# Synthesis and Evaluation of Analogues of the Partial Agonist 6-(Propyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester (6-PBC) and the Full Agonist 6-(Benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester (Zk 93423) at Wild Type and Recombinant $GABA_A$ Receptors

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A pharmacophore and an alignment rule have previously been reported for BzR agonist ligands. The design and synthesis of 6-(propyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic acid ethyl ester (6-PBC, **24**, $IC_{50}$ = 8.1 nM) was based on this pharmacophore. When evaluated in vivo this ligand exhibited anticonvulsant/anxiolytic activity but was devoid of the muscle relaxant/ataxic effects of "classical" 1,4-benzodiazepines (i.e., diazepam). Significantly, 6-PBC **24** also reversed diazepam-induced muscle relaxation in mice. The 3-substituted analogues **40**−**46** and **48** of 6-PBC **24** and Zk 93423 **27** ($IC_{50}$ = 1 nM) were synthesized and evaluated in vitro to determine what affect these modifications would have on the binding affinity at recombinant BzR subtypes. With the exception of the 3-amino ligands **40** and **41**, all the β-carbolines were found to exhibit high binding affinity at BzR sites. The 3-propyl ether derivative **45** was also evaluated in vivo and found to be devoid of any proconvulsant or anticonvulsant activity at doses up to 40 mg/kg. The 6-(1-naphthylmethyloxy) and 6-octyloxy analogues **25**, **26**, **28**, and **29** of 6-PBC **24** were synthesized to further evaluate the proposed alignment of agonists vs inverse agonists in the pharmacophore of the BzR. In addition, ligands **26** and **29** were designed to probe the dimensions of lipophilic pocket $L_3$ at the agonist site. The activity of **29** was evaluated in vivo; however, this analogue elicited no pharmacological effects at doses up to 80 mg/kg. These and other related β-carbolines were also examined in five recombinant $GABA_A$ receptor subtypes. Ligands **52**−**61** all exhibited moderate to high affinity at $GABA_A$ receptors containing $\alpha_1$ subunits. These ligands will be useful in further defining the pharmacophore at $\alpha_1\beta_3\gamma_2$ receptors.

## Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in vertebrate brain.[1] It is estimated that almost 50% of all synapses in the brain employ this neurotransmitter.[2] There are at least three distinct classes of receptors which mediate the effects of GABA.[3,4] $GABA_A$ receptors are directly associated with a $Cl^-$ ion channel while $GABA_B$ receptors appear to be coupled to $Ca^{2+}$ or $K^+$ channels through second messenger systems.[2,3] In addition, $GABA_C$ receptors are also directly associated with a $Cl^-$ ion channel, and recent evidence suggests this receptor is structurally related to $GABA_A$ receptors.[5]

The $GABA_A$ receptor contains binding sites for a variety of ligands including barbiturates, steroids, benzodiazepines, and "cage" convulsants.[4,6] The $GABA_A$ receptor complex possesses significant molecular diversity and is thought to be a pentameric structure assembled from several classes of subunits.[1] Molecular cloning experiments have identified at least 15 different subunits ($\alpha_{1-6}$, $\beta_{1-4}$, $\gamma_{1-3}$, $\delta$, $\rho_{1,2}$),[1,6,9,10] and this molecular diversity likely explains the broad spectrum of pharmacological actions associated with ligands acting at these ligand-gated ion channels. The identification and biological evaluation of highly specific ligands at $GABA_A$ receptor subtypes is critical in assigning specific physiological and pharmacological roles to these subunits. Studies from many laboratories have centered on the design and synthesis of biologically important ligands which act at $GABA_A$/Bz receptor sites.[12−25]

To date, the composition of any single $GABA_A$ receptor ion channel is unknown; however, in situ hybridization and immunohistochemical studies have shown that ($\alpha_1\beta_{2/3}\gamma_2$) is the most abundant $GABA_A$ receptor subtype in mammalian brain.[5] In addition, Pritchett et al.[11] have shown that complexes which contain the $\alpha_1$ subunit exhibit high affinity for zolpidem **69**, CL 218872 **70**, and some β-carbolines.[4] Evidence to date has suggested that most if not all of the subunits investigated do exist in the brain. Recently the binding affinity of a series of ligands at recombinant $\alpha_5\beta_2/\beta_3\gamma_2$ receptor subtypes has been shown to directly parallel their affinities at native $\alpha_5$ subtypes isolated from hippoc-

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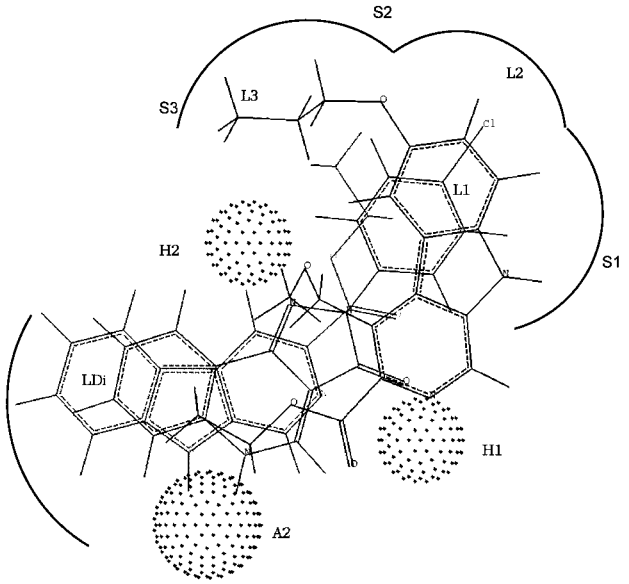



**Figure 1.** The alignment of the partial agonist 6-PBC (in black) and the inverse agonist *β*CCE (in medium black) fitted to a schematic representation of the inclusive pharmacophore with CGS 9896 at the benzodiazepine binding site. Note, the partial agonist 6-PBC is aligned in a vertical fashion, while that of the inverse agonist *β*CCE is in a horizontal orientation. The sites $H_1$ and $H_2$ designate hydrogen bond donor sites on the receptor protein while $A_2$ represents a hydrogen bond acceptor site. Interaction with the hydrophobic pocket $L_1$, as well as with $H_1$ and $A_2$ is required for potent inverse agonist activity in vivo. Agonist activity requires interaction with $H_1$, $H_2$, $L_1$, $L_2$, and/or $L_3$. Receptor descriptors $S_1$, $S_2$, and $S_3$ are regions of negative steric repulsion.

ampal tissue.[12] Thus, the use of recombinant receptor subtypes provides an excellent system to investigate ligands at the $GABA_A$ receptor complex.[12]

A comprehensive model of the pharmacophore for agonists and inverse agonists at the benzodiazepine receptor has been proposed.[13,14,18,19,24,26] This model was developed using the techniques of chemical synthesis, radioligand binding, and receptor mapping. More than 150 different ligands encompassing 15 structurally different classes of compounds were employed. This pharmacophore qualitatively accounts for the relative affinities, efficacies, and functional effects displayed by various ligands at the BzR.[12−14,16,18−27] Briefly, the pharmacophore required two hydrogen bond donating sites termed $H_1$ and $H_2$. The two sites were located about 6.7 Å from each other. The binding sites $H_1$ and $L_1$ are common to both inverse agonist and agonist pharmacophores; however, $H_2$ corresponds to the 4-position of *β*-carbolines. With respect to *β*-carbolines it was believed the oxygen atom at this position was critical for high-affinity binding by directing the ligand into the active site via formation of a hydrogen bond between the ether oxygen atom and $H_2$ on the receptor protein. There is also a lipophilic area with two zones important for agonist activity termed $L_2$ and $L_3$. Occupation of areas $L_2$ and/or $L_3$ as well as interaction at $H_1$, $H_2$, and $L_1$ are important for agonist activity.[18,19,24] In addition, there are regions of repulsive steric interaction which reduce ligand affinity for the receptor termed $S_1$, $S_2$, and $S_3$ (Figure 1).

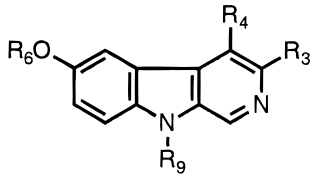
During the course of these studies the design and synthesis of the anxiolytic/anticonvulsant 6-(*n*-propyloxy)-4-(methoxymethyl)-*β*-carboline-3-carboxylic acid ethyl ester (6-PBC, **24**) was conceived.[18,19] This *β*-carboline inhibited PTZ-induced seizures in a dose-dependent fashion and exhibited anxiolytic activity when evaluated in an elevated plus-maze paradigm. This partial agonist **24** was devoid of muscle-relaxant activity and completely antagonized the myorelaxant actions of diazepam.[18,19] A recent examination of the ability to inhibit [$^3$H]Ro15-1788 binding at five recombinant $GABA_A$ receptor subtypes [$\alpha_1\beta_3\gamma_2$ ($K_i$ = 0.49 nM), $\alpha_2\beta_3\gamma_2$ ($K_i$ = 1.21 nM), $\alpha_3\beta_{3\gamma2}$ ($K_i$ = 2.20 nM), $\alpha_5\beta_3\gamma_2$ ($K_i$ = 2.39 nM), $\alpha_6\beta_3\gamma_2$ ($K_i$ = 1343 nM)] indicated 6-PBC possessed high affinity and some selectivity for the $\alpha_1\beta_3\gamma_2$ subtype, whereas the full agonist Zk 93423 **27** [$\alpha_1\beta_3\gamma_2$ ($K_i$ = 4.1 nM), $\alpha_2\beta_3\gamma_2$ ($K_i$ = 4.2 nM), $\alpha_3\beta_3\gamma_2$ ($K_i$ = 6.0 nM), $\alpha_5\beta_3\gamma_2$ ($K_i$ = 4.5 nM), $\alpha_6\beta_3\gamma_2$ ($K_i$ = >1000 nM)] was less selective (see Table 2). More recently, the synthesis and biological activity of several $\alpha_5\beta_3\gamma_2$ selective ligands (50−70-fold more selective at $\alpha_5/\alpha_1$ subtypes) has been reported from our laboratory.[12,20−23,25] These ligands will be employed to determine what role receptors which contain the $\alpha_5$ subunit may play in the human brain since these subtypes are found principally in the hippocampus.[12,21] In addition, a number of *β*-carbolines have recently been found to exhibit some subtype selectivity for the $\alpha_1\beta_3\gamma_2$ subunit.[5]

Examination of the biological activity of the partial agonist 6-PBC **24** prompted further study of *β*-carbolines via alteration of the substituent at C(3) on the *β*-carboline skeleton (as well as that of Zk 93423 **27**). This study would help to establish what effect the C(3) substituent would exert on the in vitro binding affinities of these compounds at BzR subtypes. The *β*-carbolines in Schemes 1, 2, and 3 were prepared for this study. Furthermore, several related *β*-carbolines (**25**, **26**, **28**, **29**) which contained substituents at position 6 which varied (electronically and sterically) were synthesized and evaluated. These modifications were carried out both in the agonist (i.e., $R_4 = CH_2OCH_3$) and the inverse agonist (i.e., $R_4 = CH_2CH_3$) series. In addition, the receptor subtype selectivities of a number of other *β*-carbolines were determined as well.

## Chemistry

A computer-based alignment of 6-PBC **24** in the inclusive pharmacophore of the benzodiazepine receptor site is illustrated in Figure 1 and has been previously reported.[12−14,16,18−25,27] Ligand overlap via computer modeling illustrated the 6-benzyloxy function of Zk 93423 **27** and the 5-phenyl ring of diazepam should fully occupy lipophilic pocket $L_3$; however, the partial agonists CGS 9895 and CGS 9896 and the antagonist 6-methoxy-4-(methoxymethyl)-*β*-carbolinecarboxylic acid ethyl ester[6] lacked a substituent which would interact in this region. On the basis of this evidence, it was decided to synthesize 6-PBC **24**. The 6-*n*-propyloxy substituent of **24** should only partially occupy $L_3$ and thus should provide a ligand with a partial agonist profile.[18,19,27] As expected, the *β*-carboline **24** displayed high affinity at the BzR ($IC_{50}$ = 8.1 nM). More importantly, however, **24** exhibited anxiolytic/anticonvulsant activity when evaluated in mice but was devoid of muscle relaxant activity while the ligand antagonized the muscle relaxant/ataxic activity of diazepam.[7,8] In addition, ligands **26** and **29** were prepared to further probe the dimen-

**Table 1.** Potencies of Various β-Carboline Ligands To Inhibit [$^3$H]Flunitrazepam Binding to Cortical Membranes




| compd | $R_3$ | $R_4$ | $R_6$ | $R_9$ | $IC_{50}$ (nM)[a] |
|---|---|---|---|---|---|
| **24** | $CO_2CH_2CH_3$ | $CH_2OCH_3$ | $CH_2CH_2CH_3$ | H (6-PBC) | 8.1 ± 1.5 |
| **45** | $OCH_2CH_2CH_3$ | $CH_2OCH_3$ | $CH_2CH_2CH_3$ | H | 43.2 |
| **42** | N=C=S | $CH_2OCH_3$ | $CH_2CH_2CH_3$ | H | 24.7 |
| **48** | $CO_2CH(CH_3)_2$ | $CH_2OCH_3$ | $CH_2CH_2CH_3$ | H | 30.8 |
| **40** | $NH_2$ | $CH_2OCH_3$ | $CH_2CH_2CH_3$ | H | 697 |
| **30** | $CO_2CH_2CH_3$ | $CH_2OCH_3$ | $CH_2CH_2CH_3$ | $CH_3$ | 290 |
| **26** | $CO_2CH_2CH_3$ | $CH_2OCH_3$ | $(CH_2)_7CH_3$ | H | 330 ± 30 |
| **32** | $CO_2CH_2CH_3$ | $CH_2OCH_3$ | $(CH_2)_7CH_3$ | $CH_3$ | >1000 |
| **25** | $CO_2CH_2CH_3$ | $CH_2CH_3$ | $(CH_2)_7CH_3$ | H | >1000 |
| **31** | $CO_2CH_2CH_3$ | $CH_2CH_3$ | $(CH_2)_7CH_3$ | $CH_3$ | >1000 |
| **27** | $CO_2CH_2CH_3$ | $CH_2OCH_3$ | $CH_2Ph$ | H (Zk-93423) | 1 |
| **46** | $OCH_2CH_2CH_3$ | $CH_2OCH_3$ | $CH_2Ph$ | H | 63.8 |
| **43** | N=C=S | $CH_2OCH_3$ | $CH_2Ph$ | H | 40 |
| **41** | $NH_2$ | $CH_2OCH_3$ | $CH_2Ph$ | H | 271.4 |
| **44** | $NHC(S)OCH_3$ | $CH_2OCH_3$ | $CH_2Ph$ | H | 176 |
| **29** | $CO_2CH_2CH_3$ | $CH_2OCH_3$ | $CH_2$-1-naphthyl | H | 55.5 ± 4 |
| **35** | $CO_2CH_2CH_3$ | $CH_2OCH_3$ | $CH_2$-1-naphthyl | $CH_3$ | 730 ± 60 |
| **28** | $CO_2CH_2CH_3$ | $CH_2CH_3$ | $CH_2$-1-naphthyl | H | 13.9 ± 1.9 |
| **34** | $CO_2CH_2CH_3$ | $CH_2CH_3$ | $CH_2$-1-naphthyl | $CH_3$ | >1000 |
| **49** | $CO_2CH_2CH_3$ | $CH_2CH_3$ | $CH_2Ph$ | H | 22[b] |
| **51** | $CO_2CH_2CH_3$ | $CH_2CH_3$ | $CH_2Ph$ | $CH_3$ | >5000[b] |
| **71** | $CO_2CH_2CH_3$ | $CH_2OCH_3$ | $OCH_3$ | H | 1[b] |
| **72** | $CO_2CH_2CH_3$ | H | $CH_2Ph$ | $CH_3$ | >5000[b] |

[a] Values for compounds are listed with statistical limits and represent an average of three or more experiments. Please see the Experimental Section for further details. [b] See ref 27.

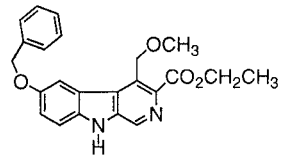
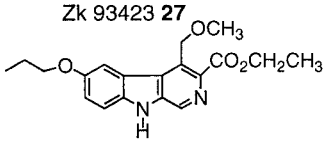
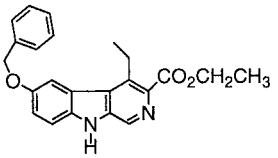
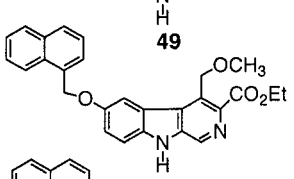
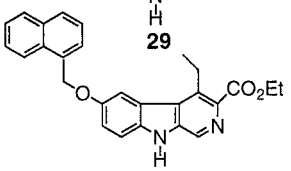
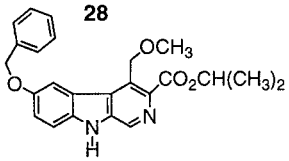
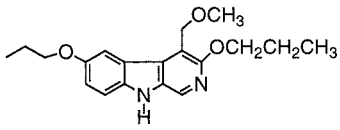
sions of $L_3$. Ligands **25** and **28** lack an oxygen substituent at the 4-position and should therefore display no affinity for the agonist site as compared to their oxygenated counterparts **26** and **29**. The $N_a$-methyl analogues **30**−**35** were prepared as negative controls to compare to their $N_a$-H analogues since the methyl function is believed to undergo negative steric interactions with $S_1$ and should be devoid of activity if the ligand alignment is correct (with respect to agonist β-carboline ligands). The synthesis of these β-carboline ligands was accomplished via the method of Neef et al.[28] and is described in detail for 6-PBC **24**.

The 5-(benzyloxy)indole **1** was treated with ammonium formate in the presence of a palladium catalyst in ethanol[10] to provide 5-hydroxyindole **2** in 92% yield (Scheme 1). Alkylation of the indole was accomplished by treating hydroxyindole **2** with propyl iodide and potassium carbonate in acetone at reflux to afford 5-(propyloxy)indole **3** in excellent yield. A Michael reaction between indole **3** and 3-hydroxy-2-nitro-5-oxahexanoic acid ethyl ester **37** in the presence of 10% acetic acid and toluene at reflux provided indole **6** in good yield. Raney nickel mediated reduction of the nitro function provided the amino ethyl ester **12**. Cyclization of **12** was achieved via a modification of the Pictet−Spengler reaction[11] to provide the tetrahydro-β-carboline **18**. Subsequent decarboxylation of **18** in xylenes at reflux followed by oxidation using sulfur in DMSO at 140 °C resulted in the fully aromatic β-carboline **24**.

Studies on β-carbolines with inverse agonist activity had demonstrated that derivatives with 3-carboxylic acid ethyl ester functions exhibit limited water solubility and are short-lived in vivo due to esterase hydrolysis.[13,14] On the basis of this, the increased water solubility (as compared to βCCE) and the inverse agonist activity of 3-ethoxy-β-carboline,[26] the 3-carboxylic acid ethyl ester function was converted into the 3-*n*-propyloxy substituent.[13,14] Consequently, the 6-propyloxy and 6-benzyloxy analogues **45** and **46** (Scheme 2) were prepared to evaluate water solubility[26] and half-life in vivo in comparison to the parent ligands **24** and **27**. Furthermore, the binding affinity of these ligands would provide further evidence to determine if the interaction of the carbonyl group at C(3) with $H_1$ was necessary for high-affinity binding at BzR subtypes. The 3-alkoxy ether group has been proposed to release electron density to the pyridine nitrogen $N_{(2)}$ and would presumably enhance interaction with hydrogen bond donor descriptor $H_1$. A similar result was observed by Allen et al.[13] in the case of inverse agonists.[12] Thus 6-PBC **24** was treated with 98% hydrazine in the presence of ethanol at reflux to provide the carbohydrazide **38** via the method of Dodd et al.[31] The carbohydrazide **38** was dissolved in concentrated aqueous HCl at 0 °C followed by the addition of sodium nitrite. Curtius rearrangement of the intermediate azido compound (not shown) was accomplished in acetic acid/water at reflux to yield 6-(propyloxy)-4-(methoxymethyl)-3-amino-β-carboline **40** as the diacetate salt. Conversion of the amino intermediate **40** into the 3-propyloxy analogue **45** was attained via diazonium chemistry using the procedure of Allen et al.[12] Treatment of amine **40** with anhydrous propanol in the presence of isoamyl nitrite, CuSCN, and KSCN provided 3,6-bis(propyloxy)-4-(methoxymethyl)-β-carboline **45**.

The isothiocyanato derivatives **42** and **43** (Scheme 2) were proposed as potential irreversible inhibitors. These ligands could be employed with the 3-substituted de-

**Table 2.** $\alpha_x\beta_3\gamma_2$ ($K_i$ Values in nM)[a]

| Compound | α1 | α2 | α3 | α5 | α6 |
|---|---|---|---|---|---|
|  Zk 93423 **27** | 4.1 | 4.2 | 6 | 4.5 | > 1000 |
|  6-PBC **24** | 0.49 | 1.21 | 2.2 | 2.4 | 1343 |
|  **49** | 20.8 | 78.3 | 58.7 | 67.3 | >10000 |
|  **29** | 15.4 | ND | 293 | 323 | >1,000 |
|  **28** | 183 | 291 | 300 | 270 | >10000 |
|  abecarnil **50** | 12.4 | 15.3 | 7.5 | 6 | >10000 |
|  **45** | 24.6 | 214 | 270 | 332 | >10000 |

[a] $K_i$ values represent the mean of two determinations which differed by less than 10%. Data were generated using Ltk$^-$ cell membranes expressing human $\alpha_x\beta_3\gamma_2$ receptors. [$^3$H]Ro15-1788 and [$^3$H]Ro15-4513 (for cells expressing $\alpha_6\beta_3\gamma_2$ receptors) were used as radioligands at a final concentration of 1−2 nM.

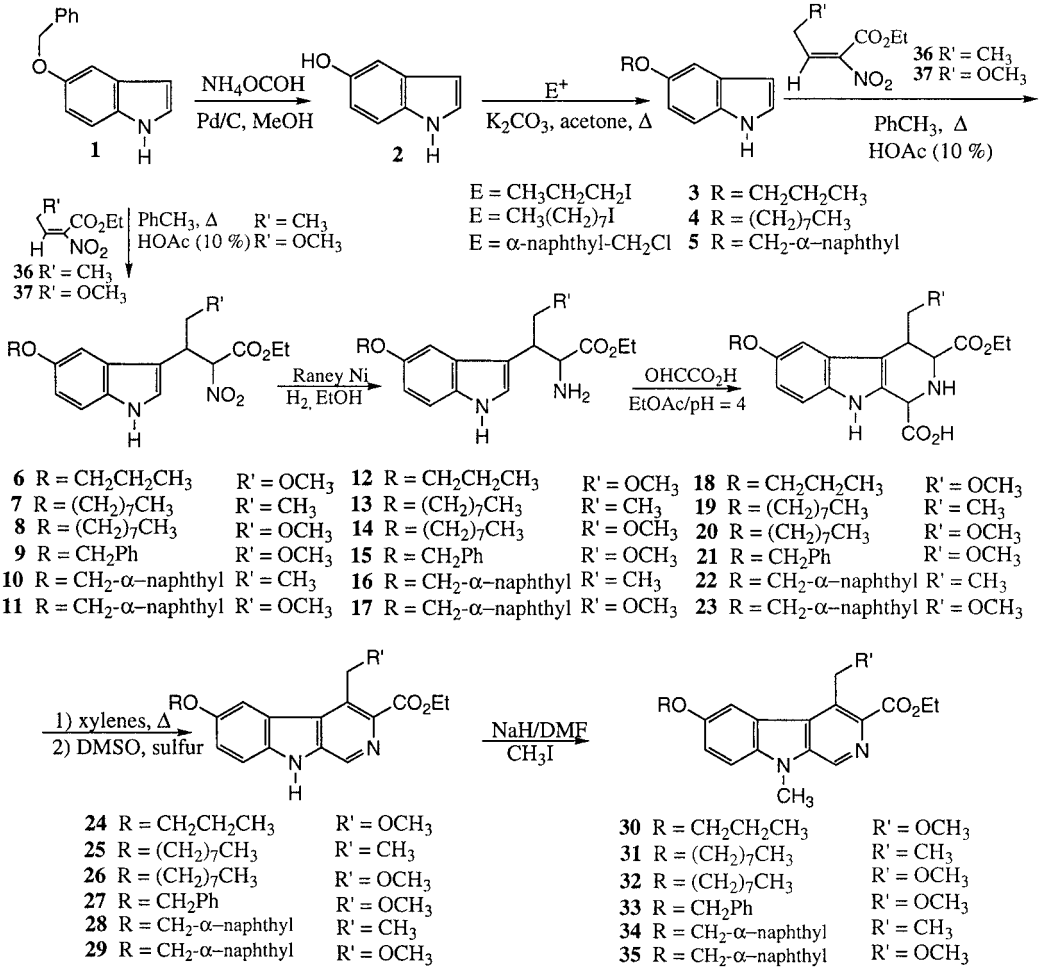
rivatives from the inverse agonist series to help characterize and understand the interaction of ligands at the GABA$_A$/Bz receptor at the molecular level.[13,14] The 3-isothiocyanato ligands **42** and **43** were prepared by treatment of the appropriate amino derivative (**40** or **41**) in chloroform with thiophosgene to provide the isothiocyanates **42** and **43** in excellent yield.

When the 3-carboxylic acid ethyl ester function of Zk 93423 **27** was replaced by a 3-carboxylic acid isopropyl ester (abecarnil **50**, Table 2), the pharmacological profile of this ligand was altered from a full agonist (see **27**) to a partial agonist (see **30**).[32,33] To investigate the role of the isopropyl ester on the agonist activity of $\beta$-carbolines related to 6-PBC **24**, the 3-ethyl ester of 6-PBC **24** was replaced by a 3-isopropyl ester function. Hence, 6-PBC **24** was hydrolyzed in the presence of 10% aqueous NaOH at reflux followed by treatment with dilute aqueous HCl to afford the carboxylic acid **47**. Subsequent esterification of acid **47** with 2-propanol in the presence of anhydrous HCl provided 6-(propyloxy)-4-(methoxymethyl)-$\beta$-carboline 3-carboxylic acid isopropyl ester **48** in 85% yield (Scheme 3).

## Results and Discussion

The potencies of various 3-substituted analogues of the parent ligands 6-PBC **24** and Zk 93423 **27** to inhibit [$^3$H]flunitrazepam to rat cortical membranes are summarized in Table 1. Replacement of the ethyl ester function of **24** or **27** with a propyl ether [**45** ($IC_{50}$ = 43.2 nM) and **46** ($IC_{50}$ = 63.8 nM)], respectively, significantly reduced affinity compared to the parent ligands. However, when evaluated in vivo the more potent analogue **45** exhibited no anticonvulsant activity at doses up to 40 mg/kg against a standard challenge of pentylenetetrazole. Furthermore, this ligand exhibited no proconvulsant activity at doses of 20 and 40 mg/kg in the same paradigm. The affinities of these two ligands at Bz binding sites are in agreement with previous studies on

**Scheme 1**

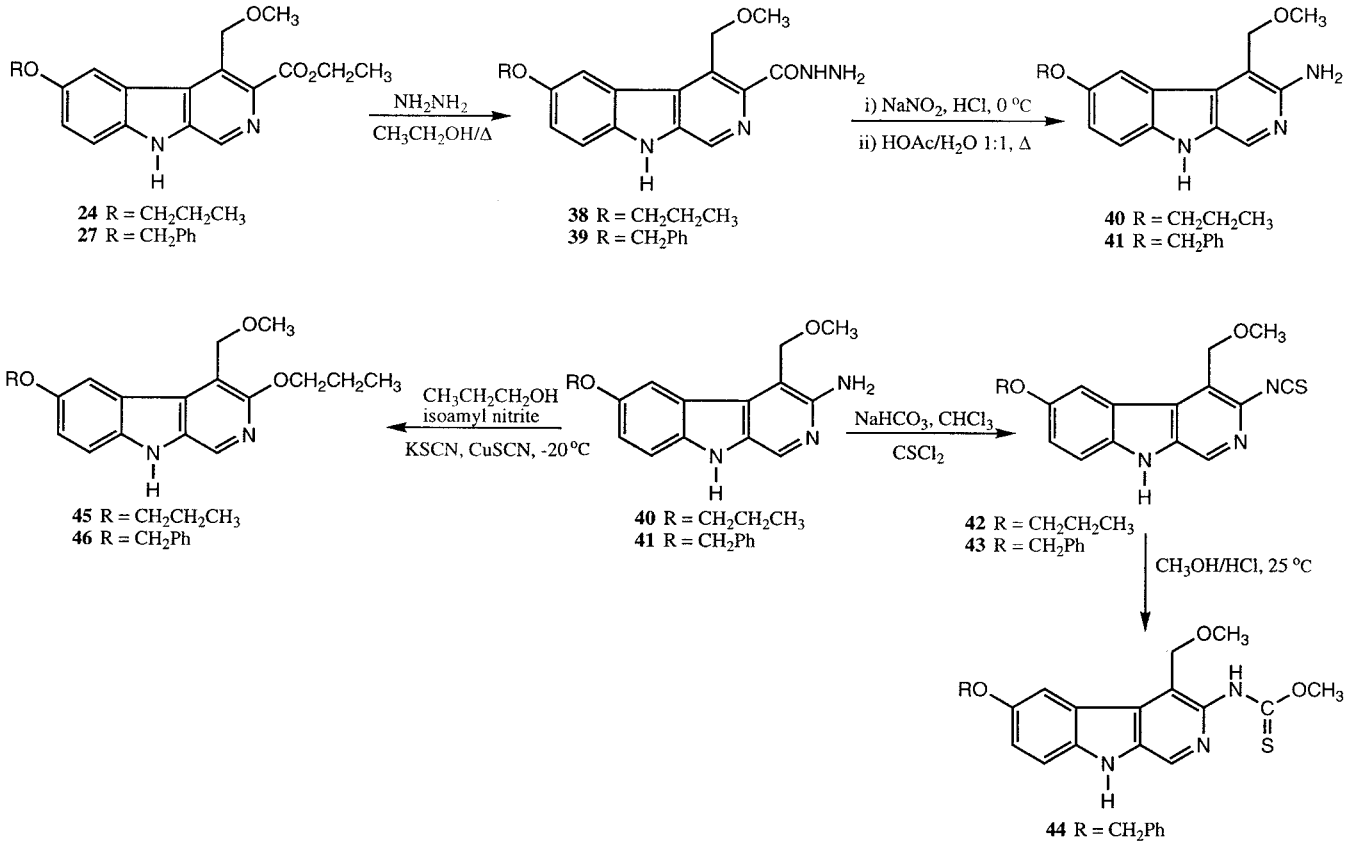



the effects of substitution at position 3 of $\beta$-carbolines.[13,14] Initially, it was believed that an ester moiety at the 3-position was required for a compound to exhibit high-affinity binding at Bz binding sites.[34−37] However, the high affinity of the partial inverse agonist 3-ethoxy-$\beta$-carboline **54** (Table 3) demonstrated this was not the case. Subsequently, further studies have suggested at least two factors affect high-affinity binding with respect to 3-alkoxy-substituted $\beta$-carbolines, one of which is the lipophilicity of the substituent which interacts at $L_1$.[13,14] The second factor is the ability of the 3-substituent to release electron density to the pyridine ring, enhancing the basicity of the nitrogen atom which results in greater ligand−receptor interaction at $H_1$. The lack of agonist and inverse agonist activity of 3-propyloxy ligands **45** and **46** in vivo may be a direct result of the length of the alkyl substituent at position 3. For example, Allen et al.[13,14] demonstrated that 3-ethoxy-$\beta$-carboline **54** displayed inverse agonist properties, while 3-(propyloxy)-$\beta$-carboline **55** elicited an antagonist response when evaluated in the pentylenetetrazole paradigm in mice.[12,13] Furthermore, the syn conformation of the carbonyl group and nitrogen function N(2) in $\beta$-carboline-3-carboxylate alkyl esters would enhance interaction at $H_1$ in vivo, and this interaction is absent in **45** and **46** which would contribute to the loss of activity in vivo as well. A direct comparison cannot be made to the work of Allen et al.[13] since the substitution pattern is complicated by the presence of the groups at position 4.

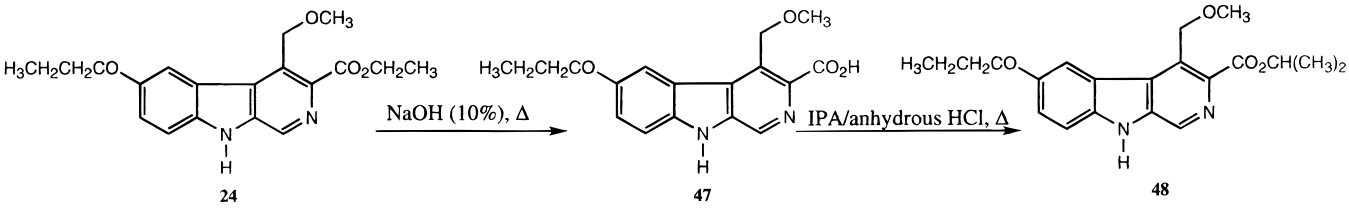
The synthesis of 3-isothiocyanato-$\beta$-carboline ($IC_{50}$ = 8 nM) by Allen et al.[13] resulted in a ligand which bound irreversibly to the Bz binding site. As discussed above, the ability of the 3-substituent to stabilize the ligand−receptor hydrogen bond $H_1$ at N(2) is important for high affinity binding at the Bz binding site. This irreversible inhibitor provided strong evidence that such an interaction was indeed necessary for high-binding affinity. As expected, the isothiocyanate derivatives **42** ($IC_{50}$ = 24.7 nM) and **43** ($IC_{50}$ = 40 nM) exhibited moderate affinity for the Bz binding site. This result further supports the work of Allen et al. in regard to interactions of ligands at $H_1$.[13,14]

Examination by molecular modeling combined with SAR studies have indicated at least three important hydrophobic receptor regions ($L_1$, $L_2$, and $L_3$) are contained within the Bz binding sites.[24] The interactions between ligands and these regions also contribute to the efficacy of these compounds. For example, studies on 3-substituted $\beta$-carbolines have shown a transition from a methyl ester to a *n*-propyl ester resulted in an inverse agonist profile in the former to an antagonist profile in the latter compound.[13] In addition, the presence of a bulky substituent such as a *tert*-butyl ester ($\beta$CCt) provided an antagonist with good $Bz_1$ selectivity.[16] Furthermore, when the 3-ethyl ester moiety of Zk 93423 **27** was replaced with an isopropyl ester (abecarnil **50**) the biological profile was altered from that of a full agonist to a partial agonist. In a complementary manner, replacement of the 3-ethyl ester of 6-PBC **24**

**Scheme 2**




**Scheme 3**



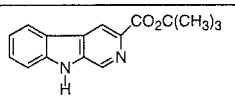
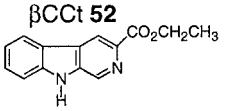
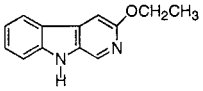
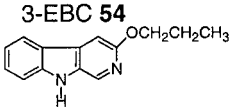
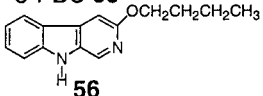
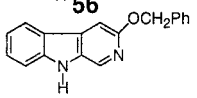
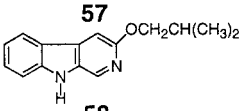
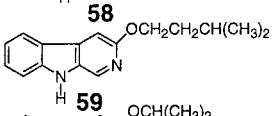
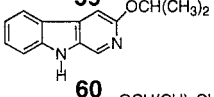
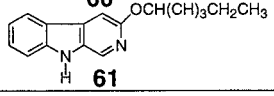

with an isopropyl ester moiety **48** ($IC_{50}$ = 30.8 nM) provided a ligand with moderate affinity in vitro; however, agonist activity was no longer observed in vivo in this series.

As expected, the 3-amino analogues **40** ($IC_{50}$ = 697 nM) and **41** ($IC_{50}$ = 271 nM) exhibited reduced affinities when compared to their parent ligands (**24** and **27**). This reduction is believed to be a result of two unfavorable interactions.[12,13] First, the lack of sufficient lipophilicity at C-3 may lead to unfavorable ligand−receptor interactions. Second, Allen et al.[13,14] have shown the amino function to exist as the iminopyridine tautomer which results in diminution of the necessary interaction between the pyridine nitrogen atom N(2) on the ligand with the hydrogen bond acceptor portion ($H_1$) of the receptor site.

The $N_a$-methyl congeners **30**, **32**, **33**, and **35** ($IC_{50}$ values of 290, >1000, 985, 730 nM, respectively) were synthesized and evaluated as negative controls. As expected, these ligands exhibited a significant decrease in affinity to the BzR as compared to their $N_a$-H counterparts. It is believed the methyl substituents of compounds **30** and **35** are interacting with $S_1$ (see Figure 1), leading to an unfavorable steric interaction. Ligands **32** and **33** also interact with $S_1$.[19,24]

The inverse agonist and agonist inclusive pharmacophore illustrated in Figure 1 has been developed to correlate essential pharmacophoric descriptors to binding affinity in vitro as well as biological activity in vivo. Evidence from molecular modeling suggests that inverse agonists ($A_2$, $H_1$, and $L_1$) and agonists ($H_1$, $H_2$, $L_1$, $L_2$, and/or $L_3$) bind to the same domain of the BzR.[24,38] Although $H_1$ and $L_1$ are common to both models, the remaining points of receptor interaction are clearly different. With respect to the agonist pharmacophore, it is believed that an oxygen atom corresponding to the 4-position of the $\beta$-carboline nucleus (i.e., the 4-methoxymethyl substituent) will align the ligand into the active site on the protein complex via generation of a hydrogen bond between the molecule and a donor site on the protein ($H_2$). As stated earlier, the ligand must form hydrogen bonds with $H_1$ (the pyridine nitrogen of the $\beta$-carboline) and $H_2$ as well as interact with $L_1$, $L_2$, and/or $L_3$ in order to elicit an agonist response with respect to $\beta$-carbolines. The phenyl group of the benzyloxy substituent of Zk 93423 **27** is believed to lie in the lipophilic pocket $L_3$. It is important to note that substituents which occupy $L_3$ cannot lie in the same plane as $H_1$, $H_2$, and $L_1$ since they would interfere with the hydrogen-bonding protein−ligand interactions nec-

**Table 3.** $\alpha_x\beta_3\gamma_2$ ($K_i$ Values in nM)[a]

| Compound | α1 | α2 | α3 | α5 | α6 |
|---|---|---|---|---|---|
|  | 0.72 | 15 | 18.9 | 111 | >5000 |
| βCCt **52**  | 1.2 | 4.9 | 5.7 | 26.8 | >1000 |
| BCCE **53**  | 6.43 | 25.1 | ND | 868 | >1000 |
| 3-EBC **54**  | 5.3 | 52.3 | 68.8 | 591 | >1000 |
| 3-PBC **55**  | 36.9 | 194 | 245 | ~1000 | >1000 |
| **56**  | 830 | >3000 | >3000 | >10000 | >10000 |
| **57**  | 24.9 | 124 | 139 | ~1000 | >10000 |
| **58**  | 350.2 | >3000 | >3000 | >3000 | >10000 |
| **59**  | 283 | >3000 | >3000 | >10000 | >10000 |
| **60**  | 245 | 818 | 869 | >10000 | >10000 |
| **61** | | | | | |

[a] $K_i$ values represent the mean of two determinations which differed by less than 10%. Data were generated using Ltk$^-$ cell membranes expressing human $\alpha_x\beta_3\gamma_2$ receptors. [$^3$H]Ro15-1788 and [$^3$H]Ro15-4513 (for cells expressing $\alpha_6\beta_3\gamma_2$ receptors) were used as radioligands at a final concentration of 1−2 nM.

essary for high-affinity binding. Thus, the alignment of the $\beta$-carboline nucleus ring A (containing the 6-benzyloxy substituent) is directed toward lipophilic regions $L_1$ and $L_2$ (see Figure 1).

Examination of Figure 1 illustrates an alignment in the pharmacophore for inverse agonist $\beta$-carboline ligands that differs from that of the agonist pharmacophore. These $\beta$-carbolines are devoid of an oxygen atom at position 4 to interact with the $H_2$ donor site. Rather, an alignment for inverse agonists, as reported earlier,[13,14,24] is obtained in which the indole N(9)−H function interacts with $A_2$ on the receptor complex.[13,14,24] This alignment directs the $\beta$-carboline ring system parallel to $H_1$ and $A_2$ rather than perpendicular to this line. This orientation no longer permits an interaction with lipophilic pocket $L_3$, resulting in an inverse agonist profile. To further study this alignment, several $\beta$-carbolines **25**, **26**, **28**−**35** were synthesized in both the agonist ($R_4 = CH_2OCH_3$) and inverse agonist series ($R_4 = CH_2CH_3$) and evaluated in vitro. These alterations permitted a change in electronic character without changing the ligand topology at C(4). In addition, ligands **26** and **29** were used to further probe the dimensions of lipophilic pocket $L_3$.

The 4-ethyl congener **49** of Zk 93423 **27** inhibits [$^3$H]-Flu binding to rat cortical membranes with an $IC_{50}$ value of 22 nM,[27] an approximately 20-fold reduction in affinity as compared to the parent ligand **27** ($IC_{50}$ = 1 nM). This ligand lacked the anticonvulsant activity associated with agonist ligands. The analogous substitution carried out in the 6-octyloxy series resulted in a similar outcome. The $\beta$-carboline ligand **26** bound with an $IC_{50}$ of 330 nM, while the 4-ethyl analogue **25** exhibited very low affinity ($IC_{50}$ = >1000 nM). While further studies are required, the high−moderate affinities of Zk 93423 **27**, 6-PBC **24**, and **29** combined with the poor affinity of **26** suggests the lipophilic pocket $L_3$ will not tolerate long-chain alkyl substituents.

The 6-(1-naphthylmethyloxy)-4-methoxymethyl derivative **29** of Zk 93423 **27** inhibited [$^3$H]flunitrazepam binding with an $IC_{50}$ value of 55.5 nM. Although considerably less potent than the parent ligand **27**, this

$\beta$-carboline exhibited a positive GABA shift of 1.5 which is consistent with a partial agonist profile of activity. However, when evaluated in vivo, **29** elicited no convulsant, proconvulsant, or anticonvulsant activity at doses up to 80 mg/kg. The decrease in potency in vitro of **29** when compared to Zk 93423 **27** has resulted in a ligand with no detectable in vivo activity at the doses studied. Examination of the affinities at recombinant receptor subtypes illustrated in Table 2 indicated ligand **29** is nearly 20-fold more selective for the $\alpha_1\beta_3\gamma_3$ site compared to the other subtypes evaluated. The full agonist Zk 93423 **27** [$\alpha_1\beta_3\gamma_2$ ($K_i$ = 4.1 nM), $\alpha_2\beta_3\gamma_2$ ($K_i$ = 4.2 nM), $\alpha_3\beta_3\gamma_2$ ($K_i$ = 6.0 nM), $\alpha_5\beta_3\gamma_2$ ($K_i$ = 4.5 nM), $\alpha_6\beta_3\gamma_2$ ($K_i$ = >1000 nM)] exhibited no remarkable subtype selectivity for diazepam sensitive (DS) $GABA_A$ receptor isoforms while the partial agonist 6-PBC **24** [$\alpha_1\beta_3\gamma_2$ ($K_i$ = 0.49 nM), $\alpha_2\beta_3\gamma_2$ ($K_i$ = 1.21 nM), $\alpha_3\beta_3\gamma_2$ ($K_i$ = 2.20 nM), $\alpha_5\beta_3\gamma_2$ ($K_i$ = 2.39 nM), $\alpha_6\beta_3\gamma_2$ ($K_i$ = 1343 nM)] displayed moderate $\alpha_1\beta_3\gamma_3$ selectivity. These data, coupled with a GABA shift of 1.5, suggest **29** may exhibit a partial agonist profile. The lack of biological activity may therefore be due to the inability of this ligand to penetrate the blood/brain barrier. Further studies are needed to fully address this issue.

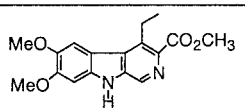
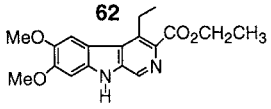
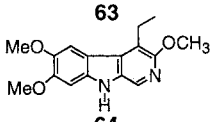
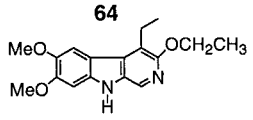
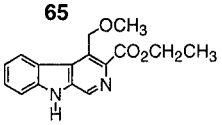
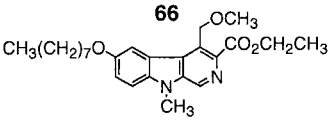
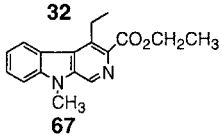
Replacement of the 4-methoxymethyl moiety of **29** with an ethyl substituent **28** resulted in a 4-fold increase in affinity ($IC_{50}$ = 13.9 nM). This is in agreement with the proposed alignment rule at the Bz site;[24] thus if the 6-substituted benzyloxy moiety of Zk 93423 **27** fully occupies lipophilic pocket $L_3$ as has been previously proposed,[24] then the naphthyl analogue **29** should exhibit a significant reduction in affinity at the Bz binding site. Furthermore, the 4-ethyl analogue **28** should exhibit potent binding affinity. Ligands which possess a 4-ethyl substituent are devoid of agonist activity; therefore the ligand was not screened in vivo. As expected, the $N_a$-methyl analogues **31**, **32**, **33**, and **34** exhibited drastically decreased affinities in vitro in agreement with the previous alignment of agonist and inverse agonist $\beta$-carbolines.[13,14,18,19,24,27]

The affinities of selected $\beta$-carboline ligands at recombinant $GABA_A$ receptors are illustrated in Tables 2−5. The proposed alignment for agonist $\beta$-carboline ligands is thought to be in a vertical orientation whereas that for inverse agonists is believed to be in a horizontal fashion with respect to $A_2$ and $H_1$ (see Figure 1).[24] The data from receptor subtype selectivity studies further supports this hypothesis. Several issues must be addressed when comparing the data for Zk 93423 **27** to that for the 4-ethyl analogue **49**, the first of which stems from the difference between the activity of these two ligands in vivo. The $\beta$-carboline Zk 93423 **27** elicited anticonvulsant and anxiolytic as well as ataxic and muscle relaxant activity, while ligand **49** was devoid of any anticonvulsant activity when evaluated against a standard challenge of pentylenetetrazole.[27] The only difference between these two ligands is the substituents at the 4-position. The 4-methoxymethyl moiety of **27** is thought to be interacting at the pharmacophoric descriptor $H_2$, resulting in the formation of a hydrogen bond interaction between the protein complex and the ligand. This interaction is believed to direct the agonist ligand into the agonist binding site, resulting in a vertical orientation of the $\beta$-carboline ligand. The 4-ethyl congener (horizontal/inverse agonist alignment) is devoid of an oxygen atom at this position, thus negating a vertical alignment. Furthermore, ligand **49** exhibited a very similar selectivity for $\alpha_1$-containing receptors as the partial agonist 6-PBC **24** when compared to affinities at the $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_5\beta_3\gamma_2$ receptor subtypes. If these compounds bound in the same orientation with very similar receptor subtype selectivity, one would have expected similar biological activity.

Further support for the proposed alignment of $\beta$-carboline ligands at the Bz binding site is illustrated in Table 3. The 3-substituted $\beta$-carboline ligands $\beta$CCE **53** and 3-EBC **54** as well as 3-PBC **55** all exhibit moderate to good selectivity for the $\alpha_1$ subtype. The partial agonist 6-PBC **24** exhibited moderate $\alpha_1\beta_3\gamma_2$ selectivity compared to the three other DS subtypes evaluated. The selectivity of these ligands for $GABA_A$ receptors containing the $\alpha_1$ subunit is similar in vitro; however, there is a large difference among them when evaluated in vivo. The 3-substituted ligands $\beta$CCE **50** and 3-EBC **52** elicit either proconvulsant or convulsant activity while 6-PBC **24** exhibits anticonvulsant/anxiolytic actions in rodents. Furthermore **24** antagonized the ataxic/muscle relaxant activity of diazepam.[18,19] The similar affinities for the $\alpha_1$ subunit of these ligands when compared to a very different biological profile for these compounds (see **24**, **50**, and **52**) further supports an alignment for inverse agonists $\beta$-carbolines different from that of agonist $\beta$-carbolines. Recently, 12 different 3,4,6-trisubstituted $\beta$-carboline ligands with a 4-methoxymethyl substituent were evaluated using computer modeling techniques.[39] When these ligands were evaluated using a vertical alignment, a cross-validated $r^2$ value of 0.5 (in a CoMFA analysis, see 6-PBC) was obtained. These results, when taken together with the affinities at recombinant $GABA_A$ receptors, further support the proposed alignment for 4-methoxymethyl-substituted $\beta$-carboline ligands at the BzR; nonetheless, further studies are required to address this issue.

Several studies using $GABA_A$ receptor subtypes have been carried out with 3-substituted $\beta$-carboline ligands,[13−15] some of which are illustrated in Table 3 (see below). The potent inverse agonist $\beta$CCE **53** bound to rat synaptosomal membranes with an $IC_{50}$ value of 5 nM. Examination of data from these studies have shown that in the 3-alkoxy series there is a steady increase in potency in vitro as chain length is increased from a 3-methoxy moiety (not shown) ($IC_{50}$ = 124 nM), to 3-ethoxy **54** ($IC_{50}$ = 24 nM), to a 3-*n*-propyloxy group **55** ($IC_{50}$ = 11 nM). When the chain length is further increased to provide the *n*-butyloxy analogue **56** ($IC_{50}$ = 98 nM), the potency is decreased by almost an order of magnitude. Furthermore, side chains with $\beta$ or $\delta$ branching (ligands **58**, **59**, and **60** with $IC_{50}$ values of 93, 500, and 471 nM, respectively) also resulted in a significant decrease in affinity. As expected, the subtype binding affinities at the $\alpha_1\beta_3\gamma_2$ subtype mimics the in vitro binding affinities to rat cortical membranes since the $\alpha_1\beta_{2/3}\gamma_2$ subtype is the most abundant receptor subtype in rodent cortex.[1] For example, 3-EBC **54** and 3-PBC **55**, as well as ethers **56**, **58**, and **60**, exhibit some $\alpha_1$ subtype selectivity. In addition, $\beta$CCt **52** is more selective for $\alpha_1$ subtypes than $\beta$CCE **53**, indicating that interactions in the pharmacophore at region $L_1$ have

**Table 4.** $\alpha_x\beta_3\gamma_2$ ($K_i$ Values in nM)[a]

| Compound | α1 | α2 | α3 | α5 | α6 |
|---|---|---|---|---|---|
|  **62** | 5.7 | 8.3 | 4.0 | 1.0 | 134 |
|  **63** | 6.2 | 18.7 | 4.0 | 3.3 | 74.9 |
|  **64** | >300 | >300 | >300 | >300 | >300 |
|  **65** | >300 | >300 | >300 | >300 | >300 |
|  **66** | 0.6 | 2.8 | 4.8 | 10.4 | 1150 |
|  **32** | >3,000 | >3,000 | >3,000 | >3,000 | >3,000 |
|  **67** | >1,000 | >1,000 | >1,000 | >1,000 | >10,000 |

[a] $K_i$ values represent the mean of two determinations which differed by less than 10%. Data were generated using Ltk$^-$ cell membranes expressing human $\alpha_x\beta_3\gamma_2$ receptors. [$^3$H]Ro15-1788 and [$^3$H]Ro15-4513 (for cells expressing $\alpha_6\beta_3\gamma_2$ receptors) were used as radioligands at a final concentration of 1−2 nM.

played a role in $\alpha_1$ selectivity. This property may be exploited in the future to design $\beta$-carbolines even more selective for $\alpha_1$ isoforms.

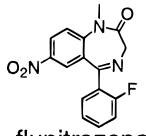
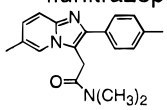
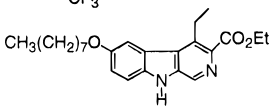
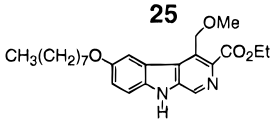
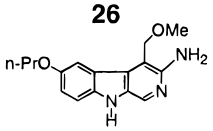
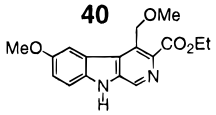
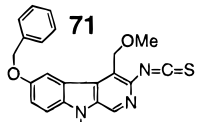
The potent inverse agonist DMCM **62** and its 3-carbethoxy analogue **63** are illustrated in Table 4. Both ligands exhibit high affinity for DS receptor subtypes. In addition, both $\beta$-carbolines display moderate affinity for the $\alpha_6\beta_3\gamma_2$ (DI) subtype. In an effort to create a longer-lived, more water soluble analogue of **62**, the 3-methoxy **64** and 3-ethoxy **65** DMCM analogues were synthesized and evaluated. These modifications resulted in significant reductions in ligand affinity. The orientation of the carbonyl group in ligands **60** and **61** has been proposed[12−15] to align in a syn conformation which results in the formation of a three-centered hydrogen bond with pharmacophoric descriptor $H_1$. This interaction leads to increased binding affinity. The 3-alkoxy analogues are unable to generate this interaction which may account for the decreased affinity at the recombinant receptor subtypes.

The negative controls **32** and **65** are shown in Table 4. Methylation of the indole $N_a$-H moiety rendered both ligands inactive at the five major receptor subtypes studied. These data are in agreement with previously published results.[13−15,24]

Recently, Liu et al. have synthesized and evaluated imidazobenzodiazepines at the five recombinant $GABA_A$ receptor subtypes illustrated here.[12,20−23] Several of the ligands examined exhibited potent $\alpha_5\beta_3\gamma_2$ subtype selectivity (~65−70-fold) compared to the other receptor subtypes. These imidazobenzodiazepines may be useful in discerning the underlying mechanisms associated with memory and learning since the $\alpha_5$ subtype is located primarily in the hippocampus.[12] These results combined with computer modeling of these congeners may provide a detailed analysis of this receptor subtype. As illustrated in Tables 2−5, many of the $\beta$-carboline ligands shown here exhibit moderate to high selectivity for the $\alpha_1\beta_3\gamma_2$ receptor subtype. The receptor subtype selectivity of these ligands, when combined with SAR data as well as computer modeling techniques, may provide detailed information regarding the topography of the $\alpha_1$-containing receptor subtypes.

In conclusion, the in vitro evaluation of ligands **24**, **26**−**29**, **40**−**46**, and **48** has shown these $\beta$-carbolines all bind with high affinity to the Bz binding sites on $GABA_A$ receptors. The receptor subtype selectivity data illustrated in Tables 2−5 has provided further evidence that the proposed alignment for inverse agonist $\beta$-carboline ligands is different than that of agonist $\beta$-carbolines. Analysis of the recombinant receptor subtype selectivity data for ligands **52**−**61** demonstrated that these ligands all exhibit moderate to high affinity for the $\alpha_1$ subtype. The data illustrated in Tables 2−5, combined with SAR studies and computer modeling analysis, may provide detailed information describing the pharmacophore for receptor subtypes containing the $\alpha_1$ subunit. In addition, the receptor subtype selectivity data has provided further evidence that the diazepam insensitive (DI) site $\alpha_6\beta_3\gamma_2$ is devoid of the lipophilic

**Table 5.** $\alpha_x\beta_3\gamma_2$ ($K_i$ Values in nM)[a]

| Compound | α1 | α2 | α3 | α5 | α6 |
|---|---|---|---|---|---|
|  flunitrazepam **68** | 2.2 | 2.5 | 4.5 | 2.1 | >2000 |
|  zolpidem **69** | 26.7 | 156 | 383 | >10000 | >10000 |
|  CL 218,872 **70** | 57 | 1964 | 1161 | 561 | >10000 |
|  **25** | 3,650 | 29,700 | 35,000 | 12,700 | >30,000 |
|  **26** | >1000 | >3000 | >3000 | >3000 | >3000 |
|  **40** | 45 | 540 | 700 | 380 | >3000 |
|  **71** | 0.14 | 1.19 | 1.72 | 4.0 | >10000 |
|  **43** | 25 | 82.6 | 58.8 | 159 | >10000 |

[a] $K_i$ values represent the mean of two determinations which differed by less than 10%. Data were generated using Ltk$^-$ cell membranes expressing human $\alpha_x\beta_3\gamma_2$ receptors. [$^3$H]Ro15-1788 and [$^3$H]Ro15-4513 (for cells expressing $\alpha_6\beta_3\gamma_2$ receptors) were used as radioligands at a final concentration of 1−2 nM.

pocket $L_3$ found in the diazepam sensitive (DS) sites ($\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$).[24]

## Experimental Section

**Radioligand Binding to Recombinant GABA$_A$ Receptors.** In brief, the affinity of compounds at GABA$_A$ subtypes was measured by competition with [$^3$H]Ro15-1788 (83 Ci/mmol; NEN) to Ltk$^-$ cells expressing human GABA$_A$ receptors composed of $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, and $\alpha_6\beta_3\gamma_2$.[41,42] Cells were removed from culture by scraping into phosphate-buffered saline, centrifuged at 3000*g* and resuspended in 10 mL of phosphate buffer (10 mM $KH_2PO_4$, 100 mM KCl, pH 7.4 at 4 °C) for each tray (25 cm$^2$) of cells. Radioligand binding assays were carried out in a volume of 500 $\mu$L which contained 100 mL of cells, [$^3$H]Ro15-1788 at a concentration of 1−2 nM and test compound in the range $10^{-9}$−$10^{-5}$ M. Nonspecific binding was defined by $10^{-5}$ M diazepam and typically represented less than 5% of the total binding. For cells expressing $\alpha_6\beta_3\gamma_2$, [$^3$H]Ro15-4513 was used as radioligand. Assays were incubated to equilibrium for 1 h at 4 °C and harvested onto GF/B filters (Brandel) by filtration using a Tomtec cell harvester and washing with ice-cold assay buffer. After drying, filter-retained radioactivity was detected by liquid scintillation counting. $K_i$ values were calculated using the least-squares iterative fitting routine of RS/l analysis software (BBN Research System, Cambridge, MA) and are the means of two determinations which differed by less than 10%.

**Radioligand Binding in Rat Brain Membranes.** The potencies of test compounds to displace [$^3$H]flunitrazepam from benzodiazepine binding sites were determined through a modification of previously described procedures.[27] In brief, rats were killed by decapitation, and the cerebral cortex was removed. Tissues were disrupted in 100 volumes of Tris-citrate buffer (50 mM, pH 7.4) using a Brinkman polytron (15 s, setting 6). Tissues were centrifuged for 20 min (4 °C) at 20000*g*. The supernatant was discarded and the tissue pellet resuspended in an equal volume of buffer. This "washing" procedure was repeated three times. Tissues were either used fresh or stored at −70 °C until used. Incubations (0.5 mL) consisted of tissue suspension (0.1 mL, ∼0.1 mg of protein), 0.05 mL of NaCl solution (2.5M), 0.05 mL of [$^3$H]flunitrazepam (final concentration, ∼1 nM), and drugs and/or buffer to volume. Nonspecific binding was determined using Ro15-1788 (final concentration 10 $\mu$M). Incubations (0−4 °C) were initiated by addition of radioligand and terminated after 60 min by rapid filtration under vacuum through GF/B filters

with two 5-mL washes of ice-cold buffer. $IC_{50}$ values were estimated using InPlot 4.0 (GraphPAD, San Diego, CA) with at least six concentrations of inhibitor. Values represent $X \pm$ SEM of at least three determinations. Compounds with potencies >1000 nM were generally only tested twice.

**In Vivo.** Adult male NIH/Swiss mice (~30 g) were injected intraperitoneally (ip) with graded doses of the compounds (0.1 mL, diluted Emulphor/saline, 1:9) or an equal volume of vehicle (0.1 mL, diluted Emulphor/saline, 1:9). Groups of 5−10 mice were injected in graded doses and 10 min later were suspended by their forepaws on a 1.5-mm-thick wire 60 cm above the benchtop to assess muscle relaxation; three falls in <1 min was considered positive for muscle relaxation. At 15 min postinjection, consistent with previous work in this paradigm,[12] mice were injected with PTZ (80 mg/kg) to assess anticonvulsant activity, or 40 mg/kg to assess proconvulsant activity. Tonic and clonic convulsions with loss of righting reflex were considered positive for seizure activity; mild myoclonic jerks and straub tail were not counted as seizures.[12,27]

**Materials.** Melting points were taken on a Thomas-Hoover melting point apparatus or an Electrothermal Model IA8100 digital melting point apparatus and are reported uncorrected. Proton NMR spectra were recorded on a Bruker 250-MHz multiple-probe instrument or a GE 500-MHz spectrometer. Infrared spectra were recorded on a Nicolet DX FTIR BX V5.07 spectrometer or a Mattson Polaris IR-10400 instrument. Low-resolution mass spectral data (EI/CI) were obtained on a Hewlett-Packard 5985 B GC−mass spectrometer, while high-resolution mass spectral data were obtained on a Finnigan HR mass spectrometer. Microanalyses were performed on a Perkin-Elmer 240C carbon, hydrogen, and nitrogen analyzer. Analytical TLC plates employed were E. Merck Brinkman UV active silica gel (Kieselgel 60 F254) on plastic, and silica gel 60b for flash chromatography was purchased from E. M. Laboratories. All chemicals were purchased from Aldrich Chemical Co. unless otherwise stated. All reactions were carried out under an atmosphere of nitrogen, and all solvents were dried according to literature procedures.

The synthesis of ligands **49**,[27] **50**,[27] **51**,[16] **52**,[16] **53**,[13] **54**,[13] **55**,[13] **56**,[14] **57**,[15] **58**,[15] **59**,[15] **60**,[14] **61**,[15] **62**,[17] **63**,[17] **64**,[17] **65**,[17] **66**,[27] **67**,[27] and **71**[27] have been previously reported.

**5-Hydroxyindole (2).** To a solution of 5-benzyloxyindole **1** (10.0 g, 0.044 mol) in dry EtOH (600 mL) were added ammonium formate (5.9 g, 0.094 mol) and 10% Pd/C (1.0 g), and the solution was stirred at room temperature under an atmosphere of $N_2$ until analysis by TLC indicated the complete consumption of starting material. The catalyst was removed by vacuum filtration over a bed of Celite. The Celite was rinsed with hot EtOH (500 mL). The organics were combined and removed under reduced pressure. The residue was taken up in $CH_2Cl_2$ and washed with water. The aqueous layer was then washed with $CH_2Cl_2$ (3 × 100 mL). The combined organic layers were then washed with brine and dried ($K_2CO_3$), and the solvent was removed under reduced pressure to provide 5-hydroxyindole **2** (5.4 g) in 90% yield. **2**: mp 107 °C (lit.[40] mp 107−108 °C); IR (KBr) 3402, 3226, 1623 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 6.22 (d, 1H), 6.61 (dd, 1H, $J$ = 3.7 and 10 Hz), 6.86 (d, 1H $J$ = 2 Hz), 7.19 (dd, 1H, $J$ = 3.7 and 10 Hz), 7.20 (s, 1H), 8.61 (s, 1H), 10.79 (s, 1H); MS (CI) *m/e* 134 ($M^+ + 1$, 100).

**5-(Propyloxy)indole (3).** To a slurry of 5-hydroxyindole **2** (29 g, 0.218 mol) and solid $K_2CO_3$ (5 equiv, 1.1 mol, 150 g) in acetone (600 mL) was added 1-iodopropane (5 equiv, 1.1 mol, 185.3 g, 106.3 mL). The resulting solution was heated to reflux under an atmosphere of $N_2$ and monitored by TLC (silica gel, 9:1 $CHCl_3$:$CH_3OH$). The reaction was cooled to room temperature, and the solids were removed by filtration. The solvent was removed under reduced pressure to provide 5-(propyloxy)indole **3** as a dark brown oil (38 g) in 98% yield. **3**: IR (neat) 3411, 1474, 1454 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.04 (t, 3H, $J$ = 7.4 Hz), 1.82 (sextet, 2H, $J$ = 6.6, 7.4 Hz), 3.95 (t, 2H, $J$ = 6.6 Hz), 6.45 (s, 1H), 6.86 (dd, 1H, $J$ = 2, 10 Hz), 7.12 (d, 4H, $J$ = 8.3 Hz), 7.25 (d, 1H, $J$ = 8.3 Hz), 8.5 (s, 1H); MS (CI) *m/e* 176 ($M^+ + 1$, 100), 134 (35); HRMS *m/e* 175.0997 ($C_{11}H_{13}NO$ requires 175.0991).

**5-(Octyloxy)indole (4).** 5-Hydroxyindole **2** (5.1 g, 0.038 mol) was added to a solution containing acetone (100 mL) and 1-iodooctane (37.2 g, 0.155 mol). To the stirred solution was added potassium carbonate (26.9 g, 0.195 mol), at which time the mixture was brought to reflux under an atmosphere of $N_2$. After 48 h, analysis by TLC indicated the absence of starting material. The potassium carbonate was removed by filtration and the acetone removed under reduced pressure. The residue was taken up in $CH_2Cl_2$ (350 mL) and washed with water. The aqueous layer was extracted (3 × 150 mL) with $CH_2Cl_2$. The combined organic layers were washed with brine and dried ($K_2CO_3$). Flash chromatography (silica gel, $CH_2Cl_2$/hexanes) provided 5-(octyloxy)indole **4** (7.43 g) in 80% yield. **4**: mp 95 °C; IR (KBr) 3256 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.89 (t, 3H, $J$ = 6.5 Hz), 1.32 (m, 12 H), 1.81 (m, 2H), 4.00 (t, 2H, $J$ = 6.5 Hz), 6.47 (t, 1H, $J$ = 2.0 Hz), 6.66 (dd, 1H, $J$ = 2.4, 8.7 Hz), 7.13 (m, 2H), 7.29 (m, 1H), 8.03 (s, 1H, br); MS (CI) *m/e* 245 ($M^+ + 1$, 100), 162 (12), 133 (48). Anal. ($C_{16}H_{23}NO$) C, H, N.

**5-(1-Naphthylmethyloxy)indole (5).** Into a 500 mL round-bottom flask were added 5-hydroxyindole **2** (9.6 g, 0.072 mol), 1-(chloromethyl)naphthalene (60 mL, 70.8 g, 0.4 mol), potassium carbonate (45 g, 4.5 equiv), and acetone (75 mL). The reaction mixture was stirred at reflux under an atmosphere of $N_2$ for 18 h at which time analysis by TLC indicated the absence of starting material. The potassium carbonate was filtered off and the acetone removed under reduced pressure. The excess 1-(chloromethyl)naphthalene was removed using a wash column (silica gel, hexanes). Flash chromatography (silica gel, $CH_2Cl_2$, hexanes, 1:1) provided the 5-methoxynaphthylindole **5** (15.5 g) in 78% yield. **5**: mp 104−105 °C; IR (KBr) 3416 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 5.54 (s, 2H), 6.51 (s, 1H), 7.02 (dd, 1H, $J$ = 2.3, 8.9 Hz), 7.20 (m, 1H), 7.31 (m, 2H), 7.53 (m, 3H), 7.87 (m, 2H), 8.15 (m, 2H); MS (CI) *m/e* 274 ($M^+ + 1$, 35), 141 (100). Anal. ($C_{19}H_{15}NO$) C, H, N.

**3-[5-(1-Naphthylmethyloxy)indol-3-yl]-2-nitro-4-(methoxymethyl)butanoic Acid Ethyl Ester (11) (Procedure a).** Into a 250 mL round-bottom flask equipped with a reflux condenser was added indole **5** (5.1 g, 18.6 mmol), hydroxy nitro ethyl ester **37** (10.75 g), toluene (125 mL), and glacial acetic acid (12 mL). The solution was heated to reflux under an atmosphere of $N_2$. After 1.25 h, analysis by TLC (silica gel, EtOAc/hexanes, 1:1) indicated the absence of starting material. The toluene was removed under reduced pressure, and the excess acetic acid was removed azeotropically through several distillations using toluene under reduced pressure. The brown oil was chromatographed (silica gel, EtOAc/hexanes, 1:1) to provide **11** as a mixture of diastereomers (8.4 g, 97%). **11**: IR (neat) 3423, 2987, 2930, 1750 (C═O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.28 (t, 1H, $J$ = 7.0 Hz), 1.71 (m, 2H), 3.72 (m, 2H), 3.86 (s, 3H), 6.06 (s, 2H), 6.48 (t, 1H, $J$ = 10.2 Hz), 7.35 (d, 1H, $J$ = 8.5 Hz), 7.74 (s, 1H), 7.76 (s, 1H), 7.86 (s, 1H), 8.06 (m, 3H), 8.23 (d, 1H, $J$ = 7.0 Hz), 8.46 (m, 3H), 8.66 (d, 1H, $J$ = 6.7 Hz), 11.45 (br s, 1H); MS (EI) *m/e* 462 ($M^+$, 1.2), 141 (100). This material was employed without further purification in a later step.

**3-[5-(1-Naphthylmethyloxy)indol-3-yl]-2-nitropentanoic Acid Ethyl Ester (10).** A solution of indole **5** (7.39 g, 0.027 mol), hydroxy nitro ester **36** (10.35 g) in toluene (180 mL), and glacial acetic acid (18 mL) was treated as described in procedure a. Flash chromatography (silica gel, $CH_2Cl_2$/hexanes, 1:1) provided **10** as a mixture of diastereomers (7.25 g) in 60% yield. **10**: IR (neat) 3420, 2982, 1748 (C═O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.26 (t, 1H, $J$ = 7 Hz), 1.75 (m, 2H), 3.72 (m, 2H), 5.96 (s, 2H), 6.48 (m, 1H), 7.32 (d, 1H, $J$ = 8.1 Hz), 7.71 (s, 1H), 7.77 (s, 1H), 7.88 (s, 1H), 8.06 (m, 3H), 8.20 (d, 1H, $J$ = 6.9 Hz), 8.46 (m, 3H), 8.63 (d, 1H, $J$ = 6.7 Hz); MS (EI) *m/e* 446 ($M^+$, 3), 141 (100). This material was employed without further purification in a later step.

**3-(5-(Octyloxy)indol-3-yl)-2-nitro-4-(methoxymethyl)-butanoic Acid Ethyl Ester (8).** Into a solution of toluene (175 mL) and glacial acetic acid (17 mL) were added 5-(octyloxy)indole **4** (6.4 g, 0.026 mol) and hydroxy nitro ester **37** (16.3 g). The solution was treated as described in procedure a. Flash chromatography (silica gel, EtOAc/hexanes, 25:75)

provided **8** as a mixture of diastereomers (4.85 g) in 43% yield. **8**: IR (film) 3374, 2931, 1736 (C=O) $cm^{-1}$; MS (CI) *m/e* 435 ($M^+ + 1$, 35), 302 (100). This material was employed without further purification in a later step.

**3-(5-(Octyloxy)indol-3-yl)-2-nitropentanoic Acid Ethyl Ester (7).** 5-(Octyloxy)indole **4** (7.12 g, 29 mmol) was treated as described in procedure a to provide **7** as a mixture of diastereomers (6.56 g) in 55% yield. **7**: IR (neat) 3430, 2938, 2860, 1757 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.86 (m, 8H), 1.30 (m, 11H), 1.50 (m, 2H), 1.82 (m, 2H), 3.87 (t, 2H, $J$ = 7.0 Hz), 4.01 (t, 2H, $J$ = 6.5 Hz), 4.29 (q, 1H, $J$ = 7.1 Hz), 5.45 (m, 1H), 6.85 (m, 1H), 7.05 (m, 1H), 7.24 (m, 1H), 7.99 (s, 1H, br); MS (EI) *m/e* 418 ($M^+$, 80), 298 (60), 286 (51), 186 (100). This material was employed without further purification in a later step.

**3-(5-(Propyloxy)indol-3-yl)-2-nitro-4-(methoxymethyl)-butanoic Acid Ethyl Ester 6.** A solution composed of 5-(propyloxy)indole **3** (38.0 g, 0.22 mol), hydroxy nitro ester **36** (45.6 g), toluene (400 mL), and glacial acetic acid (38 mL) was treated as described in procedure a. A wash column (silica gel, EtOAc) furnished the ester **6** as a mixture of diastereomers (73.1 g) in 95% yield. HRMS (Finnigan HR) *m/e* 364.1634 ($C_{18}H_{26}N_2O_6$ requires 364.1625). This material was employed without further purification in a later step.

**3-(5-(Benzyloxy)indol-3-yl)-2-nitro-4-(methoxymethyl)-butanoic Acid Ethyl Ester 9.** A solution composed of 5-(benzyloxy)indole **1** (200 g, 0.22 mol), hydroxy nitro ester **37** (40.0 g), toluene (300 mL), and glacial acetic acid (50 mL) was treated as described in procedure a. A wash column (silica gel, EtOAc) furnished the ester **9** as a mixture of diastereomers (30 g) in 82% yield. **9**: IR (KBr) 1748 (C=O) $cm^{-1}$; MS (CI) *m/e* 413 ($M^+ + 1$, 100). This material was employed without further purification in a later step.

**3-[5-(1-Naphthylmethyloxy)indol-3-yl]-2-amino-4-(methoxymethyl)butanoic Acid Ethyl Ester (17) (Procedure b).** Into a 500 mL round-bottom flask were added nitro ethyl ester **11** (8.1 g, 17 mmol), ethanol (250 mL), and activated Raney nickel (7 g). The air in the flask was removed under vacuum and an atmosphere of hydrogen placed over the solution. After 36 h, analysis by TLC indicated the absence of starting material. The Raney nickel was removed by filtration over a bed of Celite, and the ethanol was removed under reduced pressure. A wash column (silica gel, EtOAc/hexanes, 1:1) provided the amino compound **17** as a mixture of diastereomers (4.85 g) in 67% yield. **17**: IR (neat) 3382, 2984, 1733 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.14 (m, 3H), 3.32 (s, 2H), 3.39 (s, 1H), 3.69−3.79 (m, 4H), 3.96 (s, 1H), 4.03−4.14 (m, 3H), 5.53 (s, 2H), 6.95 (dd, 1H $J$ = 2.3, 4.7 Hz), 7.12 (d, 1H, $J$ = 2.5 Hz), 7.30 (m, 2H), 7.49 (m, 3H), 7.63 (d, 1H, $J$ = 6.9 Hz), 7.66 (m, 2H), 8.10 (d, 1H, $J$ = 6.9 Hz), 8.12 (m, 1H); MS (EI) *m/e* 432 ($M^+$, 10), 332 (40) 141 (100). This material was employed without further purification in a later step.

**3-[5-(1-Naphthylmethyloxy)indol-3-yl]-2-aminopentanoic Acid Ethyl Ester (16).** Into a 250 mL round-bottom flask were placed nitro ethyl ester **10** (7.02 g, 0.015 mol), ethanol (200 mL), and activated Raney nickel (6.2 g), and the resulting mixture was treated as described in procedure b. A wash column (silica gel, EtOAc/hexanes, 1:1) provided the amino compound **16** (4.1 g) in 66% yield as a mixture of diastereomers. **16**: IR (neat) 3382, 2983, 1740 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.93 (m, 3H), 1.20 (m, 5H), 1.85 (m, 1H), 3.72 (t, 2H, $J$ = 7.1 Hz), 4.07 (m, 2H), 5.51 (s, 2H), 6.96 (dd, 1H, $J$ = 2.3, 7.0 Hz), 7.06 (m, 1H), 7.27 (m, 2H), 7.51 (m, 3H), 7.62 (d, 1H, $J$ = 6.9 Hz), 7.87 (m, 2H), 8.13 (m, 1H); MS (EI) *m/e* 416 ($M^+$, 2), 314 (100), 141 (78). This material was employed in a later step.

**3-(5-(Octyloxy)indol-3-yl)-2-amino-4-(methoxymethyl)-butanoic Acid Ethyl Ester (14).** The nitro ethyl ester **8** (4.85 g, 0.012 mol) was treated as described in procedure b to provide **14** as a light brown oil (3.52 g) in 75% yield. **14**: IR (neat) 3374, 2917, 1736 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.89 (m, 3H), 1.15 (t, 3H, $J$ = 7.1 Hz) 1.32 (m, 9H), 1.48 (m, 1H), 1.80 (m, 2H), 3.35 (s, 3H), 3.41 (s, 2H), 3.76 (m, 3H), 4.07 (m, 3H), 4.11 (m, 2H), 6.83 (m, 1H), 7.04 (d, 1H, $J$ = 2.3 Hz), 7.11 (s, 1H), 7.23 (m, 1H), 7.97 (s, 1H, br); MS (EI) *m/e* 404 ($M^+$, 3), 302 (100), 190 (36), 146 (38) 102 (30). This material was employed in a later step.

**3-(5-(Octyloxy)indol-3-yl)-2-aminopentanoic Acid Ethyl Ester (13).** The nitro ethyl ester **7** (6.25 g, 15 mmol) was treated as described in procedure b to provide **13** as a mixture of diastereomers (4.52 g) in 80% yield. **13**: IR (neat) 3381, 2931, 2860, 1736 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.89 (m, 8H), 1.15 (t, 3H, $J$ = 7.1 Hz), 1.29 (m, 8H), 1.81 (m, 5H), 3.24 (m, 1H), 3.76 (t, 1H, $J$ = 6.6 Hz), 4.04 (m, 5H), 6.85 (m, 1H), 7.06 (m, 1H), 7.25 (m, 1H), 7.94 (s, 1H, br); MS (CI) *m/e* 388 ($M^+ + 1$, 100), 372 (42), 286 (48). This material was employed in a later step.

**3-(5-(Propyloxy)indol-3-yl)-2-amino-4-(methoxymethyl)butanoic Acid Ethyl Ester 12.** The nitro ethyl ester **6** (7.0 g, 0.021 mol) was treated as described in procedure b to provide **12** as a mixture of diastereomers (5.0 g) in 79% yield. **12**: $^1H$ NMR ($CDCl_3$) $\delta$ 1.04 (t, 3H, $J$ = 7.5 Hz), 1.79 (sextet, 2H, $J$ = 7 Hz), 3.15 (s, 3H), 3.90 (t, 2H, $J$ = 7 Hz), 6.7 (s, 1H), 7.2 (d, 1H, $J$ = 8 Hz), 7.27 (d, 1H, $J$ = 8 Hz), 9.48 (s, 1H); MS (CI) *m/e* 335 ($M^+ + 1$, 100); HRMS (Finnigan HR) *m/e* 334.1892 ($C_{18}H_{24}N_2O_6$ requires 334.1889). This material was employed without further purification in a later step.

**3-(5-(Benzyloxy)indol-3-yl)-2-amino-4-(methoxymethyl)butanoic Acid Ethyl Ester 15.** The mixture of nitro ethyl ester **9** (4.0 g, 9.7 mmol) and Raney nickel (8 g) in ethanol (400 mL) was treated as described in procedure b to provide **15** as a mixture of diastereomers (3.1 g) in 83% yield. **15**: IR (neat) 3339, 2987, 2938, 1742 $cm^{-1}$; MS (CI) *m/e* 413 ($M^+ + 1$, 100). This material was employed in a later step without further characterization.

**6-(1-Naphthylmethyloxy)-4-(methoxymethyl)-3-(ethoxycarbonyl)-1,2,3,4-tetrahydro-$\beta$-carboline-1-carboxylic Acid (23) (Procedure c).** Into a 250 mL round-bottom flask was added aminoindole **17** (5.07 g, 0.012 mol) in ethyl acetate (29 mL). A solution of glyoxylic acid monohydrate (1.03 g, 0.014 mol) in water (14 mL) was added dropwise to the solution. The pH was adjusted to 5 with 10% aqueous potassium carbonate, and the mixture was allowed to stir for 16 h at room temperature. The aqueous and organic phases were separated, and the aqueous phase was extracted (2 × 25 mL) with ethyl acetate. The organic phases were combined, and the solvent was removed under reduced pressure to yield the carboxylic acid **23** as a dark orange oil which solidified upon standing (5.72 g) in 92% yield. **23**: mp >300 °C; IR (KBr) 3360−3200 br, 2924, 1736 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.11 (t, 3H, $J$ = 7 Hz), 3.26 (s, 3H), 3.52 (m, 4H), 4.01 (t, 2H, $J$ = 6.7 Hz), 4.08 (s, 1H), 4.94 (s, 1H), 5.52 (s, 2H), 6.75 (dd, 1H, $J$ = 2.3, 7.2 Hz), 7.23 (m, 2H), 7.54 (m, 3H), 7.68 (d, 1H, $J$ = 7.2 Hz), 7.92 (m, 2H), 8.15 (d, 1H, $J$ = 7.2 Hz), 10.6 (s, 1H); MS (EI) *m/e* 488 ($M^+$, 0), 141 (100). This material was employed in a later step.

**6-(1-Naphthylmethyloxy)-4-ethyl-3-(ethoxycarbonyl)-1,2,3,4-tetrahydro-$\beta$-carboline-1-carboxylic Acid (22).** A solution of glyoxylic acid monohydrate (1.078 g, 0.117 mol) in $H_2O$ (12 mL) was added dropwise to a vigorously stirred solution of amino ethyl ester **16** (3.96 g, 0.0095 mol) in ethyl acetate (25 mL). The reaction was treated as described in procedure c to provide the carboxylic acid **22** (3.2 g) as a mixture of diastereomers in 80% yield. **22**: mp >300 °C; IR (KBr) 3402, 2980, 2938, 1743 (C=O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 0.87 (m, 3H), 1.05 (t, 3H, $J$ = 7.2 Hz), 1.71 (m, 2H), 4.01 (m, 3H), 4.22 (q, 2H, $J$ = 7.0 Hz), 4.89 (s, 1H), 5.51 (s, 2H), 6.78 (dd, 1H, $J$ = 2.3, 8.8 Hz), 7.22 (m, 2H), 7.53 (m, 3H), 7.66 (d, 1H, $J$ = 7.0 Hz), 7.93 (m, 2H), 8.14 (d, 1H, $J$ = 8.7 Hz), 10.6 (s, 1H); MS (EI) *m/e* 444 ($M^+$, 0), 141 (100). This material was employed in a later step.

**6-(Octyloxy)-4-(methoxymethyl)-3-(ethoxycarbonyl)-1,2,3,4-tetrahydro-$\beta$-carboline-1-carboxylic Acid (20).** The amino ethyl ester **14** (2.4 g, 6.0 mmol) was treated as described in procedure c to provide **20** as an orange solid (2.53 g) in 92% yield. **20**: mp >300 °C; IR (KBr) 2924, 1743 (C=O), 1651, 1384, 1216 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.89 (t, 3H, $J$ = 6.8 Hz), 1.26 (m, 12 H), 1.46 (m, 1H), 1.77 (m, 2H), 3.15 (s, 3H), 3.33

(m, 2H), 3.66 (m, 2H), 3.95 (m, 2H), 4.29 (m, 1H), 6.61 (m, 1H), 6.69 (m, 1H), 7.20 (m, 1H), 9.47 (s, 1H); MS (CI) *m/e* 460 ($M^+ + 1$, 0), 418 (100), 385 (24), 315 (26). This material was employed in a later step.

**6-(Octyloxy)-4-ethyl-3-(ethoxycarbonyl)-1,2,3,4-tetrahydro-*β*-carboline-1-carboxylic Acid (19).** The amino ethyl ester **13** (1.24 g, 3.2 mmol) was treated as described in procedure c to provide **19** as a mixture of diastereomers (1.29 g) in 91% yield. **19**: mp >300 °C; IR (KBr) 2924, 2854, 1743 (C═O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.89 (t, 3H, $J = 6.2$ Hz), 0.99 (m, 2H), 1.30 (m, 12H), 1.47 (m, 2H), 1.79 (m, 2H), 3.68 (m, 1H), 3.70 (m, 3H), 4.08 (m, 2H), 4.28 (m, 2H), 4.49 (m, 1H), 6.78 (m, 1H), 6.96 (m, 1H), 7.16 (m, 1H), 7.32 (s, 1H); MS (CI) *m/e* 444 ($M^+ + 1$, 3), 430 (20), 402 (100). This material was employed in a later step.

**6-(Propyloxy)-4-(methoxymethyl)-3-(ethoxycarbonyl)-1,2,3,4-tetrahydro-*β*-carboline-1-carboxylic Acid (18).** The amino ethyl ester **12** (15 g, 4.5 mmol) was treated as described in procedure c to provide the carboxylic acid **18** as a mixture of diastereomers (10.2 g) in 90% yield. **18**: mp 160−163 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 3.27 (s, 3H), 3.73 (m, 2H), 3.90 (m, 2H), 4.0 (m, 2H), 6.77 (d, 1H), 6.92 (s, 1H), 6.96 (s, 1H), 7.04 (s, 1H), 7.07 (d, 1H), 7.23 (s, 1H), 9.12 (s, 1H); MS (CI) *m/e* 347 ($M^+ + 1$, 100). This material was employed in a later step.

**6-(Benzyloxy)-4-(methoxymethyl)-3-(ethoxycarbonyl)-1,2,3,4-tetrahydro-*β*-carboline-1-carboxylic Acid (21).** The amino ethyl ester **15** (2.9 g, 7.6 mmol) was treated as described in procedure c to provide the carboxylic acid **21** as a mixture of diastereomers (2.7 g) in 87% yield. **21**: mp 156−157 °C; MS (CI) *m/e* 395 ($M^+ - 44$, 100). This material was employed in a later step without further characterization.

**6-(1-Naphthylmethyloxy)-4-(methoxymethyl)-*β*-carboline-3-carboxylic Acid Ethyl Ester (29) (Procedure d).** Into a 100 mL round-bottom flask were added carboxylic acid **23** (3.23 g, 6.61 mmol) and a mixture of xylenes (65 mL). The solution was heated at reflux for 1 h and the solvent removed under reduced pressure. The residue was dissolved in DMSO (18 mL), followed by the addition of sulfur powder (0.47 g), and this mixture was stirred at a bath temperature of 144 °C for 1.5 h. The DMSO was removed by high vacuum distillation, followed by a wash column (silica gel, EtOAc), and then purification by flash chromatography (silica gel, EtOAc/hexanes, 80:20) provided *β*-carboline **29** (642 mg) in 22% yield. **29**: mp 174−176 °C; IR (KBr) 2931, 1694 (C═O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.33 (t, 3H, $J = 7.1$ Hz), 3.27 (s, 3H), 4.34 (q, 2H, $J = 7.1$ Hz), 5.10 (s, 2H), 5.65 (s, 2H), 7.39 (dd, 1H, $J = 2.3$, 8.9 Hz), 7.48−7.62 (m, 4H), 7.71 (d, 1H, $J = 6.7$ Hz), 7.85 (s, 1H), 7.95 (m, 2H), 8.17 (d, 1H, $J = 8.2$ Hz), 8.85 (s, 1H); MS (CI) *m/e* 440 ($M^+ + 1$, 100), 299 (33), 143 (30) 111 (16). Anal. ($C_{27}H_{24}N_2O_4$) C, H, N.

**6-(1-Naphthylmethyloxy)-4-ethyl-*β*-carboline-3-carboxylic Acid Ethyl Ester (28).** The *β*-carboline **22** (3.12 g, 6.61 mmol) in xylenes at reflux was subjected to decarboxylation, followed by oxidation in DMSO (7 mL) with sulfur powder (0.156 g) as described in procedure d. The black residue was purified using flash chromatography (silica gel, EtOAc/hexanes, 80:20) to provide **28** (456 mg) in 16% yield. **28**: mp 195.3−196.5 °C; IR (KBr) 2973, 1694 (C═O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.18 (t, 3H, $J = 7.3$ Hz), 1.29 (t, 3H, $J = 7.1$ Hz), 3.24 (q, 2H, $J = 7.3$ Hz), 4.29 (q, 2H, $J = 7.1$ Hz), 5.64 (s, 2H), 7.36 (dd, 1H, $J = 2.2$, 9.0 Hz), 7.43−7.69 (m, 6H), 7.92 (m, 2H), 8.18 (d, 1H, $J = 7.6$ Hz), 8.69 (s, 1H); MS (EI) *m/e* 424 ($M^+$, 0.9), 141 (100). Anal. ($C_{27}H_{24}N_2O_3$) C, H, N.

**6-(Octyloxy)-4-(methoxymethyl)-*β*-carboline-3-carboxylic Acid Ethyl Ester (26).** The *β*-carboline **20** (2.40 g, 0.0052 mol) in xylenes (28 mL) at reflux was subjected to decarboxylation, followed by oxidation in DMSO (13 mL) with sulfur powder (0.33 g) as described in procedure d. The DMSO was removed by high vacuum distillation, and a wash column (silica gel, $CH_2Cl_2$) was run on the black residue. Crystallization from hot ethyl acetate provided the fully aromatic *β*-carboline **26** (820 mg) in 38% yield. **26**: mp 147.3−149 °C; IR (KBr) 3198 (NH), 2931, 1701 (C═O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.89 (t, 2H, $J = 6.9$ Hz), 1.29−1.57 (m, 16H), 1.84 (m, 2H), 3.52 (s, 3H), 4.09 (t, 2H, $J = 6.5$ Hz), 4.52 (q, 2H, $J = 7.1$ Hz), 5.38 (s, 2H), 7.24 (dd, 1H, $J = 2.4$, 8.9 Hz), 7.46 (d, 1H, $J = 9.0$ Hz), 7.80 (d, 1H, $J = 2.2$ Hz), 8.62 (br s, 1H), 8.87 (s, 1H); MS (CI) *m/e* 414 ($M^+ + 1$, 100), 301 (10). Anal. ($C_{24}H_{32}N_2O_4$) C, H, N.

**6-(Octyloxy)-4-ethyl-*β*-carboline-3-carboxylic Acid Ethyl Ester (25).** The *β*-carboline **19** (3.89 g, 0.0085 mol) in xylenes (50 mL) was heated at reflux, followed by oxidation in DMSO (21 mL) with sulfur powder (0.55 g) as described in procedure d. A wash column (silica gel, $CH_2Cl_2$) was run on the black residue followed by crystallization from hot ethyl acetate to provide **25** as light beige crystals (1.01 g, 30%). **25**: mp 163−165 °C; IR (KBr) 2931, 1701 (C═O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.89 (t, 3H, $J = 6.9$ Hz), 1.30−1.56 (m, 16H), 1.86 (m, 2H), 3.56 (q, 2H, $J = 7.3$ Hz), 4.08 (t, 2H, $J = 6.5$ Hz), 4.5 (q, 2H, $J = 7.1$ Hz), 7.25 (dd, 1H, $J = 2.3$, 8.9 Hz), 7.49 (d, 1H, $J = 8.9$ Hz), 7.71 (d, 1H, $J = 2.2$ Hz), 8.82 (s, 1H), 8.95 (br s, 1H); MS (EI) *m/e* 396 ($M^+$, 52), 367 (35), 350 (17), 255 (19), 238 (38), 211 (100), 181 (36). Anal. ($C_{24}H_{32}N_2O_3$) C, H, N.

**6-(Propyloxy)-4-(methoxymethyl)-*β*-carboline-3-carboxylic Acid Ethyl Ester (24).** The carboxylic acid **18** (1.2 g, 2.98 mmol) was suspended in xylenes, and the mixture was heated to reflux under an atmosphere of $N_2$ for 1 h. Then 10% Pd/C (1.33 g) was added to the yellow solution and the mixture heated to reflux for 3 h. The mixture was filtered and the catalyst washed with hot xylenes (100 mL). The xylenes were combined and removed under reduced pressure to provide a crude solid. Flash chromatography provided the *β*-carboline **24** (512 mg) in 54% yield. **24**: mp 250−251 °C; (IR (KBr) 2936, 1706 (C═O) $cm^{-1}$; $^1H$ NMR (G.E. 500 MHz, $CDCl_3$) $\delta$ 1.06 (t, 3H, $J = 7$ Hz), 1.32 (t, 3H, $J = 7.4$ Hz), 1.85 (sextet, 2H, $J = 6.6$, 7.4 Hz), 3.52 (s, 3H), 4.02 (t, 2H, $J = 6.6$ Hz), 4.43 (q, 2H, $J = 7$ Hz), 5.37 (s, 2H), 7.19 (dd, 1H, $J = 1.5$, 9 Hz), 7.41 (d, 1H, $J = 9$ Hz), 7.75 (s, 1H), 8.74 (s, 1H), 10.2 (s, 1H); HRMS *m/e* 342.1581 ($C_{19}H_{22}N_2O_4$ requires 342.1579). Anal. ($C_{19}H_{22}N_2O_4$) C, H, N.

**6-(Benzyloxy)-4-(methoxymethyl)-*β*-carboline-3-carboxylic Acid Ethyl Ester (27).** The *β*-carboline **21** (3.05 g, 6.97 mmol) in xylenes (200 mL) at reflux was subjected to decarboxylation, followed by oxidation in DMSO (100 mL) with sulfur powder (0.56 g) as described in procedure d. Flash chromatography of the black residue (silica gel, 9:1 $CHCl_3$:MeOH) followed by crystallization from ethyl acetate/ether furnished the *β*-carboline **27** (1.45 g) in 31% yield whose spectral properties were identical to those previously published.[27,28] **27**: mp 187 °C (lit.[27,28] mp 187 °C).

**9-Methyl-6-(1-naphthylmethyloxy)-4-(methoxymethyl)-*β*-carboline-3-carboxylic Acid Ethyl Ester (35) (Procedure e).** Into a 50 mL flame-dried round-bottom flask was added *β*-carboline **29** (126 mg, 0.285 mmol) in dry DMF (12 mL). To this solution were added 2.5 equiv of iodomethane and sodium hydride (16 mg, 60% dispersion in mineral oil, 0.39 mmol), and the solution was stirred at room temperature for 12 h under an atmosphere of $N_2$. The DMF was removed by heating in vacuo and the residue taken up in $CHCl_3$ and washed with water. The aqueous layer was extracted with $CHCl_3$ (3 × 25 mL). The combined organic layers were washed with brine and dried ($K_2CO_3$), and the solvent was removed under reduced pressure. A wash column (silica gel, ethyl acetate) provided **35** as an off-white solid (77 mg, 60%). **35**: mp 138−140 °C; IR (KBr) 2931, 1708 (C═O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.32 (t, 3H, $J = 7.1$ Hz), 3.25 (s, 3H), 3.98 (s, 3H), 4.33 (q, 2H, $J = 7.1$ Hz), 5.09 (s, 2H), 5.66 (s, 2H), 7.43−7.58 (m, 5H), 7.64 (m, 2H), 7.92 (m, 2H), 7,15 (d, 1H, $J = 7.7$ Hz), 8.97 (s, 1H); MS (EI) *m/e* 454 ($M^+$, 21), 141 (100), 115 (17). Anal. ($C_{28}H_{26}N_2O_4$) C, H, N.

**9-Methyl-6-(1-naphthylmethyloxy)-4-ethyl-*β*-carboline-3-carboxylic Acid Ethyl Ester (34).** A solution of *β*-carboline **28** (350 mg, 0.824 mmol) in dry DMF (18 mL) was methylated as described in procedure e to provide **34** (306 mg, 84%) as a white solid. **34**: mp 153−156 °C; IR (KBr) 2980, 1715 (C═O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.24 (t, 3H, $J = 7.0$ Hz), 1.33 (t, 3H, $J = 7.1$ Hz), 3.23 (q, 2H, $J = 6.5$ Hz), 3.97 (s, 3H), 4.33 (q, 2H, $J = 7.6$ Hz), 5.71 (s, 2H), 7.46−7.62 (m, 4H),

7.73 (m, 3H), 7.95 (m, 2H), 8.22 (d, 1H, $J$ = 7.5 Hz), 8.87 (s, 1H); MS (EI) *m/e* 438 ($M^+$, 2), 141 (100), 115 (29). Anal. ($C_{28}H_{26}N_2O_3$) C, H, N.

**9-Methyl-6-(octyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester (32).** A solution of β-carboline **26** (50 mg, 0.121 mmol) in dry DMF (8 mL) was methylated as described in procedure e to provide **32** as an off-white solid (41 mg, 80%). **32**: mp 96.2−97.4 °C; IR (KBr) 2925, 1708 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.89 (t, 3H, $J$ = 6.8 Hz), 1.26−1.56 (m, 16H), 1.84 (m, 2H), 3.51 (s, 3H), 3.96 (s, 3H), 4.10 (t, 2H, $J$ = 6.5 Hz), 4.52 (q, 2H, $J$ = 7.1 Hz), 5.37 (s, 2H), 7.31 (dd, 1H, $J$ = 2.4, 8.9 Hz), 7.42 (d, 1H, $J$ = 8.9 Hz), 7.84 (d, 1H, $J$ = 2.3 Hz), 8.86 (s, 1H); MS (CI) *m/e* 428 ($M^+ + 1$, 100), 396 (12), 381 (7), 315 (11). Anal. ($C_{25}H_{34}N_2O_4$) C, H, N.

**9-Methyl-6-(octyloxy)-4-ethyl-β-carboline-3-carboxylic Acid Ethyl Ester (31).** A solution of β-carboline **25** (0.119 g, 0.299 mmol) in dry DMF (12 mL) was methylated using 2.5 equiv of iodomethane and sodium hydride (14 mg, 60% dispersion in mineral oil, 0.35 mmol) as described in procedure e. A wash column (silica gel, ethyl acetate) on the residue provided **31** (103 mg, 84%) as an off-white solid. **31:** mp 94−95 °C; IR (KBr) 2924, 1708 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.89 (t, 2H, $J$ = 6.7 Hz), 1.32 (m, 8H), 1.51 (m, 8H), 1.81 (m, 2H), 3.58 (t, 2H, $J$ = 7.5 Hz), 3.96 (s, 3H), 4.09 (t, 2H, $J$ = 6.6 Hz), 4.52 (q, 2H, $J$ = 7.1 Hz), 7.31 (dd, 1H, $J$ = 2.4, 8.9 Hz), 7.44 (d, 1H, $J$ = 8.9 Hz), 7.75 (d, 1H, $J$ = 2.2 Hz), 8.81 (s, 1H); MS (CI) *m/e* 412 ($M^+ + 1$, 100), 299 (12). Anal. ($C_{25}H_{34}N_2O_3$) C, H, N.

**9-Methyl-6-(propyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Ethyl Ester (30).** A solution of β-carboline **24** (60 mg, 0.175 mmol) in dry DMF (6 mL) was methylated as described in procedure e. Flash chromatography provided the $N_a$-methyl-β-carboline **30** (35 mg) in 56% yield. **30**: mp 112−113 °C; IR (KBr) 2936, 1706 (C=O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.23 (t, 3H, $J$ = 7 Hz), 1.47 (t, 3H, $J$ = 7.4 Hz), 1.87 (sextet, 2H, $J$ = 6.6 Hz), 3.49 (s, 3H), 4.07 (t, 2H, $J$ = 6.6 Hz), 4.51 (q, 2H, $J$ = 7 Hz), 5.32 (s, 2H), 7.31 (d, 1H, $J$ = 9 Hz), 7.41 (d, 1H, $J$ = 9 Hz), 7.83 (s, 1H), 8.84 (s, 1H); MS (CI) *m/e* 357 ($M^+ + 1$, 100); HRMS *m/e* 356.1729 ($C_{20}H_{24}N_2O_4$ requires 356.1736).

**6-(Propyloxy)-4-(methoxymethyl)-β-carboline-3-carbohydrazide (38).** A solution of β-carboline **24** (500 mg, 1.48 mmol) in EtOH (20 mL) which contained 98% anhydrous hydrazine (5 mL) was heated at reflux under an atmosphere of $N_2$ for 2.5 h at which time analysis by TLC (silica gel, 9.5:0.5 EtOAc:MeOH) indicated the disappearance of starting material. As the solution cooled, white crystals formed. The crystals were collected by filtration, washed with EtOH, and dried to furnish the carbohydrazide **38** (300 mg) in 81% yield. **38**: mp 230−231 °C; IR (KBr) 3270, 1638 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, $CF_3COOD$, 500 MHz) $\delta$ 1.10 (t, 3H, $J$ = 7.4 Hz), 1.91 (sextet, 2H, $J$ = 6.6,7.4 Hz), 3.62 (s, 3H), 4.12 (t, 2H, $J$ = 6.6 Hz), 5.42 (s, 2H), 7.63 (s, 1H), 7.73 (d, 1H, $J$ = 9.5 Hz), 7.81 (s, 1H), 9.29 (s, 1H), 10.81 (s, 1H); MS (CI) *m/e* 329 ($M^+ + 1$, 100), 297 (21). Anal. ($C_{17}H_{20}N_4O_3$) C, H, N.

**6-(Benzyloxy)-4-(methoxymethyl)-β-carboline-3-carbohydrazide (39).** A solution of β-carboline **27** (800 mg, 2.0 mmol) in EtOH (30 mL) which contained 98% anhydrous hydrazine (6 mL) was heated at reflux under an atmosphere of $N_2$ for 7 h at which time analysis by TLC (silica gel, 9.5:0.5 EtOAc:MeOH) indicated the disappearance of starting material. The solution was cooled, and the resulting precipitate which formed was collected by filtration, washed with EtOH, and dried to provided β-carboline **39** (730 mg) in 95% yield. **39**: mp 243−244 °C; IR (KBr) 3303 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, $CF_3COOD$, 250 MHz) $\delta$ 3.45 (s, 3H), 5.30 (s, 2H), 7.25 (s, 2H), 7.40 (m, 6H), 7.70 (m, 3H), 9.28 (s, 1H), 11.15 (s, 1H); MS (CI) *m/e* 377 ($M^+ + 1$, 100), 345 (32). Anal. ($C_{21}H_{20}N_4O_3$) C, H, N.

**6-(Propyloxy)-4-(methoxymethyl)-3-amino-β-carboline (40).** A suspension of the carbohydrazide **38** (380 mg, 1.1 mmol) in $H_2O$ (50 mL) and THF (3 mL) was dissolved by the dropwise addition of aqueous concentrated HCl. The yellow solution which resulted was cooled to 0 °C followed by the addition of $NaNO_2$ (87 mg, 1.26 mmol, 1.1 equiv) in $H_2O$ (2 mL). After being stirred at 0 °C for 35 min, the solution was brought to alkaline pH with saturated aqueous $NaHCO_3$. The precipitate which formed was collected by filtration and brought up in acetic acid/water (1:1, 60 mL). The suspension was then heated at reflux during which time $N_2$ was evolved. After 45 min a new component was observed by TLC which turned brown some time later under UV light. The solvent was removed under reduced pressure to furnish an oil which was taken up in ethanol to precipitate **40** as a yellow solid as the diacetate salt. After recrystallization from ether the β-carboline **40** (275 mg, 88% yield) was obtained as the diacetate salt. **40**: mp 267−268 °C; IR (KBr) 3448, 1642, 1562, 1410 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.07 (t, 3H, $J$ = 7.4 Hz), 1.85 (m, 2H), 3.45 (s, 3H), 3.99 (t, 2H, $J$ = 6.6 Hz), 4.73 (s, 1H), 5.0 (s, 2H), 7.15 (s, 1H), 7.28 (m, 2H), 7.63 (s, 1H), 7.97 (s, 1H), 8.27 (s, 1H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 10.61, 22.76, 57.76, 67.80, 70.72, 76.60, 108.26, 109.21, 112.15, 118.75, 121.16, 128.48, 128.56, 131.70, 137.69, 153.06; MS (CI) *m/e* 286 ($M^+ + 1$, 100), 154 (65); high-resolution mass spectrum, *m/e* 285.1481 ($C_{16}H_{19}N_3O_2$ requires 285.1477).

**6-(Benzyloxy)-4-(methoxymethyl)-3-amino-β-carboline (41).** A suspension of the carbohydrazide **39** (700 mg, 1.86 mmol) in $H_2O$ (50 mL) and THF (3 mL) was dissolved by the dropwise addition of aqueous concentrated HCl. The yellow solution which resulted was cooled to 0 °C followed by the addition of $NaNO_2$ (141 mg, 2.04 mmol) in $H_2O$ (2 mL). After 35 min of stirring at 0 °C, the solution was brought to alkaline pH with saturated aqueous $NaHCO_3$. The precipitate which formed was collected by filtration and brought up in acetic acid/water (1:1, 100 mL). The suspension was then heated at reflux during which time nitrogen was evolved. After 45 min a new component was observed by TLC which turned brown some time later under UV light. The solvent was removed under reduced pressure and the crude solid recrystallized from EtOH to furnish the amine **41** as the diacetate salt (492 mg) in 60% overall yield. **41**: mp 175−176 °C; IR (KBr) 1634, 1559 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 3.39 (s, 3H), 4.95 (s, 2H), 5.14 (s, 2H), 7.30 (m, 6H), 7.50 (m, 3H), 7.68 (d, 1H, $J$ = 8.7 Hz), 8.07 (s, 1H), 8.28 (s, 1H); MS (EI 15 eV) *m/e* 333 ($M^+$, 67), 242 (100). Anal. ($C_{20}H_{19}N_3O_2$) C, H, N.

**6-(Propyloxy)-4-(methoxymethyl)-β-carboline-3-isothiocyanate (42).** A suspension of 3-amino β-carboline **40** as the diacetate salt (50 mg, 0.124 mmol) in a saturated aqueous solution of $NaHCO_3$ (5 mL) was added to $CHCl_3$ (5 mL). After 10 min of vigorous stirring at room temperature, thiophosgene (0.011 mL, 0.017 g, 0.148 mmol) was syringed into the chloroform solution and the mixture was allowed to stir for 1 h. The reaction progress was monitored by TLC (silica gel, $CHCl_3$/MeOH, 9.5:0.5). The organic layer was then separated and the aqueous layer extracted with $CHCl_3$ (3 × 10 mL). The organic layers were combined, and the solvent was removed under reduced pressure. The crude solid was then purified by flash chromatography to furnish the isothiocyanate **42** (14 mg, 34%). **42**: mp 166−168 °C; IR (KBr) 3460, 2924 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.07 (t, 3H, $J$ = 7.4 Hz), 1.85 (m, 2H), 3.49 (s, 3H), 4.02 (t, 2H, $J$ = 6.6 Hz), 5.03 (s, 2H), 7.19 (m, 1H), 7.40 (m, 1H), 7.68 (d, 1H, $J$ = 9 Hz), 8.30 (s, 1H), 8.61 (s, 1H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 49.72, 55.89, 62.05, 76.99, 108.26, 109.21, 112.15, 118.75, 121.16, 128.48, 128.56, 148.24, 158.32, 175.48, 207.28; MS (CI) *m/e* 328 ($M^+ + 1$, 28), 296 (100); HRMS *m/e* 327.1043 ($C_{17}H_{17}N_3O_2S$ requires 327.1041).

**6-(Benzyloxy)-4-(methoxymethyl)-β-carboline-3-isothiocyanate (43).** To a stirred solution of the amine **41** as the diacetate salt (100 mg, 0.220 mmol) in a saturated aqueous solution of $NaHCO_3$ (10 mL) was added chloroform (10 mL). After 15 min of vigorous stirring at ambient temperature, thiophosgene (0.028 g, 0.019 mL, 0.25 mmol) was syringed into the chloroform solution, and stirring was continued for another hour. The reaction progress was monitored by TLC (silica gel, EtOAc/MeOH, 9:1). The chloroform layer was separated and the water layer extracted with $CHCl_3$ (3 × 60 mL). The organic layers were combined and dried ($K_2CO_3$), and the

solvent was removed under reduced pressure. The crude solid was purified by flash chromatography (silica gel, EtOAc) and crystallized from benzene to yield the isothiocyanate **43** (69 mg, 83%). **43**: mp 189 °C; IR (KBr) 2140 (NCS), 1662 (N═C), 1559, 1498, 1381 (C═S) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 3.43 (s, 3H), 4.98 (s, 2H), 5.16 (s, 2H), 7.30 (m, 5H), 7.46 (s, 1H), 7.48 (d, 1H, $J$ = 8.5 Hz), 7.74 (s, 1H), 8.60 (s, 1H); MS (EI) *m/e* 375 ($M^+$, 62), 284 (60) 254 (100). Anal. ($C_{21}H_{17}N_3O_2S$) C, H, N.

**6-(Benzyloxy)-4-(methoxymethyl)-β-carboline-3-thiocarbamate (44).** To a solution of isothiocyanate **43** (30 mg, 0.08 mmol) in dry $CH_3OH$ (10 mL) was added a solution of dry $CH_3OH$ in which anhydrous HCl (10 mL) had been dissolved. Examination of the solution by TLC (silica gel, EtOAc/MeOH, 9.5:0.5, $NH_4OH$ fumes) indicated the formation of a new component which exhibited an intense purple color under UV light. The solution was stirred for 2 min and the solvent removed under reduced pressure at room temperature. The oil which remained was washed with dry ether (2 × 10 mL) and purified by flash chromatography (silica gel, EtOAc) to furnish the thiocarbamate **44** (20 mg, 61%). **44**: mp 177 °C; IR (KBr) 1655 (N═C), 1559, 1381 (C═S), 1217 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 3.39 (s, 3H), 4.05 (s, 3H), 4.88 (s, 2H), 5.16 (s, 2H), 7.23 (m, 4H), 7.35 (m, 2H), 7.48 (m, 2H), 7.68 (s, 1H), 8.40 (s, 1H), 8.60 (s, 1H); MS (EI) *m/e* 375 (56), 284 (58), 254 (100). Anal. ($C_{22}H_{20}N_3O_3S$) C, H, N.

**6-(Propyloxy)-4-(methoxymethyl)-β-carboline 3-*n*-Propyl Ether (45).** To a solution of 3-amino-β-carboline **40** (100 mg, 0.248 mmol), dry 1-propanol (25 mL), and isoamyl nitrite (0.034 g, 0.0399 mL, 0.287 mmol, 1.2 equiv) was added concentrated $H_2SO_4$ (0.5 mL) dropwise until the β-carboline **40** dissolved. The mixture was stirred at room temperature for $^1/_2$ h followed by heating at reflux for $^1/_2$ h. The reaction was monitored by TLC (silica gel, $CHCl_3$/MeOH, 9.5:0.5). After the mixture was allowed to cool to room temperature, $H_2O$ (250 mL) was added followed by a saturated aqueous solution of $NaHCO_3$ (25 mL). The mixture was then extracted with $CHCl_3$ (3 × 25 mL) and the solvent removed under reduced pressure. Flash chromatography (silica gel, $CHCl_3$/MeOH, 9.5:0.5) provided the 3-*n*-propyl ether **45** (30 mg, 52%). This material was homogeneous on TLC in two different solvent systems. **45**: mp 173−174 °C; IR (KBr) 3433, 3221 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.06 (t, 6H, $J$ = 7.4 Hz), 1.26 (t, 3H, $J$ = 7.4 Hz), 1.76 (m, 4H), 3.97 (t, 2H, $J$ = 6.6 Hz), 5.37 (s, 2H), 7.04 (dd, 1H, $J$ = 2.5, 9 Hz), 7.41 (d, 1H, $J$ = 2.5 Hz), 7.48 (d, 1H, $J$ = 9 Hz), 8.29 (s, 1H), 10.20 (s, 1H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 10.58, 14.30, 22.68, 58.17, 61.65, 67.83, 70.40, 107.85 112.60, 119.16, 121.67, 128.74, 129.13, 132.94, 136.14, 137.22, 137.65, 153.99, 167.23; MS (CI) *m/e* 329 ($M^+$ + 1, 48), 297 (100); HRMS *m/e* 328.1787 ($C_{19}H_{24}N_2O_3$ requires 328.1786).

**6-(Benzyloxy)-4-(methoxymethyl)-β-carboline 3-*n*-Propyl Ether (46).** To a solution of amine **41** (100 mg, 0.202 mmol) in anhydrous 1-propanol (25 mL) at −20 °C (dry ice/$CCl_4$) was added isoamyl nitrite (0.485 mL, 3.62 mmol). The solution was allowed to stir for 2 min, followed by the addition of KSCN (0.714 g, 7.35 mmol), and $Cu^ISCN$ (4.473 g, 0.0367 mol) in anhydrous propanol (15 mL) at −20 °C. After 4 h of stirring, analysis by TLC (silica gel, EtOAc/MeOH, 9.5:0.5) indicated the absence of starting material. The inorganic salts were filtered from the medium and the solvent removed under reduced pressure. The residue was then dissolved in aqueous $NaHCO_3$ and extracted with EtOAc (3 × 10 mL). The organic layer was removed under reduced pressure and the crude solid purified by flash chromatography (silica gel, EtOAc) to yield the ether **46** (48.6 mg, 58%) which was directly converted into the HCl salt. **46**: mp 143−144 °C; IR (KBr) 1595 $cm^{-1}$; $^1H$ NMR ($CF_3CO_2D$) $\delta$ 1.58 (t, 3H, $J$ = 7.0 Hz), 1.78 (m, 2H), 3.69 (s, 3H), 4.24 (t, 2H, $J$ = 7.0 Hz), 5.19 (s, 1H), 5.25 (s, 2H), 7.4 (m, 7H), 7.58 (s, 1H), 7.7 (s, 1H), 8.7 (s, 1H); MS (EI) *m/e* 376 ($M^+$, 53), 285 (100) 255 (35) 213 (64). Anal. ($C_{23}H_{24}N_2O_3$) C, H, N.

**6-(Propyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic Acid (47).** The β-carboline **24** was suspended in 10% aqueous NaOH (10 mL) and the slurry heated at reflux. The suspension was held at reflux until the solution became homogeneous (3 h). The reaction was cooled to 5 °C and the pH adjusted to 4 with aqueous HCl (10%). The yellow solid which formed was filtered from the medium and washed with $H_2O$ (5 mL). The solid was air-dried to provide **47** (90 mg) in 98% yield. **47**: mp 262 °C dec; IR (KBr) 3408, 3070, 2970, 1616, 1350, 1210, 1098 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$ 1.01 (t, 3H, $J$ = 7.4 Hz), 1.78 (sextet, 2H, $J$ = 6.6, 7.4 Hz), 2.48 (s, 3H), 3.37 (s, 3H), 4.02 (t, 2H, $J$ = 6.6 Hz), 5.30 (s, 2H), 7.25 (d, 1H, $J$ = 8.5 Hz), 7.56 (d, 1H, $J$ = 8.5 Hz), 7.88 (s, 1H), 8.84 (s, 1H), 12.01 (s, 1H); MS (CI) *m/e* 315 ($M^+$ + 1, 100); HRMS *m/e* 314.1254 ($C_{17}H_{18}N_2O_4$ requires 314.1266). This material was employed directly in the next step.

**6-(Propyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic Acid Isopropyl Ester (48).** The carboxylic acid **47** (90 mg, 0.286 mmol) was dissolved in a solution of 2-propanol (50 mL) which had been previously saturated with a solution of anhydrous hydrogen chloride. The reaction mixture was held at reflux under nitrogen until analysis by TLC (silica gel, $CHCl_3$/MeOH, 9.5:0.5) indicated the absence of starting material. The solvent was removed under reduced pressure and the residue dissolved in $H_2O$ (40 mL). The pH of the aqueous solution was adjusted to 8 with concentrated aqueous $NH_4OH$. The aqueous layer was then extracted with $CHCl_3$ (6 × 20 mL). The $CHCl_3$ was removed under reduced pressure to yield a yellow solid. Flash chromatography (silica gel, $CHCl_3$/MeOH, 9.5:0.5) on the crude product gave the isopropyl ester (60 mg, 85%) as a fine yellow powder. **48**: mp 243−244 °C; IR (KBr) 3409, 3156, 2966, 1602 (C═O) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.02 (t, 6H, $J$ = 7.4 Hz), 1.39 (m, 3H), 1.85 (m, 2H), 3.31 (s, 3H), 4.05 (t, 2H, $J$ = 6.6 Hz), 5.92 (s, 2H), 7.31 (d, 1H, $J$ = 9.0 Hz), 7.59 (s, 1H), 7.65 (d, 1H, $J$ = 9 Hz), 9.06 (s, 1H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 10.58, 22.21, 67.26, 67.82, 71.67, 83.07, 107.12, 107.45, 113.45, 114.02, 115.21, 118.23, 119.74, 121.54, 133.20, 136.98, 137.40, 137.94, 153.73, 169.45; MS (CI) *m/e* 357 ($M^+$ + 1, 100) 313 (50); HRMS *m/e* 356.1725 ($C_{20}H_{25}N_2O_4$ requires 356.1736).

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