Synthetic Routes to 4-Amino-3-carboxy-β-carboline Derivatives: Incidental Formation of Novel Furo[3,4-c]-β-carbolin-2-ones Displaying High Affinities for the Benzodiazepine Receptor

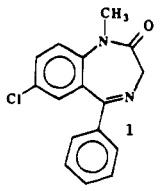
Gilbert Dorey,† Laurent Dubois,† Lia Prado de Carvalho,‡ Pierre Potier,† and Robert H. Dodd*,†

Institut de Chimie des Substances Naturelles and Institut Alfred Fessard, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette Cedex, France

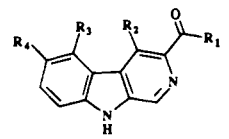
Received July 25, 1994®

The synthesis of the first 4-amino-3-carboxy-β-carboline derivative (**35**) is described. This synthesis is based on ozonolysis of the 4-vinyl-β-carboline-3-carboxamide **17** to give the 4-aldehyde **20** and potassium permanganate oxidation of the latter to the 4-carboxylic acid **34** followed by a DPPA-promoted Curtius rearrangement. During the course of these transformations, a number of furo[3,4-c]-β-carbolin-2-ones, differing in substituents at the C-10 position, were formed. While these β-carboline lactones (**15, 25, 26, 33**) generally displayed good affinities for the central type benzodiazepine receptor *in vitro* (IC_{50}'s in the 10–50 nM range), one compound, **29**, demonstrated an exceptionally high binding affinity (IC_{50} = 0.2 nM). Compound **29** was shown in electrophysiological and behavioral studies to act as a benzodiazepine receptor antagonist. The unusually high binding affinity of compound **29** corroborates the hypothesis that the benzodiazepine receptor preferentially recognizes the C-3 carbonyl function of 3-carboxy-β-carbolines in an *s-cis* conformation (i.e., the carbonyl oxygen on the same side as the pyridinyl nitrogen).

The benzodiazepine receptor of the mammalian central nervous system is known to mediate, via GABAergic mechanisms, the anticonvulsant, anxiolytic, and sedative hypnotic actions of the 1,4-benzodiazepines such as diazepam (Valium, **1**).[1–3] Among the wide variety of non-benzodiazepine molecules now known to bind with high affinity to the benzodiazepine receptor,[4] a particularly well-studied class is that of the 3-carboxy-β-carbolines, for example, β-CCM (**2a**)[5–10] and FG 7142 (**3**).[11–14]



1



2a $R_1 = OCH_3$; $R_2 = R_3 = R_4 = H$

2b $R_1 = OCH_2CH_3$; $R_2 = CH_2OCH_3$; $R_3 = OCH_2Ph$; $R_4 = H$

2c $R_1 = OCH_2CH_3$; $R_2 = CH_2OCH_3$; $R_3 = H$; $R_4 = OCH_2Ph$

3 $R_1 = NHCH_3$; $R_2 = R_3 = R_4 = H$

These compounds, however, demonstrate activities *in vivo* which are opposite to those of the clinically useful benzodiazepines, **2a** and **3** being anxiogenic in animals and man and respectively convulsant and proconvulsant in animals. The term inverse agonist is generally used to distinguish these types of benzodiazepine receptor ligands from the agonist benzodiazepines. This fundamental difference in activity between the simple β-carbolines **2a** and **3** and benzodiazepines of type **1** was the first indication that, though both classes of compound mutually and competitively displace each other from their binding site on the receptor, the binding site for inverse agonist β-carbolines may be distinct from, though possibly overlapping with, that of the benzodiazepines. Additional evidence points to the same conclusion. Thus, irreversible labeling of the benzodiazepine receptor with a photolabile[15–19] or alkylating[20] benzodiazepine diminishes subsequent binding of benzodiazepines but not of β-carbolines, while, conversely, photoaffinity labeling of the receptor with a β-carboline[21] diminishes β-carboline binding but spares that of benzodiazepines. *In vitro* binding experiments have, moreover, shown that addition of GABA to the incubation medium decreases the binding affinities of inverse agonist β-carbolines[22–24] but increases those of agonist benzodiazepines,[25,26] suggesting a very distinct allosteric coupling between the GABA and benzodiazepine binding sites on one hand and those of GABA/β-carboline binding sites on the other. It has also been observed that some substances (e.g., an octodecapeptide derived from the putative endogenous benzodiazepine receptor ligand DBI which displays inverse agonist properties) inhibit [^{3}H]-β-CCM more than [^{3}H]flunitrazepam binding to the receptor.[27]

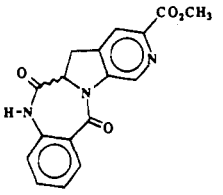
The possibility of distinct but overlapping binding sites for inverse agonist β-carbolines and agonist benzodiazepines has in fact now been integrated in some of the more recent models of ligand–receptor interactions.[28–31] These models, which owe much to the discovery of agonist β-carbolines (e.g., ZK 91296,[32] **2b**, and ZK 93423,[33] **2c**) suggest that the transformation of inverse agonist β-carbolines into agonist β-carbolines appears to be the result of extension of the 4-methoxymethyl and 5-(or 6-)benzyloxy groups of **2b,c** into the receptor binding domains generally occupied by the agonist benzodiazepines.

One way of testing these models is to build "hybrids" of β-carbolines and benzodiazepines, i.e., molecules

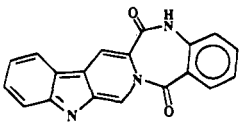
† Institut de Chimie des Substances Naturelles.
‡ Institut Alfred Fessard.
® Abstract published in *Advance ACS Abstracts*, December 1, 1994.

0022-2623/95/1838-0189$09.00/0

incorporating a portion of the important structural elements of each type of compound and thus able to interact with most or all of the receptor binding points of each class of compound. Such hybrids, by binding to both receptor domains at once, could be expected to exhibit very high affinities and/or selectivities of pharmacological action. It was these considerations which led us to synthesize a first hybrid molecule, compound **4**.[34] Its lack of binding affinity prompted the preparation of a second β-carboline–benzodiazepine hybrid, compound **5**, which this time displayed high affinity for the benzodiazepine receptor *in vitro*.[35]



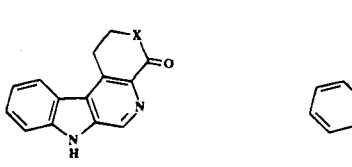
4



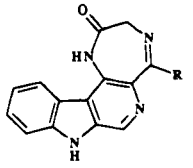
5

However, in addition to the relative instability of the tertiary amide bond of **5**, several structural features of this compound are not those which are known to favor interactions with the benzodiazepine receptor. Thus, contrary to the active β-carbolines of types **2a** and **3**, the β-carboline portion of **5** has neither an indolic NH[6,36] nor an accessible pyridinyl nitrogen atom.[36,37] Moreover, we have recently demonstrated, using the rigid analogues **6** and **7** of the active β-carbolines **2a** and **3**, respectively, that the *s-cis* conformation of the 3-carboxy group (i.e., in which the oxygen atom of the carbonyl group is *cis* to the pyridinyl nitrogen atom) is preferentially recognized by the benzodiazepine receptor.[38] The rigid *s-trans* geometry of the corresponding carbonyl function of compound **5** is thus another of its structural features which mitigates against an optimal interaction with the benzodiazepine receptor.

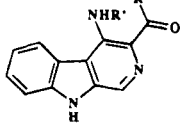
In an effort to remedy these problems, we have initiated the synthesis of hybrids of type **8**, in which the 1,4-benzodiazepine moiety is fused to the 3-4 face of the β-carboline. With this configuration, the indolic NH is restored, the pyridinyl nitrogen atom is disengaged, and, in the case of R=OH, the 3-carboxy group resulting from tautomerization assumes the receptor-preferred *s-cis* conformation. Moreover, the relative positions of the benzodiazepine and β-carboline moieties in **8** conform closely to the proposed models of ligand–receptor interactions for each type of molecule cited above.



6 X = O
7 X = NH



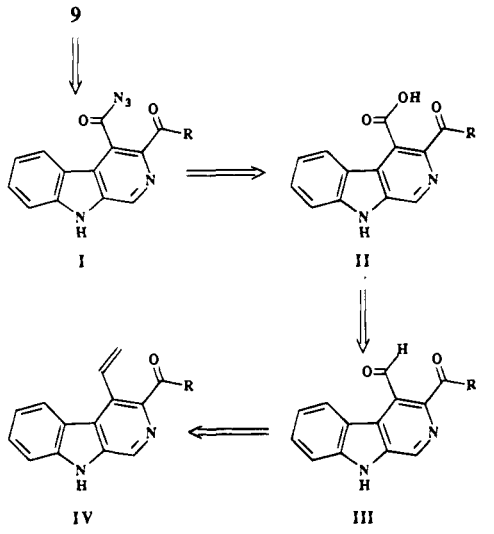
8 R = H, OH, aryl ...



9 R = H, O-alkyl, NH-alkyl, aryl ...
R' = protecting group

An obvious strategy for the synthesis of hybrids of type **8** would be condensation of a glycine derivative with a 3-carboxy-4-amino-β-carboline (e.g., **9**). In this report, we describe routes to the preparation of protected 3-carboxy-4-amino-β-carbolines as a prelude to the preparation of novel hybrid molecules **8**. Moreover, the various routes employed have led to the preparation of a number of furo[3,4-*c*]-β-carbolin-2-ones, one of which, **29**, possesses an exceptionally high affinity for the benzodiazepine receptor. The significance of the biological activites of these furocarbolines will be discussed in the context of existing models for ligand–receptor interactions at the benzodiazepine receptor.

Scheme 1



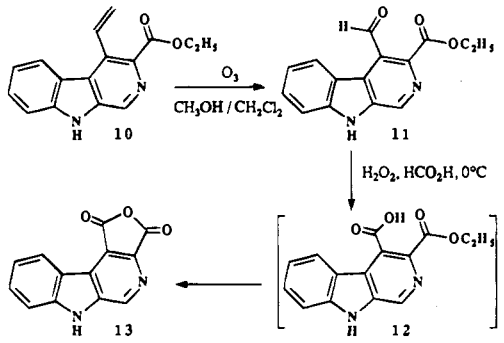
Chemistry

A preparation of 4-amino-β-carboline (i.e., having no substituent at C-3) starting from 1,2,3,4-tetrahydro-β-carboline has been described by Cook.[39] This procedure, based on a technique developed by Yonemitsu for carbazole derivatives,[40] relies on hydride abstraction at C-4 by DDQ followed by incorporation of hydrazine at this position. This methodology cannot be extended to 3-carboxy-1,2,3,4-tetrahydro-β-carbolines since treatment of these with DDQ leads to aromatization of the pyridinyl ring before a nucleophile (e.g., water or hydrazine) can attack the C-4 position.[41] An alternative method of introducing an amine function at C-4 of 3-carboxy-β-carbolines was thus required.

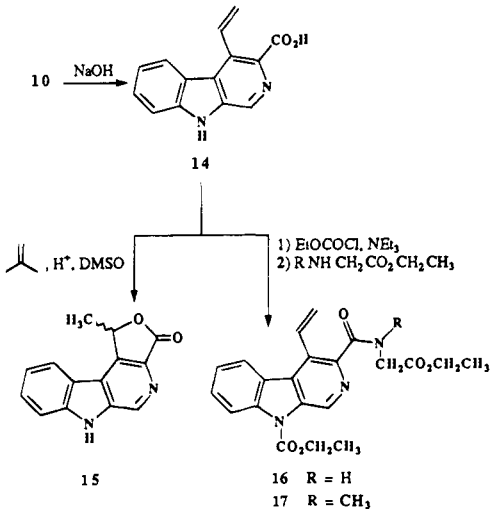
Retrosynthesis (Scheme 1) suggested that the desired 4-amino-β-carboline derivative **9** could potentially be obtained via a Curtius-type rearrangement of a 4-acyl-azide derivative, **I**, itself prepared from the 4-carboxylic acid precursor **II**. This type of reaction has previously been successfully employed for the synthesis of 3-amino-β-carbolines.[42] Although the diester derivative of **II** has been reported,[43] it was felt that selective hydrolysis of the ester at C-4 would not be feasible. Instead, the free carboxylic acid **II** could presumably be obtained by oxidation of the 4-aldehyde **III** which, in turn, could be formed by ozonolysis of a vinylic compound of type **IV**. Since such a starting compound (**10**) has also been described,[43] this appeared to be a viable synthetic route to the desired 4-amino derivative **9**.

Thus, ozonolysis of **10** in methanol–dichloromethane at −78 °C followed by trimethyl phosphite workup yielded 58% of the 4-aldehyde **11** (Scheme 2). The aldehyde group was characterized by a singlet at 10.92 ppm in the proton NMR as well as by absorption at 1695

Scheme 2



Scheme 3



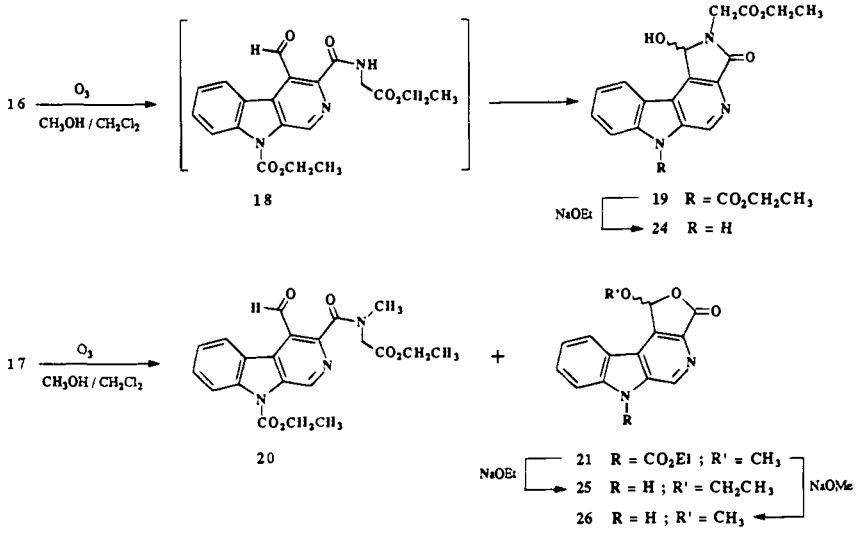
cm^{-1} in the infrared. Oxidation of the aldehyde **11** to the carboxylic acid derivative **12** was then attempted using performic acid (formed *in situ* from formic acid and aqueous hydrogen peroxide), a mild reagent previously employed in our laboratory for this type of transformation.[44] However, in this case, a 95% yield of the anhydride derivative **13** was obtained, apparently via intramolecular cyclization of the transient carboxylic acid **12** (though see below). The anhydride **13** was characterized by the disappearance of the ethyl moiety in the proton NMR and the appearance of cyclic anhydride carbonyl absorptions in the infrared.

Attempted selective opening of the anhydride **13** using 2-propanol under conditions described by Ornstein[45] were unsuccessful, only starting material being recovered. It was thus decided to replace the ethyl ester of **10** by a *tert*-butyl ester in order to minimize the possibility of intramolecular cyclization. Thus, treatment of the acid **14**, obtained by base hydrolysis of the ethyl ester **10** (Scheme 3), with isobutylene and sulfuric acid in DMSO yielded, instead of the expected *tert*-butyl ester, the methyl γ-lactone **15** as a result of intramolecular attack of the exocyclic bond at C-4 by the carboxylic acid group at C-3. That attack had occurred at the benzylic position of the vinyl group rather than at the terminal methylene was evident from the proton NMR which distinctly showed a doublet methyl group coupled to a CH (quartet).

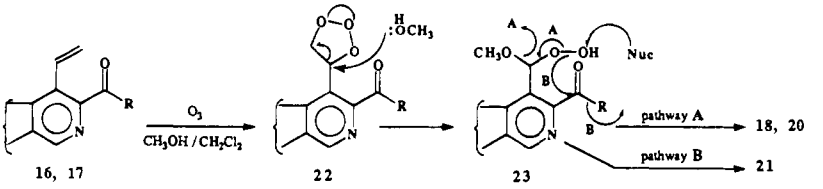
Alternatively, intramolecular cyclization of **12** during the course of aldehyde oxidation could conceivably be prevented by replacement of the ester bond at C-3 by a less labile amide bond. Moreover, since our ultimate goal, hybrid **8**, may be considered as a C-3 amide of glycine, it was decided to incorporate this amino acid directly onto **14** before resubmitting the vinyl group to oxidative transformations. Thus, by the method of mixed anhydrides previously described[46] for synthesis of β-carboline-3-carboxamides, the two glycinyl β-carboline derivatives **16** and **17** were prepared from glycine ethyl ester and sarcosine ethyl ester, respectively (Scheme 3). Each of these was then subjected to ozonolysis (Scheme 4). In the case of **16**, only the lactam **19**, the product of intramolecular cyclization of the initially formed 4-aldehyde **18**, could be isolated. The ^{1}H-NMR spectrum of **19** showed a D_2O-exchangeable, one-proton doublet at δ 5.15 coupled to a one-proton doublet at δ 6.33. This is in accord with the presence of a secondary alcohol. The ^{13}C-NMR spectrum indicated three carbonyl groups, one of which was shown to be part of a γ-lactam functionality due to an absorption at 1680 cm^{-1} in the infrared. A two-dimensional ^{1}H-^{13}C-NMR spectrum further corroborated the structure of **19**.

Ozonolysis of the *N*-methyl derivative **17**, in which cyclization to give a compound of type **19** cannot occur,

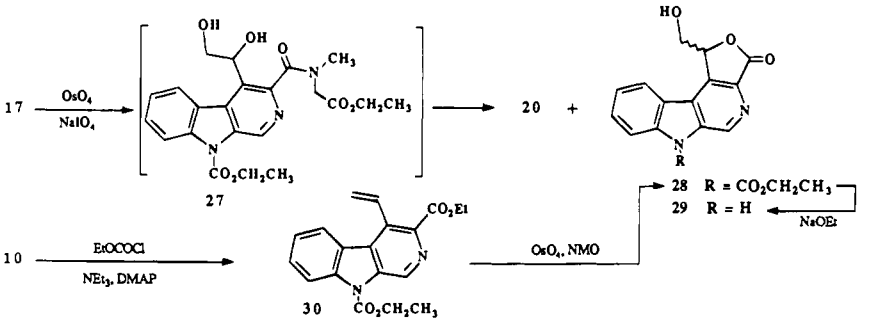
Scheme 4



Scheme 5



Scheme 6



indeed allowed isolation of the 4-aldehyde **20**, though in only 5% yield. The major product (42%) was the methoxy γ-lactone **21**, unambiguously identified by ^{1}H-NMR and mass and infrared spectra. This result may be explained by the initial attack of methanol on the ozonide **22** to give the stable α-methoxy hydroperoxide **23** (Scheme 5). In the presence of trimethyl phosphite, the hydroperoxide is reduced with subsequent elimination of the methoxy group, giving aldehyde **20** (path A). Alternatively, the reduced hydroperoxide may attack the carbonyl group at C-3, leading to elimination of sarcosine ethyl ester and formation of lactone **21** (path B). It is interesting to note that no lactone formation was observed after ozonolysis of the ethyl ester derivative **10** under the same conditions; the sarcosine side chain is thus apparently a better leaving group than ethoxy in this reaction.

For the purposes of biological evaluation, compounds **19** and **21** were N-deprotected using sodium in ethanol to give **24** and **25**, respectively, the acetal nature of **21** being responsible for the facile methoxy to ethoxy exchange seen with this compound. The methoxy group of **21** could be maintained by conducting the deprotection in methanol instead of ethanol, yielding **26**.

It was then decided to study the possibility of preparing the desired β-carboline-4-aldehyde by vicinal dihydroxylation of the 4-vinyl group of **17** followed by oxidative cleavage of the diol. Thus, treatment of compound **17** with catalytic osmium tetraoxide and excess sodium periodate led to a 62% yield of the expected aldehyde **20** (Scheme 6).

Moreover, a small quantity (4%) of the hydroxymethyl γ-lactone **28**, presumably formed by intramolecular cyclization of the intermediate diol **27**, was isolated. The formation of a five- rather than six-membered lactone was indicated by the ^{1}H-NMR spectrum of **28** in which the 6-Hz coupling between the hydroxy and methylene groups disappeared upon addition of D_2O. Moreover, the mass spectrum of **28** showed a major fragment corresponding to loss of CH_2OH. Alternatively, lactone **28** (a racemic mixture) was the only product formed when the N-protected 4-vinyl-β-carboline ester **30** (prepared from **10** using ethyl chloroformate and triethylamine) was treated with catalytic osmium tetraoxide and 4-methylmorpholine *N*-oxide (NMO) as co-oxidant.[47] The latter, unlike sodium periodate, does not promote vicinal diol cleavage. Treatment of **28** with a catalytic quantity of sodium in ethanol gave the deprotected β-carboline γ-lactone **29**.

Oxidation of the 4-aldehyde group of **20** to the carboxylic acid was next studied. However, in contrast to the oxidation of the aldehyde function of β-carboline **11** with performic acid, in which the anhydride **13** was the only product isolated, the carboxamide **20** gave, under the same conditions, the hydroxy γ-lactone **32** in 76% yield (Scheme 7). The formation of **32** may be rationalized by an initial hydration of the aldehyde group in the aqueous acidic medium to give **31** which subsequently undergoes intramolecular cyclization to **32**. This apparent difference in reactivity between ester **11** and amide **20** may be most easily explained by the differing solubilities of the reaction products. Whereas **32** precipitates directly in the reaction mixture, protecting it from further reaction, the corresponding derivative issuing from the unprotected ester **11** (i.e., compound **33**) is likely to be quite soluble in the aqueous reaction medium. As a result, the hydroxyl group of **33** can undergo further oxidation, leading to the anhydride **13** (which now precipitates). This probable sequence of events was verified by exposing aldehyde **11** to aqueous formic acid in the absence of hydrogen peroxide; only the hydroxy lactone **33** could be isolated from the reaction mixture. This would thus suggest that anhydride **13** is not simply the result of intramolecular cyclization of the 4-carboxylic acid **12**.

Formation of the hydrate **31** could theoretically be avoided by conducting the oxidation in a nonacidic medium. Choï[48] has recently reported the oxidation of an alkyl aldehyde to the corresponding carboxylic acid by use of hydrogen peroxide in THF. This technique, applied to aldehyde **20**, gave a mixture of many products which were not pursued. Finally, treatment of **20** with potassium permanganate in acetone–water[49] afforded the 4-carboxylic acid **34** (isolated as its potassium salt) in 84% yield.

Conversion of the carboxylic acid group of **34** into the BOC-amino derivative **35** via a Curtius-type rearrangement then proceeded uneventfully, though in low yield, by treatment with diphenyl phosphorazidate (DPPA)[50] in refluxing *tert*-butyl alcohol for 24 h. The ^{1}H-NMR

Scheme 7

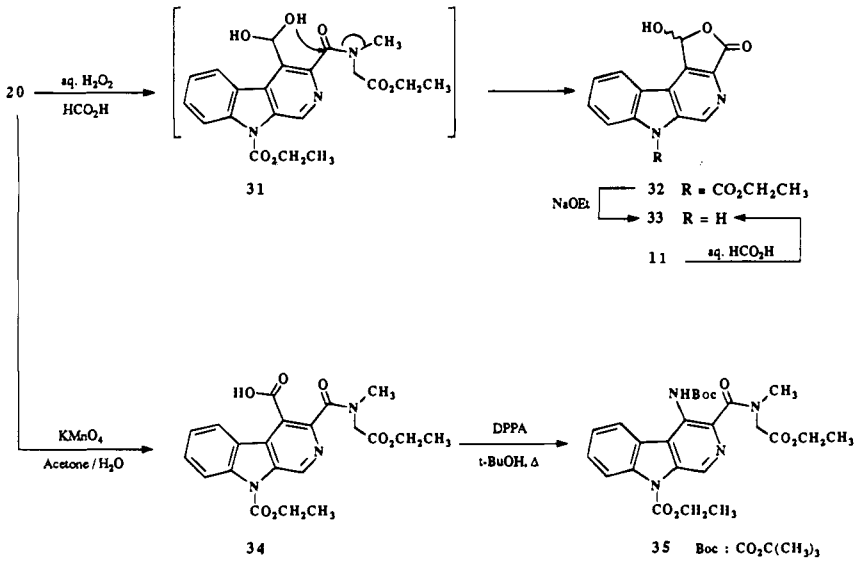
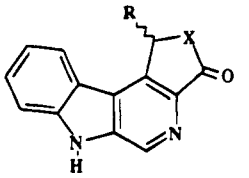


Table 1. *In Vitro* Benzodiazepine Receptor Affinities of the β-Carboline γ-Lactones



compound	X	R	IC_{50}[a] (nM)
2a (β-CCM)			2
13	O	=O	150
15	O	CH_3	20
24	NCH_2CO_2Et	OH	70
25	O	OCH_2CH_3	20
26	O	OCH_3	12
29	O	CH_2OH	0.2
33	O	OH	47

[a] Concentration of compound required to inhibit 50% of [^{3}H]flunitrazepam specific binding to *in vitro* preparations of rat cerebral cortex membranes at 0 °C as determined by the technique of ref 51; average of triplicate determinations with a maximum variance of 6%.

spectrum of **35** indicated the presence of two stable rotameric forms for this compound, each displaying D_2O-exchangeable carbamate NH's at 7.02 and 7.17 ppm, as well as the high-field resonances due to the *tert*-butyl group.

Biological Results and Discussion

The affinities of the various β-carboline γ-lactones synthesized (as well as of lactam **24**) for the benzodiazepine receptor were determined *in vitro* by the method of Rehavi[51] and are given in Table 1. Compound **29** shows an exceptionally high affinity for the benzodiazepine receptor (IC_{50} = 0.2 nM), being 10 times more active than β-CCM, **2a**. This high affinity is very much dependent on the presence of the hydroxy group since its replacement by hydrogen (to give the methyl derivative **15**) increases the IC_{50} by a factor of 100. Moreover, removal of the methylene group of **29** to give lactone **33** also results in an almost 250-fold decrease in receptor affinity. Although methylation of the hydroxy function of **33** to give **26** results in a modest 4-fold increase in affinity, this gain in affinity is less pronounced when **33** is instead ethylated to give **25**. Anhydride **13** and lactam **24** were the least active compounds in the series.

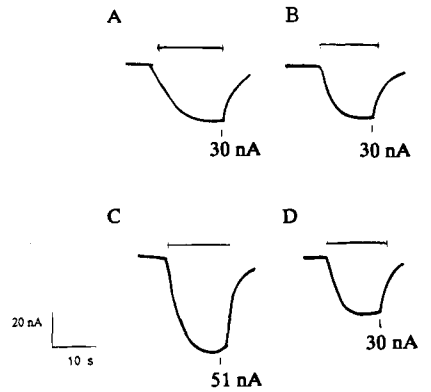


Figure 1. Membrane currents in mRNA-injected *Xenopus* oocytes in response to A, GABA (10 μM); B, GABA (10 μM) + **29** (10 μM); C, GABA (10 μM) + diazepam (1 μM); D, GABA (10 μM) + diazepam (1 μM) + **29** (10 μM). Oocytes were voltage clamped at −60 mV.

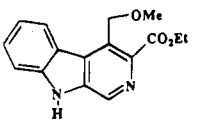
The pharmacological profile of the most active compound synthesized, lactone **29**, was first investigated electrophysiologically by voltage clamp measurements of GABA-induced currents in the *Xenopus* oocyte expression system. Oocytes injected with mRNA extracted from chick embryo brains have been shown to synthesize functional GABA receptors and associated ion channels.[52-54] Application of GABA produces currents which are sensitive to the allosteric modulation produced by drugs acting at the benzodiazepine recognition site of the GABA receptor complex. Thus, GABA-induced currents are enhanced by coapplication of benzodiazepine receptor agonists (e.g., diazepam, **1**) and diminished by inverse agonists (e.g., β-CCM, **2a**).[54,55] Benzodiazepine receptor antagonists such as flumazenil, on the other hand, produce little or no effects on currents induced by GABA but are capable of blocking the effects of benzodiazepine receptor agonists (and inverse agonists) on GABA responses.

Xenopus oocytes were thus injected with mRNA extracted from the optic lobe of chick embryos, as described,[56-58] and electrophysiological recordings using a voltage clamp were performed. A 10-s application of GABA (10 μM) to the oocyte resulted in generation of a 30-nA current (Figure 1A). This current was consistently reproduced by sequential applications of GABA (10 μM) at 3-min intervals (data not shown). Coappli-

cation of compound **29** (10 μM) with GABA (10 μM) led to no change in the GABA response (Figure 1B), suggesting that **29** is neither a benzodiazepine receptor agonist nor an inverse agonist. In order to verify whether **29** was a benzodiazepine receptor antagonist, the effect of this compound on the potentiating effect of diazepam on GABA-induced responses was tested. Thus, coapplication of GABA (10 μM) and diazepam (1 μM) led to the expected stimulation of the current produced by GABA alone (51 nA vs 30 nA; Figure 1C). This stimulation was completely blocked by coapplication of both diazepam (1 μM) and compound **29** (10 μM), the GABA response now dropping back down to the initial value (i.e., GABA alone) of 30 nA (Figure 1D). This clearly establishes **29** to be a benzodiazepine receptor antagonist.

This electrophysiologically-determined antagonist profile of **29** was verified *in vivo* in mice. Thus, the sedation and ataxia produced by diazepam (7.5 mg/kg, sc), as measured by the inability of mice to maintain themselves on a rotating rod (the rotarod test),[8] was completely reversed by administration of compound **29** (5 or 10 mg/kg, sc) 10 min after diazepam. By itself, compound **29** (5 and 10 mg/kg, sc) produced neither sedation nor convulsant effects in mice, nor were effects seen on motor coordination or muscle tone.

With respect to ligand–receptor interactions, while the restricted *s-cis* conformation of the lactone carbonyl function no doubt contributes to the exceptionally high affinity of compound **29**, the fact that the other lactone derivatives synthesized are much less active (Table 1) suggests that the hydroxymethyl group of **29** is also very important. The structure of **29** may in fact be compared to that of 4-(methoxymethyl)-β-CCE (**36**) which has also been shown[28] to antagonize the effects of diazepam but which possesses an IC_{50} which is 10-fold less than that of **29**. Compound **36** has been hypothesized to bind to



36

the benzodiazepine receptor by way of a three-centered hydrogen bond between a hydrogen bond-donating site on the receptor and the hydrogen bond-accepting oxygen atom of the C-4 ether function and the non-carbonyl oxygen atom of the C-3 ester (Figure 2). The importance of the oxygen atom in the C-4 side chain for receptor binding is unequivocally demonstrated by the 10-fold loss of binding affinity which occurs when this atom is absent as in the 4-ethyl analogue of **36**.[38] While lactone **26** is isosteric with **36**, the methoxy group of the former is probably too far removed and rigidly placed to allow participation in a three-centered hydrogen bond as with **36**. However, in lactone **29**, though the oxygen atom of the C-4 side chain is one carbon farther than in **36**, its extra freedom, as compared to **26**, allows it in fact to lie closer to the oxygen atom of the γ-lactone ring, thereby restoring the possibility of a three-point interaction with the receptor. Alternatively, the hydroxy function of compound **29** may also be considered to be a hydrogen bond donor and, as such, may interact with a completely different, and as yet uncharacterized, site

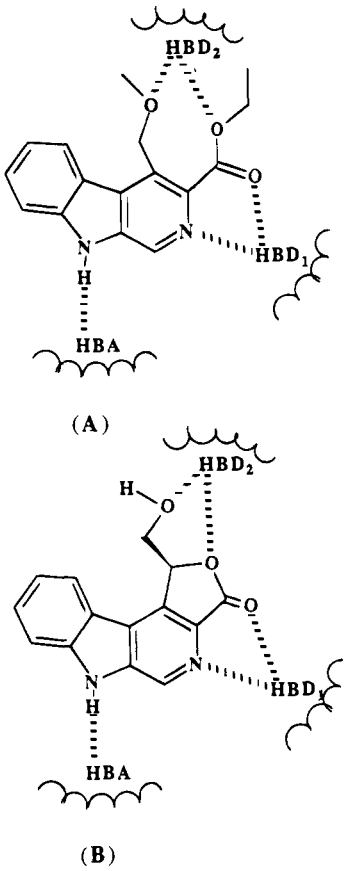


Figure 2. A—Proposed binding interaction of compound **36** with the benzodiazepine receptor.[28] B—Interaction of compound **29** with the same receptor site. HBA: hydrogen bond acceptor site. HBD: hydrogen bond donor site.

on the receptor. The synthesis and testing of an O-methylated derivative of **29** should help to resolve this question.

In conclusion, this report describes the synthesis of the first known 4-amino-3-carboxy-β-carboline derivative (**35**), a starting material which should allow the preparation of many potentially interesting benzodiazepine receptor ligands as well as permit testing of current benzodiazepine–β-carboline receptor interaction models. Moreover, the various routes which were used in attempting to synthesize this compound gave rise to a number of novel β-carboline derivatives possessing a five-membered lactone fused to the 3,4 position. One of these, racemic **29**, has been shown to possess one of the highest known affinities of the β-carboline family for the central type benzodiazepine receptor and, moreover, is the first chiral example of this class of ligand. Electrophysiological and *in vivo* studies indicate that **29** is a benzodiazepine receptor antagonist. The stereospecific synthesis and complete pharmacological profile of each enantiomer of **29** will be reported separately, as will the elaboration of the 4-amino derivative **35** into β-carboline–benzodiazepine hybrids of type **8**.

Experimental Section

Chemistry. Melting points were determined on a Büchi apparatus and are uncorrected. IR spectra of samples were obtained either as KBr pellets (for solids) or as films (for oils) with a Nicolet 205 FT-IR spectrometer. ^{1}H-NMR spectra were determined on a Bruker WP 200 MHz instrument. Chemical shifts are given as δ values with reference to Me_4Si as internal standard. Electron impact (EI) mass spectra and chemical

ionization (CI) mass spectra were recorded on AEI MS-50 and AEI MS-9 spectrometers, respectively. Fast atom bombardment mass spectra (FABMS) and high-resolution mass spectra (HRMS) were obtained using a Kratos MS-80 spectrometer. Thin-layer chromatography was performed on Merck silica gel 60 plates with fluorescent indicator. The plates were visualized with UV light (254 nm). All column chromatography was conducted on Merck 60 silica gel (230–400 mesh) at atmospheric or medium (200 mbar) pressure. Elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette, France.

Ethyl 4-Formyl-β-carboline-3-carboxylate (11). A solution of β-carboline **10**[43] (510 mg, 1.92 mmol) in a 1:1 mixture of dichloromethane and methanol (80 mL) was cooled to −78 °C, and ozone was bubbled in for 5 min. Excess ozone was removed by purging with nitrogen, and trimethyl phosphite (0.5 mL, 4.2 mmol) was then added to the reaction mixture. The solution was allowed to stand at −78 °C for 30 min and at room temperature for 2.5 h. The solvents were removed under vacuum, and the resulting oily residue was purified by chromatography on silica gel using toluene–ethanol (9:1) as developer. Aldehyde **11**, obtained as a white solid (300 mg, 58%), was crystallized from ethanol for analytical purposes: mp 190–192 °C; IR (υ_{max}, cm^{-1}) 1695 (CHO), 1710 (CO_2Et), 3250 (NH); EIMS m/z 268 (M^+), 239 ($M^+ - CHO$); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.45 (t, 3H, J = 7.0 Hz, CH_3), 4.55 (q, 2H, CH_2), 7.33 (t, 1H, J = 8.0 Hz, H-5), 7.62 (m, 2H, H-6, H-7), 8.50 (d, 1H, J = 8.0 Hz, H-8), 9.06 (s, 1H, H-1), 9.83 (br s, 1H, exchangeable with D_2O, NH), 10.92 (s, 1H, CHO). Anal. ($C_{15}H_{12}N_2O_3$) C, H, N.

β-Carboline-3,4-dicarboxylic Acid Anhydride (13). A solution of compound **11** (91 mg, 0.34 mmol) in formic acid (0.4 mL) containing hydrogen peroxide (0.2 mL of a 30% aqueous solution) was allowed to stand at 4 °C for 12 h. The resulting precipitate was collected by filtration, washed with water, and dried, yielding anhydride **13** as a yellow powder (77 mg, 95%): mp >270 °C; IR (υ_{max}, cm^{-1}) 1730–1780 (cyclic anhydride), 3200 (NH); CIMS m/z 239 (MH^+); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 7.48 (t, 1H, J = 8.0 Hz, H-5), 7.80 (m, 2H, H-6, H-7), 8.26 (d, 1H, J = 8.0 Hz, H-8), 9.00 (s, 1H, H-1), 11.95 (br s, 1H, exchangeable with D_2O); HREIMS calcd for $C_{13}H_6N_2O_3$ m/z 238.0355, found m/z 238.0378. Anal. ($C_{13}H_6N_2O_3$) C, H, N.

4-Vinyl-β-carboline-3-carboxylic Acid (14). A solution of ester **10** (1.13 g, 4.2 mmol) in a mixture of ethanol (35 mL) and aqueous sodium hydroxide (5 mL of a 3 M solution) was refluxed for 1.5 h. The reaction mixture was then concentrated under reduced pressure to a volume of 5–10 mL, and the solution was neutralized with acetic acid, whereupon a solid began to precipitate. The mixture was left at 0 °C for 1.5 h, and the solid was collected by filtration, washed with water, and dried. Recrystallization of the material from ethanol gave pure **14** (0.94 g, 93%) as a white powder: mp 306–309 °C dec; IR (υ_{max}, cm^{-1}) 1660 (C=O), 3000 (OH), 3400 (NH); FABMS m/z 239 (MH^+); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.51 (br s, ~2H, exchangeable with D_2O, OH + H_2O), 5.64 (d, 1H, J_{trans} = 16.0 Hz, CH=CH_cH_t), 5.78 (d, 1H, J_{cis} = 10.0 Hz, CH=CH_cH_t), 7.25 (t, 1H, J = 8.0 Hz, H-6), 7.61 (m, 3H, H-5, H-7, CH=CH_2), 8.39 (d, 1H, H-8), 8.84 (s, 1H, H-1), 12.14 (br s, 1H, exchangeable with D_2O, NH). Anal. ($C_{14}H_{10}N_2O_2 \cdot H_2O$) C, H, N.

(*R*,*S*)-10-Methylfuro[3,4-*c*]-β-carbolin-2(10*H*)-one (15). Compound **14** (22 mg, 0.09 mmol) was dissolved in a mixture of dioxane (2 mL) and DMSO (1 mL). The mixture was warmed slightly to aid dissolution and allowed to come to room temperature before addition of concentrated sulfuric acid (0.2 mL). Isobutylene was then bubbled through the solution for 5 min, after which the reaction vessel was sealed hermetically and the reaction was allowed to proceed for 12 h at room temperature. At the end of this period, the solution was diluted with ethyl acetate (10 mL) and washed with 1 M aqueous sodium hydroxide (4 mL). The organic phase was separated, the aqueous phase was extracted with ethyl acetate (5 mL), and the combined organic extracts were dried over sodium sulfate. Removal of the solvents under reduced pressure left **15** as a solid which was crystallized from DMSO and water (5.5 mg, 24%): mp >300 °C dec; CIMS m/z 239 (MH^+); ^{1}H-NMR (200 MHz, $CDCl_3$ + DMSO-d_6) δ 1.93 (d, 3H, J = 7.0 Hz, CH_3), 6.13 (q, 1H, J = 7.0 Hz, C$H$$CH_3$), 7.42 (d, 1H, J = 8.0 Hz, H-9), 7.70 (m, 2H, H-7, H-8), 8.08 (d, 1H, H-6), 9.15 (s, 1H, H-4), 9.82 (br s, 1H, exchangeable with D_2O, NH). HRMS calcd for $C_{14}H_{10}N_2O_2$ m/z 238.0742, found m/z 238.0744. Anal. ($C_{14}H_{10}N_2O_2$) C, H, N.

9-*N*-(Ethoxycarbonyl)-3-[*N*-(ethoxyacetyl)]-4-vinyl-β-carboline-3-carboxamide (16). A suspension of compound **14** (200 mg, 0.84 mmol) in anhydrous THF (50 mL) containing triethylamine (1 mL) was refluxed for 1.5 h, and the mixture was cooled to 0 °C. Ethyl chloroformate (340 mg, 3.14 mmol) was then added dropwise, and the reaction mixture was stirred at room temperature for 2.5 h. The solvent and excess reagents were removed under reduced pressure, the residue was taken up in THF (50 mL), and glycine ethyl ester (180 mg, 1.75 mmol) was added. The reaction mixture was refluxed for 3.5 h, the solid material was removed by filtration, and the filtrate was concentrated under reduced pressure. The oily residue was dissolved in chloroform (50 mL), the solution was washed with saturated, aqueous sodium chloride and dried over sodium sulfate, and the solvent was removed *in vacuo*. The resulting crude product was purified by chromatography on silica gel using toluene–ethanol (8:0.5) as developer yielding **16** as a solid (166 mg, 50%) which was crystallized from ethanol: mp 143–145 °C; IR (υ_{max}, cm^{-1}) 1660 (amide C=O), 1730 (ester C=O), 1745 (carbamate C=O), 3400 (amide NH); EIMS m/z 395 (M^+), 322 ($M^+ - CO_2Et$); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.32 (t, 3H, J = 6.0 Hz, C$H_3$$CH_2O$), 1.61 (t, 3H, J = 6.0 Hz, C$H_3$$CH_2O$), 4.28 (m, 4H, CH_3CH_2O, NHCH_2), 4.67 (q, 2H, J = 6.0 Hz, CH_3CH_2O), 5.54 (dd, 1H, J_{gem} = 1.0 Hz, J_{trans} = 17.0 Hz, CH=CH_cH_t), 5.80 (dd, 1H, J_{cis} = 11.0 Hz, CH=CH_cH_t), 7.37 (m, 2H, H_{arom}, NH), 7.68 (m, 2H, H_{arom}, CH=CH_2), 8.40 (d, 1H, J = 8.0 Hz, H-5), 8.62 (d, 1H, J = 8.0 Hz, H-8), 9.49 (s, 1H, H-1). Anal. ($C_{21}H_{21}N_3O_5$) C, H, N.

9-*N*-(Ethoxycarbonyl)-3-[*N*-methyl-*N*-(ethoxyacetyl)]-4-vinyl-β-carboline-3-carboxamide (17). A suspension of compound **14** (684 mg, 2.87 mmol) in anhydrous THF (100 mL) containing triethylamine (4 mL) was refluxed for 1.5 h and then cooled to 0 °C. Ethyl chloroformate (783 mg, 6.4 mmol) was added dropwise, and the mixture was stirred at room temperature for 2.5 h. The solvent and excess reagents were removed under reduced pressure, the residue was taken up in THF (100 mL), and sarcosine ethyl ester hydrochloride (530 mg, 3.5 mmol) and triethylamine (0.7 mL) were added. The reaction mixture was refluxed for 1.5 h, after which the solvent was removed under reduced pressure and the residue was taken up in ethyl acetate (100 mL). The organic solution was washed successively with saturated aqueous sodium hydrogen carbonate and water, dried over sodium sulfate, and concentrated under vacuum. The resulting solid was crystallized from dichloromethane and ethyl ether, affording pure **17** (819 mg, 70%) as a white powder: mp 150–152 °C; IR (υ_{max}, cm^{-1}) 1630 (amide C=O), 1720 (ester and carbamate C=O); EIMS m/z 409 (M^+), 336 ($M^+ - CO_2Et$); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.16 and 1.26 (2 × t, 3H, J = 7.0 Hz, C$H_3$$CH_2O$ of two rotamers), 1.53 (t, 3H, J = 7.0 Hz, $NCO_2CH_2CH_3$), 2.90 and 3.10 (2 × s, 3H, NCH_3 of two rotamers), 4.00 and 4.10 (2 × q, 2H, CH_3CH_2O of two rotamers), 3.93 and 4.19 (2 × s, 2H, CH_2-CO_2Et), 4.49 (q, 2H, $NCO_2CH_2CH_3$), 5.65 (m, 2H, CH=CH_2), 7.15 (m, 2H, CH=CH_2, H-6), 7.38 (t, 1H, J = 8.0 Hz, H-7), 7.99 (d, 1H, H-5), 8.30 (2 × d, 1H, H-8 of two rotamers), 9.12 and 9.19 (2 × s, 1H, H-1 of two rotamers). Anal. ($C_{22}H_{23}N_3O_5 \cdot {}^1/_3H_2O$) C, H, N.

Ozonolysis of Compounds 16 and 17. Following the same procedure described for the preparation of **11**, compound **16** was submitted to ozonolysis, yielding, after chromatography on silica gel (dichloromethane–ethanol, 8:0.2), lactam **19** as a solid (86%) which was crystallized from dichloromethane–hexane: mp 194–196 °C; IR (υ_{max}, cm^{-1}) 1680 (lactam C=O), 1730 (ester and carbamate C=O); CIMS m/z 398 (MH^+); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.32 (m, 6H, 2 × C$H_3$$CH_2O$), 4.22 (q, 2H, J = 7.0 Hz, CH_3CH_2O), 4.47 (m, 4H, CH_3CH_2O, CH_2-CO_2Et), 5.15 (br d, 1H, exchangeable with D_2O, J = 11.0 Hz, OH), 6.33 (d, 1H, J = 11.0 Hz, CHOH), 7.57 (t, 1H, J = 7.0 Hz, H_{arom}), 7.77 (t, 1H, H_{arom}), 8.43 (d, 2H, J = 8.0 Hz, 2 × H_{arom}), 8.84 (s, 1H, CH=N). Anal. ($C_{20}H_{19}N_3O_6$) C, H, N.

Similarly, ozonolysis of compound **17** gave two compounds, aldehyde **20** (5%) and lactone **21** (42%), which were separated by HPLC on a reverse phase silica gel column (methanol–water, 7:3).

Compound 20: mp 113–114 °C (ethanol); IR (υ_{max}, cm^{-1}): 1630 (amide C=O), 1695 (CH=O), 1735 (ester and carbamate C=O); EIMS *m/z* 411 (M^+), 338 ($M^+ - CO_2Et$); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.18 and 1.33 (2 × t, 3H, J = 7.0 Hz, CH_3CH_2O of two rotamers), 1.58 (t, 3H, J = 7.0 Hz, $NCO_2CH_2CH_3$), 2.93 and 3.16 (2 × s, 3H, NCH_3 of two rotamers), 3.99 and 4.15 (2 × q, 2H, CH_3CH_2O of two rotamers), 4.09 and 4.25 (2 × s, 2H, CH_2CO_2Et of two rotamers), 4.52 (q, 2H, $NCO_2CH_2CH_3$), 7.20 (m, 1H, H-6), 7.43 (m, 1H, H-7), 8.06 and 8.12 (2 × d, 1H, J = 8.0 Hz, H-5 of two rotamers), 9.40 and 9.49 (2 × s, 1H, H-1 of two rotamers), 10.23 (s, 1H, CHO). Anal. ($C_{21}H_{21}N_3O_6$) C, H, N.

Compound 21: mp 180 °C dec (methanol); IR (υ_{max}, cm^{-1}) 1745 (carbamate C=O), 1795 (γ-lactone C=O); EIMS *m/z* 326 (M^+), 295 ($M^+ - OCH_3$); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.62 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 3.77 (s, 3H, OCH_3), 4.73 (q, 2H, OCH_2CH_3), 6.77 (s, 1H, $CHOCH_3$), 7.55 (t, 1H, J = 8.0 Hz, H_{arom}), 7.77 (m, 1H, H_{arom}), 8.10 (d, 1H, J = 8.0 Hz, H_{arom}), 8.47 (d, 1H, H_{arom}), 9.90 (s, 1H, CH=N). Anal. ($C_{17}H_{14}N_2O_5 \cdot {}^1/_4H_2O$) C, H, N.

(*R*,*S*)-5-*N*-(Ethoxycarbonyl)-10-(hydroxymethyl)furo[3,4-*c*]-β-carbolin-2(10*H*)-one (28). To a solution of compound **17** (700 mg, 1.7 mmol) in dioxane (75 mL) and water (30 mL) was added solid osmium tetraoxide (13 mg, 0.05 mmol). The mixture was stirred for 30 min at room temperature before sodium periodate (2 g, 9.35 mmol) was added, and stirring was continued for 21 h. The solid material was removed by filtration and washed with dioxane. The combined filtrate and washings were then concentrated under reduced pressure, and the residue was purified by chromatography on silica gel (dichloromethane–ethanol, 8:0.2). Compound **20** (382 mg, 62%), identical in all respects to that formed by ozonolysis, was first eluted, followed by unreacted starting material **17** (88 mg, 13%) and finally by lactone **28** (20 mg, 4%). Compound **28** was crystallized from DMSO–water: mp 202–204 °C; IR (υ_{max}, cm^{-1}) 1720 (carbamate C=O), 1745 (γ-lactone C=O), 3400 (OH); EIMS *m/z* 326 (M^+), 295 ($M^+ - CH_2OH$); ^{1}H-NMR (200 MHz, $CDCl_3$ + DMSO-d_6) δ 1.57 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 4.07 (ddd, 1H, J_{gem} = 13.0 Hz, $J_{Ha,OH}$ = 6.0 Hz, $J_{Ha,H10}$ = 5.0 Hz, CH_aH_bOH), 4.35 (ddd, 1H, $J_{Hb,OH}$ = 3.0 Hz, CH_aH_bOH), 4.65 (s, 2H, OCH_2CH_3), 5.20 (t, 1H, exchangeable with D_2O, CH_2OH), 6.07 (dd, 1H, J = 5.0 and 3.0 Hz, $CHCH_2OH$), 7.49 (t, 1H, J = 7.0 Hz, H_{arom}), 7.70 (t, 1H, H_{arom}), 8.09 (d, 1H, J = 8.0 Hz, H_{arom}), 8.43 (d, 1H, H_{arom}), 9.85 (s, 1H, CH=N); HRMS calcd for $C_{17}H_{14}N_2O_5$ *m/z* 326.0903, found *m/z* 326.0906. Anal. ($C_{17}H_{14}N_2O_5$) C, H, N.

Ethyl 9-*N*-(Ethoxycarbonyl)-4-vinyl-β-carboline-3-carboxylate (30). To a solution of compound **12** (1 g, 3.7 mmol) and *N*,*N*-dimethyl-4-aminopyridine (146 mg, 0.37 mmol) in anhydrous THF (25 mL) was added triethylamine (1.05 mL, 8.3 mmol) and ethyl chloroformate (0.53 mL, 7.5 mmol). The reaction mixture was stirred at room temperature for 1 h, water (50 mL) was added, and the solution was made basic with saturated aqueous sodium hydrogen carbonate. The solution was then extracted with dichloromethane (3 × 20 mL), the combined organic extracts were dried over sodium sulfate, and the solvents were removed under reduced pressure. The resulting crude product was purified by chromatography on silica gel (ethyl acetate–heptane, 6:4), yielding pure **30** as pale yellow crystals (633 mg, 49%): mp 109–110 °C; IR (υ_{max}, cm^{-1}) 1715 (ester C=O), 1739 (carbamate C=O); EIMS *m/z* 338 (M^+), 265 ($M^+ - CO_2Et$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.49 (t, 3H, J = 7.5 Hz, OCH_2CH_3), 1.52 (t, 3H, J = 7.5 Hz, OCH_2CH_3), 4.47 (q, 2H, OCH_2CH_3), 4.65 (q, 2H, OCH_2CH_3), 5.59 (dd, 1H, J_{trans} = 19.7 Hz, J_{gem} = 1.4 Hz, $CH=CH_cCH_t$), 5.79 (dd, 1H, J_{cis} = 11.4 Hz, $CH=CH_cCH_t$), 7.37 (m, 1H, J = 8.2 Hz, H-6), 7.45 (dd, 1H, $CH=CH_2$), 7.62 (m, 1H, H-7), 8.38 (d, 1H, J = 6.8 Hz, H-5), 8.43 (d, 1H, H-8), 9.60 (s, 1H, H-1). Anal. ($C_{19}H_{18}N_2O_4 \cdot 0.2H_2O$) C, H, N.

Preparation of Lactone 28 from Compound 30. To a solution of compound **30** (58 mg, 0.17 mmol) and 4-methylmorpholine *N*-oxide (30 mg, 0.26 mmol) in a 10:1 mixture of acetone–water (15 mL) was added a 4% solution of osmium tetraoxide in water (20 μL, 3.2 μmol). The reaction mixture was stirred for 4 days at room temperature. A 20% aqueous solution of $Na_2S_2O_3$ (10 mL) was then added, and the mixture was extracted with dichloromethane (3 × 10 mL). The combined organic fractions were dried over sodium sulfate, and the solvents were removed under reduced pressure, leaving compound **28** as a white solid (35 mg, 61%) identical in all respects with that obtained as a byproduct from **17**.

(*R*,*S*)-5-*N*-(Ethoxycarbonyl)-10-hydroxyfuro[3,4-*c*]-β-carbolin-2(10*H*)-one (32). A solution of aldehyde **20** (13 mg, 0.03 mmol) in formic acid (0.5 mL) was held at 0 °C and treated with 30% aqueous hydrogen peroxide solution (0.2 mL). The reaction mixture was left to stand at 4 °C for 12 h during which time a precipitate formed. The solid was collected by filtration, washed with water, and dried, giving lactone **32** (7.5 mg, 75%) as fluffy white crystals: mp 192–194 °C; IR (υ_{max}, cm^{-1}) 1770 (γ-lactone C=O), 1740 (carbamate C=O); FABMS *m/z* 313 (MH^+); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.56 (t, 3H, J = 7.0 Hz, CH_3CH_2O), 4.66 (q, 2H, CH_3CH_2O), 7.16 (d, 1H, J = 10.0 Hz, CHOH), 7.59 (t, 1H, J = 8.0 Hz, H_{arom}), 7.83 (t, 1H, H_{arom}), 8.21 (d, 1H, J = 8.0 Hz, H_{arom}), 8.34 (d, 1H, H_{arom}), 8.64 (d, 1H, exchangeable with D_2O, OH), 9.71 (s, 1H, CH=N); FAB-HRMS calcd for $C_{16}H_{13}N_2O_5$ *m/z* 313.0825, found *m/z* 313.0830. Anal. ($C_{16}H_{12}N_2O_5$) C, H, N.

9-*N*-(Ethoxycarbonyl)-3-[*N*-methyl-*N*-(ethoxyacetyl)-carbamoyl]-β-carboline-4-carboxylic Acid (34). To a solution of compound **20** (145 mg, 0.35 mmol) in acetone (30 mL) and water (6 mL) was added a solution of potassium permanganate (139 mg, 0.88 mmol) in water (2 mL). The reaction mixture was stirred for 3 h at room temperature, and 30% aqueous formaldehyde solution was then slowly added dropwise until the purple color of the permanganate had disappeared. The precipitate of MnO_2 was removed by filtration through a pad of Celite. The solid and pad were washed copiously with acetone, and the combined filtrate and washings were evaporated to dryness under vacuum. The solid residue was triturated with dichloromethane and collected by filtration, affording compound **34** (127 mg, 84%) as its potassium salt sufficiently pure for use in the following step: FABMS *m/z* 466 ($M^+ + K$); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.24 (m, 3H, CH_3CH_2O), 1.53 (m, 3H, $NCO_2CH_2CH_3$), 2.88 and 3.09 (2 × s, 3H, NCH_3 of two rotamers), 3.40 (br s, 1H, partly buried under H_2O signal, exchangeable with D_2O, CO_2H), 3.97 and 4.28 (m, 4H, CH_3CH_2O, CH_2CO_2Et), 7.40 (t, 1H, J = 7.0 Hz, H-6), 7.66 (t, 1H, H-7), 8.31 (2 × d, 1H, J = 8.0 Hz, H-5 of two rotamers), 8.68 (2 × d, 1H, H-8 of two rotamers), 9.40 and 9.34 (2 × s, 1H, H-1 of two rotamers).

9-*N*-(Ethoxycarbonyl)-3-[*N*-methyl-*N*-(ethoxyacetyl)]-4-[(*tert*-butyloxycarbonyl)amino]-β-carboline-3-carboxamide (35). To a suspension of crude compound **34** (127 mg, 0.3 mmol) in *tert*-butyl alcohol held under a nitrogen atmosphere were added triethylamine (53 μL, 0.4 mmol) and diphenyl phosphorazidate (77 μL, 0.36 mmol). The reaction mixture was refluxed for 9 h and then stirred at room temperature for 20 h. The solvents were removed under reduced pressure, and the residue was purified by chromatography on silica gel (toluene–ethanol, 8:1) affording compound **35** (38 mg, 25%); CIMS *m/z* 499 (MH^+); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.27 (m, 6H, CH_3CH_2O, $NCO_2CH_2CH_3$), 1.62 (s, 9H, $C(CH_3)_3$), 3.15 and 3.29 (2 × s, 3H, NCH_3 of two rotamers), 4.05 and 4.40 (m, 4H, CH_3CH_2O, CH_2CO_2Et), 4.69 (q, 2H, J = 7.0 Hz, $NCO_2CH_2CH_3$), 7.02 and 7.17 (2 × s, 1H, exchangeable with D_2O, $NHCO_2$ of two rotamers), 7.50 (t, 1H, J = 7.0 Hz, H-6), 7.63 (m, 1H, H-7), 8.05 and 8.17 (2 × d, 1H, J = 8.0 Hz, H-5 of two rotamers), 8.46 (2 × d, 1H, H-8 of two rotamers), 9.42 and 9.52 (2 × s, 1H, H-1 of two rotamers). Anal. ($C_{25}H_{30}N_4O_7$) C, H, N.

General Procedure for Deprotection of 9-*N*-Ethoxycarbonyl Derivatives 19, 21, 28, and 32. A solution of the carbamate in anhydrous ethanol was treated at room temperature with 0.1 equiv of sodium. The reaction mixture was stirred for 2.5–3 h (TLC monitoring) and then neutralized by the addition of a small excess of acetic acid. The solution was concentrated under reduced pressure, whereupon the product precipitated. The latter was collected by filtration, washed with water to remove inorganic salts, and dried.

Compound **24** was thus obtained in 49% yield from **19**: mp 240 °C (DMSO–water); IR (υ_{max}, cm^{-1}) 1680 (γ-lactam C=O), 1735 (ester C=O); EIMS m/z 325 (M^+), 252 ($M^+ - CO_2Et$); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.20 (t, 3H, J = 7.0 Hz, CH_3), 4.08 (q, 2H, CH_2CH_3), 4.17 (d, 1H, J_{gem} = 18.0 Hz, NCH_aH_b), 4.47 (d, 1H, NCH_aH_b), 6.24 (d, 1H, J = 9.5 Hz, CHOH), 7.02 (d, 1H, exchangeable with D_2O, OH), 7.31 (t, 1H, J = 8.0 Hz, H_{arom}), 7.61 (m, 2H, H_{arom}), 8.23 (d, 1H, H_{arom}), 8.97 (s, 1H, CH=N), 12.12 (br s, 1H, exchangeable with D_2O, NH); HRMS calcd for $C_{17}H_{15}N_3O_4$ m/z 325.1062, found m/z 325.1064. Anal. ($C_{17}H_{15}N_3O_4$) C, H, N.

Compound **25** was obtained in 88% yield from **21**: mp >310 °C dec (DMSO–water); IR (υ_{max}, cm^{-1}) 1760 (lactone C=O), 3300 (NH); EIMS m/z 268 (M^+), 223 ($M^+ - OEt$); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 1.33 (t, 3H, J = 7.0 Hz, CH_3), 3.93 (dd, 1H, CH_aH_b), 4.10 (dd, 1H, CH_aH_b), 7.08 (s, 1H, CHOEt), 7.38 (t, 1H, J = 7.0 Hz, H_{arom}), 7.61 (t, 1H, H_{arom}), 7.73 (d, 1H, J = 8.0 Hz, H_{arom}), 8.10 (d, 1H, H_{arom}), 9.12 (s, 1H, CH=N), 12.42 (br s, 1H, exchangeable with D_2O, NH); HRMS calcd for $C_{15}H_{12}N_2O_3$ m/z 268.0847, found m/z 268.0848. Anal. ($C_{15}H_{12}N_2O_3$) C, H, N.

Deprotection of **21** using sodium in methanol instead of in ethanol gave compound **26** in 63% yield: mp 314–315 °C (DMSO–water); IR (υ_{max}, cm^{-1}) 1770 (γ-lactone C=O), 3200 (NH); EIMS m/z 254 (M^+), 223 ($M^+ - OCH_3$); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.70 (s, 3H, CH_3), 7.10 (s, 1H, CH-O), 7.45 (t, 1H, J = 7.0 Hz, H_{arom}), 7.74 (m, 2H, H_{arom}), 8.08 (d, 1H, J = 8.0 Hz, H_{arom}), 9.19 (s, 1H, CH=N), 12.55 (br s, 1H, exchangeable with D_2O, NH); HRMS calcd for $C_{14}H_{10}N_2O_3$ m/z 254.0691, found m/z 254.0724. Anal. ($C_{14}H_{10}N_2O_3$) C, H, N.

Compound **29** was obtained in 72% yield from **28**: mp 278–280 °C dec (DMSO–water); IR (υ_{max}, cm^{-1}) 1760 (γ-lactone C=O), 3200 (NH); EIMS m/z 254 (M^+), 223 ($M^+ - CH_2OH$); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 4.13 (m, 2H, CH_2), 5.07 (t, 1H, J = 6.0 Hz, exchangeable with D_2O, OH), 6.10 (dd, 1H, $J_{H,Ha}$ = 5.0 Hz, $J_{H,Hb}$ = 3.0 Hz, $CHCH_2$), 7.28 (t, 1H, J = 8.0 Hz, H_{arom}), 7.57 (m, 2H, H_{arom}), 8.11 (d, 1H, H_{arom}), 9.85 (s, 1H, CH=N), 12.18 (br s, 1H, exchangeable with D_2O, NH); HRMS calcd for $C_{14}H_{10}N_2O_3$ m/z 254.0691, found m/z 254.0693. Anal. ($C_{14}H_{10}N_2O_3$) C, H, N.

Compound **33** was obtained in 83% yield from **32**: mp 241 °C (ethanol); IR (υ_{max}, cm^{-1}) 1760 (γ-lactone C=O), 3200 (NH, OH); FABMS m/z 241 (MH^+); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 7.10 (d, 1H, J = 10.0 Hz, CHOH), 7.34 (t, 1H, J = 8.0 Hz, H_{arom}), 7.67 (m, 2H, H_{arom}), 8.17 (d, 1H, H_{arom}), 8.44 (d, 1H, exchangeable with D_2O, OH), 9.09 (s, 1H, CH=N), 12.38 (br s, 1H, exchangeable with D_2O, NH); HRMS calcd for $C_{13}H_9N_2O_3$ (MH^+) m/z 241.0613, found m/z 241.0600. Anal. ($C_{13}H_8N_2O_3$) C, H, N.

Preparation of Lactone 33 from Aldehyde 11. A solution of compound 11 (8.7 mg, 0.032 mmol) in formic acid (1 mL) and water (0.3 mL) was stirred at room temperature for 3 days. The precipitate which formed was collected by filtration, washed with water, and dried, yielding compound **33** (5.5 mg, 72%) identical in all respects with that prepared from **32**, as described above.

Biological Methods. *In Vitro* Benzodiazepine Receptor Binding Assays. Male Sprague–Dawley rats were decapitated, the brains were excised, and the cortex was dissected. Each cortex was homogenized in 5 mL of ice-cold Tris-HCl (50 mM, pH 7.4) with a Polytron homogenizer. The homogenate was centrifuged a first time at 460g for 3 min, and the supernatant was recentrifuged at 22400g for 20 min. The resulting supernatant was discarded, and each pellet was resuspended in 3 mL of buffer and centrifuged again at 22400g for 20 min. The resulting pellet was again suspended in 3 mL of buffer, rehomogenized, divided into 1-mL fractions, and stored at −20 °C for at least 24 h before use. For the inhibition studies, the thawed membrane preparations were diluted with 20 volumes of ice-cold buffer and 900-μL aliquots containing 60 μg of protein were incubated at 0 °C for 60 min with [^{3}H]-flunitrazepam (76.9 Ci/mmol, NEN, final concentration of 1 nM) and varying concentrations of the test compound ranging from 10^{-5} to 5×10^{-10} M (final concentrations for a total volume of 1 mL). Nonspecific binding was measured in the presence of 1 μM nonradioactive flunitrazepam and represented 10–15% of the total binding. Incubations were terminated by adding 3 mL of cold buffer to each incubation tube, filtering through Whatman GF/B glass fiber filters, and washing each filter four times with 3 mL of ice-cold buffer. Radioactivity retained on the filters was counted in 10 mL of Aquasol scintillation solution with an LKB Wallac 1215 Rackbeta 2 counter. Each value was determined in triplicate. IC_{50} values (the concentration of ligand inhibiting 50% of flunitrazepam binding) were determined by Hofstee analysis. Results are given in the table.

Electrophysiological Studies Using *Xenopus* Oocytes. Total RNAs were purified from the optic lobe of 19-day-old chick embryos by homogenization in guanidinium thiocyanate followed by a cesium chloride gradient and phenol–chloroform extractions.[56,57] Poly(A)-RNAs were then selected by affinity chromatography on oligo(dT)-cellulose.[58] *Xenopus* oocytes were injected with 50 ng of poly(A)-RNA in water and incubated at 18 °C in sterile Barth's solution for 3 days. Electrophysiological recordings were performed using a conventional two-microelectrodes voltage clamp technique at room temperature in Ringer solution. The oocytes were constantly perfused with Ringer solution (10 mL/min), and substances (GABA, diazepam, compound **29**), dissolved in Ringer solution, were applied in 10-s bursts by switching the perfusion solution to one containing the ligand(s) with intervals of 3 min between each application.

Rotarod Studies with Compound 29. The rotarod deficit was evaluated on a 2.5-cm diameter wooden rod rotating at 4 rpm. Mice were placed backwards on the rotating rod in such a way that they had to turn around to face the movement. Mice were pretrained for a 5-min period 30 min before the beginning of the experiment. The ability of each mouse to stay on the rod for 1 min was then pretested just before the first drug treatment. The occasional mouse not succeeding this pretest was eliminated. Mice were then injected with diazepam (7.5 mg/kg, sc) (Hoffmann-La Roche; suspended in Tween 90) followed 10 min later by compound **29** (5 or 10 mg/kg, sc) or saline and tested every 10 min during the next hour. Each experiment was repeated on 10 mice.

Acknowledgment. We thank DRET for fellowships (G.D., L.D.) and financial support (Contract No. 91/121).

References

(1) Squires, R.; Braestrup, C. Benzodiazepine Receptors in Rat Brain. *Nature* **1977**, *226*, 732–734.
(2) Möhler, H.; Okada, T. Benzodiazepine Receptors: Demonstration in the Central Nervous System. *Science* **1977**, *198*, 849–851.
(3) Möhler, H.; Okada, T. Biochemical Identification of the Site of Action of Benzodiazepines in Human Brain by [^{3}H]-Diazepam Binding. *Life Sci.* **1978**, *22*, 985–996.
(4) For comprehensive reviews, see: (a) Haefely, W.; Kyburz, E.; Gerecke, M.; Möhler, H. Recent Advances in the Molecular Pharmacology of Benzodiazepine Receptors and in the Structure-Activity Relationships of Their Agonists and Antagonists. *Adv. Drug Res.* **1985**, *14*, 165–322. (b) Gardner, C. R.; Tully, R. W.; Hedgecock, C. J. R. The Rapidly Expanding Range of Neuronal Benzodiazepine Receptor Ligands. *Prog. Neurobiol.* **1993**, *40*, 1–61.
(5) Braestrup, C.; Nielsen, M.; Olsen, C. E. Urinary and Brain β-Carboline-3-carboxylate as Potent Inhibitors of Brain Benzodiazepine Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2288–2292.
(6) Cain, M.; Weber, R. W.; Guzman, F.; Cook, J. M.; Barker, S. A.; Rice, K. C.; Crawley, J. N.; Paul, S. M.; Skolnick, P. β-Carbolines: Synthesis and Neurochemical and Pharmacological Actions on Brain Benzodiazepine Receptors. *J. Med. Chem.* **1982**, *25*, 1081–1091.
(7) Oakley, N. R.; Jones, B. J. Differential Pharmacological Effects of β-Carboline-3-carboxylic Acid Esters. *Neuropharmacology* **1982**, *21*, 587–589.
(8) Prado de Carvalho, L.; Grecksch, G.; Cavalheiro, E. A.; Dodd, R. H.; Chapouthier, G.; Rossier, J. Characterization of Convulsions Induced by Methyl β-Carboline-3-carboxylate in Mice. *Eur. J. Pharmacol.* **1984**, *103*, 287–293.
(9) Prado de Carvalho, L.; Grecksch, G.; Chapouthier, G.; Rossier, J. Anxiogenic and Non-Anxiogenic Benzodiazepine Antagonists. *Nature* **1983**, *301*, 64–66.
(10) Venault, P.; Chapouthier, G.; Prado de Carvalho, L.; Simiand, J.; Morre, M.; Dodd, R. H.; Rossier, J. Benzodiazepine Impairs and β-Carboline Enhances Performance in Learning and Memory Tasks. *Nature* **1986**, *321*, 864–866.

(11) Petersen, E. N.; Paschelke, G.; Kehr, W.; Nielsen, M.; Braestrup, C. Does the Reversal of the Anticonflict Effect of Phenobarbital by β-CCE and FG 7142 Indicate Benzodiazepine Receptor-Mediated Anxiogenic Properties? *Eur. J. Pharmacol.* **1982**, *82*, 217–221.
(12) Ninan, P. T.; Insel, T. M.; Cohen, R. M.; Cook, J. M.; Skolnick, P.; Paul, S. M. Benzodiazepine Receptor-Mediated Experimental "Anxiety" in Primates. *Science (Washington, D. C.)* **1982**, *218*, 1332–1334.
(13) Rossier, J.; Dodd, R. H.; Feldblum, S.; Valin, A.; Prado de Carvalho, L.; Potier, P.; Naquet, R. Methylamide β-Carboline (FG 7142), an Anxiogenic Benzodiazepine Antagonist, is Also a Proconvulsant. *Lancet* **1983**, *8342*, 77.
(14) Dorow, R.; Horowski, R.; Paschelke, G.; Amin, M.; Braestrup, C. Severe Anxiety Produced by FG 7142, a β-Carboline Ligand for Benzodiazepine Receptors. *Lancet* **1983**, *8341*, 98–99.
(15) Möhler, H.; Battersby, M. K.; Richards, J. G. Benzodiazepine Receptor Protein Identified and Visualised in Brain Tissue by a Photoaffinity Label. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1661–1670.
(16) Möhler, H. Benzodiazepine Receptors: Differential Interaction of Benzodiazepine Agonists and Antagonists after Photoaffinity Labelling with Flunitrazepam. *Eur. J. Pharmacol.* **1982**, *80*, 435–436.
(17) Thomas, J. W.; Tallman, J. F. Photoaffinity Labelling of Benzodiazepine Receptors Causes Altered Agonists-Antagonist Interactions. *J. Neurosci.* **1983**, *3*, 433–440.
(18) Brown, C. L.; Martin, I. L. Photoaffinity Labelling of the Benzodiazepine Receptor Cannot be Used to Predict Ligand Efficacy. *Neurosci. Lett.* **1983**, *35*, 37–40.
(19) Hirsch, J. D.; Kochman, R. L.; Sumner, P. R. Heterogeneity of Brain Benzodiazepine Receptors Demonstrated by (^{3}H)-Propyl β-Carboline-3-carboxylate Binding. *Mol. Pharmacol.* **1982**, *21*, 618–628.
(20) Skolnick, P.; Schweri, M.; Kutter, E.; Williams, E.; Paul, S. Inhibition of [^{3}H]-Diazepine and [^{3}H]-3-Carboethoxy-β-carboline Binding by Irazepine: Evidence for Multiple "Domains" of the Benzodiazepine Receptor. *J. Neurochem.* **1982**, *39*, 1142–1146.
(21) Dellouve-Courillon, C.; Lambolez, B.; Potier, P.; Dodd, R. H. First Use of a β-Carboline as Photoaffinity Label for the Benzodiazepine Receptor. *Eur. J. Pharmacol.* **1989**, *166*, 557–562.
(22) Braestrup, C.; Schmiechen, R.; Neef, G.; Nielsen, M.; Petersen, E. N. Interaction of Convulsive Ligands with Benzodiazepine Receptors. *Science (Washington, D. C.)* **1982**, *216*, 1241–1243.
(23) Petersen, E. N. DMCM: A Potent Convulsive Benzodiazepine Receptor Ligand. *Eur. J. Pharmacol.* **1983**, *94*, 117–124.
(24) Braestrup, C.; Nielsen, M. GABA Reduces Binding of ^{3}H-Methyl β-Carboline-3-carboxylate to Brain Benzodiazepine Receptors. *Nature* **1981**, *294*, 472–474.
(25) Tallman, J. F.; Thomas, J. W.; Gallager, D. W. GABAergic Modulation of Benzodiazepine Binding Site Sensitivity. *Nature* **1978**, *274*, 383–385.
(26) Karobath, M.; Sperk, G. Stimulation of Benzodiazepine Receptor Binding by GABA. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 1004–1006.
(27) Ferrero, P.; Santi, M. R.; Conti-Tronconi, B.; Costa, E.; Guidotti, A. Study of an Octadecaneuropeptide Derived from Diazepam Binding Inhibitor (DBI): Biological Activity and Presence in Rat Brain. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 827–831.
(28) Hollinshead, S. P.; Trudell, M. L.; Skolnick, P.; Cook, J. M. Structural Requirements for Agonist Actions at the Benzodiazepine Receptor: Studies with Analogues of 6-(Benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester. *J. Med. Chem.* **1990**, *33*, 1062–1069.
(29) Diaz-Arauzo, H.; Evoniuk, G. E.; Skolnick, P.; Cook, J. M. The Agonist Pharmacophore of the Benzodiazepine Receptor. Synthesis of a Selective Anticonvulsant/Anxiolytic. *J. Med. Chem.* **1991**, *34*, 1754–1756.
(30) Borea, P. A.; Gilli, G.; Bertolasi, V.; Ferretti, V. Stereochemical Features Controlling Binding and Intrinsic Activity Properties of Benzodiazepine-Receptor Ligands. *Mol. Pharmacol.* **1987**, *31*, 334–344.
(31) Tebib, S.; Bourguignon, J.-J.; Wermuth, C.-G. The Active Analog Approach Applied to the Pharmacophore Identification of Benzodiazepine Receptor Ligands. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 153–170.
(32) Petersen, E. N.; Jensen, L. H.; Honoré, T.; Braestrup, C.; Kehr, W.; Stephens, D. N.; Wachtel, H.; Seidelman, D.; Schmiechen, R. ZK 91296, a Partial Agonist at Benzodiazepine Receptors. *Psychopharmacologia* **1984**, *83*, 240–248.
(33) Stephens, D. N.; Shearman, G. T.; Kehr, W. Discriminative Stimulus Properties of β-Carbolines Characterised as Agonists and Inverse Agonists at Central Benzodiazepine Receptors. *Psychopharmacologia* **1984**, *83*, 233–239.
(34) Dodd, R. H.; Doisy, X.; Potier, P.; Potier, M.-C.; Rossier, J. Synthesis and Pharmacological Activity of a Pyrido [3′,4′:5,4]-pyrrolo[1,2-c]benzodiazepine-3,10-dione, a New Benzodiazepine-β-Carboline Type Hybrid Molecule. *Heterocycles* **1989**, *28*, 1101–1113.
(35) Dodd, R. H.; Ouannès, C.; Potier, M.-C.; Prado de Carvalho, L.; Rossier, J.; Potier, P. Synthesis of β-Carboline-Benzodiazepine Hybrid Molecules: Use of the Known Structural Requirements for Benzodiazepine and β-Carboline Binding in Designing a Novel, High-Affinity Ligand for the Benzodiazepine Receptor. *J. Med. Chem.* **1987**, *30*, 1248–1254.
(36) Dodd, R. H.; Poissonnet, G.; Venault, P.; Potier, M.-C.; Raffalli-Sebille, M.-J.; Chapouthier, G.; Potier, P. Modulation of the Benzodiazepine Receptor Activities of 3-Carboxy-β-carbolines by Formation of Their Anhydrobases. *Pharmacol. (Life Sci. Adv.)* **1990**, *9*, 135–143.
(37) Plate, R.; Nivard, R. J. F.; Ottenheijm, H. C. J. Synthesis and Pharmacological Activity of C(1)- and N(2)-Substituted β-Carboline Derivatives. *Heterocycles* **1986**, *24*, 3105–3114.
(38) Dorey, G.; Poissonnet, G.; Potier, M.-C.; Prado de Carvalho, L.; Venault, P.; Chapouthier, G.; Rossier, J.; Potier, P.; Dodd, R. H. Synthesis and Benzodiazepine Receptor Affinities of Rigid Analogues of 3-Carboxy-β-carbolines: Demonstration That the Benzodiazepine Receptor Recognizes Preferentially the s-Cis Conformation of the 3-Carboxy Group. *J. Med. Chem.* **1989**, *32*, 1799–1804.
(39) Cain, M.; Mantei, R.; Cook, J. M. Dichlorodicyanoquinone Oxidations in the Indole Area. Synthesis of Crenatine. *J. Org. Chem.* **1982**, *47*, 4933–4936.
(40) Oikawa, Y.; Yonemitsu, O. Selective Oxidation of the Side Chain at C-3 of Indoles. *J. Org. Chem.* **1977**, *42*, 1213–1216.
(41) Unpublished results.
(42) Dodd, R. H.; Ouannès, C.; Prado de Carvalho, L.; Valin, A.; Venault, P.; Chapouthier, G.; Rossier, J.; Potier, P. 3-Amino-β-carboline Derivatives and the Benzodiazepine Receptor. Synthesis of a Selective Antagonist of the Sedative Action of Diazepam. *J. Med. Chem.* **1985**, *28*, 824–828.
(43) Neef, G.; Eder, U.; Huth, A.; Rahtz, D.; Schmiechen, R.; Seidelmann, D. Synthesis of 4-Substituted β-Carbolines. *Heterocycles* **1983**, *20*, 1295–1313.
(44) Dodd, R. H.; Le Hyaric, M. The Oxidation of Aromatic Aldehydes to Carboxylic Acids Using Hydrogen Peroxide in Formic Acid. *Synthesis* **1993**, 295–297.
(45) Ornstein, P. L.; Schaus, J. M.; Chambers, J. W.; Huser, D. L.; Leander, J. D.; Wong, D. T.; Paschal, J. W.; Jones, N. D.; Deeter, J. B. Synthesis and Pharmacology of a Series of 3-and 4-(Phosphonoalkyl)pyridine- and-piperidine-2-carboxylic Acids. Potent N-Methyl-D-aspartate Receptor Antagonists. *J. Med. Chem.* **1989**, *32*, 827–833.
(46) Dellouve-Courillon, C.; Dorey, G.; Poissonnet, G.; Doisy, X.; Potier, P.; Dodd, R. H. Synthesis of β-Carboline-Benzodiazepine Hybrid Molecules and Their "Amputated" Analogues as Novel Ligands of the Benzodiazepine Receptor. *Tetrahedron* **1990**, *46*, 3245–3266.
(47) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. An Improved Catalytic OsO_4 Oxidation of Olefins to cis-1,2-Glycols Using Tertiary Amine Oxides as the Oxidant. *Tetrahedron Lett.* **1976**, 1973–1976.
(48) Choi, J. K.; Hart, D. J. Free Radical Cyclizations in Alkaloid Synthesis: (+)-Heliotridine and (+)-Hastanecine. *Tetrahedron* **1985**, *41*, 3959–3971.
(49) Roemmele, R. C.; Rapoport, H. Chirospecific Synthesis of β-Hydroxy α-Amino Acids. *J. Org. Chem.* **1989**, *54*, 1866–1875.
(50) Shioiri, T.; Ninomiya, K.; Yamada, S. Diphenylphosphoryl Azide. A New Convenient Reagent for a Modified Curtius Reaction and for the Peptide Synthesis. *J. Am. Chem. Soc.* **1972**, *94*, 6203–6205.
(51) Rehavi, M.; Skolnick, P.; Paul, S. M. Effects of Tetrazole Derivatives on [^{3}H]Diazepam Binding *In Vitro*: Correlation with Convulsant Potency. *Eur. J. Pharmacol.* **1982**, *78*, 353–356.
(52) Miledi, R.; Parker, I.; Sumikawa, K. Synthesis of Chick Brain GABA Receptors by Frog Oocytes. *Proc. R. Soc. London* **1982**, *B216*, 509–515.
(53) Smart, T. G.; Constanti, A.; Bilbe, G.; Brown, D. A.; Barnard, E. A. Synthesis of Functional Chick Brain GABA-Benzodiazepine-Barbiturate/Receptor Complexes in mRNA-Injected Xenopus Oocytes. *Neurosci. Lett.* **1983**, *40*, 55–59.
(54) Sigel, E.; Baur, R. Allosteric Modulation by Benzodiazepine Receptor Ligands of the $GABA_A$ Receptor Channel Expressed in Xenopus Oocytes. *J. Neurosci.* **1988**, *8*, 289–295.
(55) Prado de Carvalho, L.; Curutchet, P.; Stinnakre, J.; Rossier, J. Modulation of $GABA_A$ Receptor Channel Expressed in Xenopus Oocytes by Full and Partial Agonists of the Benzodiazepine Receptor. 19th Annual Meeting, The Society for Neuroscience, Phoenix, 1989; Abstract 335.8.
(56) Chirgwin, J. M.; Przybyla, A. E.; MacDonald, R. J.; Rutter, W. J. Isolation of Biologically Active Ribonucleic Acid from Sources Enriched in Ribonuclease. *Biochemistry* **1979**, *18*, 5294–5299.
(57) Civelli, O.; Birnberg, N.; Herbert. E. Detection and Quantitation of Pro-opiomelanocortin mRNA in Pituitary and Brain Tissues from Different Species. *J. Biol. Chem.* **1982**, *257*, 6783–6787.
(58) Aviv, H.; Leder, P. Purification of Biologically Active Globin Messenger RNA by Chromatography on Oligothymidylic Acid Cellulose. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 1408–1412.

JM940476R