# GABA Agonists and Uptake Inhibitors. Synthesis, Absolute Stereochemistry, and Enantioselectivity of (*R*)-(−)- and (*S*)-(+)-Homo-β-proline

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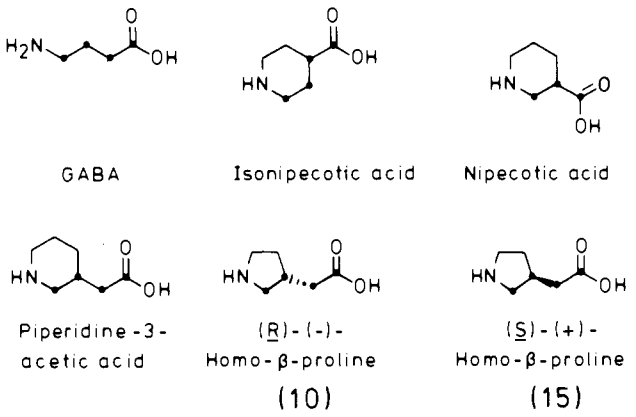
The cyclic analogue of 4-aminobutyric acid (GABA), 3-pyrrolidineacetic acid (homo-β-proline), is a potent agonist at $GABA_A$ receptors, it interacts effectively with GABA-uptake mechanisms, and it is a moderately potent inhibitor of $GABA_B$ receptor binding. (*R*)-(−)- (**10**) and (*S*)-(+)-homo-β-proline (**15**) were synthesized via methyl (3*S*)-1-[(*R*)-1-phenylethyl]-5-oxo-3-pyrrolidinecarboxylate (**5**) and its 3*R* diastereomer (**4**), respectively. The mixture **3** consisting of **4** and **5** was synthesized via addition–cyclization reactions between (*R*)-1-phenylethylamine and itaconic acid (**1**). The diastereomers **5** and **4**, which were separated chromatographically, were converted into (*R*)- (**10**) and (*S*)-homo-β-proline (**15**), respectively. The absolute stereochemistry of **10** and **15** was established on the basis of an X-ray analysis of compound **5**. The enantiomers **10** and **15** were shown to bind to $GABA_A$ and $GABA_B$ receptor sites with opposite stereoselectivity. Thus, (*R*)-homo-β-proline (**10**) proved to be more than 1 order of magnitude more potent than the *S* enantiomer (**15**) as an inhibitor of $GABA_A$ receptor binding, whereas the $GABA_B$ receptor affinity of homo-β-proline was shown to reside exclusively in (*S*)-homo-β-proline (**15**). In contrast to the stereoselective receptor affinities of **10** and **15**, these enantiomers were approximately equieffective as inhibitors of synaptosomal GABA uptake.

4-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system (CNS).[1-3] GABA is involved in the regulation of a variety of physiological mechanisms,[4-8] and impaired transmission at central GABA-operated synapses may be important in a number of neurological disorders.[2,9-13] As a consequence of these observations, there is an interest in specific agonists at postsynaptic $GABA_A$ receptors and in inhibitors of the GABA-uptake mechanisms.[3,14-16]

Extensive structure–activity studies on conformationally restricted analogues of GABA have shown that different structural constraints are imposed on agonists acting at $GABA_A$ or $GABA_B$ receptors and on inhibitors of GABA uptake.[14,16] Thus, isonipecotic acid is a potent and specific $GABA_A$ agonist,[12,17] whereas nipecotic acid is a very effective inhibitor/substrate for the GABA-uptake systems but shows no detectable affinity for $GABA_A$ or $GABA_B$ receptors[15,18,19] (Chart I). Both compounds have been further developed into highly potent, specific, and orally active $GABA_A$ agonists and GABA-uptake inhibitors, respectively.[12,14,16,20,21]

**Chart I**




In continuation of these studies, we are examining the correlation between conformational mobility, absolute stereochemistry, and biological activity of chiral compounds related to GABA. While GABA receptor sites and GABA-uptake mechanisms normally bind the enantiomers of conformationally restricted GABA analogues with a high degree of stereoselectivity, enantiomers of flexible chiral GABA analogues usually interact with these recognition sites with similar affinity.[22-25]

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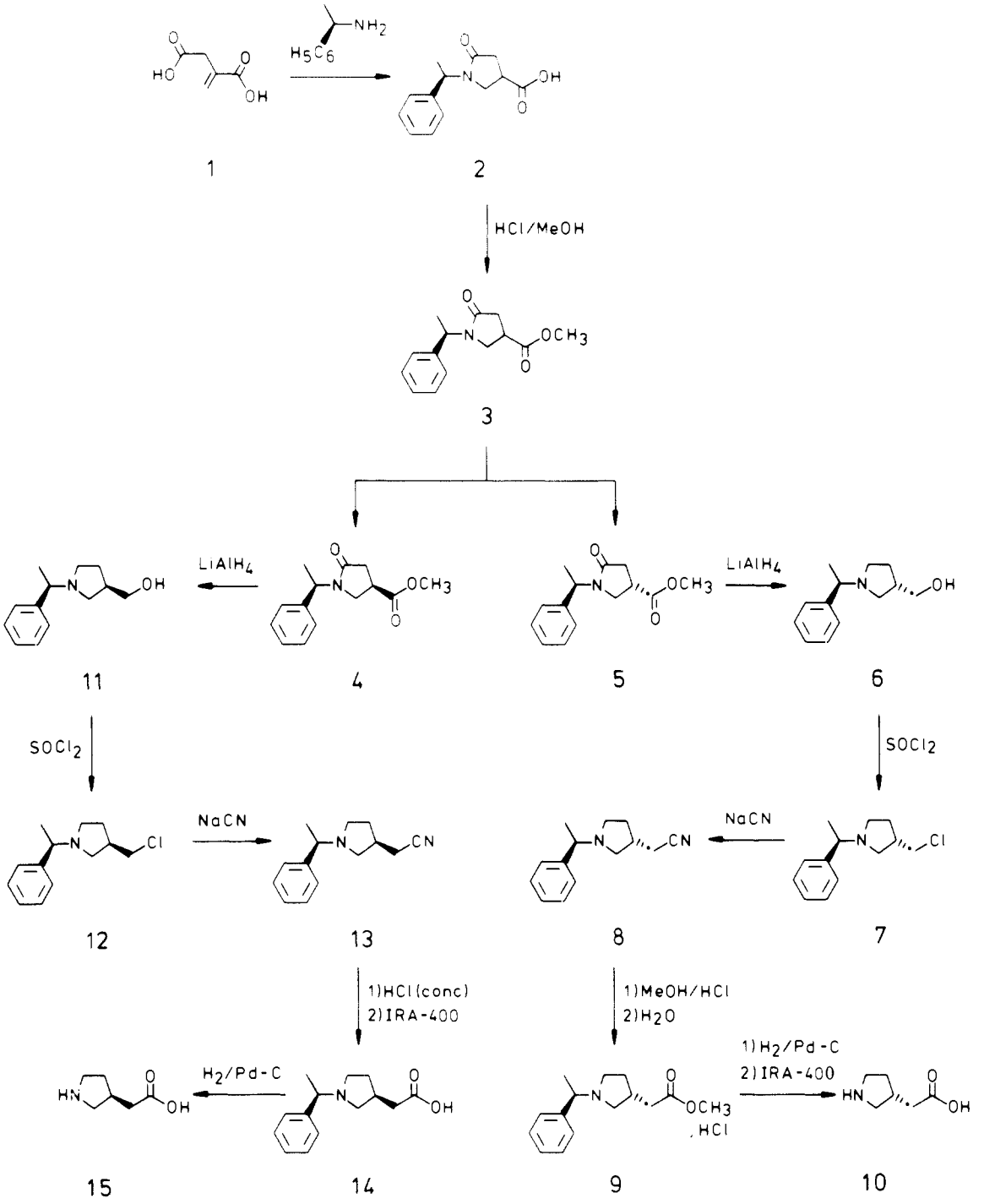
**Scheme I**




The structure–activity relationships for the cyclic GABA analogues (*RS*)-piperidine-3-acetic acid and (*RS*)-homo-β-proline emphasize the pronounced ligand/inhibitor specificity of GABA synaptic mechanisms. Thus, the former compound does not show significant affinity for any of these mechanisms, whereas (*RS*)-homo-β-proline is an inhibitor of $GABA_A$ as well as $GABA_B$ receptor binding and of GABA uptake.[15,26] This paper describes the synthesis and the determination of the absolute stereochemistry and in vitro effects on GABA synaptic mechanisms of (*R*)-(−)- (**10**) and (*S*)-(+)-homo-β-proline (**15**).

## Results

**Synthesis.** Conjugate addition of (*R*)-(+)-1-phenylethylamine to itaconic acid (**1**) and subsequent cyclization gave a diastereomeric mixture of carboxylic acids **2**, which were converted into methyl esters **3** and then separated by preparative HPLC to give **4** and **5** (Scheme I). The purity and stereochemical homogeneity of **4** and **5** were established by TLC analysis and by GC/MS on the basis of the reconstructed ion chromatograms (*m*/*z* 247). The absolute stereochemistry of **4** and **5** was determined by an X-ray crystallographic analysis of **5**. The LAH reduction of **4** and **5** to **11** and **6**, respectively, occurred without any detectable epimerization at the centers α to the ester groups as monitored by $^1H$ NMR spectroscopy (see the

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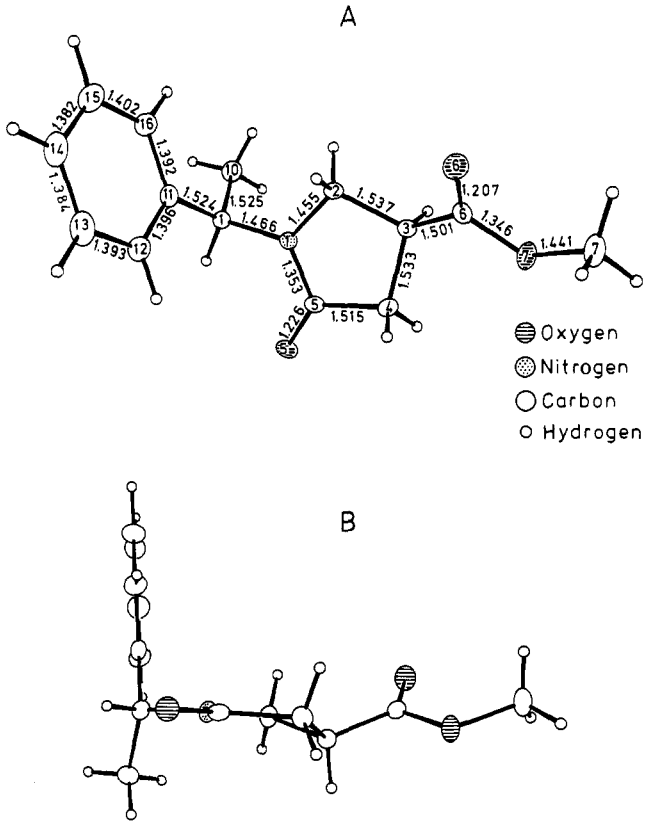


**Figure 1.** Perspective drawings from two different views of the molecular structure of methyl (3*S*)-1-[(*R*)-1-phenylethyl]-5-oxo-3-pyrrolidinecarboxylate (**5**) in the crystalline state, showing atom numbering and bond distances (Å) between non-hydrogen atoms. Esd's are 0.002–0.003 Å. Thermal ellipsoids for non-hydrogen atoms are scaled to 50% probability; hydrogen atoms are represented as spheres of arbitrary radius.

Experimental Section). Optical rotations are given for the compounds **4–15** except for chloromethyl compounds **7** and **12** and nitriles **8** and **13**. These compounds were isolated as oils, which could not be purified by chromatography to absolute TLC homogeneity. Attempts to establish the optical purity of the final products **10** and **15** chromatographically were unsuccessful. The assumed optical purity of **10** and **15** is based on (1) the numerically very similar optical rotations for **10** and **15** and (2) the fact that none of the synthetic steps between the optically homogeneous intermediates **6** and **11** and the final products **10** and **15**, respectively, are likely to involve the chiral centers of the molecules.

The compounds **11** and **6** were converted in two steps into their amino nitriles **13** and **8**, respectively. Two different synthetic routes were used to convert these intermediates into (*S*)-(+)-homo-β-proline (**15**) and (*R*)-(−)-homo-β-proline (**10**). Thus, amino acid **14**, prepared from **13** by treatment with concentrated hydrochloric acid and isolated by ion exchange, was deprotected by hydrogenolysis to give **15**. The preparation of **10** from **8** via the corresponding imino ester dihydrochloride and compound **9** followed by hydrogenolysis of **9** and hydrolysis of the reaction product on a strongly basic ion-exchange resin did, however, prove to be a more convenient synthetic route. Amino acids **10** and **15** were highly hygroscopic. Only **15** could be isolated in a semicrystalline form, and all attempts to recrystallize **15** were unsuccessful.

The structures of all new compounds were established on the basis of spectroscopic methods and supported by elemental analyses.

**X-ray Analysis.** In order to establish the absolute configurations of **10** and **15** an X-ray analysis of **5** was carried out.

Perspective drawings of the molecule of **5** with atomic numbering scheme and bond lengths between non-hydrogen atoms are shown in Figure 1. The absolute configuration of **5** at C3 was assigned (*S*) on the basis of the known stereochemistry at C1 (*R*) (Figure 1). The torsion angles within the pyrrolidine ring show that the ring adopts a flattened-envelope conformation ($C_s$ symmetry). The envelope "flap" C3 deviates 0.36 Å from the best plane through the remaining ring atoms (plane I). The methoxycarbonyl group at C3 is in an equatorial position. The benzene and the pyrrolidine rings face each other; the dihedral angle between the least-squares plane of the benzene ring and plane I is 84°.

Valency angles, torsion angles, and intermolecular distances between non-hydrogen atoms less than 3.3 Å and the geometry of the shortest C–H···O bonding interactions[27] are given as supplementary material.

**Table I.** Some in Vitro Effects of GABA and Some GABA Analogues

| compound | in vitro effects: $IC_{50}$,[a] μM | | |
|---|---|---|---|
| | $GABA_A$ binding | $GABA_B$ binding | GABA uptake |
| GABA | 0.033 | 0.030 | 3.0 |
| (*RS*)-homo-β-proline | 0.2 | 14.1 | 2.5 |
| (*R*)-(−)-homo-β-proline (**10**) | 0.07 | >100 | 1.6 |
| (*S*)-(+)-homo-β-proline (**15**) | 0.9 | 7.8 | 2.7 |

[a] $IC_{50}$ values for $GABA_A$ receptor binding were determined by incubation of rat brain synaptic membranes for 15 min at 4 °C in 0.05 M Tris–citrate buffer (pH 7.1) containing 0.005 μM [$^3$H]-GABA and the analogues at different concentrations (maximum 100 μM). In the $GABA_B$ binding assay the synaptic membranes were incubated for 15 min at 20 °C in 0.05 M Tris–HCl buffer (pH 7.4) containing 0.0025 M $CaCl_2$ and 0.005 μM [$^3$H]GABA. In the GABA uptake assay the synaptosome suspensions were preincubated for 10 min at 25 °C with 1.9 mL of phosphate medium containing the inhibitor. Then [$^3$H]GABA was added to give a final GABA concentration of 0.05 μM, and the incubation was continued for a further 10 min. $IC_{50}$ values were determined as described earlier.[39] The value at each concentration of analogue was the average of three experiments, and SEM's were less than 10%.

**In Vitro Pharmacology.** The in vitro affinities of (*R*)-(−)- (**10**) and (*S*)-(+)-homo-β-proline (**15**) for $GABA_A$ and $GABA_B$ receptor sites and for synaptosomal (neuronal) GABA uptake mechanisms were determined and compared with those of GABA and (*RS*)-homo-β-proline (Table I). The moderately potent affinity of (*RS*)-homo-β-proline for $GABA_B$ receptor sites was shown to reside exclusively in the *S* enantiomer (**15**). Interestingly, the enantiomers of homo-β-proline were found to interact with the $GABA_A$ receptor sites with the opposite stereoselectivity. Thus, (*R*)-(−)-homo-β-proline (**10**) was more than 1 order of magnitude more potent than the (*S*)-(+) isomer (**15**), showing an affinity comparable to that of GABA at $GABA_A$ receptor sites (Table I). (*RS*)-Homo-β-proline binds to the neuronal GABA uptake system with an affinity similar to that of GABA itself[15,26] (Table I). In light of the high affinity of this GABA analogue for the neuronal GABA transport carrier, the small potency difference, and thus low eudismic ratio,[28] observed for **10** and **15** with respect to binding to this system is remarkable.[29,30]

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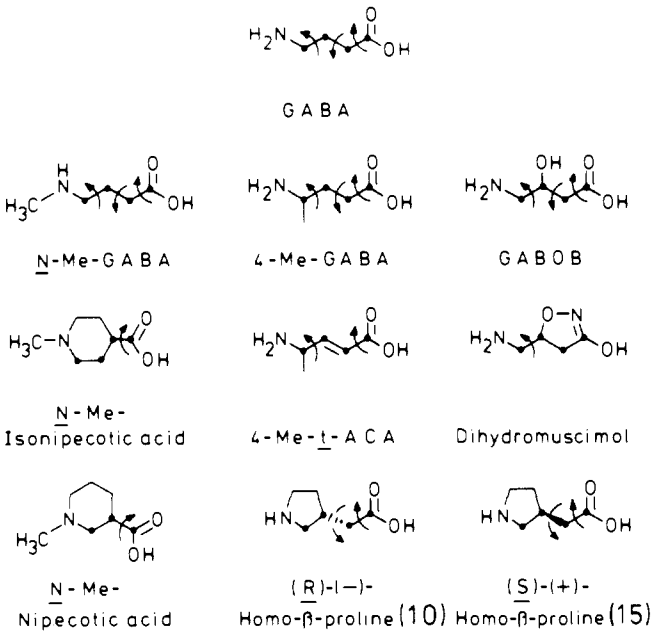
Chart II




**Discussion.** Structure–activity studies on model compounds in which different parts of the backbone and/or the basic and acid groups of GABA have been incorporated into a variety of ring structures have shed some light on the active conformations of GABA required for binding to different GABA synaptic mechanisms in the CNS.[14-16,25,31-33] A number of these conformationally restricted GABA analogues interact specifically with certain synaptic GABA recognition sites, probably reflecting that GABA adopts different conformations during its interaction with these synaptic sites. All of these conformationally restricted model compounds do, however, contain groups or ring residues in addition to the GABA or GABA-like structure elements. The specificity of such model compounds probably reflects that the biomolecules of the GABA-operated mechanisms under study have distinctly different abilities to accomodate these additional structure elements of the model compounds.

Earlier structure–activity studies on chiral GABA analogues with established absolute configuration have shown a correlation between the degree of stereoselectivity and conformational mobility of these compounds.[25] Thus, the *S* and *R* enantiomers of *trans*-4-aminopent-2-enoic acid (4-Me-*t*-ACA), but not the more flexible analogues (*S*)- and (*R*)-4-aminovaleric acid (4-Me-GABA) (Chart II), show a high degree of stereoselectivity with respect to interaction with GABA synaptic mechanisms.[12,19,34] Similarly, (*S*)- and (*R*)-dihydromuscimol, but not the enantiomers of 3-hydroxy-4-aminobutyric acid (GABOB), show very different effects on GABA receptors and GABA-uptake mechanisms.[23] It is reasonable to assume that the flexible GABA analogues such as the enantiomers of 4-Me-GABA and GABOB, in contrast to their conformationally restricted analogues, can relatively easily relieve the strain caused by unfavorable steric arrangements of groups during the interaction with receptors and transport carriers.

In 4-Me-*t*-ACA and dihydromuscimol the structure elements corresponding to the C2–C3 and the C1–C2–C3 bonds, respectively, of the backbone of GABA are conformationally constrained. In homo-β-proline the structure element corresponding to the C3–C4–N bonds of GABA is conformationally restricted (Chart II). Whereas the affinity of (*RS*)-homo-β-proline for $GABA_B$ receptor sites was shown to reside exclusively in the *S* enantiomer (**15**), $GABA_A$ receptor sites proved capable of binding both enantiomers, although (*R*)-homo-β-proline (**10**) interacts with this population of receptor sites with substantially higher affinity (Table I). These studies have disclosed considerable differences between $GABA_A$ and $GABA_B$ receptor sites in terms of capacity for accomodating steric bulk in the vicinity of the amino group of GABA. (*R*)-Homo-β-proline (**10**) apparently is capable of adopting conformation(s) which especially reflect the $GABA_A$-receptor-active conformation of GABA. Whereas the ring structure of (*S*)-homo-β-proline (**15**) to some extent can be accommodated by $GABA_B$ receptor sites, this structure element of the *R* enantiomer (**10**) effectively prevents binding to these sites (Table I).

The enantiomers of homo-β-proline (**10** and **15**) (Table I) and of 4-Me-GABA[12,19,25] show very similar structure–activity relationships with respect to the GABA-uptake systems. In both cases there are only minor differences between the effects of the enantiomers, and all four compounds show potencies comparable with that of GABA itself. These studies are consistent with the view that the GABA transport carrier, or rather the structure element of this system which binds the amino group of GABA, shows a higher degree of steric and stereochemical tolerance than, for example, the GABA receptor sites. This conclusion is supported by the observations that N-methylation of GABA and isonipecotic acid results in almost complete loss of affinity of these analogues for $GABA_A$ receptors, whereas *N*-Me-GABA and *N*-Me-nipecotic acid are effective inhibitors of GABA uptake, though not quite as potent as the parent compounds.[15,33,35] Furthermore, some very potent GABA-uptake inhibitors have been developed by incorporation of certain bulky substituents on the amino group of nipecotic acid.[15,20,21,36]

## Experimental Section

**Chemistry. General Procedures.** Melting points are corrected and were determined in capillary tubes. Elemental analyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Copenhagen, Denmark and by P. Hansen, Chemical Laboratory II, University of Copenhagen. IR spectra were recorded on a Perkin-Elmer grating infrared spectrophotometer (Model 781). The 60-MHz $^1H$ NMR spectra were recorded on a Varian 360L spectrometer. The 90-MHz $^1H$ NMR spectra were recorded on a JEOL FX 90Q spectrometer. The 250-MHz $^1H$ NMR and $^{13}C$ NMR spectra were recorded on a Bruker AM 250 instrument. $Me_4Si$ was used as an internal standard except for the compounds dissolved in $D_2O$, where, unless otherwise stated, 3-(trimethylsilyl)propanesulfonate was used. A Perkin-Elmer polarimeter 241 was used. MS spectra were recorded on a Finnigan MAT system (Model 4515B) by F. Vester, Danish Civil Defence Analytical-Chemical Laboratory. FAB-MS

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were recorded by J. Møller, Chemical Institute, University of Odense on a Varian (Palo Alto, CA) MAT 311A mass spectrometer fitted with an Ion Tech FAB-11 NF saddle field atom gun using argon atoms at 8 keV. Positive-ion FAB spectra were recorded into a Varian SS 200 data system, calibrated in the electron-impact mode. Samples were dissolved in glycerol. Thin-layer chromatography (TLC) and flash chromatography were performed with silica gel $F_{254}$ plates (Merck) and silica gel (60 H), respectively. Unless otherwise stated, evaporations were performed at ca. 15 mmHg on a rotatory evaporator.

**(3*RS*)-1-[(*R*)-1-Phenylethyl]-5-oxo-3-pyrrolidinecarboxylic Acid (2).** A mixture of itaconic acid (51 g, 392 mmol) and (*R*)-(+)-phenylethylamine (Fluka Chemical 77880, 50 mL, 393 mmol) was heated at 160 °C for 4 h to give crude **2** (88.5 g, ca. 97%). An analytical sample was recrystallized (EtOH–$H_2O$). Mp: 198–202 °C. IR (KBr): 3600–3300 (m), 3300–2700 (m), 1735 (s), 1640 (s), 1490 (s), 1450 (s) $cm^{-1}$. $^1H$ NMR [60 MHz, $CDCl_3$–DMSO (1:4)]: $\delta$ 7.2 (5 H, s), 5.3 (1 H, q), 3.7–2.9 (3 H, m), 2.7–2.3 (2 H, m), 1.45 (3 H, d). Anal. ($C_{13}H_{15}NO_3$): C, H, N.

**Methyl (3*RS*)-1-[(*R*)-1-Phenylethyl]-5-oxo-3-pyrrolidinecarboxylate (3).** A mixture of **2** (97.5 g, 418 mmol) and acetyl chloride (120 mL) in methanol (600 mL) was refluxed with stirring for 16 h. The solution was concentrated by evaporation, and upon addition of saturated aqueous sodium bicarbonate solution (500 mL), the mixture was extracted with ethyl acetate (3 × 500 mL). The combined ethyl acetate phases were dried ($MgSO_4$) and evaporated to give an oil (74 g, ca. 76%). $R_f$ 0.20 and 0.13 [toluene–ethyl acetate (2:1)].

**Methyl (3*R*)-1-[(*R*)-1-Phenylethyl]-5-oxo-3-pyrrolidinecarboxylate (4) and Methyl (3*S*)-1-[(*R*)-1-Phenylethyl]-5-oxo-3-pyrrolidinecarboxylate (5).** Diastereomeric mixture **3** (20 g, 80 mmol) was subjected to preparative HPLC on a Waters Prep-LC system 500 A with a silica gel column (PrepPak(R)-500/silica, flow rate 150 mL/min of toluene–ethyl acetate (2:1)).

Compound **4** was isolated as a TLC-pure oil (8.5 g, ca. 43%). $R_f$: 0.20 [toluene-ethyl acetate (2:1)]. $^1H$ NMR (90 MHz, $CDCl_3$): $\delta$ 7.26 (5 H, s), 5.45 (1 H, q), 3.67 (3 H, s), 3.53–3.25 (1 H, m), 3.16–2.97 (2 H, m), 2.68–2.58 (2 H, m), 1.50 (3 H, d). $^{13}C$ NMR ($CDCl_3$): 172.73, 172.23, 139.49, 128.24, 127.20, 126.62, 78.43, 75.57, 51.90, 48.84, 44.10, 35.64, 34.08, 15.61. $[\alpha]^{25}_D$: +51.3° (*c* 2.1, ethyl acetate). MS (70 eV): *m/z* 247 (61, M + H), 232 (70), 160 (25), 156 (30), 146 (22), 132 (13), 118 (34), 105 (100), 104 (62), 103 (20), 91 (26), 79 (28), 77 (37). Anal. ($C_{14}H_{17}NO_3$): C, H, N.

Compound **5** was isolated as TLC-pure crystals (9.0 g, 45%) (ethyl acetate–light petroleum) mp: 70 °C. IR (KBr): 3650–3150 (m), 3100–2800 (m), 1735 (s), 1670 (s), 1500 (m), 1425 (s) $cm^{-1}$. $^1H$ NMR (90 MHz, $CDCl_3$): $\delta$, 7.26 (5 H, s), 5.44 (1 H, q), 3.60 (3 H, s), 3.51–3.32 (1 H, m), 3.26–3.03 (2 H, m), 2.70–2.60 (1 H, m), 1.50 (3 H, d). $^{13}C$ NMR ($CDCl_3$): $\delta$ 172.73, 171.62, 139.30, 128.24, 127.27, 126.75, 78.43, 75.57, 51.96, 48.84, 44.36, 35.84, 34.08, 16.00. $[\alpha]^{25}_D$= +109.6° (*c* 2.6, ethyl acetate). MS (70 eV): *m/z* 247 (61, M + H), 232 (65), 160 (30), 156 (29), 146 (24), 132 (14), 118 (33), 105 (100), 104 (57), 103 (21), 91 (26), 79 (23), 77 (39). Anal. ($C_{14}H_{17}NO_3$): C, H, N.

**(3*S*)-1-[(*R*)-1-Phenylethyl]-3-(hydroxymethyl)pyrrolidine (6).** A solution of **5** (5.0 g, 20 mmol) in anhydrous ether (10 mL) was added slowly to a slurry of lithium aluminum hydride (1.15 g, 30 mmol) in anhydrous ether (30 mL) and the mixture was refluxed with stirring for 3.5 h. The reaction mixture was cooled on ice and the lithium aluminum complex was decomposed by the slow addition of ether–water (4:1, 20 mL). The resulting slurry was stirred for 1 h and then filtered by suction. The reaction mixture was evaporated and the residue was subjected to flash chromatography. Elution with ethyl acetate gave **6** (3.5 g, ca. 85%) as an oil. $[\alpha]^{25}_D$: +44.0° (*c* 2.3, ethyl acetate). $^1H$ NMR (250 MHz, $CDCl_3$): $\delta$ 7.26 (5 H, m), 3.73 (1 H, br s), 3.63 (1 H, dd, $J_{gem}$ = −10.0 Hz, $J_{vic}$ = 4.5 Hz), 3.47 (1 H, dd, $J_{gem}$ = −10.0 Hz, $J_{vic}$ = 5.1 Hz), 3.17 (1 H, q, $J$ = 6.6 Hz), 2.67 (1 H, dd), 2.50 (2 H, m), 2.33 (2 H, m), 1.94 (1 H, m), 1.58 (1 H, m), 1.37 (3 H, d, $J$ = 6.6 Hz). Anal. ($C_{13}H_{19}NO$): C, H, N. The complete absence of a resonance signal centered at $\delta$ 2.87 (see $^1H$ NMR data for **11**) in the $^1H$ NMR spectrum of **6** indicated that it did not contain detectable amounts of the diastereomeric compound **11**.

**(3*S*)-1-[(*R*)-1-Phenylethyl]-3-(chloromethyl)pyrrolidine (7).** To a refluxing solution of **6** (3.0 g, 14.6 mmol) in chloroform (40 mL) was slowly added a solution of thionyl chloride (60 mL, 126 mmol) in chloroform (10 mL). When the addition was completed, reflux was continued for 2 h. The reaction mixture was evaporated, and the residue was dissolved in water (25 mL). The pH was adjusted to 8 by addition of 2 M sodium hydroxide (ca. 20 mL), and the resulting mixture was extracted with ethyl acetate (3 × 125 mL). The combined ethyl acetate extracts were dried ($MgSO_4$) and evaporated and then subjected to flash chromatography. Elution with ethyl acetate–toluene (1:1) gave **7** (3.0 g, ca. 92%) as an oil. $^1H$ NMR (60 MHz, $CCl_4$): 7.2 (5 H, s), 3.5–3.3 (2 H, b), 3.1 (1 H, q), 2.6–2.2 (5 H, m), 1.5–1.2 (5 H, m). Anal. ($C_{13}H_{18}NCl$): C, H, N.

**(3*R*)-1-[(*R*)-1-Phenylethyl]-3-(cyanomethyl)pyrrolidine (8).** A mixture of **7** (2.5 g, 11.2 mmol), tricaprylmethylammonium chloride (150 mg), sodium cyanide (2.9 g, 59.2 mmol), and water (5 mL) was stirred at 100 °C for 48 h. The mixture was extracted with ethyl acetate (3 × 30 mL), and the combined and dried extracts were evaporated. The residue was subjected to flash chromatography (gradient eluted with toluene to toluene–ethyl acetate (4:1)) to give **8** (1.6 g, ca. 67%) as an oil. $^1H$ NMR (60 MHz, $CCl_4$): $\delta$ 7.2 (5 H, s), 3.1 (1 H, m), 3.6–2.0 (7 H, m), 1.4–1.0 (5 H, m). Anal. ($C_{14}H_{18}N_2$): C, H, N.

**(3*R*)-Methyl 1-[(*R*)-1-Phenylethyl]pyrrolidine-3-acetate Hydrochloride (9).** A solution of acetyl chloride (15 mL, 210 mmol), methanol (50 mL), and **8** (1.5 g, 7 mmol) was left at room temperature for 48 h. Upon evaporation, the residue was dissolved in water (10 mL) and stirred for 1 h. Upon addition of saturated aqueous sodium bicarbonate (200 mL), the mixture was extracted with ethyl acetate (3 × 200 mL). The combined and dried ($MgSO_4$) extracts were evaporated. The residue was purified with charcoal in acetone (50 mL) and recrystallized (2-propanol–ether), yielding **9** (1.1 g, 56%). Mp: 131 °C. $[\alpha]^{25}_D$: +33.7° (*c* 2.1, ethanol). $^1H$ NMR (60 MHz, $CDCl_3$): $\delta$ 7.25 (5 H, s), 3.6 (3 H, s), 3.15 (1 H, q), 2.9–1.7 (7 H, m), 1.5–1.0 (5 H, m). Anal. ($C_{15}H_{21}NO_2$,HCl): C, H, N.

**(*R*)-Pyrrolidine-3-acetic Acid [(*R*)-(−)-Homo-β-proline] (10).** A solution of **9** (0.5 g, 1.8 mmol) in methanol (20 mL) was hydrogenated (294 kPa) in a Parr hydrogenation apparatus using Pd–C (10%, 100 mg) as a catalyst. The reaction mixture was filtered and evaporated to dryness. The residue was dissolved in water (2 mL) and transferred to a column containing an ion-exchange resin [Amberlite IRA-400 (OH)] for 2 h. Elution with aqueous acetic acid (6%) gave **10** (140 mg, 62%) as an amorphous product. Mp: 160–165 °C. $^1H$ NMR (90 MHz, $D_2O$): $\delta$ 3.47–3.16 (3 H, m), 2.96–2.43 (2 H, m), 2.36–2.05 (3 H, m), 1.70–1.45 (1 H, m). $[\alpha]^{25}_D$: −9.3° (*c* 1.0, $H_2O$). The IR spectrum (KBr) of **10** was virtually identical with that of **15**.

**(3*R*)-1-[(*R*)-1-Phenylethyl]-3-(hydroxymethyl)pyrrolidine (11).** Compound **11** was synthesized from **4** (5.0 g, 20 mmol) as described above for **6**. Instead of purifying crude **11** by flash chromatography, the product was recrystallized (ethyl acetate–light petroleum) to give **11** (3.3 g, 80%). Mp: 88 °C. $[\alpha]^{25}_D$: +65.6° (*c* 2.3, ethyl acetate). IR (KBr): 3600–3300 (s), 3300–3100 (s), 2960 (s), 2920 (s), 2860 (s), 2800 (s), 1490 (s), 1450 (s) $cm^{-1}$. $^1H$ NMR (250 MHz, $CDCl_3$): $\delta$ 7.27 (5 H, m), 3.77 (1 H, br s), 3.54 (1 H, dd, $J_{gem}$ = −10.0 Hz, $J_{vic}$ = 4.5 Hz), 3.44 (1 H, dd, $J_{gem}$ = −10.0 Hz, $J_{vic}$ = 5.1 Hz), 3.16 (1 H, q, $J$ = 6.6 Hz), 2.87 (1 H, dt), 2.49 (1 H, dd, $J_{gem}$ = −9.4 Hz, $J_{vic}$ = 7.2 Hz), 2.37 (1 H, dd, $J_{gem}$ = −9.4 Hz, $J_{vic}$ = 3.6 Hz), 2.27 (2 H, m), 1.94 (1 H, m), 1.66 (1 H, m), 1.36 (3 H, d, $J$ = 6.6 Hz). Anal. ($C_{13}H_{19}NO$): C, H, N. The complete absence of a resonance signal centered at $\delta$ 2.67 (see $^1H$ NMR data for **6**) in the $^1H$ NMR spectrum of **11** indicated that it did not contain detectable amounts of the diastereomeric compound **6**.

**(3*R*)-1-[(*R*)-1-Phenylethyl]-3-(chloromethyl)pyrrolidine (12).** Compound **12** was prepared from **11** (3.0 g, 14.6 mmol) following a procedure analogous with that described above for **7**. Obtained was **12** (3.2 g, ca. 98%) as an oil. The IR spectrum (film) of **12** was virtually identical with that of **7**. Anal. ($C_{13}H_{18}NCl$): C, H, N.

**(3*S*)-1[(*R*)-1-Phenylethyl]-3-(cyanomethyl)pyrrolidine (13).** Compound **13** was prepared from **12** (3.0 g, 13.4 mmol) following a procedure analogous with that described above for **8**. Obtained was **13** (1.7 g, ca. 60%) as an oil. The IR spectrum was virtually identical with that of **8**. Anal. ($C_{14}H_{18}N_2$): C, H, N.

**(3*S*)-1-[(*R*)-1-Phenylethyl]pyrrolidine-3-acetic Acid (14).** A solution of **13** (950 mg, 4.4 mmol) in concentrated hydrochloric acid (10 mL) was refluxed for 5 h and then evaporated. Water (10 mL) was added, and the solution was passed through a column containing an ion-exchange resin [Amberlite IRA 400 (OH)] using acetic acid (6%) as an eluent. The eluate was evaporated to give **14** (430 mg, ca. 42%) as an oil. $[\alpha]^{25}_D$: +53.1° (*c* 2.0, ethanol). $^{13}C$ NMR ($D_2O$): δ 13.40, 24.00, 29.25, 35.95, 47.81, 52.57, 60.77, 123.08, 124.82, 125.14, 131.63, 175.29. MS: *m/z* 234 (87%, M + H), 174 (5), 130 (27), 128 (6), 106 (12), 105 (100), 91 (6), 68 (6), 30 (6). Anal. ($C_{14}H_{19}NO_2$): C, H, N.

**(*S*)-Pyrrolidine-3-acetic Acid [(*S*)-(+)-Homo-β-proline] (15).** A solution of **14** (360 mg, 2.8 mmol) in water (10 mL) was hydrogenated (294 kPa) in a Parr hydrogenation apparatus using Pd–C as a catalyst (5%, 150 mg). The reaction mixture was filtered and evaporated to give **15** as a very hygroscopic semicrystalline product. Mp: 178 °C. IR (KBr): 2980 (m), 2750 (m), 2450 (m), 1550 (s), 1390 (s) $cm^{-1}$. $^1H$ NMR (90 MHz, $D_2O$): δ 3.48–3.18 (3 H, m), 2.97–2.43 (2 H, m), 2.32–2.07 (3 H, m), 1.72–1.48 (1 H, m). $[\alpha]^{27}_D$: +9.6° (*c* 1.0, $H_2O$). Anal. ($C_6H_{11}NO_2 \cdot H_2O$): C: calcd, 48.96; found, 49.68; H: calcd, 8.90; found, 8.46; N: calcd, 9.52; found, 10.06.

**X-ray Crystallographic Analysis of Methyl (3*S*)-1-[(*R*)-1-Phenylethyl]-5-oxo-3-pyrrolidinecarboxylate (5).** The colorless crystals used for X-ray examination were obtained from an ethyl acetate–light petroleum solution. Crystal data are as follows: $C_{14}H_{17}NO_3$, $M_r$ = 247.29, monoclinic, space group $P2_1$ (No. 4), *a* = 10.572 (3) Å, *b* = 5.974 (4) Å, *c* = 10.127 (2) Å, β = 91.53 (2)°, *V* = 639.4 Å$^3$, *Z* = 2, $D_c$ = 1.284 g $cm^{-3}$, μ(Mo Kα) = 0.84 $cm^{-1}$.

A single crystal of the size 0.10 × 0.25 × 0.35 mm was used for the determination of the unit-cell parameters and for the collection of intensity data. The measurements were performed at −110 (±5) K on an Enraf-Nonius CAD-4 diffractometer. The crystal was cooled in a stream of nitrogen gas provided by an Enraf-Nonius low-temperature device. The temperature was kept constant within 1 K during the experiment. Graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) was used. Intensities of 2710 reflections ($\theta < 33°$, $h\ 0 \rightarrow 16$, $k\ 0 \rightarrow 9$, $l\ -15 \rightarrow 15$) were measured using the ω scan mode. Intensities of three reflections measured every $10^4$ s showed no unusual variation over the course of the experiment. The 2710 reflections were merged resulting in 2597 reflections of which the 1875 with $|F_o|^2 \geq 3\sigma(|F_o|^2)$, $\sigma(|F_o|^2) = [\sigma_c^2(|F_o|^2) + (0.05|F_o|^2)^2]^{1/2}$ were classified as observed reflections and used in the subsequent structure determination and refinement. The $\sigma_c(|F_o|^2)$ was calculated from counting statistics.

The structure was solved by use of the program MULTAN 11/82 and refined by full-matrix least-squares methods. The quantity minimized was $\sum w(|F_o| - k|F_c|)^2$, where weights were initially taken as unity. The positions of all hydrogen atoms were obtained from intermediate difference maps.

In subsequent full-matrix least-squares calculations, an overall scale factor, atomic coordinates for all atoms, and anisotropic thermal parameters for the non-hydrogen atoms were refined. The *B* values for each hydrogen atom were fixed at values 1.3 times $B_{eq}$ for the carbon atom to which it is attached. The weights used in the final cycle of refinement were $w = 1/\sigma^2(|F_o|)$, where $\sigma(|F_o|) = \sigma(|F_o|^2)/2|F_o|$. On the last cycle of least-squares refinement the maximum shift/error was 0.01. The final *R* and $R_w$ values are 0.038 and 0.044, respectively. Residual electron density in final difference Fourier maps is within −0.19 to 0.21 e Å$^{-3}$.

Neutral atomic scattering factors were those incorporated in the program system. All calculations were carried out by using the Enraf-Nonius Structure Determination Package.[37]

Table II lists the final positional and equivalent isotropic or isotropic thermal parameters. Lists of structure factors and anisotropic thermal parameters of the non-hydrogen atoms are available as supplementary material.

**Preparation of Synaptic Membranes.** Rat brain membranes were prepared as described earlier.[38,39] Whole brains from Sprague–Dawley male and female rats killed by decapitation were rapidly homogenized in 10 volumes of 0.32 M sucrose at 0–4 °C in a glass homogenizer fitted with a Teflon pestle (1000 rpm). The homogenate was centrifuged for 10 min at 1000*g*. The pellet was discarded and the supernatant was centrifuged for 20 min at 20000*g*. The pellet was resuspended in water and dispersed with a 100-W MSE Ultrasonic disintegrator (setting 6) for 2 × 15 s. The suspension was centrifuged at 8000*g* for 20 min, and the supernatant and the upper buffy coat of the pellet were centrifuged at 48000*g* for 20 min. The resulting pellet was homogenized in water, centrifuged at 48000*g* for 20 min, resuspended in water, and frozen at −70 °C. After storage for at least 2 days at −20 °C the membranes were thawed and twice washed by suspension (Ultra-Turrax, Ika-Werke, F.R.G.) in water followed by centrifugation and then homogenized in water and frozen again.

**Binding Assays.** [$^3$H]GABA binding ($GABA_A$ binding) was studied as described earlier.[39] Aliquots of synaptic membranes (0.8–1.2 mg of protein) were incubated in triplicate in 2 mL of 50 mM Tris–citrate (pH 7.1) containing 5 nM [$^3$H]GABA. Different concentrations of GABA agonists were added. Nonspecific binding in the presence of 1 mM GABA was always subtracted. The specific binding was 80 ± 5% of the total binding. The $IC_{50}$ values were estimated by measuring the inhibition of at least four different concentrations of inhibitor and performing log-probit analysis of the results.

[$^3$H]GABA binding to bicuculline-insensitive binding sites in rat brains ($GABA_B$ binding) was studied by using essentially the method described earlier.[40] For the binding assay 1200-μL

**Table II.** Atomic Positions and Equivalent Isotropic or Isotropic Thermal Parameters (Å$^2$) for Methyl (3*S*)-1-[(*R*)-1-Phenylethyl]-5-oxo-3-pyrrolidinecarboxylate (5)[a]

| atom[b] | *x* | *y* | *z* | $B_{eq}/B_{iso}$[c] |
|---|---|---|---|---|
| N1 | 0.6680 (1) | 0.262[d] | 0.1328 (2) | 0.99 (2) |
| C2 | 0.6546 (2) | 0.0492 (3) | 0.2014 (2) | 1.06 (3) |
| C3 | 0.5117 (2) | 0.0368 (3) | 0.2245 (2) | 0.94 (3) |
| C4 | 0.4650 (2) | 0.2795 (3) | 0.2154 (2) | 1.06 (3) |
| C5 | 0.5652 (2) | 0.3963 (3) | 0.1363 (2) | 0.94 (3) |
| O5 | 0.5562 (1) | 0.5814 (3) | 0.0846 (1) | 1.25 (2) |
| C6 | 0.4777 (2) | −0.0824 (4) | 0.3491 (2) | 1.02 (3) |
| O6 | 0.5491 (1) | −0.1774 (3) | 0.4253 (1) | 1.48 (2) |
| O7 | 0.3517 (1) | −0.0716 (3) | 0.3643 (1) | 1.36 (2) |
| C7 | 0.3050 (2) | −0.1708 (4) | 0.4833 (2) | 1.66 (3) |
| C1 | 0.7826 (2) | 0.3201 (4) | 0.0621 (2) | 1.03 (3) |
| C10 | 0.7965 (2) | 0.1682 (4) | −0.0577 (2) | 1.37 (3) |
| C11 | 0.8960 (2) | 0.3239 (4) | 0.1582 (2) | 1.15 (3) |
| C12 | 0.9031 (2) | 0.4917 (4) | 0.2539 (2) | 1.52 (3) |
| C13 | 1.0035 (2) | 0.4988 (4) | 0.3458 (2) | 1.91 (4) |
| C14 | 1.0984 (2) | 0.3397 (5) | 0.3419 (2) | 1.86 (4) |
| C15 | 1.0928 (2) | 0.1726 (4) | 0.2477 (2) | 1.86 (4) |
| C16 | 0.9917 (2) | 0.1642 (4) | 0.1555 (2) | 1.48 (3) |
| H21 | 0.682 (2) | −0.072 (5) | 0.145 (2) | 1.4 |
| H22 | 0.705 (2) | 0.044 (5) | 0.283 (2) | 1.4 |
| H3 | 0.475 (2) | −0.046 (5) | 0.155 (2) | 1.2 |
| H41 | 0.386 (2) | 0.291 (5) | 0.173 (2) | 1.4 |
| H42 | 0.465 (2) | 0.348 (5) | 0.300 (2) | 1.4 |
| H71 | 0.335 (2) | −0.090 (5) | 0.555 (2) | 2.2 |
| H72 | 0.331 (2) | −0.328 (5) | 0.488 (2) | 2.2 |
| H73 | 0.215 (2) | −0.160 (5) | 0.475 (2) | 2.2 |
| H1 | 0.765 (2) | 0.470 (5) | 0.029 (2) | 1.3 |
| H101 | 0.721 (2) | 0.174 (5) | −0.114 (2) | 1.8 |
| H102 | 0.803 (2) | 0.017 (5) | −0.029 (2) | 1.8 |
| H103 | 0.869 (2) | 0.211 (5) | −0.102 (2) | 1.8 |
| H12 | 0.835 (2) | 0.597 (5) | 0.261 (2) | 2.0 |
| H13 | 1.006 (2) | 0.618 (5) | 0.412 (3) | 2.5 |
| H14 | 1.166 (2) | 0.339 (6) | 0.404 (3) | 2.5 |
| H15 | 1.162 (2) | 0.060 (6) | 0.239 (3) | 2.5 |
| H16 | 0.985 (2) | 0.047 (5) | 0.091 (3) | 1.9 |

[a] Estimated standard deviations are given in parentheses. [b] The first or first two digits in the identification number of a hydrogen atom refers to the atom to which the hydrogen is attached. [c] The hydrogen atoms were refined with fixed $B_{iso}$ values. $B_{eq} = {}^4/_3\sum_i\sum_j \beta_{ij}\mathbf{a}_i\cdot\mathbf{a}_j$. [d] This parameter was held constant to define the origin.

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portions of the membrane suspension were added to 100 μL of [$^3$H]GABA (final concentration 5 nM) and 100 μL of isoguvacine (final concentration 40 μM) followed by addition of 100 μL of the test compounds at various concentrations. Nonspecific binding, measured in the presence of 100 μM (*RS*)-baclofen, was always subtracted. The specific binding was 60 ± 4% of the total binding.

**Inhibition of Synaptosomal GABA Uptake.** A crude synaptosomal preparation was prepared from rat brains as described elsewhere indetail.[41] The synaptosome suspensions (500 μL) were preincubated for 10 min at 25 °C with 1.9 mL of phosphate medium containing the inhibitor. Then [$^3$H]GABA (100 μL) was added to give a final GABA concentration of 50 nM, and the incubation was continued for a further 10 min. The $IC_{50}$ values listed in Table I are the averages of at least two independent experiments in which the $IC_{50}$ values differed <10%.

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**Registry No.** 1, 97-65-4; (3*R*)-**2**, 99735-43-0; (3*S*)-**2**, 99735-44-1; **4**, 99735-45-2; **5**, 99735-46-3; **6**, 109960-55-6; **7**, 109960-56-7; **8**, 122408-84-8; **9**, 122408-85-9; **10**, 122442-01-7; 11, 99735-47-4; **12**, 99735-48-5; **13**, 122423-40-9; 14, 122408-86-0; **15**, 122442-02-8; GABA, 56-12-2; (*R*)-(+)-PhCH($NH_2$)$CH_3$, 3886-69-9.

**Supplementary Material Available:** Tables listing valency and torsion angles, intermolecular distances, bond lengths, and thermal parameters for **5** (5 pages). Ordering information is given on any current masthead page.

# Cephalosporin 3′-Quinolone Esters with a Dual Mode of Action[1]

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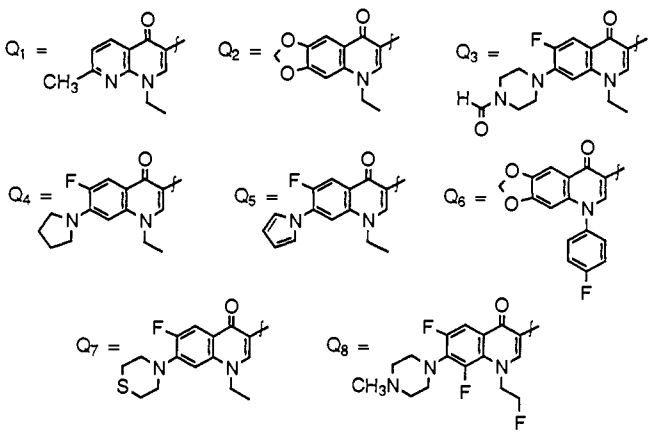
According to the generally accepted mechanism by which bacterial enzymes react with cephalosporins, opening of the β-lactam ring can lead to the expulsion of a 3′-substituent. A series of dual-action cephalosporins was prepared in which antibacterial quinolones were linked to the cephalosporin 3′-position through an ester bond in the expectation that, in addition to exerting their own β-lactam activity, these cephalosporins would act as prodrugs for the second antibacterial agent. Compared to parent cephalosporins in which the 3′-substituent was acetoxy, the bifunctional cephalosporins exhibited a broadened antibacterial spectrum, suggesting that a dual mode of action may indeed be operative.

β-Lactam antibiotics exert their biological activity by acylating active-site serine residues of the transpeptidases responsible for cross-linking peptidoglycan.[2,3] Similarly, acylation of active-site serine residues by β-lactams is a key step in the mechanism by which most β-lactamases inactivate these antibiotics through hydrolysis of the β-lactam ring.[4,5] In either case, when a cephalosporin contains a potential leaving group (X) at the 3′-position, that group is eliminated (Scheme I).[6]

Published evidence suggests that opening of the β-lactam ring correlates with elimination of the nucleofugal group, although the reaction is probably not concerted.[7-13] When the leaving group possesses intrinsic antibacterial activity, the cephalosporin should exhibit a dual mode of action.[14-17] In addition to providing its own β-lactam activity, the cephalosporin should act as a targeted prodrug for the second antibacterial agent, delivering it close to its site of action. The term "dual action" has been used to describe such cephalosporins.[14] This mechanism presents an opportunity to expand the antibacterial spectrum to include oranisms which are resistant to the third-generation cephalosporins. Thus, it may be possible to design cephalosporins with significant advantages over those currently in use.

The quinolones provide a broad class of antibacterials well-suited to the role of second agent for a number of reasons.

**Chart I.** Reference Structures




(1) The antibacterial spectra of the two classes are complementary, with quinolones being active against β-

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(1) A preliminary report of this work was presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, CA, October 23-26, 1988, Abstracts 441 and 442.
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