Novel 17β-Substituted Conformationally Constrained Neurosteroids that Modulate $GABA_A$ Receptors

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The goal of this study was to develop a series of allopregnanolone analogues substituted by conformationally constrained 17β side chains to obtain additional information about the structure−activity relationship of 5α-reduced steroids to modulate $GABA_A$ receptors. Specifically, we introduced alkynyl-substituted 17β side chains in which the triple bond is either directly attached to the 17β-position or to the 21-position of the steroid skeleton. Furthermore, we investigated the effects of C22 and C20 modification. The in vitro binding affinity for the $GABA_A$ receptor of the new analogues was measured by allosteric displacement of the specific binding of [^{3}H]4′-ethynyl-4-*n*-propyl-bicycloorthobenzoate (EBOB) to $GABA_A$ receptors on synaptosomal membranes of rat cerebellum. An allosteric binding model that has been successfully applied to ionotropic glycine receptors was employed. The most active derivative is (20*R*)-17β-(1-hydroxy-2,3-butadienyl)-5α-androstane-3-ol (**20**), which possesses low nanomolar potency to modulate cerebellar $GABA_A$ receptors and is 71 times more active than the control compound allopregnanolone. Theoretical conformational analysis was employed in an attempt to correlate the in vitro results with the active conformations of the most potent of the new analogues.

Introduction

Neurosteroids are synthesized in the central and peripheral nervous system, particularly in myelinating glial cells, but also in astrocytes and neurons, and act on the nervous system.[1] Research over the past decade has elucidated their multiple effects on various neurotransmitter systems.[2] Many studies have demonstrated that they positively or negatively modulate the function of members of the ligand-gated ion channel superfamily,[3] and most of these studies have focused on the positive allosteric actions on $GABA_A$ receptors.[4] GABA is the major inhibitory neurotransmitter in the mammalian central nervous system, and rapid synaptic inhibition is mediated through the opening of $GABA_A$ receptor−ionophores. A number of therapeutically important drugs, including benzodiazepines and sedative and anesthetic barbiturates, act to enhance the interaction of GABA with this receptor. The $GABA_A$ receptor complex can exist in multiple isoforms and can demonstrate a variety of pharmacological profiles that arise from their pentameric structure and the diversity of their subunits. It has been suggested that, as for other allosteric modulators, there may be specific neurosteroid binding sites on $GABA_A$ receptors, a theory supported by previous reports that the interaction of neurosteroids with this receptor is enantioselective[5] and is dependent on subunit composition.[6] Potentiation by the neuroactive steroids alphaxalone and allotetrahydrodeoxycorticosterone (alloTHDOC) requires regions before the second and beyond the fourth transmembrane segments of the α1 subunit, respectively.[7] The presence of a γ subunit is not required, unlike the benzodiazepine site where this is a prerequisite.[8] Recent evidence has implicated the enhanced potentiation of δ subunit-containing $GABA_A$ receptors.[8c,9] However, a study on the neurosteroid effects on recombinant human α4β3δ $GABA_A$ receptors concluded that there is not a single structural motif within steroid molecules that would account for the different effects on $GABA_A$ receptor subtypes, a finding that may be consistent with steroid interactions at multiple sites on the receptor.[9a] The physiological and pharmacological actions of neurosteroids are topics of widespread interest. Neurosteroids have been implicated in premenstrual syndrome,[10] cognitive and psychiatric dysfunctions, neuroprotection,[11] and $GABA_A$ receptor plasticity.[12] Withdrawal from chronic exogenous neurosteroids may be associated with increased seizure susceptibility due to alterations in the expression of $GABA_A$ receptor subunits.[13] The neurosteroids allopregnanolone and alloTHDOC act as efficacious allosteric agents of $GABA_A$ receptors and potentiate their Cl^- ionophore function.[14] They have been shown to be potent anticonvulsants, anxiolytics, and antistress agents and have been shown to possess sedative, hypnotic, and anesthetic activities.[15] Preg-

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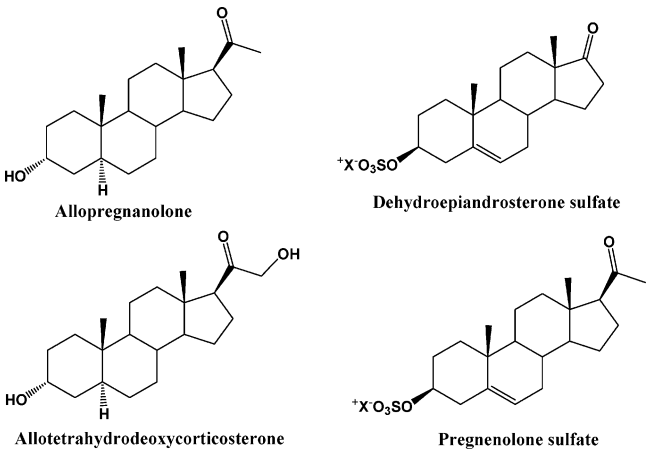
nenolone sulfate and dehydroepiandrosterone sulfate, however, have been shown to inhibit $GABA_A$ receptor−ionophore function[4b,16] and enhance memory performance in rodents.[17]



Previous in vitro and in vivo structure−activity relationship (SAR) studies indicated that although 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, certain other 17β substituents that are hydrogen-bond acceptors, such as 17β-CN, retain activity.[5,18] A number of modifications to the steroid nucleus have been made to study the SAR of neuroactive steroids, including substitutions at the 3β-, 11-, 17-, or 21-positions[19] as well as 17a-aza-D-homosteroid analogues[20] and benz[*e*]indene derivatives.[21] In an attempt to identify steroids with improved activity against $GABA_A$ receptors, we introduced alkynyl-substituted 17β side chains. Thus, in the present report we describe the synthesis and activity of two series of 3α-hydroxy-substituted steroids in which the triple bond is directly attached to either the 17β-position or the 21-position of the steroid skeleton. Furthermore, we investigated the effects of C22 and C20 modification. In vitro binding studies of the SAR of neuroactive steroids on $GABA_A$ receptor−ionophores have frequently used [^{35}S]*tert*-butylbicyclophosphorothionate (TBPS), which is a cage convulsant.[14,18,19] The channel-blocking radioligands [^{35}S]TBPS, [^{3}H]*tert*-butylbicycloorthobenzoate (TBOB), and [^{3}H]4′-ethynyl-4-*n*-propyl-bicycloorthobenzoate (EBOB)[22] bind within the ion channel.[23] Consequently, these binding studies have revealed several allosteric binding interactions of pharmacological relevance.[14,18,24,25] Here, we use an allosteric binding model[26] that has been successfully applied to glycine receptors belonging to the same superfamily of ionotropic receptors.[27] $GABA_A$ receptors of rat cerebellum were used for binding because (1) their subunit composition has been clarified: α1$\beta\gamma$2, α1α6$\beta\gamma$2, α6$\beta\gamma$2, α6$\beta\delta$, and α1α6$\beta\delta$[6d] and (2) the presence of δ subunits possibly confers high affinity to neuroactive steroids.[8c,9a] This report provides a description of the SAR for the interaction of the $GABA_A$ receptor with new pregnane steroids.

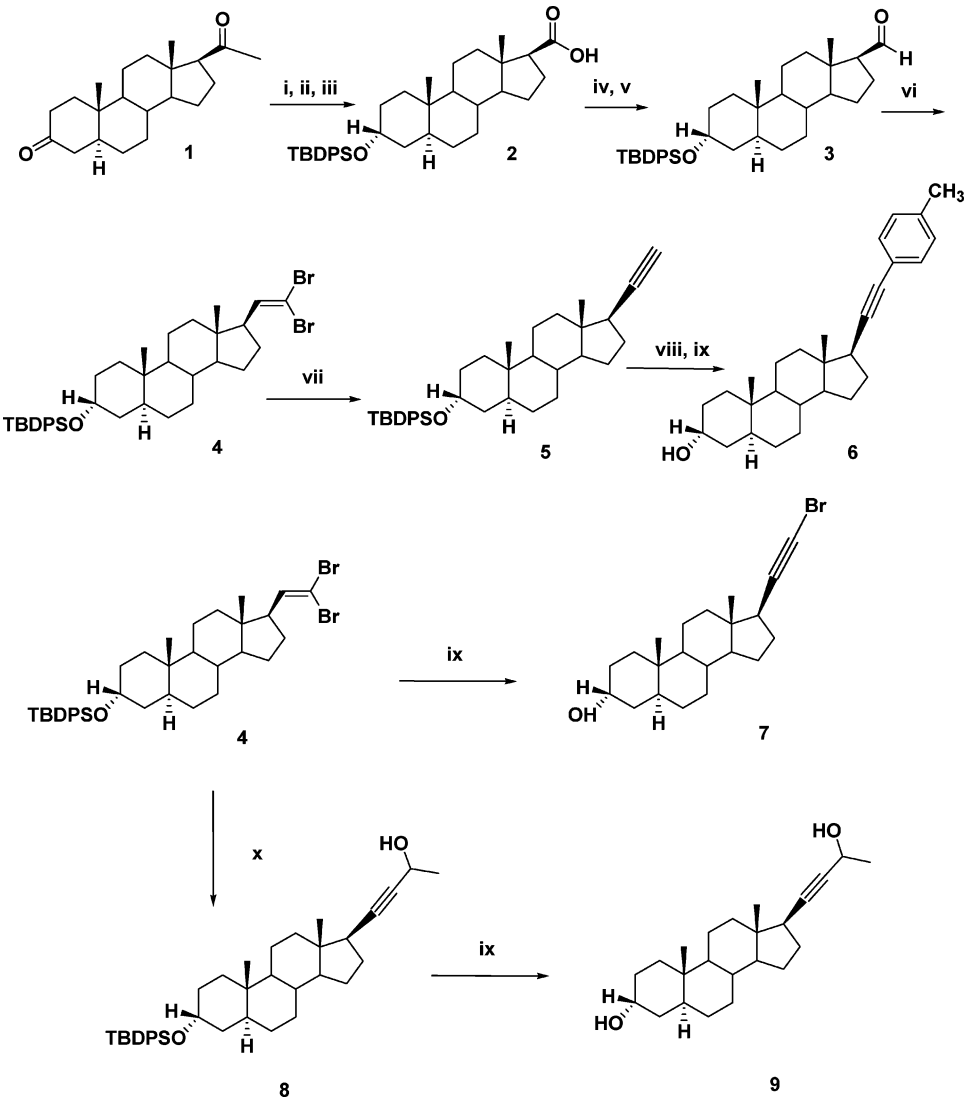
Chemistry

The synthetic strategy followed for the preparation of the neurosteroid analogues **6**, **7**, **9**, **13**−**16**, **18**−**21**, and **23** is depicted in Schemes 1−4. The synthetic route for the synthesis of the 17β-alkynyl-substituted derivatives is described in Scheme 1. Thus, regioselective and stereoselective reduction of the carbonyl group at C3 of 5α-3,20-pregnanedione (**1**) with K−Selectride in THF at −78 °C gave 3α-hydroxy-5α-pregnane-20-one. Protection of the hydroxyl group as the *tert*-butyldiphenylsilyl ether and treatment with NaOBr afforded the corresponding acid **2**. The reduction of the acid **2** to the respective alcohol using $LiAlH_4$ and the subsequent oxidation with pyridinium chlorochromate afforded the 17β-formyl derivative **3**, which in turn was transformed to the dibromo compound **4** upon treatment with CBr_4 and PPh_3 using the Corey−Fuchs methodology.[28] The addition of BuLi in THF at −78 °C to *gem*-dibromide **4** afforded alkyne **5**. Sonogashira coupling of alkyne **5** with 4-iodotoluene, using $(PPh_3)_2PdCl_2$ as a catalyst in the presence of CuI and pyrrolidine,[29] followed by the deprotection of the C3-hydroxyl group using TBAF in THF, yielded analogue **6**. Furthermore, the treatment of **4** with TBAF in THF resulted in alkynyl bromide **7**. The addition of *n*-BuLi to *gem*-dibromide **4** at −78 °C and the quenching of the resulting alkynyllithium intermediate with acetaldehyde afforded the corresponding alkynyl alcohol **8**, which, upon deprotection of the C3-hydroxyl, yielded derivative **9**.

The synthesis of the 21-alkynyl-substituted derivatives is described in Schemes 2−4. The addition of lithium trimethylsilylacetylide to the aldehyde **3** afforded a mixture of the epimeric alcohols, **10a** (20*R*) and **10b** (20*S*) in a ratio of 3 to 1, respectively, which were easily separated by flash column chromatography (Scheme 2). The less polar diastereomer **10a** was attributed the 20*R* absolute configuration on the basis of Cram's rules[30] and previous analogous reactions with 3β-hydroxy-17β-formylandrost-5-ene.[31]

To obtain compounds **10a** and **10b** stereoselectively, we employed another route starting from the acid **2** (Scheme 2). Thus, the formation of the corresponding Weinreb amide[32] and the subsequent treatment with lithium triisopropylsilylacetylide afforded the alkynyl ketone **11**. The asymmetric reduction of ketone **11** with catecholborane in the presence of (*S*)-3,3-diphenyl-1-ethyltetrahydro-3*H*-pyrrolo[1,2-c][1,3,2]oxazaborol as a catalyst[33] afforded the 20*S* epimeric alcohol **12b** as the only product. Alternatively, the stereoselective reduction of ketone **11** with BH_3-Me_2S in the presence of (*S*)-3,3-diphenyl-1-ethyltetrahydro-3*H*-pyrrolo[1,2-c][1,3,2]-oxazaborol as a catalyst[34] afforded the 20*R* epimeric alcohol **12a** as the only product, and reduction with $NaBH_4$ afforded a 1:1 mixture of epimeric alcohols **12a** and **12b**. The treatment of the alkynyl ketone **11** with a hydrogen fluoride−pyridine complex in methylene chloride resulted in the removal of the *tert*-butyldiphenylsilyl protecting group as well as the C22-triisopropylsilyl group to afford the ynone **13**. The synthesis of derivatives **14**−**16** and **18** is depicted in Scheme 3. Thus, the removal of both silyl groups in compound **12a** with TBAF in THF yielded diol **14**. The formation of the allene derivative **15** was effected by the reaction of **14** with paraformaldehyde, CuI, and diisopropylamine in refluxing dioxane[35] (compound **15** has a 20*S* configuration because of a change in the priorities of the C20 substituents). Furthermore, alcohol **12a** was treated with one equivalent of sodium hydride, followed by the

Scheme 1[a]



[a] (i) K−Selectride, THF, −78 °C; (ii) TBDPSiCl, DMF, imidazole, 55 °C; (iii) NaOBr, dioxane, H_2O, rt; (iv) $LiAlH_4$, THF, reflux; (v) PCC, CH_2Cl_2, rt; (vi) CBr_4, PPh_3, CH_2Cl_2, 0 °C; (vii) *n*-BuLi, THF, −78 °C; (viii) $(PPh_3)_2PdCl_2$, CuI, pyrrolidine, 4-iodotoluene, rt; (ix) TBAF, THF, rt; (x) *n*-BuLi, CH_3CHO, THF, −78 °C.

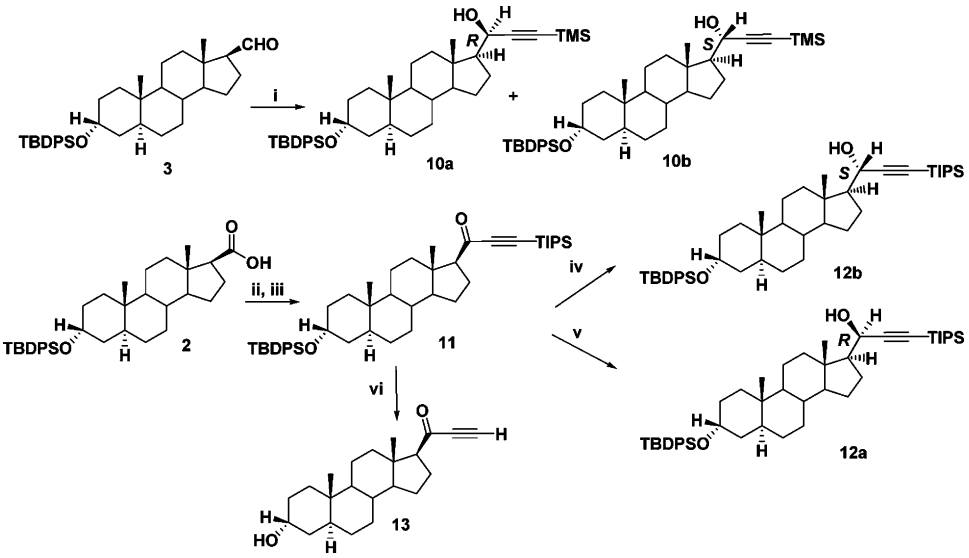
addition of methyl iodide to yield the respective 20*R*-methoxy derivative, which, upon the addition of TBAF in THF, yielded the 3α-hydroxy-(20*R*)-methoxy alkynyl derivative **16**. The selective removal of the C22-triisopropylsilyl group in compound **12a**, using 1.5 equiv of TBAF in THF, yielded alkyne **17**. The Sonogashira coupling of alkyne **17** with 4-iodoanisole in the presence of $(Ph_3P)_4Pd$, CuI, and pyrrolidine, followed by treatment with TBAF in THF, yielded diol **18**. Reactions analogous to those described in Scheme 3 using the (20*S*)-alcohol **12b** afforded the steroid analogues **19**, **20**, **21**, and **23** (Scheme 4).

Results and Discussion

The results of the allosteric displacement of [^{3}H]-EBOB binding from GABA$_A$ receptors on synaptosomal membranes of rat cerebellum for the compounds under study are reported in Table 1.

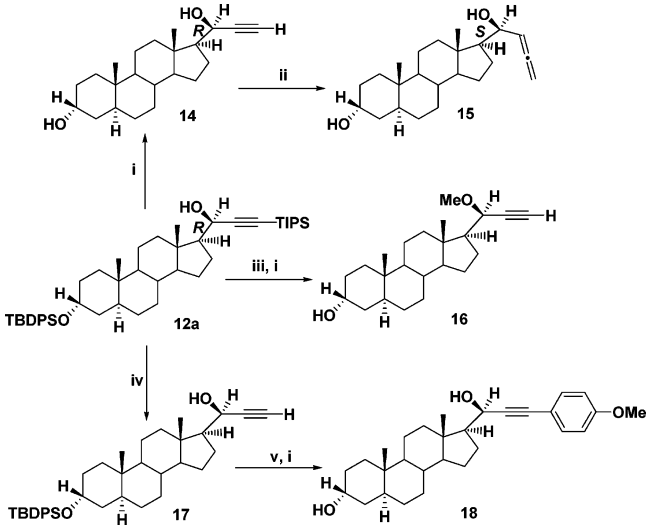
Because 3α-hydroxy-5α-androstane-17β-carbonitrile was reported to produce great potentiation of GABA$_A$ receptor function,[5] we prepared 17β-alkynyl derivatives of 3α-hydroxy-5α-androstane and, specifically, compounds **6**, **7**, and **9**. Among these analogues, only the 22-bromoalkynyl derivative **7** had a potency similar to the control compound allopregnanolone as a displacer (K_s = 350 ± 72 and 198 ± 47 nM, respectively). Substitution of the triple bond at C22 by a tolyl group is deleterious to GABA$_A$ receptor activity, rendering the resulting compound **6** 20 times less active (K_s = 4100 ± 265 nM). Analogue **9**, substituted by a C22-hydroxyethyl group, is ∼8 times less active (K_s = 946 ± 404 nM) than the prototype, 5α-reduced steroid allopregnanolone. We then set out to explore the activity of compounds containing a triple bond at position C21 of the pregnane system in conjunction with either a keto function, a hydroxyl function, or an ether function at C20. The replacement of the C21 methyl group in allopregnanolone by an alkynyl functionality, analogue **13**, resulted in a 4-fold reduction of activity (844 ± 205 nM). As mentioned previously, GABA$_A$ergic steroids contain a hydrogen-bond-acceptor group located at C17. Even though adding a triple bond in conjugation with the carbonyl group should improve its hydrogen-bond-acceptor properties to a small extent, we observe a

Scheme 2[a]



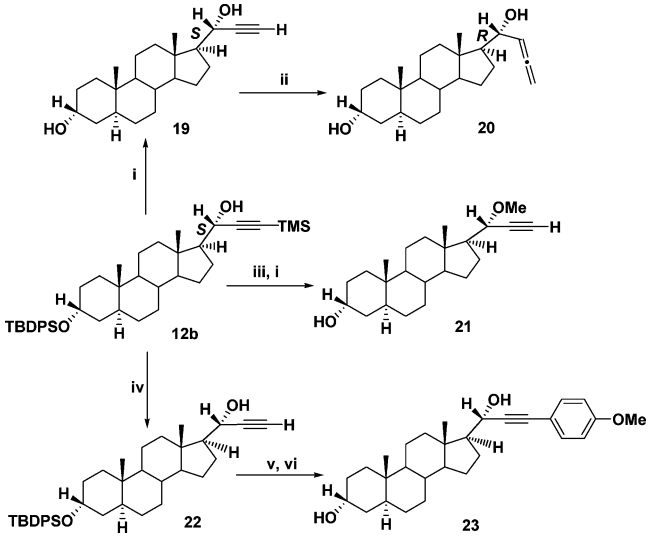
[a] (i) *n*-BuLi, trimethylsilylacetylene, THF, −78 °C; (ii) $CH_3ONH(CH_3)\cdot HCl$, DMAP, EDCI, CH_2Cl_2, rt; (iii) *n*-BuLi, (triisopropylsilyl)-acetylene, THF, −78 °C; (iv) (*S*)-3,3-diphenyl-1-ethyltetrahydro-3*H*-pyrrolo[1,2-c][1,3,2]oxazaborol, catecholborane, CH_2Cl_2, −78 °C; (v) (*S*)-3,3-diphenyl-1-ethyltetrahydro-3*H*-pyrrolo[1,2-c][1,3,2]oxazaborol, BMS, CH_2Cl_2, −78 °C; (vi) $C_5H_5N\cdot(HF)_x$, CH_2Cl_2, rt.

Scheme 3[a]



[a] (i) TBAF, THF, rt; (ii) formaldehyde, CuI, diisopropylamine, dioxane, reflux; (iii) NaH, MeI, THF, rt; (iv) 1.5 equiv of TBAF, THF, rt; (v) 4-iodoanisole, $(PPh_3)_4Pd$, CuI, pyrrolidine, rt.

Scheme 4[a]



[a] (i) TBAF, THF, rt; (ii) formaldehyde, CuI, diisopropylamine, dioxane, reflux; (iii) NaH, MeI, THF, rt; (iv) 1.5 equiv of TBAF, THF, rt; (v) 4-iodoanisole, $(PPh_3)_4Pd$, CuI, pyrrolidine, rt.

decrease in displacing activity. Therefore, we speculate that the decrease in activity in analogue **13** is not related to the hydrogen-bond-accepting capacity of the C20 keto function and that the alkynyl group is unfavorable for interaction with the steroid site of the receptor. Additionally, we examined the activity of the C22-alkynyl-substituted pregnanediols. Because we were interested in investigating the impact of the orientation (α or *β*) of the reduced substituent at the C20 position on the modulating activity of the $GABA_A$ receptor, we stereoselectively synthesized both C20 epimers. Interestingly, alkynyl pregnanediols possess higher displacing potency than ynone **13**. Furthermore, the activity was also dependent on the C20 stereochemistry, with the 20*S* isomer **19** being 3.4 times more active (K_s = 145 ± 49 nM) than the corresponding 20*R* epimer **14** (K_s = 494 ± 158 nM) and slightly more potent than allopregnanolone. Methylation of the free hydroxyl group in compounds **14** and **19** adversely affects the potency of the corresponding analogues **16** and **21**, which possess K_s = 924 ± 490 and 802 ± 227 nM, respectively. The hydroxyl functionality in analogues **14** and **19** can interact with the receptor, both as a hydrogen-bond acceptor and as a hydrogen-bond donor, whereas the methyl ethers of **16** and **21** are only hydrogen-bond-accepting substituents. It has been reported previously that 5α-androstane-3α,17*β*-diol is a relatively poor $GABA_A$ receptor modulator, whereas 17*β*-methoxy-5α-androstane-3α-ol, which possesses a hydrogen-bond-accepting substituent at C17, is an active, positive allosteric modulator.[19f] Therefore, hydrogen bonding is not the only parameter that controls the affinity of the receptor for C22-alkynyl pregnanediols. Furthermore, the substitution of the alkynyl functionality at C22 in alcohols **18** and **23** by a 4-methoxyphenyl substituent results in a more pronounced decrease in

Table 1. Allosteric Displacement of [^{3}H]EBOB Binding to Synaptosomal Membranes of Rat Cerebellum[a]

compound	K_S (nM)	α
6 ($n = 3$)	4100 ± 265	2.4 ± 0.1
7 ($n = 3$)	350 ± 72	5.8 ± 1.1
9 ($n = 3$)	946 ± 404	4.3 ± 0.4
13 ($n = 5$)	844 ± 205	5.3 ± 1.4
14 ($n = 5$)	494 ± 158	3.3 ± 0.7
15 ($n = 6$)	810 ± 230	2.6 ± 0.1
16 ($n = 6$)	924 ± 490	3.0 ± 0.3
18 ($n = 3$)	1640 ± 234	$\gg 10^b$
19 ($n = 5$)	145 ± 49	2.6 ± 0.3
20[c]	2.8 ± 1.2	1.7 ± 0.1
21 ($n = 5$)	802 ± 227	4.0 ± 0.5
23 ($n = 5$)	870 ± 292	$> 10^b$
allopregnanolone ($n = 4$)	198 ± 47	$> 10^b$

[a] Dissociation constants (K_S) of the neuroactive steroids and cooperativity factors (α) with [^{3}H]EBOB binding were determined via eq 1 and the ternary allosteric model (Scheme 5). Data are the mean (±SEM) of n experiments. [b] Cannot be determined accurately because full displacement approached nonspecific [^{3}H]EBOB binding. [c] Determined from fitting to the pooled data of five experiments. Some experiments could not be fitted separately.

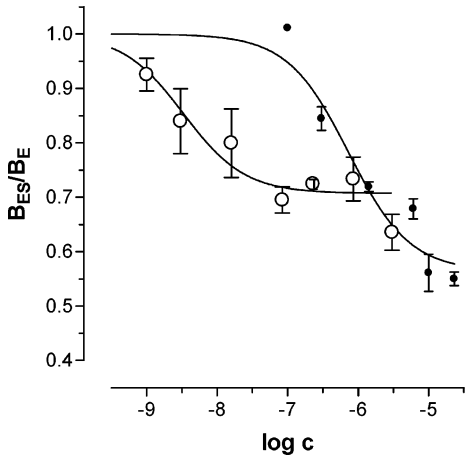


Figure 1. Displacement of [^{3}H]EBOB binding to rat cerebellar GABA$_A$ receptors by the epimeric neurosteroids **20** (○) and **15** (●). Specific binding in the presence of the steroids (B_{ES}) over the control (B_E). Points are mean ± SEM of 3−5 experiments. Curve fitting parameters are summarized in Table 1.

activity (3.3- and 6-fold, respectively; compound **18**: K_s = 1640 ± 234 nM and compound **23**: K_s = 870 ± 292 nM). The replacement of the alkynyl group in alcohols **18** and **23** by a propadienyl functionality affected the displacing potency of the respective compounds **15** and **20** very dramatically. In analogue **15**, there is a 1.6-fold decrease in affinity (K_s = 810 ± 230 nM) with respect to alcohol **14**, whereas in steroid **20**, to our delight, there is a 52-fold increase in affinity (K_s = 2.8 ± 1.3 nM) (Figure 1). Analogue **20** is the most potent compound of the derivatives described in the present study, being ∼71 times more active than allopregnanolone. As we have seen from the pregnanediols described, the 20*S* isomers are more active than the 20*R* congeners. This observation corroborates with previous studies concerning the modulation of human recombinant GABA$_A$ receptors by the endogenous 5α- or 5β-pregnan-3,20-diol.[25b] In particular, for the 5α series, the introduction of the 20-hydroxyl group in the α orientiation produced only a modest (approximately 2-fold) decrease in potency, whereas the 20β-hydroxyl steroid was ∼24 times less potent than the parent allopregnanolone. Furthermore, these pregnanediols were shown to be behaviorally active in the rat Vogel test,[36] which is a paradigm that is predictive of anxiolytic activity, and the 20α-ol shows good selectivity for anxiolytic activity versus motor impairment.

Additionally, 3α-hydroxy-pregnan-20-ols have partial agonist activity at GABA$_A$ receptors.[37] Functional data reported by Belelli et al.[25a] confirm previous biochemical,[25b] neurochemical,[37a] and behavioral studies[36b] that demonstrate that certain endogenous pregnanediols produce a more subtle modulation of GABA$_A$ receptor function compared with that of anesthetic steroids.

As an initial step to correlate the in vitro results with the active conformations of pregnanediols **14**, **19**, **15**, and **20**, a combination of minimization algorithms with stochastic and systematic conformational analysis was employed assuming a normal chair conformation for ring A.[5] As shown in Figure 2, only one lowest-energy conformer was found for each compound. The two diastereomers, **15** and **20**, differ in the orientation of both the allene and the hydroxyl groups, whereas compounds **14** and **19** differ only in the orientation of the hydroxyl group. These differences are better observed in Figure 3 in which superimposition of the lowest-energy conformers of the two diastereomeric pairs is shown. According to previous reports,[19f,20] steroids containing hydrogen-bond-accepting groups at C17 above the plane defined by the D-ring on the β side of the steroid exhibit improved GABA$_A$ receptor activity. For the four pregnanediols under study, the C20 hydroxyl group, which could serve as a hydrogen-bond acceptor, lies above the plane of the D-ring, in the global minimum-energy conformers. This is also the case for the majority of the conformers with energies up to 6 kcal/mol higher than the global minimum. This suggests that the direction adopted by the hydroxyl group is not the determining factor for optimizing the interactions of pregnanediols with their receptor binding site.

To explore the conformational space, which might influence productive binding to the receptor, the contour plots for alkynyl diols **14** and **19** and allenyl diols **15** and **20** were obtained, and they are depicted in Figure 4. Each grad scan analysis for the molecules under study was performed around the τ_1 [C(17)−C(20)−C(21)−C(22)] and τ_2 [C(17)−C(20)−O(20)−H] dihedral angles by rotating each angle in increments of 10°. As can be seen in Figure 4, for the two active compounds **19** and **20**, τ_1 and τ_2 occupy common low-energy conformational space. The same is observed for the other pair of compounds, **14** and **15**, which possess lower activity.

Previous studies[5,20] have noted the importance of O−O distance (3α-OH H-donor and side chain H-acceptor) for several anesthetic steroids that modulate GABA$_A$ receptors. The O_3-O_{20} distance in the active compounds **19** and **20** is 9.97 and 10.04 Å, respectively, whereas, for the less-potent analogues **14** and **15**, the distance is 10.64 and 10.66 Å, respectively. However, only the O_3-O_{20} distance of the less-active analogues is within the range of the corresponding distance values (10.19−11.22 Å) found in active allopregnanolone analogues.[5,20] Hence, we propose that for pregnanediols, the favorable distances between the hydrogen-bonding groups do not fall within the range of the anesthetic steroid allopregnanolone but are shorter. However, additional

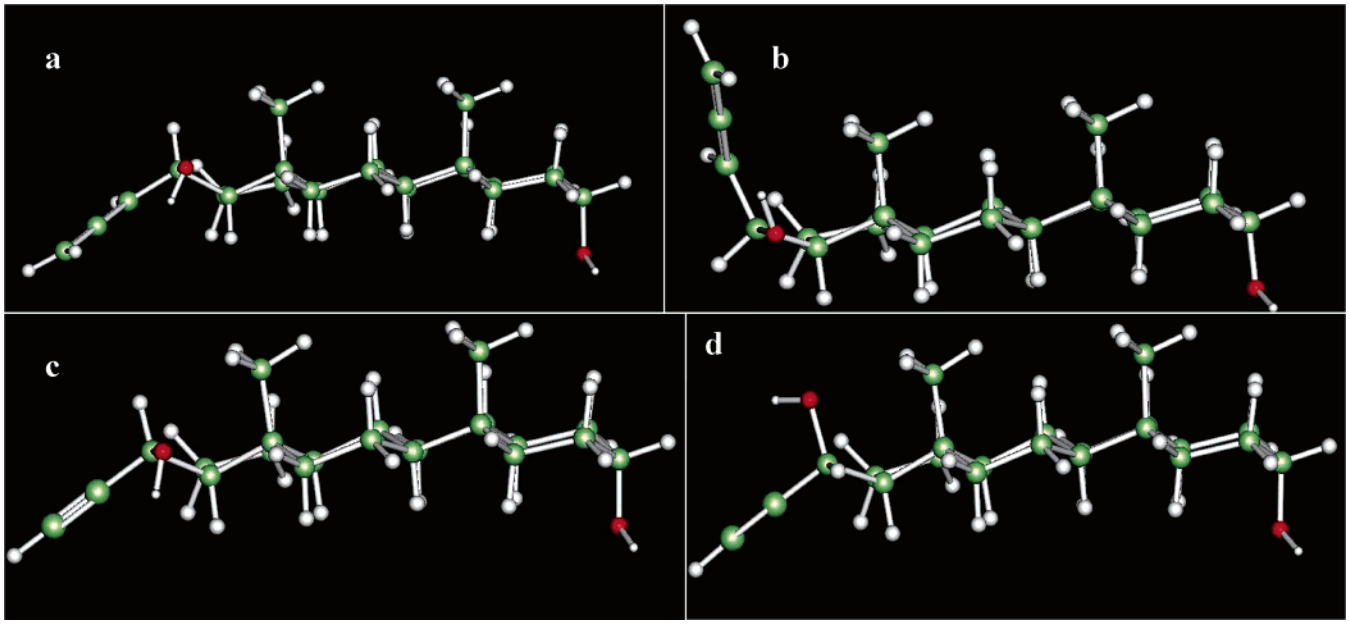


Figure 2. Lowest-energy conformers of compounds **20** (a), **15** (b), **19** (c), and **14** (d).

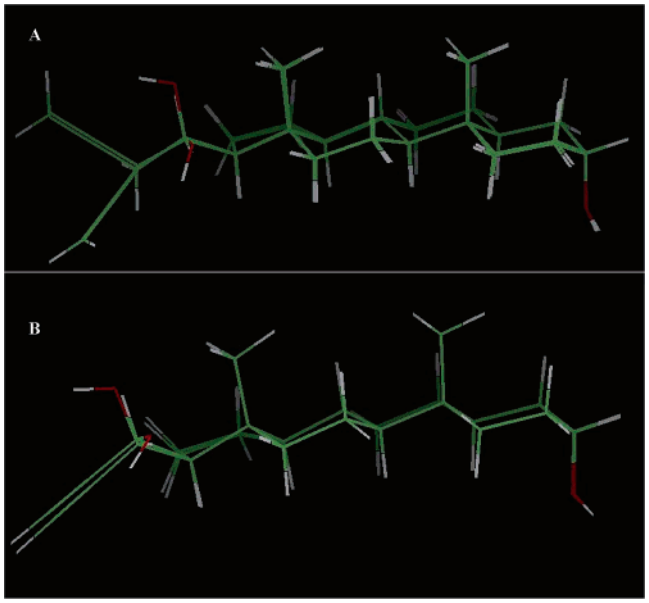


Figure 3. (A) Superimposition of compounds **20** and **15**. (B) Superimposition of compounds **14** and **19**.

studies with other pregnanediols will be needed to refine the SAR of this group of compounds.

The ternary allosteric model of Ehlert[26] has been successfully applied to ionotropic glycine receptors[27] but not to [^{3}H]EBOB binding to $GABA_A$ receptors. Therefore, the binding data need to be discussed. Full maximal displacement of [^{3}H]EBOB binding means strong negative cooperativity between steroidal and [^{3}H]-EBOB binding in the allosteric model. The resultant cooperativity factors cannot be determined exactly ($\alpha > 10$). On the other hand, the majority of our neuroactive steroids can be characterized by partial maximal displacement and weaker cooperativity with α values between 1 and 10 (Table 1). However, partial maximal displacement can also be interpreted with a subpopulation of cerebellar $GABA_A$ receptors that are insensitive for that steroid. Accordingly, allopregnanolone metabolites with 20-hydroxylated pregnanediol structures have shown heterogeneous displacement of [^{35}S]TBPS binding in rat brain regions.[25b] Three of our findings cannot be reconciled with receptor heterogeneity. (1) Three compounds (pregnanediols **18** and **23** and allopregnanolone) out of the thirteen closely related steroids of this study produced full maximal displacement. Accordingly, allopregnanolone has shown full, homogeneous displacement of [^{35}S]TBPS binding as well.[14] (2) The α values of the other 10 steroids vary between 1.7 and 5.8 (Table 1), which would correspond to different receptor subpopulations with a high affinity for the steroids. (3) Lower-affinity populations showing the displacement of [^{3}H]EBOB binding could not be observed within the solubility limits of the steroids. K_S values do not significantly depend on the binding models (allostery or heterogeneity), and they correlate with the pharmacological potencies of neuroactive steroids to modulate $GABA_A$ receptor−ionophore activity for [^{35}S]-TBPS binding.[14] On the other hand, α values do not show an apparent correlation with any known GABAergic pharmacological parameters of neurosteroids.

Conclusions

The goal of this study was to develop a series of allopregnanolone analogues substituted by conformationally constrained 17β side chains to obtain additional information about the SAR of 5α-reduced steroids to modulate $GABA_A$ receptors. Specifically, we investigated the presence of alkynyl or propadienyl functionalities at positions C17 and C20 of the steroid skeleton. The most active derivative is (20*R*)-17β-(1-hydroxy-2,3-butadienyl)-5α-androstane-3-ol (**20**), which possesses low nanomolar potency to modulate cerebellar $GABA_A$ receptors. Theoretical conformational analysis showed that the two active compounds **19** and **20** adopt similar defined critical dihedral angle values, which could be an indication of conformational space preference.

Experimental Section

NMR spectra were recorded on a Bruker AC 300 spectrometer operating at 300 MHz for ^{1}H and 75.43 MHz for ^{13}C. ^{1}H NMR spectra are reported in units of δ relative to internal

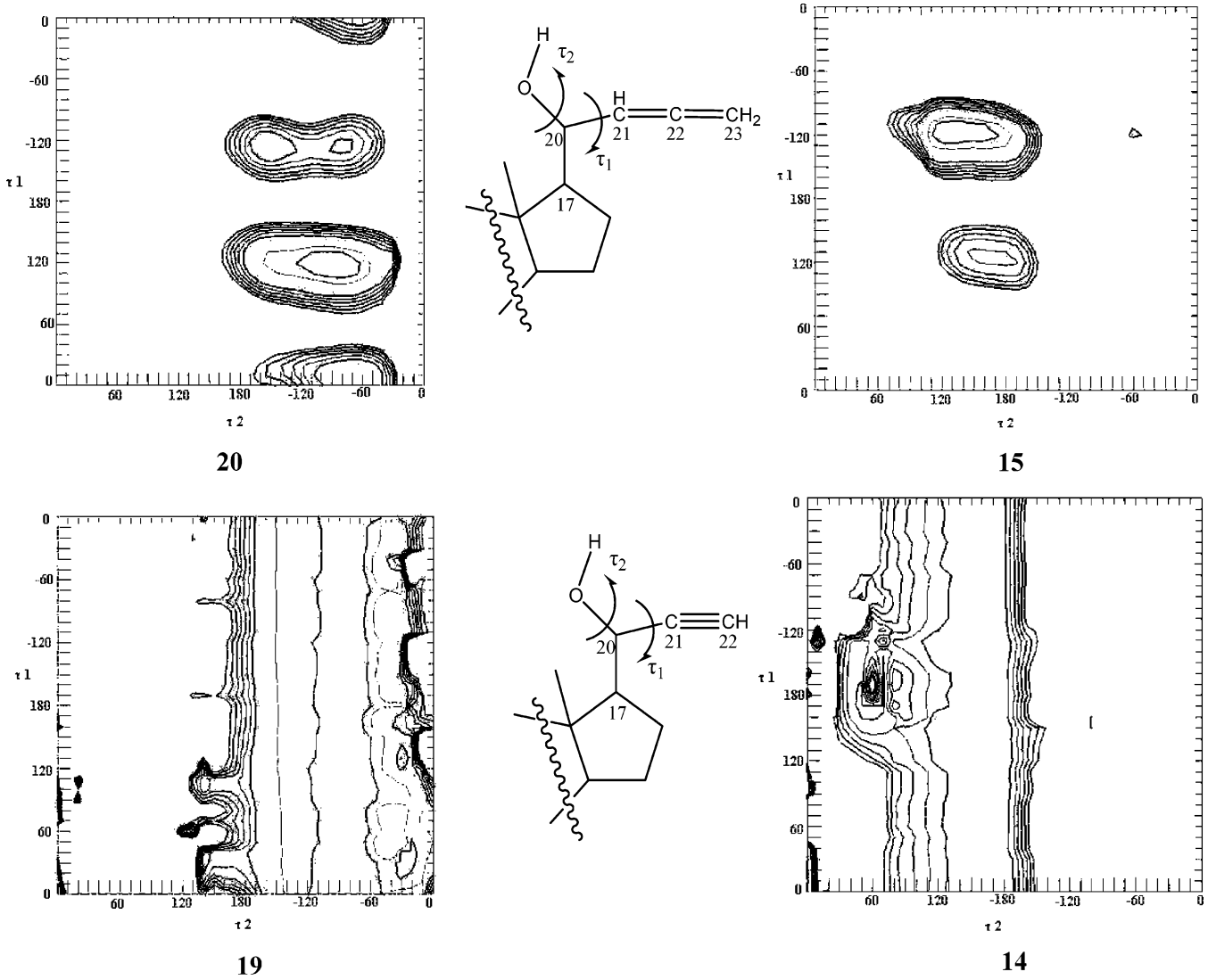


Figure 4. Contour plots generated for compounds **14**, **15**, **19**, and **20** by rotating around the τ_1 and τ_2 dihedral angles in increments of 10°. The contour levels, which are up to 6 kcal mol^{-1} higher than the lowest-energy minimum, are shown.

$CHCl_3$ at 7.24 ppm. ^{13}C NMR shifts are expressed in units of δ relative to $CDCl_3$ at 77.0 ppm. ^{13}C NMR spectra were proton noise decoupled. All NMR spectra were recorded in $CDCl_3$. Silica gel plates (Merck F254) were used for thin-layer chromatography. Chromatographic purification was performed with silica gel (200−400 mesh). Analyses, indicated by the symbols of the elements, were carried out by the microanalytical section of the Institute of Organic and Pharmaceutical Chemistry of the National Hellenic Research Foundation.

1,1-Dibromo-2-[3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17*β*-yl]-ethylene (4). Triphenylphosphine (3.14 g, 6 mmol) was added to a solution of tetrabromomethane (1.99 g, 6 mmol) in anhydrous methylene chloride (31 mL) at 0 °C, and the resulting mixture was stirred for 10 min. Subsequently, a solution of 3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17*β*-carboxaldehyde (**3**) (540 mg, 1 mmol) in methylene chloride (6 mL) was added, and the mixture was stirred at 0 °C for an additional 10 min. The reaction mixture was diluted with ethyl acetate, and the organic layer was washed with a water/saturated aqueous NaCl solution and was dried with anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the residue was purified by flash column chromatography using petroleum ether 40−60 °C/ether 95/5 as eluent to afford 560 mg (93%) of compound **4** as a solid. mp: 69−71 °C; Anal. ($C_{37}H_{50}OSiBr_2$) C, H.

17*β*-Ethynyl-3α-(*tert*-butyldiphenylsilyloxy)-5α-androstane (5). A solution of *n*-BuLi (1.6 M) in hexanes (0.45 mL, 0.72 mmol) was added to a solution of **4** (230 mg, 0.33 mmol) in anhydrous THF (8 mL) at −78 °C, and the resulting mixture was stirred at this temperature for 1 h. The mixture was quenched by the addition of saturated aqueous NH_4Cl solution. Ethyl acetate was added, and the organic layer was extracted with water and brine and was dried with anhydrous Na_2SO_4. Evaporation of the solvent in vacuo followed by purification of the residue by flash column chromatography [petroleum ether 40−60 °C/ether (97/3)] afforded 160 mg (89%) of the desired compound **5** as a solid. mp: 60−62 °C; Anal. ($C_{37}H_{50}$-OSi) C, H.

3α-(*tert*-Butyldiphenylsilyloxy)-17*β*-[2-(4-tolyl)ethynyl]-5α-androstane. A solution of **5** (120 mg, 0.23 mmol) in pyrrolidine (2 mL) was added to a mixture of tetrakis triphenylposphine palladium (0) (13.28 mg, 0.0115 mmol) and CuI (4.4 mg, 0.023 mmol) in pyrrolidine (1 mL). 4-Iodotoluene (150 mg, 0.69 mmol) was added to the resulting mixture and was stirred at rt for 24 h. Subsequently, saturated aqueous NH_4Cl solution was added, followed by the addition of ethyl acetate. The organic layer was extracted with water and brine and was dried with anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the residue was purified by flash column chromatography [petroleum ether 40−60 °C/acetone (80/20)] to afford 100 mg (72%) of 3α-(*tert*-butyldiphenylsilyloxy)-17*β*-[2-(4-tolyl)ethynyl]-5α-androstane as a solid. mp: 79−81 °C; Anal. ($C_{44}H_{56}OSi$) C, H.

17*β*-[2-(4-Tolyl)ethynyl]-5α-androstane-3α-ol (6). A solution of 3α-(*tert*-butyldiphenylsilyloxy)-17*β*-[2-(4-tolyl)ethynyl]-5α-androstane (0.074 mmol) in anhydrous THF (3 mL)

was treated with a 1 M solution of $(n\text{-Bu})_4N^+F^-$ (1.48 mL, 1.48 mmol), and the resulting solution was stirred at rt for 48 h. The reaction was cooled to 0 °C and was quenched with a saturated aqueous NH_4Cl solution. Ethyl acetate was added, and the organic layer was extracted with water and brine and was dried with anhydrous Na_2SO_4. Evaporation of the solvent in vacuo, followed by purification of the residue by flash column chromatography (methylene chloride), afforded the desired compound **6** in 50% yield as a solid. mp: 178−180 °C; ^{1}H NMR (δ): 0.77 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 0.86−2.08 (m, 23H), 2.30 (s, 3H, CH_3 aromatic), 4.02 (bs, 1H, 3β-H), 7.03−7.06 (d, J = 7.97 Hz, 2H), 7.25−7.27 (d, J = 7.97 Hz, 2H); ^{13}C NMR (δ): 11.18, 13.77, 20.57, 21.31, 24.61, 28.74, 28.99, 29.28, 32.05, 32.19, 35.84, 36.11, 36.16, 37.48, 39.14, 42.88, 44.44, 54.40, 54.83, 66.51, 82.43, 90.94, 121.18, 128.81, 131.38, 137.17; Anal. ($C_{28}H_{38}O$) C, H.

17β-(2-Bromoethynyl)-5α-androstane-3α-ol (7). A solution of **4** (0.074 mmol) in anhydrous THF (3 mL) was treated with a 1 M solution of $(n\text{-Bu})_4N^+F^-$ (1.48 mL, 1.48 mmol), and the resulting solution was stirred at rt for 48 h. The mixture was quenched with a saturated aqueous NH_4Cl solution. Ethyl acetate was added, and the organic layer was extracted with water and brine and was dried with anhydrous Na_2SO_4. Evaporation of the solvent in vacuo, followed by purification of the residue by flash column chromatography [methylene chloride/ethyl acetate (95/5)], afforded the desired compound **7** (70%) as a solid. mp: 165−167 °C; ^{1}H NMR (δ): 0.75 (s, 3H, CH_3), 0.76 (s, 3H, CH_3), 0.89−1.79 (m, 22H), 2.15 (t, J = 9.5 Hz, 1H, 17α-H), 4.01 (bs, 1H, 3β-H); ^{13}C NMR (δ): 11.18, 13.74, 20.52, 24.52, 28.45, 28.73, 29.02, 32.01, 32.19, 35.84, 36.03, 36.17, 37.37, 39.02, 39.14, 43.22, 44.32, 54.34, 54.60, 66.52, 81.89; Anal. ($C_{21}H_{31}OBr$) C, H.

4-[3α-(*tert*-Butyldiphenylsilyloxy)-5α-androstan-17β-yl]-3-butyn-2-ol (8). A solution of *n*-BuLi (1.6 M) in hexanes (2.76 mL, 4.4 mmol) was added to a solution of **4** (308 mg, 0.44 mmol) in anhydrous THF (11 mL) at −78 °C, and the mixture was stirred for 2 h at this temperature. Acetaldehyde (0.032 mL, 0.57 mmol) was added at −78 °C, and the temperature was permitted to rise to 25 °C. The addition of a 2 M HCl solution at 0 °C was followed by an extraction with ethyl acetate. The organic layer was washed with $NaHCO_3$ and brine and was dried with anhydrous Na_2SO_4, and the solvent was evaporated in vacuo. Subsequent purification of the residue by flash column chromatography [using petroleum ether 40−60 °C/ethyl acetate (90/10) as eluent] afforded 160 mg (62%) of the desired compound **8** as a solid. mp: 68−70 °C; Anal. ($C_{39}H_{54}O_2Si$) C, H.

17β-(3-Hydroxy-1-butynyl)-5α-androstane-3α-ol (9). Removal of the protecting group from **8** was accomplished following the same procedure as that used for compound **6** to afford 83% of the desired product **9** after purification by flash column chromatography [dichloromethane/ethyl acetate (90/10)]. mp: 169−171 °C; ^{1}H NMR (δ): 0.72 (s, 3H, CH_3), 0.76 (s, 3H, CH_3), 0.83−1.99 (m, 22H), 1.39 (s, 3H, CH(OH)CH_3), 2.14 (t, J = 9.36 Hz, 1H, 17α-H), 4.01 (bs, 1H, 3β-H), 4.5 (m, 1H, CH(OH)CH_3); ^{13}C NMR (δ): 11.19, 13.68, 20.53, 24.53, 24.95, 28.46, 29.01, 29.15, 32.04, 32.20, 35.84, 36.06, 36.17, 37.38, 39.15, 42.10, 44.02, 54.38, 54.77, 58.68, 66.52, 84.22, 85.94. Anal. ($C_{23}H_{36}O_2$) C, H.

3-Trimethylsilyl-1-[3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17β-yl]-2-propyn-1-ol (10a,b). A solution of 1.6 M *n*-BuLi in hexanes (1.07 mL, 1.715 mmol) was added to a solution of trimethylsilylacetylene (0.24 mL, 1.715 mmol) in anhydrous THF (5.7 mL) at 0 °C, and the resulting mixture was stirred for 2 h. Subsequently, the mixture was cooled to −78 °C, a solution of **3** (310 mg, 0.57 mmol) in anhydrous THF (5.7 mL) was added, and the mixture was stirred at this temperature for 3 h. The quenching of the reaction by the addition of NH_4Cl was followed by an extraction with ethyl acetate. The organic layer was washed with water and brine and dried with anhydrous Na_2SO_4, and the solvent was evaporated in vacuo. Subsequent purification of the residue by flash column chromatography [petroleum ether 40−60 °C/acetone (92/8)] afforded, in 85% total yield, the two diastereomers **10a** and **10b** in a 3:1 ratio, respectively, each as a solid.

(20*R*)-1-[3α(*tert*-Butyldiphenylsilyloxy)-5α-androstan-17β-yl]-3-trimethylsilyl-2-propyn-1-ol (10a) Less-polar diastereomer: 233 mg, mp: 68−71 °C; ^{1}H NMR (δ): 0.15 (s, 9H, $Si(CH_3)_3$), 0.71 (s, 6H, 18,19-CH_3), 0.84−2.07 (m, 23H), 1.06 (s, 9H, $C(CH_3)_3$), 3.99 (bs, 1H, 3β-H), 4.27 (d, J = 9.54 Hz, 1H, 20-H), 7.32−7.42 (m, 6H), 7.63−7.66 (m, 4H); ^{13}C NMR (δ): 0.09, 11.39, 12.39, 19.36, 20.62, 24.30, 25.42, 27.06, 28.55, 29.32, 32.23, 32.66, 35.47, 36.05, 36.17, 39.36, 39.56, 42.54, 54.56, 56.00, 56.75, 65.21, 68.08, 91.48, 107.43, 127.42, 129.37, 134.90, 135.77; Anal. ($C_{41}H_{59}O_2Si_2$) C, H.

(20*S*)-1-[3α(*tert*-Butyldiphenylsilyloxy)-5α-androstan-17β-yl]-3-trimethylsilyl-2-propyn-1-ol (10b) More-polar diastereomer: 78 mg. mp: 62−65 °C; ^{1}H NMR (δ): 0.15 (s, 9H, $Si(CH_3)_3$), 0.68 (s, 3H, CH_3), 0.71 (s, 3H, CH_3), 0.75−2.23 (m, 23H), 1.05 (s, 9H, $C(CH_3)_3$), 3.99 (bs, 1H, 3β-H), 4.10−4.15 (m, 1H, 20-H), 7.31−7.39 (m, 6H), 7.62−7.65 (m, 4H); ^{13}C NMR (δ): 0.17, 11.49, 12.78, 19.39, 20.51, 23.91, 26.62, 27.08, 28.58, 29.37, 32.30, 32.75, 35.25, 36.08, 36.21, 38.61, 39.42, 41.92, 54.62, 56.20, 56.72, 64.92, 68.11, 90.23, 107.31, 127.46, 129.40, 134.83, 135.80; Anal. ($C_{41}H_{59}O_2Si_2$) C, H.

3α-(*tert*-Butyldiphenylsilyloxy)-5α-androstan-17β-carboxylic acid methoxy methyl amide. A solution of 3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17β-carboxylic acid (**2**) (490 mg, 0.88 mmol) in anhydrous dichloromethane (5 mL) was treated with *N*,*O*-dimethylhydroxylamine hydrochloride (129 mg, 1.32 mmol), EDCI (161 mg, 1.32 mmol), and DMAP (253 mg, 1.32 mmol), and the resulting mixture was stirred at rt for 2h. The reaction was quenched by the addition of saturated aqueous NaCl, which was followed by an extraction with ethyl acetate. The organic layer was washed with 5% HCl and brine, and was then dried over anhydrous Na_2SO_4. Evaporation of the solvent in vacuo and purification of the residue by flash column chromatography [petroleum ether 40°−60 °C/acetone (85:15)] afforded the corresponding Weinreb amide as a white solid (520 mg, 98% yield).

1-[3α-(*tert*-Butyldiphenylsilyloxy)-5α-androstan-17β-yl]-3-triisopropylsilyl-propynone (11) A solution of *n*-BuLi (1.6 M) in hexanes (1.3 mL, 2.06 mmol) was added to a solution of triisopropylsilyl acetylene (0.7 mL, 3.09 mmol) in anhydrous THF (10 mL) dropwise at −40 °C. The resulting solution was stirred for 1h at −40 °C, and subsequently, a solution of 3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17β-carboxylic acid methoxy methyl amide (620 mg, 1.03 mmol) in anhydrous THF (1.3 mL) was added dropwise to the acetylide solution at −10 °C under an argon atmosphere. The reaction mixture was further stirred for 2 h at −10 °C and was quenched by the addition of saturated aqueous NH_4Cl, which was followed by an extraction with diethyl ether. The organic layer was washed with brine and was then dried over anhydrous Na_2SO_4. Evaporation of the solvent and purification of the residue by flash column chromatography [petroleum ether 40°−60 °C/acetone (98:2)] afforded ketone **11** as a viscous oil (520 mg, 70% yield).

(20*S*)-1-[3α(*tert*-Butyldiphenylsilyloxy)-5α-androstan-17β-yl]-3-triisopropyllsilyl-2-propyn-1-ol (12b). A solution of ketone **11** (120 mg, 0.16 mmol) in anhydrous dichloromethane was treated with (*S*)-3,3-diphenyl-1-methyltetrahydro-3*H*-pyrrolo[1,2-c][1,3,2]oxazaborole (1 M) in toluene (0.024 mL, 0.024 mmol). The resulting mixture was cooled to −78 °C, and a solution of catecholborane (0.02 mL, 0.192 mmol) in anhydrous dichloromethane was then added dropwise. After the mixture had been stirred for 5h at −78 °C, methanol (1 mL) was added, and the solution was warmed to rt and diluted with diethyl ether. The organic layer was first washed with a mixture of 1N NaOH-saturated aqueous $NaHCO_3$ (1:2) until it became colorless, then washed with brine, and then was dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by flash column chromatography [petroleum ether 40°−60 °C/acetone (90:10)] to afford **12b** as a white solid (110 mg, 95% yield, and 100% ee). ^{1}H NMR (δ): 0.72 (s, 3H, CH_3), 0.73 (s, 3H, CH_3), 1.09 (s, 21H), 1.23 (s, 9H),

2.26−1.25 (m, 22H), 4.01 (bs, 1H, 3*β*-H), 4.23 (bs, 1H, 20*H*), 7.41−7.33 (m, 6H), 7.68−7.64 (m, 4H).

(20*R*)-1-[3α(*tert*-Butyldiphenylsilyloxy)-5α-androstan-17*β*-yl]-3-triisopropyllsilyl-2-propyn-1-ol (12a). A solution of(*S*)-3,3-diphenyl-1-methyltetrahydro-3H-pyrrolo[1,2-c][1,3,2]-oxazaborole (1 M) in toluene (0.084 mL, 0.084 mmol) was added to a solution of ketone **11** (30 mg, 0.042 mmol) in 3 mL of THF. The resulting solution was cooled to −30 °C, and then 0.02 mL (0.21 mmol) of borane methyl sulfide complex was added. After the reaction was completed, it was quenched by the slow addition of methanol (1.0 mL). The reaction mixture was diluted with diethyl ether, and the organic layer was washed with saturated NH_4Cl, 5% $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4 and was concentrated in vacuo. The residue was purified by flash column chromatography [petroleum ether 40°−60 °C/acetone (90:10)] to afford **12a** as a white solid (28 mg, 93% yield, and 100% ee). ^{1}H NMR (*δ*): 0.71 (s, 6H, 18,19-CH_3), 0.84−2.07 (m, 23H), 1.06 (s, 9H, C(CH_3)$_3$), 3.99 (bs, 1H, 3*β*-H), 4.27 (d, J = 9.54 Hz, 1H, 20-H), 7.32−7.42 (m, 6H), 7.63−7.66 (m, 4H).

17*β*-(3-Oxo-1-propynyl)-5α-androstan-3α-ol (13). A solution of **11** (155 mg, 0.215 mmol) in anhydrous dichloromethane (9 mL) was treated with HF-pyridine (0.86 mmol, 0.024 mL) at 0 °C, and the resulting mixture was stirred at rt for 2 h. The addition of water at 0 °C was followed by an extraction with methylene chloride. The organic layer was washed with brine and was dried with anhydrous Na_2SO_4, and the solvent was evaporated in vacuo. Subsequent purification of the residue by flash column chromatography [dichloromethane/ethyl acetate (95/5)] afforded the desired compound **13** in 76% yield as a solid. mp:148−150 °C; ^{1}H NMR (*δ*): 0.67 (s, 3H, CH_3), 0.77 (s, 3H, CH_3), 0.80−2.27 (m, 22H), 2.62 (t, J = 8.8 Hz, 1H, 17α-H), 3.21 (s, 1H, acetylenic), 4.04 (bs, 1H, 3*β*-H); ^{13}C NMR (*δ*): 11.23, 13.49, 20.64, 22.39, 24.26, 28.46, 29.05, 31.97, 32.22, 35.53, 35.90, 36.17, 38.69, 39.15, 54.29, 56.84, 64.81, 66.52, 73.85, 78.65, 97.77, 197.91; Anal. ($C_{22}H_{32}O_2$) C, H.

(20*R*)-17*β*-(1-Hydroxy-2-propynyl)-5α-androstan-3-ol (14). Compound **12a** (200 mg, 0.31 mmol) was treated with a solution of 1 M (n-Bu)$_4$N$^+$F$^-$ (7.8 mL, 7.8 mmol) in hexanes at rt for 24 h. The reaction was completed using the same procedure as that used for compound **6** to afford, after purification by flash column chromatography using dichloromethane/ethyl acetate 90/10 as elution solvent, alcohol **14** in 80.5% yield. mp: 197−199 °C; ^{1}H NMR (*δ*): 0.73 (s, 3H, 18-CH_3), 0.77 (s, 3H, 19-CH_3), 0.80−2.10 (m, 23H), 2.41 (d, J = 1.81 Hz, 1H), 4.03 (bs, 1H, 3*β*-H), 4.24−4.72 (m, 1H, 20-H). ^{13}C NMR (*δ*): 11.23, 12.24, 20.59, 24.30, 25.34, 28.53, 29.03, 32.03, 32.20, 35.43, 35.91, 36.15, 39.16, 39.63, 42.56, 54.45, 56.07, 56.67, 64.68, 66.01, 72.50, 85.44; Anal. ($C_{22}H_{34}O_2$) C, H.

(20*S*)-17*β*-(1-Hydroxy-2,3-butadienyl)-5α-androstane-3-ol (15). Paraformaldehyde (20 mg), copper hypobromide (4 mg, 0.027 mmol), and diisopropylamine (0.07 mL, 0.5 mmol) were sequentially added to a solution of alcohol **14** (83 mg, 0.25 mmol) in anhydrous dioxane (3 mL). The resulting mixture was refluxed for 24 h. The addition of HCl 2 M and extraction with ethyl acetate were followed by an extraction of the organic layer with $NaHCO_3$, water, and brine and a drying with anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the residue was purified by flash column chromatography, using dichloromethane/ethyl acetate 85/15 as elution solvent, to afford allenic alcohol **15** (45 mg, 52%). mp: 184−187 °C.; ^{1}H NMR (*δ*): 0.76 (s, 3H, CH_3), 0.78 (s, 3H, CH_3), 0.85−2.10 (m, 23H), 4.03 (bs, 1H, 3*β*-H), 4.07 (m, 1H, 20-H), 4.82 (dd, J = 1.23 Hz, 2H, 23-H), 5.20 (q, J = 6.6 Hz, 1H, 21-H).; ^{13}C NMR (*δ*): 11.18, 12.42, 20.64, 24.43, 25.29, 28.54, 29.03, 32.06, 32.18, 35.39, 35.88, 36.13, 39.15, 39.85, 42.74, 54.41, 56.03, 56.75, 66.59, 72.56, 77.16, 94.89, 207.05.; IR(cm^{-1}): 1995 (C=C=CH_2), 1255 (C−O); Anal. ($C_{23}H_{36}O_2$) C, H.

(20*R*)-3-Triisopropyl-1-methoxy-1-[3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17*β*-yl]-2-propyne. Sodium hydride (60% dispersion in mineral oil, 12 mg, 0.28 mmol) was washed with dry hexane (2 × 2 mL), and then 1.5 mL of THF was added. The slurry was cooled to 0 °C, and a solution of alcohol **12a** (100 mg, 0.14 mmol) in THF (2 mL) was added. The reaction mixture was stirred at rt, and, after 30 min, MeI (0.02 mL, 0.28 mmol) was added; stirring was then continued for 5 h. The reaction was quenched by saturated aqueous NH_4-Cl and was diluted with ethyl acetate, and the organic layer was washed with brine and was dried over anhydrous Na_2-SO_4. After the evaporation of the solvent, the residue was purified by flash column chromatography [petroleum ether 40−60 °C/dietyl ether (98:2)] to afford (20*R*)-3-triisopropyl-1-methoxy-1-[3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17*β*-yl]-2-propyne as a white solid (70 mg, 70% yield).

(20*R*)-17*β*-(1-Methoxy-2-propynyl)-5α-androstan-3α-ol (16) The deprotection of 3α-OH and the removal of the triisopropylsilyl group in 3-triisopropylsilyl-1-methoxy-1-[3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17*β*-yl]-2-propyne were accomplished in one step by adding 1 M (n-Bu)$_4$N$^+$F$^-$ solution in THF (1.9 mL, 1.9 mmol) to a solution of the above compound (50 mg, 0.076 mmol) in anhydrous THF (3 mL). The resulting solution was stirred at rt for 24 h and was then taken through the same process used to obtain compound **6** to afford, after purification by flash column chromatography using petroleum ether 40−60 °C/acetone 82/18 as the elution solvent, methoxy derivative **16**. mp: 161−163 °C; ^{1}H NMR (*δ*): 0.69 (s, 3H, 18-CH_3), 0.77 (s, 3H, 19-CH_3), 0.79−2.24 (m, 23H), 2.44 (d, J = 1.83 Hz, 1H), 3.37 (s, 3H, OCH_3), 3.70 (dd, J = 9.8 Hz, 1H, 20-H), 4.03 (bs, 1H, 3*β*-H); ^{13}C NMR (*δ*): 10.74, 11.94, 20.44, 23.89, 26.62, 28.54, 29.02, 32.03, 32.16, 35.17, 35.89, 36.11, 38.63, 39.14, 41.74, 54.28, 54.37, 55.90, 56.13, 66.58, 73.11, 74.59, 82.95; Anal. ($C_{23}H_{35}O_2$) C, H.

(20*R*)-1-[3α-(*tert*-Butyldiphenysilyloxy)-5α-androstan-17*β*-yl]-2-propyn-1-ol (17) A solution of 1 M (n-Bu)$_4$N$^+$F$^-$ in THF (0.72 mL, 0.72 mmol) was added to a solution of **12a** (310 mg, 0.48 mmol) in THF (10 mL), and the resulting mixture was stirred at rt for 0.5 h. The reaction mixture was taken through the same process used to obtain compound **6** to afford, after purification by flash column chromatography using petroleum ether 40−60 °C/acetone 82/18 as the elution solvent, compound **17** (230 mg, 84%). Anal. ($C_{38}H_{52}O_2Si$) C, H.

(20*R*)-1-{3α-(*tert*-Butyldiphenysilyloxy)-5α-androstan-17*β*-yl-[3-(4-methoxyphenyl)-2-propynyl]}-1-ol. A solution of **17** (100 mg, 0.176 mL) in pyrrolidine (2.5 mL) was added to a solution of 4-iodoanisole (82.38 mg, 0.35 mmol), CuI (19 mg, 0.1 mmol), and tetrakis(triphenylphosphine) palladium (0) (58 mg, 0.05 mmol) in pyrrolidine (1.5 mL). The resulting mixture was stirred for 24 h at rt. The quenching of the reaction by the addition of NH_4Cl was followed by an extraction with ethyl acetate. The organic layer was washed with water and brine and dried with (Na_2SO_4), and the solvent was evaporated in vacuo. Subsequent purification of the residue with flash column chromatography using petroleum ether 40−60 °C/ acetone 80/20 as the eluent afforded, in 84% yield, (20*R*)-1-{3α-(*tert*-butyldiphenysilyloxy)-5α-androstan-17*β*-yl-[3-(4-methoxy phenyl)-2-propynyl]}-1-ol as a solid. mp: 63−65 °C; Anal. ($C_{45}H_{57}O_3Si$) C, H.

(20*R*)-17*β*-[1-Hydroxy-3-(4-methoxyphenyl)-2-propynyl]-5α-androstan-3α-ol (18). The removal of the protecting group in (20*R*)-1-{3α-(*tert*-butyldiphenysilyloxy)-5α-androstan-17*β*-yl-[3-(4-methoxyphenyl)-2-propynyl]}-1-ol was accomplished following the same procedure as that followed for compound 6 to afford, after flash column purification using dichloromethane/ethyl acetate (90/10) as the elution solvent, compound **18** in 77% yield. mp: 185−188 °C.; ^{1}H NMR (*δ*): 0.76 (s, 3H, 19-CH_3), 0.78 (s, 3H, 18-CH_3), 0.81−2.08 (m, 23H), 3.79 (s, 1H, OCH_3), 4.03 (bs, 1H, 3*β*-H), 4.45−4.49 (m, 1H, 20-H), 6.80 (d, J = 8.8 Hz, 2H), 7.32 (d, J = 8.8 Hz, 2H).; ^{13}C NMR (*δ*): 11.19, 12.38, 20.58, 24.30, 25.45, 28.52, 29.02, 32.02, 32.16, 35.40, 35.87, 36.14, 39.14, 39.61, 42.56, 54.42, 55.26, 56.07, 57.07, 65.28, 66.60, 84.40, 89.22, 113.86, 114.99, 133.03, 159.52; Anal. ($C_{29}H_{39}O_3$) C, H.

(20*S*)-17*β*-(1-Hydroxy-2-propynyl)-5α-androstan-3-ol (19). Compound **12b** (200 mg, 0.31 mmol) was treated with a solution of 1 M (n-Bu)$_4$N$^+$F$^-$ (7.8 mL, 7.8 mmol) in hexanes at rt for 24 h. The reaction was completed using the same

procedure as that used for compound **6** to afford, after purification by flash column chromatography using dichloromethane/ethyl acetate 90/10 as the elution solvent, alcohol **19** in 80.5% yield. mp: 187−190 °C. ^{1}H NMR (δ): 0.69 (s, 3H, 18-CH_3), 0.77 (s, 3H, 19-CH_3), 0.79−2.21 (m, 23H), 2.49 (d, J = 2.02 Hz, 1H), 4.03 (bs, 1H, 3β-H), 4.15 (d, J = 8.6 Hz, 1H, 20-H). ^{13}C NMR (δ): 11.17, 12.73, 20.40, 23.84, 26.32, 28.51, 29.02, 32.02, 32.16, 35.13, 35.87, 36.11, 38.51, 39.15, 41.87, 54.38, 56.19, 56.48, 64.15, 66.58, 85.28; Anal. ($C_{22}H_{34}O_2$) C, H.

(20*R*)-17β-(1-Hydroxy-2,3-butadienyl)-5α-androstane-3-ol (20). Paraformaldehyde (20 mg), copper hypobromide (4 mg, 0.027 mmol), and diisopropylamine (0.07 mL, 0.5 mmol) were sequentially added to a solution of alcohol **19** (83 mg, 0.25 mmol) in anhydrous dioxane (3 mL). The resulting mixture was refluxed for 24 h. The addition of HCl 2 M and extraction with ethyl acetate was followed by an extraction of the organic layer with $NaHCO_3$, water, and brine and a drying with anhydrous Na_2SO_4. The solvent was evaporated in vacuo, and the residue was purified by flash column chromatography using dichloromethane/ethyl acetate 85/15 as the elution solvent to afford allenic alcohol **20** (45 mg, 52%). mp: 174−177 °C. ^{1}H NMR (δ): 0.66 (s, 3H, 18-CH_3), 0.77 (s, 3H, 19-CH_3), 0.79−1.91 (m, 23H), 4.03 (bs, 2H, 3β-H, 20-H), 4.79 (dd, J = 0.90 Hz, 2H, 23-H), 5.20 (q, J = 6.4 Hz, 1H, 21-H). ^{13}C NMR (δ): 11.17, 12.98, 20.48, 24.11, 25.95, 28.53, 29.02, 32.00, 32.19, 35.14, 35.88, 36.21, 38.92, 39.14, 41.96, 54.42, 56.17, 56.91, 66.57, 72.92, 77.19, 94.76, 207.60; IR(cm^{-1}): 1995 (C=C=CH_2), 1255 (C−O); Anal. ($C_{23}H_{36}O_2$) C, H.

(20*S*)-1-Methoxy-1-[3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17β-yl]-2-propyne. Sodium hydride (60% dispersion in mineral oil, 12 mg, 0.28 mmol) was washed with dry hexane (2 × 2 mL), and 1.5 mL THF was added. The slurry was cooled to 0 °C, and a solution of alcohol **12b** (100 mg, 0.14 mmol) in THF (2 mL) was added. The reaction mixture was stirred at rt, and, after 30 min, MeI (0.02 mL, 0.28 mmol) was added; stirring was then continued for 5 h. The reaction was quenched by saturated aqueous NH_4Cl and was diluted with ethyl acetate, and the organic layer was washed with brine and was dried over anhydrous Na_2SO_4. After the evaporation of the solvent, the residue was purified by flash column chromatography [petroleum ether 40°−60 °C/diethyl ether (98:2)] to afford (20*S*)-1-methoxy-1-[3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17β-yl]-2-propyne as a white solid (70 mg, 70% yield).

(20*S*)-17β-(1-Methoxy-2-propynyl)-5α-androstan-3α-ol (21) The deprotection of 3α-OH and the removal of the triisopropylsilyl group in (20*S*)-3-triisopropylsilyl-1-methoxy-1-[3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17β-yl]-2-propyne was accomplished in one step by adding 1 M (n-Bu)$_4$N$^+$F$^-$ solution in THF (1.9 mL, 1.9 mmol) to a solution of the above compound (50 mg, 0.076 mmol) in THF (3 mL). The resulting solution was stirred at rt for 24 h and was then taken through the same process used to obtain compound **6** to afford, after purification by flash column chromatography using petroleum ether 40−60 °C/acetone 82/18 as the elution solvent, methoxy derivative **21**. mp: 171−173 °C; ^{1}H NMR (δ): 0.66 (s, 3H, 18-CH_3), 0.77 (s, 3H, 19-CH_3), 0.86−2.34 (m, 23H), 2.37 (d, J = 1.7 Hz, 1H), 3.36 (s, 3H, OCH_3), 3.82 (dd, J = 10.37 Hz, 1H, 20-H), 4.02 (bs, 1H, 3β-H); ^{13}C NMR (δ): 11.20, 12.36, 20.55, 24.27, 25.36, 28.53, 29.03, 32.04, 32.15, 35.43, 35.88, 36.12, 39.13, 39.32, 42.58, 54.09, 54.41, 55.90, 56.13, 66.58, 73.34, 73.46, 82.89; Anal. ($C_{23}H_{35}O_2$) C, H.

(20*S*)-1-[3α-(*tert*-Butyldiphenysilyloxy)-5α-androstan-17β-yl]-2-propyn-1-ol (22). A solution of 1 M (n-Bu)$_4$N$^+$F$^-$ in THF (0.72 mL, 0.72 mmol) was added to a solution of **12b** (310 mg, 0.48 mmol) in THF (10 mL), and the resulting mixture was stirred at rt for 0.5 h. The reaction mixture was taken through the same process used to obtain compound **6** to afford, after purification by flash column chromatography using petroleum ether 40−60 °C/acetone 82/18 as the elution solvent, compound **22** in 84% yield (230 mg). Anal. ($C_{38}H_{52}O_2$-Si) C, H.

(20*S*)-1-{3α-(*tert*-Butyldiphenysilyloxy)-5α-androstan-17β-yl-[3-(4-methoxyphenyl)-2-propynyl]}-1-ol. A solution of **22** (100 mg, 0.176 mL) in pyrrolidine (2.5 mL) was added to a solution of 4-iodoanisole (82.38 mg, 0.35 mmol), CuI (19 mg, 0.1 mmol), and tetrakis(triphenylphosphine) palladium (0) (58 mg, 0.05 mmol) in pyrrolidine (1.5 mL). The resulting mixture was stirred for 24 h at rt. The quenching of the reaction by the addition of NH_4Cl was followed by an extraction with ethyl acetate. The organic layer was washed with water and brine and dried with anhydrous Na_2SO_4, and the solvent was evaporated in vacuo. Subsequent purification of the residue with flash column chromatography using petroleum ether 40−60 °C/acetone 80/20 as the eluent afforded, in 84% yield, (20*S*)-1-{3α-(*tert*-butyldiphenysilyloxy)-5α-androstan-17β-yl-[3-(4-methoxyphenyl)-2-propynyl]}-1-ol as a solid. mp: 50−53 °C; Anal. ($C_{45}H_{57}O_3Si$) C, H.

(20*S*)-17β-[1-Hydroxy-3-(4-methoxyphenyl)-2-propynyl]-5α-androstan-3α-ol (23) The removal of the protecting group of (20*S*)-1-{3α-(*tert*-butyldiphenysilyloxy)-5α-androstan-17β-yl-[3-(4-methoxyphenyl)-2-propynyl]}-1-ol was accomplished using the same procedure as that used for compound **6** to afford, after flash column purification using dichloromethane/ethyl acetate (90/10) as the elution solvent, compound **23** in 77% yield. mp: 159−161 °C; ^{1}H NMR (δ): 0.73 (s, 3H, 18-CH_3), 0.76 (s, 3H, 19-CH_3), 0.85−2.25 (m, 23H), 3.79 (s, 1H, OCH_3), 4.03 (bs, 1H, 3β-H), 4.33−4.36 (m, 1H, 20-H), 6.81 (d, J = 8.8 Hz, 2H), 7.33−7.36 (d, J = 8.8 Hz, 2H).; ^{13}C NMR (δ): 11.20, 12.78, 20.56, 24.02, 26.62, 28.53, 29.02, 32.03, 32.19, 35.16, 35.87, 36.13, 38.57, 39.15, 42.65, 54.39, 55.27, 56.17, 57.03, 65.03, 66.58, 85.41, 89.17, 113.92, 114.98, 132.94, 159.61; Anal. ($C_{29}H_{39}O_3$) C, H.

Theoretical Calculations. Compounds **14**, **15**, **19**, and **20** were subjected to energy minimization using steepest descents, conjugate gradients, and Newton−Raphson algorithms embedded in the Quanta/Charmm software of Accelrys and using an SG O2 workstation. The minimization procedure was ended using energy convergence criterion between successive steps (ΔE < 0.001 kcal mol^{-1}). The dielectric constant during the calculations was set to 1. Monte Carlo conformational search analysis was applied to obtain low-energy conformers for the compounds under study. These would serve as initial structures for grid-scan analysis. The application of Monte Carlo analysis, which allows full angular window specification and the random change of all flexible dihedral angles of flexibility, generated 1000 conformers, which were consequently subjected to energy minimization using 1000 iterations and the steepest descents algorithm. The four lowest-energy structures were then used as starting structures for grid-scan analysis. Each grid-scan analysis for the four compounds was performed around the τ_1 and τ_2 dihedral angles by rotating each angle in increments of 10°. Thus, 1296 (36 × 36) conformers were generated. None of these conformers had a lower value than the initial low-energy conformer derived by Monte Carlo analysis. The energy of the conformers versus the two dihedral angles τ_1 and τ_2 were plotted as contours using 12 contour levels, each one increasing by 0.5 kcal mol^{-1} from the lowest-energy minimum. Therefore, contour levels reach up to 6 kcal mol^{-1} from the lowest-energy minimum.

Receptor Binding. Crude synaptosomal membranes were prepared from the cerebella of male Wistar rats. Cerebellar homogenates in 0.32 M sucrose were centrifuged at 1.000*g* for 10 min. The supernatant was centrifuged at 45000*g* for 30 min. The pellet was homogenized in distilled water, centrifuged at 45000*g* for 30 min, washed by suspension in Tris HCl buffer (pH 7.4), and after two centrifugations as above was frozen. The thawed membranes were centrifuged in 50 mM Tris HCl buffer containing 0.2 M NaCl at 10000*g* for 10 min. For displacement studies, membrane suspensions were incubated with 1 nM [^{3}H]EBOB (30 Ci/mmol, Dupont-NEN) in the above buffer for 2 h at 25 °C. For nonspecific binding, 50 μM picrotoxin was applied. Duplicate 1-mL samples were filtered on Whatman GF/B filters under vacuum with a Brandel harvester and washed with 3 × 3 mL of buffer.

Scheme 5

$$\begin{array}{ccc} & K_E & \\ [E] + [R] + [S] & \leftrightarrow & [ER] + [S] \\ \updownarrow K_S & & \updownarrow \alpha K_S \\ [E] + [RS] & \leftrightarrow & [ERS] \\ & \alpha K_E & \end{array}$$

R: GABA$_A$ receptor
E: [^{3}H]EBOB
S: steroid
α: cooperativity factor

$$\frac{B_{ES}}{B_E} = \frac{[E] + K_E}{[E] + \dfrac{K_E(K_S + [S])}{K_S + [S]/\alpha}} \qquad (1)$$

Binding data were evaluated according to the ternary allosteric binding model of Ehlert[26] shown in Scheme 5. Ratios of specific binding in the presence (B_{ES}) and absence (B_E) of the steroids were fitted to eq 1 (Scheme 5) via nonlinear regression with GraphPad Prism 4.02 (San Diego, CA). Saturation analysis of [^{3}H]EBOB binding previously revealed a homogeneous population of binding sites in rat cerebellum with $K_E = 1.2 \pm 0.2$ nM. Three steroids (**18**, **23**, and allopregnanolone) resulted in apparently full maximal displacement of [^{3}H]EBOB binding, and the remaining binding could not be quantitatively distinguished from nonspecific binding. Therefore, cooperativity factors could not be exactly determined ($\alpha > 10$). On the other hand, steroid **20** resulted in low maximal displacement (~30%, Figure 1), and some displacement curves could not be fitted separately. Therefore, curve fitting was applied for the pooled experimental data for **20**.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **2** and **3**. Spectroscopic data for compounds **4**, **5**, **8**, **11**, **17**, **22**, 3α-(*tert*-butyldiphenylsilyloxy)-17β-[2-(4-tolyl)ethynyl]-5α-androstane, 3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17β-carboxylic acid methoxy methyl amide, (20*R*)-3-triisopropyl-1-methoxy-1-[3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17β-yl]-2-propyne, (20*R*)-1-{3α-(*tert*-butyldiphenysilyloxy)-5α-androstan-17β-yl-[3-(4-methoxyphenyl)-2-propynyl]}-1-ol, (20*S*)-1-methoxy-1-[3α-(*tert*-butyldiphenylsilyloxy)-5α-androstan-17β-yl]-2-propyne, (20*S*)-1-{3α-(*tert*-butyldiphenysilyloxy)-5α-androstan-17β-yl-[3-(4-methoxyphenyl)-2-propynyl]}-1-ol. Geometry optimization data and low-energy conformers for compounds **14**, **15**, **19**, and **20**. Analytical data for compounds **4**−**23**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Paul, S. M.; Purdy, R. H. Neuroactive steroids. *FASEB J.* **1992**, *6*, 2311−2322. (b) Robel, R.; Baulieu, E. E. Neurosteroids biosynthesis and function. *Trends Endocrinol. Metab.* **1994**, *5*, 1−8. (c) Kulkarni, S. K.; Reddy, D. S. Neurosteroids: a new class of neuromodulators. *Drugs Today* **1995**, *31*, 433−455. (d) Compagnone, N. A.; Mellon, S. H. Neurosteroids: biosynthesis and function of these novel neuromodulators. *Front. Neuroendocrinol.* **2000**, *21*, 1−56.

(2) (a) Schumacher, M.; Robel, P.; Baulieu, E. E. Development and regeneration of the nervous system: a role for neurosteroids. *Dev. Neurosci. (Basel, Switz.)* **1996**, *18*, 16−21. (b) Majewska, M. D. Neurosteroids: endogenous bimodal modulators of the GABA$_A$ receptor. Mechanism of action and physiological significance. *Prog. Neurobiol. (Amsterdam, Neth.)* **1992**, *38*, 379−395.

(3) Rupprecht, R.; Holsboer, F. Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. *Trends Neurosci.* **1999**, *22*, 410−416.

(4) (a) Lambert, J. J.; Belelli, D.; Hill-Venning, C.; Peters, J. A. Neurosteroids and the GABA$_A$ receptor function. *Trends Pharmacol. Sci.* **1995**, *16*, 95−303. (b) Lambert, J. J.; Peters, J. A.; Harney, S.; Belelli, D. Steroid modulation of GABA$_A$ receptors. In *Handbook of Experimental Pharmacology*; Mohler, H., Ed.; Springer: Berlin, 2001, pp 117−140.

(5) Purdy, R. H.; Morrow, A. L.; Blinn, J. R.; Paul, S. M. Synthesis, metabolism, and pharmacological activity of 3α-hydroxy steroids which potentiate GABA-receptor-mediated chloride ion uptake in rat cerebral cortical synaptoneurosomes. *J. Med. Chem.* **1990**, *33*, 1572−1581.

(6) (a) Hill-Venning, C.; Belleli, D.; Peters, J. A.; Lambert, J. J. Subunit dependent interaction of the general anaesthetic etomidate with the gamma-aminobutyric acid type A receptor. *Br. J. Pharmacol.* **1997**, *120*, 749−756. (b) Belleli, D.; Casula, A.; Ling, A.; Lambert, J. J. The influence of subunit composition on the interaction of neurosteroids with GABA(A) receptors. *Neuropharmacology* **2002**, *4*, 651−661. (c) Lambert, J. L.; Belelli, D.; Peden, D. R.; Vardy, A. W.; Peters, J. A. Neurosteroid modulation of GABA$_A$ receptors. *Prog. Neurobiol. (Oxford)* **2003**, *71*, 67−80. (d) Pöltl, A.; Hauer, B.; Fuchs, K.; Tretter, V.; Sieghart, W. Subunit composition and quantitative importance of GABAA receptor subtypes in the cerebellum of mouse and rat. *J. Neurochem.* **2003**, *87*, 1444−1455.

(7) (a) Ueno, S.; Tsutsui, M.; Toyohira, Y.; Minami, K.; Yanagihara, N. Sites of positive allosteric modulation by neurosteroids on ionotropic γ-aminobutyric acid receptor subunits. *FEBS Lett.* **2004**, *566*, 213−217. (b) Rick, C. E.; Ye, Q.; Finn, S. E.; Harrison, N. L. Neurosteroids act on the GABA$_A$ receptor at sites on the N-terminal side of the middle of TM2. *NeuroReport* **1998**, *9*, 379−383.

(8) (a) Luddens, H.; Korpi, E. R.; Seeburg, P. H. GABA$_A$/benzodiazepine receptor heterogeneity: neurophysiological implications. *Neuropharmacology* **1995**, *34*, 245−254. (b) Siegel, E.; Buhr, A. The benzodiazepine binding site of GABA$_A$ receptors. *Trends Pharmacol. Sci.* **1997**, *18*, 425−429. (c) Wohlfarth, K. M.; Bianchi, M. T.; Macdonald, R. L. Enhanced neurosteroid potentiation of ternary GABA$_A$ receptors containing the delta subunit. *J. Neurosci.* **2002**, *22*, 1541−1549.

(9) (a) Pillai, G. V.; Smith, A. J.; Hunt, P. A.; Simpson, P. B. Multiple structural features of steroids mediate subtype-selective effects on human α4β3δ GABA$_A$ receptors. *Biochem. Pharmacol.* **2004**, *68*, 819−831. (b) Puia, G.; Santi, M. R.; Vicini, S.; Pritchett, D. B.; Purdy, R. H.; Paul, S. M.; et al. Neurosteroids act on recombinant human GABA$_A$ receptors. *Neuron* **1990**, *4*, 759−765.

(10) Britton, K. T.; Koob, G. F. Neuropharmacology: premenstrual steroids? *Nature* **1998**, *392*, 869−870.

(11) (a) Dubal, D. B.; Wilson, M. E.; Wise, P. M. Estradiol: a protective and trophic factor in the brain. *J. Alzheimer's Dis.* **1999**, *1*, 265−274. (b) Guarneri, P.; Cascio, C.; Russo, D.; D'Agostino, S.; Drago, G.; Galizzi, G.; De Leo, G.; Piccoli, F.; Guarneri, M.; Guarneri, R. Neurosteroids in the retina: neurodegenerative and neuroprotective agents in retinal degeneration. *Ann. N.Y. Acad. Sci.* **2003**, *1007*, 117−128.

(12) (a) Mayo, W.; Lemaire, V.; Malaterre, J.; Rodriguez, J. J.; Cayre, M.; Stewart, M. G.; Kharouby, M.; Rougon, G.; Le Moal, M.; Piazza, P. V.; Abrous, D. N. Pregnenolone sulfate enhances neurogenesis and PSA-NCAM in young and aged hippocampus. *Neurobiol. Aging* **2005**, *26*, 103−114. (b) Fritschy, J. M.; Brunig, I. Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. *Pharmacol. Ther.* **2003**, *98*, 299−323.

(13) Smith, S. S.; Gong, Q. H.; Hsu, F. C.; Markowitz, R. S.; Ffrench-Mullen, J. M.; Li, X. GABA$_A$ receptor alpha4 subunit suppression prevents withdrawal properties of an endogenous steroids. *Nature* **1998**, *392*, 926−930.

(14) (a) Lan, N. C.; Gee, K. W. Neuroactive steroid actions at the GABA$_A$ receptor. *Horm. Behav.* **1994**, *28*, 537−544. (b) Hawkinson, J. E.; Kimbrough, C. L.; McCauley, L. D.; Bolger, M. B.; Lan, N. C.; Gee, K. W. The neuroactive steroid 3α-hydroxy-5β-pregnan-20-one is a two-component modulator of ligand binding to the GABA$_A$ receptor. *Eur. J. Pharmacol.* **1994**, *269*, 157−163. (c) Gee, K. W.; McCauley, L. D.; Lan, N. C. A putative receptor for neurosteroids on the GABA$_A$ receptor complex: the pharmacological properties and therapeutic potential of epalons. *Crit. Rev. Neurobiol.* **1995**, *9*, 207−227.

(15) (a) Mellon, S. H.; Griffin, L. D. Neurosteroids: biochemistry and clinical significance. *Trends Endocrinol. Metab.* **2002**, *13*, 35−43. (b) Zorumski, C. F.; Mennerick, S.; Isenberg, K. E.; Covey, D. F. Potential clinical uses of neuroactive steroids. *Curr. Opin. Invest. Drugs (Thomson Sci.)* **2000**, *1*, 360−369.

(16) Mienville, J. M.; Vicini, S. Pregnenolone sulfate antagonizes GABA$_A$ receptor-mediated currents via a reduction of channel opening frequency. *Brain Res.* **1989**, *489*, 190−194.

(17) (a) Floods, J. F.; Smith, G. E.; Roberts, E. Dehydroepiandrosterone and its sulfate enhance memory retention in mice. *Brain Res.* **1988**, *447*, 269−278. (b) Mayo, W.; Dellu, F.; Robel, P.; Cherkaoui, J.; Le Moal, M.; Baulieu, E. E.; Simon, H. Infusion of neurosteroids into the nucleus basalis magnocellularis affects cognitive processes in the rat. *Brain Res.* **1993**, *607*, 324−328.

(18) Gee, K. W.; Bolger, M. B.; Brinton, R. E.; Coirini, H.; McEwen, B. S. Steroid modulation of the chloride ionophore in rat brain: Structure−activity requirements, regional dependence, and

mechanism of action. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 803−812. (b) Harrison, N. L.; Majewska, M. D.; Harrington, J. W.; Barker, J. L. Structure−activity relationships for steroid interaction with the γ-aminobutyric acid$_A$ receptor complex. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 346−353.

(19) (a) Han, M.; Zorumski, C. F.; Covey, D. F. Neurosteroid analogues. 4. The effect of methyl substitution at the C-5 and C-10 positions of neurosteroids on electrophysiological activity at GABA$_A$ receptors. *J. Med. Chem.* **1996**, *39*, 4218−4232. (b) Hogenkamp, D. J.; Tahir, S. H.; Hawkinson, J. E.; Upasani, R. B.; Alauddin, M.; Kimbrough, C. L.; Acosta-Burruel, M.; Whittemore, E. R.; Woodward, R. M.; Lan, N. C.; Gee, K. W.; Bolger, M. B. Synthesis and in vitro activity of 3β-substituted-3α-hydroxypregnan-20-ones: allosteric modulators of the GABA$_A$ receptor. *J. Med. Chem.* **1997**, *40*, 61−72. (c) Anderson, A.; Boyd, A. C.; Byford, A.; Campbell, A. C.; Gemmell, D. K.; Hamilton, N. M.; Hill, D. R.; Hill-Venning, C.; Lambert, J. J.; Maidment, M. S.; May, V.; Marshall, R. J.; Peters, J. A.; Rees, D. C.; Stevenson, D.; Sundaram, H. Anesthetic activity of novel water-soluble 2β-morpholinyl steroids and their modulatory effects at GABA$_A$ receptors. *J. Med. Chem.* **1997**, *40*, 1668−1681. (d) Upasani, R. B.; Yang, K. C.; Acosta-Burruel, M.; Konkoy, C. S.; McLellan, J. A.; Woodward, R. M.; Lan, N. C.; Carter, R. B.; Hawkinson, J. E. 3α-Hydroxy-3β-(phenylethynyl)-5β-pregnan-20-ones: synthesis and pharmacological activity of neuroactive steroids with high affinity for GABA$_A$ receptors. *J. Med. Chem.* **1997**, *40*, 73−84. (e) Nilsson, K. R.; Zorumski, C. F.; Covey, D. F. Neurosteroid analogues. 6. The synthesis and GABA$_A$ receptor pharmacology of enantiomers of dehydroepiandrosterone sulfate, pregnenolone sulfate, and (3α,5β)-3-hydroxypregnan-20-one sulfate. *J. Med. Chem.* **1998**, *41*, 2604−2613. (f) Anderson, A.; Boyd, A. C.; Clark, J. K.; Fielding, L.; Byford, A.; Gemmell, D. K.; Hamilton, N. M.; Maidment, M. S.; May, V.; McGuire, R.; McPhail, P.; Sansbury, F. H.; Sundaram, H.; Taylor, R. Conformationally constrained anesthetic steroids that modulate GABA$_A$ receptors. *J. Med. Chem.* **2000**, *43*, 4118−4125. (g) Jiang, X.; Manion, B. D.; Benz, A.; Rath, N. P.; Evers, A. S.; Zorumski, C. F.; Mennerick, S.; Covey, D. F. Neurosteroid analogues. 9. Conformationally constrained pregnanes: structure−activity studies of 13,24-cyclo-18,21-dinorcholane analogues of the GABA modulatory and anesthetic steroids (3α,5α)- and (3α,5β)-3-hydroxypregnan-20-one. *J. Med. Chem.* **2003**, *46*, 5334−5348.

(20) Covey, D. F.; Han, M.; Kumar, A. S.; Antonette, M.; de la Cruz, M.; Meadows, E. S.; Hu, Y.; Tonnies, A.; Nathan, D.; Coleman, M.; Benz, A.; Evers, A. S.; Zorumski, C. F.; Mennerick, S. Neurosteroid analogues. 8. Structure−activity studies of *N*-acylated 17a-aza-D-homosteroid analogues of the anesthetic steroids (3α,5α)- and (3α,5β)-3-hydroxypregnan-20-one. *J. Med. Chem.* **2000**, *43*, 3201−3204.

(21) (a) Hu, Y.; Zorumski, C. F.; Covey, D. F. Neurosteroid analogues: structure−activity studies of benz[*e*]indene modulators of GABA$_A$ receptor function. 1. The effect of 6-methyl substitution on the electrophysiological activity of 7-substituted benz-[*e*]indene-3-carbonitriles. *J. Med. Chem.* **1993**, *36*, 3956−3967. (b) Han, M.; Hu, Y.; Zorumski, C. F.; Covey, D. F. Neurosteroid analogues. 3. The synthesis and electrophysiological evaluation of benz[*e*]indene congeners of neuroactive steroids having the 5β-configuration. *J. Med. Chem.* **1995**, *38*, 4548−4556.

(22) Maksay, G.; Korpi, E. R.; Uusi-Oukari, M. Bimodal action of furosemide on convulsant [^{3}H]EBOB binding to cerebellar and cortical GABA$_A$ receptors. *Neurochem. Int.* **1998**, *33*, 353−358.

(23) Perret, P.; Sarda, X.; Wolff, M.; Wu, T. T.; Bushey, D.; Goeldner, M. Interaction of noncompetitive blockers within the γ−aminobutyric acid type A chloride channel using chemically reactive probes as chemical sensors for cysteine mutants. *J. Biol. Chem.* **1999**, *274*, 25350−25354.

(24) Maksay, G. Commentary. From kinetics and thermodynamics of GABA$_A$ receptor binding to ionophore function. *Neurochem. Int.* **1996**, *29*, 361−370.

(25) (a) Belelli, D.; Lambert, J. J.; Peters, J. A.; Gee, K. W.; Lan, N. C. Modulation of human recombinant GABA$_A$ receptors by pregnanediols. *Neuropharmacology* **1996**, *35*, 1223−1231. (b) McCauley, L. D.; Liu, V.; Chen, J. S.; Hawkinson, J. E.; Lan, N. C.; Gee, K. W. Selective actions of certain neuroactive pregnanediols at the gamma-aminobutyric acid type A receptor complex in rat brain. *Mol. Pharmacol.* **1995**, *47*, 354−362.

(26) Ehlert, F. J. Estimation of the affinities of allosteric ligands using radioligand binding and pharmacological null methods. *Mol. Pharmacol.* **1988**, *33*, 187−194.

(27) Maksay, G.; Bíró, T. Dual cooperative allosteric modulation of binding to ionotropic glycine receptors. *Neuropharmacology* **2002**, *43*, 1087−1098.

(28) Corey, E. J.; Fuchs, P. L. A synthetic method for formyl → ethynyl conversion (RCHO → RCCH or RCCR′) *Tetrahedron Lett.* **1972**, *13*, 3769−3772.

(29) (a) Chemin, D.; Linstrumelle, G. Palladium-catalyzed reaction of (E) and (Z)-dichloroethenes with 1-alkynes. An efficient stereospecific synthesis of (E) and (Z)-enediynes. *Tetrahedron* **1994**, *50*, 5335−5344. (b) Alami, M.; Ferri, F.; Linstrumelle, G. An efficient palladium-catalysed reaction of vinyl and aryl halides or triflates with terminal alkynes. *Tetrahedron Lett.* **1993**, *34*, 6403−6406. (c) Sonogashira, K.; Tohda, Y.; Hagihara, N. A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines. *Tetrahedron Lett.* **1975**, *16*, 4467−4470.

(30) (a) Piatak, D. M.; Wicha, J. Various approaches to the construction of aliphatic side chains of steroids and related compounds. *Chem. Rev.* **1978**, *78*, 199−241. (b) Chaudhuri, N. K.; Williams, J. G.; Nickolson, R.; Gut, M. Stereochemistry of the addition reactions of Grignard reagents to 20-keto steroids. Synthesis of 17α,20α-dihydrocholesterol. *J. Org. Chem.* **1969**, *34*, 3759−3766. (c) Chaudhuri, N. K.; Gut, M. Ethynylation of 17.alpha.-hydroxypregnenolone 3-acetate. Stereochemistry and acid-catalyzed rearrangement of the reaction product. *J. Org. Chem.* **1969**, *34*, 3754−3758.

(31) Mauvais, A.; Burger, A.; Roussel, J. P.; Hetru, C.; Luu, B. Acetylenic inhibitors of C-22 hydroxylase of ecsydone biosynthesis. *Bioorg. Chem.* **1994**, *22*, 36−50.

(32) Singh, J.; Satyamurthi, N.; Aidhen, I. S. The growing synthetic utility of Weinreb's amide. *J. Prakt. Chem. (Weinheim, Ger.)* **2000**, *342*, 340−347.

(33) (a) Corey, E. J.; Helal, C. J. Reduction of carbonyl compounds with chiral oxazaborolidine catalysts: A new paradigm for enantioselective catalysis and a powerful new synthetic method. *Angew. Chem., Int. Ed.* **1998**, *37*, 1986−2012. (b) Helal, C. J.; Magriotis, P. A.; Corey, E. J. Direct catalytic enantioselective reduction of achiral α,β-ynones. Strong remote steric effects across the C−C triple bond. *J. Am. Chem. Soc.* **1996**, *118*, 10938−10939. (c) Corey, E. J.; Helal, C. J. Novel electronic effects on remote substituents on the oxazaborolidine-catalysed enantioselective reduction of ketones. *Tetrahedron Lett.* **1995**, *36*, 9153−9156. (d) Wallbaum, S.; Martens, J. Asymmetric syntheses with chiral oxazaborolidines. *Tetrahedron: Asymmetry* **1992**, *3*, 1475−1504. (e) Corey, E. J.; Bakshi, R. K.; Shibata, S. J. Highly enantioselective borane reduction of ketones catalyzed by chiral oxazaborolidines. Mechanism and synthetic implications. *J. Am. Chem. Soc.* **1987**, *109*, 5551−5553.

(34) Parker, K. A.; Lederboer, M. W. Asymmetric reduction. A convenient method for the reduction of alkynyl ketones. *J. Org. Chem.* **1996**, *61*, 3214−3217.

(35) Crabbe, P.; Andre, D.; Fillion, H. Synthesis of homo-dinordrin, allenyl A-nor and dinor-steroids. *Tetrahedron Lett.* **1979**, *10*, 893−896. (b) Fillion, H.; Andre, D.; Luche, J. L. Mecanisme de l'homologation directe d'acetyleniques en allenes. *Tetrahedron Lett.* **1980**, *21*, 929−930. (c) Grabbe, P.; Nassim, B.; Robert-Lopes, M. T. One-step homologation of acetylenes to allenes: 4-hydroxynona-1,2-diene. *Org. Synth.* **1984**, *63*, 203−205. (d) Searles, S.; Li, Y.; Nassim, B.; Robert-Lopez, M. T.; Tran, P. T.; Crabbe, P. Observation on the synthesis of allenes by homologation of alk-1-ynes. *J. Chem. Soc., Perkin Trans. 1* **1984**, 747−751.

(36) Carboni, E.; Wieland, S.; Lan, N. C.; Gee, K. W. Anxiolytic properties of endogenously occurring pregnanediols in two rodent models of anxiety. *Psychopharmacology (Berlin)* **1996**, *126*, 173−178. (b) Gee, K. W.; McCauley, L. D.; Lan, N. C. A putative receptor for neurosteroids on the GABA$_A$ receptor complex: the pharmacological properties and therapeutic potential of epalons. *Crit. Rev. Neurobiol.* **1995**, *9*, 207−227.

(37) (a) Belelli, D.; Gee, K. W. 5α-Pregnan-3α,20α-diol behaves like a partial agonist in the modulation of GABA-stimulated chloride in uptake by synaptoneurosomes. *Eur. J. Pharmacol.* **1989**, *167*, 173−176. (b) Bolger, M. C.; Gee, K. W.; Lan, N. T.-Y.; Belelli, D.; Mirsadeghi, S.; Purdy, R. H.; Tahir, S. H. GABA receptor modulators. EP0554436, 1993.

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