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Neurosteroid Analogues. 17. Inverted Binding Orientations of Androsterone Enantiomers at the Steroid Potentiation Site on γ-Aminobutyric Acid Type A Receptors

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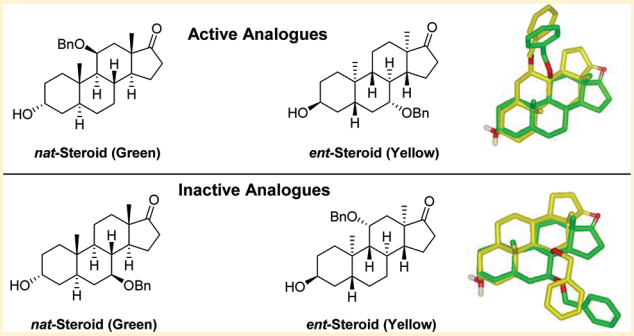
Supporting Information

ABSTRACT: The enantiomer pair androsterone and *ent*-androsterone are positive allosteric modulators of γ-aminobutyric acid (GABA) type A receptors. Each enantiomer was shown to bind at the same receptor site. Binding orientations of the enantiomers at this site were deduced using enantiomer pairs containing OBn substituents at either C-7 or C-11. 11β-OBn-substituted steroids and 7α-OBn-substituted *ent*-steroids potently displace [^{35}S]-*tert*-butylbicyclophosphorothionate, augment GABA currents, and anesthetize tadpoles. In contrast, 7β-OBn-substituted steroids and 11α-OBn-substituted *ent*-steroids have diminished actions. The results suggest that the binding orientations of the active analogues are inverted relative to each other with the 7α- and 11β-substituents similarly located on the edges of the molecules not in contact with the receptor surface. Analogue potentiation of the GABA current was abrogated by an α_1 subunit Q241L mutation, indicating that the active analogues act at the same sites in $\alpha_1\beta_2\gamma_{2L}$ receptors previously associated with positive neurosteroid modulation.



INTRODUCTION

Endogenous steroids such as allopregnanolone (**1**) and androsterone (**2**) as well as other endogenous 3α-hydroxysteroids and synthetic analogues potentiate γ-aminobutyric acid (GABA)-mediated chloride currents at GABA type A ($GABA_A$) receptors (Chart 1).[1−4] One consequence of potentiation of $GABA_A$ receptor function is an increase in neuronal inhibition in the brain. Consequently, steroids having this action are of interest as anxiolytic, anticonvulsant, sedative/hypnotic, and anesthetic agents.[5,6] Site-directed mutagenesis experiments have shown that the binding sites for this type of steroid modulation are located in the transmembrane domains of the α_1 subunits of the pentameric $\alpha_1\beta_2\gamma_2$ subtype of the mouse and rat receptor.[7,8] Similarly located sites exist on other forms of the α subunits.[9]

Because of the lack of $GABA_A$ protein crystal structure data, the molecular details of steroid binding to the receptor have not been established. Nevertheless, numerous analogue studies have established some general features of the structure−activity relationships (SARs) for steroid potentiation.[1,10,11] For example, highly active steroids nearly always contain a 3α-hydroxyl group on the steroid A-ring and, for the most active compounds, a 17β-hydrogen bond acceptor group on the steroid D-ring. Only a few *ent*-steroids have been evaluated for their effects at $GABA_A$ receptors, and much remains to be learned about their SARs at this class of receptors.[12−14]

In this study, we investigate SARs related to the enantioselectivity observed for steroid modulation of $GABA_A$ receptors by naturally occurring steroid **2** and its unnatural enantiomer *ent*-**2**. Both of these steroids have been shown previously to potentiate $GABA_A$ receptors.[14] Unexpectedly, the activity of the unnatural enantiomer is greater than that of the natural enantiomer. This same activity relationship (unnatural > natural) is also true for the more potent spiroepoxide analogues **3** and *ent*-**3**.[14]

The first goal of this study was to establish that potentiators such as steroids **2** and **3** and their enantiomers *ent*-**2** and *ent*-**3**, respectively, exerted their effects at the same potentiation site previously identified on the α_1 subunits (one site on each of the two α_1 subunits) in the pentameric $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptor. The second goal was to gain a better understanding of how active natural and enantiomeric steroids are oriented relative to each other at these receptor sites. Our strategy for achieving these two goals was to initially assume, and then later confirm, that there was indeed a common binding site for potentiating

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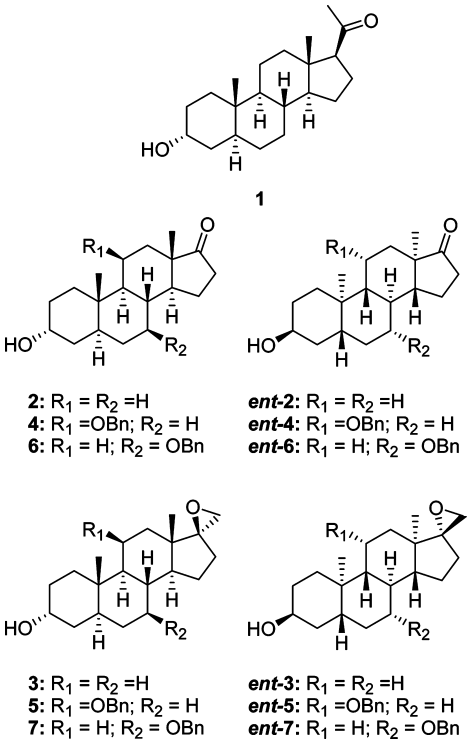
Chart 1



steroids and *ent*-steroids on $GABA_A$ receptors. This assumption allowed us to design analogues that would inform us as to how natural and enantiomeric steroids were aligned in a common binding site.

It seemed likely to us that the active steroids and *ent*-steroids would have the 3α-hydroxy group similarly positioned in the receptor binding sites since this group is critical for high potentiation activity. This is only possible if the active steroids and active *ent*-steroids are bound upside down relative to each other. Less immediately apparent is one consequence of this relative orientation of a pair of enantiomers. When oriented in this way, the C-6, C-7 edge of the natural steroids and the C-11, C-12 edge of the *ent*-steroids can be located proximate to each other (see the Discussion for the alignment model) and a testable prediction can be made about the effect that substituents at C-11 or C-7 will have on the activity of pairs of enantiomeric analogues.

Accordingly, since it is known that a steroid analogue with both an 11β-OBn and an 11α-Me substituent is a strong potentiator at $GABA_A$ receptors and steroid analogues with either a 7α- or a 7β-Me group are not,[15,16] it can be confidently predicted that analogues **4** and **5** will have high activity and analogues **6** and **7** will not. Less confidently predicted, but of more interest, are the activities of the enantiomers of these analogues. If the *ent*-steroids are bound upside down relative to the natural steroids, then the 11α-substituents in *ent*-**4** and *ent*-**5** will be located proximate to the 7β-substituents in steroids **6** and **7**, and *ent*-**4** and *ent*-**5** are predicted to be inactive. Conversely, the 7α-substituents in analogues *ent*-**6** and *ent*-**7** will be located proximate to the 11β-substituents in steroids **4** and **5**, and *ent*-**6** and *ent*-**7** are predicted to be active.

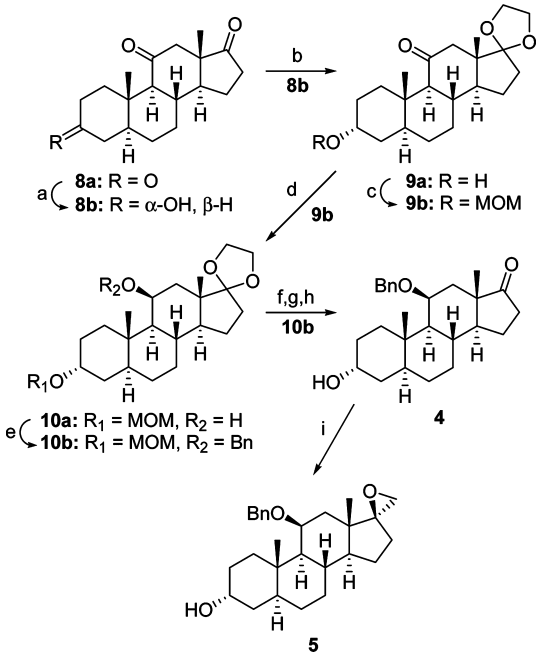
Hence, to accomplish our goals, we prepared steroids **4**–**7** and their enantiomers *ent*-**4**–*ent*-**7**. We evaluated these agents for their ability to noncompetitively displace [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS) from the picrotoxin binding site on $GABA_A$ receptors, potentiate GABA-mediated chloride currents of rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes, and cause loss of righting reflex (LRR) and loss of swimming reflex (LSR) in tadpoles. We then carried out site-directed mutagenesis studies to determine if analogues that were active in all three bioassays acted at the same sites on the $GABA_A$ receptor.

We found that steroids **4** and **5**, which have an 11β-benzyloxy group, have high activity whereas their enantiomers *ent*-**4** and *ent*-**5** do not. Conversely, we found that steroids **6** and **7**, which have a 7β-benzyloxy group, have weak activity whereas their enantiomers *ent*-**6** and *ent*-**7** have high activity. We found the potentiation effects of the active steroids and *ent*-steroids were either eliminated or greatly reduced when the known potentiation sites on the α_1 subunits were rendered ineffective by the Q241L mutation that is known to disrupt steroid actions at rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors.[8]

CHEMISTRY

The synthesis of analogues **4** and **5** is summarized in Scheme 1. The starting material was purchased triketone **8a**. Regio- and

Scheme 1[a]



[a]Reagents: (a) (i) K(*s*-Bu)$_3$BH, THF, −78 °C; (ii) 3 N NaOH, 30% H_2O_2 (72%); (b) PTSA, $HO(CH_2)_2OH$, benzene, reflux (70%); (c) (MOM)Cl, (*i*-Pr)$_2$EtN, DMAP, CH_2Cl_2 (91%); (d) LAH, Et_2O (80%); (e) KH, BnBr, THF, reflux (75%); (f) MeOH, 6 N HCl (89%); (g) Ac_2O, pyridine, DMAP (95%); (h) K_2CO_3, MeOH (96%); (i) $(CH_3)_3SI$, DMF, KO-*t*-Bu (61%).

stereoselective reduction of the 3-ketone using K(*s*-Bu)$_3$BH yielded after workup predominately the 3α-hydroxysteroid **8b** (72%). The 17-ketone was then selectively protected as the cyclic ketal in the standard manner (**9a**, 70%), and the 3α-OH group was protected with a methoxymethyl (MOM) group (**9b**, 91%). The 11-ketone is then reduced with lithium aluminum hydride (LAH) to yield the 11β-OH of steroid **10a** (80%), and the 11β-OH is alkylated with BnBr to yield steroid **10b** (75%). Removal of the MOM protecting group yielded target compound **4** (89%), which contained an impurity that was

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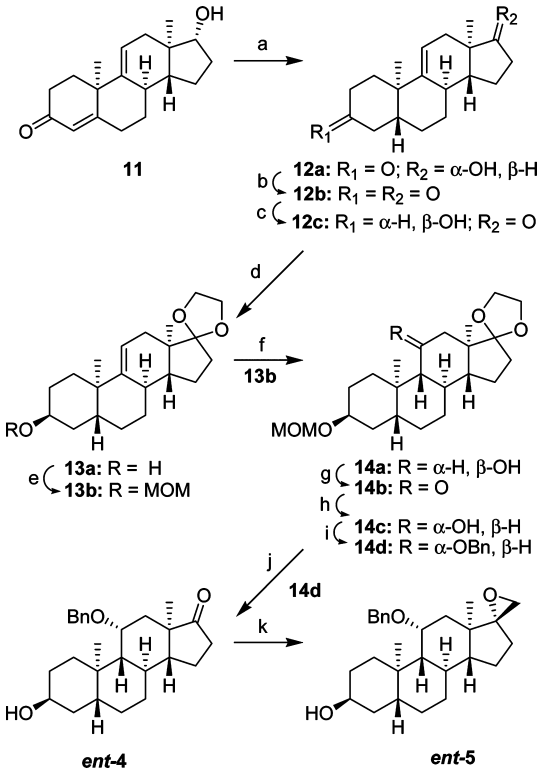
not easily removed. Acetylation of the 3α-OH group (95%), purification of the acetate derivative by flash column chromatography, and removal of the acetyl group (96%) allowed pure steroid **4** to be prepared. Epoxidation of the 17-ketone using $(CH_3)_3SI$, KO-*t*-Bu in dimethylformamide (DMF) gave the 17-spiroepoxide **5** (61%). This epoxidation is highly stereoselective with a minor amount (ca. 5%) of the epimeric epoxide being removed by a combination of flash column chromatography and recrystallization. The stereochemistry at all new chiral centers formed in Scheme 1 and synthetic Schemes 2−4 was assigned on the basis of well-known literature precedents and ^{1}H NMR data.

The preparation of analogues *ent*-**4** and *ent*-**5** is presented in Scheme 2. The starting material, *ent*-steroid **11**, was prepared

Scheme 2[a]

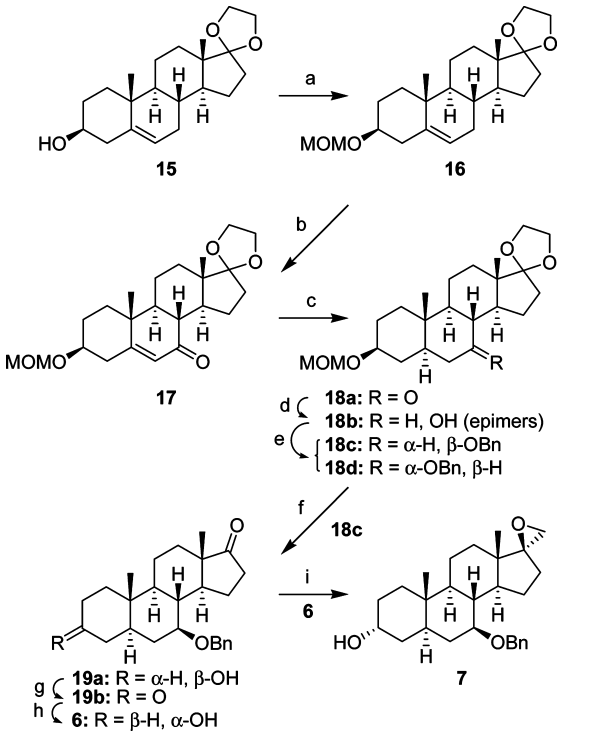


[a]Reagents: (a) Li, liquid NH_3, toluene, THF, −78 °C (81%); (b) Jones reagent, acetone (92%); (c) (i) K(*s*-Bu)$_3$BH, THF, −78 °C; (ii) 3 N NaOH, 30% H_2O_2 (65%); (d) PPTS, HO(CH$_2$)$_2$OH, benzene, reflux (87%); (e) (MOM)Cl, DMAP, (*i*-Pr)$_2$EtN, CH_2Cl_2 (97%); (f) (i) BH_3·THF, THF, 0 °C; (ii) 3 N NaOH, 30% H_2O_2 (67%); (g) PCC, CH_2Cl_2 (96%); (h) LAH, Et_2O (95%); (i) KH, THF, BnBr (69%); (j) MeOH, 6 N HCl (92%); (k) $(CH_3)_3SI$, DMF, KO-*t*-Bu (61%).

by our previously described method.[17] Li/liquid NH_3 reduction of the Δ^4-3-ketone group yielded the *trans* A,B ring fused product **12a** (81%). Jones oxidation of *ent*-steroid **12a** gave the diketone **12b** (92%). The 3-ketone was then reduced with K(*s*-Bu)$_3$BH to the 3β-OH group of *ent*-steriod **12c** (97%). The 17-ketone was next protected as the cyclic ketal to yield product **13a** (87%), and the 3β-OH group was protected with a MOM group (**13b**, 97%). The $\Delta^{9(11)}$ double bond was then removed by hydroboration to give *ent*-steroid **14a** (67%), which contained the 9β-H and 11β-OH group. The stereochemistry of the 11-OH group was changed from the β configuration to the desired α configuration by a two-step procedure: PCC oxidation (**14b**, 96%) followed by LAH reduction (**14c**, 95%). Benzylation of the 11α-OH group gave *ent*-steroid **14d** (69%), and removal of the MOM protecting group gave the required analogue *ent*-**4** (92%). The minor impurity that complicated purification of steroid **4** was not present in enantiomer *ent*-**4**, making purification of the enantiomer possible without conversion to its 3β-acetate derivative. Epoxidation of 17-ketone *ent*-**4** using $(CH_3)_3SI$, KO-*t*-Bu in DMF gave the 17-spiroepoxide *ent*-**5** (61%).

The synthesis of analogues **6** and **7** is shown in Scheme 3. Dehydroepiandrosterone was purchased, converted in the usual

Scheme 3[a]



[a]Reagents: (a) (MOM)Cl, DMAP, (*i*-Pr)$_2$EtN, CH_2Cl_2 (91%); (b) 70% aqueous *t*-BuOOH, $NaClO_2$, aqueous ACN, 50 °C (34%); (c) 10% Pd/C, EtOAc (80%); (d) LAH, Et_2O (86%); (e) KH, THF, BnBr (68%); (f) MeOH, 6 N HCl (91%); (g) PCC, CH_2Cl_2 (86%); (h) (i) K(*s*-Bu)$_3$BH, THF, −78 °C; (ii) 3 N NaOH, 30% H_2O_2 (67%); (i) $(CH_3)_3SI$, DMF, KO-*t*-Bu (57%).

manner to its cyclic ketal derivative **15**, and used as the starting material. The 3β-OH group was protected with a MOM group to yield steroid **16** (91%). Allylic oxidation of the Δ^5 double bond using $NaClO_2$ and aqueous *t*-BuOOH gave the Δ^5-6-ketone **17** in adequate, but not high, yield (34%). Catalytic hydrogenation of steroid **17** using Pd/C gave product **18a** in high yield (80%). LAH reduction of the C-7 ketone group gave an inseparable mixture of epimeric 7α- and 7β-alcohols **18b** (86%). The decision was made to benzylate the 7-OH groups (68%) to determine if the benzylated epimers were separable. This turned out to be the case, and the desired 7β-OBn epimer **18c**, which was formed by benzylation of the minor LAH reduction product, was obtained after flash column chromatography in 23% yield. The undesired 7α-OBn epimer **18d** was obtained in 45% yield. Removal of the MOM protecting group from steroid **18c** gave product **19a** (91%), PCC oxidation of

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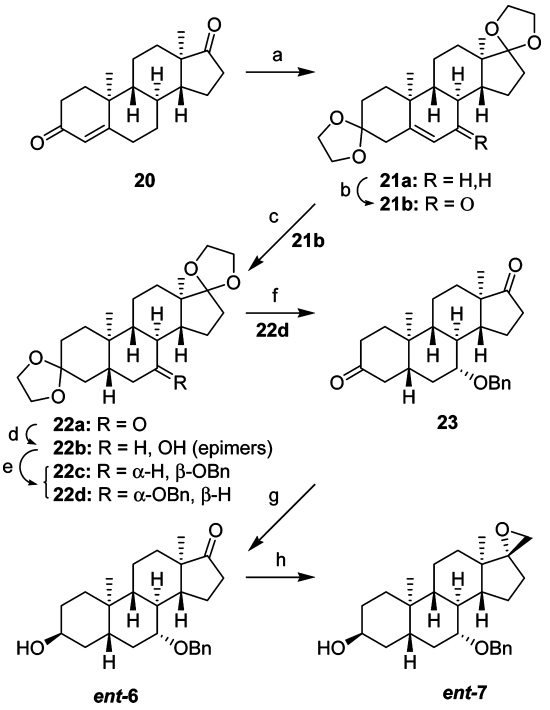
steroid **19a** gave steroid **19b** (86%), and K(*s*-Bu)$_3$BH reduction of the 3-ketone group of steroid **19b** gave target compound **6** (67%). Epoxidation of the 17-ketone using $(CH_3)_3SI$, KO-*t*-Bu in DMF gave the 17-spiroepoxide **7** (61%). As observed for the epoxidation of the compounds described in Schemes 1 and 2, this epoxidation was also highly stereoselective and yielded almost exclusively the desired spiroepoxide product.

The preparation of analogues *ent*-**6** and *ent*-**7** is demonstrated in Scheme 4. The starting material was *ent*-androstenedione

Scheme 4[a]



[a]Reagents: (a) PTSA, $HO(CH_2)_2OH$, benzene, reflux (83%); (b) 70% aqueous *t*-BuOOH, $NaClO_2$, aqueous ACN, 50 °C (31%); (c) 10% Pd/C, H_2 (50 psi), EtOAc (76%); (d) Raney Ni, H_2 (60 psi), *i*-PrOH (96%); (e) KH, THF, BnBr (72%); (f) MeOH, 6 N HCl (89%); (g) (i) K(*s*-Bu)$_3$BH, THF, −78 °C; (ii) 3 N NaOH, 30% H_2O_2 (73%); (h) $(CH_3)_3SI$, DMF, KO-*t*-Bu (68%).

(**20**), which was prepared as we described earlier.[18] This *ent*-steroid was first converted into its Δ^5-3,17-bis(cyclic ketal) **21a** in the usual manner (83%) and subjected to allylic oxidation, in the manner described earlier for the preparation of steroid **17**, to obtain *ent*-steroid **21b** (31%). Catalytic hydrogenation of enone **21b** gave the 7-ketone product **22a** (76%). Because the LAH reduction of the 7-ketone group of steroid **18a** described in Scheme 3 gave, after benzylation, the undesired 7α-OBn product **18b** as the major product, a different method was used for the reduction of the 7-ketone in *ent*-steroid **22a** in an attempt to increase the amount of the desired epimer. Raney Ni reduction[19] of the 7-ketone in *ent*-steroid **22a** gave product **22b** (96%) as an inseparable mixture of 7α- and 7β-alcohols, and benzylation (72%) gave the readily separated 7β-OBn product **22c** (22%) and 7α-OBn product **22d** (50%). Presumably, steroid approach to the surface of the Raney Ni from the *ent*-steroid β face (i.e., opposite the C-19 Me group on the α face) explains why the desired 7α-OH product was the major epimer formed in the Raney Ni reduction. For natural steroids, this means that the major alcohol product of Raney Ni reduction of a 7-ketone group would be the 7β-alcohol. Removal of the 3,17-bis(cyclic ketal) groups from *ent*-steroid **22d** gave product **23** (89%), K(*s*-Bu)$_3$BH reduction of *ent*-steroid **23** gave target compound *ent*-**6** (73%), and epoxidation of product *ent*-**6** gave target compound *ent*-**7** (68%).

[^{35}S]TBPS DISPLACEMENT RESULTS

The enantiomer pairs shown in Chart 1 were evaluated as noncompetitive displacers of [^{35}S]TBPS from the picrotoxin binding site on the heterogeneous $GABA_A$ receptors found in rat brain membranes. The IC_{50} displacement values are given in Table 1. The values reported for the reference compounds **2**, *ent*-**2**, **3**, and *ent*-**3** are our previously reported values.[14] Relative to steroid **2**, the reported IC_{50} values indicate that the axial 11β-OBn substituent present in steroid **4** slightly increases the displacement potency whereas the equatorial 7β-OBn substituent present in steroid **6** drastically decreases the displacement potency. Relative to compound *ent*-**2**, the 11α-OBn group present in enantiomer *ent*-**4** drastically reduces the binding potency and the 7α-OBn substituent present in enantiomer *ent*-**6** increases the binding potency.

Table 1. Inhibition of [^{35}S]TBPS Binding by Steroids 1−7 and Steroid Enantiomers *ent*-2−*ent*-7[a]

compd	IC_{50} (nM)	n_{Hill}
1[b]	74 ± 7	0.89 ± 0.06
2[c]	410 ± 130	0.89 ± 0.20
ent-**2**[c]	311 ± 36	1.00 ± 0.10
3[c]	105 ± 12	1.32 ± 0.16
ent-**3**[c]	467 ± 93	4.28 ± 1.90
4	285 ± 37	0.90 ± 0.09
ent-**4**	>10 μM[d]	
5	15 ± 2	1.1 ± 0.1
ent-**5**	2050 ± 400	2.04 ± 0.7
6	>10 μM[e]	
ent-**6**	168 ± 8	1.10 ± 0.05
7	6750 ± 1000	1.4 ± 0.2
ent-**7**	52 ± 5	1.12 ± 0.10

[a]The results presented are from duplicate experiments performed in triplicate. Error limits are calculated as the standard error of the mean. [b]Literature values.[27] [c]Literature values.[14] [d]No inhibition was observed at 10 μM. [e]Partial inhibition (~35%) was observed at 10 μM.

Binding results obtained with the 17-spiroepoxide enantiomer pairs **5**, *ent*-**5** and **7**, *ent*-**7** followed the same pattern observed for the binding results obtained with enantiomer pairs **4**, *ent*-**4** and **6**, *ent*-**6**. Relative to steroid **3**, the 11β-OBn substituent present in steroid **5** increases the displacement potency whereas the 7β-OBn substituent present in steroid **7** decreases the displacement potency. Relative to analogue *ent*-**3**, the 11α-OBn substituent present in analogue *ent*-**5** reduces the displacement potency whereas the 7α-OBn substituent present in analogue *ent*-**7** increases the displacement potency. Both compounds **5** and *ent*-**7** are more potent displacers of [^{35}S]TBPS than the endogenous neurosteroid **1**.

ELECTROPHYSIOLOGY RESULTS

Enantiomer pairs were evaluated for their ability to potentiate chloride currents mediated by 2 μM GABA at rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors expressed in *X. laevis* oocytes (Table 2). This GABA concentration gates, on average, ~4% of the maximum response recorded from an oocyte. The maximum achievable

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Table 2. Modulation of Rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ Receptor Function by Steroids 1−7 and Steroid Enantiomers *ent*-2−*ent*-7

	oocyte electrophysiology[a]			
compd	0.1 μM	1 μM	10 μM	10 μM (gating)
1[b]	1.26 ± 0.14	3.89 ± 1.34	9.65 ± 3.87	0.37 ± 0.07
2[c]	0.97 ± 0.02	1.41 ± 0.01	5.44 ± 0.19	0.02 ± 0.01
ent-**2**[c]	1.27 ± 0.29	3.66 ± 0.89	18.87 ± 2.38	0.03 ± 0.21
3[c]	3.11 ± 0.17	21.92 ± 1.30	33.73 ± 2.04	0.22 ± 0.02
ent-**3**[c]	2.62 ± 0.29	15.89 ± 3.89	26.28 ± 7.90	0.10 ± 0.02
4	1.08 ± 0.03	2.96 ± 0.14	24.37 ± 1.47	0.20 ± 0.03
ent-**4**	0.89 ± 0.03	0.85 ± 0.02	0.60 ± 0.01	0.00 ± 0.01
5	2.99 ± 0.45	21.19 ± 2.59	31.15 ± 2.87	0.42 ± 0.06
ent-**5**	1.01 ± 0.05	1.30 ± 0.09	3.21 ± 0.65	0.05 ± 0.03
6	0.97 ± 0.02	0.90 ± 0.04	0.76 ± 0.02	0.00 ± 0.02
ent-**6**	1.25 ± 0.03	5.74 ± 0.94	25.23 ± 7.16	0.11 ± 0.02
7	0.83 ± 0.05	0.88 ± 0.05	0.77 ± 0.20	0.09 ± 0.16
ent-**7**	3.47 ± 0.13	20.29 ± 2.75	32.39 ± 5.42	0.22 ± 0.03

[a]The GABA concentration used for the control response was 2 μM. Each compound was evaluated on at least four different oocytes at the concentrations indicated, and the results reported are the ratio of currents measured in the presence/absence of added compound. Gating represents direct current gated by 10 μM compound in the absence of GABA, and this current is reported as the ratio of compound-only current to 2 μM GABA current. Error limits are calculated as the standard error of the mean ($N \geq 4$). [b]Literature values.[27] [c]Literature values.[14]

potentiation of currents by an analogue varies for different batches of oocytes. Hence, since the different compounds in Table 2 were evaluated on different batches of oocytes, it is not possible to confidently compare the absolute potentiation caused by each analogue shown in Table 2. The low aqueous solubility of these compounds also prevented us from determining the maximal potentiation. Inspection of the concentration effect data in Table 2 suggests that there may be differences in both potency and efficacy for the different compounds. However, the conclusions we draw from the results presented in Table 2 do not require a comparison of absolute potentiation values.

As reported previously, steroids **2** and **3** and their enantiomers *ent*-**2** and *ent*-**3** all cause a concentration-dependent increase in GABA-gated chloride currents.[14] Steroids **4** and **5**, which contain 11β-OBn substituents, also cause a concentration-dependent increase in chloride currents. Steroids **6** and **7**, which contain 7β-OBn substituents, do not. For the analogues *ent*-**4** and *ent*-**5**, the 11α-OBn substituents either do not potentiate chloride currents (*ent*-**4**) or only weakly potentiate at the highest concentration, 10 μM (*ent*-**5**). Analogues *ent*-**6** and *ent*-**7**, which contain 7α-OBn substituents, both cause a concentration-dependent increase in GABA-gated chloride currents. The active benzyloxy-substituted compounds (**4**, **5**, *ent*-**6**, *ent*-**7**) at a concentration of 10 μM also directly gate a chloride current in the absence of GABA, a well-known effect of active neurosteroid analogues.

The results reported in Table 2 correlate with the results presented in Table 1. Compounds that potently displace [^{35}S]TBPS also strongly potentiate GABA-mediated chloride currents. Compounds that either weakly displace or do not displace [^{35}S]TBPS at a concentration of 10 μM either weakly potentiate or do not potentiate GABA-mediated chloride currents.

We next addressed the question of whether the steroid and *ent*-steroid potentiators act at the potentiation site previously identified for neurosteroid **1** by site-directed mutagenesis studies.[8] We did this to rule out the possibility that the steroid and *ent*-steroid potentiators act at different sites on the rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptor. Accordingly, residue 241 in the α_1 subunit was changed from glutamine to leucine. As reported previously[20] and shown in Figure 1, this amino acid change

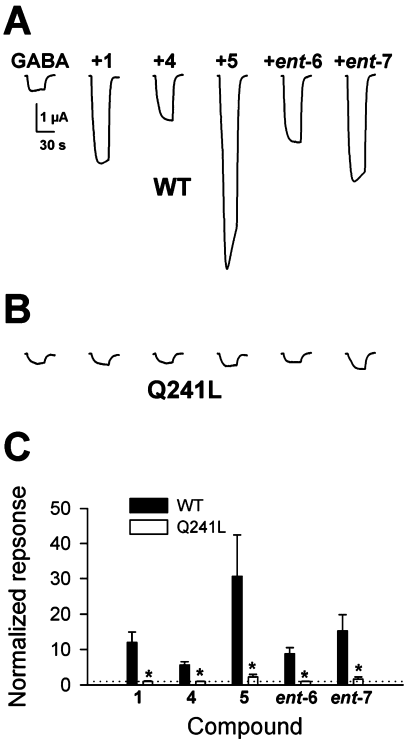

Figure 1. Effects of a steroid-insensitive α_1(Q241L) subunit on potentiation of GABA currents by analogues. (A) Oocytes expressing wild-type (WT) $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptor subunits were exposed to GABA alone or GABA coapplied with the indicated analogues at a membrane potential of −70 mV. The concentrations of the compounds were as follows: GABA, 2 μM; compound **1**, 0.5 μM; compound **4**, 1 μM, compound **5**, 0.5 μM; compound *ent*-**6**, 1 μM; compound *ent*-7, 0.5 μM. The concentrations were adjusted to partially account for different potencies observed in initial screening and to thereby yield reasonably similar potentiation values. (B) Same sequence of compound presentation in oocytes injected with RNA encoding a point mutation (Q241L) in the α_1 subunit that renders receptors insensitive to compound **1**.[8] WT β_2 RNA and γ_{2L} RNA were coinjected. (C) Summary of normalized responses of four WT oocytes and four α_1(Q241L)$\beta_2\gamma_{2L}$ oocytes in experiments like those depicted in (A) and (B). The normalizing response to GABA alone is denoted by a dotted line at $y = 1$. The asterisk indicates $p < 0.05$ by an independent sample t test.

eliminates the potentiation caused by neurosteroid **1**. It also eliminates or nearly eliminates the potentiation caused by the active analogues prepared in this study. The results support the conclusion that the potentiation caused by all the active compounds is mediated through a common site of action on the $GABA_A$ receptor. Figure 1 also provides information on the relative activity of the potentiating compounds. The 17-spiroepoxides **5** (0.5 μM) and *ent*-**7** (0.5 μM) potentiate more than the 17-ketones **4** (1 μM) and *ent*-**6** (1 μM) when compared on the same oocyte. This result is consistent with the

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greater potency of the 17-spiroepoxides for [^{35}S]TBPS displacement (Table 1).

TADPOLE LRR AND LSR RESULTS

The anesthetic effects in tadpoles of the compounds are reported in Table 3. The EC_{50} values for LRR and LSR reported in Table 3 for steroids **2** and **3** and their enantiomers *ent*-**2** and *ent*-**3** are the values we reported previously.[14] Steroids **4** and **5**, which contain axial 11β-OBn substituents, are both more potent in causing LRR and LSR in tadpoles than the reference steroids **2** and **3**, respectively. The 17-ketosteroid **6** (7β-OBn substituent) does not have an EC_{50} value of <10 μM for either LRR or LSR. The results correlate with the actions of this analogue in the other two bioassays (Tables 1 and 2). The corresponding 17-spiroepoxide **7** with the 7β-OBn substituent does have weak anesthetic activity as it produces both LRR (EC_{50} = 3.22 μM) and LSR (EC_{50} = 7.94 μM). This result for analogue **7** is consistent with its weak ability to displace [^{35}S]TBPS (Table 1), but not with its failure to potentiate GABA-mediated chloride currents (Table 2).

The enantiomeric 17-ketosteroid *ent*-**4** (11α-OBn substituent), which neither displaces [^{35}S]TBPS (Table 1) nor potentiates GABA-mediated chloride currents (Table 2) at concentrations up to 10 μM, unexpectedly causes both LRR and LSR at concentrations below 10 μM. The corresponding enantiomeric 17-spiroepoxysteroid *ent*-**5** with the 11α-OBn substituent also causes both LRR and LSR at concentrations below 10 μM. However, this analogue is also weakly active in the other two bioassays (Tables 1 and 2), so its ability to cause LRR and LSR in tadpoles is not surprising.

The enantiomeric analogues *ent*-**6** and *ent*-**7** (7α-OBn substituents) both cause, as expected, LRR and LSR in tadpoles. The enantiomeric 17-ketoanalogue *ent*-**6** is less potent than the enantiomeric 17-spiroepoxide analogue *ent*-**7**. This order of potency for their anesthetic effects correlates with their order of potency for [^{35}S]TBPS displacement (Table 1) and electrophysiological actions at $GABA_A$ receptors (Figure 1).

Table 3. Effects of Steroids 1−7 and Steroid Enantiomers *ent*-2−*ent*-7 on Tadpole Righting and Swimming Reflexes[a]

compd	tadpole LRR EC_{50} (μM)	tadpole LRR n_{Hill}	tadpole LSR EC_{50} (μM)	tadpole LSR n_{Hill}[b]
1[c]	0.42 ± 0.04	−1.83 ± 0.32	5.5 ± 0.5	−7.5 ± 1.1
2[d]	3.38 ± 0.9	−2.83 ± 2.66	none[e]	
ent-**2**[d]	1.42 ± 0.18	−2.17 ± 0.48	5.48 ± 0.12	−33.3 ± 0.1
3[d]	1.35 ± 0.01	−3.69 ± 0.08	2.76 ± 0.01	−21.1 ± 0.7
ent-**3**[d]	1.08 ± 0.01	−18.1 ± 1.3	2.71 ± 0.01	−21.8 ± 1
4	0.20 ± 0.00	−2.61 ± 0.07	0.87 ± 0[f]	−20.4 ± 0.4
ent-**4**	1.96 ± 0.02	−3.27 ± 0.07	3.45 ± 0[g]	−21.1 ± 0
5	0.07 ± 0.01	−1.58 ± 0.21	0.27 ± 0[h]	−21.4 ± 0.5
ent-**5**	2.64 ± 0.01	−22.8 ± 1.0	2.74 ± 0[i]	−23.2 ± 0
6	>10[j]		none[e]	
ent-**6**	1.21 ± 0.4	−4.27 ± 6.44	2.93 ± 0	−22.0 ± 0.5
7	3.22 ± 0.03	−15.6 ± 1.8	7.94 ± 0[k]	−27.3 ± 0
ent-**7**	0.31 ± 0.04	−2.19 ± 0.61	0.55 ± 0.01	−33.3 ± 0.1

[a]Error limits are calculated as the standard error of the mean (N = 10 or more tadpoles at each of five or more different concentrations (ranging from 0.01 to 30 μM). Unless stated otherwise, all tadpoles regained LRR and LSR after overnight recovery. [b]LSR for compounds with weak activity typically has a very steep concentration−response curve. When n_{Hill} values are in the range of −20 to −33, the n_{Hill} values reflect the fact that at one concentration nearly all tadpoles had a swimming reflex and at the next highest concentration tested nearly all or all tadpoles did not. [c]Literature values.[27] [d]Literature values.[14] [e]"None" indicates that all tadpoles had a swimming response at 10 μM. [f]Recovery from LRR and/or LSR was partial at concentrations ≥1 μM. At 10 μM, 10 of 10 tadpoles died without recovering overnight from LSR. [g]At 10 μM, 10 of 10 tadpoles died without recovering overnight from LSR. [h]At 10 μM, 5 of 10 tadpoles died without recovering overnight from LSR. [i]At 10 μM, 6 of 10 tadpoles died without recovering overnight from LSR. [j]Some tadpoles had LRR at 10 μM, but the EC_{50} was above this value. [k]At 10 μM, 4 of 10 tadpoles died without recovering overnight from LSR.

DISCUSSION

The prior observation that steroids **2** and **3** and their enantiomers *ent*-**2** and *ent*-**3** all potentiated GABA-mediated chloride currents at $GABA_A$ receptors raised two interesting questions: Are the pairs of enantiomers (steroid and corresponding *ent*-steroid) acting at the same sites on the $GABA_A$ receptor? How are the pairs of enantiomers aligned relative to each other if they act at the same sites on the receptors? The goal of this study was to provide answers to these two questions.

An analogue approach, used in conjunction with site-directed mutagenesis methodology, was chosen to address both questions. The analogues chosen were designed on the basis of an initial assumption that all enantiomer pairs acted at a common site on the receptor and on previous steroid SAR results. It was shown previously that modifying neurosteroid **1** by introducing an 11α-Me substituent together with an 11β-OBn substituent gave an analogue with increased potentiation activity.[15] Hence, we expected that new steroid analogues having 11β-OBn substituents (**4**, **5**) would have activity. Since it was also shown previously that adding a group as small as either a 7α- or 7β-Me group to neurosteroid **1** produced an analogue with greatly diminished activity,[16] we also expected that new steroid analogues having 7β-OBn substituents (**6**, **7**) would have little, if any, activity.

Expected activity outcomes for the enantiomeric compounds *ent*-**4**−*ent*-**7** depend on the choice made to align each steroid with its corresponding enantiomer. We made this alignment choice on the basis of the assumption that the critical 3-OH groups will be similarly positioned in three-dimensional space if enantiomer pairs are bound to the same $GABA_A$ receptor binding sites. To satisfy this assumption, active *ent*-steroids must be oriented upside down relative to active steroids. When so oriented, an 11β-OBn substituent in a natural steroid and a 7α-OBn substituent in an *ent*-steroid can occupy similar regions of three-dimensional space (Figure 2A). Hence, we expected that new *ent*-steroids having 7α-OBn substituents (*ent*-**6**, *ent*-**7**) would, like the 11β-OBn steroids **4** and **5**, have potentiation activity. Similarly, since a 7β-OBn substituent in a natural steroid and an 11α-OBn substituent in an upside down oriented *ent*-steroid can occupy similar regions of three-dimensional space (Figure 2B), we expected that new *ent*-steroid analogues having 11α-OBn substituents (*ent*-**4**, *ent*-**5**) would, like the 7β-OBn steroids **6** and **7**, have greatly diminished potentiation activity.

Other factors also influenced our choice of analogues chosen for this study. First, since the enantiomer of neurosteroid **1** is essentially inactive,[12] these studies could not have been done with analogues of neurosteroid **1** containing benzyloxy groups at C-7 or C-11 since no active enantiomers of neurosteroid **1** bearing benzyloxy groups at either position would be possible.

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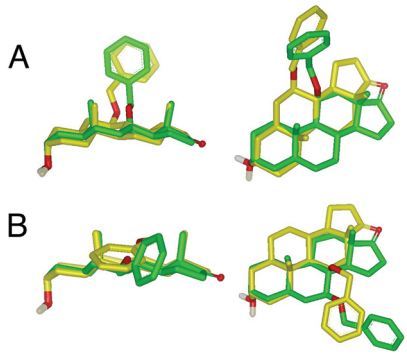

Figure 2. (A) Molecular superimpositions of active compounds **4** (green) and *ent*-**6** (yellow) and (B) inactive compounds **6** (green) and *ent*-**4** (yellow). In both panels a low-energy conformation of the analogue with the absolute configuration of natural steroids was used as the template and is shown in green, and the best result from fitting a library of conformers of the analogues with the absolute configuration opposite that of natural steroids (*ent*-steroids) is shown in yellow. The alignments maximize the overlap of the molecular volumes as well as structural features such as hydrogen bond donors and acceptors. Edge views are shown on the left, and views from above the molecules are shown on the right. Hydrogens other than that of the hydroxyl group are removed for clarity. Alignments were made using the software program ROCS (OpenEye Scientific Software).

Second, 11β-substituents in the steroids have the axial configuration, whereas the 7α-subsituents in the *ent*-steroids have the equatorial configuration. Thus, a relatively large and flexible group such as a benzyloxy group was considered advantageous for maximizing possible overlap of substituents having different configurations at the C-7 and C-11 positions. Third, we considered it important to have the C-11- and C-7-substituents be identical to avoid significant differences in log *P* for the analogues used in the study. log *P* can have a major effect on the observed [^{35}S]TBPS displacement and potentiation activity because the amount of steroid that accumulates in the cell membrane will determine its effective concentration at the transmembrane steroid binding sites located on the $GABA_A$ receptor.[21,22]

As shown by the [^{35}S]TBPS displacement results (Table 1) and electrophysiology results (Table 2), our expected outcomes were observed in these two bioassays. Two unexpected outcomes were found in the tadpole behavioral bioassay (Table 3). The analogues *ent*-**4** and **7** both caused LRR and LSR at concentrations below 10 μM even though the compounds neither effectively displace [^{35}S]TBPS nor potentiate GABA-mediated chloride currents. We suggest that the anomalous behavioral effects of these two compounds result from actions on receptors other than $GABA_A$ receptors.

The site-directed mutagenesis study (Figure 1) provides evidence that the two active steroids (**4**, **5**) and the two active *ent*-steroids (*ent*-**6**, *ent*-**7**) are acting at the same $GABA_A$ receptor binding sites. The Q241L mutation abolishes, completely or nearly completely, the activity of all four active compounds at a concentration of either 0.5 μM (the 17-spiroepoxides) or 1 μM (the 17-ketones). The $GABA_A$ receptor sites of action were not determined for the analogues *ent*-**4**, *ent*-**5**, **6**, and **7**. These analogues neither strongly displace [^{35}S]TBPS nor strongly potentiate GABA-mediated currents. It is interesting to note that compounds *ent*-**4**, **6**, and **7** appear to weakly inhibit GABA-mediated currents. We have observed previously that 3β-hydroxysteroids can show a use-dependent block of $GABA_A$ receptors that is removed by a V256S mutation of the α_1 subunit.[23] Future studies will be required to determine whether the effects of *ent*-**4**, **6**, and **7** can be explained by this mechanism, which is independent of potentiation.

This enantioselectivity study examined only 5α-reduced analogues. An earlier study examined the effect of a Q241 mutation in the α_1 subunit on the actions of a 5β-reduced steroid potentiator and its active enantiomer.[24] It was reported that mutation of the Q241 residue eliminated the actions of the 5β-reduced steroid and reduced the actions of its enantiomer, consistent with the present results. Because weak potentiation remained in the previous study and was statistically significant for analogue *ent*-**6** in the present study, the possibility of additional binding sites cannot be excluded.

The modeling results in Figure 2 were obtained with the program ROCS (OpenEye Scientific Software), which maximizes the volume overlap between a query molecule (low-energy conformers of compounds **4** and *ent*-**6**) and a library of other molecules or conformers while also maximizing the overlap of selected chemical features such as ring centroids and hydrogen bond donors and acceptors.[25] The alignments should not be interpreted to imply the precise arrangements shown, as the modeling is only intended to demonstrate that when steroids and *ent*-steroids are oriented as shown in Figure 2, the substituents that adversely affect activity are located along one edge of the aligned molecules and those that do not adversely affect activity are located on the opposite edge of the aligned molecules. Data are currently insufficient to interpret the alignments in a more precise manner.

CONCLUSION

On the basis of studies of newly prepared enantiomeric pairs of steroid analogues, we conclude that androsterone and *ent*-androsterone bind at the same sites on $GABA_A$ receptors. We further conclude that *ent*-androsterone is bound in an upside down orientation relative to androsterone at these sites. We also provide the first information describing the effects of C-7- or C-11-substituents on the GABAergic effects of *ent*-steroids. The results obtained will be useful for the design of new potentiators of $GABA_A$ receptors with a structure based on that of an *ent*-steroid.

EXPERIMENTAL SECTION

General Methods. Solvents were either used as purchased or dried and purified by standard methodology. Extraction solvents were dried with anhydrous Na_2SO_4 and, after filtration, removed on a rotary evaporator. Flash column chromatography was performed using silica gel (32−63 μm) purchased from Scientific Adsorbents (Atlanta, GA). Melting points were determined on a Kofler micro hot stage and are uncorrected. FT-IR spectra were recorded as films on a NaCl plate. NMR spectra were recorded in $CDCl_3$ at ambient temperature at 300 MHz (^{1}H) or 74 MHz (^{13}C). A purity of >95% was determined for all evaluated compounds by combustion analysis for C, H performed by M-H-W Laboratories (Phoenix, AZ). Steroids **8a** and dehydroepiandrosterone were purchased from Steraloids (Newport, RI). K(*s*-Bu)$_3$BH (K-Selectride) was purchased from Aldrich Chemical Co. (Milwaukee, WI).

(3α,5α,11β)-11-(Benzyloxy)-3-hydroxyandrostan-17-one (4). A mixture of steroid **10b** (242 mg, 0.5 mmol), MeOH (15 mL), and 6 N HCl (3 mL) was stirred at room temperature for 36 h. The MeOH was removed under reduced pressure, and the residual solution was extracted with EtOAc, dried, and concentrated to give a viscous liquid which was purified by flash column chromatography (silica gel eluted with 30−40% EtOAc in hexanes) to give crude product **4** (176 mg,

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89%). This crude product was acetylated (95% yield) in the standard manner (Ac_2O, pyridine, 4-(dimethylamino)pyridine (DMAP), room temperature) and purified by column chromatography to give the pure acetate derivative: mp 117−119 °C; IR ν_{max} 2927, 2856, 1736, 1452, 1359, 1260, 1244 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 5.00 (m, 1H), 4.65 (d, 1H, J = 11.3 Hz), 4.26 (d, 1H, J = 11.3 Hz), 4.01 (m, 1H), 2.55−2.35 (m, 2H), 2.04 (s, 3H), 1.08 (s, 3H), 1.01 (s, 3H); ^{13}C NMR δ 219.9, 170.5, 138.4, 128.1 (2C), 127.7(2C), 127.2, 74.6, 70.2, 69.8, 58.6, 53.2, 47.1, 40.8, 36.1, 35.3, 32.8, 32.7, 32.3, 31.3 (2C), 27.5, 25.7, 21.5, 21.4, 14.9, 14.3.

The acetate derivative was then hydrolyzed using K_2CO_3/MeOH to give product **4** (152 mg, 96% yield): mp 62−65 °C; $[\alpha]_D^{20}$ +82.9 (*c* 0.1, $CHCl_3$); IR ν_{max} 3436, 2922, 1738, 1452, 1354 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.65 (d, 1H, J = 11.3 Hz), 4.26 (d, 1H, J = 11.3 Hz), 4.03 (br s, 1H), 4.02 (br s, 1H), 2.51−2.37 (m, 2H), 1.07 (s, 3H), 0.99 (s, 3H); ^{13}C NMR δ 220.1, 138.5, 128.1 (2C), 127.6 (2C), 127.1, 74.7, 70.2, 66.2, 58.7, 53.2, 47.2, 39.9, 36.3, 35.3, 35.3, 32.9, 32.0, 31.5, 31.3, 28.6, 27.6, 21.5, 15.0, 14.2. Anal. ($C_{26}H_{36}O_3$) C, H.

(3*β*,5*β*,8*α*,9*β*,10*α*,11*α*,13*α*,14*β*)-11-(Benzyloxy)-3-hydroxyandrostan-17-one (*ent*-4). A mixture of compound **14d** (121 mg, 0.25 mmol), MeOH (15 mL), and 6 N HCl (3 mL) was stirred at room temperature for 36 h. The MeOH was removed under reduced pressure, and the residual solution was extracted with EtOAc, dried, and concentrated to give an oily liquid which was purified by flash column chromatography (silica gel eluted with 30−40% EtOAc in hexanes) to give product *ent*-**4** (92 mg, 92%): mp 62−64 °C; $[\alpha]_D^{20}$ −84.6 (*c* 0.08, $CHCl_3$); IR ν_{max} 3401, 2921, 1738, 1452, 1354 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.66 (d, 1H, J = 11.2 Hz), 4.27 (d, 1H, J = 11.2 Hz), 4.04 (br s, 1H), 4.03 (br s, 1H), 2.54−2.37 (m, 2H), 1.08 (s, 3H), 1.01 (s, 3H); ^{13}C NMR δ 220.1, 138.5, 128.1 (2C), 127.6 (2C), 127.1, 74.7, 70.2, 66.1, 58.7, 53.2, 47.1, 39.9, 36.3, 35.3, 35.2, 32.8, 32.0, 31.4, 31.3, 28.6, 27.6, 21.5, 14.9, 14.2. Anal. ($C_{26}H_{36}O_3$) C, H.

(3*α*,5*α*,11*β*,17*β*)-Spiro[11-(benzyloxy)androstane-17,2′-oxiran]-3-ol (5). Trimethylsulfonium iodide (40.5 mg, 0.2 mmol) followed by KO-*t*-Bu (16.5 mg, 0.15 mmol) was added to steroid **4** dissolved in DMF (4 mL), and the mixture was stirred at room temperature for 2.5 h. Brine was added, and the product was extracted into EtOAc (20 mL × 3). The combined extracts were dried and concentrated to give a viscous liquid which was purified by flash column chromatography (silica gel eluted with 30−40% EtOAc in hexanes) to give product **5** (25 mg, 61%): mp 144−146 °C; $[\alpha]_D^{20}$ +49.6 (*c* 0.05, $CHCl_3$); IR ν_{max} 3400, 2921, 2854, 1587, 1455, 1355 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.60 (d, 1H, J = 11 Hz), 4.17 (d, 1H, J = 11 Hz), 4.02 (br s, 1H), 3.96 (m, 1H), 2.90 (d, 1H, J = 4.9 Hz), 2.60 (d, 1H, J = 4.9 Hz), 1.11 (s, 3H), 0.98 (s, 3H); ^{13}C NMR δ 138.8, 128.1 (2C), 127.7 (2C), 127.1, 74.6, 71.1, 70.2, 66.4, 58.5, 54.7, 53.7, 40.1, 39.5, 36.3, 35.4, 35.1, 32.19, 32.09, 32.03, 28.8, 28.7, 27.8, 23.4, 15.7, 14.2. Anal. ($C_{27}H_{38}O_3$) C, H.

(3*β*,5*β*,8*α*,9*β*,10*α*,11*α*,13*α*,14*β*,17*α*)-Spiro[11-(benzyloxy)-androstane-17,2′-oxiran]-3-ol (*ent*-5). Compound *ent*-**4** (40 mg, 0.10 mmol) was converted into steroid *ent*-**5** using the procedure reported for the preparation of steroid **5**. Flash column chromatography (silica gel eluted with 20−35% EtOAc in hexanes) gave product *ent*-**5** (25 mg, 61%): mp 143−146 °C; $[\alpha]_D^{20}$ −44.5 (*c* 0.05, $CHCl_3$); IR ν_{max} 3369, 2921, 2853, 1595, 1454, 1357 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.61 (d, 1H, J = 11.3 Hz), 4.18 (d, 1H, J = 11.3 Hz), 4.03 (s, 1H) 3.97 (m, 1H), 2.91 (d, 1H, J = 5.0 Hz), 2.61 (d, 1H, J = 5.0 Hz), 1.11 (s, 3H), 0.98 (s, 3H); ^{13}C NMR δ 138.8, 128.1 (2C), 127.7 (2C), 127.1, 74.6, 71.1, 70.2, 66.5, 58.5, 54.7, 53.7, 40.1, 39.5, 36.3, 35.4, 35.1, 32.1, 32.0, 32.0, 28.8, 28.7, 27.8, 23.4, 15.7, 14.2. Anal. ($C_{27}H_{38}O_3$) C, H.

(3*α*,5*α*,7*β*)-7-(Benzyloxy)-3-hydroxyandrostan-17-one (6). Steroid **19b** (59 mg, 0.15 mmol) was dissolved in THF (5 mL) and cooled to −78 °C, and a 1 M solution of K-Selectride in THF (0.15 mL) was added. The reaction was stirred at −78 °C for 1.5 h and quenched by adding a few drops of water. Then 3 N aqueous NaOH (5 mL) followed by 30% H_2O_2 (5 mL) was added, and stirring was continued at room temperature for 1.5 h. The product was extracted with Et_2O (2 × 30 mL) followed by EtOAc (2 × 40 mL). The combined extracts were washed with brine, dried, and concentrated to give an off-white solid which was purified by flash column chromatography (silica gel eluted with 20−40% EtOAc in hexanes) to give product **6** (40 mg, 67%): mp 130−132 °C; $[\alpha]_D^{20}$ +131.5 (*c* 0.04, $CHCl_3$); IR ν_{max} 3400, 2928, 2858, 1736, 1453, 1361 cm^{-1}; ^1H NMR δ 7.40−7.22 (m, 5H), 4.62 (d, 1H, J = 11 Hz), 4.38 (d, 1H, J = 11 Hz), 4.08 (br s, 1H), 3.26 (m, 1H), 0.88 (s, 3H), 0.83 (s, 3H); ^{13}C NMR δ 221.7, 138.6, 128.4 (2C), 127.8 (2C), 127.5, 82.0, 70.2, 66.2, 53.2, 51.4, 48.4, 41.0, 36.1, 36.0, 35.7 (2C), 33.5, 32.2, 31.6, 29.1, 25.0, 20.3, 14.2, 11.3. Anal. ($C_{27}H_{36}O_3$) C, H.

(3*β*,5*β*,7*α*,8*α*,9*β*,10*α*,13*α*,14*β*)-7-(Benzyloxy)-3-hydroxyandrostan-17-one (*ent*-6). Compound **23** (105 mg, 0.27 mmol) was converted into compound *ent*-**6** using the procedure reported for the preparation of steroid **6**. Flash column chromatography (silica gel eluted with 20−40% EtOAc in hexanes) gave product *ent*-**6** (78 mg, 73%): mp 136−138 °C; $[\alpha]_D^{20}$ −135.5 (*c* 0.07); IR ν_{max} 3401, 2927, 2857, 1736, 1453, 1361 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.62 (d, 1H, J = 11 Hz), 4.38 (d, 1H, J = 11 Hz), 4.08 (br s, 1H), 3.26 (1H, m), 0.88 (s, 3H), 0.83 (s, 3H); ^{13}C NMR δ 221.8, 138.6, 128.4 (2C), 127.8 (2C), 127.5, 82.0, 70.2, 66.2, 53.2, 51.4, 48.4, 41.0, 36.14, 36.06, 35.7 (2C), 35.6, 33.5, 32.1, 31.6, 29.1, 25.0, 20.3, 14.2, 11.3. Anal. ($C_{27}H_{36}O_3$) C, H.

(3*α*,5*α*,7*β*,17*β*)-Spiro[7-(benzyloxy)androstane-17,2′-oxiran]-3-ol (7). Steroid **6** (60 mg, 0.15 mmol) was converted into steroid **7** using the procedure reported for the preparation of steroid **5**. Flash column chromatography (silica gel eluted with 20−35% EtOAc in hexanes) gave product **7** (35 mg, 57%): mp 146−148 °C; $[\alpha]_D^{20}$ +55.5 (*c* 0.06, $CHCl_3$); IR ν_{max} 3436, 2927, 2855, 1497, 1454, 1358, 1265 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.58 (d, 1H, J = 11 Hz), 4.37 (d, 1H, J = 11 Hz), 4.07 (br s, 1H), 3.18 (m, 1H), 2.90 (d, 1H, J = 5.2 Hz), 2.59 (d, 1H, J = 5.2 Hz), 0.89 (s, 3H), 0.82 (s, 3H); ^{13}C NMR δ 138.8, 128.3 (2C), 127.8 (2C), 127.3, 82.4, 70.2, 66.2, 53.7, 53.1, 52.8, 41.5, 40.9, 36.2 (2C), 35.7 (2C), 34.0, 33.6, 32.2, 29.4, 29.1, 26.6, 20.4, 14.7, 11.3. Anal. ($C_{27}H_{38}O_3$) C, H.

(3*β*,5*β*,7*α*,8*α*,9*β*,10*α*,13*α*,14*β*,17*α*)-Spiro[7-(benzyoxy)-androstane-17,2′-oxiran]-3-ol (*ent*-7). Compound *ent*-**6** (39 mg, 0.1 mmol) was converted into compound *ent*-**7** using the procedure reported for the preparation of steroid **5**. Flash column chromatography (silica gel eluted with % EtOAc in hexanes) gave product *ent*-**7** (28 mg, 68%): mp 145−148 °C; $[\alpha]_D^{20}$ −59.5 (*c* 0.05, $CHCl_3$); IR ν_{max} 3435, 2926., 2855, 1454, 1358, 1265 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.59 (d, 1H, J = 11 Hz), 4.37 (d, 1H, J = 11 Hz), 4.08 (br s, 1H), 3.18 (m, 1H), 2.91 (d, 1H, J = 5 Hz), 2.60 (d, 1H, J = 5 Hz), 0.89 (s, 3H), 0.82 (s, 3H); ^{13}C NMR δ 138.8, 128.3 (2C), 127.8 (2C), 127.4, 82.4, 70.2, 66.3, 53.7, 53.1, 52.8, 41.5, 40.9, 36.2 (2C), 35.7 (2C), 34.0, 33.6, 32.2, 29.5, 29.1, 26.6, 20.4, 14.7, 11.3. Anal. ($C_{27}H_{38}O_3$) C, H.

(3*α*,5*α*)-3-Hydroxyandrostane-11,17-dione (8b). Steroid **8a** (3.18 g, 10.4 mmol) was converted into compound **8b** using the procedure reported for the preparation of steroid **6**. After flash column chromatography (silica gel eluted with 20−40% EtOAc in hexanes) product **8b** (2.28, 72%) gave the following data: mp 146−149 °C; IR ν_{max} 3461, 2923, 1741, 1705, 1454, 1233 cm^{-1}; ^1H NMR δ 4.05 (br s, 1H), 1.02 (s, 3H), 0.82 (s, 3H); ^{13}C NMR δ 217.5, 209.0, 66.1, 64.9, 50.6 (2C), 50.4, 38.9, 36.1, 36.0, 35.9, 35.2, 31.3, 30.8, 28.8, 27.6, 21.5, 14.6, 11.0. Anal. ($C_{19}H_{28}O_3$) C,H.

(3*α*,5*α*)-3-Hydroxyandrostane-11,17-dione, Cyclic 17-(1,2-Ethanediyl acetal) (9a). A mixture of **8b** (609 mg, 2 mmol), ethylene glycol (1 mL), and *p*-toluenesulfonic acid (PTSA; 100 mg) in benzene (100 mL) was heated at reflux using a Dean−Stark apparatus for 16 h. The reaction mixture was cooled and basified with aqueous saturated $NaHCO_3$ and extracted with EtOAc (50 mL × 4). The combined extracts were dried and concentrated to give a white solid which was purified by flash column chromatography (silica gel eluted with 20−35% EtOAc in hexanes) to give product **9a** (488 mg, 70%): mp 92−95 °C; IR ν_{max} 3420, 2923, 1704, 1456, 1385, 1313, 1275, 1203 cm^{-1}; ^1H NMR δ 4.03 (br s, 1H), 4.00−3.78 (m, 4H), 2.62 (d, 1H, J = 11.5 Hz), 1.00 (s, 3H), 0.79 (s, 3H); ^{13}C NMR δ 211.3, 117.8, 66.2, 65.3, 64.5, 64.3, 50.2, 49.7, 49.5, 39.0, 37.1, 35.7, 35.3, 34.3, 31.9, 30.9, 28.8, 27.8, 22.1, 14.8, 10.9; HRMS (EI) m/z calcd for $C_{21}H_{32}O_4$ 348.2301, found 348.2296.

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(3α,5α)-3-[[(Methyloxy)methyl]oxy]androstane-11,17-dione, Cyclic 17-(1,2-Ethanediyl acetal) (9b). Steroid **9a** (452 mg, 1.3 mmol), chloromethyl methyl ether (0.53 mL, 7 mmol), *N*,*N*-diisopropylethylamine (1.74 mL, 10 mmol), and a catalytic amount of DMAP (40 mg) dissolved in CH_2Cl_2 (15 mL) were stirred at room temperature for 16 h. The reaction was poured into an aqueous saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (100 mL × 3). The combined extracts were dried and concentrated to give a viscous liquid which was purified by flash column chromatography (silica gel eluted with 20−25% EtOAc in hexanes) to give product **9b** (464 mg, 91%) as an oil: IR ν_{max} 2924, 1705, 1455, 1385, 1365, 1314, 1275, 1204 cm^{-1}; ^1H NMR δ 4.64 (dd, 2H, J = 8.5, 6.6 Hz), 3.98−3.75 (m, 4H), 3.35 (s, 3H), 2.61 (d, 1H, J = 11.8 Hz), 1.01 (s, 3H), 0.79 (s, 3H); ^{13}C NMR δ 211.0, 117.6, 94.4, 71.3, 65.2, 64.4, 64.2, 54.9, 50.0, 49.5, 49.4, 39.5, 36.9, 35.4, 34.2, 33.1, 31.7, 31.4, 27.7, 25.9, 22.0, 14.7, 11.0; HRMS (FAB) m/z calcd for $C_{23}H_{36}O_5Na$ 415.2460, found 415.2466.

(3α,5α,11β)-3-[[(Methyloxy)methyl]oxy]-11-hydroxyandrostan-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (10a). Steroid **9b** (392 mg, 1 mmol) dissolved in dry Et_2O was added to an ice cold, stirred suspension of LAH (114 mg, 3 mmol) in Et_2O. The reaction was warmed to room temperature, and stirring was continued for 2 h. Water (0.5 mL) was added, then a drop of aqueous 5 N NaOH (0.5 mL) was added, and the mixture was stirred for 0.5 h. Water (1 mL) was again added, and stirring was continued for another 0.5 h. The Et_2O was decanted, and the remaining solid was washed with Et_2O. The combined Et_2O solutions were dried and concentrated to give product **10a** as a liquid (315 mg, 80%): IR ν_{max} 3503, 2927, 1454, 1279, 1212 cm^{-1}; ^1H NMR δ 4.58 (dd, 2H, J = 6.3, 5.5 Hz), 4.31 (br s, 1H), 3.90−3.70 (m, 4H), 3.30 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H); ^{13}C NMR δ 119.3, 94.4, 71.3, 67.8, 64.9, 64.3, 57.8, 55.0, 51.8, 45.0, 40.4, 39.5, 35.9, 33.9, 32.9, 32.4, 31.5, 31.3, 27.8, 25.9, 22.5, 16.8, 14.3. Anal. ($C_{23}H_{38}O_5$) C, H.

(3α,5α,11β)-11-(Benzyloxy)-3-[[(methyloxy)methyl]oxy]-androstan-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (10b). KH (400 mg, 40% in mineral oil, 4 mmol) and then benzyl bromide (0.31 mL, 2.5 mmol) were added to steroid **10a** dissolved in THF (10 mL), and the mixture was refluxed for 2 h. The reaction was cooled to 0 °C, and the excess KH was carefully destroyed by MeOH (5 mL) addition. Water (20 mL) was then added, and the product was extracted with EtOAc. The EtOAc was washed with brine, dried, and concentrated to give a residue which was purified by flash column chromatography (silica gel eluted with 20−35% EtOAc in hexanes) to give product **10b** as a liquid (294 mg, 75%): IR ν_{max} 2926, 1454, 1279, 1210 cm^{-1}; ^1H NMR δ 7.40−7.18 (m, 5H), 4.70−4.52 (m, 3H), 4.20 (d, 1H, J = 11.3 Hz), 4.00−3.70 (m, 5H), 3.35 (s, 3H), 1.07 (s, 3H), 0.98 (s, 3H); ^{13}C NMR δ 139.0, 128.0 (2C), 127.5 (2C), 126.9, 119.4, 94.4, 75.1, 71.5, 70.1, 65.0, 64.3, 58.2, 55.0, 52.1, 45.3, 40.5, 36.0, 33.9, 33.0, 32.6, 31.9, 31.8, 31.6, 27.9, 26.0, 22.5, 15.7, 14.4; HRMS (FAB) m/z calcd for $C_{30}H_{44}O_5Na$ 507.3086, found 507.3083.

(5β,8α,10α,13α,14β,17α)-17-Hydroxyandrost-9(11)-en-3-one (12a). NH_3 (150 mL) was condensed into a cold (−78 °C) two-necked flask (250 mL) fitted with a Dewar condenser. Toluene (15 mL) and THF (20 mL) were added. Lithium (294 mg, 42.3 mmol), in small pieces, was added to the solution, and the mixture was stirred for 10 min, resulting in the formation of a deep blue colored solution. Compound **11**[17] (2.4 g, 8.45 mmol) in THF (20 mL) was added, and the mixture was stirred at −78 °C for 45 min. Solid ammonium chloride (6 g) was added, and the ammonia was allowed to evaporate. Water (200 mL) was added, and the reaction mixture was extracted with EtOAc (100 mL × 3). The combined EtOAc extracts were washed with brine, dried, and concentrated to give a white solid which was purified by flash column chromatography (silica gel eluted with 20−35% EtOAc in hexanes) to give product **12a** (1.97 g, 81%): mp 166−168 °C; IR ν_{max} 3391, 2921, 2869, 1713, 1596, 1445, 1374, 1271 cm^{-1}; ^1H NMR δ 5.41 (m, 1H), 3.74 (t, 1H, J = 9.1 Hz), 1.16 (s, 3H), 0.72 (s, 3H); ^{13}C NMR δ 211.6, 146.1, 116.7, 81.9, 48.4, 45.0, 44.6, 41.4, 38.3, 38.1, 37.8, 37.0, 36.9, 32.2, 30.7, 28.7, 24.2, 17.3, 10.5. Anal. ($C_{19}H_{28}O_2$) C, H.

(5β,8α,10α,13α,14β)-Androst-9(11)-ene-3,17-dione (12b). Compound **12a** (1.9 g, 6.6 mmol) was dissolved in acetone (50 mL) and stirred. Jones reagent was added dropwise until the orange color persisted, and stirring was continued for 1 h. The excess Jones reagent was quenched by adding a few drops of isopropyl alcohol, and the acetone was removed under reduced pressure to give a green liquid. Water was added, and the resulting solution was extracted with EtOAc. The EtOAc was dried and removed to give a white solid. Flash column chromatography (silica gel eluted with 20−35% EtOAc in hexanes) yielded product **12b** (1.73 g, 92%): mp 147−148 °C; IR ν_{max} 2925, 2852, 1739, 1713, 1595, 1450, 1404, 1370, 1244, 1210 cm^{-1}; ^1H NMR δ 5.43 (m, 1H), 1.18 (s, 3H), 0.85 (s, 3H); ^{13}C NMR δ 221.4, 211.1, 146.4, 116.2, 48.8, 45.9, 44.8, 44.6, 38.1, 36.9, 36.2 (3C), 33.3, 31.7, 28.5, 22.7, 17.3, 13.8. Anal. ($C_{19}H_{26}O_2$) C, H.

(3β,5β,8α,10α,13α,14β)-3-Hydroxyandrost-9(11)-en-17-one (12c). Compound **12b** (1.69 g, 5.94 mmol) was converted into compound **12c** using the procedure reported for the preparation of steroid **6**. After flash column chromatography (silica gel eluted with 20−40% EtOAc in hexanes) product **12c** (1.1 g, 65%) gave the following data: mp 186−188 °C; IR ν_{max} 3401, 2921, 2854, 1738, 1596, 1451, 1370, 1279, 1211 cm^{-1}; ^1H NMR δ 5.38 (m, 1H), 4.06 (br s, 1H), 0.94 (s, 3H), 0.82 (s, 3H); ^{13}C NMR δ 222.1, 148.0, 114.7, 66.3, 49.1, 46.0, 38.6, 37.7, 36.3, 36.2, 36.1, 33.3, 32.2, 30.6, 29.1, 28.1, 22.7, 17.0, 13.8. Anal. ($C_{19}H_{28}O_2$) C, H.

(3β,5β,8α,10α,13α,14β)-3-Hydroxyandrost-9(11)-en-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (13a). Compound **12c** (1 g, 3.49 mmol) was converted into compound **13a** using the procedure reported for the preparation of steroid **9a** (except that pyridinium *p*-toluenesulfonate (PPTS) was used as the catalyst instead of PTSA). Flash column chromatography (silica gel eluted with 20−40% EtOAc in hexanes) gave product **13a** (1 g, 87%): mp 138−140 °C; IR ν_{max} 3350, 2968, 2920, 2873, 1595, 1454, 1374, 1308, 1274, 1204 cm^{-1}; ^1H NMR δ 5.35 (m, 1H), 4.04 (br s, 1H), 4.00−3.80 (m, 4H), 2.38 (d, 1H, J = 16.5 Hz), 0.92 (s, 3H), 0.78 (s, 3H); ^{13}C NMR δ 147.0, 119.2, 115.4, 66.4, 65.1, 64.5, 48.1, 44.2, 38.4, 37.9, 37.2, 36.2, 34.0, 32.6, 32.2, 30.7, 29.1, 28.3, 23.6, 16.9, 14.1; HRMS (EI) m/z calcd for $C_{21}H_{32}O_3$ 332.2351, found 332.2352.

(3β,5β,8α,10α,13α,14β)-3-[[(Methyloxy)methyl]oxy]androst-9(11)-en-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (13b). Compound **13a** (1 g, 3 mmol) was converted into compound **13b** using the procedure reported for the preparation of steroid **9b**. Flash column chromatography (silica gel eluted with 15−25% EtOAc in hexanes) gave product **13b** (1.1 g, 97%): mp 73−75 °C; IR ν_{max} 2968, 2923, 1595, 1454, 1374, 1308, 1275, 1207 cm^{-1}; ^1H NMR δ 5.33 (d, 1 H, J = 6.1 Hz), 4.65 (dd, 2H, J = 9.1, 6.9 Hz), 3.96−3.80 (m, 4H), 3.36 (s, 3H), 2.37 (d, 1H, J = 16.2 Hz), 0.93 (s, 3H), 0.79 (s, 3H); ^{13}C NMR δ 147.1, 119.2, 115.3, 94.5, 71.5, 65.1, 64.5, 55.1, 48.1, 44.3, 28.4, 38.2, 37.2, 34.0, 33.9, 32.56, 32.2, 31.4, 28.4, 26.5, 23.6, 17.2, 14.1. Anal. ($C_{23}H_{36}O_4$) C, H.

(3β,5β,8α,9β,10α,11β,13α,14β)-3-[[(Methyloxy)methyl]oxy]-11-hydroxyandrostan-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (14a). Compound **13b** was dissolved in THF (10 mL) and stirred at 0 °C. A 1 M borane−THF complex in THF (3 mL, 3 mmol) was added, and the reaction was stirred at room temperature for 8 h. The reaction was cooled to 0 °C, and excess borane complex was carefully quenched with a few drops of water. Aqueous NaOH (3 N, 10 mL) followed by 30% H_2O_2 (10 mL) was added, and the reaction was stirred at room temperature for 16 h. The mixture was extracted with Et_2O (3 × 75 mL) followed by EtOAc (2 × 50 mL). The combined extracts were dried and concentrated to give an oil which was purified by flash column chromatography (silica gel eluted with 20−40% EtOAc in hexanes) to give product **14a** as a colorless liquid (351 mg, 67%): IR ν_{max} 3435, 2923, 1595, 1457, 1380, 1312, 1282, 1212 cm^{-1}; ^1H NMR δ 4.59 (dd, 2H, J = 10.7, 6.6 Hz), 3.93−3.68 (m, 5H), 3.30 (s, 3H), 0.88 (s, 3H), 0.78 (s, 3H); ^{13}C NMR δ 118.7, 94.2, 71.1, 69.2, 65.0, 64.4, 60.4, 54.9, 49.3, 46.2, 42.5, 39.5, 37.6, 34.9, 34.5, 34.1, 34.1, 31.1, 28.9, 26.2, 22.5, 15.0, 11.7. Anal. ($C_{23}H_{38}O_5$) C, H.

(3β,5β,8α,9β,10α,13α,14β)-3-[[(Methyloxy)methyl]oxy]-androstane-11,17-dione, Cyclic 17-(1,2-Ethanediyl acetal) (14b). Compound **14a** (250 mg, 0.63 mmol) was dissolved in stirred

CH_2Cl_2 (8 mL), solid NaOAc (154 mg, 1.89 mmol) and pyridinium chlorochromate (PCC; 407.4 mg, 1.89 mmol) were added, and the reaction was stirred at room temperature for 3 h. The CH_2Cl_2 solution was directly purified by flash column chromatography (silica gel eluted with 10−35% EtOAc in hexanes) to give product **14b** (237 mg, 96%) as an oil: IR ν_{max} 2924, 1705, 1594, 1456, 1385, 1204 cm^{-1}; ^1H NMR δ 4.63 (dd, 2H, J = 8.2, 6.9 Hz), 3.94−3.70 (m, 5H), 3.32 (s, 3H), 2.57 (d, 1H, J = 12 Hz), 0.98 (s, 3H), 0.75 (s, 3H); ^{13}C NMR δ 211.4, 117.7, 94.4, 71.4, 65.2, 64.5, 64.2, 55.0, 50.1, 49.6, 49.5, 39.6, 37.0, 35.5, 34.3, 33.2, 31.8, 31.5, 27.8, 25.9, 22.1, 14.8, 11.1. Anal. ($C_{23}H_{36}O_5$) C, H.

(3β,5β,8α,9β,10α,11α,13α,14β)-3-[[(Methyloxy)methyl]oxy]-11-hydroxyandrostan-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (14c). Compound **14b** (208 mg, 0.53 mmol) was converted into compound **14c** using the procedure reported for the preparation of steroid **10a**. The reaction time was 3 h. Flash column chromatography (silica gel eluted with 35% EtOAc in hexanes) gave product **14c** (199 mg, 95%) as an oil: IR ν_{max} 3504, 2925, 1455, 1279, 1212 cm^{-1}; ^1H NMR δ 4.58 (dd, 2H, J = 8.0, 6.6 Hz), 4.31 (br s), 3.90−3.70 (m, 4H), 3.29 (s, 3H), 1.01 (s, 3H), 0.96 (s, 3H); ^{13}C NMR δ 119.4, 94.4, 71.3, 67.9, 65.0, 64.4, 57.8, 55.0, 51.8, 45.1, 40.4, 39.6, 35.9, 33.9, 32.9, 32.4, 31.6, 31.3, 27.8, 25.9, 22.6, 16.8, 14.3; HRMS (FAB) m/z calcd for $C_{23}H_{38}O_5Na$ 417.2617, found 417.2617.

(3β,5β,8α,9β,10α,11α,13α,14β)-11-(Benzyloxy)-3-[[(Methyloxy)methyl]oxy]androstan-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (14d). Compound **14c** (200 mg, 0.51 mmol) was converted into compound **14d** using the procedure reported for the preparation of steroid **10b**. Flash column chromatography (silica gel eluted with 20−35% EtOAc in hexanes) gave product **14d** (170 mg, 69%) as an oil: IR ν_{max} 2924, 1454, 1278 cm^{-1}; ^1H NMR δ 7.40−7.18 (m, 5H), 4.70−4.52 (m, 3H), 4.20 (d, 1H, J = 11.2 Hz), 4.02−3.69 (m, 5H), 3.36 (s, 3H), 1.06 (s, 3H), 0.98 (s, 3H); ^{13}C NMR δ 139.0, 128.0 (2C), 127.6 (2C), 127.0, 119.5, 94.5, 75.2, 71.5, 70.1, 65.1, 64.4, 58.2, 55.1, 52.1, 45.3, 40.6, 36.0, 34.0, 33.1, 32.7, 31.9, 31.9, 31.7, 27.9, 26.0, 22.6, 15.8, 14.4; HRMS (FAB) m/z calcd for $C_{30}H_{44}O_5Na$ 507.3087, found 507.3091.

(3β)-3-[[(Methyloxy)methyl]oxy]androst-5-en-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (16). Steroid **15**[26] (3 g, 9 mmol) was converted into steroid **16** using the procedure reported for the preparation of steroid **9b**. Flash column chromatography (silica gel eluted with 15−20% EtOAc in hexanes) gave product **16** (3.1 g, 91%): mp 94−96 °C; IR ν_{max} 2926, 2893, 2823, 1667, 1466, 1437, 1381, 1305, 1277 cm^{-1}; ^1H NMR δ 5.33 (d, 1H, J = 5.2 Hz), 4.66 (s, 2H), 4.00−3.78 (m, 4H), 3.36 (m, 1H), 3.34 (s, 3H), 0.99 (s, 3H), 0.83 (s, 3H); ^{13}C NMR δ 140.6, 121.4, 119.4, 94.6, 76.8, 65.1, 64.5, 55.1, 50.5, 49.9, 45.6, 39.5, 37.2, 36.7, 34.1, 32.1, 31.2, 30.5, 28.8, 22.7, 20.4, 19.3, 14.1. Anal. ($C_{23}H_{36}O_4$) C, H.

(3β)-3-[[(Methyloxy)methyl]oxy]androst-5-ene-7,17-dione, Cyclic 17-(1,2-Ethanediyl acetal) (17). A mixture of steroid **16** (2.26 g, 6 mmol), 70% aqueous *t*-BuOOH (4.32 mL, 30 mmol), and $NaOCl_2$ (813 mg, 7.2 mmol) in premixed acetonitrile (60 mL) and water (20 mL) was heated at 50 °C for 36 h. The reaction was cooled, poured into aqueous 10% Na_2SO_3 (100 mL), and stirred for 0.5 h. The product was extracted into Et_2O (100 mL × 4), and the combined extracts were washed with brine, dried, and concentrated to give a viscous liquid which was purified by flash column chromatography (silica gel eluted with 15−25% EtOAc in hexanes) to yield recovered steroid **16** (450 mg, 20%) and product **17** (800 mg, 34%): mp 136−138 °C; IR ν_{max} 2946, 2880, 1668, 1629, 1461, 1382, 1295, 1215 cm^{-1}; ^1H NMR δ 5.70 (s, 1H), 4.69 (dd, 2H, J = 8.0, 6.9 Hz), 4.00−3.80 (m, 4H), 3.56 (m, 1H), 3.38 (s, 3H), 1.20 (s, 3H), 0.87 (s, 3H); ^{13}C NMR δ 201.2, 165.3, 125.9, 118.5, 94.9, 75.5, 65.1, 64.3, 55.2, 49.8, 46.0, 45.2, 44.3, 39.3, 38.3, 36.2, 34.0, 29.5, 28.5, 25.0, 20.5, 17.2, 14.3. Anal. ($C_{23}H_{34}O_5$) C, H.

(3β,5α)-3-[[(Methyloxy)methyl]oxy]androstane-7,17-dione, Cyclic 17-(1,2-Ethanediyl acetal) (18a). Steroid **17** (586 mg, 1.5 mmol), 10% Pd/C (300 mg), and EtOAc (80 mL) were placed in a Parr hydrogenation flask and hydrogenated (50 psi) for 18 h. The Pd/C was filtered under vacuum through a pad of Celite 454 on a sintered glass funnel. The filter cake was washed with EtOAc. The combined filtrate and washings were concentrated to give an off-white solid which was purified by flash column chromatography (silica gel eluted with 15−25% ethyl acetate in hexanes) to give product **18a** (471 mg, 80%): mp 117−119 °C; IR ν_{max} 2944, 2882, 1708, 1448, 1379, 1292, 1223 cm^{-1}; ^1H NMR δ 4.67 (s, 2H), 4.00−3.76 (m, 4H), 3.50 (m, 1H), 3.36 (s, 3H), 1.08 (s, 3H), 0.84 (s, 3H); ^{13}C NMR δ 210.9, 118.6, 94.7, 75.7, 65.2, 64.4, 55.1, 50.0, 46.4, 45.9, 45.7, 43.4, 36.2, 36.0, 35.2, 34.1, 29.7, 28.4, 23.8, 21.3, 14.4, 11.7. Anal. ($C_{23}H_{36}O_5$) C, H.

(3β,5α)-7-Hydroxy-3-[[(methyloxy)methyl]oxy]androstan-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (18b). Steroid **18a** (498 mg, 1.27 mmol) was converted into an inseparable mixture of the 7α- and 7β-hydroxysteroids **18b** using the procedure reported for the preparation of steroid **10a**. Filtration through a short column of silica gel eluted with 40% EtOAc in hexanes gave product **18b** (431 mg, 86%), which was then converted without characterization into the separable steroids **18c** and **18d**.

(3β,5α,7β)-7-(Benzyloxy)-3-[[(methyloxy)methyl]oxy]-androstan-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (18c) and (3β,5α,7α)-7-(Benzyloxy)-3-[[(methyloxy)methyl]oxy]-androstan-17-one, Cyclic 17-(1,2-Ethanediyl acetal) (18d). Steroid **18b** (431 mg, 1.09 mmol) was converted into products **18c** and **18d** using the procedure reported for the benzylation of steroid **10b**. Flash column chromatography (silica gel eluted with 10−35% EtOAc in hexanes) gave product **18c** (120 mg, 23%), product **18d** (240 mg, 45%), and recovered steroid **18b** (120 mg, 23%). Product **18d** eluted from the column before product **18c**. Data for **18c**: oil; IR ν_{max} 2936, 1454, 1307 cm^{-1}; ^1H NMR δ 7.40−7.19 (m, 5H), 4.68 (s, 2H), 4.55 (d, 1H, J = 11 Hz), 4.34 (d, 1H, J = 11 Hz), 4.00−3.79 (m, 4H), 3.49 (m, 1H), 3.37 (s, 3H), 3.13 (m, 1H), 0.86 (s, 3H), 0.84 (s, 3H); ^{13}C NMR δ 138.9, 128.3 (2C), 127.9 (2C), 127.3, 119.0, 94.6, 82.7, 76.1, 70.4, 65.2, 64.5, 55.1, 52.9, 50.2, 46.7, 42.0, 41.5, 37.0, 35.2, 35.1, 34.3, 33.8, 30.4, 28.7, 25.4, 20.9, 14.7, 12.4. Anal. ($C_{30}H_{44}O_5$) C, H. Data for **18d**: oil; IR ν_{max} 2935, 1453, 1306 cm^{-1}; ^1H NMR δ 7.43−7.20 (m, 5H), 4.68 (s, 2H), 4.57 (d, 1H, J = 12 Hz), 4.32 (d, 1H, J = 11.8 Hz), 3.97−3.76 (m, 4H), 3.48 (m, 1H), 3.46 (br s, 1H), 3.37 (s, 3H), 0.81 (s, 3H), 0.81 (s, 3H); ^{13}C NMR δ 139.5, 128.2 (2C), 127.4 (2C), 127.2, 119.4, 94.6, 76.4, 74.5, 70.4, 65.1, 64.6, 55.1, 46.3, 46.0, 44.2, 40.4, 37.2, 36.8, 35.7, 35.0, 34.3, 31.6, 30.4, 28.7, 22.4, 20.6, 14.1, 11.4. Anal. ($C_{30}H_{44}O_5$) C, H.

(3β,5α,7β)-7-(Benzyloxy)-3-hydroxyandrostan-17-one (19a). Steroid **18c** (120 mg, 0.25 mmol) was converted into steroid **19a** using the procedure reported for the preparation of compound *ent*-**4**. Flash column chromatography (silica gel eluted with 30−40% EtOAc in hexanes) gave product **19a** (90 mg, 91%): mp 175−177 °C; IR ν_{max} 3447, 2930, 2855, 1735, 1497, 1453, 1405, 1362, 1202 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.64 (d, 1H, J = 11 Hz), 4.38 (d, 1H, J = 11 Hz), 3.60 (m, 1H), 3.23 (m, 1H), 0.87 (s, 3H), 0.86 (s, 3H); ^{13}C NMR δ 221.6, 138.5, 128.4 (2C), 127.7 (2C), 127.5, 81.9, 70.9, 70.2, 53.0, 51.3, 48.4, 41.8, 40.9, 37.9, 36.9, 36.1, 35.2, 33.6, 31.6, 31.4, 24.9, 20.8, 14.1, 12.4. Anal. ($C_{26}H_{36}O_3$) C, H

(5α,7β)-7-(Benzyloxy)androstane-3,17-dione (19b). Steroid **19a** (71 mg, 0.18 mmol) was converted into compound **19b** using the procedure reported for the preparation of steroid **14b**. Flash column chromatography (silica gel eluted with 25% EtOAc in hexanes) gave product **19b** (61 mg, 86%): mp 181−183 °C; IR ν_{max} 2945, 2921, 2856, 1733, 1716, 1454, 1367, 1289, 1257, 1227 cm^{-1}; ^1H NMR δ 7.40−7.24 (m, 5H), 4.63 (d, 1H, J = 11 Hz), 4.40 (d, 1H, J = 11 Hz), 3.26 (m, 1H), 1.07 (s, 3H), 0.90 (s, 3H); ^{13}C NMR δ 221.2, 210.9, 138.3, 128.4 (2C), 127.8 (2C), 127.6, 81.5, 70.5, 52.2, 51.2, 48.3, 44.2, 43.7, 40.8, 38.2, 37.9, 36.0, 35.3, 33.8, 31.5, 24.9, 21.2, 14.1, 11.6. Anal. ($C_{26}H_{34}O_3$) C, H.

(8α,9β,10α,13α,14β)-Androst-5-ene-3,17-dione, Cyclic 3,17-Bis(1,2-Ethanediyl acetal) (21a). Compound **20**[18] (1.6 g, 5.6 mmol) was converted into compound **21a** using the procedure reported for the preparation of compound **9a**. Flash column chromatography (silica gel eluted with 15−25% EtOAc in hexanes) gave product **21a** (1.74 g, 83%): mp 158−160 °C; IR ν_{max} 2943, 2885, 1431, 1381, 1305 cm^{-1}; ^1H NMR δ 5.34 (m, 1H), 4.00−3.80 (m, 8H), 1.04 (s, 3H), 0.86 (s, 3H); ^{13}C NMR δ 140.0, 121.8, 119.3, 109.3, 65.0,

64.4, 64.3, 64.1, 50.4, 49.4, 45.6, 41.7, 36.6, 36.2, 34.1, 32.1, 31.0, 30.9, 30.5, 22.7, 20.3, 18.7, 14.1. Anal. ($C_{23}H_{34}O_4$) C, H.

(8α,9β,10α,13α,14β)-Androst-5-ene-3,7,17-trione, Cyclic 3,17-Bis(1,2-Ethanediyl acetal) (21b). Compound **21a** (1.73 g, 4.62 mmol) was converted into compound **21b** using the procedure reported for the preparation of steroid **17**. Flash column chromatography (silica gel eluted with 15−25% EtOAc in hexanes) gave recovered compound **21a** (500 mg, 28%) and product **21b** (560 mg, 31%): mp 198−200 °C; IR ν_{max} 2953, 2882, 1672, 1634, 1457, 1383, 1295 cm^{-1}; ^1H NMR δ 5.66 (s, 1H), 4.03−3.78 (m, 8H), 2.69 (d, 1H, J = 14.8 Hz), 1.22 (s, 3H), 0.88 (s, 3H); ^{13}C NMR δ 201.2, 164.7, 126.5, 118.6, 108.8, 65.1, 64.5, 64.5, 49.5, 46.2, 45.3, 44.4, 41.7, 38.2, 35.5, 34.1, 31.0, 29.6, 25.1, 20.6, 17.0, 14.4. Anal. ($C_{23}H_{32}O_5$) C, H.

(5β,8α,9β,10α,13α,14β)-Androstane-3,7,17-trione, Cyclic 3,17-Bis(1,2-Ethanediyl acetal) (22a). Compound **21b** (500 mg, 1.28 mmol) was converted into compound **22a** using the procedure reported for the preparation of steroid **18a**. Flash column chromatography (silica gel eluted with 20−30% EtOAc in hexanes) gave product **22a** (380 mg, 76%): mp 208−210 °C; IR ν_{max} 2952, 2880, 1705, 1456, 1386, 1293 cm^{-1}; ^1H NMR δ 4.00−3.70 (m, 8H), 1.08 (s, 3H), 0.84 (s, 3H); ^{13}C NMR δ 210.8, 118.6, 108.6, 65.1, 64.4, 64.3, 64.1, 54.7, 50.0, 45.7, 45.6, 45.2, 43.4, 37.7, 35.9, 35.2, 34.1, 31.1, 29.7, 23.8, 21.3, 14.4, 10.9. Anal. ($C_{23}H_{34}O_5$) C, H.

(5β,8α,9β,10α,13α,14β)-7-Hydroxyandrostane-3,17-dione, Cyclic 3,17-Bis(1,2-Ethanediyl acetal) (22b). Compound **22a** (140 mg, 0.36 mmol) dissolved in 2-propanol (30 mL) and Raney nickel were placed in a Parr hydrogenation flask and hydrogenated (H_2, 60 psi) for 18 h. The Raney nickel was removed by filtration under vacuum through Celite 454 in a sintered glass funnel. The Celite 454 was washed with methanol, and the filtrate was evaporated to yield a solid product which was purified by flash column chromatography (silica gel eluted with 40% EtOAc in hexanes) to give the inseparable mixture of 7α- and 7β-alcohol products **22b** (136 mg, 96%). This product was not characterized.

(5β,7β,8α,9β,10α,13α,14β)-7-(Benzyloxy)androstane-3,17-dione, Cyclic 3,17-Bis(1,2-Ethanediyl acetal) (22c) and (5β,7α,8α,9β,10α,13α,14β)-7-(Benzyloxy)androstane-3,17-dione, Cyclic 3,17-Bis(1,2-Ethanediyl acetal) (22d). The inseparable epimeric products **22b** (130 mg, 0.33 mmol) were converted into separable compounds **22c** (35 mg, 22%) and **22d** (80 mg, 50%) using the procedure reported for the preparation of steroids **18c** and **18d** from the inseparable 7α- and 7β-alcohols **18b**. Data for **22c**: mp 141−143 °C; IR ν_{max} 2943, 2872, 1496, 1456, 1362, 1306, 1286, 1245 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.57 (d, J = 12.1 Hz), 4.33 (d, J = 12.4 Hz), 3.96−3.78 (m, 8H), 3.45 (br s, 1H), 0.81 (s, 6H); ^{13}C NMR δ 139.7, 128.1 (2C), 127.4 (2C), 127.0, 119.4, 109.2, 74.5, 70.4, 65.0, 64.5, 64.0 (2C), 45.9, 45.9, 44.1, 40.3, 37.6, 36.1, 35.7, 35.5, 34.2, 31.4, 31.2, 30.4, 22.3, 20.5, 14.0, 10.5. Anal. ($C_{30}H_{42}O_5$) C, H. Data for **22d**: mp 145−147 °C; IR ν_{max} 2943, 2871, 1586, 1454, 1361, 1307, 1205 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.54 (d, 1H, J = 11 Hz), 4.34 (d, 1H, J = 11 Hz), 3.96−3.80 (m, 8H), 3.15 (m, 1H), 0.86 (s, 3H), 0.84 (s, 3H); ^{13}C NMR δ 138.8, 128.3 (2C), 127.8 (2C), 127.7, 118.9, 109.1, 82.7, 70.4, 65.1, 64.5, 64.1 (2C), 52.4, 50.4, 46.6, 41.4, 40.8, 37.8, 35.9, 35.1, 34.3, 33.6, 31.1, 30.4, 25.4, 20.9, 14.7, 11.5. Anal. ($C_{30}H_{42}O_5$) C, H.

(5β,7α,8α,9β,10α,13α,14β)-7-(Benzyloxy)androstane-3,17-dione (23). Compound **22d** (193 mg, 0.4 mmol) was converted into compound **23** using the procedure reported for the preparation of steroid **4**. Flash column chromatography (silica gel eluted with 30−40% EtOAc in hexanes) gave product **23** (140 mg, 89%): mp 183−185 °C; IR 2921, 2855, 1733, 1716, 1453, 1367 cm^{-1}; ^1H NMR δ 7.40−7.20 (m, 5H), 4.63 (d, 1H, J = 10.7 Hz), 4.40 (d, 1H, J = 11 Hz), 3.26 (m, 1H), 1.07 (s, 3H), 0.91 (s, 3H); ^{13}C NMR 221.2, 210.9, 138.3, 128.4 (2C), 127.8 (2C), 127.6, 81.4, 70.4, 52.2, 51.2, 48.3, 44.2, 43.7, 40.8, 38.2, 37.9, 36.0, 35.3, 33.8, 31.5, 24.9, 21.2, 14.1, 11.6. Anal. ($C_{26}H_{34}O_3$) C, H.

[^{35}S]TBPS Binding Methods. The methods used were described previously.[27]

***Xenopus* Oocyte Electrophysiological Methods.** cRNA encoding rat $GABA_A$ receptor subunits was injected into stage V−VI oocytes harvested from sexually mature female *X. laevis* frogs (Xenopus 1, Northland, MI). The frogs were anesthetized with 0.1% tricane (3-aminobenzoic acid ethyl ester) and oocytes obtained by partial ovariectomy. The follicular layer was removed from the oocytes by shaking for 20 min at 37 °C in 2 mg/mL collagenase dissolved in a calcium-free solution containing 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 5 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES), pH 7.4. Capped RNA for α_1, β_2, and γ_{2L} subunits was prepared in vitro (mMESSAGE mMachine kit, Ambion, Austin, TX) from linearized pBluescript vectors containing subunit coding regions. The α_1 Q241L mutation was introduced as described previously.[20] Subunit RNA was injected in equal parts (20−40 ng of total RNA), after which the oocytes were incubated at 18 °C in ND96 solution containing 96 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.4. ND96 was supplemented with pyruvate (5 mM), penicillin (100 U/mL), streptomycin (100 μg/mL), and gentamycin (50 μg/mL).

Oocyte responses were recorded 2−5 days following RNA injection. GABA currents were recorded using a two-electrode voltage clamp (OC725C amplifier, Warner Instruments) at a membrane potential of −70 mV. The bath solution was ND96 solution, and glass recording pipets (~1 MΩ resistance) were filled with 3 M KCl. Compounds were applied to the oocytes using a multibarrel pipet with a common output tip. Data acquisition and analysis were performed with pCLAMP software (Molecular Devices, Sunnyvale, CA). Statistical differences were assessed with one-tailed or two-tailed paired or unpaired t tests as indicated.

Tadpole Behavioral Methods. The methods used were described previously.[27]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table of elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

GABA, γ-aminobutyric acid; $GABA_A$, γ-aminobutyric acid type A; [^{35}S]TBPS, [^{35}S]-*tert*-butylbicyclophosphorothionate; SAR, structure−activity relationship; LRR, loss of righting reflex; LSR, loss of swimming reflex

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