

Design, Synthesis and Subtype Selectivity effects of 3,6-Disubstituted β -carbolines at Bz/GABA(A)ergic Receptors. SAR and Studies Directed Toward Agents For Treatment of Alcohol Abuse.

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Abstract:

A series of 3,6-disubstituted β -carboline was synthesized and evaluated for their *in vitro* affinities at $\alpha_x\beta_3\gamma_2$ GABA_A/benzodiazepine receptor subtypes by radioligand binding assays in search of α_1 subtype selective ligands to treat alcohol abuse. Analogues of β -carboline-3-carboxylate-*t*-butyl ester (β CCt, **1**) were synthesized *via* a CDI-mediated process and the related 6-substituted β -carboline-3-carboxylates **6** including WYS8 (**7**) were synthesized *via* a Sonogashira or Stille coupling processes from 6-iodo β CCt (**5**). The bivalent ligands of β CCt (**32** and **33**) were also designed and prepared *via* a palladium-catalyzed homocoupling process to expand the SAR to larger ligands. Based on the pharmacophore/receptor model, a preliminary structure-activity relationship (SAR) study on 34 analogues illustrated that large substituents at position -6 of the β -carboline were well tolerated. As expected, these groups are proposed to project into the extracellular domain (L_{Di} region) of GABA_A/Bz receptors (see **32** and **33**). Moreover, substituents located at position -3 of the β -carboline nucleus exhibited a conserved stereo interaction in lipophilic pocket L_1 , while N(2) presumably underwent a hydrogen bonding interaction with H_1 . Three novel β -carboline ligands (β CCt, 3PBC and WYS8), which preferentially bound to α_1 BzR subtypes permitted a comparison of the physiological efficacies with a range of classical BzR antagonists (flumazenil, ZK93426) from several different structural groups and indicated these betacarbolines were “near GABA neutral antagonists”. Based on the SAR, the most potent (*in vitro*) α_1 selective ligand was the 6-substituted acetylenyl β CCt (WYS8, **7**). Both β cct and 3PBC reduced alcohol administration in alcohol preferring (P) and high alcohol drinking (HAD) rats but had little or no effect on sucrose self-administration. Moreover, these two betacarbolines were orally active, and in addition, were anxiolytic in P rats but were only weakly anxiolytic in rodent.¹⁻³ These data prompted the synthesis of the betacarbolines presented here.

Introduction:

Alcoholism

Alcohol addiction and dependence remain a significant public health concern, impacting physical and mental well-being, family structure and occupational stability.⁴ While advances have been made in the development of novel therapies to treat alcoholism,⁵⁻⁸ alcohol-dependent individuals represent a heterogeneous group,⁹⁻¹¹ and it is unlikely that a single pharmacological treatment will be effective for all alcoholics. Hence, a better understanding of the neuromechanisms which regulate alcohol seeking behaviors and the design of clinically safe and effective drugs that reduce alcohol addiction and dependence remain a high priority.^{7,12} While the precise neuromechanisms regulating alcohol-seeking behaviors remain unknown, there is now compelling evidence that the GABA_A receptors within the striatopallidal and extended amygdala system are involved in the "acute" reinforcing actions of alcohol.¹³⁻¹⁵ The striatopallidal and extended amygdala system include the sublenticular extended amygdala [substantia innominata/ventral pallidum (VP)], shell of the nucleus accumbens (NAcc), and central nucleus of the amygdala.¹⁶⁻¹⁸ Among the potential GABA_A receptor isoforms within the VP regulating alcohol-seeking behaviors, GABA receptors containing the $\alpha 1$ receptor subtype (GABA $\alpha 1$) appear preeminent. Thus, Criswell observed that acute alcohol administration selectively enhanced the effects of iontophoretically applied GABA in the ventral pallidum (VP).^{19,20} However, no effects were seen in the septum, ventral tegmental area (VTA), and

CAI hippocampus. Further, a positive correlation was observed between alcohol-induced GABA_A enhancement and [³H] zolpidem binding (an α₁ subtype selective agonist). These data suggest the α₁ Bz/Gaba(A)ergic receptor plays an important role in alcohol-motivated behaviors.

Research on the neuroanatomical basis of alcohol reward has shown that the NACC, VTA, VP, central amygdala (CeA), and hippocampus are involved in GABAergic regulation of ethanol (EtoH) reinforcement.^{2, 21-24}

Other investigators have identified a dense reciprocal projection from the VP to the NACC,²⁵⁻²⁷ and many of these have been found to be GABAergic neurons.²⁸⁻³⁰ The NACC is now well established as a substrate that regulates the reinforcing properties of abused drugs.¹³

Finally, immunohistochemical^{31, 32} and in situ hybridization studies³³⁻³⁵ have demonstrated that the VP contains one of the highest concentrations of mRNA encoding the α₁ subunit in the CNS. These findings, together with pharmacological studies suggesting the VP plays a role in reward-mediated behaviors of psychostimulants and opiates,^{28, 36-39} suggest a possible role of the VP-α₁ receptors in the euphoric properties of alcohol.

Animal Models

To model the human condition of alcohol abuse, rodents from the alcohol ‘preferring’ (P) line

were studied. The P rat line has been shown to satisfy all criteria for an animal model of human alcohol abuse.^{9, 15, 40, 41} Findings of previous studies concluded that activation of VP- α_1 receptors by the α_1 preferring antagonist 3-PBC produced marked reductions on alcohol-maintained responding (June et al patent). The α_1 -mediated suppression at the VP level by 3-PBC showed a high degree of neuroanatomical specificity. Specifically, the α_1 -mediated suppression was not observed with the more dorsal placements in the NACC or caudate putamen. The failure of 3-PBC to alter alcohol self-administration in the NACC/striatum is in agreement with previous research which has consistently reported a lack of expression of the α_1 transcript in the NACC and caudate;^{31-34, 42} and weak binding of [³H] zolpidem binding, the α_1 selective agonist. Thus, Criswell and his colleagues^{19, 20, 35} have suggested zolpidem binding sites may be predictive of loci where ethanol activates GABAergic receptors in the CNS.

An understanding of the neuromechanisms that regulate alcohol drinking is key in the development of drugs to treat alcohol addiction and dependence in humans.² In recent years, much evidence has accumulated in favor of the GABA system.^{22, 23, 43, 44} However, despite the growing body of evidence in favor of the GABA system, much remains unknown about the role of specific GABA_A receptor subtypes in regulating ethanol reinforcement. This is due to both a lack of high-affinity and selective ligands capable of discriminating among the GABA_A receptor subunits and the heterogeneity

of various subunits within the known alcohol reward circuitry.^{31,34} Of the potential GABA_A receptors involved in the reinforcing properties of alcohol, evidence suggests the α 1 subtype within the VP may play an important role in regulating alcohol-seeking behaviors, as mentioned above. The VP contains one of the highest distributions of α 1 subunits in the mesolimbic system.³²⁻³⁵ Finally, acute ethanol administration has been reported to selectively enhance the effects of iontophoretically applied GABA in the VP. These effects correlate highly with [³H] zolpidem binding (an α 1-subtype selective agonist).^{19,20} Together, the above findings suggest a possible role for the VP α 1 receptors in the reinforcing properties of alcohol.

The GABA_A receptor is the major inhibitory neurotransmitter receptor of the central nervous system (CNS) and the site of action of a variety of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, neuroactive steroids, anesthetics and convulsants.⁴⁵ It is now clear that these receptors regulate the excitability of the brain, anxiety, muscle tone, circadian rhythms, sleep, vigilance, memory, and learning.⁴⁵ There are several disease states associated with the improper functioning of this protein, including anxiety, epilepsy,⁴⁶ insomnia,⁴⁷ depression and bipolar disorder,⁴⁸ schizophrenia,⁴⁹ as well as mild cognitive impairment and Alzheimer's disease.⁵¹ A role of GABA_A receptors in drug and alcohol abuse has also been reported.⁵²⁻⁵⁴ Briefly, GABA_A receptors are composed of 5 subunits that form a central chloride channel and can belong to different subunit classes.

A total of 19 subunits (6α , 3β , 3γ , 1ϵ , 1π , 1θ , 3ρ) of the GABA_A receptor have been cloned and sequenced from the mammalian nervous system.^{55, 56} All these polypeptides possess an approximate molecular mass of ~ 50 kD and are structurally related.

The existence of multiple GABA_A receptor subunits can give rise to a large number of different GABA_A receptor subtypes.⁵⁷ The majority of GABA_A receptors, however, are composed of 1 γ and 2 α and 2 β subunits. The presence of a γ subunit within a GABA_A receptor is necessary for the formation of a benzodiazepine binding site that is located at the interface of an α and γ subunit. Whereas the classical benzodiazepines, such as diazepam or flunitrazepam, exhibit a high affinity for receptors composed of $\alpha1\beta\gamma2$, $\alpha1\beta\gamma2$, $\alpha3\beta\gamma2$, or $\alpha5\beta\gamma2$ subunits (diazepam sensitive (DS) receptors), as well as for their less intensively investigated analogues containing the $\gamma3$ subunit, other benzodiazepine binding site ligands are also able to interact with $\alpha4\beta\gamma2$ or $\alpha6\beta\gamma2$ receptors (diazepam insensitive (DI) receptors), or with receptors containing $\gamma1$ subunits.⁴⁵ Receptors containing $\gamma1$ or $\gamma3$ subunits exhibit quite a low abundance in the brain⁵⁸⁻⁶⁰ and their contribution to the “*in vivo*” effects of benzodiazepine binding site (BZ BS) ligands currently is unclear.

To evaluate the role of the $\alpha1$ receptor in regulating alcohol reinforcement, 3-propoxy- β -carboline hydrochloride (3-PBC), a mixed benzodiazepine (BDZ) agonist-antagonist with binding selectivity at the $\alpha1$ receptor was developed.² Compared with the prototype BDZ agonist zolpidem, 3-

PBC exhibits a slightly higher binding selectivity for the $\alpha 1$ receptor.^{61,62} Preliminary behavioral studies in several species (e.g., rats, mice, and primates) show that 3-PBC is a BDZ antagonist, exhibiting competitive binding-site interactions with BDZ agonists at low to moderate doses (2.5-15 mg/kg).^{61,63,64} At higher doses (15-60 mg/kg), 3-PBC produces anxiolytic effects in the plus maze that are comparable with those of chlordiazepoxide.⁶³ Hence, 3-PBC displays an agonist or antagonist profile depending on both the dose and the task employed. To determine the capacity of 3-PBC to modulate physiological GABAergic effects, 3-PBC was evaluated in recombinant $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$ and $\alpha 5$ receptors. Next, it was determined whether the *in vitro* binding affinity of 3-PBC in wild-type synaptosomal cortical membranes would mimic the actions of 3-PBC at recombinant $\alpha 1$ receptors. In addition the *in vivo* capacity of 3-PBC to selectively reduce alcohol-maintained responding in a rodent model of the human condition of alcohol abuse after systemic and intra-VP infusions was determined.^{22,65}

The evidence suggests both a regional and receptor subtype specificity for GABA_A-containing receptors in regulating alcohol-motivated behaviors.⁶⁶ Specifically, the $\alpha 1$ subtype in the anterior and medial VP² and the $\alpha 5$ isoform in the CA1 CA3 hippocampus²² have both been shown to be important mediators of alcohol reinforcement. Unlike the VP and hippocampus, unequivocal research exists on the primary type of isoform within the CeA. For example, while some investigators contend that the

CeA is comprised primarily of receptors of the $\alpha 1$ subtype,⁶⁷ others suggest the $\alpha 2$ and $\alpha 3$ receptors are the most predominant isoforms.^{31, 32, 34} Thus given the proposed subunit composition of the GABA receptors within the CeA, pharmacological compounds capable of exploiting the $\alpha 1$, $\alpha 2$, and $\alpha 3$ subunit-containing GABA_A receptors represent optimal tools to evaluate the role of the GABA_A receptors in alcohol reinforcement and better understand neurobehavior and ethanol responding.

$\alpha 1$ Subtype Selective Ligands

The β -carboline-3-carboxylate-t-butyl ester (β cct) is a mixed benzodiazepine agonist- antagonist ligand with binding selectivity at the $\alpha 1$ receptors,^{2, 63, 68} β cct also exhibits some affinity (albeit lower) for both the $\alpha 2$ and $\alpha 3$ receptors. Behavioural studies in several species (eg, rats, mice, primates) show that β cct is a BDZ antagonist exhibiting competitive binding site interaction with BDZ agonists over a broad range of doses.^{61, 63, 69-73} Other studies show that β cct produces anxiolytic effects in rodents⁶³ and potentiates the anticonflict response induced by $\alpha 1$ subtype agonists in primates.⁷¹ Thus, β cct displays a weak agonist or antagonist profile depending on the behavioral task, species, and dose employed.

In studies involving the $\alpha 1$ subtype, β CCT and 3-PBC were observed to selectively reduce alcohol-motivated behaviors in a variety of experiments.^{2, 74} However, unlike the $\alpha 5$ selective inverse agonist RY-23, both the β -carboline antagonists β CCT and 3-PBC displayed mixed weak agonist-antagonist profiles *in vivo* in alcohol P and HAD rats. Therefore, in addition to being able to study the

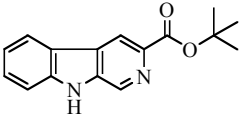
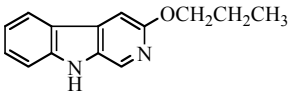
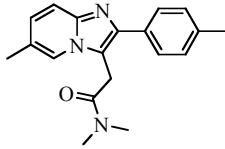
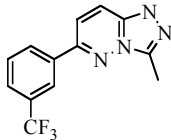
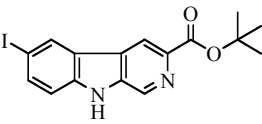
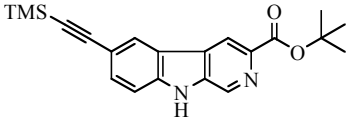
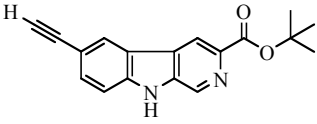
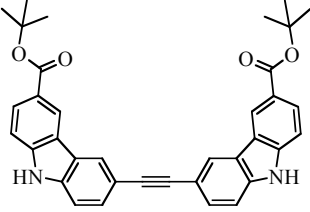
molecular basis of alcohol reinforcement, α_1 Bz beta-carboline ligands which display mixed agonist-antagonist pharmacology in alcohol P and HAD rats may be capable of reducing alcohol intake while eliminating or greatly reducing the anxiety associated with habitual alcohol, abstinence or detoxification. Thus, these types of ligands may be ideal clinical agents for the treatment of alcohol dependent individuals.^{2, 74}

Consequently, several series of structurally different compounds have been synthesized which possess some α_1 subtype selectivity.⁷⁵⁻⁷⁹ The discovery of high affinity, saturable, and stereospecific ligands for BzR has been coupled with the demonstration that β -carbolines exhibited an affinity for the BzR.⁸⁰⁻⁸⁷ Some of these agents act on the BzR to induce effects that are functionally opposite (inverse agonists/antagonists) to those of classical BDZs. Consequently, the affinities of a wide variety of β -carbolines have been reported in synaptosomal membranes from this laboratory,^{80, 82, 83, 88-94} and the laboratories of others,⁹⁵⁻¹⁰⁰ and this prompted the study of the binding affinities of a series of β -carbolines⁷⁵ at 5 recombinant GABA_A/BzR subtypes ($\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$) expressed from recombinant human cell lines.^{95, 101, 102} In general, this series of β -carboline ligands exhibited some selectivity at α_1 receptor subtypes including β CCt (**1**) and 3-PBC (**2**).^{2, 3} These two ligands displayed a 20-fold and 10-fold selectivity, respectively, for the α_1 subtype over the α_2 and α_3 receptors, as well as over 150-fold selectivity for the α_1 site over the α_5 subtype.^{2, 3} β CCt (**1**) was more selective at the α_1 subtype *in vitro* than the classical α_1 selective agonists zolpidem (**3**) and CL 218872 (**4**) (Figure 1).^{88, 103, 104} A number of *in vitro* and *in vivo* studies employing α_1 (e.g., zolpidem, CL 218872,¹⁰⁵ β CCt, and 3-PBC¹⁰⁶) selective ligands suggest the α_1 -containing GABA_A/Bz receptors of the ventral pallidum (VP) play an important role in regulating alcohol's neurobehavioral effects; particularly alcohol's reinforcing properties.^{2, 106, 20, 35, 107}

Structure Activity Relationships:

A predictive 3-D QSAR pharmacophore/receptor model for inverse agonist/antagonist β -carbolines was initially developed *via* Comparative Molecular Field Analysis (CoMFA) and later refined.^{108,109} Affinities of ligands from 15 different structural classes have been evaluated.¹¹⁰ Based on this CoMFA study of a series of β -carbolines, Huang et al. reported that β -carbolines bind to all diazepam sensitive (DS) sites of the BzR with some selectivity at the α_1 containing receptor isoform and this was confirmed by *in vitro* binding affinity of these ligands.¹¹¹ A lipophilic region (L_{Di}) of the pharmacophore receptor model appears to be larger in the α_1 , α_2 and α_3 -containing receptor isoforms and important for α_1 subtype selectivity.¹¹⁰ More recently, during the design and synthesis of β CCt-related bivalent ligands,¹¹²⁻¹¹⁵ it was found that a series of 3,6-disubstituted β -carbolines (see Figure 1), including 6-iodo- β CCt (**5**) and 6-trimethylsilyl-ethynyl- β CCt (**6**) (Figure 1) possessed α_1 subtype selectivity.¹¹²

Figure 1. *In vitro* binding affinities of a series of α_1 selective ligands (K_i in nM).

		
1 β CCt	2 3PBC	3 zolpidem
α_1 α_2 α_3 α_5 α_6	α_1 α_2 α_3 α_5 α_6	α_1 α_2 α_3 α_5 α_6
0.72 15 18.9 110.8 >5,000	5.3 52.3 68.8 591 >1000	26.7 156 383 >10,000 >10,000
		
4 CL 218872	5 6-iodo- β CCt	6 6-trimethylsilanylethynyl- β CCt
α_1 α_2 α_3 α_5 α_6	α_1 α_2 α_3 α_5 α_6	α_1 α_2 α_3 α_5 α_6
57 1964 1161 561 >10,000	14.4 44.9 123 65.3 >4000	6.8 30 36 108 1000
		
7 WYS8 (6-ethynyl- β CCt)	31 β CCt bivalent ligand	
α_1 α_2 α_3 α_5 α_6	α_1 α_2 α_3 α_5 α_6	
0.972 111 102 1473 1980	30 124 100 >300 >4000	

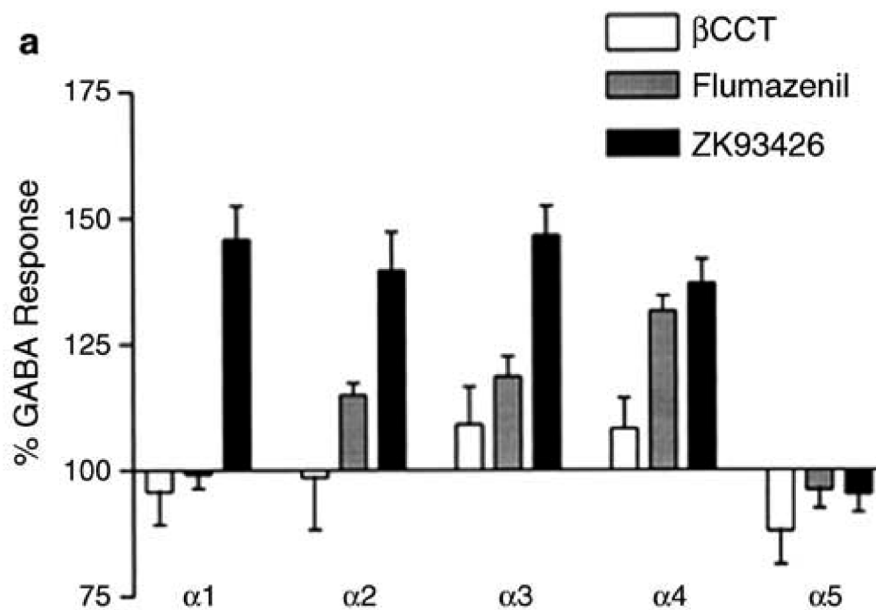
The rigidly linked linear bivalent ligands of β CCt at position “6” did bind to BzR receptors with some α_1 subtype selectivity and may provide the desired α_1 selectivity through specific occupation of the L_{Di} region of the pharmacophore/receptor model.¹¹² Although the two 3,6-disubstituted- β -carbolines **5** and **6** are less potent than β CCt (**1**), the potent binding affinities observed for **5** and **6** at the α_1 subtype has stimulated the synthesis of the β CCt analogs: 3-substituted- β -carbolines as well as 3,6-disubstituted- β -carbolines.

On the other hand, these studies also indicated that the selectivity of GABA_A/BzR site ligands

could be described in relation to binding and physiological efficacy *in vitro*. The physiological efficacy was based on the capacity of a ligand to modulate GABAergic function.¹¹⁶ BzR ligands act on chloride flux over a continuum from positive to negative modulation, with antagonists acting theoretically, at a point on the continuum, with zero intrinsic efficacy (bind to a receptor but exhibit no action).⁷⁶ Consequently, the pharmacological profiles of β CcT and 3-PBC at recombinant $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_4\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ receptor subtypes expressed in *Xenopus oocytes* were investigated.^{2,3,65}

Figure 2. Efficacy of β CcT in modulating GABA at recombinant GABA_A1-5 receptors

in *Xenopus oocytes*: comparison with other BzR antagonists.



Modulation of GABA_A $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_4\beta_3\gamma_2$ and $\alpha_5\beta_3\gamma_2$ receptor subunit combinations expressed in *Xenopus oocytes* by β CcT (open bars), flumazenil (shaded bars), and ZK 93426 (black bars). A saturating concentration (1-10 μ M) was coapplied over voltage-clamped oocytes along with an EC₅₀ of GABA.

The results of this study illustrated that β CcT was a near “neutral” antagonist (i.e., little or no efficacy) at all receptor subtypes. In fact, the level of intrinsic efficacy of β CcT in oocytes was less at

some receptor subtypes than the classical nonselective antagonist flumazenil (Ro 15-1788, for which intrinsic efficacy at all BZ-sensitive GABA_A subtype was relatively low, but not zero). To date, no compound has been characterized that exhibits zero efficacy at all BzR subtypes, raising the possibility that a compound labeled as an “antagonist” may indeed exhibit functional activity given the right circumstances. For example, more recently, the efficacies of both βCCt and 3-PBC in the selective reduction of alcohol responding and production of anxiolytic effects were demonstrated in P and HAD rats following oral administration.¹¹⁷ When compared with the classical Naltrexone treatment, these reductions in alcohol responding were more selective and longer in duration.¹¹⁷ It is important to note that similar reductions with βCCt and 3-PBC have now also been reported in primates by our collaborators, Drs Elise Weerts from John Hopkins and Donna Platt from Harvard Medical School (unpublished results). These studies in primates were initially investigated based on the original studies in the alcoholic rats. In summary, the antagonist βCCt exhibited either a neutral or low-efficacy agonist response at GABA receptors in oocytes. Although there has been some debate in the literature at present as to whether a ligand’s binding or efficacy selectivity was “the more salient factor” in determining a ligand’s capacity to function as an alcohol antagonist,^{3, 75, 117} the knowledge of the efficacy of an individual putative anti-alcohol reward ligand across all GABA_A receptors was indeed critical to the knowledge of their mode of action in the CNS.

Based on the limited availability of data on the series of α₁ “binding” and “efficacy” selective β-carbolines (βCCt, 3PBC) as anti-alcohol agents¹¹⁸ the present study was designed to expand the SAR and search for better α₁ subtype selective ligands. These compounds may be promising modulators of alcohol-related co-morbid behaviors in alcohol dependence *via* the GABA_A/BzR system. Although recent evidence suggests a salient role for GABAergic mechanisms in the regulation of excessive alcohol drinking and the negative affective states associated with abstinence, decreased GABAergic tone stemming from chronic alcohol use and withdrawal may serve to generate anxiety.¹¹⁹ Thus, compounds that enhance GABAergic tone may be effective and safe treatments for both excessive

alcohol drinking and the negative affective states associated with abstinence and may represent novel pharmacotherapies to treat alcoholism.

In this regard, the chemistry and pharmacological evaluation of a series of structurally modified analogues of β CCt (**1**) as selective and potent α_1 subtype-preferring ligands are described. The synthesis of the α_1 selective compound **7** (WYS8) and the structure-activity relationships (SAR) of 3,6-disubstituted β -carbolines are also presented. The established pharmacophore/receptor model^{110, 120} of BDZ binding sites was employed to design ligands with respect to the L_{Di} region at position-6, as well as characterize the binding pocket L_1 at position-3. Protein-ligand docking of the α_1 subtype GABA_A receptor protein and WYS8 illustrated the agreement between the pharmacophore/receptor model and BzR site prediction based on homology modeling.¹²⁰⁻¹²²

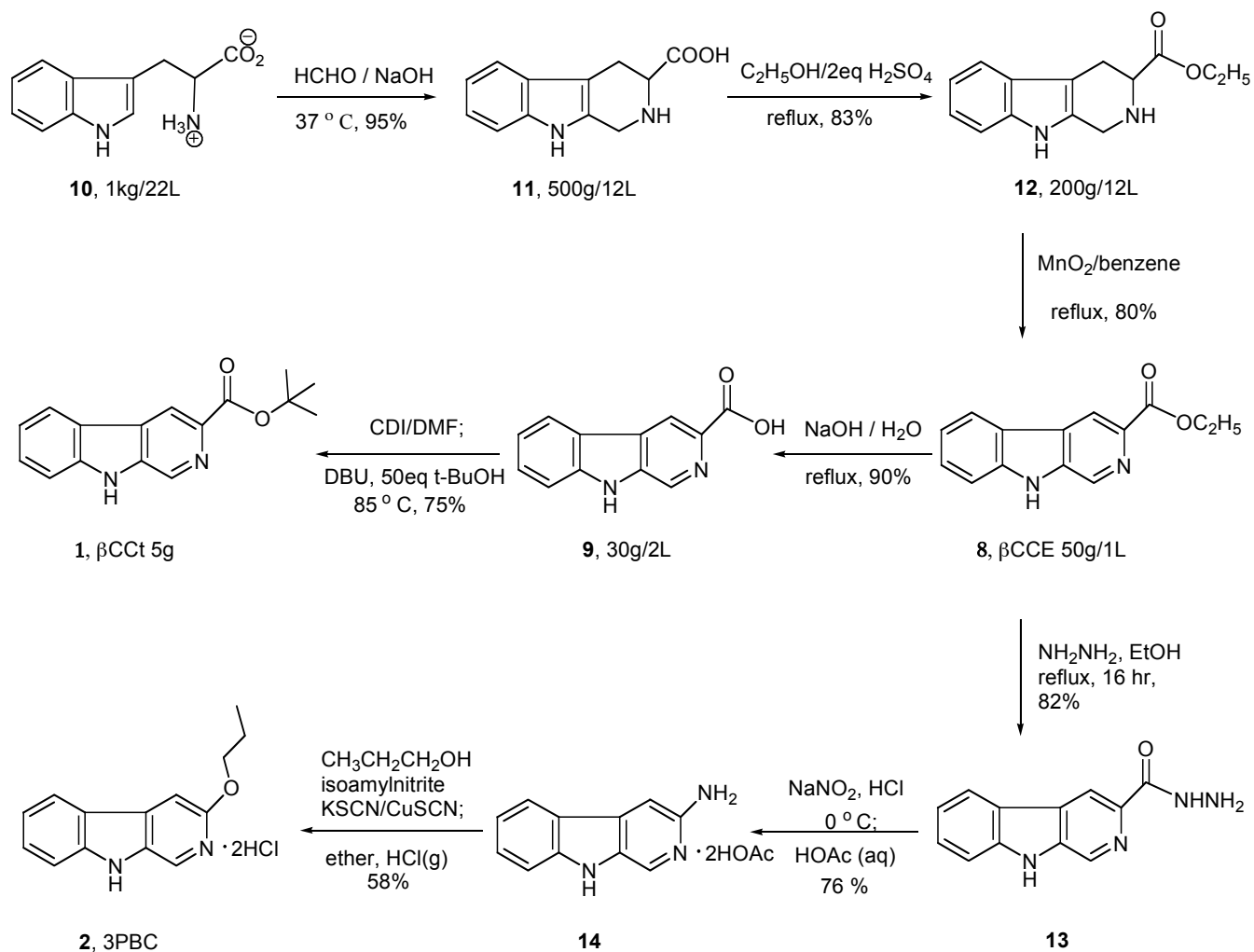
Chemistry:

The synthesis of the ligands under study is outlined in Schemes 1, 2 and 3. The important precursor β -carboline-3-carboxylate-ethyl ester (β CCE, **8**) and its corresponding acid (**9**) were the intermediates required for large-scale synthesis of β CCt (**1**), as well as an intermediate required for the synthesis of the new β -carbolines. As outlined in Scheme 1, DL tryptophan **10** was converted into tetrahydro- β -carboline (**11**) *via* a Pictet-Spengler reaction. Fischer esterification of **11**, followed by oxidation with activated MnO_2 provided the intermediate BCCE (**8**) on 200 gram scale. Hydrolysis of ester **8** to provide the acid **9**, was then followed by esterification in *t*-butanol with CDI to provide BCCT (**1**). The synthesis of 3PBC·HCl (**1**) was more difficult to scale up due to the complex last step (**14**→**2**).

It began with β CCE (**8**) from Scheme 1, which was heated with hydrazine to furnish hydrazide (**13**) in 82% yield. The hydrazide (**13**) was stirred with nitrous acid to provide an azide, which was unstable, and was converted into 3-amino- β -carboline (**14**) when stirred with acetic acid (Scheme 1) via a Curtius rearrangement. The last step, originally developed on a 100 mg scale, has now been scaled up to 4 gram levels to furnish 3PBC hydrochloride salt in reasonable yield.

In Scheme 2, the β -carboline alkyl esters **16-20** as well as chiral β CCt analogs **21-24**, and **25, 26** were prepared *via* the CDI-mediated process described above (see Scheme I).¹²³ Briefly, when β -carboline-3-carboxylic acid **9** was treated with 1,1-carbonydiimidazole (CDI) in dry DMF, the imidazole derivative **27** which resulted was subsequently transformed into the desired esters by treating it with the corresponding alcohols (individually) in the presence of DBU in a one-pot sequence. The key potential α_1 chiral selective analogs CMD-30 R/S isomers (**21 & 22**) can be synthesized by the CDI method in 90% yield (individually) on 10 gram scale. The required starting chiral alcohols were obtained by asymmetric reduction of the corresponding trifluoromethyl ketones with (+)-DIP-chloride.^{124, 125} or the (-)-DIP-enantiomer. The 6-substituted-iodo- β -carboline-3-carboxylates **28** were then prepared as intermediates to generate different functionality at position-6 through a palladium-mediated cross coupling process. For example, as illustrated in Scheme 2, β CCt (**1**) was treated with I_2/CF_3COOAg in chloroform to provide 6-iodo- β CCt

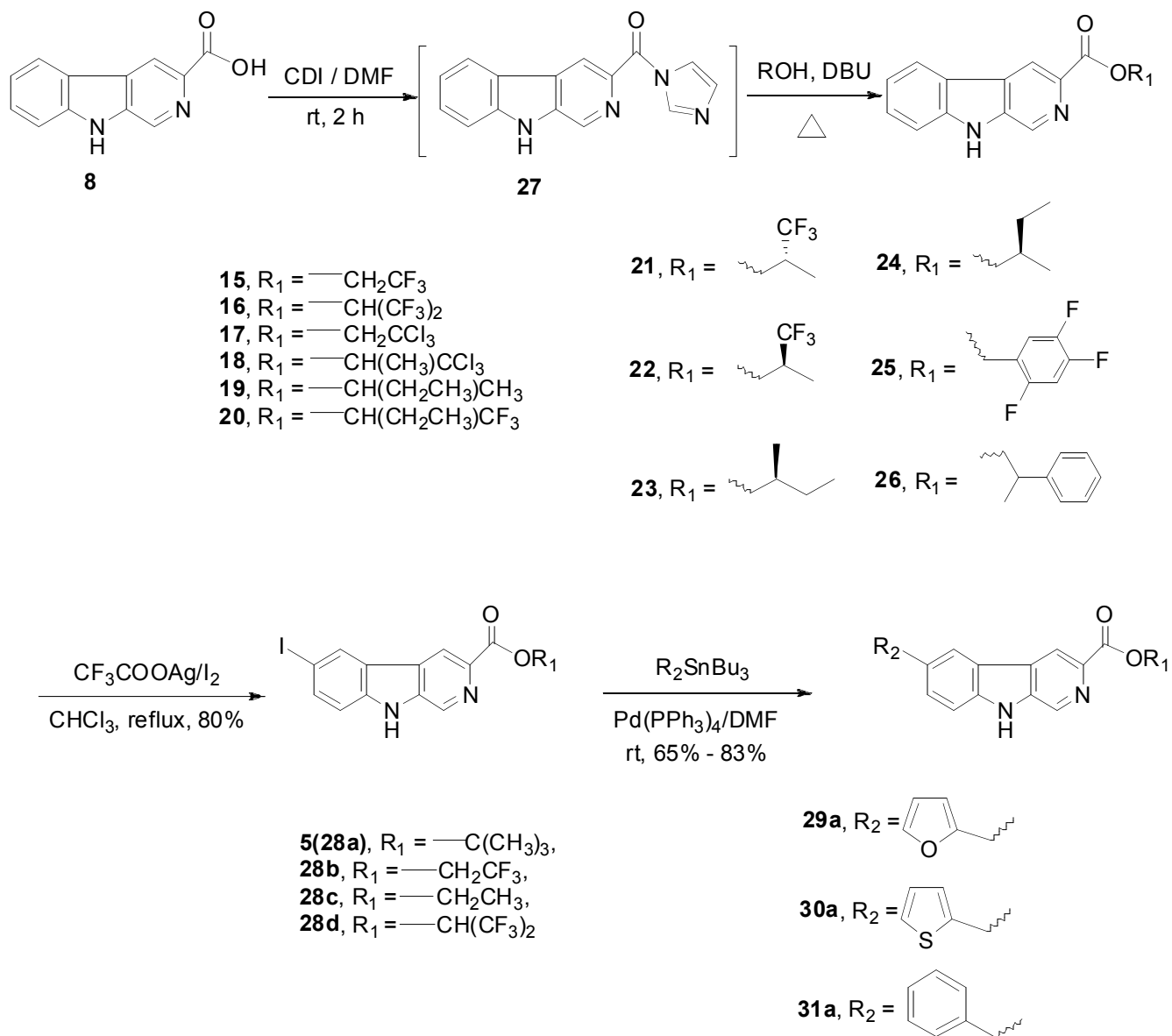
Scheme 1. Large scale synthesis of β CCt and synthesis of 3PBC.



5 (see also **28a**) in 80% yield and the 6-substituted targets **29-31** were obtained in 65%-83% yields *via* a Stille coupling process employing commercially available substituted tributyl-stannanes. The substitution by halogen occurred at position-6 *via* NMR analysis of **5** (or **28a**) with One Dimensional Nuclear Overhauser Effect (NOE) experiments.

Depicted in Scheme 3 are the synthetic routes for the β CCT related bivalent ligands **32** and **33**.¹¹² In order to efficiently affect a palladium mediated Sonogashira process at position-6 of β -carboline (a reactive electron-rich indole heterocycle), protection/deprotection of the indole N_a-H

Scheme 2. CDI-mediated esterification of 3-substituted β -carboline-3-carboxylic acids followed by the conversion into 3,6-disubstituted β -carboline-3-carboxylic acid *t*-butyl esters.

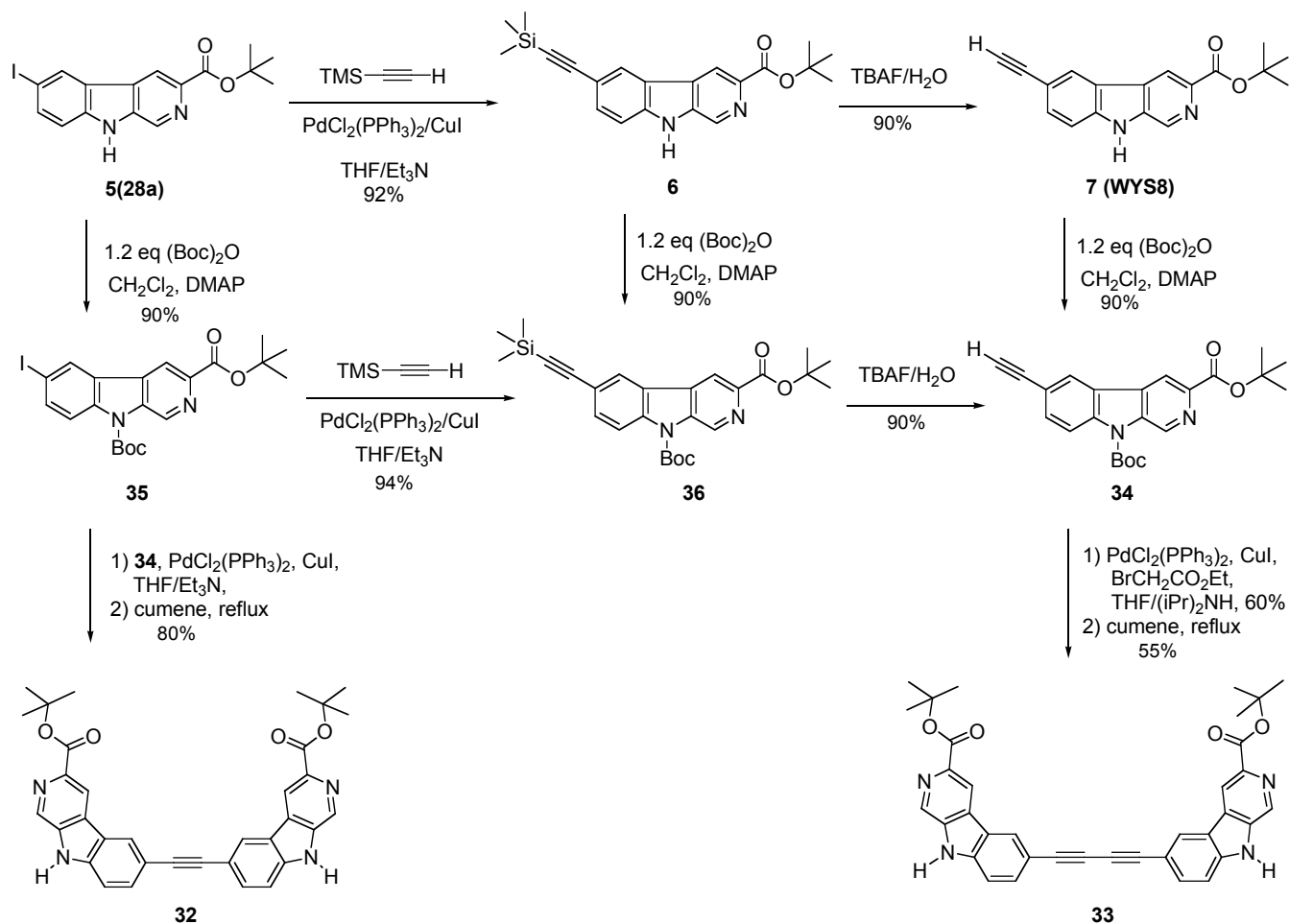


group in β -carboline **34** and **35** was necessary. The Boc protected 6-ethynyl- β -carboline-3-carboxylic acid *t*-butyl ester (**34**) was prepared directly from 6-substituted acetylenyl β CCt (**7**), which was initially termed **WYS8**. The common intermediate iodo- β CCt **5** (see also **28a**) was then converted into the 6-substituted trimethylsilylacetylenyl β CCt (**6**) *via* a Sonogashira coupling process.^{126, 127} At this point,

TBAF was employed to remove the trimethylsilyl group to provide the 6-substituted acetylenyl β CCt analog **WYS8** (**7**) as well.

The ester **7**, was then protected with a Boc group at the N(1) position to afford **34** under standard conditions. A Sonogashira process was then employed to couple **34** with Boc protected iodo- β CCt (**35**) to provide the rigid two carbon linked bivalent ligand **32** of β CCt. The Boc protecting group was removed thermally by heating in cumene at high dilution and bivalent ligand **32** was obtained. The bisacetylenic bridged ligand **33** was synthesized from the Boc protected 6-ethynyl- β CCt **34** via a homocoupling process,¹²⁸ followed by the removal of the Boc group under thermal conditions in cumene at high dilution.

Scheme 3. Preparation of 6-substituted acetylenyl β CCt (**7**, **WYS8**) and related bivalent ligands.



Biological & Pharmacological Testing

The affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]Ro15-1788 (83 Ci/mmol; NEN) binding to Ltk⁻ cell membranes expressing human receptors of composition $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_4\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$.^{95, 101} K_i values represent the mean of two determinations which differed by less than 10%. The values are reported in nM and 1.8 nM [³H]Ro15-1788 and 8 nM [³H]Ro 15-4513 (for cells expressing $\alpha_4\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$) were used as radioligands. The results are summarized in Tables 1-3.

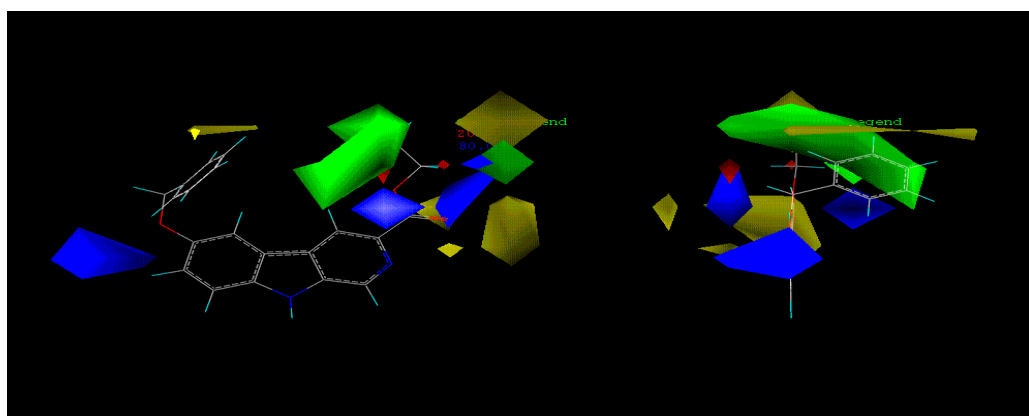
The electrophysiological analyses of all selective compounds were performed with whole cell variation of the patch-clamp-technique, in HEK cells employing GABA concentrations around the subtype-specific EC₂₀¹²⁹ to depict the quantitative efficacy difference [i.e., GABA modulation] and qualitative subunit modulation [i.e., subunit type] of these ligands relative to diazepam.

Results and Discussion

(1) β CCt bivalent ligands. Although the $\alpha_1\beta_3\gamma_2$ BzR/GABAergic subtype is very similar in structure to the α_2 and α_3 subtypes, there are slight differences.^{110, 130} One major difference is in region L_{Di}, which appears larger in the α_1 subtype than in either the α_2 , or α_3 or α_5 subtypes. This is located near position -6 of β CCt (**1**) and can be seen in the model of the Comparative Molecular Field Analysis (CoMFA) study for the α_1 subtype (Figure 3).^{120, 131} In particular, blue contours in the western region of the pharmacophore/receptor model imply positive lipophilic interactions in this area that corresponds to region L_{Di} (a region in the pharmacophore adjacent to the extracellular domain of the receptor) of the unified pharmacophore/receptor model. In this region, bulky substituents are tolerated and occupation of this area with substituents appears to enhance affinity at α_1 subtypes. This knowledge provided an opportunity to introduce a linker between two pharmacophoric β -carboline-3-carboxylate residues in order to design selective and rigid bivalent ligands. As described in the Introduction, initial efforts to

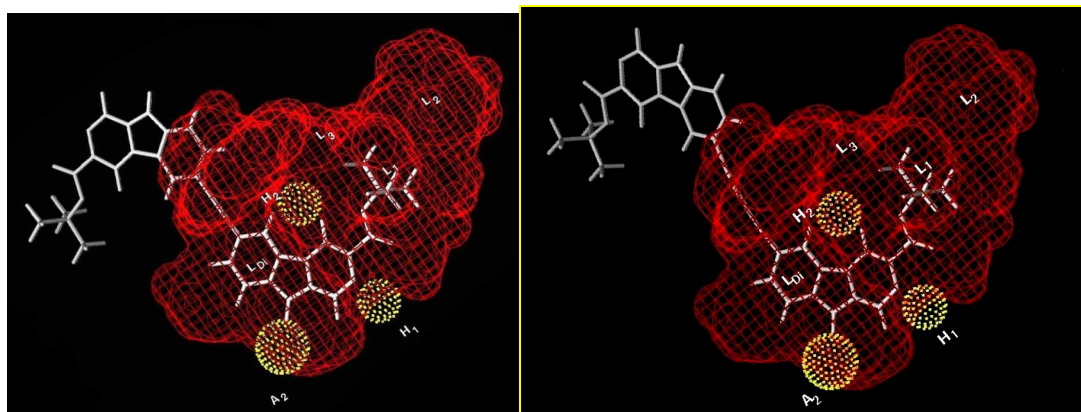
find a novel series of α_1 -preferring ligands focused on design and synthesis of β CCt bivalent ligands. Although the α_1 subtype selectivity was not amplified with the particular acetylenyl linked bivalent ligand **32**, the ligand does bind preferentially at α_1 subtypes (Table 1). It was proposed the two-carbon linker was not long enough and that crowding between the second β CCt unit and the receptor protein decreased the binding affinity at the α_1 subtype, thereby negating some of the potential selectivity. However, these rigidly linked linear bivalent ligands **32** and **33** fit the GABA_A/BzR pharmacophore/receptor model very well (Figure 4).¹²⁰ The unit at C-6, presumably, protrudes into the extracellular domain of the BzR, as previously expected,^{111, 112} and bound to BzR with some α_1 subtype selectivity.^{111, 112} To our knowledge these are the first two bivalent ligands in the β -carboline series, which bind to BzR. Further pharmacological evaluation *in vivo* of the β CCt bivalent ligand with the longer rigid linker should shed light on the above hypothesis and this would also provide some tools to determine the size and exact location of the L_{D1} region.

Figure 3. Orthogonal views of CoMFA contour maps for the affinity of 6-benzyl-substituted β -carbolines at the α,β,γ_2 BzR.



Orthogonal view of CoMFA contour maps for the α,β,γ_2 receptor subtype with 6-benzyl-substituted β -carbolines modeled by Huang.⁸⁸ Green contours represent areas of positive steric interaction at a contribution level of 85%, which would result in reduced binding affinity. Blue contours represent areas of positive charge interaction at a level of 85%, which would increase the affinity of a ligand.

Figure 4. Alignment of bivalent ligands **32** and **33** within the included volume of the $\alpha_1\beta_3\gamma_2$ subtype.

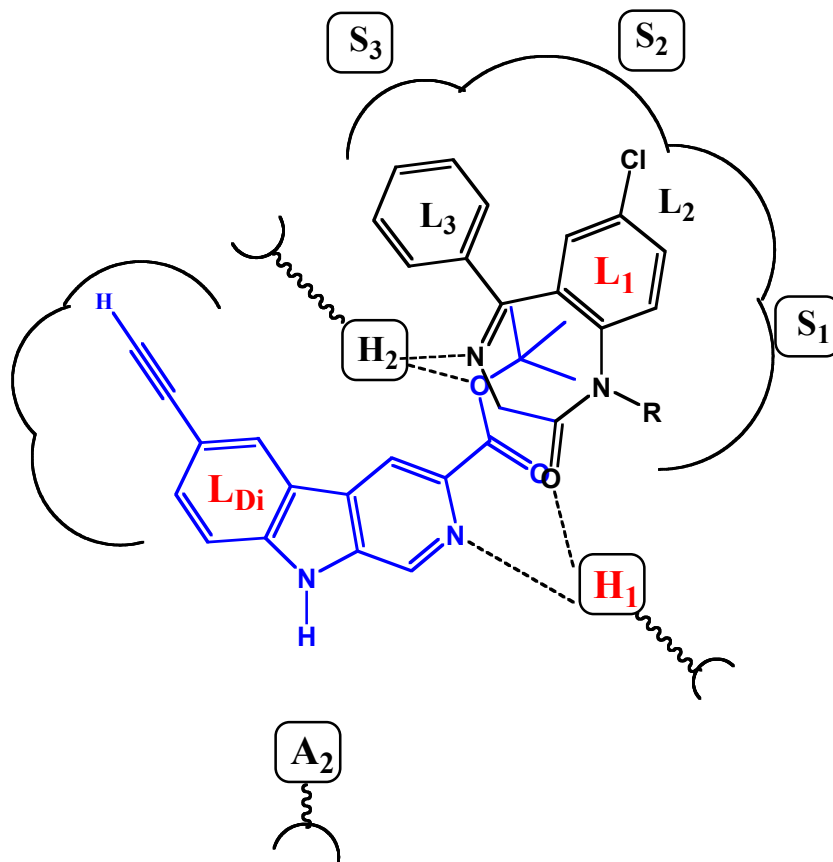


(2) **WYS8.** A series of 6-substituted- β -carboline-3-carboxylates have been synthesized and bound *in vitro* to the $\alpha_1\beta_3\gamma_2$ BzR subtype preferentially as compared to other subtypes (see Tables 1-3).^{110,111} These ligands have also been modeled in the GABA_A/BzR pharmacophore model, and the 6-substituents align well in the L_{Di} region.¹³² Occupation of this region should lead to enhanced selectivity of a ligand at the α_1 containing isoform. Among the new 3,6-disubstituted- β -carbolines, 6-trimethylsilanylethynyl- β CCt **6** has been recently synthesized and found *in vitro* to prefer the α_1 subtype. However, the most selective ligand for the α_1 subtype was, to date, WYS8 (**7**). This α_1 subtype selective ligand was 100 fold more selective over the other subtypes. This was the most α_1 subtype selective ligand reported, to date, to these authors knowledge. This 6-substituted acetylenyl β CCt **7** was 214 fold more selective for α_1 isoforms over α_5 isoforms. Studies of SAR in Table 1 confirmed the occupation of region L_{Di} of the receptor pharmacophore model did enhance α_1 selectivity in comparison to the affinity of the non-selective ligand diazepam or the α_5 selective ligand, RY080. As illustrated in

the two dimensional Figure 5, full occupation of the L_{Di} lipophilic region by β -carboline may account for the potency/selectivity of this class of ligands at the α_1 subunit. Analysis of the *in vitro* binding data for this series of bulky 6-substituted β -carboline (Table 1) has shown some selectivity for the α_1 receptor subtype. In addition, it is important to note that binding affinity in this series of ligands of greater than 400nM usually results in zero efficacy at the subtype at pharmacologically relevant concentrations.

Homology models employed here of the GABA_A receptor were as described previously^{121, 133} except that a number of alternative models were considered for loop C, which was two residues shorter than the template and hence built from a loop database. The final model was selected based on assessment of model quality¹³⁴ and consistency with published mutational data,¹³⁵⁻¹³⁹ particularly with the T207 side-chain appropriately positioned facing the benzodiazepine-binding pocket. Positioning of WYS8 in the BzR was executed using a genetic algorithm (FlexiDock®). Flexible docking provides a means of docking ligands into protein active sites.

Figure 5a. Overlap of diazepam and β CCt in the pharmacophore/receptor model.



The structure of WYS8 and diazepam in a simple representation of the pharmacophore model. WYS8 (7) (blue line) and diazepam (black line) fitted to the inclusive pharmacophore model for the BzR. Sites H₁ and H₂ represent hydrogen bond donor sites on the receptor protein complex, while A₂ represents a hydrogen bond acceptor site necessary for potent inverse activity in vivo. L₁, L₂, L₃ and L_{Di} are four lipophilic regions in the binding pharmacophore. Descriptors S₁, S₂, and S₃ are regions of negative steric repulsion.

Figure 5b. WYS8 docked in the BzR site of the $\alpha 1$ subtype GABA_A receptor. The $\alpha 1$ and $\gamma 2$ subunits are rendered in yellow and blue, respectively.

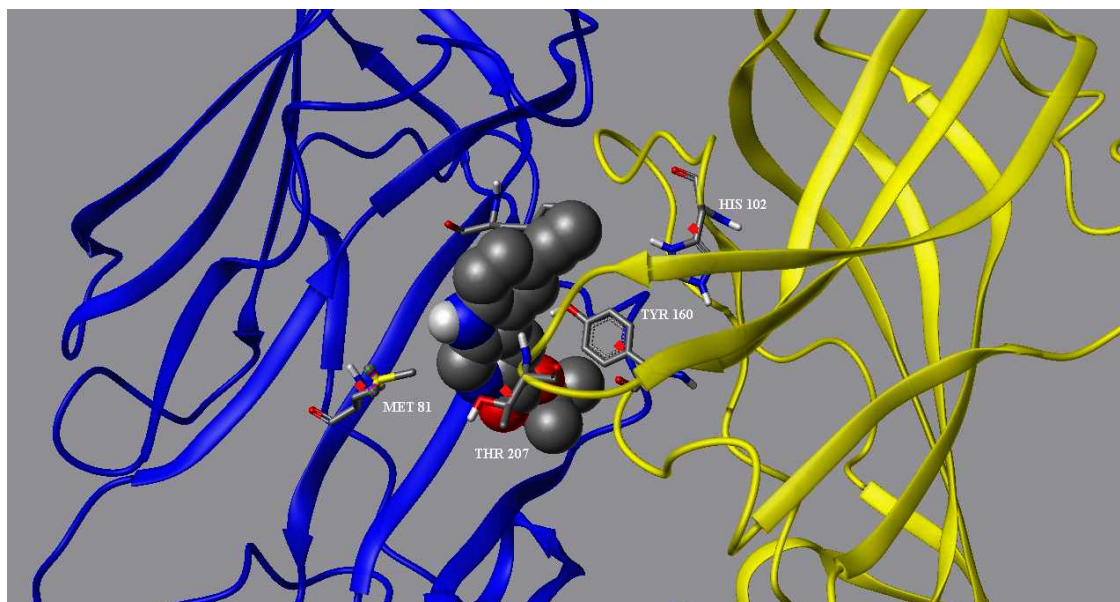
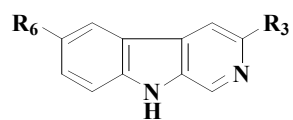








Table 1. Affinities (K_i =nM) of 3,6-disubstituted β -carbolines at $\alpha_x\beta_x\gamma_x$ ($x=1-3,5,6$) receptor subtypes.

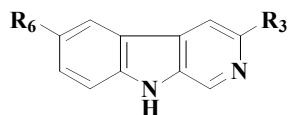


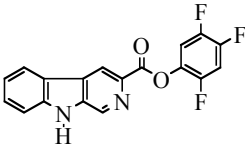
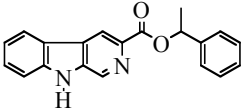
Ligands	R ₆	R ₃	α ₁	α ₂	α ₃	α ₄	α ₅	α ₆
1(βCCt)	H	CO ₂ tBu	0.72	15	18.9	1000	111	>5,000
8(βCCe)	H	CO ₂ Et	1.2	4.9	5.7	ND	26.8	2,700
2(3-PBC)	H	OnPr	5.3	52.3	68.8	1000	591	>1,000
6(WYB14)	TMS—≡	CO ₂ tBu	6.8	30	36	2000	108	1000
6b(WY-B-25)	TMS—≡	CO ₂ CH ₂ CF ₃	17	59	88	200	1444	>3000
6c(WY-B-99-1)	TMS—≡	CO ₂ Et	4.4	4.5	5.58	2000	47	2000
7(WYS8)	H—≡	CO ₂ tBu	0.972	111	102	2000	1473	1980
7b(WY-B-26-2)	H—≡	CO ₂ CH ₂ CF ₃	4.5	44.6	42.7	2000	124	2000
28a(iodo-βCCt)	I	CO ₂ tBu	14.4	44.9	123	>4000	65.3	>4000
28b(WY-B-20)	I	CO ₂ CH ₂ CF ₃	12	39	47	2000	122	3000
28c(iodo-βCCE)	I	CO ₂ Et	4.8	31	34	1000	286	1000
28d(WY-B-08)	I	CO ₂ CH(CF ₃) ₂	78	301	131	3000	681	3000
29a(WYS13)		CO ₂ tBu	2.4	13	27.5	NA	163	5000
29b(WYB27-1)		CO ₂ CH ₂ CF ₃	26	143	117	3000	127	2000
30a(WYS12)		CO ₂ tBu	37	166	314	NA	2861	5000
30b(WYB27-2)		CO ₂ CH ₂ CF ₃	9.2	13	72	2000	449	2000
31a(WYS15)		CO ₂ tBu	3.63	2.02	44.3	NA	76.5	5000
31b(WYB29-2)		CO ₂ CH ₂ CF ₃	25	137	125	2000	299	2000
CM A57	F	COC ₃ H ₇	3.7	27	40	NA	254	>2500
CM-A-82a	C(CH ₃) ₃	CO ₂ tBu	2.78	8.93	24.5	1000	7.49	1000
CM-A-87	F	CO ₂ tBu	1.62	4.54	14.7	1000	4.61	1000
32(WYS2)	Bcct—≡—Bcct		30	124	100	>300	>300	>4000
33(WYS6)	Bcct—≡—≡—Bcct		120	1059	3942	5000	5000	5000

The affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam binding to HEK cell membranes expressing human receptors of composition α₁β₂γ₂, α₂β₂γ₂, α₃β₂γ₂, α₄β₂γ₂, α₅β₂γ₂ and α₆β₂γ₂.¹⁴⁰

(3) 3-Substituted β-carbolines. It was initially believed by Braestrup, Loew, and others that an

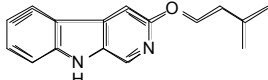
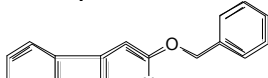
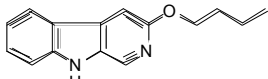
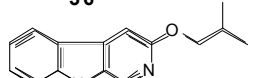
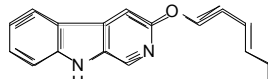
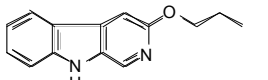
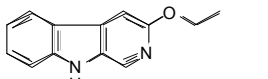
ester moiety at position-3 of β -carboline was required for a ligand to exhibit high affinity binding at Bz binding sites.^{86, 99, 141, 142} However, high affinity binding of β -carboline including the antagonist 3-propoxy β -carboline (3-P β C, Table 2b) demonstrated this was not the case.^{83, 92, 93} Examination of data from additional studies^{80, 92, 93} have suggested that at least two factors affected high affinity binding at BzR with respect to 3-alkoxy substituted β -carboline,^{81, 88, 132, 143} one of which was the lipophilicity of the substituent which interacted at L₁. The L1 pocket tolerates linear groups up to 4 carbons in length. From ligands **1** and **6** it can be seen that binding affinity is lost, illustrating that the substituent at the 3-position is too large to allow the ligand to bind. Likewise, the 3-benzyloxy β -carboline is also too bulky to fit the L1 pocket despite its lipophilic nature. The second factor was the ability of the substituent at position-3 to release electron density to the pyridine ring. This enhanced the basicity of the nitrogen atom at N(2) which resulted in a greater ligand-receptor interaction at H₁. Analysis of the binding affinities of the novel trifluoroalkyl esters of β -carboline-3-carboxylic acid further supported this hypothesis (**Table 2a**). The trifluoroalkyl esters exhibited reduced binding affinity at all receptor subtypes when compared to their corresponding alkyl esters (**20** vs. **19**, **15** vs. β CCE). Since the trifluoromethyl was a strong electron-withdrawing group, when compared to the corresponding alkoxy carbonyl moiety, the 3-trifluoroalkoxy carbonyl substituent would decrease electron density to the pyridine (N2) ring reducing the basicity of the nitrogen atom. This would result in a weaker ligand-receptor interaction at H₁. In addition, the trifluoroalkyl group was less lipophilic than the corresponding alkyl moiety, which may result in a weaker interaction at L₁. Ramachandran and Hanzawa have reported that trifluoromethyl groups are nearly as large as isopropyl or t-butyl functions.^{124, 144} It was possible, the trifluoromethyl substituted ligands are simply too large to exert high affinity binding; however, β CCT (**1**), WY-B-24 (**25**) and CM-A-77 (**26**) all bound with good potency to α 1 BzR subtypes (see Tables 1 and 2), and these ester functions occupy a large molecular volume.

Table 2a. Affinities (K_i =nM) of 3-substituted β -carbolines at $\alpha_x\beta_y\gamma_z$ ($x=1-3,5,6$) receptor subtypes

Ligands	R ₆	R ₃	α_1	α_2	α_3	α_4	α_5	α_6
BCCE	H	CO ₂ Et	1.2	4.9	5.7	1000	26.8	2700
15	H	CO ₂ CH ₂ CF ₃	3.0	24.5	41.7	>500	125.7	>2000
16(WYB09-1)	H	CO ₂ CH(CF ₃) ₂	3.99	8	32	1000	461	2000
17(WYB23-1)	H	CO ₂ CH ₂ CCl ₃	10	33	43	1000	189	2000
18(WYB17)	H	CO ₂ CH(CH ₃)CCl ₃	2000	2000	2000	3000	2000	5000
19(CMA64)	H	CO ₂ CH(CH ₃)C ₂ H ₅	18	60	116	NA	216	>2000
20(CMA69)	H	CO ₂ CH(CF ₃)C ₂ H ₅	1000	1000	1000	NA	1000	>2000
25(WY-B-24)			22.0	177	44.8	3000	422	3000
26(CM-A-77)			33.5	1000	1000	1000	1000	3000

The affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam binding to HEK cell membranes expressing human receptors of composition $\alpha\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, $\alpha_4\beta_2\gamma_2$, $\alpha_5\beta_2\gamma_2$ and $\alpha_6\beta_2\gamma_2$.¹⁴⁰

Table 2b. Affinities (K_i =nM) of Ether-substituted β -carbolines at $\alpha_x\beta_y\gamma_z$ ($x=1-3,5,6$) receptor subtypes

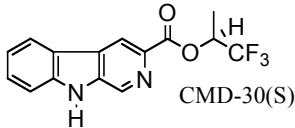
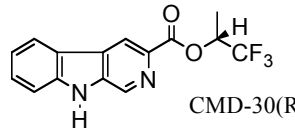
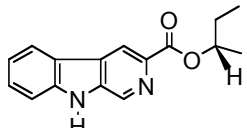
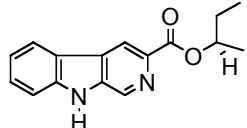
Ligands	α_1	α_2	α_3	α_5	α_6
 1	350.2	3000	3000	3000	10000
 2	830	3000	3000	10000	10000
 36	36.9	194	245	1000	1000
 38	24.9	123.6	139.2	1000	10000
 39	245	818	859	10000	10000
 2	5.3	52.3	68.8	591	1000
 40	6.43	25.1	28.2	826	1000

The affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam binding to HEK cell membranes expressing human receptors of composition $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, $\alpha_4\beta_2\gamma_2$, $\alpha_5\beta_2\gamma_2$ and $\alpha_6\beta_2\gamma_2$.¹⁴⁰

(4) Chiral 3-substituted β -carboline. Examination of the binding data for the enantiomeric pair of β -carboline sec-butyl esters **23** and **24** (Table 3) indicated that the (R)-enantiomer **24** bound tighter to the receptor subtypes than the (S) isomer **23**. Although both enantiomers exhibited approximately a 4-fold selectivity for the $\alpha_1\beta_3\gamma_2$ subtype, the (R) isomer remained more potent *in vitro* at all 5 sites. Because the receptor subtype selectivity remained about the same for the (R) and (S) isomers, this indicated the stereoenvironment in lipophilic pocket L₁ was highly conserved across the entire series of

BzR subtypes in agreement with earlier work on the binding affinities of the enantiomers of the framework-constrained 4,5-substituted pyrroloimidazobenzodiazepines.¹⁴⁵ It was possible that lipophilic pocket L₁ was simply a large area in the pharmacophore/receptor model with only small steric differences between receptor subtypes. More work will be required to determine if this is the case. A similar result was observed in the case of (R) and (S) isomers of CMD-30. The (R)-enantiomer CMD-30 R (**22**) bound slightly tighter to the receptor subtype than the (S) isomer (**21**) with almost 70 fold more selectivity for the α_1 subtype over the α_5 isoform. In addition, previously it was reported that a hydrogen bond between the N (9) H atom of a β -carboline and the secondary site A₂ in the receptor pharmacophore was required for potent inverse agonist activity *in vivo*.^{80,81} Therefore, a series of ligands with the Boc protection at position-9 such as **34-36** were evaluated and were not α_1 subtype selective ligands. In fact, they did not bind to BzR at all in agreement with previous work.¹³²

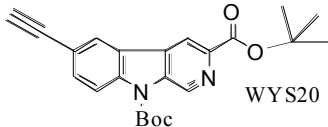
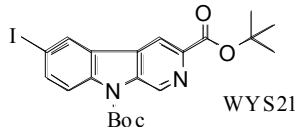
Table 3. Affinities (K_i=nM) of chiral 3-substituted β -carbolines at $\alpha_x\beta_y\gamma_z$ (x=1-3,5,6) receptor subtypes

Ligands	α_1	α_2	α_3	α_4	α_5	α_6
21  CMD-30(S)	90	931	172	>3000	1847	>2000
22  CMD-30(R)	27.0	343.3	453	>3000	1847	>2000
23 	17	59	88	NA	1444	>3000
24 	7.7	32.5	43	NA	69	>2000

The affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam

binding to HEK cell membranes expressing human receptors of composition $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, $\alpha_4\beta_2\gamma_2$, $\alpha_5\beta_2\gamma_2$ and $\alpha_6\beta_2\gamma_2$.¹⁴⁰

Table 3. Affinities (K_i =nM) of Boc-protected 3-substituted β -carbolines at $\alpha_x\beta_2\gamma_2$ ($x=1-3,5,6$) receptor subtypes.

Ligands	α_1	α_2	α_3	α_4	α_5	α_6
<p>34</p>  <p>WYS20</p>	450	5000	ND	ND	5000	5000
<p>35</p>  <p>WYS21</p>	ND	ND	ND	ND	1847	ND

ND = not determined yet

(5) Efficacy of α_1 Preferring Ligands in oocytes at GABA_A Receptor Channels. The physiological efficacy of β CCt, as compared to other Bz antagonists, was investigated across all diazepam sensitive (DS) receptor subunits at recombinant α_1 , α_2 , α_3 , and α_5 receptor subunits in the *Xenopus* oocytes assay and is depicted in Figure 2 by Harvey et al.^{2,65} In comparison to other BzR antagonists such as flumazenil and ZK 93426, as mentioned, β CCt exhibited either a neutral or low-efficacy agonist response at GABA α_1 ($96\pm 7\%$), α_2 ($99\pm 10\%$), α_3 ($108\pm 6\%$), and α_4 ($107\pm 5\%$) receptors. However, a low-efficacy partial inverse agonist response was observed at the α_5 receptor ($88\pm 7\%$ of the GABA response). Flumazenil exhibited an efficacy profile that was qualitatively similar to β CCt at the α_1 ($99\pm 5\%$), α_3 ($118\pm 7\%$), and α_5 ($96\pm 6\%$) subtypes. At the α_2 receptor, flumazenil produced a low-efficacy agonist response ($115\pm 4\%$), while β CCt was GABA neutral ($99\pm 10\%$). Flumazenil also produced a qualitatively similar response to β CCt at the α_4 receptor, albeit the

magnitude of GABA potentiation by flumazenil far exceeded that of β CCt (132 ± 6 vs. $108\pm 6\%$, respectively). However, it is important to note, with regard to $\alpha_4/\alpha_6\beta_2\gamma_2$ subtypes, the agonist effect was observed at $10\ \mu\text{M}$, far above that required for agonist efficacy at the DS subtypes. In contrast, ZK 93426 produced a clear agonist profile, potentiating GABAergic activity by 137 ± 8 – $148\pm 11\%$ across the α_1 – α_4 subtypes, but was GABA neutral at the α_5 receptor ($96\pm 6\%$). These findings suggested that β CCt had no appreciable intrinsic efficacy. The rationale for referring to this agent as a “mixed agonist-antagonist” was based on the fact that, despite the ability to potentiate GABA at certain receptor subtypes, it was “GABA neutral” at select doses. In addition, at select doses, β CCt and 3-P β C were capable of competitive antagonism of classical benzodiazepine agonists,^{88, 90, 106} therefore, the development of subtype-selective antagonists for GABA_A receptors, such as β CCt, which targeted the GABA_A α_1 receptor as a weak agonist-like antagonist,⁷⁶ can facilitate efforts to understand the antialcohol action of β -carbolines in nonhuman and human primates alike.

In the NIMH supported PDSP screen (Roth, Evans, et al., UNC), neither β CCt, 3PBC, nor WYS8 exhibited significant interactions at other receptors (see <http://pdsp.med.unc.edu> for details).

Conclusion

Ethanol allosterically modulates the GABA receptor complex to open the chloride channel and hyperpolarize cells. At the pharmacological level, the effects of ethanol can be antagonized with GABA antagonists.¹¹⁹ Unfortunately, the paucity of high affinity subtype selective ligands capable of discriminating among the various GABA_A receptor subtypes has, thus far, precluded study of the precise role GABA_A subunits play in mediating EtOH-maintained responding.

A series of β -carboline ligands described here has exhibited some selectivity at the α_1 receptor subtype which included β -carboline-3-carboxylate-*t*-butyl ester (β CCt, **1**) and 3-propoxy- β -carboline hydrochloride (3-PBC, **2**). These ligands displayed a 20-fold and 10-fold selectivity, respectively, for the α_1 subtype over the α_2 and α_3 receptors, as well as over 150-fold selectivity for the α_1 site over the α_5

subtype.^{2,3} β CCt (**1**) was more selective at the α_1 subtype *in vitro* than the classical α_1 selective agonists zolpidem (**3**) and CL 218872 (**4**).^{88,103,104} β CCt and 3-PBC are capable of competitive antagonism of classical benzodiazepine agonists,^{88,90,106} therefore, the development of subtype-selective antagonists for GABA_A receptors which targets the GABA_A α_1 receptor as a weak agonist-like antagonist,⁷⁶ can facilitate antialcohol efforts to understand the action of β -carbolines in nonhuman and human primates alike. Compared with Naltrexone, the reductions in alcohol responding were more selective and longer in duration.¹¹⁷ The goal of this study was to identify novel α_1 GABA_A subtype-preferring ligands that may serve as prototypes for further evaluation of clinical efficacy. These types of compounds may provide treatments for excessive alcohol drinking and the negative affective states associated with abstinence. Ligands that enhance GABAergic tone may be effective and safe treatments for both excessive alcohol drinking and the negative affective states associated with abstinence. This may represent novel, new pharmacotherapies to treat alcoholism.

Studies of the structure-activity relationships confirmed that occupation of region L_{Di} of the receptor pharmacophore model did enhance α_1 selectivity in comparison to the affinity of the non-selective ligands or the α_5 selective ligands. Full occupation of the L_{Di} lipophilic region by β -carbolines may account for the potency/selectivity of this class of ligands at the α_1 subunit. Based on the SAR, the most potent α_1 selective ligand was 6-substituted acetylenyl β CCt (WYS8, **2**). It was suggested the attenuation of EtOH-motivated responding effected by WYS8 (**2**) will be mediated *via* the α_1 selective antagonism of the GABA_A/BzR receptor.² In regard to ester functions at C(3), although both (R) and (S) enantiomers exhibited approximately a 4-fold selectivity for the $\alpha_1\beta_3\gamma_2$ subtype, the (R) isomer remained more potent *in vitro* at all 5 BzR sites. Two factors affected high affinity binding at BzR with respect to β -carbolines, one of which was the lipophilicity of the substituent which interacted at L₁. The second factor was the ability of the substituent at position-3 to release electron density to the pyridine ring.

The most selective ligand for α_1 subtypes, to date, to these authors' knowledge was WYS8 (**7**).

This 6-substituted acetylenyl β CCt (**7**) was 214 fold more selective for α_1 isoforms over α_5 isoforms. WYS8 can clearly be differentiated from nonselective BDZs by its selective binding affinity at the α_1 receptor subunit and reduced capacity to potentiate GABA in *Xenopus* oocytes.

Innate elevations of the α_1 and α_2 subunits of the HAD rat may contribute to the capacity of novel β -carboline ligands to function as both anxiolytic agents and alcohol antagonists in this genetic rat line.¹¹⁷ These differences may explain the capacity of these novel β -carboline ligands to block alcohol drinking and exhibit anxiolytic actions in the P and HAD alcoholic rats. WYS8 may be a suitable ligand to evaluate as a preclinical agent to reduce alcohol dependence. Its reduced efficacy at the α_{1-2} subunits in potentiating GABA may render it a safe BDZ receptor ligand devoid of synergistic interactions with alcohol.

Experimental Section:

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- or 300-MHz multiple-probe instrument. 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 5985B GC-mass spectrometer, while high resolution mass spectral data were taken on a VG autospectrometer (Double Focusing High Resolution GC/Mass Spectrometer, UK). Microanalyses were performed on a CE Elantech EA1110 elemental 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 solvents were dried according to the published procedures.

1,2,3,4-Tetrahydro-9H-pyrido [3,4-b] indole-3-carboxylic acid (11). D, L-tryptophan (1000 g, 4.9 mol) was added to a solution of aq sodium hydroxide (12 L, 0.4 N) after which the mixture was

stirred until it dissolved. Formaldehyde (560 mL of a 37% aq solution, 6.9 mol) was added and the solution was allowed to stir for three days at 37 °C. Glacial acetic acid (400 mL) was added which resulted in the precipitation of a solid as a fine suspension. The mixture was allowed to stir for two days, after which additional solid formed. The solid was filtered from the medium, washed with water (4 x 1000 mL), and dried to give **37** (953g, 90.0%). **11**: mp 295 °C (lit mp 293 °C)¹⁴⁶ (lit mp 286 °C);¹⁴⁷ (IR (KBr) 3600-2300, 1630 cm⁻¹; MS (CI, CH₄), m/z (relative intensity) 217 (M⁺ + 1, 50), 216(62), 169(59), 144(100). This material was employed directly in the next step.

Ethyl 1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole-3-carboxylate (12). The 1,2,3,4-tetra-hydro-9H-pyrido[3,4-b]indole-3-carboxylic acid **11** (500 g, 2.3 mol) was dissolved in anhydrous ethanol (9 L) in a 12L (3 neck) flask, and conc sulfuric acid (98%, 245 mL, 4.6 mol) was carefully added to the solution until most of the solid dissolved. The reaction mixture was heated to reflux under nitrogen until the starting material was no longer detected by TLC on silica gel (48 h), and the solution became homogeneous. The reaction solution was cooled and the solvent removed under reduced pressure. The residue was dissolved in H₂O (6.4 L) and the pH of the solution adjusted to 8 with cold aq NH₄OH (conc.) after which a precipitate formed. This mixture was then extracted with CHCl₃ (6 x 2.5 L). The combined organic layers were dried (Na₂SO₄) and the solvent was removed under reduced pressure to yield a light tan solid which was dried in a vacuum oven at 100 °C to provide **12** (465 g, 83%). **11**: mp 150 °C (lit. mp 149-150 °C);^{98, 148} ¹H (300MHz, CDCl₃) δ 1.33 (t, *J* = 7.32 Hz, 3H), 2.45 (s(br), 1H), 2.88 (dd, *J* = 9.70 Hz, *J* = 15.37 Hz, 1H), 3.13 (dd, *J* = 4.76 Hz, *J* = 15.5 Hz, 1H), 3.77 (dd, *J* = 4.76 Hz, *J* = 9.70 Hz, 1H), 4.07 (s(br), 2H), 4.26 (q, *J* = 7.14 Hz, 2H), 7.18-7.08 (m, 2H), 7.29 (d, *J* = 7.87 Hz, 1H), 7.48 (d, *J* = 7.68 Hz, 1H), 8.17 (s(br), 1H); MS (CI CH₄) m/e 144 (97.3), 245 (M+1, 87), 244 (100), 183 (6), 171 (33), 144 (83). This material was employed directly in the next step.

Ethyl 9H-pyrido[3,4-b]indole-3-carboxylate (8). Into a round bottom flask (12 L) equipped with a reflux condenser and an overhead stir was added 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester **39** (200g, 0.86 mol) and dry benzene (8 L). The solution was allowed to heat to reflux at which time activated MnO₂ (200 g) was added to the flask. Additional quantities of activated MnO₂

were added until analysis by TLC (silica gel/ethyl acetate) indicated the absence of starting material. The hot solution was filtered through a bed of celite to remove the MnO₂ and the filter cake was washed with hot benzene. The benzene layers were allowed to cool. A precipitate formed and was collected by vacuum filtration, which provided (100-120g, 50%-60%) of pure β -carboline-3-carboxylic acid ethyl ester **8** (β CCE). The benzene which remained in the filtrate was removed under reduced pressure to provide 25-35 g of additional β CCE, but as crude material. The crude material could be purified by recrystallization from ethanol. **8**: mp 225-227 °C (lit. 224-229 °C);^{148, 149} ¹H (300MHz, DMSO-d₆) δ 1.36 (t, J = 6.95 Hz, 3H), 4.37 (q, J = 6.95 Hz, 2H), 7.37-7.24 (m, 1H), 7.68-7.57 (m, 2H), 8.38 (d, J = 7.87 Hz, 1H), 8.90 (s, 1H), 8.97 (s, 1H), 10.7 (br, 1H); MS (CI, CH₄) m/e 241 (M⁺ + 1, 47), 195 (22), 168 (100), 140 (9). This material was employed directly in the next step.

β -Carboline-3-carboxylic acid (9). β -Carboline-3-carboxylic acid ethyl ester **8** (30.0 g, 0.126 mol) was suspended in aq NaOH (10%, 1.5L) and heated to reflux until all the material had gone into solution (1 h). The heating was continued for an additional 3 h. The reaction mixture was cooled to rt and acidified by addition of ice cold aq conc HCl to pH 4. The precipitate which resulted was stirred overnight. The solid was collected by vacuum filtration and washed with H₂O (2 x 150 mL). The product was dried at 80 °C under vacuum for 24 h to provide **9** (26.1 g, 99%). **9**: mp 220-221 °C (lit. mp 220 °C);⁶⁸ IR (KBr) 3260, 2970, 1710 cm⁻¹; ¹H NMR (CDCl₃) δ 7.31 (t, J = 7.32 Hz, 1H), 7.69-7.57 (m, 2H), 8.38 (d, J = 7.87 Hz, 1H), 8.90 (s, 1H), 8.96 (s, 1H), 12.10 (s, 1H, NH). (s, 1H); MS (CI, CH₄), m/e (M⁺ + 1, 269). Anal. Calcd. for C₁₆H₁₆N₂O₂ (0.55 H₂O): C, 69.07; H, 6.19; N, 10.07. Found: C, 68.81; H, 5.77; N, 10.00.

β -Carboline-3-carboxylic acid t-butyl ester (1). To a solution of carbonyl diimidazole (28.2 g, 0.177 mol) in anhydrous DMF (1.2 L) was added dry β -carboline-3-carboxylic acid **9** (25 g, 0.118 mol) under argon. The reaction mixture was initially a pale yellow-colored suspension, but after stirring for 30min, a purple or red-colored solution resulted. The reaction mixture was stirred for an additional 2h at rt and carbon dioxide was released during the reaction. Analysis by TLC (silica gel) indicated the

absence of starting material on the baseline. To this reaction mixture was added dry DBU (18 g, 0.118 mol) and dry freshly distilled t-butyl alcohol (437 g/560 mL, 50 eq). The mixture was heated at 85°C for 18h until analysis by TLC indicated the disappearance of the imidazole intermediate. The solvent was then removed under reduced pressure. The residue was partitioned between CH₂Cl₂ (1.2 L) and H₂O (800 mL). The organic layer was separated and the H₂O layer was extracted with CH₂Cl₂ (2 x 500 mL). The combined organic layer was washed with an aq solution of 10% K₂CO₃, water, brine and dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 1:1) to provide βCCt (20 g, 65%) as a white solid. βCCt can be recrystallized from EtOAc to provide white crystals **1**: mp 301-303°C (lit. mp 298-300);⁸² IR (KBr) 3500-3400, 3200-3000, 1610, 1560, 1370, 1340 cm⁻¹; ¹H (300MHz, DMSO-d₆) δ 1.36 (t, 3H, J=6.95Hz), 4.37 (q, 2H, J=6.95Hz), 7.37-7.24 (m, 1H), 7.68-7.57 (m, 2H), 8.38 (d, 1H, J=7.87Hz), 8.90 (s, 1H), 8.97 (s, 1H), 10.7 ((br), 1H); The spectral data for **1** were identical to those reported in the literature.⁹²

2,2,2-Trifluoroethyl β-carboline-3-carboxylate 15 was prepared from β-carboline-3-carboxylic acid **9** and 2,2,2-trifluoroethyl alcohol following the procedure employed for the preparation of **1**. **15**: mp 264-266 °C; IR (NaCl) 3275, 1735 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 4.87 (m, 2H), 7.42 (m, 1H), 7.65 (m, 2H), 8.24 (d, *J* = 7.9 Hz, 1H), 8.93 (s, 1H), 9.09 (s, 1H), 9.10 (s, br, 1H); δ 60.4, 60.8, 112.8, 118.9, 120.7, 121.2, 122.7, 127.8, 129.2, 134.5, 135.2, 138.1, 141.3, 164.5; MS (EI) *m/e* (relative intensity) 294(M⁺, 30), 195(7), 168(100), 167(42). Anal. Calcd. for C₁₄H₉F₃N₂O₂: C, 57.15; H, 3.08; N, 9.52. Found: C, 57.22; H, 3.14; N, 9.23.

9H-β-Carboline-3-carboxylic acid 2,2,2-trifluoro-1-trifluoromethyl-ethyl ester 16 was prepared following the procedure employed for the preparation of **1**. **16**: ¹H NMR (300 MHz, DMSO-d₆) δ 7.05-7.21 (m, 1H), 7.33-7.44 (t, 1H), 7.61-7.72 (m, 2H), 8.48 (d, *J* = 7.89 Hz, 1H), 9.09 (s, 2H), 12.31 (s, 1H); ¹³C NMR (75.5 MHz, DMSO-d₆) δ 65.9, 66.8, 112.9, 120.1, 120.9, 122.8, 127.8, 129.4,

133.3, 134.9, 138.4, 141.3, 162.9. This material was pure by TLC (silica gel).

9H- β -Carboline-3-carboxylic acid 2,2,2-trichloro-ethyl ester 17 was prepared following the procedure employed for the preparation of **1**. **17**: ^1H NMR (300 MHz, CDCl_3) δ 5.17 (s, 2H), 7.38-7.44 (m, 1H), 7.63-7.74 (m, 2H), 8.27 (d, $J = 7.95$ Hz, 1H), 8.95 (s, 1H), 9.21 (s, 1H), 9.66 (s, 1H). This material was pure by TLC (silica gel).

9H- β -Carboline-3-carboxylic acid 2,2,2-trifluoro-1-methyl-ethyl ester 18 was prepared following the procedure employed for the preparation of **1**. **18**: m.p. 247–249 °C; IR (NaCl) 2359, 1729, 1345, 1251, 1092, 729, 450 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 1.82 (d, $J = 6.1$ Hz, 3H), 5.96 (q, 1H), 7.42 (t, 1H), 7.67 (m, 2H), 8.26 (d, $J = 7.6$ Hz, 1H), 8.8 (s, 1H), 8.9 (s, 1H), 9.5 (br, 1H); ^{13}C NMR (75.5 MHz, CDCl_3) δ 16.5, 79.1, 112.3, 118.5, 121.2, 121.5, 121.9, 128.8, 129.2, 133.9, 137.4. This material was pure by TLC (silica gel).

(S)-1,1,1-Trifluoroisopropyl β -carboline-3-carboxylate (21). To a solution of carbonyl diimidazole (0.168 g, 1.03 mmol) in anhydrous DMF (5 mL), β -carboline-3-carboxylic acid **9** (0.10 g, 0.47 mmol) was added. The reaction mixture which resulted was stirred for 2 h at rt until analysis by TLC (silica gel) indicated the absence of starting material on the baseline. The solution which resulted was then cooled to -6 °C and this was followed by addition of (S)-1,1,1-trifluoropropan-2-ol (2.3 eq) which was contaminated with some EtOH. The dry DBU (100 mg, 0.68 mmol) in dry DMF (0.5 mL) was slowly syringed into the reaction mixture at -6 °C. The mixture was stirred at 0 °C for 8 h until analysis by TLC (silica gel) indicated the disappearance of the imidazole intermediate. The reaction mixture was then poured into ice water (30 mL) and extracted with CH_2Cl_2 (3 x 40 mL). The combined organic layers were washed with H_2O (5 x 40 mL), brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexanes = 2:1) to provide **21** (0.113 g, 78%) as a white solid. **21**: mp 239-241 °C; $[\alpha]_{\text{D}}^{27} = -9.62^\circ$ ($c = 0.81$, in CHCl_3); IR (NaCl) 3266, 1725, 1502 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 1.63 (d, $J = 6.6$

Hz, 3H), 5.75 (m, 1H), 7.40 (t, $J = 7.5$ Hz, 1H), 7.64 (t, $J = 7.6$ Hz, 1H), 7.72 (d, $J = 8.1$ Hz, 1H), 8.25 (d, $J = 7.9$ Hz, 1H), 8.91 (s, 1H), 9.18 (s, 1H), 10.04 (s, br, 1H); MS (EI) m/e (relative intensity) 308 (M^+ , 17), 168 (100), 140 (21). Exact mass calcd. for $C_{15}H_{11}F_3N_2O_2$: 308.0773. Found: 308.0773. Anal. Calcd. for $C_{15}H_{11}F_3N_2O_2$: C, 58.45; H, 3.60; N, 9.09. Found: C, 58.15; H, 3.63; N, 8.88.

(S)-1,1,1-Trifluorobutan-2-ol was prepared following the literature procedure.¹²⁴ To an oven-dried, 25 mL round-bottom flask was transferred (-)-DIP-Chloride (10.68 g, 33 mmol) in a glove box. Then 1,1,1-trifluorobutan-2-one (4.03 g, 32 mmol) was added at rt under argon. The reaction mixture was stirred at rt for 10 h. Ethyl ether was added and the reaction solution was cooled to 0 °C followed by addition of acetaldehyde (1.6 g, 1.1 eq). The reaction mixture was allowed to warm to rt and stirring was continued for 24 h while the second equivalent of α -pinene was liberated. An aq solution of sodium hydroxide (2.5M, 30 mL) was added and the solution which resulted was extracted with ether (3 x 30 mL). The ether layer was dried (Na_2SO_4) and fractionally distilled through a Pyrex distilling column packed with glass beads. The desired alcohol was collected along with EtOH (20%). **(S)-1,1,1-Trifluorobutan-2-ol**: 1H NMR (300 MHz, $CDCl_3$) 1.04(dt, 3H, $J = 0.6$ Hz and $J = 7.4$ Hz), 1.58(m, 1H), 1.72(m, 1H), 3.82(m, 1H). This material was used in a later step without further purification.

(R)-1,1,1-Trifluoroisopropyl β -carboline-3-carboxylate 22 was prepared from the acid **9** and (R)-1,1,1-trifluoropropan-2-ol following the procedure employed for preparation of (S)-1,1,1-trifluoropropan-2-ol. **30**: mp 239-241 °C; $[\alpha]_D^{27} = 8.73^\circ$ ($c = 0.88$, in $CHCl_3$); The spectral data for **22** were identical to those for **21**; however, the optical rotation was in the opposite direction.

(S)- β -Carboline-3-carboxylic acid sec-butyl ester (23). To a solution of carbonyl diimidazole (1.53 g, 9.4 mmol) in anhydrous DMF (50 mL) was added β -carboline-3-carboxylic acid **9** (1.0 g, 4.7 mmol). The reaction mixture was stirred for 2 h at rt and carbon dioxide was released during the reaction. Analysis by TLC (silica gel) indicated the absence of starting material on the baseline. To this

reaction mixture was added dry DBU (0.72 g, 4.7 mmol) and dry (S)-butyl alcohol (1.13 g, 15.2 mmol). The mixture which resulted was heated at 55 °C for 8 h until analysis by TLC (silica gel) indicated the disappearance of the imidazole intermediate. The solvent was then removed under reduced pressure. The residue was partitioned between CH₂Cl₂ (100 mL) and H₂O (100 mL). The organic layer was separated and the H₂O layer was extracted with CH₂Cl₂ (2 x 80 mL). The combined organic layer was washed with H₂O (3 x 100 mL), brine and dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 2:1) to provide **23** (0.96 g, 76%) as a white solid. **23**: mp 212-213 °C; [α]₂₅ = 35.6° (CHCl₃, c = 1.43); IR (KBr) 3222, 1706, 1622, 1494 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.99 (t, *J* = 7.5 Hz, 3H), 1.40 (d, *J* = 6.3 Hz, 3H), 1.79 (m, 2H), 5.27 (m, 1H), 7.34 (t, *J* = 7.9 Hz, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 8.3 Hz, 1H), 8.21 (d, *J* = 7.9 Hz, 1H), 11.47 (s, br, 1H); MS (CI, CH₄) *m/e* (relative intensity) 269(M⁺ + 1, 100), 241(15), 213(41), 195(14). Anal. Calcd. for C₁₆H₁₆N₂O₂: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.33; H, 6.09; N, 10.26.

(R)- β -Carboline-3-carboxylic acid sec-butyl ester 24 was prepared in 75% yield following the procedure for preparation of **1**. **24**: [α]₂₅ = -35.2° (CHCl₃, c = 1.25) The spectral data for **22** were identical to those for **23**.

6-Iodo-9H- β -carboline-3-carboxylic acid t-butyl ester (5). Into a round bottom flask (250 mL) was added CF₃CO₂Ag (1.03 g, 4.67 mmol), β -carboline-3-carboxylic acid t-butyl ester **9** (1.02 g, 3.83 mmol), and CHCl₃ (100 mL). This was followed by addition of iodine (1.15 g, 4.66 mmol). The reaction mixture was allowed to stir at rt for 6 h after which another portion of CF₃CO₂Ag (500 mg, 2.26 mmol) was added and stirring continued for another 10 h at reflux. Analysis by TLC (silica gel) indicated that most of the β CCt had disappeared. The reaction mixture was filtered through a bed of celite to remove the solid salts and the filter cake was washed with EtOH (3 x 50 mL). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel,

EtOAc/hexane = 4:1) to provide **5** (908 mg, 65%) as a white solid. **5**: mp 347-348 °C (dec.); IR (NaCl) 3223, 1710, 1485, 1323, 1245, 1161, 1104, 1020 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 1.68 (s, 9H), 7.63 (d, $J = 8.6$ Hz, 1H), 7.85 (dd, $J = 1.5$ Hz and $J = 8.6$ Hz, 1H), 8.53 (d, $J = 1.4$ Hz, 1H), 9.31 (s, 1H), 11.47 (s, br, 1H); ^{13}C NMR (75.7 MHz, CDCl_3) δ 28.3, 80.8, 83.6, 115.1, 118.1, 123.9, 126.7, 131.2, 134.1, 136.8, 137.5, 138.7, 140.4, 164.9; $\square\text{MS}$ (EI800) m/e (relative intensity) 395 ($\text{M}^+ + 1$, 18), 367 (20), 339 (100), 268 (33). Anal. Calcd. for $\text{C}_{16}\text{H}_{15}\text{IN}_2\text{O}_2$: C, 48.75; H, 3.84; N, 7.11. Found: C, 49.01; H, 3.91; N, 6.95.

6-Trimethylsilanylethynyl-9H- β -carboline-3-carboxylic acid t-butyl ester (6). Into a round bottom flask (50 mL) which contained a solution of degassed THF/ Et_3N (10 mL/2 mL), was added 6-iodo-9H- β -carboline-3-carboxylic acid t-butyl ester **5** (400 mg, 1.02 mmol), bis[triphenylphosphine] palladium dichloride (35 mg, 5 mol%) and copper(I) iodide (7 mg, 5 mol%) Note: Practically, on a small scale, CuI could be used up to 10-15 mol% because of its lower molecular weight compared to the palladium catalyst; on a bigger scale, both $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ and CuI can be used as low as 0.5-1 mol%. The reaction mixture was then degassed 2 times with an oil pump at -78 °C, and then the trimethylsilyl acetylene (300 mg, 3.06 mmol) was added into the mixture and it was degassed one more time at -78 °C. The reaction mixture was gradually allowed to warm to rt and stirred at rt for an additional 0.5-1h until all the starting material had disappeared (TLC analysis indicated that the original red spot changed color to purple, since both s.m. and product had very similar R_f values). The solvent was removed under reduced pressure at this point and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 4:1) to provide **6** (340 mg, 92%) as a white solid. ^1H NMR (300 MHz, CDCl_3) δ 1.71 (s, 9H), 1.75 (s, 9H), 7.67 (m, 2H), 8.26 (d, $J = 7.6$ Hz, 1H), 8.8 (s, 1H), 8.9 (s, 1H), 9.5 (br, 1H); ^{13}C NMR (75.5 MHz, CDCl_3) δ 0.021, 28.3, 81.9, 92.6, 105.7, 113.2, 114.9, 117.7, 121.3, 125.5, 128.3, 132.4, 133.8, 138.2, 138.4, 141.5, 165.4; EIMS 364(M^+ , 38), 293(12), 264(100), 249(48), 124(60). This material was employed directly in the next step.

6-Ethynyl-9H- β -carboline-3-carboxylic acid t-butyl ester (7)-WYS8. To a solution of 6-trimethylsilyl- β -carboline-3-carboxylic acid t-butyl ester **5** (850 mg, 2.32 mmol) in THF (10 mL) was added 1.2 eq of TBAF (2.8 mL of 1M TBAF/THF solution) at 0 °C and then the solution was allowed to warm to rt. After consumption of the starting material as indicated by TLC, H₂O (10 mL) was added and the mixture was extracted with CH₂Cl₂ (3 x 20 mL). The combined organic layer was concentrated under reduced pressure and the residue was chromatographed on a short silica gel column (EtOAc/hexane = 4:1) to give **7** (620 mg, 92%) as a white solid. **7**: ¹H NMR (300 MHz, CDCl₃) δ 1.75(s, 9H), 3.54(s, 1H), 7.62-7.85 (m, 2H), 8.41 (s, 1H), 8.83 (s, 1H), 9.33 (s, 1H), 11.1 (s, 1H); EIMS 292 (M⁺, 25), 236(12), 192 (100), 164(30). This material was pure by TLC (silica gel) and used directly in the next step.

6-Thiophen-2-yl-9H- β -carboline-3-carboxylic acid t-butyl ester (30a). Representative Procedure for preparation of 6-substituted β -carbolines. A solution of 6-Iodo- β -carboline-3-carboxylic acid t-butyl ester **5** (265 mg, 0.67 mmol) in dry toluene (15 mL) was degassed under vacuum and purged with dry N₂ through the solution 3 times. The mixture was then heated to 140 °C under nitrogen after which tetrakis(triphenylphosphine) palladium (0) (77 mg, 0.067 mmol, 10mol%) and 2-(tributylstannyl) thiophene (718 mg, 2.01 mmol) were added in one portion. The mixture was heated to reflux under nitrogen. After 12 h, the mixture was allowed to cool to rt and the precipitate which resulted was removed by vacuum filtration. The filtrate was concentrated under reduced pressure and the residue was treated with a saturated aq solution of NaHCO₃ (30 mL) and extracted with CH₂Cl₂ (3 x 25 mL). The combined extracts were washed with brine and dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 5:1) to provide a white solid **30a** (195 mg, 83%). **30a**: ¹H NMR (300 MHz, CDCl₃) δ 2.07 (s, 9H), 7.15 (t, 1H), 7.31 (d, *J* = 3Hz, 1H), 7.40 (d, *J* = 3Hz, 1H), 7.89 (q, 2H), 8.44 (s, 1H), 8.89 (s, 1H), 9.32 (s, 1H), 11.2 (s, 1H). This material was pure by TLC (silica gel).

6-Furan-2-yl-9H- β -carboline-3-carboxylic acid t-butyl ester 29a was prepared following the procedure for preparation of **30a**. **29a**: ^1H NMR (300 MHz, CDCl_3) δ 1.63(s, 9H), 6.63 (m, 1H), 6.97 (d, $J = 6\text{Hz}$, 1H), 7.70 (d, $J = 9\text{Hz}$, 1H), 7.77 (s, 1H), 7.96 (d, $J = 9\text{Hz}$, 1H), 8.73 (s, 1H), 8.90 (s, 1H), 8.96 (s, 1H), 12.1 (s, 1H); ^{13}C NMR (75.5 MHz, CDCl_3) δ 14.3, 22.4, 28.4, 31.3, 78.9, 80.7, 102.3, 104.6, 104.9, 106.3, 112.4, 113.2, 117.4, 117.8, 121.7, 123.5, 125.1, 127.1, 127.8, 133.6, 134.1, 138, 140.6, 142.6, 154.1, 165.0, 166.0. This material was pure by TLC (silica gel).

6-Furan-2-yl-9H- β -carboline-3-carboxylic acid 2,2,2-trifluoro-ethyl ester 29b was prepared following the procedure for preparation of **30a**. **29b**: ^1H NMR (250 MHz, CDCl_3) δ 4.82-4.92 (m, 2H), 6.54 (s, 1H), 6.75 (s, 1H), 7.38 (s, 1H), 7.65 (d, $J = 10\text{Hz}$, 1H), 7.96 (d, $J = 10\text{Hz}$, 1H), 8.5 (s, 1H), 8.93 (s, 1H), 9.13 (s, 1H), 9.59 (s, 1H); ^{13}C NMR (75.5 MHz, CDCl_3) δ 60.9, 105.3, 112.5, 112.8, 113.3, 117.6, 119.2, 122.3, 123.8, 125.3, 127.9, 134.7, 135.3, 138.6, 140.6, 142.7, 154.1, 164.4. This material was pure by TLC (silica gel).

6-Iodo- β -carboline-3,9-dicarboxylic acid di-tert-butyl ester (35). To a solution of 6-iodo- β -carboline-3-carboxylic acid t-butyl ester **5** (2g, 5.06mmol) in anhydrous CH_2Cl_2 (30mL), $(\text{Boc})_2\text{O}$ (1.32g, 6.07mmol) and DMAP(123mg, 1.01mmol) were added. The reaction mixture was allowed to stir at rt for half an hour until analysis by TLC (silica gel) indicated that the starting material had been converted into the Boc protected indole **35**. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 5:95) to provide **35** (2.3 g, 92%) as a white solid. **35**: mp 317-320 $^\circ\text{C}$; IR (NaCl) 2975, 2917, 1728, 1457, 1343, 1238, 1154, 1119, 1031, 811 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 1.71 (s, 9H), 1.77 (s, 9H), 7.91 (d, $J = 8.972\text{ Hz}$, 1H), 8.22 (d, $J = 8.972\text{Hz}$, 1H), 8.42 (s, 1H), 8.61 (s, 1H), 9.61 (s, 1H); ^{13}C NMR (300MHz, CDCl_3) δ 28.1, 28.2, 82.1, 85.8, 87.2, 115.9, 118.5, 125.7, 129.9, 130.6, 135.7, 138.9, 143.0 149.6 164.1; MS (EI) m/e (relative intensity) 494 (M^+ , 42), 438 (71), 338 (100), 294 (70), 268 (8), 212 (8), 168 (35). Anal. calcd. for $\text{C}_{21}\text{H}_{23}\text{IN}_2\text{O}_4(0.1\text{ C}_6\text{H}_{14})$: C, 51.60; H, 4.85; N, 5.57; Found: C, 51.84; H, 4.88; N: 5.45.

6-Trimethylsilanylethynyl- β -carboline-3,9-dicarboxylic acid di-*tert*-butyl ester (36).

Dichlorobis(triphenylphosphine)palladium(II) (140 mg; 2 mol %), and CuI (40 mg; 2 mol %) were added to a solution of 6-iodo- β -carboline-3,9-dicarboxylic acid di-*tert*-butyl ester **35** (4.9 g; 10 mmol) in anhydrous THF (30 mL) and triethylamine (10 mL). The mixture was degassed, and back-filled three times with argon. Then (trimethylsilyl) acetylene (1.08 g; 11 mmol) was added with stirring under argon. After the mixture was allowed to stir for 1 h, the solvents were removed in vacuum and the residue was chromatographed on a short column (silica gel, hexane/CH₂Cl₂ = 7:3) to give **36** (4.36 g, 94%) as a white solid. **36**: mp 334-336 °C (dec); IR (NaCl) 2974, 2137, 1732, 1559, 1476, 1469, 1368, 1343, 1309, 1247, 1156, 1109, 872, 842, 760 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.31 (s, 9H), 1.71 (s, 9H), 1.78 (s, 9H), 7.77 (d, 2H, J=8.97), 8.25 (s, 1H), 8.41 (d, 2H, J=8.61), 8.65 (s, 1H), 9.62 (s, 1H); ¹³C NMR (300MHz, CDCl₃) δ 0.11, 28.1, 28.2, 82.1, 85.8, 94.4, 104.4, 116, 116.5, 118.7, 123.5, 124.7, 131.6, 133.7, 136.2, 138.1, 139.2, 142.9, 149.7, 164.1; MS (EI) m/e (relative intensity) 465 (M⁺, 30), 409 (100), 365 (21), 308 (80), 262 (25), 249 (40). Anal. calcd. for C₂₆H₃₂N₂O₄Si: C, 67.21; H, 6.94; N, 6.03; O, 13.77; Si, 6.04. This material was pure by TLC (silica gel) and used in the next step.

6-Ethynyl- β -carboline-3,9-dicarboxylic acid di-*tert*-butyl ester 34: To a solution of 6-trimethylsilanylethynyl- β -carboline-3,9-dicarboxylic acid di-*tert*-butyl ester **36** (2.14g, 4.6 mmol) in THF (20 mL), 1.2 eq of TBAF (5.52 ml of 1M TBAF/THF solution) at 0 °C was added and then the solution was allowed to warm to rt. After consumption of the starting material as indicated by TLC (silica gel), H₂O (10 mL) was added and the mixture was extracted with CH₂Cl₂ (20 mL, 3X). The combined organic layer was concentrated under reduced pressure and the residue was chromatographed on a short column (silica gel, hexane/CH₂Cl₂ = 4:1) to give **34** (1.66 g, 92%) as a white solid. **34**: mp 226-229 °C; IR (NaCl) 3303, 2978, 2346, 2232, 1734, 1622, 1560, 1463, 1394, 1310, 1248, 1151, 1110, 1031, 911, 836, 731 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 1.54 (s, 9H), 1.72 (s, 9H), 1.79 (s, 9H), 3.17 (s, 1H), 7.79 (d, 2H, J=8.76), 8.27 (s, 1H), 8.44 (d, 2H, J=8.94), 8.67 (s, 1H), 9.64 (s, 1H); ¹³C NMR (75.5

MHz, CDCl₃) δ 27.3, 28.1, 28.2, 67.8, 82.1, 83.0, 85.1, 85.5, 116.0, 116.7, 123.5, 124.9, 133.7, 136.2, 138.1, 139.4, 143.0, 164.2; MS (EI) m/e (relative intensity) 393 (M⁺, 32), 338 (13), 321 (26), 293 (100), 167 (28), 139 (24). Anal. Calcd. for C₂₃H₂₄N₂O₄(0.05CH₂Cl₂): C, 69.78; H, 6.12; N, 7.06; Found: C: 69.70; H: 6.10; N: 6.81.

1,2-Bis(9H-β-carboline-3-carboxylic acid tert-butyl ester) ethyne (32). Dichlorobis (triphenylphosphine)palladium(II) (60 mg, 2 mol%) and copper iodide (16 mg, 2 mol%) were added to a mixture of 6-ethynyl-β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester **34** (1.6g, 4.1mmol) and 6-iodo-β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester **35** (2.1g, 4.25mmol) in THF/ TEA(30 mL; 4:1). The reaction mixture which resulted was degassed, and back-filled three times with argon. The reaction mixture was then allowed to stir at rt for about 1h until analysis by TLC (silica gel) indicated the starting materials were absent. The solution was concentrated under reduced pressure and the residue was chromatographed on a silica gel column with CH₂Cl₂ as the eluent to give 1,2-Bis(β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester) ethyne (2.97 g, 95%) as a white solid: mp 305-307 °C; IR (NaCl) 2972, 2929, 1737, 1559, 1466, 1338, 1156, 1102, 823, 624cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 1.72 (s, 9H), 1.79 (s, 9H), 7.86 (d, *J* = 8.97 Hz, 1H), 8.32 (s, 1H), 8.47 (d, *J* = 8.79Hz, 1H), 8.68 (s, 1H), 9.63 (s, 1H); ¹³C NMR (300MHz, CDCl₃) δ 28.2, 82.1, 85.8, 89.0, 116.0, 116.7, 118.6, 123.6, 124.3, 131.5, 133.3, 136.1, 138.1, 139.1, 143.0, 149.7, 164.2; MS (FAB) 759(M⁺, 13). Anal. calcd. for C₄₄H₄₆N₄O₈(H₂O): C, 68.03; H, 6.22; N, 7.21; Found: C: 68.10; H: 6.22; N: 7.21.

The 1,2-bis(β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester) ethyne (800mg, 1.05 mmol) was added to a distilled solution of cumene (40 mL), which had been degassed. The reaction vessel was evacuated and refilled with nitrogen three times. The temperature was then brought to reflux for about 30 min until a yellow precipitate had formed. The mixture which resulted was filtered and washed with hexane to give pure dimer **32** (545 mg, 93%). **32**: m.p. >350 °C (dec.); IR (KBr) 3227, 1716, 1327, 1162, 738, 450 cm⁻¹; ¹H NMR (300 MHz, DMSO) δ 1.62 (s, 9H), 7.70-7.80 (m, 2H), 8.7 (s, 1H), 8.94 (s, 1H), 8.99

(s, 1H), 12.25 (s,1H); ^{13}C NMR (300MHz, CDCl_3) δ 28.3, 80.8, 113.3, 114.6, 117.9, 125.8, 127.5, 131.9, 134.2, 138.0, 140.8, 164.9; MS (FAB) 559(M^+ , 41). This material was pure by TLC (silica gel).

1,4-Bis(9H- β -carboline-3-carboxylic acid tert-butyl ester) buta-1,3-diyne (33). In a round bottom flask (200 mL), $\text{PdCl}_2(\text{PPh}_3)_2$ (58 mg, 2 mol%), CuI (16mg, 2 mol%), and diisopropylethylamine (534 mg, 4.92 mmol) were added and the mixture stirred under argon. The flask was evacuated (degassed) and refilled with argon. The THF (40 mL) and 6-ethynyl- β -carboline-3,9-dicarboxylic acid di-tert-butyl ester **34** (1.6 g, 4.1 mmol) were then added (under argon) to the above mixture. To this flask methyl bromoacetate (410 mg, 2.5 mmol) was added, and the reaction mixture was stirred at rt for 6-8 h. The progress of this reaction was monitored by TLC on silica gel. After the reaction was complete, 8-10 g of silica gel was added and the solvent was removed under vacuum. The solid residue (a plug) was then placed on a column and subjected to column chromatography (CH_2Cl_2) to give **1,4-Bis(β -carboline-3,9-dicarboxylic acid di-tert-butyl ester) buta-1,3-diyne** (2.08 g, 65%): ^1H NMR (300 MHz, CDCl_3) δ 1.74(s, 9H), 1.81(s, 9H), 7.82(d, $J = 8.79$ Hz, 1H), 8.34(s, 1H), 8.48(d, $J = 8.79$ Hz, 1H), 8.69(s, 1H), 9.65(s, 1H); ^{13}C NMR (300MHz, CDCl_3) δ 28.2, 74.0, 81.2, 82.2, 86.0, 116.1, 116.9, 117.2, 123.7, 125.5, 131.3, 134.0, 136.2, 138.1, 139.6, 143.2, 149.6, 164.1; MS (FAB) 783(M^+ , 100). Anal. Calcd. for $\text{C}_{46}\text{H}_{46}\text{N}_4\text{O}_8 \cdot 1.5\text{H}_2\text{O}$: C, 68.22; H, 6.10; N, 6.91; Found: C: 68.49; H: 5.87; N: 6.58.

The 1,4-Bis-(β -carboline-3,9-dicarboxylic acid di-tert-butyl ester) buta-1,3-diyne (411 mg, 0.5 mmol) was added to a distilled solution of cumene (20 mL), which had been degassed. The reaction mixture was evacuated and refilled with nitrogen three times. The temperature was then brought to reflux for about 30 min until a yellow precipitate had formed. The mixture was filtered and the solids washed with hexane to give pure dimer **33**. **33**: mp >350 °C (dec.) IR(KBr) 3424, 1708, 1627, 1466, 1369, 1302, 1251, 1154, 1107, 1025, 846, 645 cm^{-1} ; ^1H NMR (300 MHz, DMSO) δ 1.63(s, 9H), 7.60-7.69(m, 2H), 8.40(s, 1H), 8.96(s, 1H), 9.04(s, 1H), 12.5(s, 1H); ^{13}C NMR (300MHz, CDCl_3) δ 28.3, 78.8, 82.5, 112.9, 118.1, 121.0, 122.1, 129.5, 131.5, 134.8, 141.1, 163.0, 131.9, 134.2, 138.0, 140.8, 164.9; MS (FAB)

583(M⁺, 100). This material was pure by TLC (silica gel).

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