3α-Hydroxy-3β-(phenylethynyl)-5β-pregnan-20-ones: Synthesis and Pharmacological Activity of Neuroactive Steroids with High Affinity for $GABA_A$ Receptors

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Neuroactive steroids that allosterically modulate $GABA_A$ receptors have potential uses as anticonvulsants, anxiolytics, and sedative−hypnotic agents. Recently, a series of pregnanes substituted with simple alkyl groups at the 3β-position were synthesized and found to be active *in vitro*. The present report describes the synthesis of a series of substituted 3α-hydroxy-3β-(phenylethynyl)pregnan-20-ones and their *in vitro* structure−activity relationship determined by their potency for inhibition of [^{35}S]TBPS binding in rat brain membranes. Appropriate substitution of the phenyl group results in ligands with particularly high affinity for the neuroactive steroid site on $GABA_A$ receptors (e.g., 4-acetyl **28**, IC_{50} 10 nM). The potency of selected steroids was confirmed electrophysiologically in oocytes expressing cloned human $GABA_A$ α1β2γ2L receptors (e.g., compound **28**, EC_{50} 6.6 nM). Consistent with their *in vitro* activity, some of the 3β-(phenylethynyl)-substituted steroids displayed anticonvulsant activity in the pentylenetetrazol (PTZ) and maximal electroshock (MES) tests following ip administration in mice. Notably, the 3β-[(4-acetylphenyl)ethynyl]-19-nor derivative **36** demonstrated an attractive anticonvulsant profile (PTZ and MES ED_{50} values of 2.8 and 9.2 mg/kg, respectively). A new pharmacophore for the neuroactive steroid site of $GABA_A$ receptors is proposed based upon the high affinity of certain substituted 3β-(phenylethynyl) steroids.

Introduction

γ-Aminobutyric $acid_A$ ($GABA_A$) receptors are ligand-gated, chloride-permeable ion channels that mediate most of the fast postsynaptic inhibitory currents in mammalian brain.[1] $GABA_A$ receptor modulators, such as barbiturates and benzodiazepines, have been used extensively as anxiolytics, anticonvulsants, sedatives, and hypnotics.[1] More recently, additional $GABA_A$ receptor modulators, such as non-benzodiazepines acting at the central benzodiazepine site and loreclezole, have demonstrated the continued utility of this drug target.[1]

Following the discovery of the modulatory effects of neuroactive steroids on $GABA_A$ receptors,[2] considerable effort has been made toward characterizing their unique site on the receptor complex, their physiological and pharmacological effects, and evaluation of the feasibility of generating GABA-potentiating drugs with improved side-effect profiles.[3] Interactions of neuroactive steroids with the receptor complex have been explored primarily using radioligand binding, electrophysiological, and chloride flux techniques. Previous *in vitro*[4−9] and *in vivo*[10−12] structure−activity relationship studies indicated that steroidal positive allosteric $GABA_A$ receptor modulators must possess a 3α-hydroxylated A ring and a keto group at C20 of the 17β-acetyl side chain, although certain other 17β-substitutions such as 17β-CN retain activity.[6,9,12] Numerous modifications to the steroid nucleus have been made in order to study the SAR of neuroactive steroids, including substitutions at the 3β-, 11-, 17-, or 21-positions.[4−9] None of these modifications have resulted, however, in a compound with appreciably higher potency than the naturally occurring progesterone metabolite 3α-hydroxy-5α-pregnan-20-one (3α,5α-P).

In the present report, we describe the synthesis and activity of a series of novel 3β-substituted steroids with *in vitro* potencies significantly greater than that of 3α,5α-P. Consistent with their high *in vitro* potencies, certain of these steroids are potent anticonvulsants in the pentylentetrazol (PTZ) and maximal electroshock (MES) seizure models. The high potency of certain of these 3β-substituted steroids necessitated a reevaluation of neuroactive steroid SAR, resulting in the description of a new pharmacophore for the steroid binding site on $GABA_A$ receptor complexes.

Chemistry

3α-Hydroxy-3β-phenyl-5β-pregnan-20-one (**1**) was synthesized by adding phenylmagnesium bromide to the pregnan-3,20-dione derivative (**2**)[7] and purifying the epimeric mixture of 3α-hydroxy and 3β-hydroxy derivatives by column chromatography. Similarly, 3β-phenyl compounds with a spacer (**3, 4,** and **6**) were prepared by the addition of benzylmagnesium chloride, lithium phenyl acetylide, or (phenylpropynyl)lithium, respectively, to **2** as outlined in Scheme 1. The 3β-(phenylethynyl) (**4**) and 3β-(phenylpropynyl) (**6**) derivatives were then hydrogenated in the presence of Pd/C to afford the corresponding 3β-(phenylethyl) (**5**) and 3β-(phenylpropyl)pregnanes (**7**).

The synthesis of 3β-substituted phenylethynyl derivatives is depicted in Scheme 2. The Wittig reaction of the corresponding benzaldehyde (**8**) with carbon tetrabromide in the presence of 2 equiv of triphenylphosphine afforded the intermediate phenyldibromoethene (**9**).[13] The dibromoethene derivative was then allowed to react

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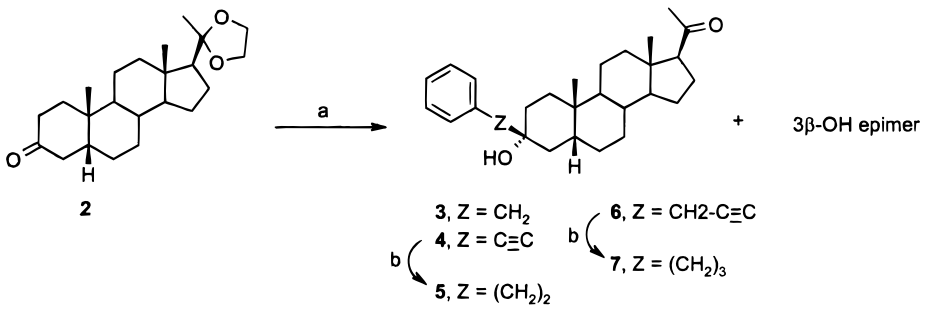
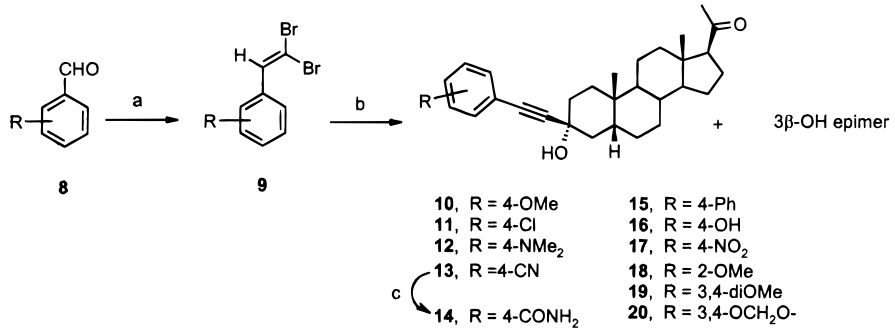
Scheme 1[a]



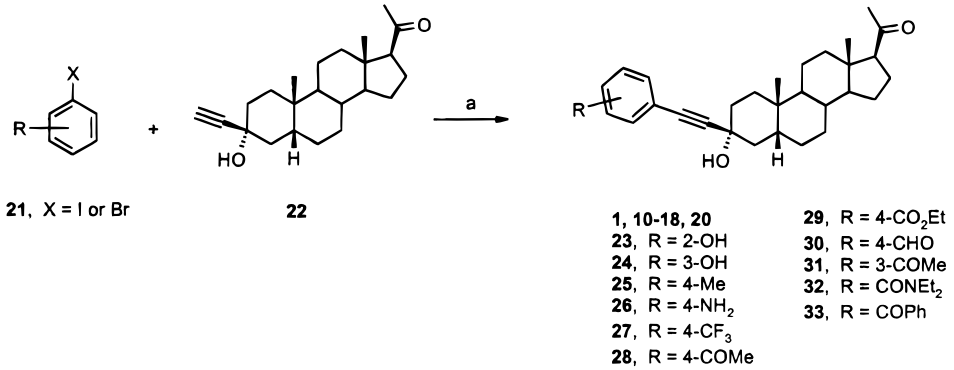
[a] Reagents: (a) (i) $PhCH_2MgCl$, PhC≡CLi, or $PhCH_2C{\equiv}CLi$, THF, −70 °C to room temperature, (ii) 2 N HCl/acetone, room temperature; (b) Pd/C, H_2, EtOAc, room temperature.

Scheme 2[a]



[a] Reagents: (a) $CBr_4/PPh_3/CH_2Cl_2$, or CBr_4/PPh_3/Zn dust/CH_2Cl_2, 0−20 °C; (b) (i) 2 equiv of *n*-BuLi/THF, (ii) **2**/THF, −70 °C to room temperature; (iii) 2 N HCl/acetone, room temperature; (iv) chromatography; (c) H_2O_2/K_2CO_3/DMSO, room temperature.

Scheme 3[a]



[a] Reagents: (a) $(PPh_3)_2PdCl_2/CuI/CH_2Cl_2/(NEt_3$ or $HNEt_2)$, room temperature.

with 2 equiv of *n*-butyllithium to generate, *in situ*, lithium phenyl acetylide, which was then reacted with the ketone **2** to give, after hydrolysis of the 20-ketal, the corresponding phenylethynyl derivatives (**10**−**20**).

An alternate synthetic route was employed to prepare certain 3*β*-(phenylethynyl) derivatives as outlined in Scheme 3. Different iodo- or bromobenzenes (**21**) were coupled with 3*β*-ethynyl derivative (**22**)[7] in the presence of catalytic amounts of $(PPh_3)_2PdCl_2$ and CuI to get the corresponding 3*β*-(phenylethynyl) derivatives (**1**, **10**−**18**, **20**, **23**−**33**).[14]

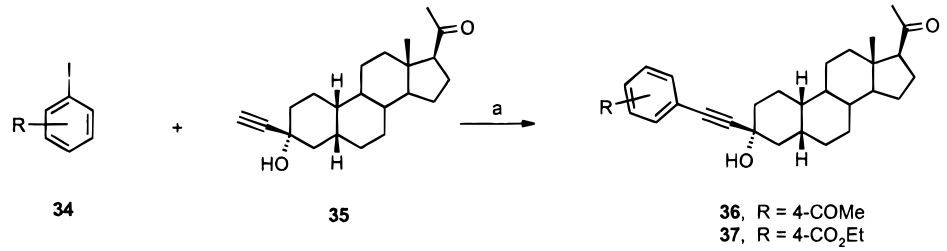
Similar coupling of iodobenzenes (**34**) with the 19-nor derivative of **22** (compound **35**) in the presence of Pd catalyst afforded the 3α-hydroxy-3*β*-(substituted phenylethynyl)-5*β*-19-norpregnanes (**36** and **37**) in 20−30% yields (Scheme 4).

Some of the 3*β*-phenyl compounds (**1**, **3**, **4**, **6**, **10**−**13**, **15**−**20**) obtained by the addition reaction of the appropriate organometallic reagents with **2**, as outlined in Schemes 1 and 2, were found to be contaminated with 3*β*-hydroxy epimers. However, the desired more polar 3α-hydroxy derivatives, in which the hydroxy group is equatorial (lower TLC R_f), were easily separated from the less polar 3*β*-hydroxy (axial, higher TLC R_f) epimers by flash chromatography using toluene:acetone mixtures. Although the stereochemistry of the 3-hydroxy group was established on the basis of the polarity (TLC R_f) of the epimers, it was further confirmed by synthesizing unambiguous authentic samples following the coupling method described in the Scheme 3. (See the Experimental Section).

Results and Discussion

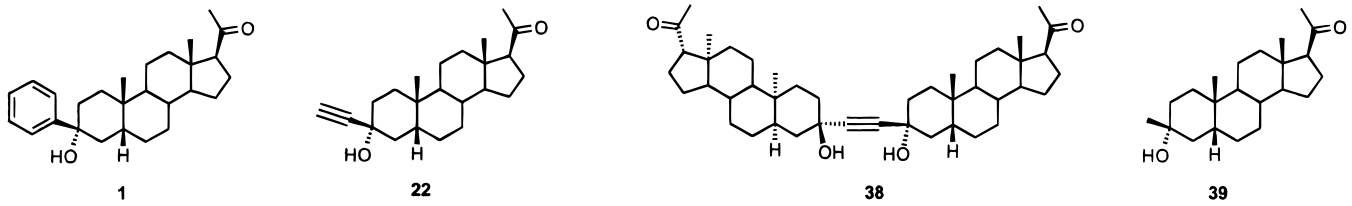
Synthetic Rationale. Naturally occurring neuroactive steroids have therapeutic limitations because they are rapidly metabolized, presumably by conjugation of the 3α-hydroxy group or oxidation to the corresponding ketone, rendering them inactive as modulators of GABAergic function. The oxidation of the 3α-hydroxy group can be blocked by the addition of a substituent

Scheme 4[a]

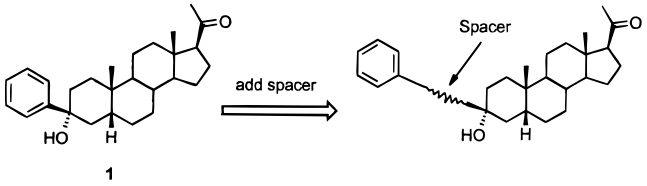


[a] Reagents: (a) $(PPh_3)_2PdCl_2/CuI/CH_2Cl_2/NEt_3$, room temperature.

Chart 1



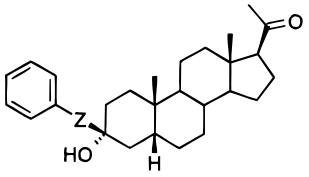
Scheme 5



at the 3β-position.[3] Recently, a series of pregnanes substituted with simple alkyl groups at the 3β-position were synthesized and found to be active *in vitro*, as indicated by their activity as allosteric inhibitors of the binding of the chloride channel-associated radioligand [^{35}S]-*tert*-butylbicyclophosphorothionate (TBPS) in rat brain cortical membranes.[7] During the synthesis of the potent 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one (**22,** TBPS IC_{50} 39 nM),[7] a byproduct with a dimeric structure (**38,** Chart 1) was isolated. Although this steroid derivative had relatively low potency in the TBPS assay (IC_{50} 2.9 μM),[17] even this level of activity was surprising considering the bulk of the 3β-substituent. Encouraged by these results it was decided to add a comparatively smaller phenyl group at the 3β-position. Although more potent than the dimeric compound **38**, substitution at the 3β-position with a phenyl group (**1,** IC_{50} 380 nM) resulted in a 10-fold loss in affinity compared to the corresponding 3β-methyl compound (**39,** IC_{50} 37 nM).[7]

Synthesis and Evaluation of Spacer Units. The relatively low activities of **1** and **38** suggested that bulky substituents adjacent to the A ring make contact with a forbidden volume in the binding site. Therefore, a further modification of the 3β-position was carried out by adding a spacer between the phenyl ring and the steroid nucleus to evaluate whether a bridging unit could provide enhanced potency (Scheme 5). The spacers used were flexible or rigid chains of 1−3 carbon atoms. The resulting phenyl derivatives with a spacer were more active than the phenyl compound **1** without the spacer (Table 1). Although large differences in potency were not observed with the different spacers examined, the affinity was generally higher with spacers containing unsaturation and was lower as the phenyl ring was moved further away from the steroid nucleus. The optimal spacer was found to be the two-carbon rigid ethynyl group (compound **4**, IC_{50} 100 nM). These

Table 1. Inhibitory Potency in the TBPS Binding Assay and Anticonvulsant Activity of 3β-Substituted Pregnane Derivatives



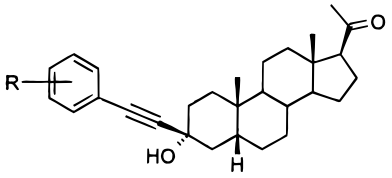
compd	Z	IC_{50} (nM)[a]	I_{max} (%)	PTZ protection (%)[b]
1		380 ± 50	89 ± 2	25
3	CH_2	110 ± 10	101 ± 3	38
4	C≡C	100 ± 10	100 ± 1	25
5	$(CH_2)_2$	150 ± 20	102 ± 7	0
6	$C{\equiv}CCH_2$	131 ± 4	92 ± 5	13
7	$(CH_2)_3$	190 ± 40	91 ± 2	13

[a] Compounds were incubated with rat brain cortical P2 membranes and 2 nM [^{35}S]TBPS in the presence of 5 μM GABA as described in the Experimental Section. Values are means and SEMs of three or four independent experiments. Hill values were 1.0 for all compounds. [b] The PTZ protection is the percent of animals (n = 8) not exhibiting PTZ-induced seizures 10 min following a 10 mg/kg ip dose of steroid.

results suggested the presence of an auxiliary binding pocket for hydrophobic groups in the neuroactive steroid binding site located close to the position occupied by the steroid A ring.

Structure−Activity Relationships of Phenylethynyls. In order to probe the nature of the binding pocket, a set of substituents on the phenyl ring of 3β-substituted steroids containing the ethynyl spacer was selected to provide systematic variation in lipophilic, electronic, steric, and hydrogen-bonding properties (Table 2). The activity of these 3β-(phenylethynyl) compounds in the TBPS binding assay was found to be highly dependent on the substitution on the phenyl ring. Substitution with an electron-withdrawing group at the 4-position of phenyl ring increased the affinity, e.g., 4-CN (**13**, IC_{50} 60 nM), 4-CF_3 (**27**, IC_{50} 56 nM), and 4-NO_2 (**17**, IC_{50} 42 nM). Furthermore, substitution at the 4-position on the phenyl ring with electron-withdrawing groups capable of accepting hydrogens to form hydrogen bonds gave the most potent compounds with the IC_{50} values ~10 nM, e.g., 4-COMe (**28,** 10 nM), 4-CO_2Et (**29,** 12 nM). Substitution with electron-donating groups capable of donating hydrogen to form

Table 2. Inhibitory Potency in the TBPS Binding Assay and Anticonvulsant Activity of 3β-(Phenylethynyl)pregnane Derivatives



compd	R	IC_{50} (nM)[a]	I_{max} (%)	PTZ protection (%)
4	H	100 ± 10	100 ± 1	25
10	4-OMe	42 ± 4	84 ± 5	25
11	4-Cl	70 ± 15	88 ± 5	0
12	4-NMe_2	40 ± 5	78 ± 5	50
13	4-CN	60 ± 9	85 ± 10	44
14	4-$CONH_2$	1800 ± 400	96 ± 1	13
15	4-Ph	54 ± 12	90 ± 3	0
16	4-OH	2300 ± 200	100 ± 4	0
17	4-NO_2	42 ± 9	98 ± 7	13
18	2-OMe	170 ± 20	89 ± 2	0
19	3,4-$(OMe)_2$	290 ± 10	102 ± 2	13
20	3,4-OCH_2O	68 ± 2	93 ± 3	13
23	2-OH	82 ± 31	30 ± 6	0
		5100 ± 500	57 ± 11	
24	3-OH	420 ± 100	100 ± 1	0
25	4-Me	83 ± 16	84 ± 4	50
26	4-NH_2	96 ± 48	17 ± 2	13
		11800 ± 1800	84 ± 1	
27	4-CF_3	56 ± 7	88 ± 4	0
28	4-COMe	10 ± 2	106 ± 2	75
29	4-CO_2Et	12 ± 3	97 ± 4	19
30	4-CHO	67 ± 44	44 ± 14	0
		1600 ± 200	54 ± 13	
31	3-COMe	45 ± 9	34 ± 3	0
		1500 ± 300	65 ± 3	
32	4-$CONEt_2$	93 ± 13	91 ± 1	13
33	4-COPh	55 ± 11	96 ± 4	38
36	4-COMe, 19-nor	12 ± 3	97 ± 2	100
37	4-CO_2Et, 19-nor	26 ± 6	94 ± 1	13

[a] See Table 1 and Experimental Section for TBPS binding and PTZ protection assays. Values listed for compounds **23**, **26**, **30**, and **31** are the high- and low-affinity components of the two-component inhibition curves. Hill values for one component inhibitors were 1.0 except for compounds **10** (1.21 ± 0.08), **12** (1.26 ± 0.17), **18** (1.25 ± 0.06), **24** (0.83 ± 0.06), and **32** (1.38 ± 0.12). Values are means and SEMs of three to five independent experiments.

hydrogen bonds rendered these compounds almost inactive, e.g., 4-OH (**16**, IC_{50} 2300 nM), 4-NH_2 (**26**, IC_{50} 11 800 nM, predominant low-affinity component). Although, the 4-OH substitution decreased the activity, a gradual increase in activity was observed in moving the hydroxy group closer to the steroid nucleus. Hence, the 3-hydroxy derivative **24** was about 5-fold more potent than the 4-hydroxy-substituted compound **16**. Although complicated by a complex two component inhibition, the 2-hydroxy-substituted compound **23**, with the hydroxy group adjacent to the steroid nucleus, inhibited TBPS binding at lower concentrations, with a high-affinity component 28-fold more potent than compound **16** (Table 2).

Thus, addition of a hydrogen bond accepting group at the 4-position of the phenyl ring generally increased the affinity and addition of a hydrogen bond donating group decreased the affinity. As predicted, replacing the hydrogens of the hydrogen bond donor hydroxy and amino groups of the relatively inactive **16** and **26** with alkyl groups to give compounds with hydrogen bond accepting methoxy (compound **10**) and dimethylamino

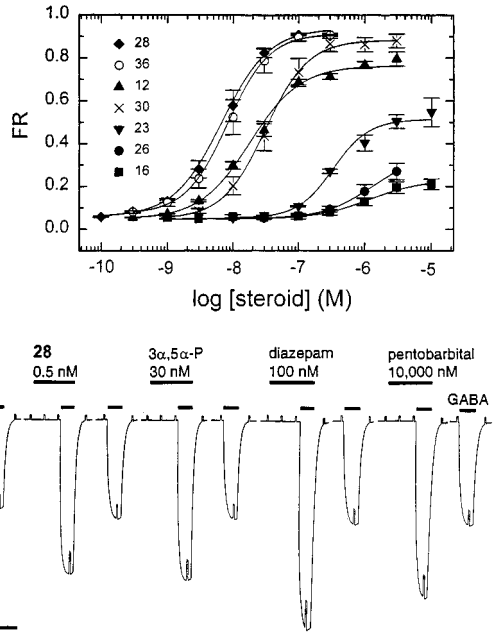


Figure 1. Functional modulation of human $GABA_A$ $\alpha1\beta2\gamma2L$ receptors by 3β-(phenylethynyl)pregnane derivatives. Top: Potentiation of GABA-activated currents by steroids. Data are plotted as mean values ± SEM, expressed as a fraction of the maximum current elicited by GABA. FR, fractional response. Bottom: Sample recordings comparing potentiation of GABA-activated currents by **28**, 3α,5α-P, diazepam, and sodium pentobarbital. Records are from a single oocyte using a holding potential of −70 mV. Drugs were applied as indicated by bars. The control GABA concentration was 8 μM. A train of depolarizing pulses (+10 mV, upward deflections) was used to time drug applications and monitor membrane conductance. Scale bars represent 100 nA and 1 min.

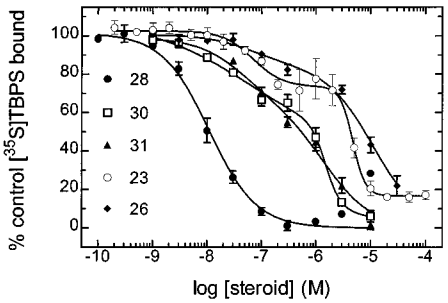
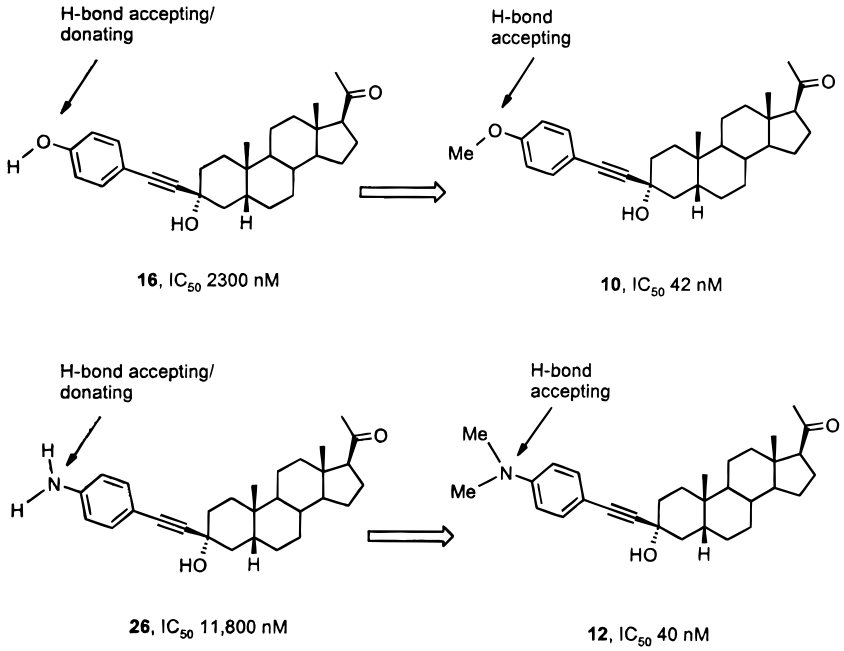


Figure 2. Inhibition of [^{35}S]TBPS binding in rat brain membranes by 3β-(phenylethynyl)pregnane derivatives: Comparison of the potent inhibitor **28** with compounds displaying complex binding curves. Concentrations of **28** > 1 μM resulted in an apparent decrease in inhibition of TBPS binding, presumably due to insolubility of this steroid at these concentrations; these data points were not included in the curve fit. Steroids were incubated with 2 nM [^{35}S]TBPS for 90 min at room temperature in the presence of 5 μM GABA.

(compound **12**) groups, respectively, resulted in several-fold increases in potency (Scheme 6).

Further evidence for the importance of the hydrogen bond accepting capability of the 4-substituent was obtained with the low-affinity compound **14**, R = 4-$CONH_2$. Since, the $CONH_2$ group can act as a hydrogen bond acceptor or donor, it was predicted that eliminating the hydrogen bond donating capability of the group should result in an active compound. To test this theory, the *N*,*N*-diethylcarboxamido derivative **32**, which does not have hydrogen bond donating capability, was synthesized and found to be 19 times more active than its parent (Scheme 7), providing additional support

Scheme 6



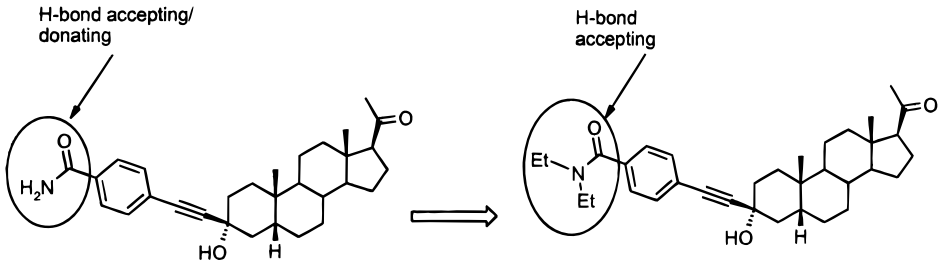
16, IC_{50} 2300 nM

10, IC_{50} 42 nM

26, IC_{50} 11,800 nM

12, IC_{50} 40 nM

Scheme 7



14, IC_{50} 1800 nM

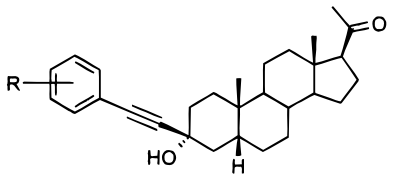
32, IC_{50} 93 nM

for the importance of hydrogen bond accepting ability of 4-substituents for receptor potency.

Electrophysiology. Functional modulation of $GABA_A$ receptors by selected steroids was evaluated electrophysiologically in *Xenopus* oocytes expressing cloned human $\alpha1\beta2\gamma2L$ $GABA_A$ receptor complexes. Oocytes injected with a mixture of $\alpha1$, $\beta2$, and $\gamma2L$ cRNAs (~1 ng each) produced strong expression of functional $GABA_A$ receptors. The range of maximal $GABA_A$-activated currents ($GABA_{MAX}$) was between 2200 and 4470 nA, and the mean maximal response was 2820 ± 130 nA ($n = 20$). GABA concentration−response curves were measured in a sample of oocytes. The EC_{50} value was 22 ± 2.2 μM, and the slope was 1.0 ± 0.01 (n = 13), consistent with a pharmacologically homogeneous population of receptors.

Modulation of GABA responses by a selected group of steroids (compounds **12, 16, 23, 26, 28, 30, 36**) is shown in Figure 1, top. Modulation was determined by adjusting the GABA concentration to produce a current approximately 5% of the $GABA_{MAX}$. The mean GABA concentration required to elicit the 5% response was 4.1 ± 1.9 μM, and the mean response was 0.048 ± 0.001, expressed as a fraction of $GABA_{MAX}$ ($n = 20$). Modulation was measured as the potentiation of the control GABA response combined with any current activated directly by the steroids. Such steroid-activated currents were small, ranging from 0 to 10% of $GABA_{MAX}$ and were only appreciable when applying micromolar concentrations of steroids.

Table 3. Functional Modulation of $GABA_A$ Receptors by 3β-(Phenylethynyl)pregnane Derivatives



compd	R	EC_{50} (nM)[a]	slope	efficacy (FR)	n
12	4-NMe_2	18 ± 1.5	1.1 ± 0.53	0.76 ± 0.002	4
16	4-OH	>1000			4
23	2-OH	340 ± 34	1.6 ± 0.11	0.51 ± 0.029	3
26	4-NH_2	> 1000			3
28	4-COMe	6.6 ± 0.69	1.2 ± 0.07	0.94 ± 0.010	3
30	4-CHO	31 ± 5.8	1.3 ± 0.20	0.88 ± 0.023	4
36	4-COMe (19-nor)	8.2 ± 1.4	1.4 ± 0.20	0.91 ± 0.06	3

[a] Steroid potentiation of 5% GABA responses in *Xenopus* oocytes expressing $\alpha1\beta2\gamma2L$ subunits of human recombinant $GABA_A$ receptors as described in the Experimental Section.

The 3β-(4-acetylphenyl)ethynyl derivatives **28** and **36** were potent, full efficacy modulators of GABA-activated responses (Table 3). The 4-(dimethylamino) and 4-aldehydo analogues **12** and **30**, respectively, were several-fold less potent than **28** and **36**. The 4-(dimethylamino) analogue **12** might also have been less efficacious.

The 2-hydroxyl analogue **23** was approximately 10-fold less potent than **12** and **30** and demonstrated intermediate efficacy modulation, whereas the 4-hydroxyl and 4-amino analogues **16** and **26**, respectively, were weak potentiators. The measurement of maximal fractional responses for **16** and **26** was hindered by solubility limitations.

In these types of assays, 0.5 nM **28** produced a level of potentiation of GABA responses comparable to a 60-fold higher concentration of the prototypical neurosteroid 3α,5α-P, a 200-fold higher concentration of the benzodiazepine diazepam, and a 20 000-fold higher concentration of the barbiturate sodium pentobarbital (Figure 1, bottom).

Correlation of *in Vitro* Assays. The rank order of potency for potentiation of GABA-evoked currents in *Xenopus* oocytes expressing $\alpha1\beta2\gamma2$L receptors was **28** ≥ **36** >**12** > **30** > **23** > **26** ∼ **16**. Similarly, the rank order potency for inhibition of TBPS binding in rat brain membranes for this set of compounds was **28** ≥ **36** > **12** ≥ **30** (high-affinity component) ≥ **23** (high-affinity component) > **16** > **26** (low-affinity component). The good correlation between electrophysiological and binding assays was expected based on previous studies[6] and indicates that allosteric inhibition of TBPS binding is a useful tool to develop steroid structure−activity relationships. However, complex binding curves were observed for four compounds in this study (**23**, **26**, **30**, and **31**) (Figure 2). Three of these were evaluated electrophysiologically (**23**, **26**, and **30**). Indeed, these compounds were selected for functional confirmation of activity because of their unusual inhibition curves in the binding assay. For compounds **23** and **30**, the high-affinity component in the binding assay correlates more closely to functional activity in the electrophysiological assays, whereas the low-affinity component for compound **26** appears most relevant. These complex binding curves may be due to receptor subtype selectivity, negative cooperativity, partial agonism, multiple sites or mechanism of action, and/or combinations of these factors. In principle, evaluation of the [^{35}S]TBPS inhibition curves for these compounds in membranes from cell lines expressing recombinant receptor complexes may help to resolve this question, although this was not addressed in the present study. In any case, steroids displaying complex binding curves that have been examined electrophysiologically all show unremarkable concentration−modulation curves. In practical terms, these results indicate that compounds with unusual profiles in the binding assay should be evaluated functionally to confirm activity.

***In Vivo* Pharmacology.** Preliminary *in vivo* pharmacological testing was conducted using the PTZ-induced seizure model. Compounds were administered in 50% hydroxypropyl-β-cyclodextrin (HP-β-CD) at a dose of 10 mg/kg, ip, 10 min prior to PTZ injection. These data, which are presented in Tables 1 and 2, belie a simple relationship between *in vitro* affinity and *in vivo* potency and/or efficacy. For example, although compounds **29** and **36** exhibit identical IC_{50} values (12 nM), **36** produces 100% protection against PTZ-induced seizures at 10 mg/kg, whereas **29** yields only 19% protection. Poor bioavailability and/or metabolic lability may contribute to these discrepancies, although these possibilities were not further investigated. Two representative high-affinity analogs with marked *in vivo* activity, compounds **28** and **36**, were evaluated for anticonvulsant potency against PTZ- and MES-induced seizures following intraperitoneal administration. The neuroactive steroid **28**, administered intraperitoneally in 50% HP-β-CD 30 min prior to the convulsant stimulus, produced a dose-dependent blockade of seizures with an ED_{50} of 8.2 mg/kg (95% confidence interval 5.1−13.3) against PTZ and an ED_{50} of 7.4 mg/kg (95% CI 5.2−10.5) against MES. Compound **36** exhibited a different *in vivo* potency profile with ED_{50} values of 2.8 mg/kg (1.7−4.5) against PTZ and 9.2 mg/kg (6.0−14.0) against MES. The degree of neurobehavioral impairment produced by **28** and **36** was evaluated using the hanging-wire test. Compound **28** produced a dose-dependent increase in hanging-wire failure with a TD_{50} of 10.0 mg/kg (6.3−15.7), whereas **36** displayed a TD_{50} of 13.2 mg/kg (9.7−17.9). The dose at which **28** produced ataxia was only 1.2 times greater than the dose required for anticonvulsant efficacy against PTZ, whereas for compound **36**, the difference was 4.7. Thus, of the pair, the 19-nor compound **36** congener displays pharmacological properties better suited to development as a potential antiepileptic medication. The generality of this finding remains to be determined.

The anticonvulsant profile of compound **36** is similar to that reported previously for the endogenous neuroactive steroids 3α,5α-P and 3α-hydroxy-5β-pregnan-20-one (3α,5β-P).[15] Endogenous neurosteroids, such as 3α,5α-P and 3α,5β-P, are unsuitable as therapeutic agents, however, as they are readily oxidized and/or conjugated at the 3α position,[12] resulting in compounds that are inactive at $GABA_A$ receptors, but potentially active at hormonal steroid receptors.[4−6] 3β-Substitution, which in part prevents metabolism of the 3α-hydroxy group, has been suggested to enhance the bioavailability of pregnane steroids without altering their primary pharmacologic properties.[3,7] Indeed, ganaxolone (CCD 1042; 3α-hydroxy-3β-methyl-5α-pregnan-20-one), a 3β-methylated synthetic analogue of the endogenous neuroactive steroid 3α,5α-P, has been shown to retain the anticonvulsant activity of the endogenous steroid 3α,5α-P, while acquiring oral bioavailability and other properties that would be expected to facilitate its use as an antiepileptic drug.[16] Thus, the 3β-[(4-acetylphenyl)ethynyl]-5β-19-nor derivative **36** would be predicted to be of similar therapeutic utility.

Pharmacophore Model

Previous studies[4−9] have demonstrated that a hydrogen bond donating a 3α-hydroxyl group is an essential element for the primary docking of the ligand to the receptor. Replacing the hydrogen of the hydroxyl with methyl, thus eliminating the ability of the steroid to donate a hydrogen to form a hydrogen bond in this region, results in 250-fold reduction in potency.[17] On the basis of the SAR of several 3β-(phenylethynyl)-pregnane derivatives, we propose that an additional hydrogen bond accepting group (e.g., carbonyl group of an acetylphenyl or carbethoxyphenyl) located at a certain distance from the A ring of the steroid can take part in secondary docking giving rise to a tightly bound ligand−receptor complex. Thus, the 3α-hydroxy group is an absolute requirement for binding (primary docking), whereas neuroactive steroid affinity can be augmented via an additional hydrogen bonding interaction

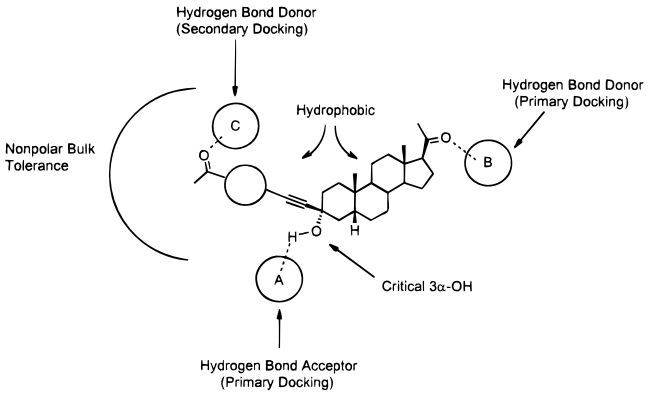


Figure 3. A schematic pharmacophore model for the neuroactive steroid binding site of GABA$_A$ receptors.

(secondary docking). This secondary docking may be the basis for the exceptionally high *in vitro* activity of some of the phenylethynyl derivatives. For example, compound **28** is 5- or 24-fold more potent than 3α,5α-P (IC_{50} 51 nM or EC_{50} 160 nM)[7] in TBPS binding or electrophysiological assays, respectively.

From the above observations it is possible to propose a pharmacophore model for the binding site. The schematic of the model is shown in Figure 3. The model entails a critical primary docking through hydrogen-bonding interactions at A and B and a secondary docking through a strong hydrogen bonding interaction at C. Although region B may well involve a hydrogen bond donor group in the binding pocket, it should be pointed out that certain other side groups besides acetyl (in particular CN) also provide for high affinity primary docking.[6,9] Although the pharmacophore model presented is useful in designing steroid derivatives with high *in vitro* potency, it does not predict their *in vivo* activities. Future work will focus on use of this model in conjunction with other approaches to design potent compounds with improved *in vivo* profiles.

Conclusions

The present report describes the synthesis of a series of substituted 3α-hydroxy-3*β*-(phenylethynyl)pregnan-20-ones and their *in vitro* structure−activity relationship determined by their potency for inhibition of [^{35}S]TBPS binding in rat brain membranes. Appropriate substitution of the phenyl group results in ligands with particularly high affinity for the neuroactive steroid site on GABA$_A$ receptors (e.g., 4-acetyl **28**, IC_{50} 10 nM). The potency of some of these steroids was confirmed electrophysiologically in oocytes expressing α1*β*2*γ*2L receptors (e.g., compound **28**, EC_{50} 6.6 nM). Consistent with their *in vitro* activity, some of these 3*β*-(phenylethynyl)-substituted steroids displayed anticonvulsant activity in the pentylenetetrazol (PTZ) and maximal electroshock (MES) tests following ip administration in mice. Notably, the 3*β*-(4-acetylphenyl)-19-nor derivative **36** provided an attractive anticonvulsant profile (PTZ and MES ED_{50} values of 2.8 and 9.2 mg/kg, respectively). A new pharmacophore for the neuroactive steroid site of GABA$_A$ receptors is proposed based upon the high affinity of certain substituted 3*β*-(phenylethynyl) steroids.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded on a Varian 200 or 300 MHz spectrometer in $CDC1_3$ with tetramethylsilane as reference. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, or Robertson Microlit Laboratories, Madison, NJ. Elemental analyses for the undesired side products, 3*β*-hydroxy epimers, were not obtained. Flash chromatography on silica gel (230−400 mesh, Mallinckrodt) was carried out as described by Still.[18] HPLC grade solvents were obtained from Baxter. THF and ether were distilled from sodium and benzophenone. Methylene chloride, Et_3N, and Et_2NH were dried over CaH_2 and distilled. Other synthetic reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI) and used as received unless otherwise specified. 3*β*-Ethynyl-3α-hydroxy-5*β*-pregnan-20-one and 5*β*-pregnane-3,20-dione cyclic 20-(1,2-ethanediyl acetal) were obtained from DioSynth BV (The Netherlands). *γ*-Aminobutyric acid (GABA) was purchased from Sigma Chemical Co. (St. Louis, MO). [^{35}S]TBPS (60−100 Ci/mmol) was obtained from New England Nuclear (Boston, MA), and unlabeled TBPS was from Research Biochemicals International (Natick, MA).

3α-Hydroxy-3*β*-phenyl-5*β*-pregnan-20-one (1). A solution of 5*β*-pregnane-3,20-dione cyclic 20-(1,2-ethanediyl acetal)[7] (**2**, 720 mg, 2 mmol) in dry THF (20 mL) was treated with phenylmagnesium bromide (3M in THF, 6 mmol, 2 mL) at −70 °C. After the mixture was stirred at this temperature for 3 h and then at room temperature for 2 h, it was quenched with 2 N HCl (10 mL). The solvent was removed, and the residue was dissolved in acetone (20 mL). After 1 N HCl (5 mL) was added, the solution was stirred at room temperature for 0.5 h. Saturated $NaHCO_3$ solution was added to neutralize the acid. The solvents were removed, and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, dilute $NaHCO_3$ solution, water, and brine. After being dried over anhydrous $MgSO_4$, the solution was filtered and evaporated to yield the crude product (1.3 g). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. On elution with toluene:acetone mixture (95:5) gave 3*β*-hydroxy-3α-phenyl-5*β*-pregnan-20-one (420 mg) as a first fraction: TLC R_f 0.52 (toluene:acetone, 95:5); mp 186−191 °C; ^{1}H NMR ($CDCl_3$) δ 7.21−7.61 (m, 5H), 2.58 (m, 1H), 2.13 (s, 3H), 1.04 (s, 3H), 0.63 (s, 3H). Further elution with the same solvent mixture yielded 3α-hydroxy-3*β*-phenyl-5*β*-pregnan-20-one (**1**, 185 mg): TLC R_f 0.45 (toluene:acetone, 95:5); mp 182−184 °C; IR 2938, 2868, 1687 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.26−7.62 (m, 5H), 2.56 (m, 1H), 2.13 (s, 3H), 0.84 (s, 3H), 0.60 (s, 3H). Anal. ($C_{27}H_{38}O_2$) C, H.

3*β*-Benzyl-3α-hydroxy-5*β*-pregnan-20-one (3). A solution of 5*β*-pregnane-3,20-dione, cyclic 20-(1,2-ethanediyl acetal)[7] (**2**, 360 mg, 1 mmol) in dry THF (20 mL) was added dropwise to a solution of benzylmagnesium chloride (2 M in THF, 3 mmol, 1.5 mL) in dry THF (20 mL) at −70 °C. After the mixture was stirred at this temperature for 40 min and then at room temperature for 2.5 h, it was quenched with saturated NH_4Cl (2 mL). The solvent was removed, and the residue was dissolved in acetone (20 mL). After 2 N HCl (2 mL) was added, the solution was stirred at room temperature for 20 min. Saturated $NaHCO_3$ solution was added to neutralize the acid. The solvents were removed, and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, dilute $NaHCO_3$ solution, water, and brine. After being dried over anhydrous $MgSO_4$, the solution was filtered and evaporated to yield the crude product (400 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. On elution with toluene:acetone mixture (97:3) gave 3α-benzyl-3*β*-hydroxy-5*β*-pregnan-20-one (250 mg) as a first fraction: TLC R_f 0.57 (toluene:acetone, 9:1); mp 150−152 °C; ^{1}H NMR ($CDCl_3$) δ 7.15−7.38 (m, 5H), 2.75 (s, 2H), 2.53 (m, 1H), 2.13 (s, 3H), 0.95 (s, 3H), 0.61 (s, 3H). Further elution with the same solvent mixture yielded 3*β*-benzyl-3α-hydroxy-5*β*-pregnan-20-one (**3**, 30 mg): TLC R_f 0.50 (toluene:acetone, 9:1); mp 133−141 °C; IR 2929, 2868, 2359, 1687 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.18−7.42 (m, 5H), 2.89 (s, 2H), 2.52 (m, 1H), 2.11 (s, 3H), 1.03 (s, 3H), 0.60 (s, 3H). Anal. ($C_{28}H_{40}O_2$) C, H.

3α-Hydroxy-3β-(phenylethynyl)-5β-pregnan-20-one (4). Method A. A solution of 5β-pregnane-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (**2**, 180 mg, 0.5 mmol) in dry THF (20 mL) was treated with lithium phenyl acetylide (1 M in THF, 1.5 mmol, 1.5 mL) at −70 °C. After being stirred the mixture at this temperature for 1 h and then at room temperature for 2 h, it was quenched with 2 N HCl (1 mL). The solvent was removed, and the residue was dissolved in acetone (20 mL). After 1 N HCl (5 mL) was added, the solution was stirred at room temperature for 0.5 h. Saturated $NaHCO_3$ solution was added to neutralize the acid. The solvents were removed, and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, dilute $NaHCO_3$ solution, water, and brine. After being dried over anhydrous $MgSO_4$, the solution was filtered and evaporated to yield the crude product (300 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (95:5) gave 3α-hydroxy-3β-(phenylethynyl)-5β-pregnan-20-one (**4**, 160 mg): TLC R_f 0.22 (toluene:acetone, 95:5); mp 188−190 °C; IR 2930, 2868, 2361, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.22−7.52 (m, 5H), 2.55 (m, 1H), 2.12 (s, 3H), 0.98 (s, 3H), 0.61 (s, 3H). Anal. ($C_{29}H_{38}O_2$) C, H.

Method B. Alternatively, this compound was prepared in 25% yield in a manner analogous to the preparation of that for **23** (see below) by the Pd-catalyzed coupling reaction of 4-iodobenzene and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (4:1) gave the product as a colorless solid, which was found to be identical to **4** (TLC R_f, mp, NMR).

3α-Hydroxy-3β-(2′-phenylethyl)-5β-pregnan-20-one (5). A solution of 3α-hydroxy-3β-(phenylethynyl)-5β-pregnan-20-one (44 mg) was dissolved in EtOAc (12 mL), Pd/C (5%, 12 mg) was added, and the mixture was hydrogenated at 45 psi of hydrogen for 15 h at room temperature. Filtration of the catalyst followed by evaporation of the solvent yielded the crude product, which was purified by chromatography over silica gel to isolate the pure **5** (33 mg): TLC R_f 0.40 (hexane:acetone, 7:3); mp 153−184 °C; IR 2929, 2868, 2349, 1687 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.12−7.39 (m, 5H), 2.61−2.78 (m, 2H), 2.54 (m, 1H), 2.12 (s, 3H), 0.95 (s, 3H), 0.60 (s, 3H). Anal. ($C_{29}H_{42}O_2$) C, H.

3α-Hydroxy-3β-(3′-phenyl-1′-propynyl)-5β-pregnan-20-one (6). A solution of 3-phenyl-1-propyne (232 mg, 2 mmol) in dry THF (7 mL) was treated under N_2 with *n*-BuLi (2.5 M in hexane, 2 mmol, 0.8 mL) at −65 °C. The mixture was stirred at this temperature for 10 min. It was then treated dropwise with a solution of 5β-pregnane-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (**2**, 206 mg, 0.6 mmol) in dry THF (10 mL) over a period of 30 min. After the resulting mixture was stirred at −78 °C for 0.5 h, it was quenched with saturated NH_4Cl solution (5 mL) at −10 °C. The solvent was removed, and the residue was then dissolved in acetone (15 mL). After 1 N HCl (8 mL) was added, the solution was stirred at room temperature for 0.5 h. A 2 N NaOH solution was added to neutralize the acid. The solvents were removed, and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, dilute $NaHCO_3$ solution, water, and brine. After being dried over anhydrous $MgSO_4$, the solution was filtered and evaporated to yield the crude product (460 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (93:7) gave 3α-hydroxy-3β-(3′-phenyl-1′-propynyl)-5β-pregnan-20-one (**6**, 175 mg): TLC R_f 0.46 (hexane:acetone, 7:3); mp 124−132 °C; IR 2929, 2868, 2359, 1697 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.18−7.42 (m, 5H), 3.66 (s, 2H), 2.55 (m, 1H), 2.11 (s, 3H), 0.96 (s, 3H), 0.60 (s, 3H). Anal. ($C_{30}H_{40}O_2$) C, H.

3α-Hydroxy-3β-(3′-phenylpropyl)-5β-pregnan-20-one (7). A solution of **6** (50 mg) was dissolved in EtOAc (12 mL), Pd/C (5%, 10 mg) was added, and the mixture was hydrogenated at 30 psi of hydrogen for 45 min at room temperature. Filtration of the catalyst followed by evaporation of the solvent yielded the crude product, which was purified by chromatography over silica gel to isolate the pure **7** (30 mg): TLC R_f 0.48 (hexane:acetone, 7:3); mp 41−46 °C; IR 2928, 2869, 1698, 1265 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.11−7.38 (m, 5H), 2.59−2.71 (m, 2H), 2.54 (m, 1H), 2.11 (s, 3H), 0.92 (s, 3H), 0.59 (s, 3H). Anal. ($C_{30}H_{44}O_2$) C, H.

General Method for the Preparation of 2,2-Dibromophenylethene Derivatives (9).[13] To a well-stirred solution of CBr_4 (2.985 g, 9 mmol) in CH_2Cl_2 (20 mL) at 0 °C were added PPh_3 (4.75 g, 18.1 mmol) and then the benzaldehyde derivative (**8**, 9 mmol). Stirring was continued at 0 °C for 20 min, at which point water (20 mL) was added. The organic layer was separated, washed with water, dried (anhydrous $MgSO_4$), filtered, and evaporated to leave the crude product. Purification of this by column chromatography on silica gel (hexane:acetone, 9:1) afforded the 2,2-dibromophenylethene derivative (**9**).

3α-Hydroxy-3β-(4-methoxyphenylethynyl)-5β-pregnan-20-one (10). Method A. A solution of 2,2-dibromo-1-(4′-methoxyphenyl)ethene[19] (prepared by the Wittig reaction of 4-methoxybenzaldehyde with carbon tetrabromide in the presence of triphenylphosphine) (875 mg, 3 mmol) in dry THF (15 mL) was treated under N_2 with *n*-BuLi (2.5M in THF, 6 mmol, 2.4 mL) at −78 °C. The mixture was stirred at this temperature for 0.5 h and then at room temperature for 1 h more. It was then recooled to −78 °C, and a solution of 5β-pregnan-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (**2**, 720 mg, 2 mmol) in dry THF (10 mL) was added dropwise over a period of 30 min. After the resulting mixture was stirred at −78 °C for 2 h, the cooling bath was removed and the stirring was continued at room temperature for another hour. It was then quenched with 2 N HCl solution (1 mL) at 10 °C. The solvent was removed, and the residue was then dissolved in acetone (25 mL). After 2 N HCl (10 mL) was added, the solution was stirred at room temperature for 2 h. Saturated $NaHCO_3$ solution was added to neutralize the acid. The mixture was concentrated until the product started crystallizing out. After the mixture stood at room temperature for 1 h, the solid was collected by filtration, washed with water, and dried. The crude product was then recrystallized from a mixture of hexane:acetone (4:1) to yield **10** as colorless rods (400 mg): TLC R_f 0.32 (toluene:acetone, 95:5); mp 190−194 °C; IR 2932, 2865, 2361, 1699 cm^{-1}, ^1H NMR ($CDCl_3$) δ 7.38 (d, 2H, J = 8.8 Hz), 6.82 (d, 2H, J = 8.8 Hz), 3.81 (s, 3H), 2.53 (m, 1H), 2.12 (s, 3H), 0.98 (s, 3H), 0.60 (s, 3H). Anal. ($C_{30}H_{40}O_3$) C, H.

Method B. Alternatively, this compound was prepared in 45% yield in a manner analogous to the preparation of that for **23** (see below) by the Pd-catalyzed coupling reaction of 4-iodoanisole and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (7:3) gave the product as a colorless solid, which was found to be identical to **10** (TLC R_f, mp, NMR).

3β-(4-Chlorophenylethynyl)-3α-hydroxy-5β-pregnan-20-one (11). Method A. This compound was prepared in 57% yield in a manner analogous to the preparation of **10** starting from 2,2-dibromo-1-(4′-chlorophenyl)ethene[13] (obtained by the Wittig reaction of 4-chorobenzaldehyde with carbon tetrabromide in the presence of triphenylphosphine) and **2**. The crude compound was purified by column chromatography on silica gel (hexane:acetone, 4:1): TLC R_f 0.24 (toluene:acetone, 95:5); mp 188−190 °C; IR 2931, 2868, 2349, 1695, 1488 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38 (d, 2H, J = 8.5 Hz), 7.27 (d, 2H, J = 8.5 Hz), 2.52 (m, 1H), 2.12 (s, 3H), 0.99 (s, 3H), 0.61 (s, 3H). Anal. ($C_{29}H_{37}ClO_2$) C, H, Cl.

Method B. Alternatively, this compound was prepared in 53% yield in a manner analogous to the preparation of **23** (see below) by the Pd-catalyzed coupling reaction of 4-chloroiodobenzene and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (3:1) gave the product as a colorless solid, which was found to be identical to **11** (TLC R_f, mp, NMR).

3β-[[4-(*N*,*N*-Dimethylamino)phenyl]ethynyl]-3α-hydroxy-5β-pregnan-20-one (12). Method A. This compound was prepared in 35% yield in a manner analogous to the preparation of **10** starting from 2,2-dibromo-1-[4′-(*N*,*N*-dimethylamino)phenyl]ethene (obtained by the Wittig reaction of 4-(*N*,*N*-dimethylamino)benzaldehyde with carbon tetrabromide in the presence of triphenylphosphine) and **2**. The crude compound was purified by column chromatography on silica gel (hexane:acetone, 9:1): TLC R_f 0.28 (hexane:acetone, 7:3);

mp 212−216 °C; IR 2920, 2855, 2361, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32 (d, 2H, J = 8.5 Hz), 6.63 (d, 2H, J = 8.5 Hz), 2.97 (s, 6H), 2.52 (m, 1H), 2.12 (s, 3H), 0.97 (s, 3H), 0.60 (s, 3H). Anal. ($C_{31}H_{43}NO_2$) C, H, N.

Method B. Alternatively, this compound was prepared in 45% yield in a manner analogous to the preparation of **23** (see below) by the Pd-catalyzed coupling reaction of 4-bromo-*N*,*N*-dimethylaniline and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (3:1) gave the product as a colorless solid, which was found to be identical to **12** (TLC R_f, mp, NMR).

3β-(4-Cyanophenylethynyl)-3α-hydroxy-5β-pregnan-20-one (13). Method A. A solution of 2,2-dibromo-1-(4-cyanophenyl)ethene[20] (prepared by the Wittig reaction of 4-cyanobenzaldehyde with carbon tetrabromide in the presence of 1 equiv of triphenylphosphine and 1 equiv of zinc dust) (824 mg, 3.33 mmol) in dry THF (15 mL) was treated under N_2 with *n*-BuLi (2.5M in THF, 6 mmol, 2.4 mL) at −78 °C. The mixture was stirred at −75 °C for 0.5 h, and then a solution of 5β-pregnane-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (725 mg, 2.014 mmol) in dry THF (10 mL) was added dropwise over a period of 10 min. After the resulting mixture was stirred at −70 °C for 2 h, it was quenched with NH_4Cl solution (3 mL). The solvent was removed, and the residue was then dissolved in acetone (25 mL). After 2 N HCl (10 mL) was added, the solution was stirred at room temperature for 1 h. Saturated $NaHCO_3$ solution was added to neutralize the acid. The solvents were removed, and the residue was extracted with CH_2Cl_2. The organic layer was washed with water and brine. After being dried over anhydrous $MgSO_4$, the solution was filtered and evaporated to yield the crude product (600 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (95:5) gave **13** as a brown solid (150 mg): TLC R_f 0.29 (toluene:acetone, 95:5); mp 200−203 °C; IR 2926, 2869, 2222, 1678 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60 (d, 2H, J = 8.4 Hz), 7.51 (d, 2H, J = 8.4 Hz), 2.53 (m, 1H), 2.12 (s, 3H), 0.99 (s, 3H), 0.60 (s, 3H). Anal. ($C_{30}H_{37}NO_2$) C, H, N.

Method B. Alternatively, this compound was prepared in 25% yield in a manner analogous to the preparation of **23** (see below) by the Pd-catalyzed coupling reaction of 4-bromobenzonitrile and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (3:1) gave the product as a colorless solid, which was found to be identical to **13** (TLC R_f, mp, NMR).

3β-[(4-Formamidophenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one (14). Method A. A solution of **13** (79 mg, 0.178 mmol) in DMSO (1.5 mL) was treated with a H_2O_2 (30%, 7 drops) and K_2CO_3 (12 mg) at 0−5 °C. The mixture was stirred at room temperature for 1 h. Water (3 mL) was added, and the precipitated solid was collected by filtration. The crude product was crystallized from MeOH as colorless prisms (48 mg): TLC R_f 0.15 (hexane:acetone, 7:3); mp 255−262 °C dec; IR 3346, 3053, 2926, 2854, 2305, 1692, 1663, 1259, 730 cm^{-1}; ^1H NMR ($CDCl_3$) 7.76 (d, 2H, J = 8.3 Hz), 7.50 (d, 2H, J = 8.3 Hz), 6.05 (bs, 1H), 6.61 (bs, 1H), 2.53 (m, 1H), 2.12 (s, 3H), 0.99 (s, 3H), 0.61 (s, 3H). Anal. ($C_{30}H_{39}NO_3$) C, H, N.

Method B. Alternatively, this compound was prepared in 15% yield in a manner analogous to the preparation of **23** (see below) by the Pd-catalyzed coupling reaction of 4-bromobenzamide and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (7:3) gave the product as a colorless solid, which was found to be identical to **14** (TLC R_f, mp, NMR).

3β-(4-Biphenylethynyl)-3α-hydroxy-5β-pregnan-20-one (15). Method A. This compound was prepared in 53% yield in a manner analogous to the preparation of **10** starting from 2,2-dibromo-1-(4-biphenylyl)ethene (obtained by the Wittig reaction of 4-phenylbenzaldehyde with carbon tetrabromide in the presence of triphenylphosphine) and **2**. The crude compound was purified by column chromatography on silica gel (hexane:acetone, 4:1): TLC R_f 0.22 (hexane:acetone, 4:1); mp 182−184 °C; ^1H NMR ($CDCl_3$) δ 7.32−7.66 (m, 9H), 2.52 (m, 1H), 2.12 (s, 3H), 1.00 (s, 3H), 0.61 (s, 3H). Anal. ($C_{35}H_{42}O_2$) C, H.

Method B. Alternatively, this compound was prepared in 15% yield in a manner analogous to the preparation of **23** (see below) by the Pd-catalyzed coupling reaction of 4-bromobiphenyl and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (4:1) gave the product as a colorless solid, which was found to be identical to **15** (TLC R_f, mp, NMR).

3β-(4-Hydroxyphenylethynyl)-3α-hydroxy-5β-pregnan-20-one (16). Method A. This compound was prepared in 46% yield in a manner analogous to the preparation of **10** starting from 2,2-dibromo-1-(4-hydroxyphenyl)ethene[21] (obtained by the Wittig reaction of 4-hydroxybenzaldehyde with carbon tetrabromide in the presence of triphenylphosphine) and **2**. The crude compound was purified by column chromatography on silica gel (hexane:acetone, 9:1): TLC R_f 0.17 (toluene:acetone, 9:1); mp 252−256 °C; IR 3404, 2927, 2873, 1683 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37 (d, 2H, J = 8.5 Hz), 6.78 (d, 2H, J = 8.5 Hz), 5.00 (bs, 1H), 2.52 (m, 1H), 2.12 (s, 3H), 0.98 (s, 3H), 0.60 (s, 3H). Anal. ($C_{29}H_{38}O_3$) C, H.

Method B. Alternatively, this compound was prepared in 35% yield in a manner analogous to the preparation of **23** (see below) by the Pd-catalyzed coupling reaction of 4-iodophenol and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (7:3) gave the product as a colorless solid, which was found to be identical to **16** (TLC R_f, mp, NMR).

3α-Hydroxy-3β-(4-nitrophenylethynyl)-5β-pregnan-20-one (17). Method A. A solution of 2,2-dibromo-1-(4-nitrophenyl)ethene[22] (prepared by the Wittig reaction of 4-nitrobenzaldehyde with carbon tetrabromide in the presence of triphenylphosphine) (296 mg, 1 mmol) in dry THF (20 mL) was treated under N_2 with *n*-BuLi (2.5M in THF, 2 mmol, 0.8 mL) at −95 °C. The mixture was stirred at −80 to −100 °C for 0.5 h, and then a solution of 5β-pregnane-3,20-dione cyclic 20-(1,2-ethanediyl acetal) (720 mg, 2 mmol) in dry THF (10 mL) was added dropwise over a period of 10 min. After the resulting mixture was stirred <−80 °C for 1 h and then at 0 °C for an additional 1 h, it was quenched with NH_4Cl solution (3 mL). The solvent was removed, and the residue was then dissolved in acetone (25 mL). After 2 N HCl (10 mL) was added the solution was stirred at room temperature for 1 h. Saturated $NaHCO_3$ solution was added to neutralize the acid. The solvents were removed, and the residue was extracted with CH_2Cl_2. The organic layer was washed with water and brine. After being dried over anhydrous $MgSO_4$ the solution was filtered and evaporated to yield the crude product (400 mg). This crude product was then dissolved in a small amount of CH_2Cl_2 and poured on a column of silica gel. Elution with toluene:acetone mixture (96:4) gave **17** as a brown solid (70 mg): TLC R_f 0.18 (toluene:acetone, 95:5); mp 241−244 °C; IR 2922, 2862, 2346, 1697, 1516, 1336, 855 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.21 (d, 2H, J = 8 Hz), 7.59 (d, 2H, J = 8 Hz), 2.52 (m, 1H), 2.12 (s, 3H), 1.00 (s, 3H), 0.61 (s, 3H). Anal. ($C_{29}H_{37}NO_4$) C, H, N.

Method B. Alternatively, this compound was prepared in 45% yield in a manner analogous to the preparation of **23** (see below) by the Pd-catalyzed coupling reaction of 4-iodonitrobenzene and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (7:3) gave the product as a colorless solid, which was found to be identical to **17** (TLC R_f, mp, NMR).

3α-Hydroxy-3β-[(2-methoxyphenyl)ethynyl]-5β-pregnan-20-one (18). Method A. This compound was prepared in 54% yield in a manner analogous to the preparation of **10** starting from 2,2-dibromo-1-(2-methoxyphenyl)ethene (obtained by the Wittig reaction of 2-methoxybenzaldehyde with carbon tetrabromide in the presence of triphenylphosphine) and **2**. The crude compound was purified by column chromatography on silica gel (hexane:acetone, 4:1): TLC R_f 0.27 (hexane:acetone, 4:1); mp 183−185 °C; IR 2928, 2868, 2361, 1697 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38 (d, 1H, J = 7.5 Hz), 7.28 (m, 1H), 6.89 (m, 2H), 3.87 (s, 3H), 2.52 (m, 1H), 2.12 (s, 3H), 0.99 (s, 3H), 0.61 (s, 3H). Anal. ($C_{30}H_{40}O_3$) C, H.

Method B. Alternatively, this compound was prepared in 45% yield in a manner analogous to the preparation of **23** (see below) by the Pd-catalyzed coupling reaction of 2-iodoanisole

and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (4:1) gave the product as a colorless solid, which was found to be identical to **18** (TLC R_f, mp, NMR).

3β-[(3,4-Dimethoxyphenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one (19). This compound was prepared in 45% yield in a manner analogous to the preparation of **10** starting from 2,2-dibromo-1-(3,4-dimethoxyphenyl)ethene[23] (obtained by the Wittig reaction of 3,4-dimethoxybenzaldehyde with carbon tetrabromide in the presence of triphenylphosphine) and **2**. The crude compound was purified by column chromatography on silica gel. Elution with toluene:acetone mixture (96:4) gave a phenylacetylene compound, which was not characterized. Further elution with the same solvent yielded 3α-[(3′,4′-dimethoxyphenyl)ethynyl]-3β-hydroxy-5β-pregnan-20-one (11%) as a first fraction and 3β-[(3′,4′-dimethoxyphenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one as a second fraction (45%): TLC R_f 0.18 (hexane:acetone, 4:1); mp 82−88 °C; IR 2929, 2867, 2361, 1697 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.05 (dd, 1H, J = 1.8 and 8.3 Hz), 6.91 (d, 1H, J = 1.8 Hz), 6.78 (d, 1H, J = 8.3 Hz), 3.89 (s, 6H), 2.52 (m, 1H), 2.12 (s, 3H), 0.99 (s, 3H), 0.61 (s, 3H). Anal. ($C_{31}H_{42}O_4$) C, H.

3α-Hydroxy-3β-[[3,4-(methylenedioxy)phenyl]ethynyl]-5β-pregnan-20-one (20). Method A. This compound was prepared in 65% yield in a manner analogous to the preparation of **10** starting from 2,2-dibromo-1-[3′,4′-(methylenedioxy)-phenyl]ethene (obtained by the Wittig reaction of 3,4-(methylenedioxy)benzaldehyde with carbon tetrabromide in the presence of triphenylphosphine) and **2**. The crude compound was purified by column chromatography on silica gel (toluene:acetone, 95:5): TLC R_f 0.43 (hexane:acetone, 7:3); mp 188−191 °C; IR 2929, 2868, 2361, 1697 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.98 (d, 1H, J = 7.95 Hz), 6.88 (s, 1H), 6.73 (d, 1H, J = 7.95 Hz), 5.97 (s, 2H), 2.52 (m, 1H), 2.12 (s, 3H), 0.98 (s, 3H), 0.60 (s, 3H). Anal. ($C_{30}H_{38}O_4$) C, H.

Method B. Alternatively, this compound was prepared in 30% yield in a manner analogous to the preparation of **23** (see below) by the Pd-catalyzed coupling reaction of 4-bromo-1,2-(methylenedioxy)benzene and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (7:3) gave the product as a colorless solid, which was found to be identical to **20** (TLC R_f, mp, NMR).

3β-(2-Hydroxyphenylethynyl)-3α-hydroxy-5β-pregnan-20-one (23). A solution of 2-iodophenol (96 mg, 0.44 mmol) and 3β-ethynyl-3α-hydroxy-5β-pregnan-20-one (**22**[7], 150 mg, 0.44 mmol) in dry degassed triethylamine (0.5 mL) was stirred under argon at room temperature. Bis(triphenylphosphine)-palladium chloride (5 mg) and CuI (5 mg) were added, and the mixture was stirred at this temperature for 45 min. CH_2Cl_2 (5 mL) was added, and the stirring was continued for another hour. The TLC showed 100% conversion of the starting material; hence, the solvent was removed and the residue was purified by chromatography on silica gel. Elution with hexane:EtOAc (4:1) gave **23** (40 mg) as a colorless solid; TLC R_f 0.34 (toluene:acetone, 95:5); mp 210−212 °C; IR 3427, 2929, 2868, 2359, 1697 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.58 (d, 1H, J = 7.5 Hz), 7.59 (d, 1H, J = 8 Hz), 7.26 (m, 2H), 6.68 (s, 1H), 2.57 (m, 1H), 2.13 (s, 3H), 0.87 (s, 3H), 0.61 (s, 3H). Anal. ($C_{29}H_{38}O_3$) C, H.

3β-[(3-Hydroxyphenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one (24). This compound was prepared in 25% yield in a manner analogous to the preparation of **23** starting from 3-iodophenol and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (4:1) gave **24** as a colorless solid: TLC R_f 0.31 (toluene:acetone, 85:15); mp 208−213 °C; IR 3500, 2928, 2358, 1677 cm^{-1}; ^{1}H NMR (MeOH-d_4) δ 7.15 (m, 1H), 6.70−6.95 (m, 3H), 2.68 (m, 1H), 2.16 (s, 3H), 1.05 (s, 3H), 0.65 (s, 3H). Anal. ($C_{29}H_{38}O_3$) C, H.

3α-Hydroxy-3β-[(4-methylphenyl)ethynyl]-5β-pregnan-20-one (25). This compound was prepared in 46% yield in a manner analogous to the preparation of **23** starting from 4-iodotoluene and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (7:3) gave **25** as a colorless solid: TLC R_f 0.25 (toluene:acetone, 95:5); mp 208−210 °C; ^{1}H NMR ($CDCl_3$) δ 7.35 (d, 2H, J = 7.4 Hz), 7.11 (d, 2H, J = 7.4 Hz), 2.52 (m, 1H), 2.35 (s, 3H), 2.12 (s, 3H), 0.98 (s, 3H), 0.60 (s, 3H). Anal. ($C_{30}H_{40}O_2$) C, H.

3β-[(4-Aminophenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one (26). This compound was prepared in 60% yield in a manner analogous to the preparation of **23** starting from 4-iodoaniline and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (4:1) gave **26** as a pale yellow solid: TLC R_f 0.22 (hexane:acetone, 95:5); mp 203−207 °C dec; IR 3441, 3352, 2923, 2859, 2345, 1686 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.25 (d, 2H, J = 8.4 Hz), 6.58 (d, 2H, J = 8.4 Hz), 3.80 (bs, 2H), 2.52 (m, 1H), 2.11 (s, 3H), 0.97 (s, 3H), 0.60 (s, 3H). Anal. ($C_{29}H_{39}NO_2$) C, H, N.

3α-Hydroxy-3β-[[4-(trifluoromethyl)phenyl]ethynyl]-5β-pregnan-20-one (27). This compound was prepared in 31% yield in a manner analogous to the preparation of **23** starting from 4-(trifluoromethyl)iodobenzene and **22**. The crude compound was purified by column chromatography on silica gel. Elution with toluene:acetone mixture (95:5) gave **27** as a colorless solid: TLC R_f 0.23 (toluene:acetone, 95:5); mp 201−203 °C; IR 2922, 2863, 2358, 1688, 1312 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.58 (d, 2H, J = 7 Hz), 7.52 (d, 2H, J = 7 Hz), 2.52 (m, 1H), 2.12 (s, 3H), 0.99 (s, 3H), 0.61 (s, 3H). Anal. ($C_{30}H_{37}F_3O_2$) C, H, F.

3β-[(4-Acetylphenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one (28). This compound was prepared in 51% yield in a manner analogous to the preparation of **23** starting from 4-iodoacetophenone and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (7:3) gave **28** as a colorless solid: TLC R_f 0.40 (toluene:acetone, 9:1); mp 192−194 °C; IR 2935, 2858, 2360, 1700, 1683 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.91 (d, 2H, J = 8.1 Hz), 7.51 (d, 2H, J = 8.1 Hz), 2.60 (s, 3H), 2.52 (m, 1H), 2.12 (s, 3H), 0.99 (s, 3H), 0.61 (s, 3H). Anal. ($C_{31}H_{40}O_3$) C, H.

3β-[(4-Carbethoxyphenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one (29). This compound was prepared in 68% yield in a manner analogous to the preparation of **23** starting from 4-iodobenzoic acid ethyl ester and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (4:1) gave **29** as a colorless solid: TLC R_f 0.31 (hexane:acetone, 4:1); mp 164−166 °C; IR 2922, 2871, 2347, 1717, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.01 (d, 2H, J = 8.1 Hz), 7.49 (d, 2H, J = 8.1 Hz), 4.39 (m, 2H), 2.52 (m, 1H), 2.12 (s, 3H), 1.42 (m, 3H), 0.99 (s, 3H), 0.61 (s, 3H). Anal. ($C_{32}H_{42}O_4$) C, H.

3β-[(4-Formylphenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one (30). This compound was prepared in 25% yield in a manner analogous to the preparation of **23** starting from 4-bromobenzaldehyde and **22**. Triethylamine was replaced with diethylamine. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (7:3) gave **30** as a colorless solid: TLC R_f 0.18 (hexane:acetone, 4:1); mp 212−215 °C; IR 2922, 2858, 2347, 1694, 1684 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 10.01 (s, 1H), 7.91 (d, 2H, J = 8.1 Hz), 7.58 (d, 2H, J = 8.1 Hz), 2.52 (m, 1H), 2.12 (s, 3H), 1.00 (s, 3H), 0.62 (s, 3H). Anal. ($C_{30}H_{38}O_3$) C, H.

3β-[(3-Acetylphenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one (31). This compound was prepared in 58% yield in a manner analogous to the preparation of **23** starting from 3-iodoacetophenone and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (4:1) gave **31** as a colorless solid: TLC R_f 0.22 (hexane:acetone, 4:1); mp 195−197 °C; IR 2927, 2859, 2361, 1699, 1684 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.01 (s, 1H), 7.91 (d, 1H, J = 7.7 Hz), 7.62 (d, 1H, J = 7.9 Hz), 7.42 (dd, 1H, J = 7.7 and 7.9 Hz), 2.62 (s, 3H), 2.54 (m, 1H), 2.12 (s, 3H), 0.99 (s, 3H), 0.61 (s, 3H). Anal. ($C_{31}H_{40}O_3$) C, H.

3β-[[4-(*N*,*N*-Diethylcarbamoyl)phenyl]ethynyl]-3α-hydroxy-5β-pregnan-20-one (32). This compound was prepared in 12% yield in a manner analogous to the preparation of **23** starting from 4-iodo-*N*,*N*-diethylbenzamide and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (3:1) gave **32** as a colorless solid: TLC R_f 0.22 (hexane:acetone, 3:1); mp

174−177 °C; IR 2930, 2867, 2361, 1699, 1693 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.48 (d, 2H, J = 7.6 Hz), 7.31 (d, 2H, J = 7.6 Hz), 3.52 (bm, 2H), 3.25 (bm, 2H), 2.52 (m, 1H), 2.12 (s, 3H), 0.99 (s, 3H), 0.61 (s, 3H). Anal. ($C_{34}H_{47}NO_3$) C, H, N.

3β-[(4-Benzoylphenyl)ethynyl]-3α-hydroxy-5β-pregnan-20-one (33). This compound was prepared in 10% yield in a manner analogous to the preparation of **23** starting from 4-iodobenzophenone and **22**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (4:1) gave **33** as a colorless solid: TLC R_f 0.54 (hexane:acetone, 7:3); mp 78−83 °C; IR 2920, 2837, 2361, 1698, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.51−7.92 (m, 9H), 2.52 (m, 1H), 2.12 (s, 3H), 1.00 (s, 3H), 0.61 (s, 3H). Anal. ($C_{36}H_{42}O_3$) C, H.

3β-[(4-Acetylphenyl)ethynyl]-3α-hydroxy-5β-19-norpregnan-20-one (36). This compound was prepared in 30% yield in a manner analogous to the preparation of **23** starting from 4-iodoacetophenone and 3β-ethynyl-3α-hydroxy-5β-19-norpregnan-20-one [**35**, obtained by the addition of lithium acetylide to 5β-19-norpregnane-3,20-dione cyclic 20-(1,2-ethanediyl acetal)].[7] The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (4:1) gave **36** as a colorless solid: TLC R_f 0.12 (hexane:acetone, 4:1); mp 64−65 °C; IR 2920, 2868, 2361, 1699, 1684 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.91 (d, 2H, J = 8.2 Hz), 7.53 (d, 2H, J = 8.2 Hz), 2.60 (s, 3H), 2.55 (m, 1H), 2.12 (s, 3H), 0.63 (s, 3H). Anal. ($C_{30}H_{38}O_3$) C, H.

3β-[4-Carbethoxyphenyl)ethynyl]-3α-hydroxy-5β-19-norpregnan-20-one (37). This compound was prepared in 20% yield in a manner analogous to the preparation of **23** starting from 4-iodobenzoic acid ethyl ester and **35**. The crude compound was purified by column chromatography on silica gel. Elution with hexane:acetone mixture (4:1) gave **37** as a colorless solid: TLC R_f 0.27 (hexane:acetone 4:1); mp 164−165 °; IR 2922, 2874, 2358, 1713, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.01 (d, 2H, J = 8.1 Hz), 7.49 (d, 2H, J = 8.1 Hz), 4.39 (m, 2H), 2.55 (m, 1H), 2.13 (s, 3H), 1.40 (m, 3H), 0.63 (s, 3H). Anal. ($C_{31}H_{40}O_4$) C, H.

Pharmacology. Steroid Inhibition of TBPS Binding. TBPS binding assays using rat brain cortical membranes in the presence of 5 μM GABA has been described.[4,6,7] Briefly, cortices were rapidly removed following decapitation of carbon dioxide-anesthetized Sprague−Dawley rats (200−250 g). The cortices were homogenized in 10 volumes of ice-cold 0.32 M sucrose using a glass/Teflon homogenizer and centrifuged at 1500*g* for 10 min at 4 °C. The resultant supernatants were centrifuged at 10000*g* for 20 min at 4 °C to obtain the P2 pellets. The P2 pellets were resuspended in 200 mM NaCl/50 mM Na−K phosphate pH 7.4 buffer and centrifuged at 10000*g* for 10 min at 4 °C. This washing procedure was repeated twice, and the pellets were resuspended in 10 volumes of buffer. Aliquots (100 μL) of the membrane suspensions were incubated with 2 nM [^{35}S]TBPS and 5 μL aliquots of test drug dissolved in dimethyl sulfoxide (DMSO) (final 0.5%) in the presence of 5 μM GABA. The incubation was brought to a final volume of 1.0 mL with buffer. Nonspecific binding was determined in the presence of 2 μM unlabeled TBPS and ranged from 15 to 25%. Following a 90 min incubation at room temperature, the assays were terminated by filtration through glass fiber filters (Schleicher and Schuell No. 32) using a cell harvester (Brandel) and rinsed three times with ice-cold buffer. Filter bound radioactivity was measured by liquid scintillation spectrometry. Nonlinear curve fitting of the overall data for each drug averaged for each concentration was done using Prism (GraphPad). The data were fit to a partial instead of a full inhibition model if the sum of squares was significantly lower by *F*-test. Similarly, the data were fit to a two component instead of a one-component inhibition model if the sum of squares was significantly lower by *F*-test. The concentration of test compound producing 50% inhibition (IC_{50}) of specific binding and the maximal extent of inhibition (I_{max}) were determined for the individual experiments with the same model used for the overall data and then the means ± SEM of the individual experiments were calculated.

Electrophysiology of $GABA_A$ Receptors Expressed in *Xenopus* Oocytes. Oocytes and cRNAs encoding $GABA_A$ receptor subunits were prepared as previously described.[24] Briefly, oocytes were microinjected with a 1:1:1 mixture of cRNAs encoding the human α1, β2, and γ2L subunits (approximately 1 ng of each cRNA per cell). Membrane currents were recorded with a two-electrode voltage clamp in frog Ringer's solution consisting of 115 mM NaCl, 2 mM KCl, 1.8 mM CaCl, and 5 mM HEPES, pH 7.4. Drug and wash solutions were applied directly to the oocyte using a microcapillary "linear array" system. Maximum GABA responses were elicited by application of 1 mM GABA. Stock solutions of steroids were dissolved in DMSO over the range of 0.01 μM to 10 mM. Stocks were diluted 300−1000-fold in frog Ringer's solution and DMSO adjusted to 0.3% by volume; 0.3% DMSO had little effect on GABA responses. Both GABA concentration−response data and steroid concentration−modulation data were fit to a four-parameter logistic equation (Origin, Microcal, Inc.). Unless indicated otherwise, data is expressed as mean values ± SEM to two significant figures.

***In Vivo* Pharmacology.** Male NSA mice weighing between 15 and 20 g were obtained from Harlan Sprague−Dawley (San Diego, CA). Upon arrival they were housed in standard polycarbonate cages (four per cage) containing a sterilized bedding material in a room of constant temperature (23.0° ± 2.5 °C) with a 12 h (0700−1900 light) light/dark cycle. Food (Teklad LM 485) and water were freely available. Mice were acclimated a minimum of 4 days prior to experimentation.

Pentylenetetrazol-Induced Seizures. Seizures were induced by administration of 85 mg/kg, sc pentylenetetrazole (30 min observation period). The dose used was previously determined to be the CD_{97}. A clonic seizure was defined as forelimb clonus of ≥3 s duration. Data were treated quantally.

Maximal Electroshock-Induced Seizures. Seizures were induced by application of current (50 mA, 60 pulses/s, 0.8 ms pulse width, 1 s duration, dc) using a Ugo Basile ECT device (Model 7801). Mice were restrained by gripping the loose skin on their dorsal surface, and saline-coated corneal electrodes were held lightly against the two cornea. Current was applied, and mice were observed for a period of up to 30 s for the occurrence of a tonic hindlimb extensor response. A tonic seizure was defined as a hindlimb extension in excess of 90° from the plane of the body. Results were treated in a quantal manner.

Hanging Wire. The hanging-wire test used a custom-built apparatus that consisted of a metal wire (2 mm diameter) suspended horizontally above a padded surface (25 cm). Mice were held by the base of the tail, their forepaws placed in contact with the wire, and then released. Animals were required to bring both hindpaws in contact with the wire within 5 s in order to be scored as a pass. Results were treated quantally.

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