Synthesis and *in Vitro* Activity of 3β-Substituted-3α-hydroxypregnan-20-ones: Allosteric Modulators of the $GABA_A$ Receptor†

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Two naturally occurring metabolites of progesterone, 3α-hydroxy-5α- and 5β-pregnan-20-one (**1** and **2**), are potent allosteric modulators of the $GABA_A$ receptor. Their therapeutic potential as anxiolytics, anticonvulsants, and sedative/hypnotics is limited by rapid metabolism. To avoid these shortcomings, a series of 3β-substituted derivatives of **1** and **2** was prepared. Small lipophilic groups generally maintain potency in both the 5α- and 5β-series as determined by inhibition of [^{35}S]TBPS binding. In the 5α-series, 3β-ethyl, -propyl, -trifluoromethyl and -(benzyloxy)methyl, as well as substituents of the form 3β-XCH_2, where X is Cl, Br, or I or contains unsaturation, show limited efficacy in inhibiting [^{35}S]TBPS binding. In the 5β-series, the unsubstituted parent **2** is a two-component inhibitor, whereas all of the 3β-substituted derivatives of **2** inhibit TBPS via a single class of binding sites. In addition, all of the 3-substituted 5β-sterols tested are full inhibitors of [^{35}S]TBPS binding. Electrophysiological measurements using α1β2γ2L receptors expressed in oocytes show that 3β-methyl- and 3β-(azidomethyl)-3α-hydroxy-5α-pregnan-20-one (**6** and **22**, respectively) are potent full efficacy modulators and that 3α-hydroxy-3β-(trifluoromethyl)-5α-pregnan-20-one (**24**) is a low-efficacy modulator, confirming the results obtained from [^{35}S]TBPS binding. These results indicate that modification of the 3β-position in **1** and **2** maintains activity at the neuroactive steroid site on the $GABA_A$ receptor. In animal studies, compound **6** (CCD 1042) is an orally active anticonvulsant, while the naturally occurring progesterone metabolites **1** and **2** are inactive when administered orally, suggesting that 3β-substitution slows metabolism of the 3-hydroxyl, resulting in orally bioavailable steroid modulators of the $GABA_A$ receptor.

Introduction

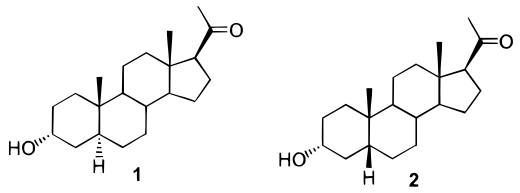
Therapeutically useful anticonvulsants, anxiolytics, and sedative−hypnotics such as benzodiazepines (BZ) and barbiturates mediate their action by binding to distinct allosteric modulatory sites on the $GABA_A$ receptor−Cl^- channel complex. There is now a large body of evidence for an additional modulatory site on $GABA_A$ receptors that binds neuroactive steroids.[1] The prototypical ligand for this binding site is epiallopregnanolone (3α-hydroxy-5α-pregnan-20-one, **1**; Chart 1) an endogenous metabolite of progesterone that demonstrates potent modulatory effects at the $GABA_A$ receptor.

Most neuroactive steroids studied for their interaction with the $GABA_A$ receptor are endogenously occurring metabolites of progesterone and deoxycorticosterone.[1] Anesthetic properties of a large number of steroids have previously been described, but whether the observed anesthetic properties are wholly mediated by $GABA_A$ receptors, or by charges in membrane fluidity, is still uncertain.[2]

Endogenously occurring neuroactive steroids such as **1**, its 5β-epimer **2,** or 3α,21-dihydroxy-5α-pregnan-20-one (5αTHDOC) have been shown to allosterically inhibit the binding of [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS), a cage convulsant which binds in close proximity to the chloride channel portion of the $GABA_A$ receptor.[3] We have recently demonstrated that the rank order potency of 15 neuroactive steroids in inhibiting [^{35}S]TBPS binding parallels their ability to potentiate GABA-induced currents in electrophysiological studies.[4] This finding suggests that the TBPS inhibition assay is generally a good measurement of the relative potencies of neuroactive steroids at the $GABA_A$ receptor. In addition, two other metabolites of **1** and **2**, 5α-pregnane-3α,20α-diol and 5β-pregnane-3α,20β-diol, are representative of neuroactive steroids that show limited inhibition of [^{35}S]TBPS binding even at high concentrations.[5,6] The limited efficacy of these neuroactive steroids has also been demonstrated electrophysiologically,[7] indicating that the [^{35}S]TBPS binding assay can also provide an estimate of the relative functional efficacies of neuroactive steroids.

Given their mechanism of action, endogenous neuroactive steroids are expected to have anticonvulsant,[8]

Chart 1. Naturally Occurring Neuroactive Steroids



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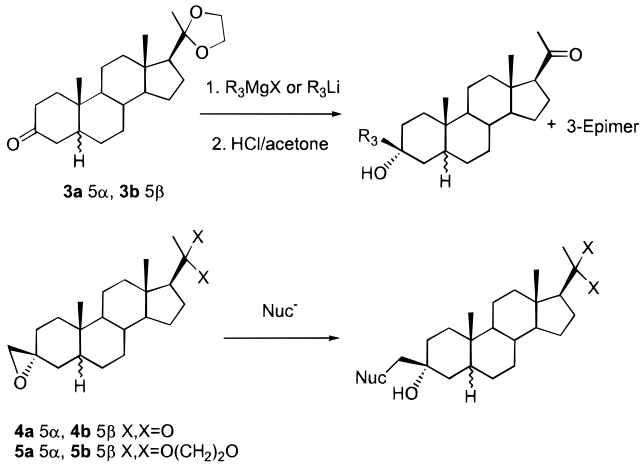
Scheme 1



3a 5α, **3b** 5β

4a 5α, **4b** 5β X,X=O
5a 5α, **5b** 5β X,X=O(CH$_2$)$_2$O

anxiolytic,[9] and sedative−hypnotic[10] activities. Indeed, these actions have been demonstrated in a variety of animal models.[8−10] Nevertheless, naturally occurring neuroactive steroids have therapeutic limitations because they are rapidly metabolized, presumably by conjugation of the 3α-hydroxyl or oxidation to the corresponding ketones.[11] Phillips *et al.*[12] have described the preparation of simple 3*β*-alkyl-substituted derivatives of **1** that increase the *in vivo* half-life compared to **1** as anesthetics. Expanding on this work, we have synthesized and characterized the *in vitro* activity of a series of 3*β*-substituted derivatives of **1** and **2**.

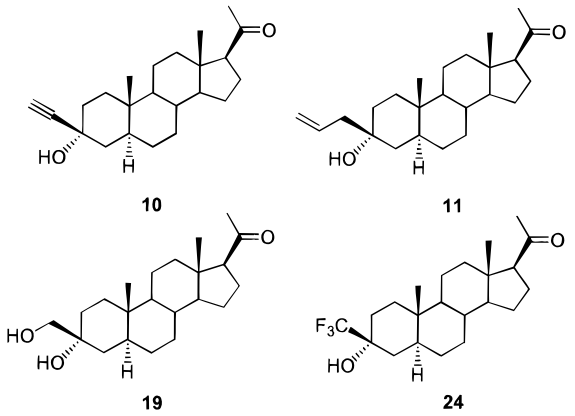
Chemistry

The preparation of the 3*β*-substituted-3α-hydroxy-5α- and -5*β*-pregnan-20-ones listed in Tables 1 and 2 was accomplished using two methods depending on the nature of the 3*β*-substituent and whether the steroid was a 5α- or 5*β*-pregnane: (1) Reaction of organometallic reagents with 20,20-ethylenedioxypregnan-3-one (**3**) or (2) addition of nucleophiles to (3*R*)-spiro[oxirane-2′,5α-pregnan]-20-one (**4**) or to a 20-ketal-protected analog (**5**; Scheme 1).[13] Compounds **6**−**8** in the 5α-series and compounds **30** and **31** in the 5*β*-series are covered by Glaxo patents.[12] Of these compounds, only the preparation and characterization of **6** was described.[12a]

For comparison with the 3*β*-substituted analogs, 3α-hydroxy-5α-pregnan-20-one (**1**) was prepared from 3*β*-hydroxypregn-5-en-20-one (hydrogenation followed by Mitsunobu reaction with trifluoroacetate and hydrolysis to the 3α-ol). In the 5α-series, the alkyne **10** was prepared from 20,20-(ethylenedioxy)-5α-pregnan-3-one (**3a**; prepared in three steps from 3*β*-hydroxypregn-5-en-20-one) and lithium acetylide−ethylenediamine complex followed by deprotection and separation of the 3α- and 3*β*-ols. Reaction of **3a** with allylmagnesium chloride and deprotection afforded **11** again accompanied by its 3-epimer. The less polar 3α-ols (axial hydroxyl) were easily separated from the more polar 3*β*-ols (equatorial hydroxyl) by flash chromatography using hexane/acetone mixtures. The 3*β*-trifluoromethyl derivative **24** was isolated as the minor isomer (ratio of 3*β*- to 3α-ol 10:1) from the fluoride-initiated addition of $TMSCF_3$[14] to **3a**.

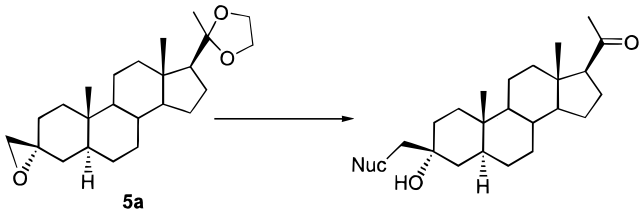
The synthesis of **15**−**18**, **20**, **22**, and **26**−**28** by the oxirane method is outlined in Table 3. Reaction of 5α-pregnane-3,20-dione with dimethylsulfoxonium methylide[15] regioselectively and stereoselectively gave the 3α-epoxide[16] **4a**. Only under forcing conditions, refluxing in THF, was any reaction with excess ylide observed. Ethers **15**−**18** were prepared by addition of epoxide **4a** to a solution of sodium in the desired alcohol. Some 17-epimerization was observed under these reaction conditions, but the 17-epimer was easily removed by recrystallization or column chromatography.

Chart 2



10 **11** **19** **24**

Scheme 2



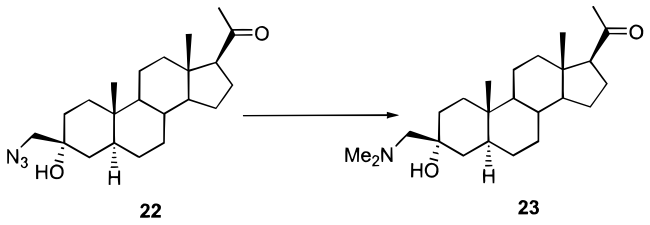
5a

12 Nuc = HCC
21 Nuc = NC
25 Nuc = F

Reaction of **4a** with tetramethylammonium chloride in DMF in the presence of an excess of acetic acid afforded the *β*-chloro alcohol **26** in 68% yield. In the absence of acetic acid, the reaction failed to go to completion even with a large excess of chloride. Presumably, an equilibrium between the epoxide and the alkoxide formed by chloride addition is driven to completion by protonation of the alkoxide with acid. Addition of tetramethylammonium bromide under similar conditions gave a mixture of bromide **27**, 3-(hydroxymethyl)-5α-pregn-2-en-20-one, and 3*β*-(acetoxymethyl)-3α-hydroxy-5α-pregnan-20-one, identified by ^{1}H NMR. Column chromatography gave **27** in 22% yield. Reaction of **4a** with NaI in THF/methanol/HOAc afforded iodide **28** in 67% yield. The addition of sodium thiomethoxide and sodium azide[13] to **4a** gave the expected opening products **20** and **22,** respectively. Hydrogenolysis of **18** gave the diol **19** in 91% yield.

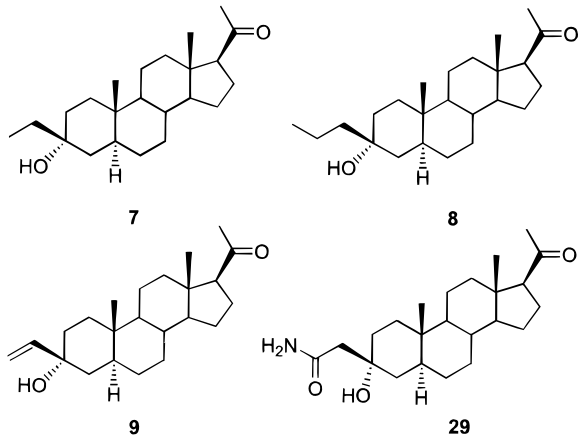
Opening of epoxide **4a** was generally effective with nucleophiles that were compatible with the 20-ketone. However, reaction of **4a** with tetrabutylammonium fluoride gave a mixture of the desired fluoride **25** and its 3- and 17-epimers. To avoid 17-epimerization, the reaction was repeated with the protected epoxide **5a** (Scheme 2). Opening of the epoxide was only observed in very concentrated reaction mixtures with carefully dried (azeotropic removal of water) tetrabutylammonium fluoride. After deprotection, **25** was isolated in 59% yield. Reaction of **5a** with potassium cyanide in EtOH gave the desired nitrile **21** in 52% yield after deprotection. Hydrolysis of the cyanide **21** gave primary

Scheme 3



22 23

Chart 3



7 8 9 29

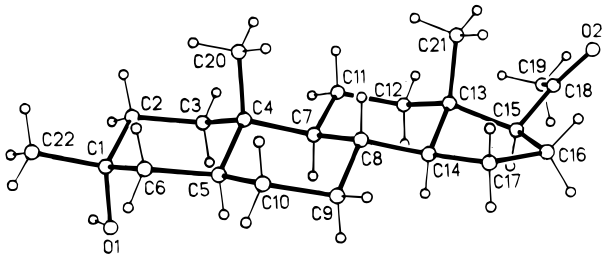
amide **29**. A DMSO suspension of **5a** reacted smoothly with an excess of lithium acetylide−ethylenediamine complex to give **12** in 93% yield after liberation of the 20-ketone. No reaction was observed between **5a** and lithium acetylide−ethylenediamine complex or lithium trimethylsilylacetylide in THF.

Hydrogenation of the azide (H_2, Pd/C) and alkylation (formalin, formic acid) afforded the tertiary amine **23** in 36% yield (Scheme 3). Hydrogenation of **12** gave the 3β-propyl derivative **8**. A procedure for the synthesis of allylic alcohols from epoxides recently reported by Alcaraz *et al.*[17] was employed for the preparation of the allylic alcohol **9** as a single epimer. Thus, addition of an excess of dimethylsulfonium methylide to epoxide **5a** followed by hydrolysis of the 20-ketal gave **9** in 70% yield. Hydrogenation of **9** (H_2, 5% Pd/C) gave the saturated analog **7**.

The syntheses of compounds **6**, **13**, and **14** from 3α-hydroxy-3β-(iodomethyl)-5α-pregnan-20-one (**28**) is given in Scheme 4. Hydrogenolysis of **28** over 5% Pd/C gave **6** in quantitative yield. Reaction of **28** under free radical conditions with allyltributylstannane or propargyltriphenylstannane gave **13** and **14** in 35% and 11% yields, respectively.[18]

A crystal structure of **6** (Figure 1) confirmed the stereochemistry of the 3β-methyl substituent. The 3β-methyl in **6** then confirms the identity of the 3α-epoxide **4a** and the 3-stereochemistry of its derivatives (compounds **7**−**9**, **12**−**23**, and **25**−**29**, Table 1). The stereochemistry of the allyl group in **11** was confirmed by hydrogenation and comparison with authentic **8** prepared from 3β-(3-propyne) **12**.

In the 5β-series, 3α-hydroxy-5β-pregnan-20-one (**2**) was prepared from 20,20-(ethylenedioxy)-5β-pregnan-3-one (**3b**) by reduction with lithium tri-*tert*-butoxyaluminum hydride and hydrolysis to unmask the 20-ketone. The 5β-pregnanes **30** and **32**−**34** (Table 2) were prepared from **3b** (Scheme 5). Addition of MeMgBr, vinylmagnesium bromide, lithium acetylide−ethylenediamine complex, or 2-propynylmagnesium bromide to



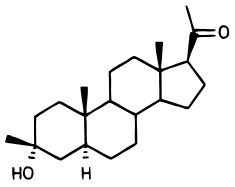
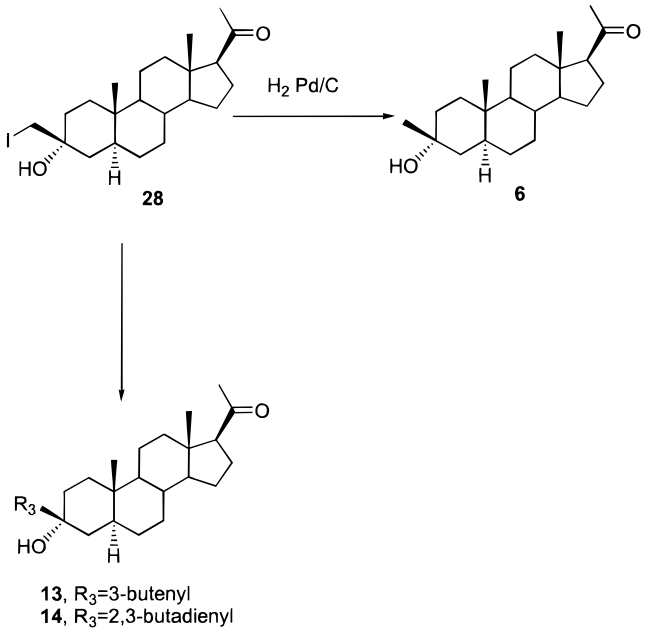


Figure 1. Crystal structure of **6**.

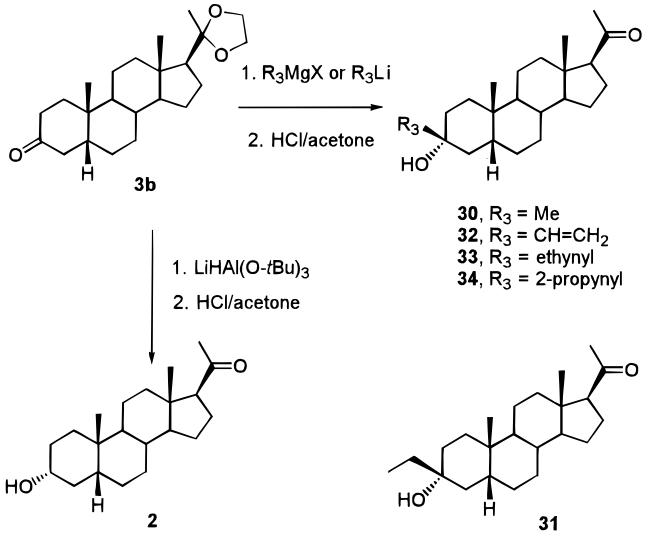
Scheme 4



28 6

13, R_3=3-butenyl
14, R_3=2,3-butadienyl

Scheme 5

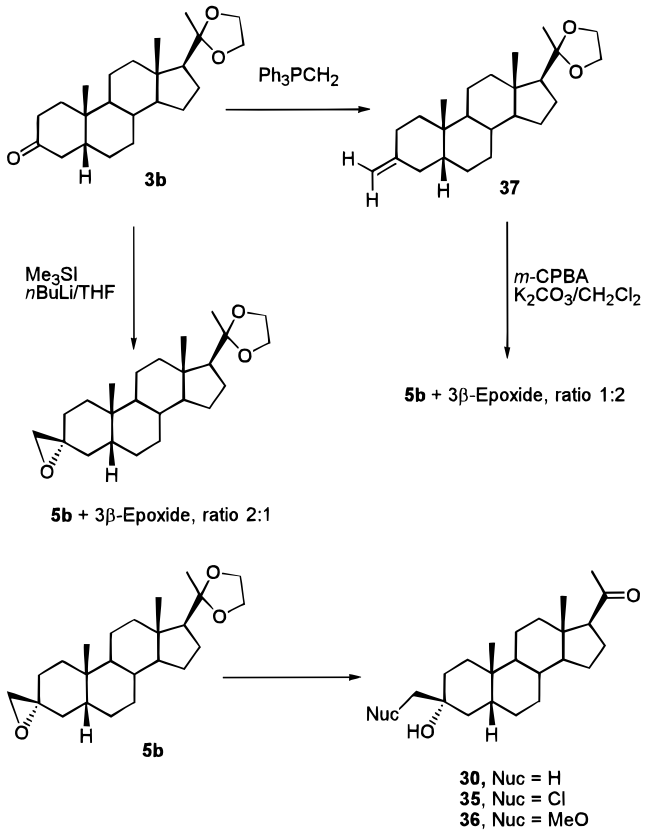


30, R_3 = Me
32, R_3 = CH=CH_2
33, R_3 = ethynyl
34, R_3 = 2-propynyl

2 31

3b gave a mixture of 3α- and 3β-ols which were separated by column chromatography (3α-ols identified as the more polar isomers). Hydrogenation of **33** (H_2, Pd/C) afforded the 3β-ethylpregnane **31** in 66% yield

Scheme 6



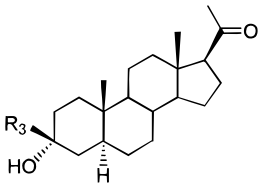
after recrystallization. The yield of 3β-methylpregnane **30** in the Grignard addition was found to be variable, and a more reproducible procedure was found using the epoxide **5b** (Scheme 6). Reaction of **5b** with an excess of lithium aluminum hydride and deketalization gave **30**, identical with the more polar isomer formed in the Grignard reaction.

(Chloromethyl)- and (methoxymethyl)pregnanes **35** and **36** were obtained from epoxide **5b** as described for the preparation of the 5α-analogs **26** and **15**. The synthesis of epoxide **5b** is given in Scheme 6. Reaction of 20,20-(ethylenedioxy)-5β-pregnan-3-one (**3b**) with dimethylsulfoxonium methylide gave exclusively the undesired 3β-epoxide. A 2:1 mixture of the 3α- and 3β-epoxides was isolated from the reaction of **3b** and dimethylsulfonium methylide. In an attempt to improve on this ratio, **3b** was converted to the exocyclic alkene **37** and subjected to epoxidation. Epoxidation of similar exocyclic alkenes with hydrogen peroxide in benzonitrile results in a 2:1 ratio of equatorial to axial attack.[19] Unfortunately, no reaction with **37** was observed under these conditions. Epoxidation of **37** with *m*-chloroperoxybenzoic acid gave a 1:2 ratio of 3α- and 3β-epoxides which could be separated by flash chromatography. The more polar 3α-epoxide (TLC with hexane/acetone mixtures) was identified by comparison with the less polar 3β-epoxide formed from dimethylsulfoxonium methylide and **3b**. More conveniently, the mixture of epoxides was separated after reaction with the desired nucleophile.

Pharmacology

[^{35}S]TBPS Binding Studies. The ability of 3β-substituted-3α-hydroxy-5α- and -5β-pregnan-20-ones to allosterically modulate the binding of [^{35}S]TBPS in rat brain cortical membranes is compared to the endogenously occurring progesterone metabolites **1** and **2** in Tables 1 and 2, respectively. The concentration of steroid inhibiting 50% specific binding (IC_{50}) and the maximal extent of inhibition (I_{max}) were calculated by fitting [^{35}S]TBPS inhibition data to a sigmodial function. Compounds with I_{max} > 90% are considered full agonists, while compounds exhibiting incomplete displacement of [^{35}S]TBPS binding at high concentration are partial agonists (I_{max} < 90% at > 1 μM). For comparison, the IC_{50} and I_{max} values for the full agonist benzodiazepine diazepam[20] and partial agonist benzodiazepine Ro 16-6028 (bretazenil)[20] are included along with data for pentobarbital.[5] The potencies of the synthetic steroids vary from more potent than **1** and **2** to completely inactive (IC_{50} > 10 000 nM). Lipophilic substituents are generally accommodated at the 3β-position, while the polar, hydrogen bond-donating groups tested (3β-hydroxymethyl **19** and 3β-carboxamidomethyl **29**) and the tertiary amine **23** reduce or abolish activity. For simple alkyl substituents at the 3β-position, there is no simple relationship between the size of the group and the ability to inhibit [^{35}S]TBPS binding. In the

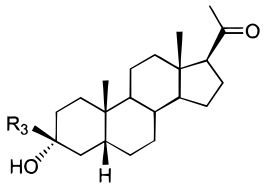
Table 1. Potency and Efficacy of 3β-Substituted-5α-pregnanes in Inhibiting [^{35}S]TBPS Binding in Rat Brain Cortical Membranes[a]



compd	R_3	IC_{50} (nM)	I_{max} (%)
diazepam		91	49
Ro 16-6028		6.1	27
pentobarbital		1700	100
1	H	51 ± 5	95 ± 1
6	Me	80 ± 18	94 ± 1
7	Et	257 ± 24	69 ± 1
8	Pr	173 ± 33	42 ± 3
9	H_2C=CH	120 ± 7	94 ± 1
10	HC≡C	64 ± 8	93 ± 1
11	H_2C=$CHCH_2$	231 ± 25	59 ± 3
12	HC≡CCH_2	50 ± 7	43 ± 1
13	H_2C=$CHCH_2CH_2$	325 ± 65	59 ± 6
14	H_2C=C=$CHCH_2$	365 ± 47	57 ± 4
15	$MeOCH_2$	76 ± 9	104 ± 1
16	$EtOCH_2$	230 ± 51	104 ± 4
17	*n*-$PrOCH_2$	655 ± 77	94 ± 3
18	$BnOCH_2$	376 ± 50	57 ± 3
19	$HOCH_2$	2134 ± 79	101 ± 5
20	$MeSCH_2$	>10000	46 at 10 μM
21	$NCCH_2$	614 ± 54	38 ± 4
22	N_3CH_2	27 ± 2	99 ± 7
23	Me_2NCH_2	>10000	0 at 10 μM
24	F_3C	266 ± 43	44 ± 4
25	FCH_2	228 ± 51	79 ± 4
26	$ClCH_2$	71 ± 11	33 ± 3
27	$BrCH_2$	224 ± 45	44 ± 2
28	ICH_2	702 ± 143	50 ± 6
29	H_2NCOCH_2	>10000	28 at 10 μM

[a] Compounds (1 nM−10 μM) 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 3-9 independent experiments except for the inactive compounds **23** and **29** (n = 2). Hill values were 1.0 for all active compounds except **16** (1.2 ± 0.2) and **18** (1.6 ± 0.1). The concentration of steroid inhibiting 50% specific binding (IC_{50}) and the maximal extent of inhibition (I_{max}) were calculated by fitting the data to the sigmodial function. Data for pentobarbital are taken from ref 5, and those for diazepam and Ro 16-6028 (bretazenil) are from ref 20. Data for compounds **1** and **6** are taken from ref 26b, and data for **24** are from ref 24.

Table 2. Potency and Efficacy of 3β-Substituted-5β-pregnanes in Inhibiting [^{35}S]TBPS Binding in Rat Brain Cortical Membranes[a]



compd	R_3	IC_{50} (nM)	I_{max} (%)
2	H	44 ± 11	65 ± 3
		12380 ± 5040	35 ± 3
30	Me	37 ± 10	104 ± 1
31	Et	135 ± 7	95 ± 4
32	H_2C═CH	43 ± 4	103 ± 1
33	HC≡C	39 ± 5	101 ± 1
34	HC≡CCH_2	214 ± 20	101 ± 2
35	$ClCH_2$	243 ± 52	103 ± 2
36	$MeOCH_2$	40 ± 5	103 ± 1

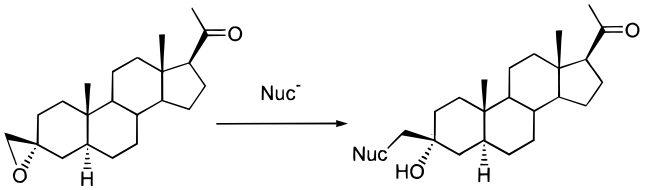
[a] See footnote to Table 1 for methods and data analysis. Compound **2** (0.2 nM−100 μM) inhibits TBPS binding with two-components. Hill values were 1.0 for all active compounds except **31** (1.3 ± 0.1), **33** (1.12 ± 0.03), **34** (1.2 ± 0.1), and **35** (1.3 ± 0.1). Data for compound **30** are taken from ref 26a.

series 3β-H, -Me, -Et, and -Pr (**1** and **6**−**8**, Table 1), **1** and **6** are equipotent while a 5-fold loss is observed for the 3β-ethylpregnane **7**. The 3β-propyl homolog **8**, however, is more potent than **7**. The addition of a methylene to alkyne **10** gives the homolog **12** with equal potency. The ethers **15**−**17** show a 3-fold loss of activity for each methylene added. The corresponding thioether (**20**) of **15** is inactive. The 3β-trifluoromethyl derivative **24** and the 3β-fluoromethyl analog **25** show substantial (4.5−5.2-fold) reduction in activity compared to **1**. The other halogens (**26**−**28**) show a decrease in activity with increasing size. The most potent compound in this series appears to be the azide **22**, which is 2-fold more potent than **1**.

In addition to exhibiting a range of potencies, the steroids examined show varied levels of efficacy in inhibiting [^{35}S]TBPS binding. In general, active 5α-compounds incorporating a 3β-group XCH_2 show limited inhibition if X contains unsaturation (compounds **11**−**14** and **21**) or if X is Cl, Br, or I (halomethyls **26**−**28**). Simple alkyl groups larger than methyl (3β-Et, **7**, and 3β-Pr, **8**) also appear to be limited efficacy inhibitors in the assay, as are the benzyloxymethyl (**18**) and trifluoromethyl (**24**) compounds. Compounds with unsaturation in the 3β-substituent directly adjacent to the 3-position of the steroid A-ring are full inhibitors, even when the corresponding saturated analog is a limited inhibitor (e.g., compounds **9** and **10** compared with compound **7**).

The *in vitro* binding results for the 5β-pregnanes examined are given in Table 2. The parent 3β-unsubstituted compound **2** is known to be a two-component modulator of [^{35}S]TBPS binding.[21] The addition of a 3β-substituent results in derivatives which inhibit [^{35}S]-TBPS binding with a single-component as do all the 5α-compounds listed in Table 1. While the 3β-methylpregnane **30** is equipotent with **2**, the addition of a methylene spacer in **31** results in a 3.6-fold loss in activity. Unsaturated groups directly attached to the steroid nucleus at the 3β-position (**32** and **33**) and the methoxymethyl derivative **36** exhibit no loss in potency compared to **2**. The 3β-(2-propyne) derivative **34** and the 3β-chloromethyl alcohol **35** result in about a 5-fold loss in activity in contrast to the same substitution in the 5α-series which results in compounds which are approximately equipotent with the parent **1**. Unlike their 5α counterparts, **31**, **34**, and **35** are full inhibitors of [^{35}S]TBPS binding.

Table 3. Reaction of (3*R*)-Spiro[oxirane-2′,5α-pregnan]-20-one (**4a**) with Nucleophiles



4a **15-18**, **20**, **22** and **26-28**

compd	reagents	yield (%)
15	MeONa	58
16	EtONa	57
17	*n*-PrONa	40
18	BnONa	52
20	MeSNa	57
22	NaN_3	73
26	Me_4NCl	68
27	Me_4NBr	22
28	NaI	67

The 3β-hydroxy isomers of compounds **1**, **6**, **24**, **30**, and **32** were tested in the [^{35}S]TBPS binding assay and found to be from 50- to 1000-fold less active than the 3α-ols. The strict stereochemical requirement of 3α-ol for activity has previously been noted.[22]

Electrophysiology. Coinjection of α1, β2, and γ2L cRNAs into oocytes resulted in strong expression of functional $GABA_A$ receptors. The mean maximum GABA-activated current was 2700 ± 100 nA (current elicited by 3 mM GABA) (n = 22), and the range of maximum responses was between 1875 and 3630 nA. To further characterize the receptors, GABA concentration−response curves were measured in a sample of cells. The EC_{50} was 40 ± 3 μM, and the slope value was 1.3 ± 0.1 (n = 13), giving no indication of a pharmacologically heterogeneous population of receptors.

Functional modulation of $GABA_A$ receptors by steroids was assayed as shown in Figure 2. A maximum response was elicited by applying 3 mM GABA. All other currents were scaled to this value. The GABA concentration was then adjusted to elicit a fractional current of approximately 0.05 (5%); the mean GABA concentration was 5.9 ± 0.6 μM, and the mean response was 0.051 ± 0.001 nA (n = 22). Potency and efficacy of modulation were assayed by measuring effects of increasing concentrations of steroid on the fixed 0.05 control response. Steroid-activated currents, *i.e.*, currents observed in the absence of GABA, were measured as the peak response elicited during preincubations (Figure 2, arrow). Five steroids were selected for comparison with the [^{35}S]TBPS inhibition experiments: **1, 6**, **19**, and **22**−**24.** Modulation was measured in terms of total current **(**Figure 3, upper panel) and steroid-activated currents (Figure 3, lower panel).

The endogenous steroid **1** was a potent, full efficacy modulator of α1β2γ2L receptors expressed in oocytes (Figure 3, Table 4). At concentrations > 30 nM, **1**, in the nominal absence of GABA, also activated inward membrane currents. These responses were sensitive to inhibition by bicuculline and picrotoxin, indicating that

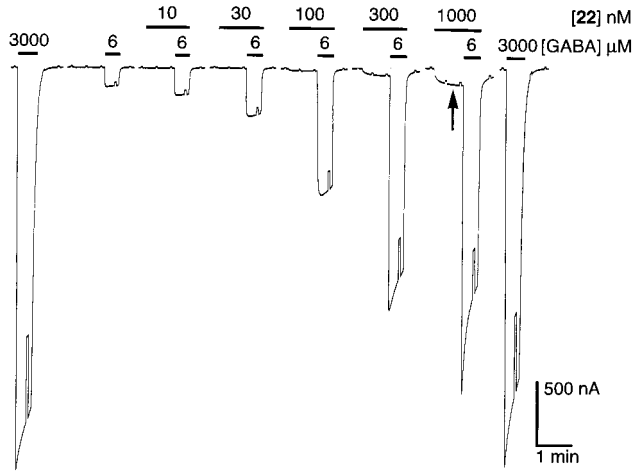


Figure 2. Sample records illustrating potentiation of GABA-activated currents by **22** in *Xenopus* oocytes expressing human $\alpha1\beta2\gamma2$L receptor subunits. For this cell the 0.05 (5%) GABA response required 6 μM GABA. Responses to 3 mM GABA (maximum currents) are included for comparison. Steroid-activated currents were apparent at higher concentrations of **22** (*e.g.*, arrow). Holding potential was −70 mV, periodically pulsed to −60 mV to help time drug applications. Inward current is indicated by downward deflection. Individual records were separated by 2−10 min intervals of wash.

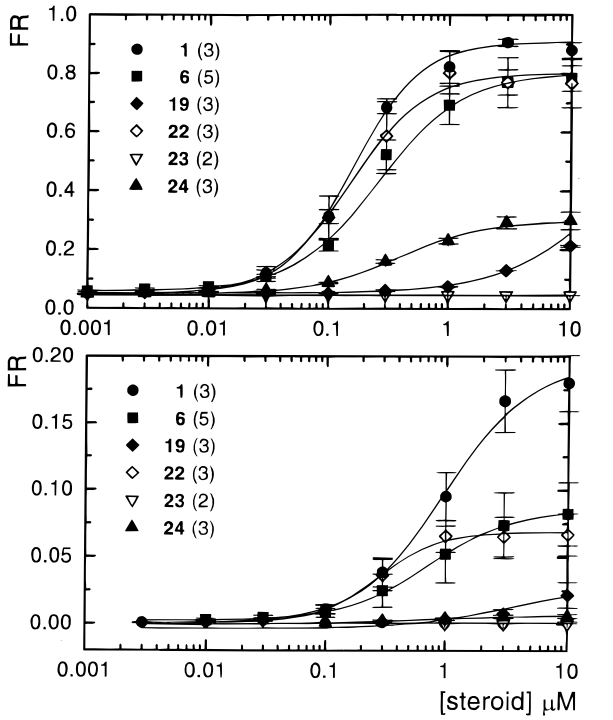
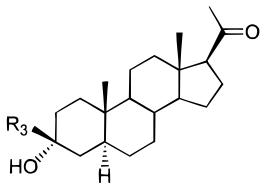


Figure 3. Functional modulation of human $\alpha1\beta2\gamma2$L receptors by 3β-substituted-5α-pregnanes: (upper panel) potentiation of GABA responses by steroids and (lower panel) steroid-activated currents in the nominal absence of GABA; note the expansion of the *y*-axis. Data are plotted as mean ±SEM, expressed as a fraction of the maximum current elicited by GABA; FR, fractional response. The number of separate experiments is given in parentheses. Smooth curves, best fits of the logistic equation to the data. Optimum EC_{50} and slope values for data in the upper panel are given in Table 3. EC_{50} (μM) and slope values for the steroid-activated currents are, respectively: **1**, 0.98, 1.2; **6**, 0.73, 1.2; and **22**, 0.27, 1.7.

the currents were due to direct steroidal activation of $GABA_A$ receptors. Maximum steroid-activated currents for **1** were between 0.15 and 0.2 of the peak GABA response, and the EC_{50} was 0.98 μM.

Table 4. Potency and Efficacy of 3β-Substituted-5α-pregnanes in Functional Modulation of Cloned $\alpha1\beta2\gamma2$L $GABA_A$ Receptors Expressed in *Xenopus* Oocytes[a]



compd	R_3	EC_{50} (nM)	slope	efficacy (FR)	*n*
1	H	160 ± 50	1.5 ± 0.1	0.91 ± 0.01	3
6	CH_3	260 ± 40	1.2 ± 0.1	0.80 ± 0.04	5
19	$HOCH_2$	∼20000			3
22	N_3CH_2	150 ± 50	1.4 ± 0.2	0.80 ± 0.05	3
23	Me_2NCH_2	>50000			2
24	F_3C	390 ± 70	1.2 ± 0.1	0.30 ± 0.02	3

[a] Potentiation of GABA-activated currents by steroids was measured as described in Results and the Experimental Section. EC_{50} and slope values are the optimum logistic fits of data presented in Figure 3. For **19** an approximate EC_{50} was estimated by extrapolation, assuming full efficacy modulation (Table 1). Efficacy is expressed in terms of a fractional response (FR) calculated with respect to the maximum GABA current elicited in the cell. Data are given as the mean ± SEM, quoted to two significant figures.

The azido analog **22** showed equal potency to **1** in modulation of GABA responses. The methyl-substituted analog **6** was 1.6 times less potent. Both steroids were full efficacy modulators and, like **1**, caused some direct activation of $\alpha1\beta2\gamma2$L receptors. EC_{50} values for steroid-activated currents were 0.28 μM for **22** and 0.73 μM for **6**. The fractional peak efficacy of responses elicited in the absence of GABA by **22** and **6** was approximately 0.07.

The (trifluoromethyl)pregnane **24** was 2.4 times less potent than **1**. As seen in the [^{35}S]TBPS binding, **24** showed distinctly low-efficacy modulation with maximum potentiation only 0.3 of the peak GABA response. Changing DMSO concentrations over the range 0.1−1% had little effect on levels of modulation induced by 1 μM **24**, suggesting that solubility was not a factor in limiting efficacy. The 3β-(hydroxymethyl)pregnane **19** potentiated GABA currents with low-potency, and the tertiary amine **23** was inactive at concentrations up to 10 μM. The latter compound was also tested for antagonism at steroid sites. At 10 μM, **23** did not inhibit modulation induced by 30 nM **6**, indicating it is not a steroid site antagonist. The low-efficacy modulator **24** and the low-potency compound **19** elicited only small steroid-activated currents that were <0.02 of the maximum GABA response.

Conclusions

The 3β-modifications made to the naturally occurring progesterone metabolites **1** and **2** generally maintain good potency at the neuroactive steroid binding site as determined by inhibition of [^{35}S]TBPS binding and confirmed in three cases by electrophysiological assay. Small lipophilic groups in this position are well accommodated, as well as larger groups incorporating unsaturation. In the 5α-series, 3β-ethyl, -propyl, -trifluoromethyl, and -(benzyloxy)methyl, as well as substituents of the form XCH_2, where X contains unsaturation or is Cl, Br, or I, show limited efficacy in inhibiting [^{35}S]TBPS binding. Limited efficacy may be due to partial agonism at the neuroactive steroid binding site and/or neuroactive steroid receptor heterogenity. The latter possibility

exists since numerous $GABA_A$ receptor subunits and variants are expressed in rat cortex.[23] Electrophysiological and binding studies using recombinant receptors have confirmed that the trifluoromethyl compound **24** (Co 2-1970) is a partial agonist for the neuroactive steroid site of the $GABA_A$ receptor.[24] It is possible that partial agonist neuroactive steroids may have superior *in vivo* profiles compared to full agonists. Partial agonist benzodiazepines have been found to have improved therapeutic profiles in preclinical studies compared to benzodiazepine full agonists, including less sedation, decreased interaction with alcohol, and diminished abuse potential.[25]

In the 5β-series, addition of a 3β-substituent to the two-component inhibitor **2** gives compounds which inhibit [^{35}S]TBPS binding via a single class of binding sites. Although the nature of the two-component inhibition of TBPS binding in rat cortical membranes has not been fully explained, $GABA_A$ receptors could contain two nonequivalent steroid binding sites per receptor complex which may interact with negative cooperativity. All of the 3β-substituted-5β-pregnanes tested (Table 2) are one-component full inhibitors of [^{35}S]TBPS binding, suggesting that these neuroactive steroids are full agonists which are nonselective and do not display negative cooperativity.

The apparent potency of steroids as measured by electrophysiological assay was consistently lower than that measured by [^{35}S]TBPS binding, with ranges of 1.5−10-fold for the compounds tested. Nonetheless, there was generally good agreement between the two assay systems.[4] In particular, the electrical assays confirmed that **1**, **6**, and **22** are potent full efficacy agonists, that **24** is a low-efficacy modulator, that **19** is a low-potency modulator, and that **23** is inactive. The potency difference found between **1** and **22** in the [^{35}S]-TBPS binding assay was not apparent in electrophysiological assays, and while **1** and **6** are equipotent in the [^{35}S]TBPS binding assay, **6** is 1.6-fold less potent than **1** with α1β2γ2L receptors. These modest differences in relative potencies may simply reflect differences between allosteric modulation of the TBPS binding site as opposed to the channel itself or may be a consequence of differences in subtype selectivity. For example, some classes of the $GABA_A$ receptor found in rat brain membranes could have different sensitivities for steroids than the cloned α1β2γ2L receptors expressed in oocytes.

In addition to maintaining *in vitro* potency, the presence of 3β-substitution in the steroids synthesized has been shown to improve oral *in vivo* activity when compared with **1** and **2**. For example, compound **6** (CCD 1042) is an orally active anticonvulsant in animals, whereas **1** and **2** are inactive.[26] Thus, appropriate 3β-substitution of neuroactive steroids results in potent $GABA_A$ receptor modulators which have activity following oral administration and should prevent conversion to metabolites with hormonal side effects. These compounds represent a clear step forward in the development of neuroactive steroids for treatment of neuropsychiatric disorders.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded on a Varian 200 or 300 MHz spectrometer in $CDCl_3$ with tetramethylsilane as reference. ^{19}F NMR spectra in $CDCl_3$ are referenced to $FCCl_3$ at 0.00 ppm. Infrared spectra were obtained as KBr pellets on a Perkin-Elmer 1600 Series FTIR instrument and are referenced to polystyrene film at 1602 cm^{-1}. 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. Flash chromatography on silica gel (230−400 mesh; Mallinckrodt) was carried out as described by Still.[27] HPLC grade solvents were obtained from Baxter. THF and ether were distilled from sodium and benzophenone. Methylene chloride and DMSO 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. 20,20-(Ethylenedioxy)-5α- and -5β-pregnan-3-ones (**3a**,**b**, respectively) and 5α-pregnane-3,20-dione were obtained from DioSynth (Oss, The Netherlands) or prepared as described in the literature.[28] 3α-Hydroxy-5α-pregnan-20-one (**1**) was obtained from Steraloids (Windham, NH) or prepared as described in the literature.[29] The 5β-isomer **2** was obtained from DioSynth. 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).

(3*R*)-Spiro[oxirane-2′,5α-pregnan]-20-one (4a). To a stirred solution of trimethylsulfoxonium iodide (5.29 g, 24.0 mmol) in 75 mL of DMSO was added NaH (97%; 488 mg, 19.7 mmol). After stirring at room temperature for 1 h, a suspension of 5α-pregnane-3,20-dione (1.54 g, 4.86 mmol) in 50 mL of DMSO was added dropwise via addition funnel. After 2.5 h, the reaction mixture was poured into ice-cold water and extracted with ether (3×). The combined ether layers were then washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. Recrystallization from 50 mL of 1:1 MeOH/acetone gave 1.18 g (73%) of **4a** as a white solid: mp 161 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.62 (s, 2H), 2.54 (t, 1H), 2.11 (s, 3H), 0.84 (s, 3H), 0.61 (s, 3H). Anal. ($C_{22}H_{34}O_2$) C, H.

20,20-(Ethylenedioxy)-(3*R*)-spiro[oxirane-2′,5α-pregnane] (5a). A mixture of trimethylsulfoxonium iodide (3.80 g, 17.3 mmol) and potassium *tert*-butoxide (95%; 1.87 g, 15.8 mmol) in 50 mL of dry THF was refluxed for 1.5 h. To the resulting mixture at room temperature was added solid **3a** (4.81 g, 13.3 mmol). After stirring at room temperature for 3 h, the solvent was removed and the residue was partitioned between CH_2Cl_2, and water. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were washed with a saturated NaCl solution, dried ($MgSO_4$), filtered, and evaporated to dryness. Recrystallization from 1:1 MeOH/acetone afforded 3.15 g (63%) of **5a** as a white solid: mp 169−171 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05−3.85 (m, 4H), 2.61 (s, 2H), 1.29 (s, 3H), 0.84 (s, 3H), 0.76 (s, 3H). Anal. ($C_{24}H_{38}O_3$) C, H

20,20-(Ethylenedioxy)-(3*R*)-spiro[oxirane-2′,5β-pregnane] (5b). To a solution of **37** (1.05 g, 2.94 mmol) in 10 mL of dry CH_2Cl_2 was added anhydrous potassium carbonate (500 mg, 3.62 mmol). The resulting mixture was cooled in an ice/water bath, and solid 3-chloroperoxybenzoic acid (50−60% by weight; 860 mg, 2.99 mmol if 60%) was added in a few portions. After stirring for 1 h, the mixture was filtered and the precipitated benzoate salt was washed with CH_2Cl_2. The solvent was removed in vacuo, and the residue was chromatographed (10% hexane/CH_2Cl_2). Along with 679 mg (62%) of the undesired epoxide (R_f 0.3, 100% CH_2Cl_2) the desired epoxide **5b** (407 mg) was isolated contaminated with 20% of the undesired epoxide. The impure desired epoxide was rechromatographed (10% hexane/CH_2Cl_2). The desired epoxide (R_f 0.23 100% CH_2Cl_2) was isolated as a white solid in 26% yield: mp 115.5−117.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.00−3.83 (m, 4H), 2.58 (s, 2H), 2.39 (t, 1H, J = 13.2 Hz), 1.30 (s, 3H), 0.98 (s, 3H), 0.75 (s, 3H). Anal. ($C_{24}H_{38}O_3$) C, H.

3α-Hydroxy-3β-methyl-5α-pregnan-20-one (6). A suspension of **4a** (10.0 g, 30.3 mmol) in 150 mL of 1:1 THF/MeOH was treated with sodium iodide (7.1 g, 45 mmol) and glacial acetic acid (4.5 mL, 45 mmol). After heating at reflux for 1.5 h, an additional 1.0 g of NaI and 0.65 mL of HOAc were added, and refluxing was continued for an additional 30 min. The resulting mixture was diluted with 175 mL of 1:1 methanol/THF, and solid NaOAc (8 g) was added followed by 2 g of 5% Pd/C. After stirring under an atmosphere of hydrogen gas for

18 h, the catalyst was removed by filtration and the solvent was removed under reduced pressure. The solution of the residue in CH_2Cl_2 was washed successively with a saturated $NaHCO_3$ solution, a 10% aqueous $Na_2S_2O_3$ solution, and a saturated NaCl solution. After drying ($MgSO_4$), the solvent was filtered and evaporated. The crude product was recrystallized twice from MeOH to give 5.1 g (51%) of **6** as a white solid: mp 190−192 °C (lit.[12a] mp 189 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.53 (t, 1H, J = 8.8 Hz), 2.11 (s, 3H), 1.20 (s, 3H), 0.75 (s, 3H), 0.60 (s, 3H). Anal. ($C_{22}H_{36}O_2$) C, H.

3β-Ethyl-3α-hydroxy-5α-pregnan-20-one (7). A solution of **9** (312 mg, 0.91 mmol) in 10 mL of EtOH was treated with 40 mg of 5% Pd/C and stirred under a hydrogen atmosphere for 24 h. Removal of the catalyst by filtration and concentration in vacuo gave crude **7**. Recrystallization from 3:1 hexane/acetone gave 222 mg (71% yield) of **7** as a white solid: mp 163−165 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.53 (t, 1H, J = 8.8 Hz), 2.11 (s, 3H), 0.91 (t, 3H, J = 7.4 Hz), 0.74 (s, 3H), 0.60 (s, 3H). Anal. ($C_{23}H_{38}O_2$) C, H.

3α-Hydroxy-3β-propyl-5α-pregnan-20-one (8). As described for the synthesis of **7**, hydrogenation of **12** afforded **8** in 32% yield after recrystallization from hexane/acetone: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.53 (t, 1H), 2.11 (s, 3H), 0.74 (s, 3H), 0.60 (s, 3H). Anal. ($C_{24}H_{40}O_2 \cdot 2/3H_2O$) C, H.

3β-Ethenyl-3α-hydroxy-5α-pregnan-20-one (9). A suspension of trimethylsulfonium iodide (365 mg, 1.79 mmol) in 4 mL of THF at −10 °C was treated with a 2.5 M solution of *n*-BuLi in hexanes (0.75 mL, 1.87 mmol). The resulting mixture was allowed to warm to 0 °C and then recooled to −10 °C, and a solution of **5a** in 3 mL of dry THF was added via syringe. The reaction mixture was allowed to warm to room temperature and stirred overnight. After partitioning between water and EtOAc, the reaction mixture was dried (Na_2SO_4), filtered, and concentrated. Removal of the 20-ketal with 1 N HCl/acetone/THF and flash chromatography (15% acetone/hexane) gave **9** in 70% yield as a white solid: mp 163−165 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.92 (dd, 1H, J = 17.3, 10.7 Hz), 5.22 (d, 1H, J = 17.3 Hz), 5.00 (d, 1H, J = 10.7 Hz), 2.53 (t, 1H, J = 8.9 Hz), 2.20−1.98 (m, 2H), 2.11 (s, 3H), 0.78 (s, 3H), 0.60 (s, 3H); IR 3083 (=CH_2), 1700 (C=O), 993 (=CH), 908 cm^{-1} (=CH). Anal. ($C_{23}H_{36}O_2$) C, H.

3β-Ethynyl-3α-hydroxy-5α-pregnan-20-one (10). Acetylene gas was bubbled through a mixture of lithium acetylide−ethylenediamine complex (2.75 g, 90%, 27.5 mmol) in dry benzene (60 mL) at a moderate rate. The mixture was then heated to 50−55 °C and treated in parts with **3a** (9 g, 25 mmol). After stirring at this temperature for 5 h and then at room temperature for another 17 h, the resulting suspension was cooled to 10 °C and was treated with saturated NaCl solution (5 mL). The solvent was removed, and the residue was taken up in water. The water insoluble product was collected by filtration, washed with water, and dried under vacuum. Recrystallization from EtOAc gave unreacted **3a** (3.35 g). The mother liquor was evaporated to dryness, and the residue was purified by column chromatography. Elution with a toluene/acetone mixture (92:8) gave unreacted **3a** (1.3 g) followed by 20-ketal-protected **10**. Removal of the ketal (aqueous HCl/THF/acetone) followed by column chromatography (92:8 toluene/acetone mixture) gave 280 mg (3%) of **10**: mp 175−177 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.52 (t, 1H), 2.42 (s, 1H), 2.11 (s, 3H), 0.81 (s, 3H), 0.60 (s, 3H). Anal. ($C_{23}H_{34}O_2$) C, H.

3α-Hydroxy-3β-(2-propenyl)-5α-pregnan-20-one (11). A 2 M solution of allylmagnesium chloride in THF (5 mL, 10 mmol) was diluted with 20 mL of THF and cooled to −75 °C. A solution of **3a** (1.00 g, 2.77 mmol) in 15 mL of dry THF was added to the reaction mixture via syringe pump over 30 min. The reaction mixture was stirred cold for 5 h and then allowed to warm to room temperature. After stirring overnight, the reaction mixture was added to ice-cold water and extracted with EtOAc (3 × 50 mL). The pooled organic layers were washed with a saturated NaCl solution, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was dissolved in 7 mL of THF and 3 mL of acetone and treated with 1 mL of a 1 M HCl solution. After stirring overnight, the reaction mixture was added to 5 mL of a saturated $NaHCO_3$ solution and 15 mL of water. The mixture was extracted with EtOAc (3 × 25 mL), and the pooled organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated. Column chromatography (elution with a gradient from 100% CH_2Cl_2 to 1.5% acetone/CH_2Cl_2) and recrystallization from hexane afforded 154 mg (12%) of the alkene as a white solid: mp 163−164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.89 (ddt, 1H, J = 16.9, 10.3 Hz), 5.15 (dd, 1H, J = 10.3, 2.1 Hz), 5.11 (dd, 1H, J = 15.9, 2.0 Hz), 2.54 (t, 1H), 2.11 (s, 3H), 0.74 (s, 3H), 0.60 (s, 3H). Anal. ($C_{24}H_{38}O_2$) C, H.

3α-Hydroxy-3β-(2-propynyl)-5α-pregnan-20-one (12). A suspension of lithium acetylide−ethylenediamine complex (2.48 g, 26.9 mmol) in 12 mL of dry DMSO was cooled in an ice/water bath, and solid **5a** (2.02 g, 5.39 mmol) was added. After stirring at room temperature for 21 h, the mixture was cooled to 0 °C and added to 100 mL of an ice-cold saturated NH_4Cl solution. After addition of 100 mL of water, the mixture was extracted with EtOAc (4 × 50 mL). The combined EtOAc extracts were washed with a saturated NaCl solution, dried (Na_2SO_4), filtered, and concentrated. The 20-ketal obtained was dissolved in 25 mL of THF and treated with 5 mL of a 1 N HCl solution and 5 mL of acetone. After stirring for 3 h, the reaction mixture was cooled in an ice/water bath. The precipitate that formed was isolated and washed with a total of 35 mL of 4:1:1 THF/water/acetone, affording 1.3 g (68%) of **12** as a white solid: mp 218−223 °C dec. The mother liquor was added to 10 mL of a saturated $NaHCO_3$ solution and extracted with EtOAc (3 × 50 mL). The organic layers were combined, washed with a saturated NaCl solution, dried, and concentrated to give an additional 487 mg of **12**: total yield 1.79 g (93%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.53 (t, 1H, J = 8.8 Hz), 2.33 (s, 1H), 2.32 (s, 1H), 2.11 (s, 3H), 2.17 (s, 1H), 0.75 (s, 3H), 0.60 (s, 3H). Anal. ($C_{24}H_{36}O_2$) C, H.

3β-(But-3-enyl)-3α-hydroxy-5α-pregnan-20-one (13). Dry benzene (3 mL) was degassed for 15 min with argon. Next were added successively 3α-hydroxy-3β-(iodomethyl)-5α-pregnan-20-one (**28**; 263 mg, 0.574 mmol), allyltributyltin (97%; 0.5 mL, 1.56 mmol), and azobis(isobutyroylnitrile) (20 mg, 0.12 mmol). The resulting mixture was refluxed for 3.5 h and allowed to cool to room temperature. After removing the solvent, the residue was subjected to flash chromatography twice, eluting with CH_2Cl_2 and 7:1 hexane/acetone. Compound **13** was obtained as a white solid (75 mg, 35%): mp 140−141 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.86 (ddt, 1H, J = 17.0, 10.2, 6.6 Hz), 5.04 (d, 1H, J = 17.1 Hz), 4.96 (d, 1H, J = 10.1 Hz), 2.53 (t, 1H), 2.12 (s, 3H), 0.75 (s, 3H), 0.61 (s, 3H). Anal. ($C_{25}H_{40}O_2$) C, H.

3β-(Butadi-2,3-enyl)-3α-hydroxy-5α-pregnan-20-one (14). Propargyl bromide (80 wt % solution in toluene; 1.7 mL, 11 mmol) was added to a mixture of Mg (471 mg, 19.4 mmol) and dry Et_2O (25 mL). $HgCl_2$ (15 mg) was then added. The resulting mixture was first stirred at room temperature and then warmed briefly. The turbid reaction mixture was stirred at <20 °C (cold water bath) for ∼1 h, after which time most of the Mg had reacted. Propargyl bromide (80 wt % in toluene; 0.4 mL, 2.69 mmol) was then added, and the resulting mixture was stirred at <20 °C for ∼1 h. A suspension of triphenyltin chloride (95%; 5.04 g, 12.4 mmol) in Et_2O (50 mL) was added dropwise, and the reaction mixture was stirred at room temperature for ∼3 h. A saturated aqueous NH_4Cl solution (10 mL) was added, and the two phases which formed were separated. The organic phase was dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a white solid. Recrystallization from hexane (50 mL) furnished triphenylprop-2-ynylstannane as white, plate-shaped crystals (3.92 g, 81%).

A mixture containing **28** (234 mg, 0.51 mmol), triphenylprop-2-ynylstannane (795 mg, 2.04 mmol), azobis(isobutyroylnitrile) (18 mg, 0.11 mmol), and dry benzene (2 mL) was degassed for ∼10 min. After 2 h at reflux, the solvent was removed, and the residue was subjected to flash chromatography, affording **14** as a white solid (20 mg, 11%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.14 (m, 1H), 4.78 (m, 2H), 2.53 (m, 1H), 2.11 (s, 3H), 0.74 (s, 3H), 0.60 (s, 3H). Anal. ($C_{25}H_{38}O_2 \cdot {}^3/_8H_2O$) C, H.

3α-Hydroxy-3β-(methoxymethyl)-5α-pregnan-20-one (15). To a solution of **4a** (5.14 g, 15.6 mmol) in CH_3OH (600 mL) was added Na metal (∼600 mg), and the resulting solution

was heated at reflux for 16 h. After the reaction mixture had come to room temperature, glacial acetic acid (3 mL) was added dropwise. The solvent was then removed under reduced pressure, and CH_2Cl_2 and water were added. The aqueous layer was back-extracted with CH_2Cl_2. The combined organics were washed with a saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a white solid (5.55 g), which was found to be a 84:16 mixture of **15** and its 17-epimer on the basis of its ^{1}H NMR spectrum. Recrystallization from 1:1 hexane/acetone furnished **15** (3.27 g, 58%) as white crystals: mp 163−164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.40 (s, 3H), 3.19 (s, 2H), 2.54 (t, 1H), 2.12 (s, 3H), 0.76 (s, 3H), 0.61 (s, 3H). Anal. ($C_{23}H_{38}O_3$) C, H.

A similar procedure was used for the preparation of 3β-(ethoxymethyl)-3α-hydroxy-5α-pregnan-20-one (**16**): mp 101−102 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.54 (q, 2H, J = 7.0 Hz), 3.22 (s, 2H), 2.55 (t, 1H, J = 8.9 Hz), 2.13 (s, 3H), 1.22 (t, 3H, J = 7.0 Hz), 0.77 (s, 3H), 0.62 (s, 3H). Anal. ($C_{24}H_{40}O_3$) C, H. 3α-Hydroxy-3β-propoxymethyl-5α-pregnan-20-one (**17**): mp 96−98 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.42 (t, 2H, J = 6.5 Hz), 3.20 (s, 2H), 2.53 (t, 1H, J = 8.7 Hz), 2.11 (s, 3H), 0.93 (t, 3H, J = 6.5 Hz), 0.75 (s, 3H), 0.60 (s, 3H). Anal. ($C_{25}H_{42}O_3$) C, H. 3β-[(Benzyloxy)methyl]-3α-hydroxy-5α-pregnan-20-one (**18**): mp 125−126 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H), 4.57 (s, 2H), 3.28 (s, 2H), 2.53 (t, 1H), 2.12 (s, 3H), 0.76 (s, 3H), 0.62 (s, 3H). Anal. ($C_{28}H_{40}O_3$) C, H.

3α-Hydroxy-3β-(hydroxymethyl)-5α-pregnan-20-one (19). Argon was passed over a solution of **18** (606 mg, 1.3 mmol) in absolute ethanol (100 mL). Palladium on activated carbon (5%; 352 mg) was added in one portion, and hydrogen gas (2−3 L) was maintained over the reaction mixture. After 60 h, the hydrogen was replaced with argon and the reaction mixture was filtered. The filtrate was evaporated, and the residue was purified by flash column chromatography (8:1 CH_2Cl_2/acetone) to give **19** as a white solid (440 mg, 91%): mp 183.5−185 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.41 (s, 2H), 2.55 (t, 1H), 2.13 (s, 3H), 0.78 (s, 3H), 0.62 (s, 3H). Anal. ($C_{22}H_{36}O_3 \cdot 0.25H_2O$) C, H.

3α-Hydroxy-3β-(thiomethoxymethyl)-5α-pregnan-20-one (20). To a mixture of **4a** (348 mg, 1.05 mmol) and dry DMF (10 mL), which became a clear solution on heating, was added $NaSCH_3$ (165 mg, 2.33 mmol). The reaction mixture was stirred at room temperature for 30 min, and 1 mL of CH_3OH was added. Subsequently CH_2Cl_2 and water were added. Brine was also added to overcome the emulsion formation. The aqueous layer was back-extracted with CH_2Cl_2. The combined organics were washed with water, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a white solid (387 mg), which was found to be a mixture of **20** and its 17-epimer on the basis of its ^{1}H NMR spectrum. Flash column chromatography (100:1 CH_2Cl_2/acetone) furnished 225 mg (57% yield) of **20**: mp 157−159 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.61 (s, 2H), 2.54 (t, 1H), 2.20 (s, 3H), 2.13 (s, 3H), 0.76 (s, 3H), 0.62 (s, 3H). Anal. ($C_{23}H_{38}SO_2$) C, H, S.

3β-(Cyanomethyl)-3α-hydroxy-5α-pregnan-20-one (21). Potassium cyanide (210 mg, 3.2 mmol) and **5a** (1.0 g, 2.7 mmol) were stirred in ethanol (175 mL) for 48 h, refluxed for 5 h, and then allowed to stir at room temperature overnight. Ether (100 mL) and brine (100 mL) were added to the reaction mixture. The separated aqueous phase was washed with ether, and the combined organic phases were washed with brine. Aqueous HCl (1 M, 6 mL) and THF (15 mL) were added to the organic phases, and the solution was stirred overnight. An aqueous $NaHCO_3$ solution (20 mL) was added and then water (50 mL). The separated aqueous phase was washed with ether, and the combined organic phases were dried over $MgSO_4$, filtered, and evaporated. The white solid residue was purified by flash column chromatography (40:20:1 CH_2Cl_2/hexane/acetone) to give **21** as a white solid (493 mg, 52%): mp 218.5−220 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.52 (t, 1H), 2.49 (s, 2H), 2.12 (s, 3H), 0.79 (s, 3H), 0.61 (s, 3H); IR 2213 (CN), 1693 cm^{-1} (C=O). Anal. ($C_{23}H_{35}NO_2$) C, H, N.

3β-(Azidomethyl)-3α-hydroxy-5α-pregnan-20-one (22). To a solution of **4a** (1.01 g, 3.06 mmol) in dry DMF (50 mL) was added solid NaN_3 (226 mg, 3.48 mmol). Glacial acetic acid (1.05 mL, 17.5 mmol) was added, and the resulting mixture was warmed in order to give a clear solution. Little reaction was observed after 4 h at room temperature, so the reaction mixture was heated at 35−50 °C for 3 days. CH_2Cl_2 and water were added. The aqueous layer was back-extracted with CH_2Cl_2. The combined organic layers were washed with a saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered, and concentrated to give a solid. Purification by flash column chromatography (100% CH_2Cl_2) afforded the azide **22** (834 mg, 73%) as a white solid: mp 147−148 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.23 (s, 2H), 2.53 (t, 1H, J = 8.7 Hz), 2.12 (s, 3H), 0.76 (s, 3H), 0.61 (s, 3H); IR 2095 (N_3), 1689 cm^{-1} (C=O). Anal. ($C_{22}H_{35}N_3O_2$) C, H, N.

3β-[(Dimethylamino)methyl]-3α-hydroxy-5α-pregnan-20-one (23). A solution of **22** (330 mg, 0.883 mmol) in absolute EtOH was flushed with Ar; 5% Pd/C (94 mg) was added, and the mixture was flushed successively with Ar and hydrogen. The reaction mixture was then stirred under an atmosphere of hydrogen at room temperature for 5 h. The catalyst was removed by filtration. The resulting filtrate was concentrated under reduced pressure to an oil to which were added formaldeyde (37% in water; 2.0 mL, 72.1 mmol) and formic acid (95−97%; 0.5 mL, 13.3 mmol). The above mixture, which became a solution on refluxing, was heated with stirring between 60 and 110 °C overnight. After the mixture had come to room temperature, 50% NaOH (~5 mL) was added dropwise. Subsequently ether and water were added, and the resulting mixture was stirred until a solution formed. The aqueous layer was back-extracted with ether. The combined organics were washed successively with water and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give a solid, which was purified by flash column chromatography. Elution with acetone gave **23** (120 mg, 36%) as a white solid: mp 126−128 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.53 (t, 1H), 2.38 (s, 6H), 2.25 (s, 2H), 2.12 (s, 3H), 0.75 (s, 3H), 0.61 (s, 3H). Anal. ($C_{22}H_{35}N_3O_2$) C, H, N.

3α-Hydroxy-3β-(trifluoromethyl)-5α-pregnan-20-one (24). To a solution of **3a** (20.5 g, 56.9 mmol) in 170 mL of dry THF was added 10 mL (9.62 g, 67.6 mmol) of neat $TMSCF_3$. The resulting solution was cooled in an ice/water bath, and solid n-$Bu_4NF \cdot xH_2O$ (115 mg, 0.44 mmol) was added in one portion. The cold bath was removed, and the reaction mixture was stirred at room temperature for 5 h. An additional 1 mL (6.77 mmol) of $TMSCF_3$ was added along with 36 mg (0.14 mmol) of solid n-$Bu_4NF \cdot xH_2O$. After stirring overnight, 35 mL of a 3 N HCl solution was added. After 5 h, the two-phase reaction mixture was separated and the aqueous layer was extracted with EtOAc (3 × 50 mL). The pooled organic layers were washed with a saturated $NaHCO_3$ solution, dried over $MgSO_4$, filtered, and concentrated. Recrystallization from 20% acetone/hexane gave 9.28 g of the undesired 3β-ol. A second crop gave an additional 2.56 g of the 3β-ol. The mother liquor from the second crop was concentrated to dryness and recrystallized from hexane. The crystals that formed where subjected to two flash columns (100% CH_2Cl_2). A total of 1.08 g (5% yield) of the 3α-ol **24** was isolated: mp 187−189 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.53 (t, 1H), 2.12 (s, 3H), 0.79 (s, 3H), 0.61 (s, 3H); ^{19}F NMR ($CDCl_3$) −14.63 ppm (s). Anal. ($C_{22}H_{33}F_3O_2$) C, H.

3β-(Fluoromethyl)-3α-hydroxy-5α-pregnan-20-one (25). A mixture of n-$Bu_4NF \cdot xH_2O$ (7.87 g) and benzene (50 mL) was refluxed with azeotropic removal of water overnight. The mixture, which was not a clear solution, was then concentrated to ~10 mL and allowed to cool to room temperature. A solution of **5a** (2.55 g, 6.81 mmol) in 20 mL of dry benzene was then added. The reaction was refluxed and made more concentrated. After the mixture had come to room temperature (giving a light-yellow solid), ether and water were added. Since all the solid did not dissolve, CH_2Cl_2 was added. The aqueous layer was back-extracted with CH_2Cl_2. The combined organic extracts were washed twice with water, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was dissolved with heating in 105 mL of 20:1 acetone/water containing 1 N HCl and allowed to stand at room temperature for 30 min. The mixture became turbid, so CH_2Cl_2 was added to obtain a clear solution. The solvent was removed under reduced pressure, giving a white solid to which were added CH_2Cl_2 and water. The aqueous layer was back-extracted with CH_2Cl_2. The combined organics were washed

with a saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. Flash column chromatography (CH_2Cl_2) provided **25** (1.41 g, 59%): mp 201−203 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.17 (d, 2H, $J_{H,F}$ = 48 Hz), 2.54 (t, 1H, J = 8.8 Hz), 2.13 (s, 3H), 0.77 (s, 3H), 0.61 (s, 3H). Anal. ($C_{22}H_{35}FO_2$) C, H.

3β-(Chloromethyl)-3α-hydroxy-5α-pregnan-20-one (26). A mixture of **4a** (1.00 g, 3.02 mmol), dry tetramethylammonium chloride (696 mg, 6.35 mmol), glacial acetic acid (0.2 mL, 210 mg, 3.49 mmol), and 50 mL of DMF was heated at 90 °C. After 2h, an additional 60 mL of DMF and 390 mg (3.56 mmol) of tetramethylammonium chloride were added. After 75 min at 100 °C, the reaction mixture was allowed to cool and added to ice-cold water containing 25 mL of a saturated $NaHCO_3$ solution. The resulting mixture was extracted with EtOAc (4 × 100 mL). The pooled organic layers were extracted with 15 mL of a saturated $NaHCO_3$ solution and a saturated NaCl solution. The solvent was dried (Na_2SO_4), filtered, and concentrated. Column chromatography (100% CH_2Cl_2) and recrystallization from EtOH afforded 495 mg (44%) of **26** as a white solid: mp 214−215 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.47 (s, 2H), 2.52 (t, 1H, J = 8.9 Hz), 2.11 (s, 3H), 0.75 (s, 3H), 0.60 (s, 3H). Anal. ($C_{22}H_{35}ClO_2$) C, H, Cl.

3β-(Bromomethyl)-3α-hydroxy-5α-pregnan-20-one (27). A mixture of **4a** (362 mg, 1.10 mmol), tetramethylammonium bromide (322 mg, 2.16 mmol), acetic acid (1.0 mL, 17.5 mmol), and dry DMF (30 mL) was heated at ∼110 °C. After 3 h the solvent was removed by distillation under vacuum. To the resulting solid were added CH_2Cl_2, water, and brine. The organic layer was washed with saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a solid (380 mg). Flash chromatography (9:1 hexane/acetone) provided the bromide **27** (98 mg, 22%) as a white solid: mp 196−197 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.42 (s, 2H), 2.52 (t, 1H), 2.12 (s, 3H), 0.76 (s, 3H), 0.63 (s, 3H). Anal. ($C_{22}H_{35}BrO_2$) C, H, Br.

3α-Hydroxy-3β-(iodomethyl)-5α-pregnan-20-one (28). To a suspension of **4a** (3.06 g, 9.26 mmol) and NaI (1.80 g, 12.0 mmol) in 50 mL of 1:1 MeOH/THF was added glacial acetic acid (1 mL), and the reaction mixture was heated to reflux. After 4h at reflux, an additional 0.5 mL of glacial acetic acid was added. The reaction mixture was maintained at reflux for an additional 2 h and then allowed to stir overnight at room temperature. The reaction mixture was then concentrated in vacuo, and the residue was partitioned between CH_2Cl_2 and water. The aqueous layer was washed twice with CH_2Cl_2, and the combined organic layers were extracted with a saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered, and concentrated. Recrystallization from 1:1 acetone/hexane gave 2.84 g (67%) of **28** as a white solid: mp 132−133 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.30 (s, 2H), 2.53 (t, 1H, J = 8.8 Hz), 2.12 (s, 3H), 0.74 (s, 3H), 0.60 (s, 3H). Anal. ($C_{22}H_{35}IO_2$) C, H.

3β-(Carboxamidomethyl)-3α-hydroxy-5α-pregnan-20-one (29). Potassium carbonate (40 mg, 0.29 mmol) was added to a solution of **21** (61 mg, 0.17 mmol) in H_2O_2 (1.5 mL, 30%) and DMSO (6 mL) at 0−5 °C. The mixture was stirred at room temperature for 6 h and poured into ice/water. The separated solid was collected by filtration, washed with water, and dried. Column chromatography (1:1 acetone/hexane) gave **29** as a colorless solid (20 mg): mp 198−202 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.71−5.91 (br s, 1H), 5.31−5.51 (br s, 1H), 3.61−3.77 (m, 1H), 2.41−2.62 (m, 1H), 0.74 (s, 3H), 0.70 (s, 3H). Anal. ($C_{23}H_{37}NO_3$) C, H, N.

3α-Hydroxy-3β-methyl-5β-pregnan-20-one (30). Synthesis from 3b. To a solution of **3b** (1.00 g, 2.77 mmol) in 60 mL of dry THF at 0 °C was added 1.72 mL (5.16 mmol) of a 3 M solution of MeMgBr in ether. The reaction mixture was allowed to warm to room temperature, and an additional 0.86 mL (2.58 mmol) of the Grignard reagent was added. After 1 h, the reaction was quenched with a saturated NH_4Cl solution and the mixture diluted with 50 mL of water and extracted with EtOAc (3 × 50 mL). The pooled organic layers were dried ($MgSO_4$), filtered, and concentrated. Column chromatography (5% acetone/hexane) gave 490 mg of a white solid. Removal of the 20-ketal (acetone/*p*-TsOH/water) and recrystallization from 3:1 hexane/acetone gave 350 mg (38% yield) of **30**: mp 160−161 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.53 (t, 1H, J = 8.7 Hz), 2.11 (s, 3H), 1.27 (s, 3H), 0.95 (s, 3H), 0.60 (s, 3H).

Synthesis from Epoxide 5b. A solution of **5b** (110 mg, 0.294 mmol) in 4 mL of THF at 0 °C was treated with solid lithium aluminum hydride (28 mg, 0.74 mmol). The resulting mixture was stirred at room temperature for 1 h and then heated at reflux for 5 min. Once at room temperature, the reaction mixture was cooled in an ice/water bath and diluted with ether. Ice-cold water was added, and the organic layer was decanted from the two-phase mixture. The aqueous phase remaining was triturated with ether (3 × 15 mL). The combined ether layers were washed with water and brine, dried with Na_2SO_4, filtered, and concentrated. Column chromatography with 20% ethyl acetate/hexane gave 67 mg of ketal-protected **30**. Deketalization in 5 mL of THF and 1 mL of a 1 M HCl solution and partitioning between a saturated $NaHCO_3$ solution and ethyl acetate gave 51 mg of crude product. Column chromatography (30% ethyl acetate/hexane) afforded 25 mg (25%) of **30** as a white solid: mp 157−158 °C. ^{1}H NMR identical with **30** prepared above. Anal. ($C_{22}H_{36}O_2$) C, H.

3β-Ethyl-3α-hydroxy-5β-pregnan-20-one (31). A solution of **33** (155 mg) was dissolved in MeOH (12 mL), Pd/C (5%, 12 mg) was added, and the mixture was hydrogenated at 200 kPa pressure overnight at room temperature. Filtration of the catalyst followed by evaporation of the solvent yielded the crude product, which was recrystallized from a hexane−acetone mixture to give **31** (103 mg, 66%): mp 115−117 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.51−2.72 (m, 1H), 2.12 (s, 3H), 0.74 (s, 3H), 0.60 (s, 3H). Anal. ($C_{23}H_{38}O_2$) C, H.

3β-Ethenyl-3α-hydroxy-5β-pregnan-20-one (32). A solution of **3b** (1.18 g, 3.3 mmol) in dry THF (20 mL) was treated with a 1 M solution of vinyl magnesium bromide in THF (3.7 mmol, 3.7 mL) at −70 °C. After the mixture was stirred for 5 min and then at room temperature for 2.5 h, the reaction was quenched with a saturated NH_4Cl solution (10 mL). The solvent was removed, and the residue was extracted with EtOAc. The organic layer was washed with water, a dilute $NaHCO_3$ solution, water, and brine. After drying over $MgSO_4$, the solution was filtered and evaporated to yield the crude product. This crude product was then dissolved in acetone (20 mL), and a 1 N HCl solution (10 mL) was added. After stirring for 15 h, the solvents were removed and the residue was extracted with CH_2Cl_2. The organic layer was washed with water, a dilute $NaHCO_3$ solution, water, and brine. After drying ($MgSO_4$), the solution was filtered and concentrated in vacuo. Column chromatography (94:6 toluene/acetone) gave 189 mg (17%) of **32**: mp 113−116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.13 (dd, 1H, J = 18.4, 11.0 Hz), 5.31 (d, 1H, J = 18.4 Hz), 5.17 (d, 1H, J = 11.0 Hz), 2.52 (m, 1H), 2.11 (s, 3H), 0.93 (s, 3H), 0.59 (s, 3H). Anal. ($C_{23}H_{36}O_2 \cdot 0.25H_2O$) C, H.

3β-Ethynyl-3α-hydroxy-5β-pregnan-20-one (33). Using the procedure given for the synthesis of the 5α-isomer **10**, the 5β-isomer **33** was prepared in 45% yield: mp 196−197 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.54 (t, 1H), 2.51 (s, 1H), 2.12 (s, 3H), 0.97 (s, 3H), 0.60 (s, 3H). Anal. ($C_{23}H_{34}O_2$) C, H.

3α-Hydroxy-3β-(prop-2-ynyl)-5β-pregnan-20-one (34). To a mixture of Mg (268 mg, 11.0 mmol), propargyl bromide (80 wt %; 1.5 mL, 10.1 mmol), and dry Et_2O (15 mL) was added $HgCl_2$ (10 mg). The resulting mixture was warmed in a water bath in order to initiate the reaction. The mixture became turbid and was stirred at <20 °C until all Mg reacted (∼1 h). A solution of **3b** (725 mg, 2.01 mmol) in dry Et_2O (20 mL) and dry THF (10 mL) was cooled to −78 °C. Subsequently, the propargylmagnesium bromide solution (∼0.6 M; 7 mL), prepared above, was added dropwise at −78 °C. The resulting mixture was stirred at −78 °C for ∼1 h. The mixture was then allowed to warm to room temperature where it was stirred for 1 h; 1 N HCl, *p*-toluenesulfonic acid monohydrate, and acetone were added. The two-phase mixture was stirred at room temperature overnight. Et_2O and water were added. The aqueous layer was back-extracted with Et_2O. The combined organics were washed with a saturated aqueous $NaHCO_3$ solution and brine, dried ($MgSO_4$), filtered, and concentrated. Flash-chromatography twice (10:1 hexane/acetone and 7:1 hexane/ethyl acetate) gave **34** as a white solid (208 mg, 29%): mp 144−145.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.53

(t, 1H), 2.52 (s, 1H), 2.51 (s, 1H), 2.15 (s, 1H), 2.11 (s, 3H), 0.94 (s, 3H), 0.59 (s, 3H). Anal. ($C_{24}H_{36}O_2$) C, H.

20,20-(Ethylenedioxy)-3-methylene-5β-pregnane (37). A suspension of methyltriphenylphosphonium bromide (3.0 g, 8.40 mmol) in 10 mL of dry THF was treated with a 1 M solution of KO-*t*-Bu in THF (8.4 mL, 8.4 mmol). The resulting red-orange mixture was heated at 45 °C for 30 min. The reaction mixture was allowed to cool to room temperature, and **3b** (2.00 g, 5.55 mmol) was added as a solid in one portion. After refluxing for 3 h, the reaction mixture was allowed to cool to room temperature and then placed in an ice/water bath. After 10 min, the reaction mixture was poured into an ether/water mixture. The aqueous layer was extracted with ether (2 × 25 mL), and the combined organic layers were washed with a saturated NaCl solution, dried (Na_2SO_4), filtered, and concentrated. This crude material was triturated with hexane (2 × 100 mL). The hexane was evaporated in vacuo and the residue (2.2 g) was subjected to column chromatography (1000 mL of 1.25% acetone/hexane and 500 mL of 1.75% acetone/hexane) affording the alkene **37** as a white solid: mp 127−128 °C, weight 1.83 g (91%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.57 (s, 2H), 4.03−3.82 (m, 4H), 2.51 (t, 1H, J = 13.4 Hz), 2.10−0.97 (m, 22H), 1.29 (s, 3H), 0.93 (s, 3H), 0.75 (s, 3H).

3β-(Chloromethyl)-3α-hydroxy-5β-pregnan-20-one (35). Reaction of **5b** with tetramethylammonium chloride as described for the synthesis of the 5α-isomer **26** afforded **35** in 90% yield after column chromatography (100% CH_2Cl_2): mp 183−185 °C. Recrystallization from 15% acetone/hexane gave an analytically pure sample: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.73 (dd, 2H, J = 11.3, 19.5 Hz), 2.53 (t, 1H, J = 8.8 Hz), 2.11 (s, 3H), 0.95 (s, 3H), 0.59 (s, 3H). Anal. ($C_{22}H_{35}ClO_2$) C, H, Cl.

3α-Hydroxy-3β-(methoxymethyl)-5β-pregnan-20-one (36). Using the procedure described for the synthesis of the 5α-isomer, the 5β-isomer **36** was isolated as a white solid in 60% yield: mp 95.5−97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.39 (s, 3H), 3.38 (dd, 2H), 2.52 (t, 1H, J = 8.7 Hz), 2.11 (s, 3H), 0.93 (s, 3H), 0.59 (s, 3H). Anal. ($C_{23}H_{38}O_3$) C, H.

Steroid Inhibition of TBPS Binding. Rat brain cortical membranes were prepared as previously described.[4,5] Briefly, cortices were rapidly removed following decapitation of carbon dioxide-anesthetized Sprague−Dawley rats (200−250 g). The cortices were homogenized in 10 vols 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 vol of buffer. Aliquots (100 μL) of the membrane suspensions were incubated with 2 nM [^{35}S]TBPS and 5 μL aliquots of test drug (nine concentrations ranging from 1 nM to 10 μM final) 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. Hill coefficients were allowed to vary from unity 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 ± SEMs of the individual experiments were calculated.

$GABA_A$ Receptor Expression and Electrophysiology in *Xenopus* Oocytes. Preparation of oocytes and cRNAs encoding $GABA_A$ receptor subunits were as described elsewhere.[30] Oocytes were microinjected with a 1:1:1 mixture of cRNAs encoding the human α1, β2, and γ2L subunits, ~1 ng of cRNA for each subunit per cell. Electrical recordings were made using a two-electrode voltage clamp in frog Ringer solution containing (in mM): NaCl, 115; KCl, 2; $CaCl_2$, 1.8; HEPES, 5; pH 7.4. Drug and wash solutions were applied directly to the oocyte using a microcapillary "linear array".[24] Steroids were made up in DMSO over the range 0.001−10 mM. Stocks were diluted 1000−3000-fold in Ringer, and unless otherwise stated, DMSO was fixed at 0.3% by vol, a concentration at which the vehicle had little effect on GABA responses.[24] Concentration−response data for GABA and steroids, and concentration-modulation data for steroids, were fit to a four-parameter logistic equation (Origin, Microcal Inc.) Data are given as mean ± SEM, all values quoted to two significant figures.

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Supporting Information Available: Crystal structure data for **6** (10 pages); tables of observed and calculated structure factors (14 pages). Ordering information is given on any current masthead page.

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