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

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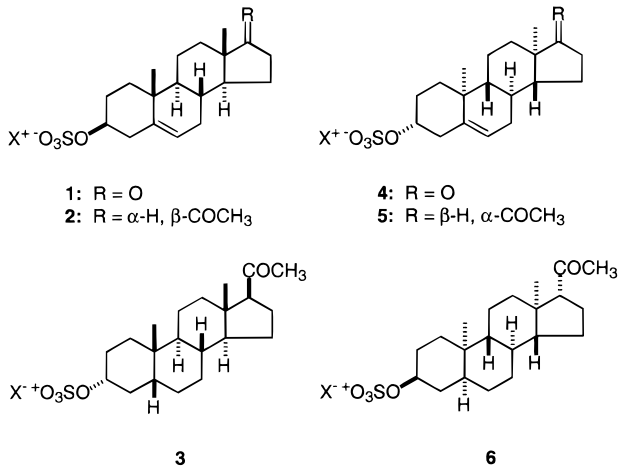
The unnatural enantiomers of dehydroepiandrosterone sulfate (**1**), pregnenolone sulfate (**2**), and (3α,5β)-3-hydroxypregnan-20-one sulfate (**3**), compounds **4**−**6**, respectively, were prepared by total steroid synthesis. The enantioselectivity of the compounds as negative modulators of the $GABA_A$ receptors present in cultured rat hippocampal neurons was examined using electrophysiological methods. Enantioselectivity was found for the inhibitory actions of the dehydroepiandrosterone enantiomers. The IC_{50}s for compounds **1** and **4** were 11 ± 1 and 80 ± 14 μM, respectively. Little, if any, enantioselectivity was found for the other two pairs of steroid sulfate inhibitors. The IC_{50}s for compounds **2** and **5** were 82 ± 12 and 76 ± 27 μM, respectively. The IC_{50}s for compounds **3** and **6** were 39 ± 7 and 46 ± 2 μM, respectively. The results suggest that the sites of action for the androstane and pregnane series of steroid sulfate blockers of GABA-mediated current are different. The observed enantioselectivity for the actions of dehydroepiandrosterone sulfate indicates that its inhibitory actions are mediated via a chiral recognition site and provides new evidence in support of the earlier hypothesis that there is a binding site for this compound on $GABA_A$ receptors. Conversely, the failure to observe enantioselectivity for the actions of pregnenolone sulfate and steroid sulfate **3** indicates that a chiral recognition site for these steroids does not exist on $GABA_A$ receptors and suggests that the effects of these compounds on this receptor's function may arise indirectly as a consequence of steroid-induced membrane perturbation.

Introduction

Steroids are potent modulators of a variety of ligand-gated and voltage-gated ion channels.[1−6] With regard to steroid modulation of $GABA_A$ receptor function, binding studies have shown that steroids that enhance GABA-mediated chloride currents (e.g., (3α,5α)- and (3α,5β)-3-hydroxypregnan-20-one) act at a site (or sites) distinct from the GABA, benzodiazepine, barbiturate, or picrotoxin binding sites.[1,7] Numerous derivatives have been examined to establish the structure−activity relationships of steroids having positive modulatory actions on $GABA_A$ receptor function.[8] Additionally, enantiomers of a few of these steroids as well as some structurally related benz[*e*]indene analogues have been investigated.[9−11] The high degree of enantioselectivity found in these latter studies provides additional evidence that, for at least some steroids, their positive modulatory actions are due to their direct interaction with the receptor and not to an indirect action arising from steroid-induced perturbation of the membrane in which the receptor resides.

In addition to the steroids that have positive modulatory actions on $GABA_A$ receptor function, there are sulfated steroids that have negative modulatory actions. Thus, dehydroepiandrosterone sulfate (**1**, Chart 1) and pregnenolone sulfate (**2**) have been shown to decrease GABA-mediated chloride currents.[12−18] Although there is general agreement that the negative modulatory actions of these two steroids are not mediated by the same binding sites responsible for the positive modulatory actions of the other nonsulfated steroids (vide supra), and further agreement that these two sulfated steroids do not exert their negative modulatory effects at a common site, the location and number of binding sites for the sulfated steroids on $GABA_A$ receptors remains speculative. Extensive structure−activity studies of steroid sulfate blockers of $GABA_A$ receptor function have not appeared in the literature. Moreover, no effort has been made to determine if the actions of these

Chart 1



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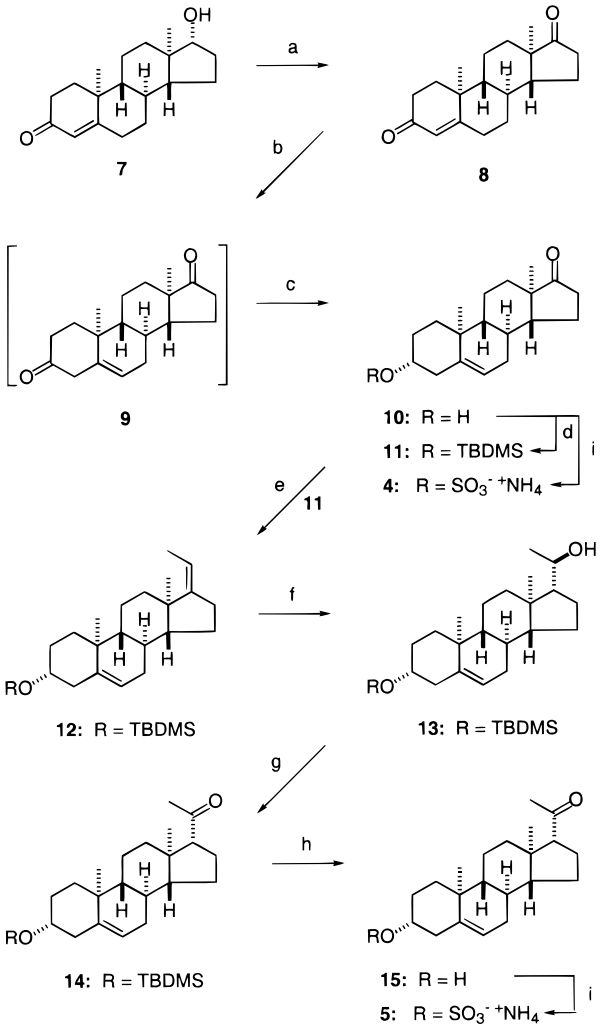
Scheme 1[a]



[a] (a) Jones reagent, acetone; (b) *t*-BuOK, *t*-BuOH; (c) LiAl(*t*-BuO)$_3$H, THF; (d) TBDMSCl, DMAP, Et_3N, THF, CH_2Cl_2; (e) $EtP(Ph)_3Br$, NaH, DMSO, THF; (f) (i) 9-BBN-H, THF; (ii) 30% H_2O_2, 10% NaOH; (g) PCC, CH_2Cl_2; (h) (*n*-Bu)$_4$NF, THF; (i) (i) $(CH_3)_3N{\cdot}SO_3$, pyridine; (ii) NH_3, CH_2Cl_2.

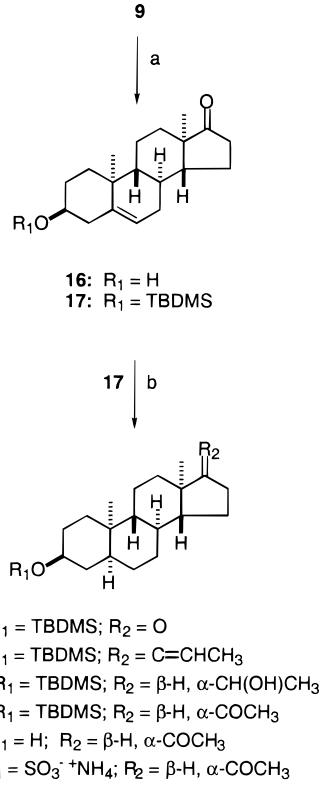
compounds on GABA$_A$ receptor function are mediated via an enantioselective recognition site.

The goal of the present study was to determine if the negative modulatory actions of sulfated steroids at GABA$_A$ receptors are enantioselective. Whereas the direct interactions of sulfated steroids with GABA$_A$ receptors would be expected to be highly enantioselective, indirect effects on receptor function due to steroid-induced membrane perturbation are not expected to be highly enantioselective. Accordingly, the actions of steroids **1**−**3** and their corresponding enantiomers **4**−**6** on the GABA$_A$ receptors found in cultured rat hippocampal neurons have been examined using electrophysiological methods.

Chemistry

The chemistry reported here was initially performed using testosterone (the naturally occurring enantiomer of steroid **7**) as a starting material to optimize reaction conditions and to provide samples of the enantiomers of all compounds described in Schemes 1 and 2. Steroid **7** was prepared according to a literature procedure,[19,20]

Scheme 2[a]



[a] (a) K-Selectride, THF; (b) Pd-C, H_2, *i*-PrOH.

and Jones oxidation of steroid **7** at 0 °C gave diketone **8** in 95% yield (Scheme 1). Deconjugation of the enone system in steroid **8** was achieved in the customary manner using *t*-BuOK in *t*-BuOH under strictly anaerobic conditions.[21] Numerous additional products are formed if oxygen is not rigorously excluded in this deconjugation reaction. Steroid intermediate **9** was characterized spectroscopically by ^{1}H NMR and ^{13}C NMR, and it was found to contain only small amounts of side products and none of the conjugated enone. Since it was found that chromatography of steroid **9** on silica gel resulted in substantial loss of the material, the compound was used in the next reaction without purification. Reduction of the carbonyl group at C-3 was accomplished regioselectively and diastereoselectively by treating steroid **9** with LiAl(*t*-BuO)$_3$H in THF at −78 °C to give product **10**. The overall yield for the conversion of steroid **8** to steroid **10** was 49% ($[\alpha]_D^{25}$ −4.68, ee 98.9%).

Using a modification of the method developed by Dusza,[22] steroid **10** was treated with sulfur trioxide trimethylamine complex in pyridine to give product **4** as its pyridinium salt. This pyridinium salt was then dissolved in CH_2Cl_2, and a stream of anhydrous ammonia was bubbled through the solution. The pure ammonium salt of steroid **4** precipitated from the CH_2Cl_2 solution and was isolated in 72% yield ($[\alpha]_D^{25}$ −13.2; for dehydroepiandrosterone sulfate ammonium salt, $[\alpha]_D^{27}$ +11.4).

Starting from steroid **10**, the remainder of the reactions shown in Scheme 1 summarize the synthetic route used for the preparation of pregnenolone sulfate enantiomer **5**. Hence, steroid **10** was converted into the known steroid **11** in 95% yield using Et_3N, DMAP, and

TBDMSCl in THF and CH_2Cl_2.[19] Also as reported previously, steroid **11** was converted into known steroid **12** in 72% yield using standard ylide chemistry (NaH, $EtP(Ph)_3Br$ in DMSO).[19] The $\Delta^{17(20)}$ double bond of steroid **12** was then selectively hydroborated using 9-BBN-H in THF, and the intermediate organoborane was oxidized with 30% H_2O_2 to give previously unreported steroid **13** in 85% yield. An analogous regioselective reaction of the $\Delta^{17(20)}$ double bond in the presence of the Δ^5 double bond with 9-BBN-H has been reported previously,[19] and the assignment of the 20*R* configuration to the hydroxyl group at C-20 is based on the fact that hydroboration of the analogous (*Z*)-$\Delta^{17(20)}$ double bond in naturally occurring steroids leads stereoselectively to the 20*S* alcohols.[23]

Oxidation of steroid **13** with PCC in CH_2Cl_2 gave the 20-ketosteroid **14** in 90% yield, and removal of the TBDMS group from compound **14** using $(n\text{-Bu})_4NF$ in THF gave the pregnenolone enantiomer **15** in 93% yield ($[\alpha]_D^{28}$ −26.0, ee 99.4%). By using the method described above for the preparation of steroid sulfate **4**, steroid **15** was converted into pregnenolone sulfate enantiomer **5** in 95% yield ($[\alpha]_D^{29}$ −20.1, ee 97.2%).

The synthesis of the steroid sulfate **6** is summarized in Scheme 2. Steroid **9** was prepared from steroid **8** as described above (Scheme 1), and regioselective reduction of its carbonyl group at C-3 with K-Selectride in THF at −78 °C gave steroid **16** in a 49% overall yield (2 steps). Silylation of the C-3 hydroxyl group gave the TBDMS ether **17** in 73% yield, and hydrogenation of the double bond in steroid **17** using Pd/C as a catalyst gave the 5α-reduced steroid **18** as the product (90% yield). The stereochemistry observed for reduction of the double bond in steroid **17** was expected because of a previous study on the stereochemistry of catalytic hydrogenation of Δ^5 steroids having 3α substituents.[24]

The remaining structures shown in Scheme 2 for the conversion of steroid **18** into steroid sulfate **6** are closely analogous to those reported in Scheme 1 for the conversion of steroid **11** to steroid sulfate **5**. Except as noted, the reagents used were identical and the yields for each reaction were as follows: olefination reaction **18** → **19** (67%); hydroboration (BH_3·THF) reaction **19** → **20** (61%); oxidation reaction **20** → **21** (98%); desilylation reaction **21** → **22** (97%); and sulfation reaction **22** → **6** (89%). Steroid **22** had $[\alpha]_D^{30}$ −104.0 (ee 94.0%) and steroid sulfate **6** had $[\alpha]_D^{31}$ −94.5 (ee 98.6%).

Electrophysiology

Voltage clamp recordings were obtained from cultured postnatal rat hippocampal neurons using whole-cell patch clamp methods.[25] As shown in Figure 1, dehydroepiandrosterone sulfate (**1**) reversibly inhibited chloride currents gated by 2 μM GABA. This inhibition was concentration-dependent with an IC_{50} of 11 ± 1 μM. A similar IC_{50} of 13 ± 3 μM was reported previously for the noncompetitive block of GABA-mediated currents in cultured neurons from rat ventral mesencephalon.[14] At concentrations > 1 mM, enantiomer **1** inhibited GABA responses completely. The unnatural dehydroepiandrosterone enantiomer, compound **4**, also inhibited GABA currents but was about 7-fold less potent than enantiomer **1** with an IC_{50} of 80 ± 14 μM. Both

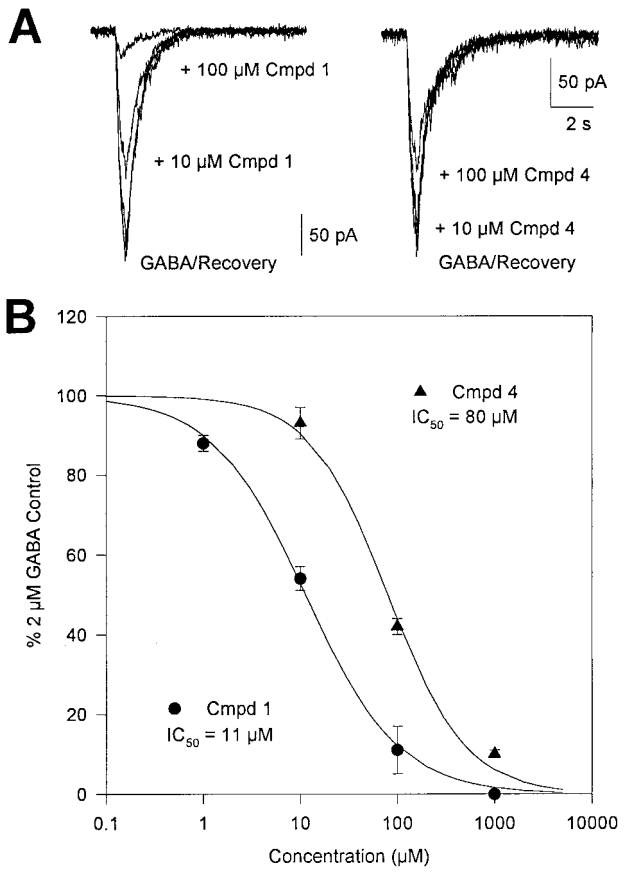


Figure 1. Inhibition of GABA-mediated currents by compounds **1** and **4**. (A) The traces show recordings from cultured hippocampal neurons voltage-clamped at −60 mV and exposed to 500 ms applications of 2 μM GABA in the absence or presence of 10 or 100 μM compound **1** or compound **4**. The inhibitory effects of these agents on GABA responses were completely reversible. The time axis displayed in the right panel applies to both recordings. (B) The graph shows concentration−response curves for the inhibition of currents gated by 2 μM GABA by various concentrations of compound **1** (●) or compound **4** (▲). The solid lines represent the best fit of the logistic equation given in Methods with IC_{50} = 11 ± 1 μM and Hill coefficient (n) = 0.9 ± 0.4 for compound **1** and IC_{50} = 80 ± 14 μM and n = 1.1 ± 0.1 for compound **4**.

enantiomers inhibited GABA currents noncompetitively in hippocampal neurons (Figure 2).

Steroid sulfate enantiomer pairs **2**,**5** and **3**,**6** were also found to inhibit currents gated by GABA (Figures 3 and 4). However, in contrast to the clear enantioselectivity shown by compounds **1** and **4**, there was little or no enantioselectivity observed with these compounds. The IC_{50}s for compounds **2** and **5** were 82 ± 12 and 76 ± 27 μM, respectively. A similar IC_{50} of around 60 μM was reported previously for the block of GABA-mediated currents by compound **2** in cultured neurons from neonatal rat cortex.[13] Compounds **3** and **6** were about twice as potent as compounds **2** and **5** and had IC_{50}s of 39 ± 7 and 46 ± 2 μM, respectively. Similar to the effects of compounds **1** and **4**, the effects of compounds **2**, **3**, **5** and **6** were reversible. Additionally, compounds **3** and **6** were shown to inhibit GABA currents in hippocampal neurons noncompetitively (Figure 5).

Discussion

The major results of this study are that the actions of dehydroepiandrosterone sulfate, but not of preg-

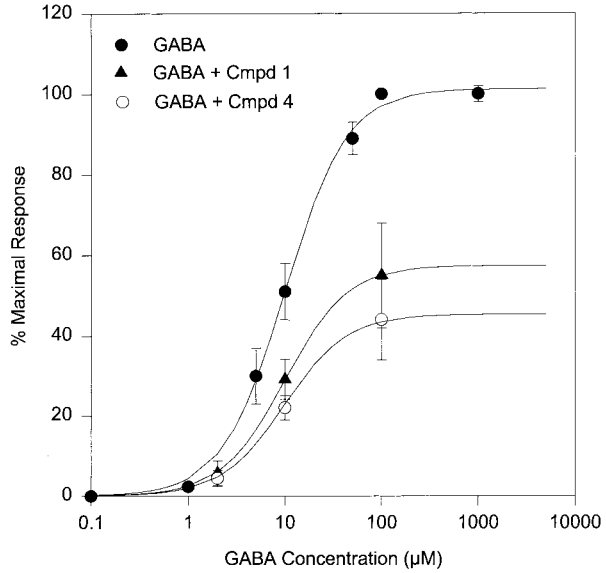


Figure 2. Compounds **1** and **4** are noncompetitive GABA receptor antagonists. The graph shows a control concentration−response curve for GABA (●) and the effect of 10 μM compound **1** (▲) and 100 μM compound **4** (○). Responses to various concentrations of GABA were normalized with respect to the response at 100 μM. The GABA concentration−response curve was fit with the logistic equation described in Methods with response$_{max}$ = 101 ± 2%, EC_{50} = 9.8 ± 0.6 μM, and Hill coefficient (n) = 1.4 ± 0.1. The curves in the presence of compound **1** and compound **4** were fit with the noncompetitive inhibition model with K_i = 13 ± 0.1 and 81 ± 2 μM, respectively. A competitive inhibition model failed to describe these data.

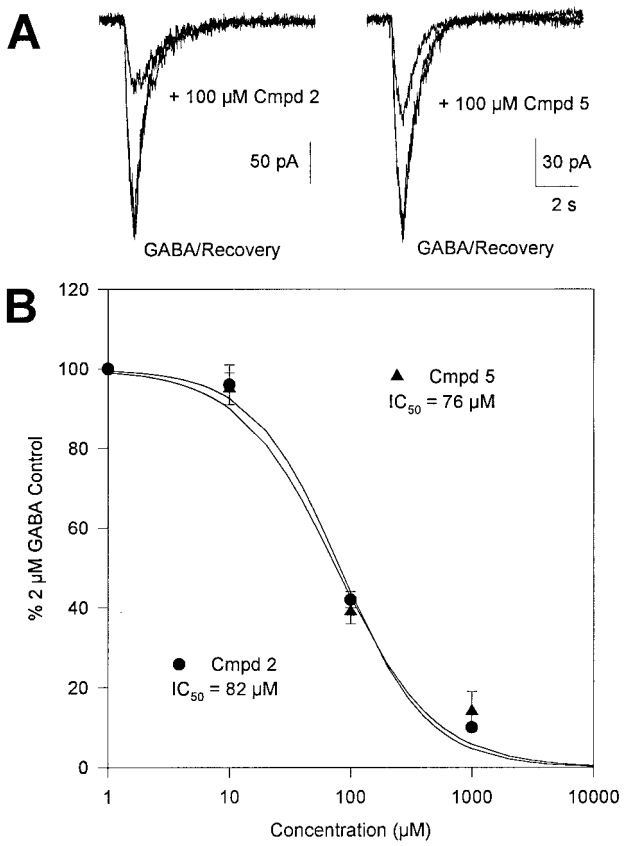


Figure 3. Inhibition of GABA-mediated currents by compounds **2** and **5**. (A) As in Figure 1, the traces show voltage clamp records obtained at −60 mV for currents gated by 2 μM GABA in the absence and presence of 100 μM compound **2** or compound **5**. (B) The graph shows concentration−response curves for the inhibition of 2 μM GABA currents by various concentrations of compound **2** (●) or compound **5** (▲). The solid lines represent the best fit of the logistic equation given in Methods with IC_{50} = 82 ± 12 μM and Hill coefficient (n) = 1.2 ± 0.2 for compound **2** and IC_{50} = 76 ± 27 μM and n = 1.1 ± 0.4 for compound **5**.

nenolone sulfate or steroid sulfate **3**, on GABA$_A$ receptor function are enantioselective. This result suggests that the mechanisms and sites of action for the androstane and pregnane series of steroid sulfate blockers of GABA-mediated current are different. The enantioselectivity observed for the actions of dehydroepiandrosterone sulfate provides the first evidence that the modulatory actions of this compound on GABA$_A$ receptor function are mediated via a chiral recognition site. This result suggests that there is a direct binding interaction between dehydroepiandrosterone sulfate and the receptor. Conversely, the failure to observe enantioselectivity for the actions of pregnenolone sulfate and steroid sulfate **3** demonstrates that a chiral recognition site does not exist for these compounds and suggests that their effects on GABA$_A$ receptor function may arise from steroid-induced membrane perturbation.

The unnatural dehydroepiandrosterone sulfate enantiomer, compound **4**, was 7-fold less potent as a blocker of GABA-mediated current than the natural enantiomer, compound **1**. With regard to potency, the degree of enantioselectivity found with the dehydroepiandrosterone sulfate enantiomers is similar to that found in previous studies of the enantioselectivity of steroids and benz[*e*]indenes having positive modulatory actions on GABA$_A$ receptor function.[9−11] However, with regard to efficacy, the results from the two studies are different. The efficacy of enantiomers **1** and **4** is equal, and the slopes of the enantiomer concentration−response curves (Figure 1) are not significantly different. These results suggest that each enantiomer binds to the same site. In the previous enantioselectivity studies, the unnatural enantiomers at concentrations up to 100 μM (the limit of solubility for the compounds) did not have the same efficacy in the electrophysiology experiments as the natural enantiomers. This difference in enantiomer efficacy would be expected if the enantiomers bound to different sites, and evidence was presented in those studies for the simultaneous binding of each enantiomer to a different binding site on the same GABA$_A$ receptor.[9]

The mechanism and site(s) of action for dehydroepiandrosterone sulfate's negative modulatory actions on GABA$_A$ receptor function have not been defined fully. Previous studies have established that dehydroepiandrosterone sulfate does not bind at the GABA or benzodiazepine binding sites of the receptors.[14,16,17] By contrast, previous studies have not produced general agreement as to whether dehydroepiandrosterone sulfate binds to the picrotoxin or barbiturate binding sites of the receptors.[12,17] The results from this study, while not addressing the possibility that dehydroepiandrosterone sulfate binds to either the barbiturate or picrotoxin sites, do impose a new requirement for enantioselectivity in the actions of this compound at these potential binding sites. The finding that dehydroepiandrosterone sulfate does not have enantioselective interactions at one or both of these sites would eliminate the site(s) from further consideration as the dehydroe-

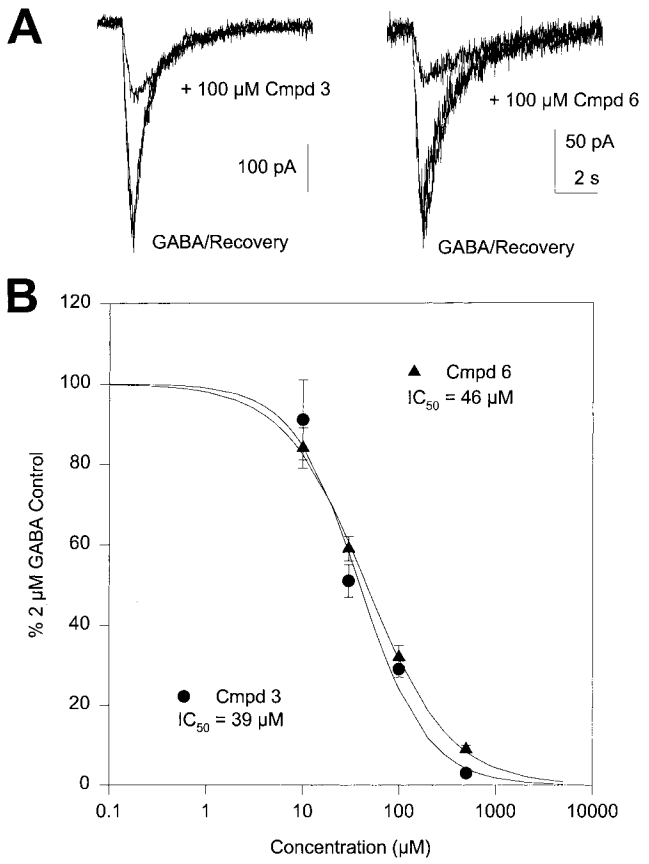


Figure 4. Inhibition of GABA-mediated currents by compounds **3** and **6**. (A) The traces show the effects of 100 μM compound **3** or compound **6** on currents activated by 2 μM GABA. (B) The graph shows concentration−response curves for the inhibition of GABA currents by various concentrations of compound **3** (●) or compound **6** (▲). The solid lines represent the best fit of the logistic equation given in Methods with $IC_{50} = 39 \pm 7$ μM and Hill coefficient (n) = 1.2 ± 0.3 for compound **3** and $IC_{50} = 46 \pm 2$ μM and $n = 1.0 \pm 0.1$ for compound **6**.

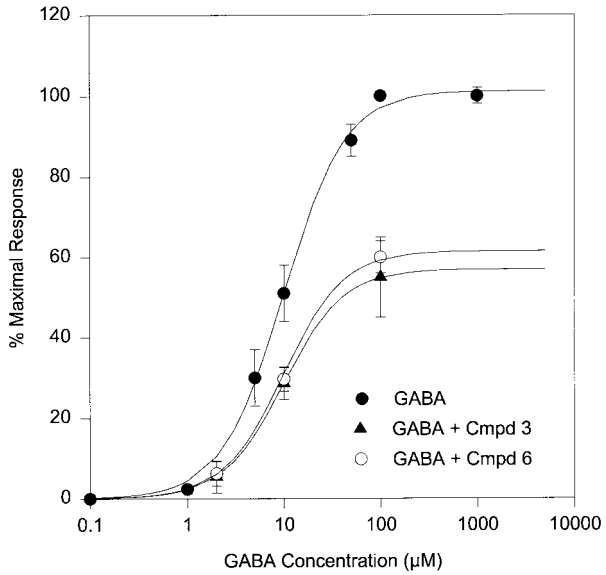


Figure 5. Compounds **3** and **6** are noncompetitive GABA receptor antagonists. The GABA concentration−response curve (●) is the same as that shown in Figure 2. The other curves show the effects of 30 μM compound **3** (▲) and 30 μM compound **6** (○) on the GABA concentration−response curve. The effects of compounds **3** and **6** were well-described by a noncompetitive inhibition model with $K_i = 39 \pm 0.4$ and 47 ± 2 μM, respectively.

piandrosterone binding site on $GABA_A$ receptors. Future studies are planned to address this issue (vide infra).

For pregnenolone sulfate, evidence from previous studies has established that this compound does not bind at the GABA, benzodiazepine, or barbiturate binding sites of $GABA_A$ receptors.[12,15,18] On the basis of a Scatchard analysis of the displacement of [^{35}S]TBPS by pregnenolone sulfate, it has been suggested that pregnenolone sulfate binds at the picrotoxin site.[15] Modeling studies for a hypothetical binding site for pregnenolone sulfate involving extracellular domains located at the amino terminus of the α_1 subunit of $GABA_A$ receptors also have been published.[2] However, since no enantioselectivity was observed for the electrophysiological actions of pregnenolone enantiomers **2** and **5** in this study, we consider it unlikely that either the picrotoxin site or the hypothetical extracellular site is a binding site for pregnenolone sulfate. Until it is demonstrated that the binding of pregnenolone sulfate to the picrotoxin site or some other putative site on $GABA_A$ receptors is not enantioselective, we consider it more likely that the effects of pregnenolone sulfate on $GABA_A$ receptor function are indirectly mediated by steroid-induced membrane perturbation.

To our knowledge, the action of steroid sulfate **3** on $GABA_A$ receptor function has not been reported previously. We find that this compound is twice as potent as pregnenolone sulfate in blocking GABA-mediated currents. As was the case for the results obtained with pregnenolone sulfate enantiomers **2** and **5**, no clear enantioselectivity was observed for steroid sulfate enantiomers **3** and **6**. Thus, it seems most likely that the effects of steroid sulfate **3** on $GABA_A$ receptor function are also indirectly mediated by steroid-induced membrane perturbation.

The results of this study have provided new evidence for the existence of a binding site for dehydroepiandrosterone sulfate, but not for pregnenolone sulfate or steroid sulfate **3**, on $GABA_A$ receptors. Recently, electrophysiological studies carried out with picrotoxin-resistant forms of $GABA_A$ receptors were used to demonstrate that the blocking actions of a convulsant γ-thiobutyrolactone are caused by binding of the compound to the picrotoxin site. Thus, whereas wild-type $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors were blocked by the convulsant γ-thiobutyrolactone, picrotoxin-resistant forms of this receptor were not blocked by the compound.[26] Similar results would be expected if any of the steroid sulfates studied here blocked GABA-mediated currents as a result of binding at the picrotoxin site. Studies of the steroid sulfate enantiomers with picrotoxin-resistant $GABA_A$ receptors are currently in progress.

Last, pregnenolone sulfate and (3α,5β)-3-hydroxypregnan-20-one sulfate also have modulatory actions on NMDA receptors.[4] Pregnenolone sulfate potentiates glutamate currents at NMDA receptors, and studies in support of the use of pregnenolone sulfate or its congeners as cognitive enhancers have been published.[2,27] Conversely, (3α,5β)-3-hydroxypregnan-20-one sulfate blocks glutamate currents at NMDA receptors, and studies in support of the use of this steroid or its congeners as neuroprotective agents for use in the treatment of stroke or other types of excitotoxic injury

have been published.[28] As is the case for the effects of these steroid sulfates on GABA$_A$ receptor function, their mechanisms and site(s) of action for modulation of NMDA receptor function are not understood fully. If, as has been found in this study of GABA$_A$ receptors, little or no enantioselectivity is found for the actions of these compounds on NMDA receptors, unnatural enantiomers **5** and **6** could be particularly attractive for future in vivo studies since it is unlikely that these compounds would be substrates for the enzymes involved in steroid hormone biosynthesis. Thus, the effects of these steroid sulfates on ion channel function could perhaps be more readily distinguished from other effects arising from the conversion of the steroid sulfates into steroids with hormonal activity. This is particularly true in the case of pregnenolone sulfate which is the precursor to all but the vitamin D class of steroid hormones in humans.

Experimental Section

General Methods. Melting points were determined on a Kofler micro hot stage and are uncorrected. NMR spectra were recorded at ambient temperature in $CDCl_3$ (unless noted otherwise) with a 5 mm probe on a Varian Gemini 2000 operating at 300 MHz (^{1}H) or 75 MHz (^{13}C). For ^{1}H NMR and ^{13}C NMR spectra, the internal references were TMS (δ 0.00) and $CDCl_3$ (δ 77.00), respectively. IR spectra were recorded as films on a NaCl plate (unless noted otherwise) with a Perkin-Elmer 1710 FT-IR spectrophotometer. Optical rotations were determined on a Perkin-Elmer Model 241 polarimeter, and ee values, when given, were determined from the optical rotation values of each steroid in the enantiomer pair under identical experimental conditions. Elemental analyses were carried out by M-H-W Laboratories, Phoenix, AZ. Solvents were either used as purchased or dried and purified by standard methodology. Extraction solvents were dried with $MgSO_4$, filtered, and removed on a rotary evaporator under water aspirator vacuum. Flash chromatography was performed using silica gel (32−63 microns) purchased from Scientific Adsorbents, Atlanta, GA. The FlashElute Chromatography System was purchased from Elution Solutions, Charlottesville, VA.

(8α,9*β*,10α,13α,14*β*)-Androst-4-ene-3,17-dione (8). Steroid **7** (5.23 g, 18.1 mmol, $[\alpha]_D^{29}$ −110.7 (c 1.06, $CHCl_3$), lit[11] $[\alpha]_D^{25}$ −114.1, lit[20] $[\alpha]_D^{20}$ −115.9) was dissolved in stirred acetone (250 mL) and cooled at 0 °C for 10 min. Jones reagent was added dropwise until the orange color persisted. After 15 min, 2-propanol (5 mL) was added to reduce any excess reagent, water was added (250 mL), and the reaction mixture was extracted with EtOAc (3 × 150 mL). The combined organic extracts were washed with brine (1 × 150 mL), dried, and filtered, and the solvents were removed to give a yellow solid. Flash chromatography (silica gel eluted with 20% EtOAc in hexanes) gave the product (4.91 g, 95%) as a white solid: mp 172−173 °C (from Et_2O and hexanes); $[\alpha]_D^{27}$ −189.7 (c 1.01, $CHCl_3$); ee 96.5%; IR 2943, 2854, 1742, 1728, 1675, 1614, 1454, 1335, 1272, 1248, 1227, 1094, 1054, 1022, 860 cm^{-1}; ^{1}H NMR δ 5.74 (s, 1H, C=CH), 1.20 (s, 3H, CH_3), 0.91 (s, 3H, CH_3); ^{13}C NMR δ 220.50, 199.44, 170.38, 124.23, 53.76, 50.79, 47.11, 38.54, 35.62 (2 × C), 35.07, 33.79, 32.44, 31.18, 30.65, 21.61, 20.20, 17.24, 13.56. Anal. ($C_{19}H_{26}O_2$) C, H.

(3α,8α,9*β*,10α,13α,14*β*)-3-Hydroxyandrost-5-en-17-one (10). Compound **8** (2.68 g, 9.37 mmol) was mixed with *t*-BuOK (10.5 g, 93.8 mmol) in a flask equipped with a rubber septum and purged with N_2 for 25 min. *t*-BuOH (82 mL) was slowly added by syringe, and the stirred reaction was allowed to proceed for 3 h. The reaction was quenched by the rapid addition of the reaction mixture to 10% aqueous acetic acid (282 mL). After stirring for 10 min, the acetic acid was neutralized by the addition of solid $NaHCO_3$, and the mixture was extracted with EtOAc (3 × 150 mL). The combined extracts were dried, filtered, and removed to give crude product **8** (3.26 g) as a yellow solid which was immediately dissolved in THF (57 mL), added to a flask equipped with a rubber septum, cooled to −78 °C in a dry ice/acetone bath, and purged with N_2 for 10 min. LiAl(*t*-BuO)$_3$H (15 mL of a 1.0 M solution in THF) was added by syringe. After 3.5 h, additional LiAl(*t*-BuO)$_3$H (8 mL of a 1.0 M solution in THF) was added, and 40 min later the mixture was poured into chilled 1 N HCl (440 mL). The mixture was extracted with EtOAc (3 × 150 mL), the combined organic extracts were washed with saturated $NaHCO_3$ (2 × 150 mL), dried, and filtered, and the solvents were removed to afford a yellow solid (3.58 g). ^{1}H NMR analysis revealed a mixture of steroids **8**−**10**. This steroid mixture was recycled following the same procedure, and the resulting yellowish solid was purified by flash chromatography to give a white solid (1.64 g). Recrystallization from EtOAc and hexanes gave product **10** (1.32 g, 49%) as a white solid: mp 139−140 °C; $[\alpha]_D^{25}$ −4.68 (c 1.58, $CHCl_3$); ee 98.9%; IR 3422, 2933, 1738, 1620, 1454, 1375, 1058, 1029 cm^{-1}; ^{1}H NMR δ 5.37 (m, 1H, C=CH), 4.09 (m, 1H, C*H*OH), 1.02 (s, 3H, CH_3), 0.87 (s, 3H, CH_3); ^{13}C NMR δ 221.49, 141.10, 120.95, 71.51, 51.67, 50.11, 47.45, 42.08, 37.06, 36.51, 35.74, 31.43, 31.35, 31.29, 30.64, 21.73, 20.21, 19.27, 13.38. Anal. ($C_{19}H_{28}O_2$) C, H.

(3α,8α,9*β*,10α,13α,14*β*)-3-(Sulfooxy)androst-5-en-17-one Ammonium Salt (4). Steroid **10** (80 mg, 0.28 mmol) was dissolved in stirred pyridine (3 mL), and sulfur trioxide trimethylamine complex (80 mg, 0.58 mmol) was added. The sulfur trioxide complex slowly dissolved in the pyridine, and after 24 h, additional sulfur trioxide complex (470 mg, 3.52 mmol) was added. After an additional 42 h, 10% HCl was added dropwise until pH 1−2 was reached. The reaction mixture was then extracted with CH_2Cl_2 (4 × 15 mL), and the combined extracts were washed with water (1 × 15 mL). The water wash was further extracted with CH_2Cl_2 (2 × 15 mL), the combined extracts were dried and filtered, and the solvent was removed to afford the impure pyridinium salt. The salt was dissolved in a minimal amount of CH_2Cl_2 (∼15 mL), and anhydrous NH_3 was bubbled through the solution for 30 min. The precipitated ammonium salt was collected by filtration and dried sequentially by aspiration and high vacuum to give product **4** (67 mg, 72%) as a white powder: mp 197−198 °C (dec); $[\alpha]_D^{30}$ −13.2 (c 0.92, MeOH); IR (KBr) 3455, 3201, 2944, 1738, 1634, 1438, 1378, 1218, 1138, 1091, 1061, 982, 952 cm^{-1}; ^{1}H NMR (CD_3OD) δ 5.44 (m, 1H, C=CH), 4.14 (m, 1H, CHOS), 2.57 (ddd, J = 2.1, 5.1, 13.2 Hz, 1H), 2.50−2.36 (m, 2H), 1.07 (s, 3H, CH_3), 0.89 (s, 3H, CH_3); ^{13}C NMR (CD_3OD) δ 224.30, 142.08, 122.93, 79.75, 53.04, 51.75, 40.34, 38.36, 37.80, 36.69, 32.74, 32.64, 31.83, 29.93, 22.73, 21.40, 19.68, 13.85. HRMS *m*/*z* calcd for $C_{19}H_{27}O_5S$: 367.1579. Found: 367.1581.

(3α,8α,9*β*,10α,13α,14*β*)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]androst-5-en-17-one (11). Steroid **10** (1.06 g, 3.68 mmol) was dissolved in THF (4.5 mL) and CH_2Cl_2 (22.5 mL), and DMAP (0.90 g, 7.37 mmol), TBDMSCl (1.12 g, 7.43 mmol), and Et_3N (1 mL) were added. The flask was purged with N_2, and the reaction was stirred overnight. Saturated NH_4Cl (25 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with brine (30 mL), dried, and filtered, and the solvents were removed to afford a slightly tan solid (1.9 g). Flash chromatography (silica gel eluted with 20% EtOAc in hexanes) gave product **11** (1.40 g, 95%) as a white solid: mp 146−147 °C; lit[19] mp 138−140 °C; IR 2948, 2928, 2890, 2858, 1747, 1668, 1254, 1091, 1060, 1030, 1007, 837 cm^{-1}; ^{1}H NMR δ 5.34 (m, 1H, C=CH), 3.48 (m, 1H, CHOSi), 2.46 (dd, J = 8.4, 19.2 Hz, 1H), 1.02 (s, 3H, CH_3), 0.89 (s, 9H, $C(CH_3)_3$), 0.88 (s, 3H, CH_3), 0.06 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR δ 221.32, 141.95, 120.46, 72.44, 51.81, 50.32, 47.50, 42.75, 37.25, 36.68, 35.77, 31.96, 31.47, 31.41, 30.76, 25.83 (3 × C), 21.79, 20.26, 19.35, 18.12, 13.43, −4.74 (2 × C).

(3α,8α,9*β*,10α,13α,14*β*,17Z)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]pregna-5,17(20)-diene (12). NaH powder (0.41 g, 17.1 mmol) was added to a dry three-necked round-bottom flask containing stirred DMSO (15 mL). The flask was

equipped with a reflux condenser, and the solution was heated at 70−80 °C for 1 h under N_2. After cooling to 0 °C, $EtP(Ph)_3$-Br (6.32 g, 17.0 mmol) in DMSO (30 mL) was added. After 10 min, steroid **11** (1.37 g, 3.40 mmol) in THF (22 mL) was added to the reddish mixture. The reaction was heated at 60 °C under N_2. After 24 h, the reaction had not gone to completion as determined by TLC analysis (5% EtOAc in hexanes). Additional reagent was generated by dissolving NaH (0.41 g) in DMSO (15 mL), heating to 70 °C for 1 h, and then adding $EtP(Ph)_3Br$ (6.32 g, 17.0 mmol) in DMSO (20 mL). The reagent mixture was cannulated into the reaction vessel, and the reaction was allowed to proceed overnight. The following morning, THF was added (25 mL) and the reaction was allowed to proceed an additional 2 h before cooling to room temperature. The reaction mixture was poured into water (500 mL) and extracted with hexanes (5 × 75 mL). The combined organic extracts were dried and filtered, and the solvents were removed to afford a noisome yellow solid (2.99 g). Flash chromatography gave product **12** (1.01 g, 72%) as a white solid: mp 133−135 °C; lit[19] mp 130−132 °C; IR 2934, 1461, 1372, 1255, 1083, 836 cm^{-1}; ^1H NMR δ 5.33 (m, 1H, C=CH), 5.14 (br q, J = 7.2 Hz, 1H, C=C*H*CH_3), 3.49 (m, 1H, CHOSi), 1.66 (br d, J = 6.9 Hz, 3H, CH_3), 1.02 (s, 3H, CH_3), 0.90 (s, 12H, CH_3 and $C(CH_3)_3$), 0.06 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR δ 150.32, 141.63, 121.07, 113.50, 72.54, 56.51, 53.30, 50.18, 43.95, 42.75, 37.24, 36.92, 36.53, 32.00, 31.65, 31.34, 25.81 (3 × C), 24.37, 21.11, 19.26, 18.09, 16.46, 12.97, −4.77 (2 × C).

(3α,8α,9*β*,10α,13α,14*β*,20*R*)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]20-hydroxypregn-5-ene (13). Steroid **12** (0.94 g, 2.28 mmol) was dissolved in stirred THF (8 mL), the reaction vessel was purged with N_2, and 9-BBN-H (22 mL of a 0.5 M solution in THF) was added. After 22 h, additional 9-BBN-H solution (14 mL) was added. After an additional 24 h, the reaction was cooled to 0 °C, and aqueous 10% NaOH (19 mL) was cautiously added. Shortly thereafter, 30% H_2O_2 (19 mL) was added dropwise over 30 min. After 2.5 h, the reaction mixture was transferred to a separatory funnel and extracted with EtOAc (4 × 30 mL). The combined organic extracts were dried and filtered, and the solvents were removed to afford a white solid (5.46 g). Flash chromatography (silica gel eluted with 10% EtOAc in hexanes) afforded product **13** (840 mg, 85%) as a white solid: mp 163−165 °C; IR 3360, 2931, 2898, 2858, 1460, 1381, 1254, 1090, 1009, 955, 888 cm^{-1}; ^1H NMR δ 5.31 (m, 1H, C=CH), 3.70 (m, 1H, C*H*OH), 3.47 (m, 1H, CHOSi), 1.22 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.88 (s, 9H, $C(CH_3)_3$), 0.67 (s, 3H, CH_3), 0.05 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR δ 141.67, 121.10, 72.57, 70.26, 58.39, 56.57, 50.12, 42.75, 41.55, 38.76, 37.32, 36.53, 31.99, 31.81, 31.49, 25.83 (3 × C), 25.57, 24.10, 23.41, 20.67, 19.30, 18.12, 12.31, −4.74 (2 × C). Anal. ($C_{27}H_{48}O_2Si$) C, H.

(3α,8α,9*β*,10α,13α,14*β*,20α)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]pregn-5-en-20-one (14). Steroid **13** (790 mg, 1.83 mmol) was dissolved in stirred CH_2Cl_2 (40 mL), and NaOAc (180 mg, 2.20 mmol) and Celite (360 mg) were added. The reaction vessel was cooled to 0 °C, and PCC (0.60 g, 2.78 mmol) was added. The reaction was maintained at 0 °C under N_2 for 30 min and then allowed to warm to room temperature. After 3.5 h, the reaction was diluted with Et_2O, applied to silica gel, and eluted with EtOAc. The EtOAc was removed to afford a slightly tan solid (0.78 g). Flash chromatography (silica gel eluted with 2% EtOAc in hexanes) gave product **14** (710 mg, 90%) as a white solid: mp 166−167 °C; IR 2939, 2853, 1702, 1669, 1471, 1433, 1383, 1356, 1245, 1194, 1078, 1024 cm^{-1}; ^1H NMR δ 5.30 (m, 1H, C=CH), 3.47 (m, 1H, CHOSi), 2.51 (t, J = 9 Hz, 1H), 2.10 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.87, (s, 9H, $C(CH_3)_3$), 0.61 (s, 3H, CH_3), 0.04 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR δ 209.59, 141.63, 120.89, 72.49, 63.68, 56.91, 50.03, 43.90, 42.70, 38.79, 37.30, 36.50, 31.94, 31.78, 31.70, 31.38, 25.80 (3 × C), 24.37, 22.72, 20.96, 19.27, 18.09, 13.05, −4.77 (2 × C). Anal. ($C_{27}H_{46}O_2Si$) C, H.

(3α,8α,9*β*,10α,13α,14*β*,17α)-3-Hydroxypregn-5-en-20-one (15). Steroid **14** (0.66 g, 1.53 mmol) was dissolved in stirred THF (9 mL), and (n-Bu)$_4$NF (9 mL of a 1 M solution in THF) was added. The following morning (∼20 h), saturated NH_4Cl (40 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried and filtered, and the solvents were removed to afford a yellow oil. Flash chromatography (silica gel eluted with CH_2Cl_2 then 20% EtOAc in hexanes) gave a white solid (490 mg) which was recrystallized from EtOAc and hexanes to give product **15** as flaky white crystals (450 mg, 93%): mp 188−191 °C; $[\alpha]_D^{28}$ −26.0 (c 1.00, EtOH); ee 99.4%; IR 3434, 2929, 2885, 1699, 1434, 1360, 1315, 1234, 1195, 1060 cm^{-1}; ^1H NMR δ 5.34 (m, 1H, C=CH), 3.51 (m, 1H, C*H*OH), 2.52 (t, J = 9 Hz, 1H), 2.11 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 0.62 (s, 3H, CH_3); ^{13}C NMR δ 209.7, 140.9, 121.4, 71.64, 63.7, 56.9, 49.9, 43.9, 42.2, 38.8, 37.2, 36.4, 31.8, 31.7, 31.5, 31.4, 24.4, 22.7, 21.0, 19.2, 13.1. Anal. ($C_{21}H_{32}O_2$) C, H.

(3α,8α,9*β*,10α,13α,14*β*,17α)-3-(Sulfooxy)pregn-5-en-20-one ammonium salt (5). Steroid **15** (376 mg, 1.19 mmol) was dissolved in stirred pyridine (6 mL), and sulfur trioxide trimethylamine complex was added (1.04 g, 7.47 mmol). The reaction vessel was purged with N_2, and after 2 days, 1 N HCl was added to acidify to pH 1−2. The reaction mixture was extracted with CH_2Cl_2 (4 × 40 mL), the combined organic extracts were dried and filtered, and the solvent was removed to afford the crude pyridinium salt (800 mg). The crude salt was dissolved in a minimal amount of CH_2Cl_2 (∼15 mL), and anhydrous NH_3 was bubbled through the solution for 30 min. The resulting white precipitate was collected and dried by vacuum aspiration affording product **5** (466 mg, 95%) as a fluffy white solid: mp 206−207 °C (dec); $[\alpha]_D^{29}$ −20.1 (c 1.04, MeOH); ee 97.2%; IR 3548, 3136, 2945, 1707, 1685, 1637, 1402, 1239, 1062, 979 cm^{-1}; ^1H NMR δ 5.39 (m, 1H, C=CH), 4.13 (m, 1H, CHOS), 2.64 (t, J = 9 Hz, 1H), 2.12 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 0.62 (s, 3H, CH_3); ^{13}C NMR δ 212.64, 141.82, 123.21, 79.84, 64.72, 58.09, 51.49, 45.10, 40.35, 39.85, 38.44, 37.66, 33.19, 32.89, 31.61, 29.95, 25.43, 23.78, 22.15, 19.68, 13.50. HRMS m/z calcd for $C_{21}H_{31}O_5S$: 395.1892. Found: 395.1893.

(3*β*,8α,9*β*,10α,13α,14*β*)-3-Hydroxyandrost-5-en-17-one (16). Steroid **8** (2.22 g, 7.76 mmol) and t-BuOK (8.69 g, 77.6 mmol) were placed in a flask equipped with a stir bar and rubber septum and purged with N_2 for 25 min. t-BuOH (68 mL) was added, and the reaction mixture was stirred under N_2 for 3 h and rapidly poured into stirred aqueous 10% HOAc (250 mL). After 10 min, the HOAc was neutralized with solid $NaHCO_3$, and the mixture was extracted with EtOAc (4 × 125 mL). The combined extracts were dried and filtered, and the solvents were removed to afford a yellow solid (2.75 g). The solid was dissolved in stirred THF (82 mL), and the reaction vessel was purged with N_2. After cooling at −78 °C for 10 min, K-Selectride (12 mmol, 12 mL of a 1.0 M solution in THF) was added. Additional amounts of the K-Selectride solution (4 and 3 mL) were added after 2.5 and 3.25 h, respectively. The reaction was quenched 45 min after the last addition of K-Selectride by the careful addition of aqueous 10% NaOH (50 mL) and then 30% H_2O_2 (20 mL). After warming to room temperature (∼30 min), the reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with brine (2 × 100 mL), dried, and filtered, and the solvents were removed to afford a white solid. Flash chromatography using the FlashElute system (cartridge size 40 L, 18% EtOAc in hexanes, 27 psi) gave product **16** (1.46 g, 65%) as a white solid: mp 220−222 °C; $[\alpha]_D^{29}$ −8.23 (c 0.74, EtOH); IR 3471, 2941, 2884, 1726, 1454, 1422, 1376, 1362, 1312, 1236, 1066, 1025 cm^{-1}; ^1H NMR δ 5.41 (m, 1H, C=CH), 4.01 (m, 1H, C*H*OH), 2.56 (dt, J = 2.7, 14.7 Hz, 1H), 1.02 (s, 3H, CH_3), 0.87 (s, 3H, CH_3); ^{13}C NMR δ 221.2, 139.06, 122.97, 66.88, 51.70, 50.34, 47.42, 39.68, 37.33, 35.71, 32.99, 31.32 (2 × C), 30.73, 28.73, 21.72, 19.94, 18.56, 13.38. Anal. ($C_{19}H_{28}O_2$) C, H.

(3*β*,8α,9*β*,10α,13α,14*β*)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]androst-5-en-17-one (17). Steroid **16** (1.43 g, 4.97 mmol) was dissolved in CH_2Cl_2 (40 mL) and freshly distilled THF (7.5 mL), and TBDMSCl (1.87 g, 12.4 mmol), DMAP (1.21 g, 9.91 mmol), and Et_3N (1.7 mL, 12.9 mmol) were added. The reaction was stirred under N_2 for 113 h, and saturated NH_4Cl

(50 mL) was added. After stirring for 30 min, the reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried and filtered, and the solvents were removed to afford a yellow oil (3.79 g). Flash chromatography (silica gel eluted with 2.5% EtOAc in hexanes) gave product **17** (1.46 g, 73%) as a white solid: mp 107−108 °C; IR 2929, 2855, 1737, 1462, 1248, 1088, 1076 cm^{-1}; ^{1}H NMR δ 5.24 (m, 1H, C=CH), 3.99 (m, 1H, CHOSi), 1.00 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.85 (s, 9H, $C(CH_3)_3$), 0.01 (s, 3H, $SiCH_3$), −0.01 (s, 3H, $SiCH_3$); ^{13}C NMR δ 221.52, 139.95, 120.67, 67.35, 51.77, 50.30, 47.54, 40.37, 37.20, 35.87, 33.07, 31.48 (2 × C), 30.84, 29.75, 25.74 (3 × C), 21.84, 20.03, 19.04, 18.04, 13.50, −4.80, −4.86. Anal. ($C_{25}H_{42}O_2Si$) C, H.

(3*β*,5α,8α,9*β*,10α,13α,14*β*)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]androstan-17-one (18). Steroid **17** (1.38 g, 3.43 mmol) was dissolved in 2-propanol (90 mL) and hydrogenated (65 psi, H_2; 0.37 g, 10% Pd/C) in a Paar hydrogenator. After 5 days, additional Pd/C (0.19 g) was added and the hydrogenation was continued until the following morning whereupon the reaction mixture was filtered through Celite and the solvent was removed to afford a yellow oil (1.56 g). Flash chromatography (silica gel eluted with 2.5% EtOAc in hexanes) gave product **18** (1.24 g, 90%) as a white solid: mp 95−98 °C; IR 2929, 2858, 1743, 1452, 1373, 1253, 1096, 1078, 1055, 1007, 901, 872 cm^{-1}; ^{1}H NMR δ 3.58 (m, 1H, CHOSi), 2.42 (dd, J = 8.7, 19.2 Hz, 1H), 0.92 (s, 3H, CH_3), 0.88 (9H, s, $C(CH_3)_3$), 0.83 (s, 3H, CH_3), 0.04 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR δ 221.58, 72.55, 51.37, 47.79, 42.14, 40.50, 36.78, 35.84, 35.48, 35.36, 34.67, 31.61, 30.87, 26.89, 25.83 (3 × C), 25.24, 23.19, 21.70, 19.95, 18.16, 13.63, −4.78 (2 × C). Anal. ($C_{25}H_{44}O_2Si$) C, H.

(3*β*,5α,8α,9*β*,10α,13α,14*β*,17*Z*)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]pregn-17(20)-ene (19). NaH (0.36 g, 15 mmol) and DMSO (14 mL) were placed in a dry three-necked round-bottom flask equipped with a stir bar, an addition funnel, and a reflux condenser. After heating at 80 °C for 50 min, the reaction mixture was cooled to 0 °C and $EtP(Ph)_3Br$ (5.62 g, 15.1 mmol) dissolved in DMSO (21 mL) was added in one portion. The bright red reaction mixture was allowed to warm to room temperature, and steroid **18** (1.20 g, 2.97 mmol) dissolved in THF (22 mL) was added. The following morning, TLC analysis (5% EtOAc in hexanes) revealed that the reaction had not gone to completion. Additional ylide was generated (NaH, 0.36 g; $EtP(Ph)_3Br$, 5.62 g; DMSO, 35 mL) in a separate flask and cannulated into the reaction vessel. THF (18 mL) was added, the reaction was continued for 3 h, and then the reaction mixture was poured into H_2O (500 mL). The aqueous layer was extracted with hexanes (5 × 100 mL). The combined organic extracts were washed with H_2O (4 × 100 mL), dried, and filtered, and the solvents were removed to afford a foul-smelling yellow solid (1.48 g). Flash chromatography using the FlashElute system (cartridge size, 40 L; 0.5% EtOAc in hexanes, 30 psi) gave product **20** (0.83 g, 67%) as a white solid: mp 79−81 °C; IR 2929, 2859, 1471, 1463, 1451, 1373, 1361, 1252, 1098, 1081, 1058, 1007, 871 cm^{-1}; ^{1}H NMR δ 5.10 (qm, J = 6.9 Hz, 1H, C=C*H*CH_3), 3.59 (m, 1H, CHOSi), 1.64 (dm, J = 6.9 Hz, 3H, C=CHC*H*$_3$), 0.91 (s, 3H, CH_3), 0.89 (s, 9H, $C(CH_3)_3$), 0.85 (s, 3H, CH_3), 0.05 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR δ 150.60, 113.23, 72.78, 56.18, 44.36, 42.23, 40.26, 37.29, 36.84, 35.48, 35.33, 34.58, 31.44, 30.94, 27.17, 26.19, 25.87 (3 × C), 24.34, 23.25, 20.88, 18.19, 16.72, 12.96, −4.74 (2 × C). Anal. ($C_{27}H_{48}OSi$) C, H.

(3*β*,5α,8α,9*β*,10α,13α,14*β*,17α,20*R*)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-20-hydroxypregnane (20). Steroid **19** (0.80 g, 1.92 mmol) was dissolved in freshly distilled THF under N_2, and BH_3·THF complex (5.5 mmol, 5.5 mL of a 1.0 M solution in THF) was added. After 24 h, TLC analysis (5% EtOAc in THF) revealed that the reaction had not gone to completion. Additional BH_3·THF complex (5.5 mL) and THF (6 mL) were added. The following morning the reaction mixture was cooled to 0 °C with an ice bath prior to the cautious addition of aqueous 10% NaOH (13 mL) and 30% H_2O_2 (18 mL). The reaction mixture was allowed to stir for 3.5 h before it was extracted with EtOAc (3 × 60 mL). The combined organic extracts were washed with brine (1 × 30 mL), dried, and filtered, and the solvents were removed to afford a white solid (1.41 g). Flash chromatography (silica gel eluted with 10% EtOAc in hexanes) gave product **20** (0.51 g, 61%) as a white solid: mp 174−176 °C; IR 3369, 2928, 2859, 1450, 1372, 1252, 1095, 1009 cm^{-1}; ^{1}H NMR δ 3.67 (m, 1H, C*H*OH), 3.57 (m, 1H, CHOSi), 1.20 (d, J = 6.0 Hz, 1H), 0.89 (s, 3H, CH_3), 0.88 (s, 9H, $C(CH_3)_3$), 0.62 (s, 3H, CH_3), 0.04 (s, 6H, $Si(CH_3)_2$); ^{13}C δ 72.74, 70.26, 58.53, 56.19, 42.19, 41.85, 40.18, 39.08, 36.81, 35.51, 35.40, 34.51, 30.91, 27.15, 26.31, 25.86 (3 × C), 25.61, 24.04, 23.37, 23.26, 20.39, 18.19, 12.46, −4.77 (2 × C). Anal. ($C_{27}H_{50}O_2Si$) C, H.

(3*β*,5α,8α,9*β*,10α,13α,14*β*,17α)-3-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]pregnan-20-one (21). Steroid **20** (450 mg, 1.04 mmol) was dissolved in stirred CH_2Cl_2 (23 mL), and NaOAc (0.10 g, 1.21 mmol) and Celite (0.20 g) were added. After cooling to 0 °C for 20 min, PCC (0.34 g, 1.57 mmol) was added. After 30 min under an N_2 atmosphere, the ice bath was removed and the reaction vessel was allowed to warm to room temperature. After 5.5 h, the reaction mixture was diluted with Et_2O (40 mL), applied to silica gel, and eluted with EtOAc. Removal of the EtOAc gave a tan solid (0.48 g) which was purified by flash chromatography using the FlashElute System (cartridge size, 40S; 5% EtOAc in hexanes; 20 psi) to give product **21** (0.44 g, 98%) as a white solid: mp 119−121 °C; IR 2933, 2857, 1707, 1472, 1450, 1373, 1360, 1290, 1252, 1216, 1193, 1176, 1095, 1080, 1059, 871 cm^{-1}; ^{1}H NMR δ 3.57 (m, 1H, CHOSi), 2.49 (t, J = 9 Hz, 1H), 2.08 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.87 (s, 9H, $C(CH_3)_3$), 0.56 (s, 3H, CH_3), −0.04 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR δ 209.32, 72.20, 63.40, 56.16, 43.78, 41.66, 39.68, 38.68, 36.36, 35.31, 35.02, 34.07, 30.96, 30.47, 26.62, 25.83, 25.40 (3 × C), 23.87, 22.76, 22.31, 20.42, 17.72, 12.79, −5.22 (2 × C). Anal. ($C_{27}H_{48}O_2Si$) C, H.

(3*β*,5α,8α,9*β*,10α,13α,14*β*,17α)-3-Hydroxypregnan-20-one (22). Steroid **21** (0.42 g, 0.97 mmol) was dissolved in stirred freshly distilled THF (7 mL), and (n-Bu)$_4$NF (5 mmol, 5 mL of a 1 M solution in THF) was added under N_2. After 20 h, saturated NH_4Cl (30 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried and filtered, and the solvents were removed to afford a yellow oil (2.19 g). Flash chromatography (silica gel eluted with 10% EtOAc in hexanes) gave product **22** (300 mg, 97%) as a white solid: mp 148−150 °C; $[\alpha]_D^{30}$ −104.0 (c 1.02, $CHCl_3$); ee 94.0%; IR 3349, 2934, 2864, 1705, 1449, 1358, 1234, 1193, 1070, 1041 cm^{-1}; ^{1}H NMR δ 3.61 (m, 1H, C*H*OH), 2.50 (t, J = 9 Hz, 1H), 2.08 (s, 3H, $COCH_3$), 0.89 (s, 3H, CH_3), 0.56 (s, 3H, CH_3); ^{13}C NMR δ 209.87, 71.57, 63.78, 56.65, 44.21, 41.88, 40.29, 39.11, 36.24, 35.71, 35.22, 34.46, 31.37, 30.33, 26.93, 26.25, 24.28, 23.16, 22.73, 20.65, 13.23. Anal. ($C_{21}H_{34}O_2$) C, H.

(3*β*,5α,8α,9*β*,10α,13α,14*β*,17α)-3-(Sulfooxy)pregnan-20-one ammonium salt (6). Steroid **22** (100 mg, 0.31 mmol) was dissolved in pyridine (1.7 mL), and sulfur trioxide trimethylamine complex (0.27 g, 1.94 mmol) was added. The reaction vessel was purged with N_2, and after 18 h, aqueous 10% HCl was added to acidify to pH 1−2. The reaction mixture was extracted with CH_2Cl_2 (3 × 30 mL), the combined organic extracts were dried and filtered, and the solvent was removed to give a yellowish solid. The solid was dissolved in a minimal amount of CH_2Cl_2 and filtered through Celite. Anhydrous NH_3 was bubbled through the eluent for 30 min. Filtration through a sintered glass funnel gave product **6** (116 mg, 89%) as a white solid with a faint pink tinge: mp 199−200 °C (dec); $[\alpha]_D^{31}$ −94.5 (c 0.99, MeOH); ee 98.6%; IR 3484, 3153, 2938, 2871, 1711, 1639, 1449, 1402, 1213, 1059 cm^{-1}; ^{1}H NMR δ 4.28 (m, 1H, CHOS), 2.67 (t, J = 9.3 Hz, 1H), 2.11 (s, 3H, $COCH_3$), 0.95 (s, 3H, CH_3), 0.59 (s, 3H, CH_3); ^{13}C NMR δ 212.78, 80.35, 64.86, 57.86, 45.42, 43.61, 41.73, 40.20, 37.21, 36.40, 35.60, 34.54, 31.59, 28.82, 28.12, 27.50, 25.39, 23.79, 23.70, 21.93, 13.69. HRMS m/z calcd for $C_{21}H_{33}O_5S$: 397.2049. Found: 397.2046.

Electrophysiology. Hippocampal cultures were prepared from 1−2 day old albino rat pups and maintained as described previously.[29] Experiments were carried out at room temperature (~22 °C) using cultures that had been kept in vitro for

3−10 days. At the time of an experiment the growth media was exchanged for a solution containing the following (in mM): 140 NaCl, 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose, 10 hydroxyethylpiperazine ethanesulfonic acid (HEPES), and 0.001 tetrodotoxin (TTX) with pH adjusted to 7.3. TTX was included to block voltage-gated Na^+ currents and to diminish spontaneous synaptic currents. Voltage clamp recordings were obtained using whole-cell patch clamp methods.[25] Recording electrodes were fashioned from 1.2 mm borosilicate glass capillaries (World Precision Instruments) using a Flaming-Brown P-87 horizontal pipet puller (Sutter Instruments) and had resistances of 3−7 MΩ when fire-polished and filled with a solution containing the following (in mM): 140 CsCl, 4 NaCl, 4 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, and 5 ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) with pH adjusted to 7.3 using CsOH. Currents were filtered at 1.5 kHz and were digitized using pCLAMP V 5.5 (Axon Instruments). Data were analyzed using pCLAMP V 5.5, Sigmaplot for Windows V 2.0 (Jandel Scientific), and routines written in Axobasic. The data in this paper are presented as the mean ±SEM.

GABA stock solutions were prepared in the extracellular solution. Test compound stock solutions were prepared in DMSO and were diluted with the extracellular solution at the time of an experiment. The final DMSO concentration was <0.2%, a concentration that does not alter GABA currents in hippocampal neurons. Compounds were applied by pressure ejection from pipets positioned within 5 μm of the recorded neuron using a 500 ms jet of compressed air at 10−20 psi. This system allows no discernible drug leakage between applications and affords reliable repeated drug delivery. The concentrations of drugs reported are those in the pipet. The actual concentration at the cell is likely to be less due to diffusion and to the fact that the entire cell is not uniformly exposed to the pipet contents.

For concentration−response studies examining the effects of steroids on GABA-mediated currents, data were fit to a logistic equation of the following form: response = $100\{1 - ([\text{drug}]^n/([\text{drug}]^n + IC_{50}^n))\}$ using a least-squares minimization routine. In this equation, [drug] is the steroid concentration, IC_{50} is the half-maximal inhibitory concentration, and *n* is the Hill coefficient.

For studies examining the effects of a fixed concentration of the steroids on the GABA concentration−response curve, the control (GABA) curve was fit with an equation of the following form: response = $\text{response}_{max}\{[\text{GABA}]^n/([\text{GABA}]^n + EC_{50}^n)\}$, where response_{max} is the maximal response, [GABA] is the GABA concentration, EC_{50} is the half-maximal effective concentration, and *n* is the Hill coefficient. The effects of the steroids on the GABA concentration response curve were evaluated using competitive and noncompetitive inhibition models. In the competitive model, the concentration−response curve in the presence of antagonist is described by the following equation: response = $\text{response}_{max}[\text{GABA}]^n/\{[\text{GABA}]^n + (EC_{50}^n(1 + ([\text{I}]^n/K_i^n)))\}$, where [I] is the concentration of the inhibition and K_i is the apparent affinity constant for the antagonist. For noncompetitive inhibition the equation is the following: response = $\{\text{response}_{max}([\text{GABA}]^n/([\text{GABA}]^n + EC_{50}^n))\}\{1 - ([\text{I}]^n/([\text{I}]^n + K_i^n))\}$.

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