, 17.2 (2C), 32.8, 36.2, 48.2, 53.9, 67.5, 69.2, 127.8 (2C), 128.3 (2C), 128.7, 136.0, 155.8, 178.5. Anal. ($C_{18}H_{25}NO_4$) C, H, N.

(*R,S*)-4,5-Dihydro-3,3-dipropyl-4-ethyloxycarbonylamino-2(3*H*)-furanone ((±)-9b). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.37 mmol) was hydrogenated and the product was treated with ethyl chloroformate (55 mg, 0.5 mmol) to afford compound (±)-**9b** as a white solid (73 mg, 77%): mp 111−112 °C; IR (KBr) 1548, 1690, 1788, 3326 cm^{-1}; CIMS *m/z* 258 $(MH)^+$; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.93 (t, 6H, J = 7.0 Hz, 2 × CH_2CH_3), 1.27 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 1.33−1.70 (m, 8H, 2 × $CH_2CH_2CH_3$), 3.84 (dt, 1H, $J_{4,5a}$ = $J_{4,5b}$ = 7.1 Hz, $J_{4,NH}$ = 1.2 Hz, H-4), 4.15 (q, 2H, J = 7.0 Hz, OCH_2CH_3), 4.45−4.60 (m, 2H, H-5), 4.82 (br s, 1H, NH); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.4, 14.6 (2C), 17.2 (2C), 32.7, 36.1, 48.2, 53.6, 61.6, 69.2, 156.2, 178.8. Anal. ($C_{13}H_{23}NO_4$) C, H, N.

(*R,S*)-4-Allyloxycarbonylamino-4,5-dihydro-3,3-dipropyl-2(3*H*)-furanone ((±)-9c). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.37 mmol) was hydrogenated and the product was treated with allyl chloroformate (60 mg, 0.5 mmol) to afford compound (±)-**9c** as a white solid (81 mg, 81%): mp 80−81 °C; IR (KBr) 1557, 1686, 1777, 3316 cm^{-1}; CIMS *m/z* 270 $(MH)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.93 (t, 3H, J = 6.8 Hz, CH_2CH_3), 0.95 (t, 3H, J = 6.6 Hz, CH_2CH_3), 1.22−1.68 (m, 8H, 2 × $CH_2CH_2CH_3$), 3.87 (m, 1H, H-4), 4.44−4.61 (m, 4H, OCH_2, H-5), 4.95 (br s, 1H, NH), 5.23−5.39 (m, 2H, CH_2=CH), 5.85−5.99 (m, 1H, CH_2=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 14.6, 17.1, 17.2, 32.6, 36.1, 48.2, 53.8, 66.2, 69.2, 118.4, 132.3, 155.6, 178.4. Anal. ($C_{14}H_{23}NO_4$) C, H, N.

(*R,S*)-4-*t*-Butyloxycarbonylamino-4,5-dihydro-3,3-dipropyl-2(3*H*)-furanone ((±)-9d). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.37 mmol) was hydrogenated and the product was treated with BOC anhydride (110 mg, 0.5 mmol) to afford compound (±)-**9d** as a white solid (73 mg, 70%): mp 134−135 °C; IR (KBr) 1540, 1683, 1768, 3340 cm^{-1}; CIMS *m/z* 286 $(MH)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 0.93 (t, 3H, J = 7.0 Hz, CH_2CH_3), 0.94 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.25−1.44 (m, 4H, 2 × CH_2CH_3), 1.45 (s, 9H, $(CH_3)_3C$), 1.52−1.65 (m, 4H, 2 × CH_2CH_2), 3.83 (m, 1H, H-4), 4.47 (m, 2H, H-5), 4.66 (br s, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.5, 14.7, 17.2 (2C), 28.4 (3C), 32.6, 36.2, 48.4, 53.6, 69.5, 69.6, 157.2, 178.5. Anal. ($C_{15}H_{27}NO_4$) C, H, N.

(*R,S*)-4,5-Dihydro-3,3-dipropyl-4-phenyloxycarbonylamino-2(3*H*)-furanone ((±)-9e). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.36 mmol) was hydrogenated and the product was treated with phenyl chloroformate (79 mg, 0.5 mmol) to afford compound (±)-**9e** as an off-white powder (73 mg, 67%): mp 138−139 °C; IR(KBr) 1542, 1705, 1768, 2960, 3312 cm^{-1}; CIMS *m/z* 306 $(MH)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 0.93 (t, 3H, J = 7.1 Hz, CH_2CH_3), 0.97 (t, 3H, J = 7.0 Hz, CH_2CH_3), 1.29−1.70 (m, 8H, 2 × $CH_2CH_2CH_3$), 3.93 (m, 1H, H-4), 4.57 (m, 2H, H-5), 5.24 (br s, 1H, NH), 7.05−7.13 (m, 2H, ArH), 7.23−7.26 (m, 1H, ArH), 7.35−7.41 (m, 2H, ArH); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 14.5, 14.7, 17.3 (2C), 32.9, 36.2, 48.5, 54.1, 69.2, 121.5, 122.9, 125.9, 129.3, 129.6, 150.7, 154.2, 178.4. Anal. ($C_{17}H_{23}NO_4 \cdot 0.2H_2O$) C, H, N.

(*R,S*)-4-Benzamido-4,5-dihydro-3,3-dipropyl-2(3*H*)-furanone ((±)-9f). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.36 mmol) was hydrogenated and the product was treated with benzoyl chloride (70 mg, 0.5 mmol) to afford compound (±)-**9f** as a white solid (59 mg, 57%): mp 130−131 °C; IR(KBr) 1547, 1637, 1767, 3306 cm^{-1}; CIMS *m/z* 290 $(MH)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, 3H, J = 7.9 Hz, CH_2CH_3), 0.95 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.26−1.74 (m, 8H, 2 × $CH_2CH_2CH_3$), 4.03 (dd, 1H, $J_{4,5a}$ = 9.7 Hz, $J_{4,5b}$ = 5.5 Hz, H-4), 4.61 (dd, 1H, $J_{4,5a}$ = 9.7 Hz, J_{gem} = 7.1 Hz, H-5a), 4.98 (m, 1H, H-5b), 6.65 (br s, 1H, NH), 7.39−7.57 (m, 3H, ArH), 7.78 (dd, 2H, J = 7.1 and 1.4 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 14.7, 17.1, 17.2, 32.3, 36.1, 48.8, 52.8, 70.2, 127.0, 128.8, 132.1, 133.7, 167.3, 178.9. Anal. ($C_{17}H_{23}NO_3$) C, H, N.

(*R,S*)-4-(*p*-Chlorophenylsulfonamido)-4,5-dihydro-3,3-dipropyl-2(3*H*)furanone ((±)-9g). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.36 mmol) was hydrogenated and the product was treated with *p*-chlorophenylsulfonyl chloride (106 mg, 0.5 mmol), affording compound (±)-**9g** as a white solid (100 mg, 84%): mp 96−97 °C; IR(KBr) 1174, 1478, 1758 cm^{-1}; CIMS *m/z* 360 (MH^+ with ^{35}Cl), 362 (MH^+ with ^{37}Cl); ^1H NMR (250 MHz, $CDCl_3$) δ 0.80 (t, 3H, J = 7.1 Hz, CH_2CH_3), 0.88 (t, 3H, J = 6.9 Hz, CH_2CH_3), 1.01−1.63 (m, 8H, 2 × $CH_2CH_2CH_3$), 3.79 (dd, 1H, J_{gem} = 9.1 Hz, $J_{4,5a}$ = 8.0 Hz, H-5a), 4.01 (dt, 1H, $J_{4,NH}$ = 9.1 Hz, $J_{4,5}$ = 8.0 Hz, H-4), 4.25 (dd, 1H, J_{gem} = 9.1 Hz, $J_{4,5b}$ = 8.0 Hz, H-5b), 5.28 (d, 1H, $J_{4,NH}$ = 9.1 Hz, NH), 7.54 (d, 2H, J = 9.1 Hz, ArH), 7.84 (d, 2H, J = 9.1 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.4, 17.0, 17.2, 33.0, 35.6, 48.0, 55.2, 68.9, 128.5, 129.8, 138.5, 140.1, 177.4. Anal. ($C_{16}H_{22}NO_4SCl \cdot 0.1C_6H_{14}$) C, H, N, S.

(*R,S*)-4,5-Dihydro-3,3-dipropyl-4-(*p*-methoxyphenylsulfonamido)-2(3*H*)-furanone ((±)-9h). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.36 mmol) was hydrogenatated and the product was treated with *p*-methoxyphenylsulfonyl chloride (104 mg, 0.5 mmol), affording compound (±)-**9h** as a white powder (107 mg, 84%): mp 103−104 °C; IR(KBr) 1168, 1323, 1754 cm^{-1}; CIMS *m/z* 356 $(MH)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.78 (t, 3H, J = 7.1 Hz, CH_2CH_3), 0.88 (t, 3H, J = 6.9 Hz, CH_2CH_3), 1.07−1.59 (m, 8H, 2 × $CH_2CH_2CH_3$), 3.76 (dd, 1H, J_{gem} = 9.1 Hz, $J_{4,5a}$ = 8.3 Hz, H-5a), 3.89 (s, 3H, OCH_3), 3.98 (q, 1H, J = 8.3 Hz, H-4), 4.21 (dd, 1H, J_{gem} = 9.1 Hz, $J_{4,5b}$ = 8.3 Hz, H-5b), 4.99 (d, 1H, $J_{4,NH}$ = 8.3 Hz, NH), 7.01 (d, 2H, J = 8.9 Hz, ArH), 7.83 (d, 2H, J = 8.9 Hz, ArH). Anal. ($C_{17}H_{25}NO_5S \cdot 0.1C_6H_{14}$) C, H, N, S.

(*R,S*)-4,5-Dihydro-3,3-dipropyl-4-(*p*-trifluoromethylphenylsulfonamido)-2(3*H*)-furanone ((±)-9i). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.5 mmol) was hydrogenated and the product was treated with *p*-trifluoromethylphenylsulfonyl chloride (127 mg, 0.5 mmol), affording compound (±)-**9i** as a white solid (81 mg, 57%): mp 106−107 °C; IR(KBr) 1169, 1323, 1753 cm^{-1}; CIMS *m/z* 394 $(MH)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 0.78 (t, 3H, J = 7.1 Hz, CH_2CH_3), 0.86 (t, 3H, J = 7.0 Hz, CH_2CH_3), 0.93−1.62 (m, 8H, 2 × $CH_2CH_2CH_3$), 3.82 (dd, 1H, J_{gem} = 9.2 Hz, $J_{4,5a}$ = 7.7 Hz, H-5a), 4.03 (dt, 1H, $J_{4,NH}$ = 9.4 Hz, $J_{4,5a}$ = 7.7 Hz, $J_{4,5b}$ = 7.5 Hz, H-4), 4.27 (dd, 1H, J_{gem} = 9.2 Hz, $J_{4,5b}$ = 7.5 Hz, H-5b), 5.60 (d, 1H, $J_{4,NH}$ = 9.4 Hz, NH), 7.83 (d, 2H, J = 8.4 Hz, ArH), 8.05 (d, 2H, J = 8.4 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 14.4, 16.9, 17.2, 32.8, 35.5, 48.1, 55.4, 69.0, 126.6, 127.4, 127.6, 135.1, 143.7, 177.4. Anal. ($C_{17}H_{22}NO_4SF_3 \cdot 0.1H_2O$) C, H, N, S.

(*R,S*)-4,5-Dihydro-3,3-dipropyl-4-(1-naphthalenesulfonamido)-2(3*H*)-furanone ((±)-9j). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.5 mmol) was hydrogenated and the product was treated with 1-naphthalenesulfonyl chloride (114 mg, 0.5 mmol), affording compound (±)-**9j** as an off-white solid (67 mg, 50%): mp 210−211 °C; IR(KBr) 1165, 1332, 1755, 2962, 3240 cm^{-1}; CIMS *m/z* 376 $(MH)^+$; ^1H NMR (250 MHz, DMSO-d_6) δ 0.69 (t, 3H, J = 7.0 Hz, CH_2CH_3), 0.72 (t, 3H, J = 6.6 Hz, CH_2CH_3), 0.84−1.48 (m, 8H, 2 × $CH_2CH_2CH_3$), 3.85 (dd, 1H, J_{gem} = 8.6 Hz, $J_{4,5a}$= 7.6 Hz, H-5a), 4.06 (m, 1H, H-4), 4.17 (s, 1H, NH), 4.26 (dd, 1H, J_{gem} = 8.6 Hz, $J_{4,5b}$ = 8.4 Hz, H-5b), 7.75−7.90 (m, 3H, ArH), 8.25 (dd, 1H, J = 1.3 and 7.7 Hz, ArH), 8.31 (d, 1H, J = 7.2 Hz, ArH), 8.39 (d, 1H, J = 7.9 Hz, ArH), 8.79 (d, 1H, J = 8.3 Hz, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.6, 13.8, 15.6, 16.2, 31.9, 34.0, 47.2, 54.3, 67.5, 124.2, 126.6, 126.9, 127.7 (2C), 128.6, 128.7, 133.5, 134.0, 135.1, 177.1. Anal. ($C_{20}H_{25}NO_4S$), C, H, N.

(*R,S*)-4,5-Dihydro-3,3-dipropyl-4-(2-thiophenesulfonamido)-2(3*H*)-furanone ((±)-9k). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.36 mmol) was hydrogenated and the product was treated with 2-thiophenesulfonyl chloride (92 mg, 0.5 mmol), affording compound (±)-**9k** as a white powder (94 mg, 79%): mp 92−93 °C; IR(KBr) 1154, 1350, 1774, 2962, 3258 cm^{-1}; CIMS *m/z* 332 $(MH)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 0.83 (t, 3H, J = 7.0 Hz, CH_2CH_3), 0.90 (t, 3H, J = 6.8 Hz, CH_2CH_3), 1.09−1.58 (m, 8H, 2 × $CH_2CH_2CH_3$), 3.82 (dd, 1H, $J_{4,5a}$ = 8.2 Hz, J_{gem} = 9.2 Hz, H-5a), 4.07−4.16 (m, 1H, H-4), 4.29 (dd, 1H, J_{gem} = 9.2 Hz, $J_{4,5b}$ = 7.8 Hz, H-5b), 5.17 (d, 1H, $J_{4,NH}$ = 8.6 Hz, NH), 7.14 (t, 1H, J = 4.6 Hz, ArH), 7.68 (d, 2H, J = 4.6 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 14.6, 17.3, 17.4, 33.4, 35.9, 48.2, 55.5, 69.1, 127.9, 133.1 (2C), 140.6, 177.2. Anal. ($C_{14}H_{21}NO_4S_2$) C, H, N, S.

(*R,S*)-4,5-Dihydro-4-(dimethylaminosulfonamido)-3,3-dipropyl-2(3*H*)-furanone ((±)-9l). Following the same procedure as for the preparation of compound (±)-**10**, compound (±)-**8** (100 mg, 0.36 mmol) was hydrogenated and the product was treated with dimethylsulfamoyl chloride (72 mg, 0.5 mmol), affording compound (±)-**9l** as an off-white powder (58 mg, 55%): mp 62−63 °C; IR(KBr) 1149, 1349, 1778, 2960, 3277 cm^{-1}; CIMS *m/z* 293 $(MH)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 0.94 (t, 6H, J = 6.9 Hz, 2 × CH_2CH_3), 1.18−1.73 (m, 8H, 2 × $CH_2CH_2CH_3$), 2.83 (s, 6H, $N(CH_3)_2$), 3.99 (m, 1H, H-5a), 4.13 (m, 1H, H-4), 4.48−4.62 (m, 2H, H-5b, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 14.6, 17.4, 17.6, 33.2, 35.9, 38.2, 48.2, 55.6, 69.5, 177.7. Anal. ($C_{12}H_{24}N_2O_4S$) C, H, N.

(*R*)-4-Benzylamino-4,5-dihydro-2(3*H*)-furanone ((*R*)-5). A solution of (*R*)-4-benzyloxycarbonylamino-4,5-dihydro-2(3*H*)-furanone ((*R*)-**10**, 150 mg, 0.64 mmol) (prepared from D-aspartic acid)[38,39] in ethyl acetate (10 mL) was stirred under hydrogen (30 psi) at room temperature for 4 h in the presence of 10% palladium on carbon (100 mg). The reaction mixture was filtered through Celite, the filter pad was washed with ethyl acetate, and the combined filtrate and washings were

evaporated under reduced pressure. The residue was taken up in methanol (3 mL), benzaldehyde (0.065 mL, 0.64 mmol) was added with cooling, and the reaction mixture was stirred for 1 h. Methanol (17 mL) and platinum oxide catalyst (100 mg) were added, and the mixture was hydrogenated at 40 psi for 3 h. After removal of the catalyst by filtration through Celite, the filtrate was evaporated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel (ethyl acetate−hexane 1:1), affording compound (*R*)-**5** as a colorless oil (70 mg, 57%) whose spectral characteristics were identical to racemic **5** prepared above: $[\alpha]^{25}_D$ +16.5° (*c* 1.0, $CHCl_3$).

(*R*)-4-Benzylamino-3,3-diallyl-4,5-dihydro-2(3*H*)-furanone ((*R*)-8). Following the same procedure as for the preparation of racemic **8**, a solution of the lithium enolate of compound (*R*)-**5** (92 mg, 0.49 mmol) in THF was treated at −78 °C with a solution of allyl bromide (0.23 mL, 2.44 mmol) in HMPA (1 mL). The reaction mixture was stirred at −78 °C and then worked up as before. Isolation of the least polar product by chromatography on silica gel (ethyl acetate−hexane 1:1) afforded compound (*R*)-**8** as an oil (68 mg, 52%) whose spectroscopic characteristics were identical to racemic **8**: $[\alpha]^{25}_D$ +1.4° (*c* 0.8, $CHCl_3$).

(*R*)-4-Benzyloxycarbonylamino-4,5-dihydro-3,3-dipropyl-2(3*H*)-furanone ((*R*)-9a). Following the same procedure as for the preparation of compound (±)-**9a**, compound (*R*)-**8** (100 mg, 0.36 mmol) was hydrogenated and the product was treated with benzyl chloroformate (86 mg, 0.5 mmol) to afford compound (*R*)-**9a** as a colorless oil (65 mg, 57%). The spectroscopic characteristics of compound (*R*)-**9a** were identical to those of racemic **9a**: $[\alpha]^{25}_D$ −0.28° (*c* 1.44, $CHCl_3$).

Separation of the Enantiomers of Compound 9a. Racemic **9a** (40 mg) was subjected to HPLC on a chiral OD column using hexane−2-propanol (9:1) as the eluent. The fraction having the shorter retention time had an optical rotation ($[\alpha]^{25}_D$ −0.28° (*c* 1.0, $CHCl_3$)) corresponding to *R*-**9a**, prepared from D-aspartic acid as described above. The fraction having the longer retention time ($[\alpha]^{25}_D$ +0.24° (*c* 1.0, $CHCl_3$)) thus corresponded to (*S*)-**9a**.

Receptor Binding Studies. The synthesized compounds were tested in vitro for their capacity to bind to different sites on the $GABA_A$ receptor, including the GABA site itself (by displacement studies of [^{3}H]-muscimol), the benzodiazepine site (by displacement of [^{3}H]-flunitrazepam), and the picrotoxin site (by displacement of [^{35}S]-TBPS). Briefly, frozen membranes from cerebellum or from brain without cerebellum were thawed, centrifuged, and resuspended in 50 mM Tris-citrate buffer, pH 7.4, at a protein concentration of about 1 mg/mL. Membranes (0.5 mL) were then incubated in a total of 1 mL of a solution containing 50 mM Tris-citrate buffer, pH 7.4, 150 mM NaCl, and 2 nM [^{3}H]-flunitrazepam or 2 nM [^{3}H]-muscimol in the absence or presence of various concentrations of the compounds to be investigated or of 10 μM diazepam or 10 μM GABA, for 90 min at 4 °C, respectively. For [^{35}S]-TBPS binding, membranes were incubated in a total of 1 mL of a solution containing 50 mM Tris-citrate buffer, pH 7.4, 200 mM NaBr, and 2 nM [^{35}S]-TBPS in the absence or presence of various concentrations of the compounds to be investigated or of 10 μM TBPS or picrotoxinin for 180 min at room temperature.[42,43]

Membranes were then filtered through Whatman GF/B filters. When [^{3}H]-flunitrazepam or [^{3}H]-muscimol binding was investigated, the filters were rinsed twice with 5 mL of ice-cold 50 mM Tris-citrate buffer. When [^{35}S]-TBPS binding was investigated, the filters were rinsed three times with 3.5 mL of this buffer. Filters were transferred to scintillation vials and subjected to scintillation counting after addition of 3.5 mL of scintillation fluid. Nonspecific binding determined in the presence of 10 μM diazepam, 10 μM GABA, or 10 μM TBPS was subtracted from total [^{3}H]-flunitrazepam, [^{3}H]-muscimol, or [^{35}S]-TBPS binding, respectively, to result in specific binding.

Electrophysiological Studies. The synthesized compounds were studied for their ability to induce opening of the $GABA_A$ receptor channel or to allosterically modulate currents elicited by GABA. For this purpose, recombinant $GABA_A$ receptors were expressed in *Xenopus* oocytes. Briefly, *Xenopus laevis* oocytes were prepared, injected, and defolliculated and currents were recorded as described.[40,41] Oocytes were injected with 50 nL of cRNA dissolved in 5 mM K-Hepes (pH 6.8). This solution contained the transcripts coding for the different subunits at a concentration of 10 nM for α1, 10 nM for β2, and 50 nM for γ2. For dual subunit combinations, a concentration of 100 nM was used for each transcript (α1, β2, and the point mutant β2N265S). RNA transcripts were synthesized from linearized plasmids encoding the desired protein using the message machine kit (Ambion) according to the recommendation of the manufacturers. A poly(A) tail of about 300 residues was added to the transcripts by using yeast poly(A) polymerase (USB or Amersham). The cRNA combinations were coprecipitated in ethanol and stored at −20 °C. Transcripts were quantified on agarose gels after staining with Radiant Red RNA stain (Bio-Rad) by comparing staining intensities with various amounts of molecular weight markers (RNA-Ladder, Gibco-BRL). Electrophysiological experiments were performed by the two-electrode voltage clamp method at a holding potential of −80 mV. The medium contained 90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM Na-Hepes (pH 7.4). GABA eliciting about 2−4% of the maximal current amplitude was applied for 20 s, and a washout period of 4 min was allowed to ensure full recovery from desensitization. The perfusion system was cleaned between drug applications by washing with dimethyl sulfoxide to avoid contamination. Drugs were applied at a concentration of 100 μM in the absence of GABA to see whether the drugs could act as a channel agonist. To study allosteric modulation, GABA was first applied alone and subsequently in combination with either 0.1 or 100 μM of the drug.

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