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

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Abstract

The enantiomer pair androsterone and *ent*-androsterone are positive allosteric modulators of γ -aminobutyric acid type A (GABA_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. 11 β -OBn substituted steroids and 7 α -OBn substituted *ent*-steroids potently displace [³⁵S]TBPS, augment GABA currents and anesthetize tadpoles. In contrast, 7 β -OBn substituted steroids and 11 α -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 7 α and 11 β substituents similarly located on the edges of the molecules not in contact with the receptor surface. Analogue potentiation of 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 like allopregnanolone (**1**) and androsterone (**2**) as well as other endogenous 3 α -hydroxysteroids and synthetic analogues potentiate GABA-mediated chloride currents at 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

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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 (SAR) for steroid potentiation.^{1,10,11} For example, highly active steroids nearly always contain a 3 α -hydroxyl group on the steroid A-ring and, for the most active compounds, a 17 β -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 SAR at this class of receptors.¹²⁻¹⁴

In this study, we investigate SAR 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 like 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 $\alpha_1\beta_2\gamma_2\delta$ 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 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 3 α -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 Discussion for 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 11 β -OBn and an 11 α -Me substituent is a strong potentiator at GABA_A receptors and steroid analogues with either a 7 α - or 7 β -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 11 α -substituents in *ent*-**4** and *ent*-**5** will be located proximate to the 7 β -substituents in steroids **6** and **7**, and *ent*-**4** and *ent*-**5** are predicted to be inactive. Conversely, the 7 α -substituents in analogues *ent*-**6** and *ent*-**7** will be located proximate to the 11 β -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 non-competitively displace [³⁵S]-*t*-butylbicyclophosphorothionate ([³⁵S]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 11 β -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 7 β -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 receptors⁸.

Chemistry

The synthesis of analogues **4** and **5** is summarized in Scheme 1. The starting material was purchased triketone **8a**. Regio- and stereoselective reduction of the 3-ketone using K(*sec*-Bu)₃BH yielded after workup predominately the 3 α -hydroxysteroid **8b** (72%). The 17-ketone was then selectively protected as the cyclic ketal in the standard manner (**9a**, 70%), and the 3 α -OH group was protected with a MOM group (**9b**, 91%). The 11-ketone is then reduced with LAH to yield the 11 β -OH of steroid **10a** (80%), and the 11 β -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 not easily removed. Acetylation of the 3 α -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 (CH₃)₃SI, KO^tBu in 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 this Scheme, and synthetic Schemes 2-4, was assigned based on well-known literature precedents and ¹H NMR data.

The preparation of analogues *ent*-**4** and *ent*-**5** is presented in Scheme 2. The starting material, *ent*-steroid **11**, was prepared by our previously described method.¹⁷ Li/liq. NH₃ 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(*sec*-Bu)₃BH to the 3 β -OH group of *ent*-steroid **12c** (97%). The 17-ketone was next protected as the cyclic ketal to yield product **13a** (87%), and the 3 β -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 9 β -H and 11 β -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%). Benzoylation of the 11 α -OH group gave *ent*-steroid **14d** (69%), and removal of the MOM protecting group gave 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 3 β -acetate derivative. Epoxidation of 17-ketone *ent*-**4** using (CH₃)₃SI, KO^tBu 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 manner to its cyclic ketal derivative **15**, and used as the starting material. The 3 β -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 7 α and 7 β -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 7 β -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 7 α -OBn epimer **18d** was obtained in 45% yield. Removal of the MOM protecting group from steroid **18c** gave product **19a** (91%), PCC oxidation of steroid **19a** gave steroid **19b** (86%), and $\text{K}(\text{sec-Bu})_3\text{BH}$ reduction of the 3-ketone group of steroid **19b** gave target compound **6** (67%). Epoxidation of the 17-ketone using $(\text{CH}_3)_3\text{SI}$, KO^tBu 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 (**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 7 α -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 reduction¹⁹ of the 7-ketone in *ent*-steroid **22a** gave product **22b** (96%) as an inseparable mixture of 7 α - and 7 β -alcohols, and benzylation (72%) gave the readily separated 7 β -OBn product **22c** (22%) and 7 α -OBn product **22d** (50%). Presumably, steroid approach to the surface of the Raney Ni from the *ent*-steroid β face (i.e., opposite to the C-19 Me group on the α face) explains why the desired 7 α -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 7 β -alcohol. Removal of the 3,17-bis cyclic ketal groups from *ent*-steroid **22d** gave product **23** (89%), $\text{K}(\text{sec-Bu})_3\text{BH}$ reduction of *ent*-steroid **23** gave target compound *ent*-**6** (73%), and epoxidation of product *ent*-**6** gave target compound *ent*-**7** (68%).

[³⁵S]-*t*-Butylbicyclophosphorothionate ([³⁵S]TBPS) Displacement Results

The enantiomer pairs shown in Chart 1 were evaluated as noncompetitive displacers of [³⁵S]TBPS from the picrotoxin binding site on the heterogeneous GABA_A receptors found in rat brain membranes. The IC₅₀ displacement values are given in Table 1. The values reported for the reference compounds **2**, *ent*-**2** and **3**, *ent*-**3** are our previously reported values.¹⁴ Relative to steroid **2**, the reported IC₅₀ values indicate that the axial 11 β -OBn substituent present in steroid **4** slightly increases displacement potency, whereas the equatorial 7 β -OBn substituent present in steroid **6** drastically decreases displacement potency. Relative to compound *ent*-**2**, the 11 α -OBn group present in enantiomer *ent*-**4** drastically reduces binding potency and the 7 α -OBn substituent present in enantiomer *ent*-**6** increases binding potency.

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 11 β -OBn substituent present in steroid **5** increases displacement potency, whereas the 7 β -OBn substituent present in steroid **7**

decreases displacement potency. Relative to analogue *ent-3*, the 11 α -OBn substituent present in analogue *ent-5* reduces displacement potency, whereas the 7 α -OBn substituent present in analogue *ent-7* increases displacement potency. Both compounds **5** and *ent-7* are more potent displacers of [³⁵S]TBPS than the endogenous neurosteroid **1**.

Electrophysiology Results

Enantiomer pairs were evaluated for their ability to potentiate chloride currents mediated by 2 TM GABA at rat $\alpha_1\beta_2\gamma_{2L}$ GABA_A receptors expressed in *Xenopus laevis* oocytes (Table 2). This GABA concentration gates, on average, ~4% of the maximum response recorded from an oocyte. The maximum achievable 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**, **3** and their enantiomers *ent-2*, *ent-3*, all cause a concentration dependent increase in GABA-gated chloride currents.¹⁴ Steroids **4** and **5**, which contain 11 β -OBn substituents, also cause a concentration dependent increase in chloride currents. Steroids **6** and **7**, which contain 7 β -OBn substituents, do not. For the analogues *ent-4* and *ent-5*, the 11 α -OBn substituents, either do not potentiate chloride currents (*ent-4*) or only weakly potentiate at the highest concentration, 10 TM (*ent-5*). Analogues *ent-6* and *ent-7*, which contain 7 α -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 TM 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 [³⁵S]TBPS also strongly potentiate GABA-mediated chloride currents. Compounds that either weakly displace or do not displace [³⁵S]TBPS at a concentration of 10 TM 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 $\alpha 1$ subunit was changed from glutamine to leucine. As reported previously²⁰ and shown in Figure 1, this amino acid change 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 TM) and *ent-7* (0.5 TM) potentiate more than the 17-ketones **4** (1 TM) and *ent-6* (1 TM) when compared on the same oocyte. This result is consistent with the greater potency of the 17-spiroepoxides for [³⁵S]TBPS displacement (Table 1).

Tadpole Loss of Righting Reflex (LRR) and Loss of Swimming Reflex (LSR) Results

The anesthetic effects in tadpoles of the compounds are reported in Table 3. The EC₅₀ values for LRR and LSR reported in Table 3 for steroids **2**, **3** and their enantiomers *ent-2*, *ent-3* are the values we reported previously.¹⁴ Steroids **4** and **5**, which contain axial 11 β -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** (7 β -OBn substituent) does not have an EC₅₀ value of <10 TM 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 7 β -OBn substituent does have weak anesthetic activity as it produces both LRR (EC₅₀, 3.22 TM) and LSR (EC₅₀, 7.94 TM). This result for analogue **7** is consistent with its weak ability to displace [³⁵S]TBPS (Table 1), but not with its failure to potentiate GABA-mediated chloride currents (Table 2).

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

The enantiomeric analogues *ent-6* and *ent-7* (7 α -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 [³⁵S]TBPS displacement (Table 1) and electrophysiological actions at GABA_A receptors (Figure 1).

Discussion

The prior observation that steroids **2**, **3** and their enantiomers *ent-2*, *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 based on 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 11 α -Me substituent together with an 11 β -OBn substituent gave an analogue with increased potentiation activity.¹⁵ Hence, we expected that new steroid analogues having 11 β -OBn substituents (**4**, **5**) would have activity. Since it was also shown previously that adding a group as small as either a 7 α - or 7 β -Me group to neurosteroid **1** produced an analogue with greatly diminished activity,¹⁶ we also expected that new steroid analogues having 7 β -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 based on 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 11 β -OBn substituent in a natural steroid and a 7 α -OBn substituent in an *ent*-steroid can occupy similar regions of three-dimensional space (Figure 2, panel A). Hence, we expected that new *ent*-steroids having 7 α -OBn substituents (*ent*-**6**, *ent*-**7**) would, like the 11 β -OBn steroids **4** and **5**, have potentiation activity. Similarly, since a 7 β -OBn substituent in a natural steroid and an 11 α -OBn substituent in an upside down oriented *ent*-steroid can occupy similar regions of three-dimensional space (Figure 2, panel B), we expected that new *ent*-steroid analogues having 11 α -OBn substituents (*ent*-**4**, *ent*-**5**) would, like the 7 β -OBn steroids **6** and **7**, have greatly diminished potentiation activity.

Other factors also influenced our choice of analogues chosen for this study. Firstly, 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. Secondly, 11 β substituents in the steroids have the axial configuration whereas the 7 α substituents in the *ent*-steroids have the equatorial configuration. Thus, a relatively large and flexible group like the benzyloxy group was considered advantageous for maximizing possible overlap of substituents having different configurations at the C-7 and C-11 positions. Thirdly, we considered it important to have the C-11 and C-7 substituents be identical to avoid significant differences in logP for the analogues used in the study. LogP can have a major effect on observed [³⁵S]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 [³⁵S]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 TM even though the compounds neither effectively displace [³⁵S]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 concentrations of either 0.5 TM (the 17-spiroepoxides) or 1 TM (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 [³⁵S]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 3 β -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 5 α -reduced analogues. An earlier study examined the effect of a Q241 mutation in the α_1 subunit on the actions of a 5 β -reduced steroid potentiator and its active enantiomer.²⁴ It was reported that mutation of the Q241 residue eliminated the actions of the 5 β -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 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

Based on 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.

General Methods

Solvents were either used as purchased or dried and purified by standard methodology. Extraction solvents were dried with anhydrous Na₂SO₄ 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 CDCl₃ at ambient temperature at 300 MHz (¹H) or 74 MHz (¹³C). 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(*sec*-Bu)₃BH (K-Selectride ®) was purchased from Aldrich Chemical Co. (Milwaukee, WI).

(3α,5α,11β)-11-Benzyloxy-3-hydroxyandrostane-17-one (**4**)

A mixture of steroid **10b** (242 mg, 0.5 mmol), MeOH (15 mL) and 6 N HCl (3mL) 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, 89%). This crude product was acetylated (95% yield) in the standard manner (Ac₂O, 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); ¹³C NMR δ 219.9, 170.5, 138.4, 128.1 (2 × C), 127.7 (2 × C), 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 (2 × C), 27.5, 25.7, 21.5, 21.4, 14.9, 14.3.

The acetate derivative was then hydrolyzed using K₂CO₃/MeOH to give product **4** (152 mg, 96% yield): mp 62–65 °C; [α]_D²⁰ +82.9 (*c* 0.1, CHCl₃); 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 (b s, 1H), 4.02 (b 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 ($2 \times \text{C}$), 127.6 ($2 \times \text{C}$), 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. ($\text{C}_{26}\text{H}_{36}\text{O}_3$) C, H.

(3 β ,5 β ,8 α ,9 β ,10 α ,11 α ,13 α ,14 β)-11-Benzyloxy-3-hydroxyandrostane-17-one (*ent*-4)

A mixture of steroid **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; $[\alpha]_{\text{D}}^{20} -84.6$ (c 0.08, CHCl_3); IR ν_{max} 3401, 2921, 1738, 1452, 1354 cm^{-1} ; ^1H NMR: 7.40–7.20 (m, 5H), 4.66 (d, 1H, $J = 11.2$ Hz), 4.27 (d, 1H, $J = 11.2$ Hz), 4.04 (b s, 1H), 4.03 (b s, 1H), 2.54–2.37 (m, 2H), 1.08 (s, 3H), 1.01 (s, 3H); ^{13}C NMR: 220.1, 138.5, 128.1 ($2 \times \text{C}$), 127.6 ($2 \times \text{C}$), 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. ($\text{C}_{26}\text{H}_{36}\text{O}_3$) C, H.

(3 α ,5 α ,11 β ,17 β)-Spiro[11-benzyloxyandrostane-17,2'-oxiran]-3-ol (5)

Trimethylsulfonium iodide (40.5 mg, 0.2 mmol) followed by $\text{K}^t\text{O}^t\text{Bu}$ (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 $\times$ 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; $[\alpha]_{\text{D}}^{20} +49.6$ (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 (b 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 ($2 \times \text{C}$), 127.7 ($2 \times \text{C}$), 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. ($\text{C}_{27}\text{H}_{38}\text{O}_3$) C, H.

(3 β ,5 β ,8 α ,9 β ,10 α ,11 α ,13 α ,14 β ,17 α)-Spiro[11-benzyloxyandrostane-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; $[\alpha]_{\text{D}}^{20} -44.5$ (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: 138.8, 128.1 ($2 \times \text{C}$), 127.7 ($2 \times \text{C}$), 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, 28.8, 28.7, 27.8, 23.4, 15.7, 14.2. Anal. ($\text{C}_{27}\text{H}_{38}\text{O}_3$) C, H.

(3 α ,5 α ,7 β)-7-Benzyloxy-3-hydroxyandrostane-17-one (6)

Steroid **19b** (59 mg, 0.15 mmol) was dissolved in THF (5 mL), cooled to -78 °C and a 1M 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) were 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; $[\alpha]_{\text{D}}^{20} = +131.5$ (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 (b s, 1H), 3.26 (m, 1H), 0.88 (s, 3H), 0.83 (s, 3H); ^{13}C NMR δ 221.7, 138.6, 128.4 ($2 \times \text{C}$), 127.8 ($2 \times \text{C}$), 127.5, 82.0, 70.2, 66.2, 53.2, 51.4, 48.4, 41.0, 36.1, 36.0, 35.7 ($2 \times \text{C}$), 33.5, 32.2, 31.6, 29.1, 25.0, 20.3, 14.2, 11.3. Anal. ($\text{C}_{27}\text{H}_{36}\text{O}_3$) C, H.

(3 β ,5 β ,7 α ,8 α ,9 β ,10 α ,13 α ,14 β)-7-Benzoyloxy-3-hydroxyandrostane-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; $[\alpha]_{\text{D}}^{20} = -135.5$ (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 (b s, 1H), 3.26 (1H, m), 0.88 (s, 3H), 0.83 (s, 3H); ^{13}C NMR δ 221.8, 138.6, 128.4 ($2 \times \text{C}$), 127.8 ($2 \times \text{C}$), 127.5, 82.0, 70.2, 66.2, 53.2, 51.4, 48.4, 41.0, 36.14, 36.06, 35.7 ($2 \times \text{C}$), 35.6, 33.5, 32.1, 31.6, 29.1, 25.0, 20.3, 14.2, 11.3. Anal. ($\text{C}_{27}\text{H}_{36}\text{O}_3$) C, H.

(3 α ,5 α ,7 β ,17 β)-Spiro-[7-Benzoyloxyandrostane-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; $[\alpha]_{\text{D}}^{20} +55.5$ (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 (b 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 ($2 \times \text{C}$), 127.8 ($2 \times \text{C}$), 127.3, 82.4, 70.2, 66.2, 53.7, 53.1, 52.8, 41.5, 40.9, 36.2 ($2 \times \text{C}$), 35.7 ($2 \times \text{C}$), 34.0, 33.6, 32.2, 29.4, 29.1, 26.6, 20.4, 14.7, 11.3. Anal. ($\text{C}_{27}\text{H}_{38}\text{O}_3$) C, H.

(3 β ,5 β ,7 α ,8 α ,9 β ,10 α ,13 α ,14 β ,17 α)-Spiro[7-Benzoyoxyandrostane-17,2'-oxiran]-3-ol (*ent-7*)

Compound *ent-6* (39 mg, 0.1 mmol) was converted into steroid *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; $[\alpha]_{\text{D}}^{20} -59.5$ (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, $J = 11\text{Hz}$), 4.08 (b 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 ($2 \times \text{C}$), 127.8 ($2 \times \text{C}$), 127.4, 82.4, 70.2, 66.3, 53.7, 53.1, 52.8, 41.5, 40.9, 36.2 ($2 \times \text{C}$), 35.7 ($2 \times \text{C}$), 34.0, 33.6, 32.2, 29.5, 29.1, 26.6, 20.4, 14.7, 11.3. Anal. ($\text{C}_{27}\text{H}_{38}\text{O}_3$) 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%) had: mp 146–149 °C; IR ν_{max} 3461, 2923, 1741, 1705, 1454, 1233 cm^{-1} ; ^1H NMR δ 4.05 (b s, 1H), 1.02 (s, 3H), 0.82 (s, 3H); ^{13}C NMR δ 217.5, 209.0, 66.1, 64.9, 50.6 ($2 \times \text{C}$), 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. ($\text{C}_{19}\text{H}_{28}\text{O}_3$) 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), 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 $\times$ 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) as solvent 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 (b 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 $\text{C}_{21}\text{H}_{32}\text{O}_4$: 348.2301. Found: 348.2296.

(3 α ,5 α)-(3-Methyloxymethyloxy)-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*-diisopropyl ethylamine (1.74 mL, 10 mmol) and a catalytic amount of DMAP (40 mg) dissolved in CH_2Cl_2 (15 mL) was 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 $\times$ 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 $\text{C}_{23}\text{H}_{36}\text{O}_5\text{Na}$: 415.2460. Found: 415.2466

(3 α ,5 α ,11 β)-(3-Methyloxymethyloxy)-11-hydroxyandrostane-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 (b 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. ($\text{C}_{23}\text{H}_{38}\text{O}_5$) C, H.

(3 α ,5 α ,11 β)-11-Benzoyloxy-3-(methyloxymethyloxy)-androstane-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 0 $^\circ\text{C}$ 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 (2 $\times$ C), 127.5 (2 $\times$ C), 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 $\text{C}_{30}\text{H}_{44}\text{O}_5\text{Na}$: 507.3086. Found: 507.3083.

(5 β ,8 α ,10 α ,13 α ,14 β ,17 α)-17-Hydroxyandrost-9(11)-en-3-one (12a)

NH_3 (150 mL) was condensed into a cold (-78 $^\circ\text{C}$) 2-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 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 $\times$ 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 $^{\circ}\text{C}$; 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. ($\text{C}_{19}\text{H}_{28}\text{O}_2$) C, H.

(5 β ,8 α ,10 α ,13 α ,14 β)-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 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 $^{\circ}\text{C}$; 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 ($3 \times \text{C}$), 33.3, 31.7, 28.5, 22.7, 17.3, 13.8. Anal. ($\text{C}_{19}\text{H}_{26}\text{O}_2$) C, H.

(3 β ,5 β ,8 α ,10 α ,13 α ,14 β)-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%) had: mp 186–188 $^{\circ}\text{C}$; IR ν_{max} 3401, 2921, 2854, 1738, 1596, 1451, 1370, 1279, 1211 cm^{-1} ; ^1H NMR δ 5.38 (m, 1H), 4.06 (b 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. ($\text{C}_{19}\text{H}_{28}\text{O}_2$) C, H.

(3 β ,5 β ,8 α ,10 α ,13 α ,14 β)-3-Hydroxyandrost-9(11)-en-17-one, cyclic-17-1,2-ethanediyl acetal (13a)

Compound **12c** (1g, 3.49 mmol) was converted into compound **13a** using the procedure reported for the preparation of steroid **9a** (except that 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 $^{\circ}\text{C}$; IR ν_{max} 3350, 2968, 2920, 2873, 1595, 1454, 1374, 1308, 1274, 1204 cm^{-1} ; ^1H NMR δ 5.35 (m, 1H), 4.04 (b 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 $\text{C}_{21}\text{H}_{32}\text{O}_3$: 332.2351. Found: 332.2352.

(3 β ,5 β ,8 α ,10 α ,13 α ,14 β)-(3-Methyloxymethyloxy)-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 $^{\circ}\text{C}$; 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. ($\text{C}_{23}\text{H}_{36}\text{O}_4$) C, H.

(3 β ,5 β ,8 α ,9 β ,10 α ,11 β ,13 α ,14 β)-(3-Methyloxymethyloxy)-11-hydroxyandrostane-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. 3 N aqueous NaOH (10 mL) followed by 30% H_2O_2 (10 mL) were 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, 31.1, 28.9, 26.2, 22.5, 15.0, 11.7. Anal. ($\text{C}_{23}\text{H}_{38}\text{O}_5$) C, H.

(3 β ,5 β ,8 α ,9 β ,10 α ,13 α ,14 β)-(3-Methyloxymethyloxy)-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 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. ($\text{C}_{23}\text{H}_{36}\text{O}_5$) C, H.

(3 β ,5 β ,8 α ,9 β ,10 α ,11 α ,13 α ,14 β)-(3-Methyloxymethyloxy)-11-hydroxyandrostane-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 (b 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 $\text{C}_{23}\text{H}_{38}\text{O}_5\text{Na}$: 417.2617. Found: 417.2617.

(3 β ,5 β ,8 α ,9 β ,10 α ,11 α ,13 α ,14 β)-11-Benzyloxy-(3-Methyloxymethyloxy)-androstane-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 ($2 \times \text{C}$), 127.6 ($2 \times \text{C}$), 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 C₃₀H₄₄O₅Na: 507.3087. Found: 507.3091.

(3 β)-(3-Methyloxymethyloxy)-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); ¹³C 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. (C₂₃H₃₆O₄) C, H.

(3 β)-(3-Methyloxymethyloxy)-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), NaOCl₂ (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% Na₂SO₃ (100 mL) and stirred for 0.5 h. The product was extracted into Et₂O (100 mL $\times$ 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); ¹³C 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. (C₂₃H₃₄O₅) C, H.

(3 β ,5 α)-(3-Methyloxymethyloxy)-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); ¹³C 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. (C₂₃H₃₆O₅) C, H.

(3 β ,5 α)-7-Hydroxy-3-(Methyloxymethyloxy)-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 7 α ,7 β -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**.

(3 β ,5 α ,7 β)-7-Benzoyloxy-3-(Methyloxymethyloxy)-androstan-17-one, cyclic-17-1,2-ethanediyl acetal (18c) and (3 β ,5 α ,7 α)-7-Benzoyloxy-3-(Methyloxymethyloxy)-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**.

18c—oil; IR $\nu_{\max}$ 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 (2 $\times$ C), 127.9 (2 $\times$ C), 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. ($\text{C}_{30}\text{H}_{44}\text{O}_5$) C, H.

18d—oil; IR $\nu_{\max}$ 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 (b, s, 1H), 3.37 (s, 3H), 0.81 (s, 3H), 0.81 (s, 3H); ^{13}C NMR δ 139.5, 128.2 (2 $\times$ C), 127.4 (2 $\times$ C), 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. ($\text{C}_{30}\text{H}_{44}\text{O}_5$) C, H.

(3 β ,5 α ,7 β)-7-Benzoyloxy-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 $^{\circ}\text{C}$; IR $\nu_{\max}$ 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 (2 $\times$ C), 127.7 (2 $\times$ C), 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. ($\text{C}_{26}\text{H}_{36}\text{O}_3$) C, H.

(5 α ,7 β)-7-Benzoyloxyandrostane-3,17-dione (19b)

Steroid **19a** (71 mg, 0.18 mmol) was converted into steroid **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 $^{\circ}\text{C}$; IR $\nu_{\max}$ 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 (2 $\times$ C), 127.8 (2 $\times$ C), 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. ($\text{C}_{26}\text{H}_{34}\text{O}_3$) C, H.

(8 α ,9 β ,10 α ,13 α ,14 β)-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 $^{\circ}\text{C}$; IR $\nu_{\max}$ 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. ($\text{C}_{23}\text{H}_{34}\text{O}_4$) C, H.

(8 α ,9 β ,10 α ,13 α ,14 β)-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, 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. ($\text{C}_{23}\text{H}_{32}\text{O}_5$) C, H.

(5 β ,8 α ,9 β ,10 α ,13 α ,14 β)-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. ($\text{C}_{23}\text{H}_{34}\text{O}_5$) C, H.

(5 β ,8 α ,9 β ,10 α ,13 α ,14 β)-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 7 α - and 7 β -alcohol products **22b** (136 mg, 96%). This product was not characterized.

(5 β ,7 β ,8 α ,9 β ,10 α ,13 α ,14 β)-7-Benzyloxyandrostane-3,17-dione, cyclic-3,17-bis(1,2-ethanediyl acetal) (22c) and (5 β ,7 α ,8 α ,9 β ,10 α ,13 α ,14 β)-7-Benzyloxyandrostane-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 7 α - and 7 β -alcohols **18b**.

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 (b s, 1H), 0.81 (s, 6H); ^{13}C NMR: 139.7, 128.1 ($2 \times \text{C}$), 127.4 ($2 \times \text{C}$), 127.0, 119.4, 109.2, 74.5, 70.4, 65.0, 64.5, 64.0 ($2 \times \text{C}$), 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. ($\text{C}_{30}\text{H}_{42}\text{O}_5$) C, H.

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 ($2 \times \text{C}$), 127.8 ($2 \times \text{C}$), 127.7, 118.9, 109.1, 82.7, 70.4, 65.1, 64.5, 64.1 ($2 \times \text{C}$), 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. ($\text{C}_{30}\text{H}_{42}\text{O}_5$) C, H.

(5 β ,7 α ,8 α ,9 β ,10 α ,13 α ,14 β)-7-Benzyloxyandrostane-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 ($2 \times \text{C}$), 127.8 ($2 \times \text{C}$), 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. ($\text{C}_{26}\text{H}_{34}\text{O}_3$) C, H.

[^{35}S]-TBPS Binding Methods

The methods used were as 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 One, Northland, MI). Frogs were anesthetized with 0.1% tricane (3-aminobenzoic acid ethyl ester), and oocytes obtained by partial ovariectomy. The follicular layer was removed from oocytes by shaking for 20 min at 37° C in 2 mg/mL collagenase dissolved in calcium-free solution containing 96 mM NaCl, 2 mM KCl, 1 mM MgCl_2 , 5 mM HEPES, pH 7.4. Capped RNA for α_1 , β_2 , and $\gamma_{2\text{L}}$ 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 total RNA), after which oocytes were incubated at 18° C in ND96 solution containing 96 mM NaCl, 2 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 5 mM HEPES, pH 7.4. ND96 was supplemented with pyruvate (5 mM), penicillin (100 U/mL), streptomycin (100 $\mu\text{g/mL}$), and gentamycin (50 $\mu\text{g/mL}$).

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

Tadpole Behavioral Methods

The methods used were as described previously.²⁷

Supplementary Material

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Abbreviations

GABA	γ -aminobutyric acid
GABA _A	γ -aminobutyric acid type A

[³⁵S]TBPS	[³⁵ S]- <i>tert</i> -butylbicyclophosphorothionate
SAR	structure-activity relationships
LRR	loss of righting reflex
LSR	loss of swimming reflex

References

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Figure 1.

Effects of a steroid-insensitive α_1 (Q241L) subunit on potentiation of GABA currents by analogues. **A.** Oocytes expressing WT $\alpha_1\beta_2\gamma_{2L}$ GABA_A receptor subunits were exposed to GABA alone or GABA co-applied with the indicated analogues at a membrane potential of -70 mV. Concentrations of compounds were as follows: GABA, 2 TM; compound **1**, 0.5 TM; compound **4**, 1 TM, compound **5**, 0.5 TM, compound *ent* **6**, 1 TM; compound *ent* **7**, 0.5 TM. 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 4 WT oocytes and 4 α_1 (Q241L) $\beta_2\gamma_{2L}$ oocytes in experiments like that depicted in A and B. Normalizing response to GABA alone is denoted by a dotted line at $y = 1$. * $p < 0.05$ by independent sample t-test.

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 to 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).

Scheme 1.

^aReagents: a) i: $\text{K}(\text{sec-Bu})_3\text{BH}$, THF, -78°C , ii: 3 N NaOH, 30% H_2O_2 (72%); b) PTSA, $\text{HO}(\text{CH}_2)_2\text{OH}$, benzene, reflux (70%); c) MOMCl, $(i\text{-Pr})_2\text{EtN}$, 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) $(\text{CH}_3)_3\text{SI}$, DMF, KO^tBu (61%).

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

Scheme 2.

^aReagents: a) Li, liq. NH₃, toluene, THF, -78 °C (81%); b) Jones reagent, acetone (92%); c) i: K(*sec*-Bu)₃BH, THF, -78 °C, ii: 3 N NaOH, 30% H₂O₂ (65%); d) PPTS, HO(CH₂)₂OH, benzene, reflux (87%); e) MOMCl, DMAP, (*i*-Pr)₂EtN, CH₂Cl₂ (97%); f) i: BH₃·THF, THF, 0 °C, ii: 3 N NaOH, 30% H₂O₂ (67%); g) PCC, CH₂Cl₂ (96%); h) LAH, Et₂O (95%); i) KH, THF, BnBr (69%); j) MeOH, 6 N HCl (92%); k) (CH₃)₃Si, DMF, KO^tBu (61%).

Scheme 3.

^a Reagents: a) MOMCl, DMAP, (*i*-Pr)₂EtN, CH₂Cl₂ (91%); b) 70% aqueous *t*-BuOOH, NaClO₂, aqueous ACN, 50 °C (34%); c) 10% Pd/C; EtOAc (80%); d) LAH, Et₂O (86%); e) KH, THF, BnBr (68%); f) MeOH, 6 N HCl (91%); g) PCC, CH₂Cl₂ (86%); h) i) K(*sec*-Bu)₃BH, THF, -78 °C, ii: 3 N NaOH, 30% H₂O₂ (67%); i) (CH₃)₃SI, DMF, KO^tBu (57%).

Scheme 4.

^aReagents: a) PTSA, HO(CH₂)₂OH, benzene, reflux (83%); b) 70% aqueous *t*-BuOOH, NaClO₂, aqueous ACN, 50 °C (31%); c) 10% Pd/C, H₂ (50 psi), EtOAc (76%); d) Raney Ni, H₂ (60 psi), *i*-PrOH (96%); e) KH, THF, BnBr (72%); f) MeOH, 6 N HCl (89%); g) i: K(*sec*-Bu)₃BH, THF, -78 °C, ii: 3 N NaOH, 30% H₂O₂ (73%); h) (CH₃)₃SI, DMF, KO^tBu (68%).

Chart 1.

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

compd	IC ₅₀ (nM)	<i>n</i> Hill
1 ^b	74 ± 7	0.89 ± 0.06
2 ^c	410 ± 130	0.89 ± 0.20
<i>ent</i> - 2 ^c	311 ± 36	1.00 ± 0.10
3 ^c	105 ± 12	1.32 ± 0.16
<i>ent</i> - 3 ^c	467 ± 93	4.28 ± 1.90
4	285 ± 37	0.90 ± 0.09
<i>ent</i> - 4	>10 μM ^d	–
5	15 ± 2	1.1 ± 0.1
<i>ent</i> - 5	2,050 ± 400	2.04 ± 0.7
6	>10 μM ^e	–
<i>ent</i> - 6	168 ± 8	1.10 ± 0.05
7	6,750 ± 1,000	1.4 ± 0.2
<i>ent</i> - 7	52 ± 5	1.12 ± 0.10

^a Results presented are from duplicate experiments performed in triplicate. Error limits are calculated as 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.

Table 2
Modulation of Rat $\alpha_1\beta_2\gamma_2\text{L}$ GABAA Receptor Function by Steroids 1–7 and Steroid Enantiomers *ent*-2–*ent*-7

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

^aThe 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/2 μM GABA current. Error limits are calculated as standard error of the mean ($N \geq 4$).

^bLiterature values.²⁷

^cLiterature values.¹⁴

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 <i>n</i> _{Hill}	Tadpole LSR EC ₅₀ (μM)	Tadpole LSR <i>n</i> _{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	—
<i>ent</i> -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
<i>ent</i> -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
<i>ent</i> -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
<i>ent</i> -5	2.64 ± 0.01	−22.8 ± 1.0	2.74 ± 0 ⁱ	−23.2 ± 0
6	>10 ^j	—	None ^e	—
<i>ent</i> -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
<i>ent</i> -7	0.31 ± 0.04	−2.19 ± 0.61	0.55 ± 0.01	−33.3 ± 0.1

^aError 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 μM to 30 μM). Unless stated otherwise, all tadpoles regained LRR and LSR after overnight recovery.

^bLSR 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.

^cLiterature values.²⁷

^dLiterature values.¹⁴

^e“None” indicates that all tadpoles had a swimming response at 10 μM.

^fRecovery from LRR and/or LSR was partial at concentrations ≥ 1 μM. At 10 μM, 10/10 tadpoles died without recovering overnight from LSR.

^gAt 10 μM, 10/10 tadpoles died without recovering overnight from LSR.

^hAt 10 μM, 5/10 tadpoles died without recovering overnight from LSR.

ⁱAt 10 μM, 6/10 tadpoles died without recovering overnight from LSR.

^jSome tadpoles had LRR at 10 μM but the EC₅₀ was above this value.

^kAt 10 μM, 4/10 tadpoles died without recovering overnight from LSR.