Neurosteroid Analogues. 18. Structure–Activity Studies of *ent*-Steroid Potentiators of γ-Aminobutyric Acid type A Receptors and Comparison of Their Activities with Those of Alphaxalone and Allopregnanolone

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Abstract

A model of the alignment of neurosteroids and *ent*-neurosteroids at the same binding site on γ-aminobutyric acid type A (GABA$_A$) receptors was evaluated for its ability to identify the structural features in *ent*-neurosteroids that enhance their activity as positive allosteric modulators of this receptor. Structural features that were identified included: 1) a ketone group at position C-16; 2) an axial 4α-OMe group and 3) a C-18 methyl group. Two *ent*-steroids were identified that were more potent than the anesthetic steroid alphaxalone in their threshold for and duration of loss of the righting reflex in mice. In tadpoles, loss of righting reflex for these two *ent*-steroids occurs with EC$_{50}$ values similar to those found for allopregnanolone. The results indicate that *ent*-steroids have considerable potential to be developed as anesthetic agents as and drugs to treat brain disorders that are ameliorated by positive allosteric modulators of GABA$_A$ receptor function.

INTRODUCTION

Currently, there is considerable interest in neurosteroid physiology and in the development of neurosteroid analogues for the treatment of a variety of disorders of central nervous system (CNS) function and as new intravenous general anesthetics.[1-4] The vast majority of

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Supporting Information Available: Table of elemental analysis results, additional views of superimposed analogues (Figure S1), and structures in Table 1 with [35S]TBPS IC$_{50}$ values (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

the medicinal chemistry done in the field of neuroactive steroids is focused on developing new neurosteroid analogues having the absolute configuration of naturally-occurring steroids.[5] The potential of *ent*-neurosteroids, steroids with an absolute configuration opposite to that of naturally-occurring steroids, to be developed for these purposes has not been addressed even though *ent*-steroids could have a different spectrum of actions in the CNS and different routes of metabolism.

As an initial approach to evaluating the potential of *ent*-steroids as new anesthetic agents and CNS drugs, we have been engaged for a number of years in exploring the molecular basis for the enantioselectivity of neurosteroid action at γ-aminobutyric acid type A (GABA$_A$) receptors.[6-10] Androsterone (**1**), a naturally occurring neurosteroid, is a weak potentiator of γ-aminobutyric acid (GABA)-mediated chloride currents at GABA$_A$ receptors (Chart 1).[11,12] We reported previously that its enantiomer, *ent*-androsterone (***ent*-1**), is a more effective positive allosteric modulator of this ion channel than steroid **1**.[9] In a subsequent study of 7-OBn and 11-OBn substituted analogues of compounds **1** and *ent*-**1** we provided evidence in support of the hypothesis that one enantiomer of androsterone is inverted relative to the other in the neurosteroid modulation site on the GABA$_A$ receptor.[13] We report herein a continuation of these enantioselectivity studies with the immediate goal of learning more about the SAR of *ent*-steroid modulators of GABA$_A$ receptors and the long term goal of identifying new *ent*-steroids with therapeutic potential.

A heuristic model for the alignment of the enantiomers of androsterone at the neurosteroid binding site in the GABA$_A$ receptor has been used to select the *ent*-steroids chosen for synthesis and evaluation (Figure 1, panel A). The model posits that the A-rings of both enantiomers are identically located in the neurosteroid binding site as inferred from our previous study.[13] This model, when considered in the context of what has been shown in previous studies of steroid modulators of GABA$_A$ receptors, was then used to make the following series of predictions for the activity of new *ent*-steroid analogues.

First, previous structure–activity (SAR) studies of steroid anesthetics acting at GABA$_A$ receptors have shown that analogues with 2β-substituents (e.g., **2**), but not 4β-substituents (e.g., **3**), are highly active.[14] Thus, as a consequence of the inverted orientation of compound ***ent*-1** relative to **1**, the model predicts that introducing a 2α-OMe group as a modification of compound ***ent*-1** will yield inactive analogue ***ent*-2**, whereas modification with a 4α-OMe group will give active analogue ***ent*-3**.

Second, the model places the oxygens in the ketone groups at C-17 in the androsterone enantiomers in nearly the same location. However, results obtained with highly active anesthetic steroid **4** suggest that the C-17 position may not be the optimal location for the carbonyl group in *ent*-steroids.[15] An alignment of the A-rings of steroid **4**, a synthetic analogue of endogenous neurosteroid allopregnanolone (**5**), with compound ***ent*-1**suggests that the carbonyl group would be better placed at C-16 (Figure 1, panel B). This prediction, along with that of an expected loss of activity for the corresponding 16-ketosteroids was evaluated using the enantiomer pairs (**7–9** and ***ent*-7–*ent*-9**).

Third, the model predicts that the C-18 and C-19 methyl groups are similarly located in the neurosteroid binding site. Either, both, or neither of these methyl groups may be important for orientation/activity of the enantiomers in the neurosteroid binding site. Previous studies have shown that the C-18 Me group can affect the number of components in TBPS binding curves (one vs. two), as well as the kinetic modes of action.[16,17] This predicts that the activity of analogues of ***ent*-1** without the C-18 methyl group will have their activities altered more than ***ent*-1** analogues lacking the C-19 methyl group. This prediction was tested with compounds ***ent*-7** and ***ent*-10–*ent*-12.**

Herein, we report results with novel steroid and *ent*-steroid analogues that are consistent with all three of these predictions. In addition, we have identified *ent*-steroids (**ent-7** and **ent-9**) with potencies comparable to those of the endogenous neurosteroid **5** and higher than those of the anesthetic steroid alphaxalone (**6**).

CHEMISTRY

Since the synthetic pathways to either enantiomer of the compounds described in Schemes 1-3 are identical, only those for the *ent*-steroids are discussed here. Physical and spectroscopic details for the corresponding steroids, when prepared, are given immediately after these data are reported for the corresponding *ent*-steroids in the Experimental Section.

The synthesis of compound ***ent*-1** was described previously.[9] The synthesis of **ent-2** is shown in Scheme 1. Starting material **ent-13** was prepared as previously reported.[9] Regio- and stereoselective reduction of this compound's 3-ketone group with Li(*t*-OBu)$_3$AlH yielded after workup predominately product **ent-14** (82%). Mesylation of the resultant 3α-hydroxyl group gave the corresponding mesylate **ent-15** (100%), and elimination of the mesylate group gave predominately the Δ^2-olefinic product **ent-16** (87%). Epoxidation of the double bond using HCOOH/H$_2$O$_2$ gave epoxide **ent-17** (100%), and opening of the epoxide with H$_2$SO$_4$/MeOH gave **ent-2** (62%).

The synthesis of **ent-3** is shown in Scheme 2. Starting material **ent-18** was prepared as previously reported.[7] Treatment of the starting material with Zn powder in HOAc gave the (5α)- and (5β)-Δ^3-olefinic products in about a 1:1 ratio as determined by NMR. Fractional recrystallization gave the pure product **ent-19** (43%). Epoxidation of the double bond using HCOOH/H$_2$O$_2$ gave epoxide **ent-20** (84%), and opening of the epoxide with H$_2$SO$_4$/MeOH gave **ent-3** (78%).

In Scheme 3, we adapted a route that we used previously to convert a steroid 3-ketone group to a 2-ketone group[18] to transform the 17-ketone groups of **ent-1**–**ent-3** and **ent-21** to the 16-ketone groups of **ent-7**–**ent-9** and **ent-11**, respectively. Each starting material was condensed with benzaldehyde, reduced to a diol using NaBH$_4$/CeCl$_3$ and acetylated using AcOAc/pyr/DMAP to give products **ent-22** (91%), **ent-25** (95%), **ent-28** (97%), and **ent-31** (91%). The 16-ketone groups were then formed by ozonolysis of the exocyclic double bonds at the 16-position to give products **ent-23** (82%), **ent-26** (90%), **ent-29** (87%), and **ent-32** (98%), respectively. In the next step, the 17α-acetate groups were removed using SmI$_2$ in THF. This reaction also results in partial reduction of the 16-ketone groups. The crude 16-ketone, 16-alcohol product mixtures were treated with Jones reagent to give the isolated products **ent-24** (94%), **ent-27** (94%), **ent-30** (84%), and **ent-33** (70%), respectively. Saponification of the 3β-acetate groups gave the target compounds **ent-7** (93%), **ent-8** (97%), **ent-9** (94%), and **ent-11** (98%), respectively.

The synthetic routes to the 18-nor compound **ent-10** (Scheme 4) and 18,19-dinor compound **ent-12** (Scheme 5) are largely derived from methods developed previously to prepare chrysene enantiomers from 19-nortestosterone or to convert 19-nortestosterone into *ent*-19-nortestosterone.[19,20] Starting material **ent-34** was prepared as described previously,[9] and converted to the 17α-tosylate **ent-35** (91%). Stereoselective reduction of the 3-ketone group of **ent-35** was achieved using K(*s*-Bu)$_3$BH to yield product **ent-36** (71%) containing the 3β-hydroxyl group. Rearrangement of compound **ent-36** to product **ent-37** (91%) was achieved using MeMgBr in refluxing toluene. Compound **ent-37** is not very soluble in the solvents used for ozonolysis of the compound's double bond, so it was converted to the MOM protected compound **ent-38** (90%) and then converted by ozonolysis to the diketone **ent-39**

(65%). Aldol condensation of compound *ent*-**39** using NaOH/aqueous MeOH gave enone *ent*-**40** (75%). Li/liq. NH$_3$ of this enone gave product *ent*-**41** (72%).

At this point, the MOM protecting group at the 3-position, which was chosen for it's stability in the Li/liq. NH$_3$ reduction step, had to be exchanged for an acetate group because the MOM group would not survive the harsh conditions of a subsequent CrO$_3$ oxidation step. The MOM group in compound *ent*-**41** was removed using 6 N HCl/MeOH, and the resultant hydroxyl group in compound *ent*-**42** (92%) was acetylated using AcOAc/pyr/DMAP to yield the 3-acetate *ent*-**43** (95%).

CrO$_3$/AcOH oxidation of compound *ent*-**43** cleaved the D-ring to yield crude dicarboxylic acid *ent*-**44,** which was isolated after esterification using AcCl/MeOH as diester *ent*-**45** (51%). Dieckmann condensation using NaOMe/THF of compound *ent*-**45** yielded the D-ring β-ketoester *ent*-**46** (71%) as the only condensation product. An analogous oxidation, esterification, ring closure sequence has been reported previously for (5α)-3-ketosteroids.[21-23] In the cited study, the hydrogen at the ring fusion and the carbomethoxy group in the product were found to be *syn* to each other. For this reason, we assigned the stereochemistry of the 17-carbomethoxy group in product *ent*-**46** the α-configuration. Decarbomethoxylation using LiCl in non-dried DMF gave the desired analogue *ent*-**10** (86%). Using carefully dried DMF gave lower yields.

For the synthesis of *ent*-**12**, steroid **47** was used as starting material.[24] CrO$_3$/AcOH oxidation of steroid **47** cleaved the A-ring to yield crude 2,3-secosteroid **48** (81%), which was purified and isolated after esterification using AcCl/MeOH as the diester **49** (61%). The 17-ketone group of 2,3-secosteroid **49** was then reduced using NaBH$_4$ to yield product **50** (74%). Dieckmann condensation of compound **50** using NaOMe/THF yielded the A-norsteroid **51** (81%) as the only condensation product and the stereochemistry of the 3-carbomethoxy group was assigned, by literature analogy,[21-23] the α-configuration. Decarbomethoxylation using LiCl in non-dried DMF gave A-norsteroid **52** (85%). This part of the synthetic sequence has the effect of converting the A-ring of a steroid into what will become the D-ring of an *ent*-steroid.

The last part of the synthesis of compound *ent*-**12** involves converting the D-ring of A-norsteroid **52** into the A-ring of *ent*-steroid *ent*-**57**. Compound **52** was converted into the tosylate **53** (91%), and the A-ring ketone ring of this tosylate was converted into the vinylidene group of A-norsteroid **54** (80%). Treatment of compound **53** with MeMgBr in refluxing toluene gave the di-olefin **55** (90%). Ozonolysis of compound **55** regenerates the ketone group from the vinylidene group and cleaves the endocyclic double bond to form trione **56** (51%). Aldol condensation of trione **56** completes the 19-norsteroid to *ent*-18,19-dinorsteroid transformation yielding product *ent*-**57** (82%). Li/liq. NH$_3$ reduction of the double bond in enone *ent*-**57** yields product *ent*-**58** (63%), and regio- and stereoselective reduction of the 3-ketone group with K(s-Bu)$_3$BH yields target compound *ent*-**12** (70%).

[^{35}S]TBPS DISPLACEMENT RESULTS

The compounds 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 (Table 1; Supplemental Figure S2 shows each analogue in the alignment orientations shown in Figure 1 along with the IC$_{50}$ values). The results demonstrate first, for reference, that the endogenous neurosteroid **5** (IC$_{50}$ = 74 ± 7 nM) and compounds *ent*-**7** and *ent*-**9** (IC$_{50}$ = 81 ± 8 and 83 ± 11 nM, respectively) are the most potent displacers of [^{35}S]TBPS. These three compounds are all more potent than the clinically used anesthetic steroid **6** (IC$_{50}$ = 226 ± 24 nM).

Comparison of the IC_{50} values for the steroid pair **1** and **7** shows that moving the D-ring ketone group from position C-17 to C-16 decreases displacement potency by about two-fold. By contrast, this same structural modification for the compound pair **ent-1** and **ent-7** increases displacement potency by about four-fold.

Introduction of an axial 2-OMe group lowers displacement potency for steroids with a 17-ketone group (compare steroids **1** and **2**) by about five-fold, but for steroids with a 16-ketone group (compare steroids **7** and **8**) the substituent lowers displacement potency only slightly (< two-fold). An axial 2-OMe substituent causes a much larger decrease in displacement potency for the corresponding *ent*-steroid pairs. For the pair **ent-1** and **ent-2**, the effect is about 21-fold. For the pair **ent-7** and **ent-8**, the effect is about 37-fold.

Addition of an axial 4-OMe group yields steroid analogues that are weak displacers of [^{35}S]TBPS. Steroids **3** (17-ketone) and **9** (16-ketone) displaced only 45% and 65% of bound [^{35}S]TPBS at the highest concentration tested (3 μM), respectively. An opposite effect on displacement potency was found for the axial 4-OMe substituent in the enantiomeric compounds **ent-3** and **ent-9**. For the 17-ketone pair **ent-1** and **ent-3**, the axial 4-OMe group increased displacement potency by about two-fold and for the 16-ketone pair **ent-7** and **ent-9** there was no significant effect of the axial 4-OMe group on displacement potency.

In summary, an axial 2-OMe substituent has a modest negative effect on displacement potency for steroids having a 17-ketone group and little, if any, effect on steroids with a 16-ketone group. A large negative effect of this substituent on the IC_{50} value is found for the corresponding *ent*-steroids having the D-ring ketone group in either position. An axial 4-OMe substituent has largely opposite effects on displacement potency. For the steroids, regardless of the position of the D-ring ketone group, displacement potency is greatly reduced by this substituent. For the corresponding *ent*-steroids, the axial 4-OMe substituent has little, if any, effect on displacement potency.

Compounds **ent-10**–**ent-12** were evaluated to assess the relative importance of the C-18 and C-19 Me groups for the high displacement potency found for compound **ent-7**. Compound **ent-10**, which lacks the C-18 Me group, is about a 19-fold weaker displacer of [^{35}S]TBPS than compound **ent-7**. Compound **ent-11**, which lacks the C-19 Me group, is only about a three-fold weaker displacer of [^{35}S]TBPS than compound **ent-7**. Compound **ent-12**, which lacks both C-18 and C-19 Me groups, has an IC_{50} value that is essentially the same as that of compound **ent-10**. These results indicate that in this series of *ent*-steroids the C-18 Me group has a substantially larger effect on [^{35}S]TBPS displacement potency than does the C-19 Me group. This difference was anticipated based on previous results obtained from similar studies carried out on steroids and discussed in the Introduction. The effect of the C-18 Me group on kinetic processes affecting the magnitude of potentiation of whole cell GABA currents is reported in the next section.

ELECTROPHYSIOLOGY RESULTS

The compounds shown in Chart 1 were initially evaluated for their ability to potentiate chloride currents elicited by 2 μM GABA (a concentration that on average gates ~4% of maximal GABA response) at rat $\alpha_1\beta_2\gamma_{2L}$ type GABA$_A$ receptors expressed in *Xenopus laevis* oocytes (Table 2). The results demonstrate that compounds with a low IC_{50} value for [^{35}S]TBPS displacement tended to exhibit detectable potentiation of GABA$_A$ receptor currents at 100 nM, the lowest concentration tested. All active compounds exhibited concentration dependent potentiation, and the weakest compounds failed to exhibit potentiation even at the highest concentration tested (10 μM), suggesting their qualitative inactivity The results shown were obtained on different batches of oocytes and reported potentiation values do not account for minor variations in GABA EC_{50} and other potential

variables (e.g., different extent of receptor phosphorylation) that may quantitatively affect GABA and neurosteroid responsiveness. Therefore, only qualitative comparisons of results for the different analogues can be made for the results reported in Table 2.

In general, results found in electrophysiological evaluations presented in Table 2 are congruent with results from [^{35}S]TBPS binding studies presented in Table 1. In Table 2, steroids **1** (17-ketone) and **7** (16-ketone) are qualitatively similar to each other, and both are modest enhancers of currents. Results in Table 2 for the axial 2-OMe substituted compounds suggest that this substituent has a small negative effect on the concentration dependent potentiation caused by the steroids (compare steroids **1** and **2**; **7** and **8**) and a large negative effect on potentiation effects of the corresponding *ent*-steroids (compare compounds ***ent*-1** and ***ent*-2**; ***ent*-7** and ***ent*-8**). For steroid analogues **3** and **9**, which have the axial 4-OMe substituent, the results in Table 2 indicate that this substituent eliminates potentiation of currents by the compounds at concentrations up to 10 μM. For analogues ***ent*-3** and ***ent*-9**, this substituent also qualitatively diminishes potentiation of current (compare analogues ***ent*-1** and ***ent*-3**; ***ent*-7** and ***ent*-9**). However, both of these *ent*-steroids still show a concentration dependent increase in potentiation, an effect not seen with steroids **3** and **9**.

Comparison of results in Table 2 for *ent*-16-ketosteroid analogues ***ent*-7** and ***ent*-10–*ent*-12** shows that absence of the C-18 Me group (***ent*-10**) has a larger negative impact on potentiation than does absence of the C-19 Me group (***ent*-11**). The analogue without the C-18 and C-19 Me groups (***ent*-12**) has little potentiating activity at concentrations up to 10 μM.

For quantitative purposes, several direct comparisons of potentiating effects of selected compounds at a fixed concentration on the same oocyte were made (Figure 2, panels A–D). In Figure 2 (panel A), the potentiation caused by 17-ketosteroids **1–3** and 16-ketosteroids **8** and **9** at 5 μM are compared. The minor effects on potentiation caused by the axial 2-OMe substituents (steroids **2** and **8**), and the major negative effects of the axial 4-OMe groups (steroids **3** and **9**) on potentiation are readily apparent when the potentiating effects of the four analogues are compared with that of reference steroid **1**.

In Figure 2 (panel B), quantitative comparisons for selected *ent*-steroids (0.5 μM) are shown. Changing the position of the C-17 ketone (***ent*-1**) group to C-16 (***ent*-7**) enhances potentiation. The enhancement obtained by moving the position of the ketone group is eliminated by the addition of an axial 2-OMe group (***ent*-8**) and further augmented by the addition of an axial 4-OMe group (***ent*-9**).

In Figure 2 (panel C), a quantitative comparison of the potentiating effects of analogue ***ent*-9** with those of the reference steroids **4-6** on the same oocyte at 0.5 μM is shown. Analogue ***ent*-9** had higher activity than either the reference anesthetic steroid **6** or its C-17 vinylcyano analogue steroid **4**, and had an activity very similar to that of the more active endogenous neurosteroid **5**.

In Figure 2 (panel D), a quantitative comparison of the series of *ent*-16-ketosteroid analogues with the different pattern of C-18 and C-19 Me group substitutions is shown. The results from direct comparisons of 5 μM compound agree with the conclusion made from the qualitative results presented in Table 2. Absence of the C-18 Me group yields compounds with lower activity than those having this group whether or not the C-19 Me group is present.

To verify that the effects of the two most active *ent*-16-ketosteroids (***ent*-7** and ***ent*-9**) and endogenous neurosteroid **5** were mediated by the same site and similar mechanisms, we

examined the effects of mutations that in previous studies had been shown to affect receptor modulation by steroids. These studies utilized mutated rat $\alpha_1\beta_2\gamma_{2L}$ receptors expressed in HEK 293 cells. Steroid **5** potentiation of rat $\alpha_1\beta_2\gamma_{2L}$ receptors was previously shown to be drastically reduced in the $\alpha_1(Q241L)\beta_2\gamma_{2L}$ mutant, possibly due to the Leu residue being unable to form a hydrogen bond with the steroid molecule.[25,26] Potentiation by compounds ***ent*-7** and ***ent*-9** was also reduced in this mutated receptor (Table 3). Receptors containing $\alpha_1(S240L)\beta_2\gamma_{2L}$ subunits that abolish potentiation by some 5β-reduced steroids, but in which potentiation by steroid **5** is maintained,[25] are also potentiated by both *ent*-steriods (Table 3). The third mutated receptor on which the compounds were tested is $\alpha_1(W245L)\beta_2\gamma_2$ receptor. This mutated receptor is not potentiated by steroids, and it has been proposed that this mutation affects a general transduction element required for steroid-induced potentiation.[25] Neither analogue ***ent*-7** nor analogue ***ent*-9** potentiates receptors carrying this mutation (Table 3). Thus, these studies with mutated receptors are consistent with all three compounds (***ent*-7**, ***ent*-9** and **5**) acting at the same potentiation sites.

Five *ent*-16-ketosteroids were examined in greater detail to determine how single-channels kinetics are affected by the axial 4-OMe substituent (***ent*-9**) or by the different C-18 and/or C-19 Me substituents (***ent*-7** and ***ent*-10–*ent*-12**). These studies were performed using rat wild type $\alpha_1\beta_2\gamma_{2L}$ receptors expressed in HEK 293 cells.

Previous single-channel studies have demonstrated that receptors activated by high concentrations of GABA produce clusters of single-channel openings, i.e., episodes of intense activity separated by long quiescent periods.[27] Coapplication of neurosteroids with GABA elicits specific changes in the intracluster open and closed time distributions. For example, in the presence of steroid **5**, the changes are: an increase in the mean duration of the longest-lived open state, an increase in the fraction of events in this open state, and a decrease in the fraction of events in the longest intracluster closed state.[28] Table 4 reports a statistical analysis of these parameters in the presence of the five novel *ent*-16-ketosteroids. Sample single-channel traces along with the open and closed time histograms for channel events in the corresponding patches are shown in Figure 3.

Comparison of compounds ***ent*-7** and ***ent*-,** compounds containing the C-18 and C-19 Me groups, reveals that the major effect of the axial 4-OMe substituent (***ent*-9**) is to increase the mean lifetime of the longest open state. Both compounds increase the prevalence of long open events and decrease the prevalence of long closed events.

Comparison of results for compounds ***ent*-7** and ***ent*-10** allows the effect of the C-18 Me group to be determined. Both analogues increase the fraction of the longest open state. By contrast, analogue ***ent*-7**, but not analogue ***ent*-10** (the analogue without the C-18 Me group), decreases the prevalence of the longest closed state. Both compounds prolong the mean duration of the longest state but the effect reaches significance only in the presence of analogue ***ent*-10**.

Comparison of results for compounds ***ent*-7** and ***ent*-11** allows the effect of the C-19 Me group on single-channel properties to be determined. Both compounds decrease the fraction of dwells in the longest closed state. However, analogue ***ent*-7** (the compound with the C-19 Me group), unlike analogue ***ent*-11** (the compound without the C-19 Me group), increases the fraction of dwells in the longest open state. Overall, the results suggest that single-channel properties are affected in different ways by each Me group. The C-19 Me group has its largest effect on the prevalence of the longest open event and the C-18 Me group has its largest effect on the prevalence of the long-lived closed state.

When neither Me group is present (***ent*-12**), the sole kinetic effect of the compound is an increase in the fraction of the longest open state. This suggests that the increase in this kinetic parameter is not controlled solely by the presence of a C-19 Me substituent. In fact, previous studies have found that even steroid analogues with other ring systems such as cyclopenta[*b*]phenanthrenes and cyclopenta[*b*]anthracenes can increase the prevalence of long openings,[29] thus indicating that this single-channel parameter is sensitive to a wide range of structural variations.

TADPOLE LOSS OF RIGHTING REFLEX (LRR) AND LOSS OF SWIMMING (LSR) RESULTS

The anesthetic effects of the compounds in tadpoles are summarized in Table 5. Comparison of EC_{50} values for the steroid pair **1** and **7** shows that moving the D-ring 17-ketone group from position C-17 to C-16 has no significant effect on tadpole LRR, but lowers LSR since only steroid **7** has an EC_{50} value below 10 μM. This same structural modification for the compound pair ***ent*-1** and ***ent*-7** led to the latter having lower EC_{50} values for both LLR and LSR (~three fold in both cases), results consistent with the same trend found in binding data reported in Table 1.

For the 17-ketosteroids, the axial 2-OMe group had a minor negative effect on potency for either LRR or LSR (compare steroids **1** and **2**). This substituent was without effect on LRR or LSR for steroids with a 16-ketone group (compare steroids **7** and **8**). All four of these steroids have very similar LRR and LSR activity profiles. For the *ent*-17-ketosteroid pair ***ent*-1** and ***ent*-2**, the latter compound has a higher LRR EC_{50} value (~two fold) and the LSR EC_{50} value is raised above 10 μM. For the enantiomer pair ***ent*-7** and ***ent*-8**, the effect of the axial 2-OMe group is very large. This substituent raises both the LRR and LSR EC_{50} values by ~five fold. No inconsistencies were found for the axial 2-OMe substituent effects between the binding (Table 1.) and tadpole reflex results.

The steroids **3** (17-ketone) and **9** (16-ketone) with the axial 4-OMe substituent have low activity as anesthetics in tadpoles. Based on the high IC_{50} values found in the [^{35}S]TBPS displacement experiments (Table 1), the result that steroid **9** had any effect on either LRR or LSR at concentrations below 30 μM is somewhat surprising. Yet again, the axial 4-OMe substituent in the enantiomeric compounds ***ent*-3** and ***ent*-9** produced analogues with high activity. The EC_{50} values for both LRR and LSR are the lowest values observed and correlate with the low IC_{50} values for [^{35}S]TBPS displacement (Table 1). Both EC_{50} values are lower than those found for anesthetic steroid **6** and are comparable to those found for the potent endogenous neurosteroid **5**. Thus, effects of the axial 2-OMe or 4-OMe groups in the LRR and LSR bioassay generally maintain the pattern wherein these substituents have opposite effects in the steroid and *ent*-steroid series.

The contribution that the C-18 and C-19 Me groups have on potency for LRR and LSR of compound ***ent*-7** was determined by comparing the EC_{50} values of this compound with those of compounds ***ent*-10**–***ent*-12**. Compound ***ent*-10**, which lacks the C-18 Me group, has a higher LRR EC_{50} value than compound ***ent*-7** by about seven-fold and does not cause LSR at concentrations up to 10 μM. Compound ***ent*-11**, which lacks the C-19 Me group, is about two-fold less potent at causing LRR and three-fold less potent at producing LSR than compound ***ent*-7**. Compound ***ent*-12**, which lacks both the C-18 and C-19 Me groups, produces neither LRR nor LSR at concentrations up to 10 μM. Overall, these results indicate that in this series of *ent*-steroids the C-18 Me group has a major effect on LRR and LSR whereas the C-19 Me group has a minor effect.

Figure 4 shows a rank order correlation for the [^{35}S]TBPS IC_{50} and tadpole LRR EC_{50} values for all 18 compounds. The Pearson correlation coefficient is 0.81 indicative of a strong correlation between these parameters. The greatest outliers on the plot are compounds

ent-**2**, *ent*-**9** and **8** all of which were more potent at causing LRR than expected from their [^{35}S]TBPS IC$_{50}$ values.

ANESTHESIA IN MICE RESULTS

The potency, rate of onset, and rate of recovery for compounds *ent*-**7** and *ent*-**9** relative to these parameters for anesthetic steroid **6** were made using tail vein injections in mice. The duration of anesthesia, defined as loss of righting reflex, observed for the compounds is shown in Figure 5. We previously found that the threshold dose for mice LRR in mice by this route of administration was 8 mg/kg for steroid **6** which caused LRR of less than 1 min duration.[15] A 16 mg/kg dose of steroid **6** caused LRR of about 4 min duration. At a dose of 8 mg/kg compound *ent*-**7** caused LRR of about 3 min duration, and at a dose of 16 mg/kg duration of LRR was increased to about 7 min. The threshold dose for compound *ent*-**7** was not determined. For compound *ent*-**9**, no LRR was observed at a dose of 1 mg/kg and LRR of about 1 min was observed at a threshold dose of 2 mg/kg. At 4 mg/kg, LRR induced by compound *ent*-**9** lasted about 5 min, and at a dose of 8 mg/kg LRR lasted for about 9 min. For all three compounds the onset of anesthesia was immediate (< 30 sec), and recovery was characterized by a rapid progression over 1–2 min from an initial return of leg movement followed by righting and subsequent walking around the cage.

DISCUSSION

This is the second SAR study to follow up on our initial observation that *ent*-androsterone and *ent*-etiocholanolone are modulators of GABA$_A$ receptors.[9,10] In the first follow up study, we presented a model for how the pair of androsterone enantiomers were aligned when bound to the site that causes steroid potentiation of GABA$_A$ receptors.[13] In this study, we further validate and refine that model (Figure 1) and also show that it is useful for identifying structural features that could lead to development of novel clinically useful intravenous general anesthetics. We identify two candidate *ent*-steroids that have this potential.

Three predictions were made based on our original model and prior SAR studies of steroids and *ent*-steroids that potentiate GABA$_A$ receptors. The first concerned the effect of an axial 4α-OMe group on the activity of compound *ent*-**1**. It was shown about 40 years ago in the published SAR studies that led to the clinical use of anesthetic steroid **6** that axial 2β-alkoxy groups were compatible with high anesthetic activity and it was stated that various axial 4β-groups were not.[14] We confirmed this SAR using steroids **2** (axial 2β-OMe substituent) and **3** (axial 4β-OMe substituent). Our model predicted that as a consequence of the inverted binding of *ent*-steroids relative to steroids at the steroid potentiation site, an *ent*-steroid with an axial 2α-OMe group would yield a compound with low activity (*ent*-**2**), whereas an *ent*-steroid with an axial 4α-OMe group would yield a compound (*ent*-**3**) with about the same anesthetic activity as steroid **2**. This was found to be correct and consistently observed in all three of our screening bioassays ([^{35}S]-TBPS displacement, oocyte electrophysiology, tadpole LRR and LSR).

The second prediction was that for active *ent*-steroids, a more favorable position for the D-ring hydrogen bond acceptor ketone group found at position C-17 in steroid potentiators, would be at position C-16 in *ent*-steroid potentiators. It also implies that moving the ketone group to the C-16 position in steroids would decrease the activity of steroid potentiators. The prediction for the *ent*-steroids was evaluated by comparing the actions of compounds *ent*-**1** and *ent*-**3** with those of *ent*-**7** and *ent*-**9**, respectively. In both cases, this structural change produced compounds with much higher activity in all three bioassays. The prediction for the steroids was evaluated with the steroid pairs **1** and **7** as well as with steroid pairs **2** and **8**.

The lower activity expected for steroids **7** and **8** was observed in the [^{35}S]TBPS displacement and oocyte electrophysiology screens which measure actions at GABA$_A$ receptors. However, these two compounds were modestly more potent in the tadpole LRR and LSR screen. This result was unexpected, but could be explained by steroid **7** and **8** modulation of other receptors that contribute to LRR and LSR in tadpoles.

The third prediction was that the activities of the *ent*-steroids would be more greatly affected by removal of the C-18 Me group than they would be by removal of the C-19 Me group. In the model shown in Figure 1, the C-18 and C-19 Me groups of both steroids and *ent*-steroids are in nearly the same place in three-dimensional space. This implies that the effects observed for C-18 and C-19 Me groups in steroids and *ent*-steroids should be the same. In steroids, their potentiation effects are little altered when the C-19 Me group is absent.[24] By contrast, removal of the C-18 Me group can have a major impact on [^{35}S]TBPS displacement potency and single channel kinetics.[16,17] Hence, we expected similar effects for the C-18 and C-19 Me group substitution pattern in active *ent*-steroids. The effect of this Me group substitution pattern was evaluated using compounds ***ent*-7** and ***ent*-10–*ent*-12**.

Compound ***ent*-11** lacks the C-19 Me group. Relative to compound ***ent*-7**, which has both the C-18 and C-19 Me groups, ***ent*-11** has diminished activity in all three screening bioassays. Removing the C-18 Me group, as in compound ***ent*-10**, causes a larger loss of activity relative to compound ***ent*-7** than does removal of the C-19 Me group (***ent*-11**) in all three screening bioassays. Compound ***ent*-12**, which lacks both the C-18 and C-19 Me groups, is not an effective potentiator in the electrophysiology screen and is inactive in the tadpole screens at or below 10 μM. This di-nor *ent*-steroid is a very weak displacer of [^{35}S]TBPS. Overall the activities of compounds ***ent*-7** and ***ent*-11**, the compounds with the C-18 Me group, are similar to each other, and compounds ***ent*-10** and ***ent*-12**, the compounds without the C-18 Me group are similar to each other. Consistent with expectations, those with the C-18 Me group have the higher activity. We attribute the effect of the C-19 Me group to be explained in terms of its effect on logP. Removing this substituent, lowers logP (***ent*-7**, logP 3.71; ***ent*-11**, logP 3.24) thereby decreasing a compound's accumulation in the membrane where the steroid potentiation sites on the GABA$_A$ receptor are located.[26,28] As a consequence of its lower effective concentration in the membrane bilayer, compound ***ent*-11** has modestly decreased activity. With regard to the large loss of activity found when the C-18 Me group is not present, we propose that this is due to removal of a structural feature that is required to meet pharmacophore requirements for high activity. The calculated logP values for compounds ***ent*-10** and ***ent*-11** are the same (3.24) indicating that logP alone does not adequately explain why compounds without a C-18 Me group have such low activity. The very low activity of compound ***ent*-12** (10 μM), which lacks both Me groups is likely due to the combination of the logP (2.77) and pharmacophore requirement effects. The correlation of logP with biological activity is further addressed later in the Discussion.

We carried out electrophysiologcal experiments with three different mutated forms of GABA$_A$ receptors to verify that potentiation effects of *ent*-16-ketosteroids (***ent*-7**, ***ent*-9**) were affected by these mutations in the same way as those of endogeneous neurosteroid **5**. The effects of all three mutations on the actions of the two *ent*-16-ketosteroids and steroid **5** were the same. We infer that the actions of the *ent*-steroids examined in this study are mediated by the previously identified steroid modulation site.[26]

We conducted single-channel measurements on the effects of analogues ***ent*-7** and ***ent*-9–*ent*-12** for two reasons. The first goal was to determine the kinetic components through which these *ent*-steroids potentiate rat α$_1$β$_2$γ$_{2L}$ receptors, for comparison with changes observed in the presence of steroid **5**. The second goal was to determine whether changes in *ent*-steroid structure correlate with the specific kinetic effects. Previous studies employing

single-channel kinetic analysis have revealed, for example, that the presence of the C-18 Me group in a steroid as well as the configuration at C-5 (α vs. β) can determine the ability of a steroid to affect the mean duration of long channel openings.[16] Data presented in this study significantly extend prior knowledge and satisfy both of these goals.

We found that compound *ent*-**9** had the identical profile of single-channel kinetic effects as the previously studied endogenous neurosteroid **5**.[28] Removing the axial 4α-OMe group (*ent*-**7**) removed the effect of increasing the mean lifetime of long open events. We then examined the effect of C-18, C-19 Me substituents on the profile of kinetic effects observed with compound *ent*-**7**. We found that the C-19 Me group's effect correlated with open time properties. By contrast, the C-18 Me group's effect correlated with decreased prevalence of the long closed state. The C-18 Me group seems to be a specific structural effect, whereas the C-19 Me group does not, since some cyclopenta[*b*]phenanthacene and cyclopenta[*b*]anthracenes analogues without a Me group placed in a position equivalent to that occupied by the steroid C-19 Me group, but containing the equivalent of a steroid C-18 Me group, produce similar effects on long open state events.[29] For this reason, we postulate that the C-19 Me group in both steroids and *ent*-steroids having a *trans* A,B-ring fusion increases analogue potentiation predominately by an effect on logP. Comparison of single channel data (Table 4) with whole-cell recordings (Table 2) indicates that the kinetic component that contributes most to enhancement of the whole-cell peak response is the reduction in the fraction of long closed events. We note that this is in agreement with our previous study examining GABA$_A$ receptor potentiation by steroid **5**.[28,30]

The correlation of logP with the biological activity of neurosteroids merits further discussion. The introduction of polar substituents at the C-11 and/or the C-21 position of neurosteroid **5** yields analogues with reduced activity at GABA$_A$ receptors. The decreased activity of the more polar analogues strongly correlates with logP.[31,32] However, the correlation does not rule out the possibility that the polar substituents yield analogues which do not satisfy the pharmacophore requirements for maximal activity and that the correlation with logP is fortuitous. More generally, it implies that making structural changes that affect logP and pharmacophore requirements are difficult to interpret when both parameters change in the same direction. There are two ways to circumvent this commonly encountered interpretation difficulty in SAR studies. One is to use enantiomers which have identical logP values. Thus, changes in pharmacological activity (in bioassays where pharmacokinetic and metabolism issues are not confounding factors) can be unambiguously correlated with fit to the pharmacophore requirements. The second way is to identify analogues wherein the logP and pharmacological activity move in opposite directions. Both approaches are exemplified in this study.

Overall, we failed to find a correlation between [^{35}S]TBPS IC$_{50}$ values and logP for those compounds which strongly displaced [^{35}S]TBPS IC$_{50}$ < 500 nM) as shown in Figure 6. As a specific example, the endogeneous neurosteroid **5** and analogue *ent*-**9** have very different logP values (**5**, logP 4.46; *ent*-9, 3.19) but essentially the same IC$_{50}$ values. In total, five enantiomeric pairs of steroids were examined in this study and in all cases the pairs of enantiomers had different activities. Since our mutagenesis data support the conclusion that the pairs of enantiomers act at the same steroid potentiation site on the GABA$_A$ receptor, this most likely reflects the ability of one enantiomer in each pair, independent of logP, to meet pharmacophore requirements better than the other enantiomer. It remains possible that the neurosteroid potentiation site has a different induced fit when bound to steroids than it does when bound to *ent*-steroids, but this does not invalidate our conclusion. Rather, it only indicates that receptor–ligand interactions should not be viewed as a rigid lock and key fit, but as an induced fit between both molecules as is now widely appreciated in SAR studies.

Finally, we examined the *in vivo* anesthetic effects of compounds ***ent*-7** and ***ent*-9** in mice (Figure 5). At equivalent doses, both compounds induced LRR for a longer period of time than did anesthetic steroid **6**. Compound ***ent*-9** was particularly potent at causing LRR. It induced about twice the length of LRR at a dose of 8 mg/kg as did anesthetic steroid **6** at the higher dose of 16 mg/kg. Our expectation is that the lower logP value of compound ***ent*-9** will increase its water solubility and that *ent*-steroids, in general, may provide a structural framework for the development of anesthetic steroid analogues with improved water solubility.

Additional efforts will be required to determine the full potential of *ent*-steroids as clinically useful drugs. As noted above, compound ***ent*-9** has the potential to be a clinically useful anesthetic. Other *ent*-steroids could be potentially useful for treating conditions in which increasing neuronal inhibition by potentiation of GABA$_A$ receptor function has proven useful (e.g., seizures, anxiety, depression).[1,33] Since enzymes and nuclear hormone receptors that bind endogeneous steroids have structurally defined chiral binding sites, there is the potential that *ent*-steroids will not be bound with high affinity to these proteins. If so, then *ent*-steroid drugs might not strongly interfere with endogenous steroid hormone biosynthesis or be agonists for steroid hormone receptors. Several examples of the failure of *ent*-steroid hormones to act as agonists at nuclear receptors are already reported in the literature.[34-36] Results from those studies led to the suggestion that *ent*-steroid drugs have the potential to treat brain disorders or traumatic brain injury without acting as hormones that stimulate the development and growth of hormone-dependent cancers.[36,37]

It is also possible that the half-lives of *ent*-steroid drugs may be quite different, and potentially longer, than those of steroid drugs used as anticonvulsants, anxiolytics, and other neuroactive agents. In this regard, liver metabolism of *ent*-steroids has received almost no attention thus far. One reported study of the glucuronidation of three *ent*-steroids showed that the enantiomers are preferred substrates for different glucuronosyltransferases than those that accept the naturally occurring enantiomers as substrates.[38] Other differences in liver metabolism are also likely to occur. Thus, it is likely that any effort to develop *ent*-steroids as drugs will require a great deal of effort with the attendant generation of a lot of new knowledge as so little is known about the *in vivo* actions and metabolism of *ent*-steroids in humans.

CONCLUSION

We conclude that a previously proposed model showing the alignment of androsterone enantiomers at the steroid potentiation site on GABA$_A$ receptors has predictive value. The model accurately predicted: 1) that the potentiating effects of compound ***ent*-1** would be increased in an analogue with the 16-ketone group (***ent*-7**); 2) that high activity would be maintained by adding by adding an axial 4α-OMe substituent (***ent*-9**), but not an axial 2α-OMe group (***ent*-8**); and 3) that the C-18 Me group in *ent*-16-ketosteroids is more important for enhancement of potentiation than the C-19 Me group. We compared the length of loss of righting reflex in mice induced by tail vein injection of anesthetic steroid **6** and analogues ***ent*-7** and ***ent*-9**. We found that both *ent*-steroids induced a longer period of loss of righting reflex at a lower dose than did steroid **6**. We conclude that the high activity of these two compounds results from their ability to satisfy pharmacophore requirements, not because of an increase in logP, since compound ***ent*-9**, has a lower logP than anesthetic steroid **6** and both of these *ent*-neurosteroids have a lower logP than endogeneous neurosteroid **5**. We propose that *ent*-steroids have high potential for development as both intravenous anesthetics and drugs to treat brain disorders that are alleviated by potentiation of GABA$_A$ receptor function.

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 or 400 MHz (^1H), 74 or 100 MHz (^{13}C). Purity of > 95% was determined for all evaluated compounds by combustion analysis for C,H performed by M-H-W Laboratories (Phoenix, AZ). Steroids **1**,**5**,**6**,**13**,**14** and **18** were purchased from Steraloids (Newport, RI). K(*sec*-Bu)$_3$BH (K-Selectride) was purchased from Aldrich Chemical Co. (Milwaukee, WI). The logP values were calculated using ChemDraw from CambridgeSoft (Cambridge, MA).

(5β,8α,9β,10α,13α,14β)-Androstane-3,17-dione (*ent*-1)

The compound was prepared as previously described.[9]

(2α,3β,5β,8α,9β,10α,13α,14β)-3-Hydroxy-2-methoxyandrostan-17-one (*ent*-2)

The 2α,3α-epoxide *ent*-**17** (250 mg, 0.86 mmol), containing minor amounts of the 2β,3β-epoxide, was dissolved in MeOH (10 mL), a drop of conc. H_2SO_4 was added and the reaction was stirred at room temperature for 3 hr. The reaction mixture was made basic with aqueous $NaHCO_3$ and the MeOH was removed under reduced pressure. The resulting residue was diluted with water and extracted with EtOAc (3×80 mL). The combined organic extracts were dried and concentrated to give a solid which was purified by column chromatography (silica gel eluted with 20–35% EtOAc in hexanes) to give *ent*-**2** as a white solid (170 mg, 62%): mp 153–155 °C; $[\alpha]_D^{23}$ –98.3 (*c* 0.23, $CHCl_3$); IR ν_{max} 3438, 2920, 1739, 1595, 1453, 1406, 1372, 1255, 1214 cm^{-1} ; ^1H NMR ($CDCl_3$) δ 3.95 (br s, 1H), 3.32 (br s, 4H), 2.45 (dd, 1H, *J* = 19.2 Hz, 8.7 Hz), 1.00 (m, 1H), 0.95 (s, 3H), 0.85 (s, 3H), 0.77 (m, 1H); ^{13}C NMR δ 221.5, 80.6, 68.1, 56.6, 55.1, 51.4, 47.8, 39.0, 36.1, 35.8, 35.5, 34.5, 32.0, 31.5, 30.8, 27.8, 21.7, 20.1, 13.8, 13.1. Anal. ($C_{20}H_{32}O_2$) C, H.

(2β,3α,5α)-3-Hydroxy-2-methoxyandrostan-17-one (2)

Steroid **2** (206 mg, 73%) was prepared from the natural enantiomer of compound *ent*-**17** using the procedure described for the preparation of compound *ent*-**2.** Flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) gave product **2**: mp 151–153 °C; $[\alpha]_D^{23}$ +92.4 (*c* 0.37, $CHCl_3$); IR ν_{max} 3439, 1738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.84–3.83 (m, 1H), 3.22 (br s, 4H), 2.54 (br s, 1H), 2.38 (dd, 1H, *J* = 19.2 Hz, 8.8 Hz), 0.86 (s, 3H), 0.76 (s, 3H), 0.71–0.64 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 221.4, 80.5, 67.7, 56.4, 54.9, 51.3, 47.6, 38.7, 35.9, 35.6, 35.2, 34.3, 31.8, 31.4, 30.6, 27.6, 21.5, 19.9, 13.6, 12.9. Anal. ($C_{20}H_{32}O_3$) C, H.

(3β,4α,5β,8α,9β,10α,13α,14β)-3-Hydroxy-4-methoxyandrostan-17-one (*ent*-3)

Compound *ent*-**3** (200 mg, 78%) was prepared from compound *ent*-**20** using the procedure described for the preparation of *ent*-**2.** Flash column chromatography (silica gel eluted with 35% EtOAc in hexanes) gave product *ent*-**3** as a white solid: mp 215–218 °C; $[\alpha]_D^{23}$ –99 (*c* 0.06, $CHCl_3$); IR ν_{max} 3510, 2917, 2838, 1735, 1594, 1443, 1375, 1242 cm^{-1} ; ^1H NMR ($CDCl_3$) δ 4.02 (br s, 1H), 3.35 (s, 3H), 3.04 (s, 1H), 2.44 (dd, 1H, *J* = 19.0 Hz, 9.0 Hz), 0.98 (s, 3H), 0.86 (s, 3H), 0.62 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 221.5, 85.4, 66.1, 59.0, 55.2, 51.5, 47.8, 44.0, 36.2, 35.8, 35.0, 31.8, 31.5, 31.1, 25.2, 25.0, 21.7, 19.6, 14.0, 13.8. Anal. ($C_{20}H_{32}O_3$) C, H.

(3α,4β,5α)-3-Hydroxy-4-methoxyandrostan-17-one (3)

Steroid **3** (270 mg, 81%) was prepared from the natural enantiomer of compound ***ent*-20** using the procedure described for the preparation of compound ***ent*-2.** Flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) gave product ***ent*-3** as a white solid : mp 223–225 °C; $[\alpha]_D^{20}$ +95 (*c* 0.06, CHCl$_3$); IR ν_{max} 3513, 1734 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.97 (br s, 1H), 3.30 (s, 3H), 2.95–2.85 (m, 1H), 2.44 (dd, 1H, *J* = 19.3 Hz, 8.8 Hz), 0.93 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 221.7, 85.7, 66.3, 59.1, 55.4, 51.7, 47.9, 44.2, 36.3, 36.0, 35.2, 32.0, 31.7, 31.3, 25.4, 25.1, 21.9, 19.8, 14.1, 14.0. Anal. (C$_{20}$H$_{32}$O$_3$) C, H.

[3α,5α,17(20)*Z*]-3-Hydroxy-11-oxopregn-17(20)-ene-21-nitrile (4)

The compound was prepared as previously described.[15]

(3β,5β,8α,9β,10α,13α,14β)-3-Hydroxyandrostan-16-one (*ent*-7)

Compound ***ent*-24** (88 mg, 0.27 mmol) was dissolved in methanol (10 mL) and water (0.5 mL) and K$_2$CO$_3$ (5 mmol, 680 mg) were added. The reaction was refluxed for 2 h, cooled to room temperature, water (30 mL) was added and the product was extracted into EtOAc (50 mL × 3). The combined extracts were dried, filtered, and the solvent removed. The residue was purified by flash column chromatography (silica gel eluted with 25–40% EtOAc in hexanes) to give product ***ent*-7** (72 mg, 93%) as a white solid: mp 152–153 °C; $[\alpha]_D^{23}$ +156.8 (*c* 0.25, CHCl$_3$); IR ν_{max} 3435, 1737 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.06 (m, 1H), 0.87 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 219.0, 66.4, 55.9, 54.2, 51.7, 39.3, 39.2, 39.0, 38.2, 36.3, 35.8, 34.9, 32.2, 31.9, 28.9, 28.3, 20.3, 18.1, 11.2. Anal. (C$_{19}$H$_{30}$O$_2$) C, H.

(3α,5α)-3-Hydroxyandrostan-16-one (7)

Steroid **7** (50 mg, 90%) was prepared from the natural enantiomer of compound ***ent*-24** using the procedure described for the preparation of compound ***ent*-7.** Product **7** was obtained as a white solid: mp 152–154 °C (lit mp[39] 153–154 °C); $[\alpha]_D^{23}$ –154.0 (*c* 0.20, CHCl$_3$); IR ν_{max} 3441, 1738 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.05 (m, 1H), 0.87 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 219.0, 66.2, 55.8, 54.2, 51.6, 39.2, 39.1, 38.9, 38.1, 36.2, 35.7, 34.8, 32.2, 31.8, 28.8, 28.2, 20.2, 18.0, 11.2. Anal. (C$_{19}$H$_{30}$O$_2$) C, H.

(2α,3β,5β,8α,9β,10α,13α,14β)-3-Hydroxy-2-methoxyandrostan-16-one (*ent*-8)

Compound ***ent*-8** (120 mg, 97%) was prepared from compound ***ent*-27** using the procedure described for the preparation of compound ***ent*-7.** Flash column chromatography (silica gel eluted with 25–40% EtOAc in hexanes) gave product ***ent*-8** as a white solid: mp 209–211 °C; $[\alpha]_D^{23}$ +144 (*c* 0.25, CHCl$_3$); IR ν_{max} 3447, 1729 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.95 (br s, 1H), 3.32 (s, 4H), 0.95 (s, 3H), 0.85 (s, 3H); ^{13}C NMR δ 218.9, 80.6, 68.0, 56.6, 55.8, 54.9, 51.7, 39.2, 39.1, 38.8, 38.2, 36.1, 35.3, 34.3, 32.2, 32.0, 27.8, 20.4, 18.1, 13.0. Anal. (C$_{20}$H$_{32}$O$_3$) C, H.

(2β,3α,5α)-3-Hydroxy-2-methoxyandrostan-16-one (8)

Steroid **8** (68 mg, 94%) was prepared from the natural enantiomer of ***ent*-27** using the procedure described for the preparation of compound ***ent*-7.** Product **8** was obtained as a white solid: mp 205–207 °C; $[\alpha]_D^{23}$ –143.5 (*c* 0.20, CHCl$_3$); IR ν_{max} 3445, 1730 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.92 (br s, 1H), 3.31 (s, 4H), 0.94 (s, 3H), 0.84 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 218.9, 80.6, 68.0, 56.6, 55.8, 54.9, 51.7, 39.2, 39.1, 38.8, 38.2, 36.1, 35.3, 34.3, 32.2, 32.0, 27.8, 20.4, 18.1, 13.0. Anal. (C$_{20}$H$_{32}$O$_3$) C, H.

(3β,4α,5β,8α,9β,10α,13α,14β)-3-Hydroxy-4-methoxyandrostan-16-one (*ent*-9)

Compound *ent*-9 (50 mg, 94%) was prepared from compound *ent*-30 using the procedure described for the preparation of compound *ent*-7. Flash column chromatography (silica gel eluted with 25–40% EtOAc in hexanes) gave product *ent*-9 as a white solid: mp 198–200 °C; $[\alpha]_D^{23}$ +152 (*c* 0.2, CHCl$_3$); IR ν_{max} 3458, 1725 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.01 (br s, 1H), 3.34 (s, 3H), 3.02 (d, 1H, *J* = 1.1 Hz), 0.98 (s, 3H), 0.87 (s, 3H); ^{13}C NMR δ 219.0, 85.4, 66.2, 59.0, 55.9, 55.1, 51.8, 43.9, 39.3, 39.3, 38.1, 36.2, 34.9, 32.6, 31.6, 25.2, 25.0, 19.9, 18.1, 14.0. Anal. (C$_{20}$H$_{32}$O$_3$) C, H.

(3α,4β,5α)-3-Hydroxy-4-methoxyandrostan-16-one (9)

Steroid 9 (62 mg, 90%) was prepared from the natural enantiomer of compound *ent*-30 using the procedure described for the preparation of compound *ent*-7. Steroid 9 was obtained as a white solid: mp 196–198 °C; $[\alpha]_D^{23}$ −141.8 (*c* 0.17, CHCl$_3$); IR ν_{max} 3445, 1725 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.99 (br s, 1H), 3.33 (s, 3H), 3.00 (m, 1H), 0.97 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 219.0, 85.4, 66.2, 59.0, 55.9, 55.1, 51.8, 43.9, 39.3, 39.1, 38.1, 36.2, 34.9, 32.5, 31.6, 25.2, 25.0, 19.9, 18.1, 14.0. Anal. (C$_{20}$H$_{32}$O$_3$) C, H.

(3β,5β,8α,9β,10α,13α,14β)-18-Norandrostan-16-one (*ent*-10)

A mixture of β-ketoester *ent*-46 (25 mg, 0.075 mmol), LiCl (100 mg) and DMF was heated at 160 °C for 30 min under N$_2$. The reaction mixture was cooled, diluted with water and extracted with EtOAc. The combined organic extracts were dried and concentrated to give an off-white solid. The crude product was purified by flash column chromatography (silica gel eluted with 30–40% EtOAc in hexanes) to give product *ent*-10 as a white solid (18 mg, 86%): mp 174–177 °C; $[\alpha]_D^{23}$ +171.9 (*c* 0.1, CHCl$_3$); IR ν_{max} 3475, 2923, 2853, 1723 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.06 (br s, 1H), 2.36 (m, 2H), 0.78 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 218.5, 66.4, 53.3, 50.2, 45.9, 43.8, 43.3, 41.7, 38.8, 36.2, 35.8, 32.2, 32.0, 31.1, 29.0, 28.1, 24.8, 11.1. Anal. (C$_{18}$H$_{28}$O$_2$) C, H.

(3β,5β,8α,9β,10α,13α,14β)-19-Norandrostan-16-one (*ent*-11)

Compound *ent*-11 (207 mg, 98%) was prepared from compound *ent*-33 using the procedure described for the preparation of compound *ent*-7. Flash column chromatography (silica gel eluted with 25–40% EtOAc in hexanes) gave product *ent*-11 as a white solid: mp 160–162 °C; $[\alpha]_D^{23}$ +162.0 (*c* 0.22, CHCl$_3$); IR ν_{max} 3401, 2915, 2861, 1743 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.08 (br s, 1H), 0.87 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 218.8, 66.3, 56.0, 50.9, 48.0, 47.0, 40.53, 40.47, 39.2, 39.1, 38.1, 35.8, 33.4, 32.9, 31.2, 25.1, 23.4, 18.1. Anal. (C$_{18}$H$_{28}$O$_2$) C, H.

(3β,5β,8α,9β,10α,13α,14β)-3-Hydroxygonan-16-one. (*ent*-12)

K-Selectride (1M in THF, 0.45 mL) was added dropwise under N$_2$ to a cooled solution (−78 °C) of compound *ent*-58 (108 mg, 0.41 mmol) in anhydrous THF (15 mL). After 2 h stirring at −78 °C, water (2 mL) was added and the mixture was allowed to reach room temperature. Then, aqueous NaOH (2 mL, 6 M), and 30% H$_2$O$_2$ (2 mL) were added and the reaction was stirred for 30 min. The product was extracted with CH$_2$Cl$_2$ (2 × 50 mL), the combined extracts were washed with aqueous HCl (1 N), saturated aqueous NaHCO$_3$, and brine. Solvent was dried and evaporated. Flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) gave product *ent*-12 (76 mg, 70%) as a white solid: mp 168–169 °C (EtOAc/hexanes); $[\alpha]_D^{23}$ +218.6 (*c* 0.17, CHCl$_3$). IR ν_{max} 3466, 2917, 2846, 1724 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.08 (1H, m), 2.30–2.41 (2H, m), 1.97–2.10 (2H, m). ^{13}C NMR (CDCl$_3$) δ 218.5, 66.5, 49.3, 47.7, 47.3, 47.2, 46.1, 43.8, 43.6, 40.7, 35.9, 33.5, 33.1, 31.4, 31.1, 29.6, 23.6. Anal. (C$_{17}$H$_{26}$O$_2$) C, H.

(5β,8α,9β,10α,13α,14β)-Androstane-3,17-dione (*ent*-13)

This compound was prepared as described previously.[9]

(3α,5β,8α,9β,10α,13α,14β)-3-Hydroxyandrostan-17-one (*ent*-14)

A 1M THF solution of Li(*t*-OBu)$_3$AlH (6.86 mL, 1.1 equiv) was added to a cold (−40 °C) solution of compound *ent*-13 (1.80 g, 6.24 mmol) in THF (15 mL), and the resulting mixture was stirred at −40 °C for 90 min. A few drops of acetone were added to consume excess hydride reagent and the mixture was stirred for a few min. Then 1M HCl was added and the reaction was warmed to room temperature. The product was extracted into EtOAc and the combined organic extracts were washed with brine, dried and the solvent removed to give an oil, which was purified by flash column chromatography (silica gel eluted with 30–40% EtOAc in hexanes) to give product *ent*-14 as a colorless solid (1.49 g, 82%): mp 171–173 °C; [α]$_D^{23}$ −95.4 (*c* 0.13, CHCl$_3$); IR ν_{max} 3470, 2928, 2853, 1732, 1450, 1374 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.59 (m, 1H), 2.41 (dd, 1H, *J* = 19.2 Hz, 9.0 Hz), 2.05 (dd, 1H, *J* = 19.2 Hz, 9.0 Hz), 0.85 (s, 3H), 0.83 (s, 3H), 0.69 (m, 1H); ^{13}C NMR (CDCl$_3$) δ 221.4, 71.1, 54.4, 51.4, 47.8, 44.8, 38.0, 36.9, 35.8, 35.6, 35.0, 31.5, 31.4, 30.9, 28.4, 21.7, 20.5, 13.8, 12.3. Anal. (C$_{20}$H$_{32}$O$_3$) C, H.

(3α,5β,8α,9β,10α,13α,14β)-3-[(Methylsulfonyl)oxy]-androstan-17-one (*ent*-15)

MeSO$_2$Cl (0.58 mL, 7.5 mmol) was added to a cold solution (0 °C) of compound *ent*-14 (1.45 g, 5 mmol) and Et$_3$N (1.4 ml, 10 mmol) in CH$_2$Cl$_2$ (15 mL) and the mixture was slowly warmed to room temperature (~15 min) and stirred for 30 min. An aqueous saturated NaHCO$_3$ solution was added and the product was extracted into CH$_2$Cl$_2$. The organic extracts were washed with brine, dried and concentrated to give an oil, which was purified by flash column chromatography (silica gel eluted with 15–30% EtOAc in hexanes) to give mesylate *ent*-15 as a white solid (1.84 g, 100%): mp 150–152 °C (dec); IR ν_{max} 2919, 1737, 1452, 1352 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.43 (m, 1H), 2.86 (s, 3H), 2.27 (dd, 1H, *J* = 19.2 Hz, 8.8 Hz), 0.71 (s, 3H), 0.70 (s, 3H), 0.57 (m, 1H); ^{13}C NMR (CDCl$_3$) δ 220.2, 81.3, 53.6, 50.7, 47.2, 44.2, 38.2, 36.2, 35.3, 34.9, 34.6, 34.4, 31.0, 30.2, 28.1, 27.7, 21.2, 20.0, 13.3, 11.7.

(3β,5α)-3-[(Methylsulfonyl)oxy]-androstan-17-one (15)

The known steroid **15**[40] (1.62 g, 99 %) was prepared from the natural enantiomer of compound *ent*-14 using the procedure described for the preparation of compound *ent*-15.

(5β,8α,9β,10α,13α,14β)-Androst-2-en-17-one (*ent*-16)

Mesylate *ent*-15 (1.60 g, 4.34 mmol) and LiBr (3.89 g, 44.7 mmol) in DMF (15 mL) were heated at 125 °C under N$_2$ for 2 h. The reaction mixture was cooled, made basic with aqueous NaHCO$_3$ and extracted with EtOAc. The combined organic extracts were washed with brine, dried and concentrated to give an oil which was purified by flash column chromatography (silica gel eluted with 5–20% EtOAc in hexanes) to give the a 5:1 mixture (determined by NMR) of Δ^2 and Δ^3 olefins as a white solid (1.03 g, 87%): IR ν_{max} 3021, 2933, 2886, 2842, 1744, 1655, 1467, 1377 cm^{-1}; (major isomer) ^{1}H NMR (CDCl$_3$) δ 5.52 (m, 2H), 2.38 (dd, 1H, *J* = 19.2 Hz, 8.8 Hz), 0.81 (s, 3H), 0.72 (s, 3H); (major isomer) ^{13}C NMR(CDCl$_3$) δ 220.8, 125.5, 125.5, 54.0, 51.2, 47.4, 41.2, 39.5, 35.6, 34.9, 34.5, 31.4, 30.4, 30.0, 28.2, 21.6, 20.0, 13.5, 11.5.

(5α)-Androst-2-en-17-one (16)

The known steroid **16**[39] (1.1 g, 91%, 7:1 mixture of Δ^2 and Δ^3 olefins) was prepared from the natural enantiomer of compound *ent*-**15** using the procedure described for the preparation of compound *ent*-**16**.

(2α,3α,5β,8α,9β,10α,13α,14β)-2,3-Epoxyandrostan-17-one (*ent*-17)

Compound *ent*-**16** (containing the unseparated Δ^3 olefin, 0.97 g, 3.56 mmol) was dissolved in CH_2Cl_2 (20 mL) and HCO_2H (1.34 mL) and then 30% H_2O_2 (3.3 mL) was added and the reaction was stirred for 4 h at room temperature. MeOH (20 mL) was added and after 3 min, 10% aqueous NaOH (15mL) was added and the mixture was stirred for 5 min. 10% HCl (20 mL) was added dropwise and the resulting mixture was stirred for 3 min. The product was extracted into CH_2Cl_2 (3×150 mL) and the organic extracts were dried and concentrated to give an oil which was purified by flash column chromatography (silica gel eluted with 20–30% EtOAc in hexanes) to give the epoxide *ent*-**17** (containing the 3,4-epoxide) as a white solid (1.06 g, 100 %): IR ν_{max} 2918, 1740, cm^{-1} ; (major isomer) ^{1}H NMR (CDCl$_3$) δ 3.10–2.98 (m, 2H), 2.40 (dd, 1H, J = 19.6 Hz, 9.0 Hz), 0.72 (s, 3H), 0.66 (s, 3H), 0.56 (m, 1H); (major isomer) ^{13}C NMR (CDCl$_3$) δ 220.4, 53.5, 51.8, 50.9, 50.4, 47.2, 37.9, 35.9, 35.4, 34.8, 33.4, 31.2, 30.2, 28.7, 27.8, 21.4, 19.8, 13.3, 12.3.

(2β,3β,5α)-2,3-Epoxyandrostan-17-one (17)

The known steroid **17**[41] (1.05 g, 91%) was prepared from the natural enantiomer of compound *ent*-**16** (containing the unseparated Δ^3 olefin) using the procedure described for the preparation of compound *ent*-**17**.

(8α,9β,10α,13α,14β)-Androst-4-ene-3,17-dione (ent-18)

This compound was prepared as previously described.[7]

(5β,8α,9β,10α,13α,14β)-Androst-3-en-17-one (*ent*-19)

To a boiling solution of compound *ent*-**18** (750 mg, 2.63 mmol) in glacial AcOH (40 mL), Zn dust (4.5g) was added in several portions during a period of 15 min and then heating was continued an additional 15 min. The reaction was cooled, and the Zn dust was filtered, and the filtrate was collected. The filter-cake was washed with AcOH and EtOAc. Solvents were removed from the combined filtrates and washings, water was added to the residue and the product was extracted into EtOAc. The combined extracts were washed with aqueous NaHCO$_3$, brine, dried and solvents were evaporated to give a white solid which is a mixture (2.3:1 by NMR) of product *ent*-**19** and the epimeric (5α)-3-ene product. The product mixture was crystallized from hexanes to give pure product *ent*-**19** (310 mg, 43%): mp 122–125 °C; IR ν_{max} 3016, 2968, 2940, 2837, 2807, 1742, 1470, 1443, 1376, 1251 cm^{-1} ; ^{1}H NMR (CDCl$_3$) δ 5.60 (m, 1H), 5.29 (m, 1H), 2.44 (dd, 1H, J = 19.0 Hz, 9.0 Hz), 0.88 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 221.3, 131.0 125.6, 53.5, 51.5, 47.9, 45.9, 35.8, 35.1, 35.0, 34.0, 31.6, 30.9, 27.1, 23.4, 21.8, 20.3, 13.9, 11.8.

(5α)-Androst-3-en-17-one (19)

The known steroid **19**[42] (350 mg, 37%) was prepared from the natural enantiomer of compound *ent*-**18** using the procedure described for the preparation of compound *ent*-**19**.

(3β,4β,5β,8α,9β,10α,13α,14β)-2,3-Epoxyandrostan-17-one (*ent*-20)

Compound *ent*-**20** (300 mg, 84%) was prepared from compound *ent*-**19** using the procedure described for the preparation of compound *ent*-**17**. The crude product was obtained as an oil and after flash column chromatography (silica gel eluted with 20–30% EtOAc in hexanes)

yielded a white solid: mp 148–150 °C; IR ν_{max} 2928, 2882, 2859, 1740, 1472, 1446, 1405, 1373, 1251 cm^{-1} ; ^{1}H NMR (CDCl$_3$) δ 3.09 (s, 1H), 2.63 (d, 1H, J = 3.9 Hz), 2.36 (dd, 1H, J = 19.6 Hz, 9.0 Hz), 0.79 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 220.7, 55.4, 52.5, 51.9, 51.0, 47.6, 46.6, 35.6, 34.8, 34.0, 31.3, 30.5, 30.2, 26.3, 21.5, 21.1, 20.4, 13.7, 13.3.

(2α,3α)-2,3-Epoxyandrostan-17-one (20)

The known steroid **20**[43] (300 mg, 95%) was prepared from the natural enantiomer of compound **ent-19** using the procedure described for the preparation of compound **ent-16**. Steroid **20** had: IR ν_{max} 2926, 1738, 1403, 1250 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.05–3.04 (m, 1H), 2.61 (d, 1H, J = 3.9), 2.35 (dd, 1H, J = 18.7, 10.2), 0.76 (s, 3H), 0.70 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 220.5, 55.4, 52.5, 51.8, 51.0, 47.5, 46.6, 35.5, 34.7, 34.0, 31.3, 30.5, 30.1, 26.3, 21.5, 21.0, 20.3, 13.7, 13.2.

(3β,5β,8α,9β,10α,13α,14β)-3-Hydroxyestran-17-one (ent-21)

Compound **ent-21** was prepared from (8α,9β,10α,13α,14β,17α)-17-hydroxyestr-4-en-3-one[37] using the methods reported previously for the preparation of compound **ent-1** from *ent*-testosterone.[9] Compound **ent-21** had: mp 158–160 °C; [α]$_D$ = −108.5 (*c* 0.24, CHCl$_3$); IR ν_{max} 3487, 2859, 1739, 1444, 1374, 1261 cm^{-1}; ^{1}H NMR (CHCl$_3$) δ 4.01 (s, 1H), 2.42 (dd, 1H, J = 19.3 Hz, 9.8 Hz), 0.86 (s, 3H); ^{13}C NMR (CHCl$_3$) δ 221.6, 66.3, 50.6, 48.2, 47.9, 47.0, 40.7, 40.5, 35.9, 35.8, 33.4, 32.9, 31.4, 29.8, 24.9, 23.7, 21.6, 13.8.

(3α,5α)-3-Hydroxyestran-17-one (21)

– The compound was prepared as previously described.[44]

(3β,5β,8α,9β,10α,13α,14β,17α)-16-(Phenylmethylene)-androstane-3,17-diol diacetate (ent-22)

Compound **ent-1** (1.25 g, 4.5 mmol) and benzaldehyde (1.4 mL, 14 mmol) were added to KOH (700 mg) dissolved in EtOH (60 mL) and the reaction was stirred at room temperature for 16 h. The reaction mixture was cooled to 0 °C and CeCl$_3$·7 H$_2$O (7.45 g, 20 mmol) and NaBH$_4$ (756 mg, 20 mmol) were added, the reaction was allowed to warm to room temperature and stirring continued for 3 h. Glacial AcOH (7 mL) was added and the product extracted into EtOAc (3 × 150 mL). The combined extracts were dried and the solvents removed to give an oil. The oil was dissolved in CH$_2$Cl$_2$ (30 mL) and AcOAc (1.4 mL, 15 mmol), NEt$_3$ (4.2 ml, 30 mmol) and DMAP (200 mg) were added and stirring was continued at room temperature for 4 h. Aqueous saturated NaHCO$_3$ was then added. After 1 h, the product was extracted into CH$_2$Cl$_2$ (3 × 125 mL). The combined extracts were dried and solvents removed to give an oil. The crude product was purified by flash column chromatography (silica gel, eluted with 25–35 % EtOAc in hexanes) to give product **ent-22** as a colorless liquid containing benzyl acetate. The benzyl acetate was removed by applying high vacuum at 60 °C for 10 h, to give product **ent-22** as a white solid (1.9 g, 91%): mp 162–164 °C; IR ν_{max} 2932, 2855, 1735, 1492, 1447, 1371, 1237 cm^{-1}; ^{1}H NMR (CHCl$_3$) δ 7.37– 7.17 (m, 5H), 6.21 (d, 1H, J = 2.4 Hz), 5.37 (s, 1H), 5.03 (s, 1H), 2.68 (dd, 1H, J = 16.8 Hz, 6.6 Hz), 2.21 (s, 3H), 2.07 (s, 3H), 0.83 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (CHCl$_3$) δ 171.2, 170.7, 141.0, 137.6, 128. 3 (2 × C), 128.2 (2 × C), 126.4, 123.5, 84.6, 70.0, 54.1, 48.9, 42.9, 40.0, 36.5, 35.9, 34.8, 32.8, 32.7, 31.5, 30.9, 28.1, 26.0, 21.5, 21.2, 20.3, 12.3, 11.3.

(3α,17β)-16-(Phenylmethylene)-androstane-3,17-diol diacetate (22)

Steroid **22** (144 mg, 90%) was prepared from the natural enantiomer of compound **ent-1** using the procedure described for the preparation of compound **ent-21**. Steroid **22** had: ^{1}H NMR (CDCl$_3$) δ 7.29–7.10 (m, 5H), 6.13 (d, 1H, J = 2.4 Hz), 5.29 (s, 1H), 4.94 (s, 1H), 2.62

(dd, 1H, J = 16.8 Hz, 6.6 Hz), 2.12 (s, 3H), 1.98 (s, 3H), 0.74 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 171.1, 170.6, 140.9, 137.5, 128.2, 128.1, 126.4, 123.5, 84.5, 69.9, 54.0, 48.8, 42.9, 39.9, 36.4, 35.8, 34.8, 32.8, 32.7, 31.5, 30.9, 28.1, 26.0, 21.5, 21.1, 20.2, 12.2, 11.3.

(3β,5β,8α,9β,10α,13α,14β,17α)-3,17-Dihydroxyandrostan-16-one diacetate (*ent*-23)

Compound *ent*-23 (110 mg, 82%, starting from compound *ent*-1) was prepared from compound *ent*-22 using the procedure described for the preparation of compound *ent*-26. Compound *ent*-23 was a white solid and had: ^{1}H NMR (CDCl$_3$) δ 4.96 (m, 2H), 2.11 (s, 3H), 2.01 (s, 3H), 0.77 (s, 6H); ^{13}C NMR (CDCl$_3$) δ 211.0, 170.5, 170.2, 85.5, 69.7, 53.8, 45.2, 41.6, 39.7, 36.2, 35.9, 35.8, 34.2, 32.6, 32.4, 31.4, 27.8, 25.8, 21.4, 20.5, 19.7, 12.3, 11.2.

(3α,5α,17β)-3,17-Dihydroxyandrostan-16-one diacetate (23)

Steroid 23 (109 mg, 91%) was prepared from the natural enantiomer of compound *ent*-22 using the procedure described for the preparation of compound *ent*-26. Steroid 23 was a white solid and had: ^{1}H NMR (CDCl$_3$) δ 4.98–4.95 (m, 2H), 2.14 (s, 3H), 2.03 (s, 3H), 0.78 (s, 6H); ^{13}C NMR (CDCl$_3$) δ 211.1, 170.6, 170.3, 85.6, 69.8, 53.9, 45.3, 41.7, 39.8, 36.2, 36.0, 35.9, 34.2, 32.6, 32.5, 31.5, 27.9, 25.9, 21.4, 20.6, 19.8, 12.4, 11.2.

(3β,5β,8α,9β,10α,13α,14β)-3-Hydroxyandrostan-16-one acetate (*ent*-24)

Freshly prepared Sm filings (1.5 mmol, 225 mg) were added to THF (10 mL) and I$_2$ (1.0 mmol, 254 mg) in THF (5 mL) was added at room temperature. The suspension was stirred under N$_2$. After 30 min, the mixture became deep blue indicating SmI$_2$ formation and stirring was continued for another 30 min. Compound *ent*-23 (0.28 mmol, 110 mg) in THF/ methanol (10/1, 11 mL) was added. After 2 h, 10% aqueous Na$_2$CO$_3$ (60 mL) was added and the product was extracted into EtOAc (3 × 50 mL). The combined extracts were washed with brine (2 × 20 mL) and dried. Solvents were removed and the residue was purified by flash column chromatography (silica gel, eluted with 20% EtOAc in hexanes) to give product *ent*-24 (48 mg, 51%) and a compound in which the 16-ketone group had been reduced to a 16-hydroxyl group of undetermined configuration (48 mg). Jones oxidation of the 16-hydroxyl group gave additional compound *ent*-24 (40 mg, 43%): ^{1}H NMR (CDCl$_3$) δ 5.00 (br s, 1H), 2.03 (s, 3H), 0.86 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 218.7, 170.6, 69.9, 55.8, 54.1, 51.7, 39.9, 39.1, 38.1, 35.9, 34.8, 32.7, 32.5, 32.1, 28.0, 26.0, 21.5, 20.3, 18.0, 11.3.

(3α,5α)-3-Hydroxyandrostan-16-one acetate (24)

Steroid 24 (60 mg, 91%) was prepared from the natural enantiomer of compound *ent*-23 using the procedure described for the preparation of compound *ent*-23 and had: ^{1}H NMR (CDCl$_3$) δ 5.00 (br s, 1H), 2.03 (s, 3H), 0.85 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 218.7, 170.6, 69.9, 55.8, 54.1, 51.7, 39.9, 39.2, 38.1, 35.9, 34.8, 32.7, 32.5, 32.1, 28.0, 26.0, 21.5, 20.3, 18.0, 11.3.

(2α,3β,5β,8α,9β,10α,13α,14β,17α)-2-Methoxy-16-(phenylmethylene)-androstane-3,17-diol diacetate (*ent*-25)

Compound *ent*-25 (441mg, 95%) was prepared as a white solid from compound *ent*-2 using the procedure described for the preparation of compound *ent*-22 and had: mp 167–169 °C; IR ν$_{max}$ 2932, 1737, 1599, 1492, 1448, 1369, 1241 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.36–7.06 (m, 5H), 6.21 (d, 1H, J = 2.4 Hz), 5.37 (s, 1H), 5.02 (br s, 1H), 3.36 (br s, 4H), 2.67 (dd, 1H, J = 16.8 Hz, 7.0 Hz), 2.21 (s, 3H), 2.08 (s, 3H), 0.96 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 171.2, 170.3, 141.0, 137.6, 128.3 (2 × C), 128.2 (2 × C), 126.4, 123.6, 84.6, 77.7,

69.9, 57.0, 54.8, 48.9, 43.0, 39.9, 37.0, 36.5, 35.7, 34.3, 31.5, 30.9, 29.0, 27.7, 21.4, 21.2, 20.3, 13.1, 12.3.

(2β,3α,17β)-2-Methoxy-16-(phenylmethylene)-androstane-3,17-diol diacetate (25)

Steroid **25** (150 mg, 95%) was prepared from the natural enantiomer of compound ***ent*-2** using the procedure described for the preparation of compound ***ent*-22** and had: IR ν_{max} 1737, 1240 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.28–7.11 (m, 5H), 6.13 (d, 1H, J = 2.3 Hz), 5.28–5.27 (m, 1H), 4.94 (br s, 1H), 3.27 (br s, 4H), 2.58–2.54 (m, 1H), 2.12 (s, 3H), 1.99 (s, 3H), 0.88 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 171.1, 170.2, 140.9, 137.5, 128.2 (2 × C), 128.1 (2 × C), 126.4, 123.5, 84.5, 77.6, 69.8, 56.9, 54.8, 48.8, 42.9, 39.9, 36.9, 36.5, 35.6, 34.2, 31.4, 30.9, 29.0, 27.6, 21.4, 21.4, 21.1, 20.3, 13.0, 12.2.

(2α,3β,5β,8α,9β,10α,13α,14β,17α)-3,17-Dihydroxy-2-methoxyandrostan-16-one diacetate (*ent*-26)

A stream of O$_3$ was bubbled through a solution of compound ***ent*-25** (345 mg, 0.70 mmol) dissolved in MeOH/EtOAc (2:1; 20 mL) at −78 °C until a blue color persisted for 10 min. The excess O$_3$ was purged from the reaction using an O$_2$ stream as evidenced by the solution becoming colorless. Me$_2$S was then added (2 mL) and the reaction was allowed to warm to room temperature and then allowed to stir for 14 h. Solvents were removed and the residue was purified by flash column chromatography (silica gel, eluted with 30–40% EtOAc in hexanes) to yield compound ***ent*-26** as a foamy white solid (265 mg, 90%): IR ν_{max} 2934, 1762, 1742, 1451, 1370, 1240 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.98 (d, 1H, J = 2.0 Hz), 4.97 (s, 1H), 3.36 (br s, 4H), 2.13 (s, 3H), 2.04 (s, 3H), 0.93 (s, 3H), 0.79 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 210.9, 170.2, 170.1, 85.6, 77.5, 69.7, 56.9, 54.6, 45.2, 41.7, 39.8, 36.7, 36.2, 36.0, 35.7, 33.7, 31.5, 28.8, 27.4, 21.3, 20.6, 19.9, 13.0, 12.4.

(2β,3α,17β)-3,17-Dihydroxy-2-methoxyandrostan-16-one diacetate (26)

Steroid **26** (120 mg, 94%) was prepared as a foamy solid from the natural enantiomer of compound ***ent*-25** using the procedure described for the preparation of compound ***ent*-26**. Steroid **26** had: IR ν_{max} 1762, 1741, 1239 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.94–4.93 (m, 2H), 3.28 (br s, 4H), 2.09 (s, 3H), 2.00 (s, 3H), 0.89 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 211.0, 170.3, 170.1, 85.6, 77.5, 69.7, 56.9, 54.6, 45.2, 41.7, 39.8, 36.7, 36.3, 36.0, 35.7, 33.7, 31.5, 28.9, 27.4, 21.3, 20.6, 19.9, 13.0, 12.4.

(2α,3β,5β,8α,9β,10α,13α,14β)-3-Hydroxy-2-Methoxyandrostan-16-one acetate (*ent*-27)

Compound ***ent*-27** (170 mg, 94%) was prepared as a foamy solid from compound ***ent*-26** using the procedure described for the preparation of compound ***ent*-24**. Compound ***ent*-27** had: IR ν_{max} 2932, 2850, 1742, 1453, 1408, 1369, 1243, 1214 cm^{-1} ; ^{1}H NMR (CDCl$_3$) δ 4.98 (s, 1H), 3.32 (s, 4H), 2.04 (s, 3H), 0.94 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 218.5, 170.1, 77.6, 69.8, 56.9, 55.8, 54.8, 51.6, 39.8, 39.2, 39.1, 38.1, 36.7, 35.7, 34.3, 32.1, 28.9, 27.6, 21.3, 20.3, 18.0, 13.0.

(2β,3α,5α)-3-Hydroxy-2-methoxyandrostan-16-one (27)

Steroid **27** (85 mg, 83%) was prepared from the natural enantiomer of compound ***ent*-26** using the procedure reported for the preparation of compound ***ent*-24**. Steroid **27** had: IR ν_{max} 1741, 1243 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.98–4.97 (m, 1H), 3.32 (s, 4H), 2.04 (s, 3H), 0.94 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 218.6, 170.1, 77.5, 69.8, 56.9, 55.8, 54.8, 51.7, 39.8, 39.2, 39.1, 38.2, 36.7, 35.7, 34.3, 32.1, 28.9, 27.6, 21.3, 20.3, 18.1, 13.1.

(3β,4α,5β,8α,9β,10α,13α,14β)-4-Methoxy-16-(phenylmethylene)-androstane-3,17-diol diacetate (*ent*-28)

Compound *ent*-28 (300 mg, 97%) was prepared as a white solid from compound *ent*-3 using the procedure described for the preparation of compound *ent*-22. Compound *ent*-28 had: mp 150–152 °C; IR ν_{max} 2935, 2859, 1738, 1599, 1492, 1448, 1371, 1241 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.30–7.08 (m, 5H), 6.13 (d, 1H, *J* = 2.2 Hz), 5.28 (s, 1H), 4.97 (d, 1H, *J* = 2.3 Hz), 3.30 (s, 3H), 3.22 (s, 1H), 2.60 (m, 1H), 2.12 (s, 3H), 2.00 (s, 3H), 0.90 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 171.2, 170.4, 141.0, 137.6, 128.3 (2 × C), 128.2 (2 × C), 126.4, 123.5, 84.6, 82.5, 68.6, 58.9, 55.0, 49.0, 45.0, 42.9, 36.4, 35.9, 34.8, 32.5, 31.8, 30.9, 25.0, 22.0, 21.4, 21.2, 19.9, 14.0, 12.3.

(3α,4β,17β)-4-Methoxy-16-(phenylmethylene)-androstane-3,17-diol diacetate (28)

Steroid **28** (126 mg, 100%) was prepared as a white semisolid from the natural enantiomer of compound *ent*-3 using the procedure described for the preparation of compound *ent*-22. Steroid **28** had: IR ν_{max} 1733, 1241 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.28–7.11 (m, 5H), 6.13 (d, 1H, *J* = 1.9 Hz), 5.28–5.26 (m, 1H), 4.97–4.96 (m, 1H), 3.29 (s, 3H), 2.62–2.57 (m, 1H), 2.11 (s, 3H), 2.00 (s, 3H), 0.90 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 171.1, 170.4, 141.0, 137.6, 128.3, 128.2, 126.4, 123.5, 84.6, 82.5, 68.6, 58.9, 55.0, 49.0, 45.0, 42.9, 36.4, 35.9, 34.8, 32.6, 31.8, 30.9, 25.0, 22.1, 21.4, 21.1, 19.9, 14.0, 12.3.

(3β,4α,5β,8α,9β,10α,13α,14β,17α)-3,17-Dihydroxy-4-methoxyandrostan-16-one diacetate (*ent*-29)

Compound *ent*-29 (205 mg, 87%) was prepared as a white solid from compound *ent*-28 using the procedure described for the preparation of compound *ent*-23. Compound *ent*-29 had: mp 158–160 °C; IR ν_{max} 2928, 1762, 1742, 1449, 1372, 1240 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.02 (d, 1H, *J* = 2.0 Hz), 4.97 (s, 1H), 3.34 (s, 3H), 3.01 (br s, 1H), 2.13 (s, 3H), 2.05 (s, 3H), 0.96 (s, 3H), 0.79 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 211.0, 170.3 (2 × C), 85.6, 82.2, 68.5, 58.8, 54.8, 45.3, 44.8, 41.6, 36.2, 36.0, 35.8, 34.3, 32.3, 31.8, 24.8, 21.9, 21.3, 20.6, 19.4, 13.9, 12.4.

(3α,4β,17β)-3,17-Dihydroxy-4-Methoxyandrostan-16-one diacetate (29)

Steroid **29** (96 mg, 96%) was prepared as a foamy solid from the natural enantiomer of compound *ent*-28 using the procedure described for the preparation of compound *ent*-26. Steroid **29** had: ^{1}H NMR (CDCl$_3$) δ 4.97–4.92 (m, 2H), 3.29 (s, 3H), 2.97 (br s, 1H), 2.08 (s, 3H), 2.00 (s, 3H), 0.90 (s, 3H), 0.74 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 221.0, 170.3, 85.6, 82.2, 68.5, 58.8, 54.8, 45.3, 44.8, 41.6, 36.2, 36.1, 35.8, 34.3, 32.3, 31.9, 24.8, 21.9, 21.4, 20.6, 19.4, 14.0, 12.4.

(3β,4α,5β,8α,9β,10α,13α,14β)-3-Hydroxy-4-Methoxyandrostan-16-one acetate (*ent*-30)

Compound *ent*-30 (72 mg, 84%) was prepared as a white solid from compound *ent*-29 using the procedure described for the preparation of compound *ent*-24. Compound *ent*-30 had: mp 159–161 °C; IR ν_{max} 2944, 2861, 1733, 1449, 1407, 1380, 1244, 1216 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.03 (d, 1H, *J* = 1.6 Hz), 3.36 (s, 3H), 3.03 (br s, 1H), 2.07 (s, 3H), 0.98 (s, 3H), 0.87 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 218.8, 170.4, 82.4, 68.6, 58.9, 55.8, 55.0, 51.8, 44.9, 39.3, 39.1, 38.1, 35.9, 34.9, 32.4, 32.4, 24.9, 22.0, 21.4, 19.9, 18.1, 14.0.

(3α,4β,5α)-3-Hydroxy-4-methoxyandrostan-16-one acetate (30)

Steroid **30** (64 mg, 78%) was prepared as a foamy solid from the natural enantiomer of compound *ent*-29 using the procedure described for the preparation of compound *ent*-24. Steroid **30** had: ^{1}H NMR (CDCl$_3$) δ 5.04–5.03 (m, 1H), 3.35 (s, 3H), 3.02 (br s, 1H), 2.06 (s,

3H), 0.98 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 218.8, 170.4, 82.4, 68.6, 58.9, 55.8, 55.0, 51.8, 44.9, 39.3, 39.1, 38.1, 35.9, 34.9, 32.5, 32.4, 25.0, 22.0, 21.4, 19.9, 18.1, 14.0.

(3β,5β,8α,9β,10α,13α,14β,17α)-19-Nor-16-(phenylmethylene)-androstane-3,17-diol diacetate (*ent*-31)

Compound *ent*-31 (670 mg, 91%) was prepared as an oil from compound *ent*-21 using the procedure described for the preparation of compound *ent*-22. Compound *ent*-31 had: IR ν$_{max}$ 2919, 2862, 1738, 1600, 1492, 1446, 1370, 1240 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.40–7.10 (m, 5H), 6.24 (b s, 1H), 5.40 (b s 1H), 5.07 (b s, 1H), 2.69 (dd, 1H, *J* = 16.7 Hz, 6.3 Hz), 2.23 (s, 3H), 2.07 (s, 3H), 0.81 (s, 3H); ^{13}C (CDCl$_3$) NMR δ 171.0, 170.6, 140.9, 137.5, 128.2 (4 × C), 126.4, 123.5, 84.5, 69.7, 48.0, 47.8, 46.5, 42.9, 40.4, 37.4, 36.6, 36.4, 33.2, 30.7, 30.4, 29.9, 25.0, 24.2, 21.4, 21.1, 12.1. Anal. (C$_{29}$H$_{38}$O$_4$) C, H.

(3β,5β,8α,9β,10α,13α,14β,17α)-19-Nor-3,17-dihydroxyandrostan-16-one diacetate (*ent*-32)

Compound *ent*-32 (530 mg, 98%) was prepared as a white solid from compound *ent*-31 using the procedure described for the preparation of compound *ent*-26. Compound *ent*-32 had: mp 116–118 °C; IR ν$_{max}$ 2919, 2861, 1763, 1736, 1445, 1371, 1239, 1216 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.91 (br s, 1H), 4.89 (s, 1H), 2.19 (dd, 1H, *J* = 18.7 Hz, 7.7 Hz), 2.03 (s, 3H), 1.92 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 210.6, 170.1, 169.9, 85.4, 69.3, 47.4, 46.1, 44.1, 41.5, 39.5, 37.1, 36.2, 35.9, 35.6, 32.8, 30.3, 29.6, 24.3, 23.7, 21.1, 20.3, 12.0. Anal. (C$_{22}$H$_{32}$O$_5$) C, H.

(3β,5β,8α,9β,10α,13α,14β)-19-Nor-3-hydroxyandrostan-16-one acetate (*ent*-33)

Compound *ent*-33 (290 mg, 70%) was prepared as a colorless oil from compound *ent*-32 using the procedure described for the preparation of compound *ent*-24. Compound *ent*-33 had: IR ν$_{max}$ 2918, 2862, 1737, 1444, 1408, 1378, 1244, 1214 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.97 (b s, 1H), 1.97 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 218.1, 170.3, 69.6, 55.8, 50.8, 47.8, 46.5, 40.4, 39.0, 38.9, 38.0, 37.3, 36.5, 36.5, 33.1, 31.0, 29.8, 25.0, 23.3, 21.2, 17.9. Anal. (C$_{20}$H$_{30}$O$_3$) C,H.

(5β,8α,9β,10α,13α,14β,17α)-17-androstane-3-one (*ent*-34)

This compound was prepared as previously described.[45]

(5β,8α,9β,10α,13α,14β,17α)-17-[[(4-Phenylmethyl)sulfonyl]oxy]-androstan-3-one (*ent*-35)

Compound *ent*-34 (1.8 g, 6.54 mmol), *p*-TsCl (2.5g, 13.08 mmol) and pyridine (60 mL) were heated at 40 °C for 18 h. The reaction mixture was cooled and poured into a beaker containing crushed ice. The mixture was extracted with CH$_2$Cl$_2$ (3×100 mL) and the combined extracts were washed with 1N HCl to remove the pyridine, washed with brine, dried and solvent removed. The crude product was purified by flash column chromatography (silica gel eluted with10% EtOAc in CH$_2$Cl$_2$) to give *ent*-35 as a white crystalline solid (2.65 g, 91%). mp 180–182 °C; ; IR ν$_{max}$ 2917, 2851, 1710, 1598, 1449, 1415, 1361, 1253, 1217 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.77 (d, 2H, *J* = 7.8 Hz), 7.32 (d, 2H, *J* = 7.8 Hz), 4.24 (t, 1H, *J* = 8.2 Hz), 2.44 (s, 3H), 0.98 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 211.7, 144.4, 134.1, 129.6 (2 × C), 127.76 (2 × C), 89.8, 53.5, 49.7, 46.5, 44.5, 43.0, 38.4, 38.0, 36.0, 35.6, 35.0, 31.0, 28.6, 27.6, 23.2, 21.6, 21.6, 20.7, 11.8. 11.4.

(3β,5β,8α,9β,10α,13α,14β,17α)-17-[[(4-Phenylmethyl)sulfonyl]oxy]-androstan-3-ol (*ent*-36)

Compound *ent*-35 (2.8 g, 6.3 mmol) was dissolved in THF (50 mL), cooled to −78 °C and K-selectride in THF (1 M, 7.2 mL, 7.2 mmol, 1.2 eq) was added dropwise. Stirring at −78 °C was continued for 2 h. Aqueous NaOH (3 M, 20 mL) and then 30% H$_2$O$_2$ (20 mL) were

added and the reaction was warmed to room temperature and stirred for 2 h. Water (150 mL) was added and the product was extracted into EtOAc. The combined EtOAc extracts were dried and the solvents removed to give an oil which was purified by flash column chromatography (silica gel eluted with 10% EtOAc in CH_2Cl_2) to give product *ent*-36 (2 g, 71%): mp 186–188 °C; IR v_{max} 3400, 2918, 2850, 1599, 1446, 1355 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.77 (d, 2H, *J* = 7.1 Hz), 7.32 (d, 2H, *J* = 7.1 Hz), 4.24 (t, 1H, *J* = 7.8 Hz), 4.03 (d, 1H, *J* = 2.3 Hz) 2.44 (s, 3H), 0.78 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 144.1, 134.2, 129.6 (2 × C), 127.8 (2 × C), 90.1, 66.4, 66.3, 54.1, 50.0, 43.0, 39.0, 36.1, 35.7, 35.1, 32.1, 31.3, 28.9, 28.2, 27.6, 23.2, 21.6, 20.0, 11.8, 11.1.

(3β,5β,8α,9β,10α,14β)-17-Methylandrost-14(17)-en-3-ol (*ent*-37)

MeMgBr (3 M in Et$_2$O, 5 mL) was added to refluxing toluene (80 mL) containing dissolved compound *ent*-36 (1.34 g, 3 mmol) and refluxing was continued for 1 h under N$_2$. The reaction was cooled, acidified with 1N HCl and extracted with CH$_2$Cl$_2$. The combined extracts were washed with brine, dried and concentrated to give an oil. The oil was dissolved in MeOH (20 mL) and 5 M HCl (prepared from concentrated HCl diluted with MeOH) was added and the reaction was stirred at room temperature for 15 h. The reaction was made basic by the addition of aqueous NaHCO$_3$ and the MeOH was removed. The aqueous residue was diluted further with water and the product extracted into EtOAc. The combined extracts were dried and concentrated to give an oil, which was purified by flash column chromatography (silica gel eluted with 15–35% EtOAc in hexanes) to give product *ent*-37 as a colorless oil (750 mg, 91%): IR v_{max} 3337, 2920, 2856, 1444, 1359, 1255 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.02 (br s, 1H), 1.58 (s, 3H), 0.68 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 136.5, 127.4, 66.4, 53.9, 52.3, 45.3, 38.7, 37.1, 36.1, 35.8, 32.3, 32.2, 28.9, 28.3, 28.1, 25.6, 25.2, 13.4, 11.1.

(3β,5β,8α,9β,10α,14β)-3-(Methoxymethoxy)-17-methylandrost-14(17)-ene (*ent*-38)

Compound *ent*-37 (823 mg, 3 mmol) was dissolved CH$_2$Cl$_2$ (80 mL) and cooled to 0 °C. (*i*-Pr)$_2$EtN (2.6 mL, 15 mmol) and ClCH$_2$OMe (0.76 ml, 10 mmol) were added and the reaction was stirred at room temperature for 6 h. The reaction mixture was made basic by adding aqueous NaHCO$_3$ solution and the product extracted into CH$_2$Cl$_2$. The combined extracts were washed with brine, dried and solvent removed to give a viscous liquid. The crude product was purified by flash column chromatography (silica gel eluted with 10–15% EtOAc in hexanes) to give product *ent*-38 as a colorless liquid (960 mg, 90%): IR v_{max} 2924, 2857, 1445, 1368, 1302, 1213 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.57 (s, 2H), 3.75 (s, 1H), 3.28 (s, 3H), 1.51 (s, 3H), 0.64 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 136.4, 127.1, 94.3, 71.4, 54.8, 53.8, 52.2, 45.3, 39.2, 37.0, 35.8, 33.5, 32.8, 32.2, 28.3, 28.0, 26.2, 25.6, 25.2, 13.2, 11.2.

(1*R*,4a*R*,4b*R*,7*S*,8a*R*,10a*S*)-7-(Methoxymethoxy)docecahydro-4b-methyl-1(3-oxobutyl-2(1*H*)-phenanthrenone (*ent*-39)

Compound *ent*-38 (2.38 g, 7.5 mmol) was dissolved in CH$_2$Cl$_2$ (100 mL) and cooled to − 78 °C. O$_3$ was passed through the solution until it remained blue for 20 min and then O$_2$ was passed through the solution until it became colorless. The reaction was warmed to 0 °C, AcOH (30 mL) was added and the CH$_2$Cl$_2$ removed on a rotary evaporator. Additional AcOH (15 mL) and Zn dust (6 g) were added and the reaction was stirred at room temperature for 3 h to decompose the ozonide intermediate. The Zn was removed by filtration through Celite and the Celite was washed with EtOAc. The combined filtrate and washings were combined and the volatile solvents removed to leave the product in AcOH. The AcOH was carefully neutralized with aqueous NaHCO$_3$ and the product extracted into EtOAc. The combined extracts were dried and solvent removed to leave the crude product as an oil which was purified by flash column chromatography (silica gel eluted with 20–35%

EtOAc in hexanes) to give product *ent*-**39** (1.7 g, 65%) as a liquid: IR ν_{max} 2928, 1712, 1447, 1367, 1213 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.57 (s, 2H), 3.75 (s, 1H), 3.28 (s, 3H), 2.03 (s, 3H), 0.65 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 212.2, 208.7, 94.3, 71.2, 54.9, 54.6, 52.1, 42.3, 41.7, 40.8, 38.7, 36.0, 33.0, 32.6, 32.0, 29.6, 28.0, 26.2, 26.1, 19.2, 10.9.

(3β,5β,8α,9β,10α,14β)-3-(Methoxymethoxy)-*D*-Homo-18-norandrost-13(17a)-en-17-one acetate (*ent*-40)

Compound *ent*-**39** (1.55g, 4.43 mmol) was dissolved in MeOH (15 mL) and water (1.5 mL), 10% methanolic NaOH (12 mL) was added and the reaction was stirred at room temperature for 1 h. The reaction was then acidified with 3N HCl and the product extracted into CH$_2$Cl$_2$. The combined extracts were washed with aq. NaHCO$_3$, brine, dried and the solvents were removed. The crude product was purified by flash column chromatography (silica gel eluted with 30–40% ethyl acetate in hexanes) to give product *ent*-**40** as a white solid. (1.10 g, 75%): mp 98–100 °C; IR ν_{max} 2926, 1674, 1622, 1449, 1364, 1259, 1209 cm^{-1} ; ^{1}H NMR (CDCl$_3$) δ 5.68 (s, 1H), 4.55 (s, 2H), 3.74 (s, 1H), 3.27 (s, 3H), 0.63 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 199.5, 166.4, 123.7, 94.3, 71.1, 54.9, 52.0, 43.6, 43.0, 38.6, 36.2, 35.8, 35.8, 35.7, 33.1, 32.5, 31.1, 27.9, 26.1, 25.9, 25.3, 10.9.

(3β,5β,8α,9β,10α,13α,14β)-3-(Methoxymethoxy)-*D*-Homo-18-norandrostan-17-one (*ent*-41)

Liquid NH$_3$ (250 mL) was condensed in a 3–necked 500 mL round bottom flask fitted with a Dewar condenser containing Dry Ice/acetone and an overhead stirrer and then placed in a cold bath at −78 °C. Li (140 mg, 20 mmol) was added and stirring continued for 30 min during which time the solution became deep blue. THF (80 mL) was added and after 10 min, compound *ent*-**40** (942 mg, 2.7 mmol) dissolved in THF (40 mL) was added and stirring continued for 1 h. Solid NH$_4$Cl (5 g) was added and the solution became colorless. The reaction was allowed to warm to room temperature and the liq. NH$_3$ was allowed to evaporate. Water was added and the product extracted into EtOAc. The combined extracts were washed with brine, dried and solvent removed to give a yellow solid which was purified by flash column chromatography (silica gel eluted with 30% EtOAc in hexanes) to give product *ent*-**41** as a white solid (650 mg, 72%): mp 85–87 °C; IR ν_{max} 2919, 2859, 1717, 1445, 1366, 1322, 1206, 1209 cm^{-1} ; ^{1}H NMR (CDCl$_3$) δ 4.65 (s, 2H), 3.82 (s, 1H), 3.36 (s, 3H), 0.72 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 211.9, 94.5, 71.4, 55.1, 52.9, 48.6, 47.4, 43.3, 41.3, 40.8, 39.1, 35.9, 34.3, 33.5. 32.6, 30.9, 30.3, 28.3, 26.2, 24.6, 11.2.

(3β,5β,8α,9β,10α,13α,14β)-3-Hydroxy-*D*-Homo-18-norandrostan-17-one (*ent*-42)

Compound *ent*-**41** (500 mg, 1.50 mmol) dissolved in MeOH (20 mL) was stirred with 6 N HCl (6 mL) at room temperature for 24 h. The HCl was neutralized by careful addition of aqueous NaHCO$_3$ solution and the MeOH removed. Water was added and the product was extracted into EtOAc. The combined extracts were washed with brine, dried and the solvents removed to give the crude product as an off-white solid which was purified by flash column chromatography (silica gel eluted with 40–50% EtOAc in hexanes). Compound *ent*-**42** was obtained as a white solid (400 mg, 92%): mp 227–229 °C; IR ν_{max} 3395, 2939, 2922, 2850, 1699, 1594, 1419, 1360, 1320, 1264, 1208 cm^{-1} ; ^{1}H NMR (CDCl$_3$) δ 4.04 (s, 1H), 0.71 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 211.9, 66.2, 52.9, 48.6, 47.4, 43.2, 41.2, 40.8, 38.4, 36.1, 35.7, 34.3, 32.0, 31.0, 30.3, 28.9, 28.2, 24.6, 11.0.

(3β,5β,8α,9β,10α,13α,14β)-3-Hydroxy-*D*-Homo-18-norandrostan-17-one acetate (*ent*-43)

Compound *ent*-**42** (370 mg, 1.27 mmol), AcOAc (0.5 mL) and Et$_3$N (1 mL) in CH$_2$Cl$_2$ (8 mL) were stirred at room temperature for 24 h. Aqueous sat. NaHCO$_3$ (5 mL) was carefully added and the mixture was stirred for 1 h. The product was extracted into CH$_2$Cl$_2$ and the combined extracts were washed with brine, dried and the solvent removed to give an off-

white solid. The crude product was purified by flash column chromatography (silica gel eluted with 20–30% EtOAc in hexanes) to give product *ent*-**43** as a white solid (400 mg, 95%): mp 141–143 °C; IR ν_{max} 2920, 2860, 2733, 1717, 1446, 1361, 1246 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.96(s, 1H), 2.00 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 211.6, 170.4, 69.8, 69.6, 52.7, 48.5, 47.3, 43.1, 41.1, 40.7, 39.2, 35.7, 34.2, 32.6, 32.5, 30.8, 30.2, 27.9, 25.9, 24.5, 21.4, 21.3, 11.1, 11.0.

(3β,5α)-3-(Acetyloxy)-16,17-seco-*D*-homo-18-norandrostane-16,17-dioic acid (*ent*-44) and (3β,5α)-3-(Hydroxy)-16,17-seco-*D*-homo-18-norandrostane-16,17-dioic acid dimethyl ester (*ent*-45)

A mixture of compound *ent*-**43** (96 mg) and a CrO$_3$ solution (0.7 mL) [prepared by mixing CrO$_3$ (500 mg), water (0.7mL) and acetic acid (0.8 mL)] and a solution of aqueous methanolic acetic acid (1 mL) [prepared by mixing acetic acid (30 mL), water (1 mL) and methanol (0.05 mL)] was stirred at 60 °C for 4.5 h. The reaction was cooled to room temperature, water (2.5 mL) was added and the reaction was stirred at room temperature for 13 h. Additional water (15 mL) was added and the product was extracted into CH$_2$Cl$_2$. The combined extracts were dried and the solvent removed to give dicarboxylic acid *ent*-**44** as a white solid which was immediately converted without characterization to diester *ent*-**45** upon stirring with dry HCl in MeOH (10 mL) [prepared by adding AcCl (3 mL) to MeOH (8 mL)] at room temperature for 15 h. The HCl was cautiously neutralized with aqueous NaHCO$_3$ and the MeOH was removed. Water was added and the product was extracted into EtOAc. The combined extracts were washed with brine, dried and solvent removed to give an oil. Purification by flash column chromatography (silica gel eluted with 40% EtOAc in hexanes) yielded compound *ent*-**45** as a colorless oil (54 mg, 51%): IR ν_{max} 3445.55, 2924, 2858, 1738, 1436, 1361, 1258 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.03 (s, 1H), 3.65 (s, 6H), 0.71 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 173.7 (2 × C), 66.3, 53.0, 51.5, 51.4, 45.0, 40.8, 39.3, 38.8, 38.3, 36.1, 35.7, 35.3, 32.7, 31.9, 31.3, 28.9, 28.4, 24.4, 11.0.

(3β,5β,8α,9β,10α,13α,14β,17α)-3-Hydroxy-16-oxo-18-norandrostane-17-carboxylic acid methy ester (*ent*-46)

A mixture of compound *ent*-**45** (53 mg, 0.14 mmol), NaOMe (27 mg, 0.5 mmol) and THF (8 mL) was heated at reflux for 0.5 h. The reaction was cooled, acidified with 1 N HCl to pH 3 and the product extracted into CH$_2$Cl$_2$ (3 × 75 mL). The combined extracts were washed with brine, dried and solvents removed to give a white solid. The crude product was purified by flash column chromatography (silica gel eluted with 40% EtOAc in hexanes) gave pure product *ent*-**46** as a white solid (34 mg, 71%): mp 136–138 °C; IR ν_{max} 3435, 2920, 2855, 1756, 1727, 1435, 1410, 1384, 1339, 1261 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.06 (t, 1H, *J* = 2.5 Hz), 3.75 (s, 3H), 2.86 (d, 1H, *J* = 2.5 Hz), 2.50 (dd, 1H, *J* = 18.0 Hz, 5.8 Hz), 0.78 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 210.2, 169.5, 66.3, 61.9, 53.1, 52.3, 47.1, 46.6, 43.2, 41.6, 38.7, 36.2, 35.7, 32.2, 31.9, 30.0, 28.9, 28.0, 24.5, 11.0.

(5α)-Estrane-3,17-dione (47)

This compound was prepared as previously described.[20]

(5α)-17-Oxo-3,4-secoestrane-2,3-dicarboxylic acid (48) and (5α)-17-Oxo-3,4-secoestrane-2,3-dicarboxylic acid dimethyl ester (49)

A solution of CrO$_3$ (10.9 g), H$_2$SO$_4$ (98%, 16 mL), and water (76 mL) was added dropwise to a stirred solution of compound **47** (7.9 g, 28.5 mmol) in AcOH (80 mL) at 65 °C. The reaction was heated at 70 °C for 1 h. Then, crushed ice and water (500 mL) were added and the mixture was stirred at room temperature for 1 h. Steroid **48** precipitated and was filtered, washed with water, and dried overnight at room temperature to give pure dicarboxylic acid

(5.53 g, 60%) as a white solid. The aqueous phase was extracted with CH_2Cl_2 (2 × 150 mL), and the combined extracts were washed with brine (2 × 100 mL), dried and evaporated to afford impure dicarboxylic acid **48** (2 g, 21%) as an oily residue. Pure steroid **48** had: ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.88 (3H, s); ^{13}C NMR (75 MHz, CDCl$_3$) δ 221.5, 179.43, 179.25, 50.6, 47.9, 47.7, 43.2, 40.6, 39.4, 38.8, 36.0, 35.3, 32.3, 31.6, 29.4, 26.0, 21.7, 13.9.

AcCl (6 mL, 23 mmol) was added to steroid **48** (7.53 g) dissolved in MeOH (100 mL) and the reaction was stirred at room temperature for 2 h. Then, water (50 mL) was added and the product was extracted into CH_2Cl_2 (2 × 100 mL). The combined extracts were washed with water, dried and the solvent removed to yield an oily residue which was purified by flash column chromatography (silica gel eluted with 15% EtOAc in hexanes) to give steroid **49** (4.43 g, 52%) as an oil. Unreacted steroid **48** was reclaimed by washing the silica gel with MeOH and the esterification procedure was repeated to give additional product **49** (780 mg, 9%) as an oil: IR v_{max} 2925, 2858, 1737 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.65 (6H, s), 0.86 (3H, s); ^{13}C NMR (CDCl$_3$) δ 220.9, 173.6 (2 × C), 51.7, 51.6, 50.6, 47.99, 47.90, 43.3, 40.6, 39.3, 39.1, 36.0, 35.3, 32.3, 31.7, 29.4, 25.9, 21.7, 13.9.

(5α,17β)-17-Hydroxy-3,4-secoestrane-2,3-dicarboxylic acid dimethyl ester (50)

Steroid **49** (1.55 g, 31.4 mmol) was dissolved in stirred EtOH (20 mL) and cooled in an ice-bath. NaBH$_4$ (218 g, 5.8 mmol) was added in small portions. After 1.5 h, a solution of water (50 mL) and AcOH (2 mL) was added and the solution was stirred for 1h. The product was extracted into CH_2Cl_2 (2 × 125 mL), washed with an aqueous 1 N HCl, sat. aqueous NaHCO$_3$, brine, dried and the solvent removed. Flash column chromatography (silica gel eluted with 15% EtOAc in hexanes) gave secosteroid **50** (1.16 g, 74%) as an oily product: IR v_{max} 3452, 2950, 2921, 2867, 1733 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.60–3.65 (7H, m), 0.73 (3H, s); ^{13}C NMR (CDCl$_3$) δ 173.7 (2 × C), 82.0, 51.68, 51.62, 50.2, 47.9, 43.5, 43.1, 41.2, 39.5, 39.2, 36.9, 35.4, 32.5, 30.7, 30.1, 26.3, 23.3, 11.2.

(3α,5α,17β)-17-Hydroxy-2-oxo-A-norestrane-3-carboxylic acid methyl ester (51)

A NaOMe solution (5 mL) was prepared by dissolving Na (1.14 g, 49 mmol) in MeOH (25 mL) followed by MeOH evaporation on a rotary evaporator. Dry THF (30 mL) was added and the flask was filled with N$_2$. Steroid **50** (5 g, 14.1 mmol) dissolved in dry THF (50 mL) was slowly added. The reaction mixture was heated at 100 °C for 1 h under N$_2$ and then allowed to attain room temperature. Then 1 N HCl (15 mL) was slowly added and the product was extracted into CH_2Cl_2 (2 × 100 mL). The combined extracts were washed with brine, dried and the solvent removed. Flash column chromatography (silica gel eluted with 15% EtOAc in hexanes) gave product **51** (3.7 g, 81%) as a white solid: mp 138–142 °C (EtOAc/hexanes); IR v_{max} 3440, 2918, 2866, 1756, 1727 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 3.75 (3H, s), 3.67 (t, 1H, J = 8 Hz), 2.88 (d, 1H, J = 12.6 Hz), 2.48 (dd, 1H, J = 6.6 Hz, 18 Hz), 0.77 (3H, s). ^{13}C NMR (CDCl$_3$) δ 210.1, 169.7, 81.9, 62.1, 52.4, 49.7, 48.7, 47.5, 45.7, 43.6, 43.3, 41.6, 36.5, 30.5, 30.2, 29.9, 27.1, 23.3, 11.4. Anal. ($C_{19}H_{28}O_4$) C, H.

(5α,17β)-17-Hydroxy-A-norestran-2-one (52)

LiCl (1.95 g, 46.1 mmol) was added to a solution of steroid **51** (3.7 g, 11.5 mmol) dissolved in DMF (50 mL) and water (0.5 mL) and the reaction was heated at 160 °C for 35 min. Then, crushed ice and water were added and the product was extracted into CH_2Cl_2 (2 × 120 mL). The combined extracts were washed with water and dried. After solvent evaporation, the residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **52** (2.6 g, 85%) as a white solid: mp 177–179 °C (EtOAc/hexanes); IR v_{max} 3450, 2915, 2861, 1732 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.77 (3H, s), 2.41–

2.31 (2H, m), 3.66 (t, 1H, J = 8 Hz). ^{13}C NMR (CDCl$_3$) δ 218.3, 82.0, 49.9, 48.9, 48.6, 46.1, 44.2, 43.9, 43.6, 41.7, 36.6, 30.9, 30.64, 30.61, 27.1, 23.4, 11.4. Anal. (C$_{17}$H$_{26}$O$_2$) C, H.

(5α,17β)-17-[[(4-Methylphenyl)sulfonyl]oxy]-A-norestran-2-one (53)

A solution of steroid **52** (2.46 g, 9.37 mmol), DMAP (57 mg, 0.49 mmol), and *p*-TsCl (6.25 g, 32.8 mmol) in anhydrous pyridine (50 mL) was heated at 65 °C overnight. The reaction mixture was poured into ice-water and the product extracted into CH$_2$Cl$_2$ (2 × 75 mL). The combined extracts were washed with aqueous HCl, aqueous NaHCO$_3$, brine, and dried. After solvent evaporation, the oily residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give steroid **53** (3.56 g, 91%) as a white solid: mp 138–139 °C (ether/hexanes); IR ν$_{max}$ 2920, 2858, 1741, 1598, 1357, 1188, 1176 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.80–7.77 (2H, m), 7.34–7.31 (2H, m), 4.28 (t, 1H, J = 8 Hz), 2.45 (3H, s), 2.33 (dd, 2H, J = 6.6 Hz, 18 Hz), 0.82 (3H, s). ^{13}C NMR (CDCl$_3$) δ 217.9, 144.6, 134.6, 129.8 (2 × C), 128.0 (2 × C), 90.0, 48.9, 48.6, 48.4, 45.9, 44.1, 43.8, 43.6, 41.2, 36.0, 30.8, 30.4, 27.7, 26.8, 23.3, 21.8, 12.0. Anal. (C$_{24}$H$_{32}$O$_4$S) C, H.

(5α,17β)-2-Methylene-17-[[4-(methylphenyl)sulfonyl]oxy]-A-norestrane(54)

A solution of *n*-BuLi (2.5 M, 24.1 mmol, 9.6 mL) was added dropwise to a solution of MeP(Ph)$_3$Br (8.85 g, 24.7 mmol) in dry benzene (70 mL) and dry THF (12 mL) under N$_2$ and the mixture was stirred for 30 min. A solution of steroid **53** (2.58 g, 6.19 mmol) in dry benzene (35 mL) was added and stirred at room temperature. After 6 h, the reaction was diluted with water (50 mL) and extracted with EtOAc (2 × 75 mL). The combined extracts were washed with brine, dried and the solvents evaporated. Flash column chromatography on (silica gel eluted with 5% EtOAc in hexanes) gave steroid **54** (2.05 g, 80%) as a white solid: mp 136–137 °C (EtOAc/hexanes); IR ν$_{max}$ 2920, 2855, 1657, 1598, 1359 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.80–7.77 (2H, m), 7.34–7.31 (2H, m), 4.83 (2H, m), 4.27 (t, 1H, J = 8 Hz), 2.45–2.36 (5H, m), 0.81 (3H, s); ^{13}C NMR (CDCl$_3$) δ 151.5, 144.5, 134.6, 129.8 (2 × C), 128.0 (2 × C), 105.9, 90.3, 51.0, 49.0, 48.9, 46.7, 43.7, 41.3, 39.6, 37.6, 36.2, 31.0, 30.8, 27.8, 26.7, 23.5, 21.8, 12.1. Anal. (C$_{25}$H$_{31}$O$_3$S) C, H.

(5α)- 17-Methyl-2-Methylene-A-norgon-13(17)-ene (55)

Steroid **54** (2 g, 4.8 mmol) was dissolved in anhydrous toluene (50 mL) and heated to 100 °C. MeMgBr (3.0 M in Et$_2$O, 8 mL, 24 mmol) was added dropwise to the stirring hot solution under an N$_2$ atmosphere and a white precipitate appeared. The reaction was heated for 1 h at 115 °C. The flask was cooled, a few pieces of crushed ice were added, and the pH of the solution was adjusted to pH 2 by dropwise addition of 2 N aqueous H$_2$SO$_4$. The toluene layer was separated and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined extracts were washed with brine, dried and the solvents evaporated. Flash column chromatography on (silica gel eluted with 1% EtOAc in hexanes) gave steroid **55** (1.04 g, 90%) as an oily product: IR ν$_{max}$ 3067, 2921, 2851, 1657, 1441 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.86 (2H, m), 1.62 (3H, s). ^{13}C NMR (CDCl$_3$) δ 151.9, 136.7, 128.3, 105.7, 100.2, 52.6, 51.5, 51.3, 47.1, 46.4, 39.7, 37.8, 37.4, 31.5, 31.2, 28.4, 25.6, 13.6.

(3a*S*,5a*R*,6*S*,9a*R*,9b*S*)-Decahydro-6-(3-oxobutyl)-1*H*-benz[*e*]indene-2,7-dione (56)

A solution of steroid **55** (760 mg, 3.13 mmol) in CH$_2$Cl$_2$ (55 mL) was treated with O$_3$ at −78 °C until a blue color persisted (ca. 1 hr). O$_2$ was passed through the solution for 30 min until the blue color disappeared. AcOH (50 mL) was added and the CH$_2$Cl$_2$ was evaporated under vacuum without heating. Then, AcOH (10 mL) and Zn dust (2.03 g, 31 mmol) were added and the reaction mixture was stirred at room temperature for 1.5 h. Zn dust was filtered off through cotton, washing with CH$_2$Cl$_2$ (100 mL). The Zn dust was stirred with EtOAc (50 mL) for 1 h. The solids were filtered off, the combined solvents were evaporated

and the product was purified by flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) to give compound **56** (390 mg, 51%) as an oil: IR ν_{max} 3406, 2919, 2859, 1742, 1709 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.09 (3H, s); ^{13}C NMR (CDCl$_3$) δ 217.0, 211.6, 209.1, 53.9, 48.4, 47.4, 46.5, 45.6, 43.6, 43.2, 41.6, 40.9, 32.0, 31.1, 30.5, 30.0, 19.5.

(8α,9β,10α,13α,14β)-Gon-4-ene-3,16-dione (*ent*-57)

A solution of NaOH [10% w/v in MeOH/water (9:1), 2 mL] was added to a solution of compound **56** (392 g, 1.41 mmol) in MeOH (20 mL) and the mixture was stirred at room temperature. After 3 h, a few pieces of crushed ice were added, the pH was adjusted to pH 2 by adding aqueous 1 N HCl, and the product was extracted into CH$_2$Cl$_2$ (2 × 70 mL). The combined extracts were washed with brine, dried and the solvent evaporated. Purification by flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) gave compound *ent*-57 (302 mg, 82%) as a white solid: mp 147–148 °C (EtOAc/hexanes); IR ν_{max} 2932, 2854, 1740, 1662, 1612 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.84 (1H, s); ^{13}C NMR (CDCl$_3$) δ 217.4, 199.8, 165.8, 124.9, 48.7, 48.6, 46.5, 45.8, 43.6, 43.2, 42.6, 36.6, 35.3, 31.4, 30.7, 30.3, 26.5. Anal. (C$_{17}$H$_{22}$O$_2$) C, H.

(5β,8α,9β,10α,13α,14β)-Gonane-3,16-dione (*ent*-58)

Anhydrous NH$_3$ (10 mL) was condensed using a Dewar condenser into a three-neck flask containing Li metal (20 mg, 2.9 mmol) at −78 °C. Then, anhydrous THF (12 mL) was added and the resulting blue solution was stirred for 0.5 h. A solution of compound *ent*-57 (150 mg, 0.58 mmol) in dry THF (6 mL) was added dropwise to the vigorously stirred solution. After 2 h of stirring, the reaction color was discharged by careful addition of solid NH$_4$Cl in portions and left overnight while the NH$_3$ evaporated. The reaction was then acidified with aqueous 1 N HCl and the product was extracted into EtOAc (2 × 50 mL). The combined organic phases were washed with brine, dried and the solvent evaporated. The residue was dissolved in acetone (50 mL) and Jones reagent was added dropwise until an orange color persisted. The course of the reaction was checked by TLC. Then, 2-propanol was added dropwise until the reaction mixture turned green. After 30 min, the reaction mixture was poured into water-ice. The product was extracted into CH$_2$Cl$_2$ (2 × 30 mL). The combined extracts were washed with brine, dried and the solvent was evaporated. Flash column chromatography (silica gel eluted with 15% EtOAc in hexanes) gave compound *ent*-58 (95 mg, 63%) as a white solid: mp 188–189 °C (EtOAc/hexanes); IR ν_{max} 2914, 2867, 1740, 1705 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.90–2.42 (24H, m); ^{13}C NMR (CDCl$_3$) δ 218.1, 211.6, 49.1, 48.7, 47.3, 46.8, 46.0, 45.9, 43.7, 43.5, 43.4, 41.4, 33.8, 31.1, 31.0, 30.4, 30.0.

[^{35}S]-TBPS Binding Methods

The methods used were as described previously.[46]

Xenopus Oocyte and HEK 293 Cell Electrophysiological Methods

Receptor expression and whole-cell and single-channel recordings were carried out as described previously.[27, 46, 47]

Tadpole Behavioral Methods

The methods used were as described previously.[46]

Mouse Behavioral Methods

The methods used were as described previously.[48]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

aAbbreviations

CNS	central nervous system
GABA	γ-aminobutyric acid
GABA$_A$	γ-aminobutyric acid type A
[^{35}S]TBPS	[^{35}S]-*tert*-butylbicyclophosphorothionate
SAR	structure–activity relationships
LRR	loss of righting reflex
LSR	loss of swimming reflex

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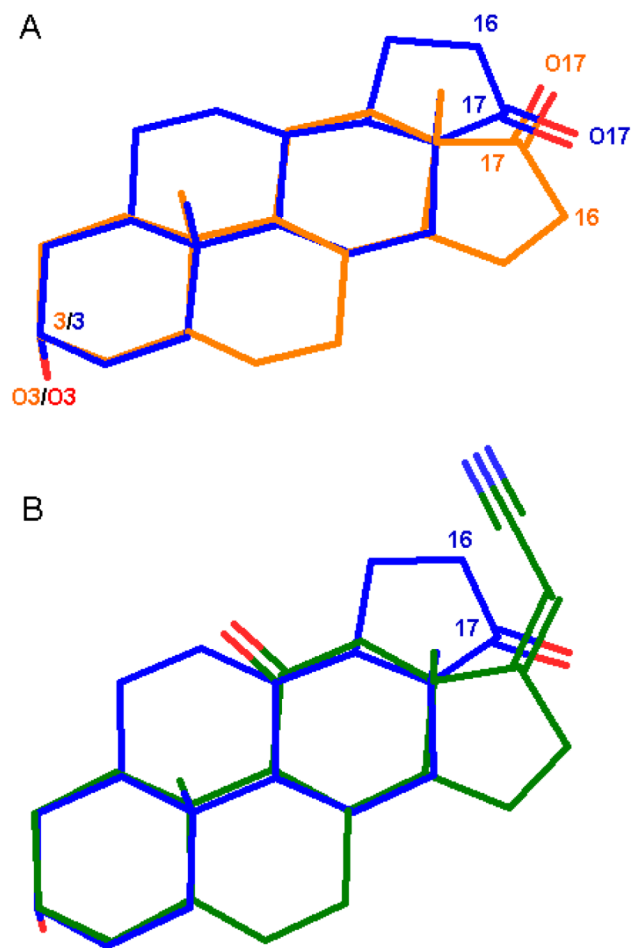
Figure 1.
Molecular modeling. (A) Proposed model of the alignment of enantiomers **1** and *ent*-**1** at the neursosteroid modulation site on GABA$_A$ receptors. (B) Proposed model of the alignment of compounds **4** and *ent*-**1** at the same site. Aligments were obtained by superimposing O-3, C-3, C-13 and C-18 for each pair of compounds. Additional views of the superimpositions from different perspectives are presented in the Supplemental Materials along with a view showing the superimposition of steroid **5** and analogue *ent*-**9** (Figure S1).
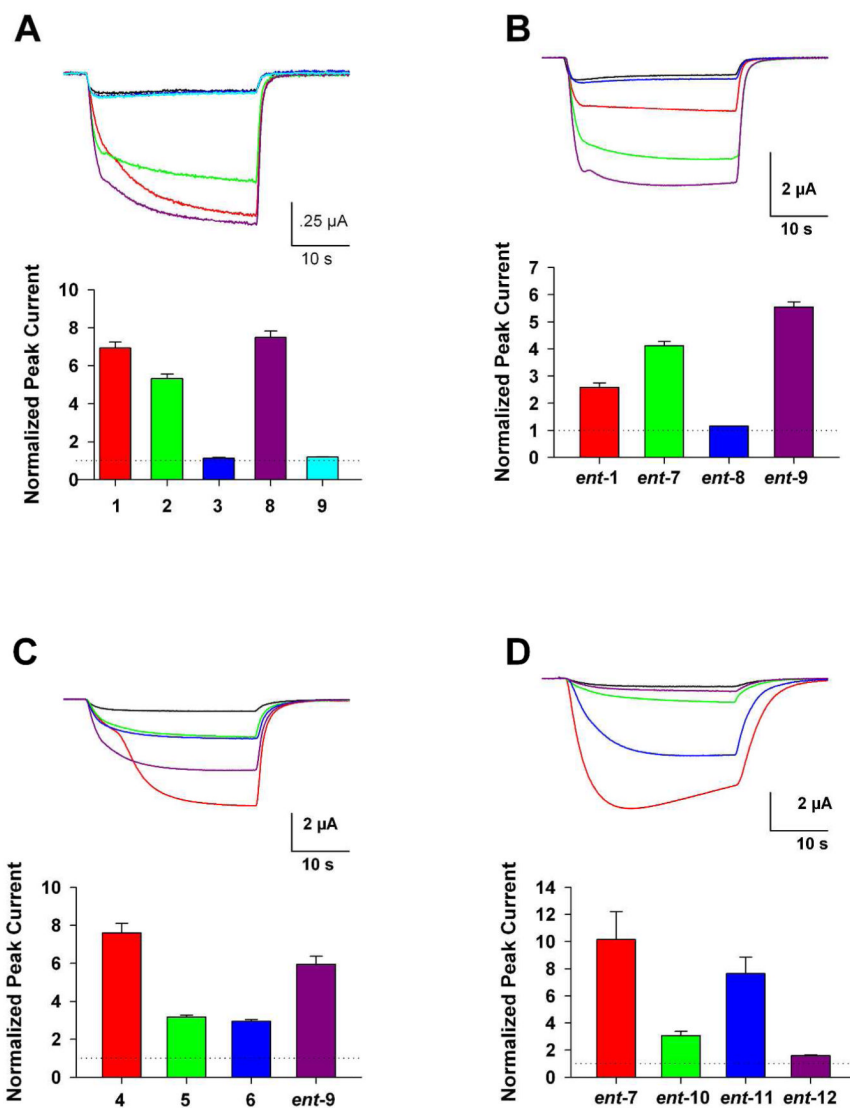
Figure 2.
Side-by-side, equimolar comparison of structurally related compounds in *Xenopus* oocytes expressing wild-type $\alpha_1\beta_2\gamma_L$ GABA$_A$ receptors. The traces in the top panels of (A–D) represent current responses of oocytes to 0.5 µM GABA alone (smallest, black trace) and GABA plus the color-indicated compound at 5 µM (A, D), or 0.5 µM (B, C). Bar graphs indicate normalized peak current relative to the response to GABA alone.
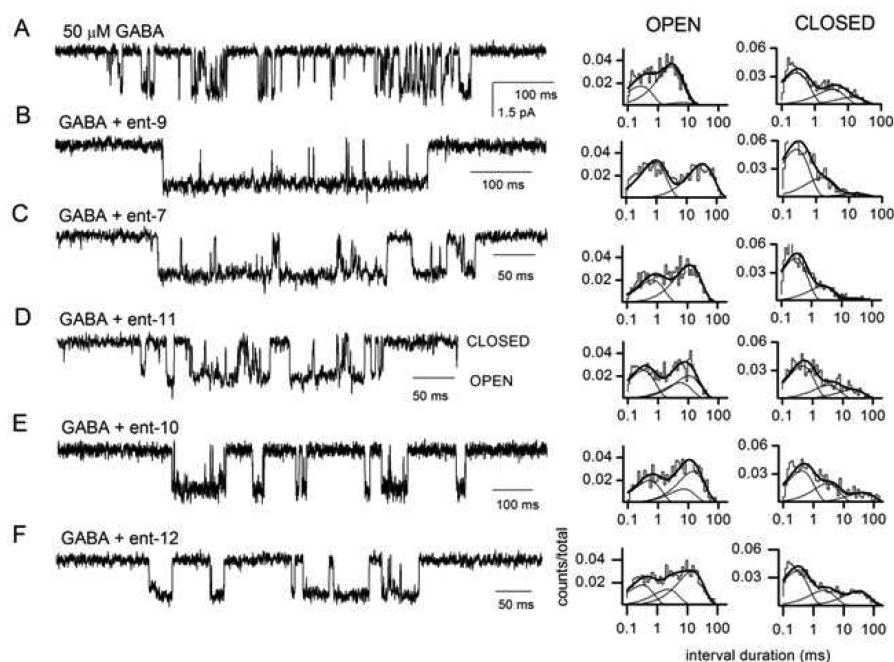
Figure 3.
Exposure to compounds **ent-9**, **ent-7**, **ent-11**, **ent-10**, **or ent-12** modulates single-channel currents from wild-type α1β2γ2 GABA$_A$ receptors. Sample single-channel currents and the respective open and closed time histograms in the presence of 50 µM GABA (A), GABA + 20 µM **ent-9** (B), GABA + 20 µM **ent-7** (C), GABA + 20 µM **ent-11** (D), GABA + 20 mM **ent-10** (E), or GABA + 20 µM **ent-12** (F). Channel openings are shown as downward deflections. In the presence of GABA alone, the open times were 0.27 ms (35%), 2.4 ms (53%), and 4.6 ms (11%), and the closed times were 0.22 ms (53%), 1.4 ms (24%), and 8.9 ms (22%). In the presence of GABA + **ent-9**, the open times were 0.80 ms (52%), and 30.3 ms (48%), and the closed times were 0.24 ms (67%), 1.4 ms (28%), and 14.3 ms (6%). In the presence of GABA + **ent-7**, the open times were 0.64 ms (39%), and 10.9 ms (61%), and the closed times were 0.27 ms (71%), 2.1 ms (24%), and 25.0 ms (5%). In the presence of GABA + **ent-11**, the open times were 0.30 ms (43%), 5.0 ms (24%), and 9.7 ms (33%), and the closed times were 0.43 ms (59%), 3.0 ms (24%), and 19.6 ms (17%). In the presence of GABA + **ent-10**, the open times were 0.48 ms (33%), 6.6 ms (21%), and 13.2 ms (46%), and the closed times were 0.38 ms (52%), 2.8 ms (31%), and 38.5 ms (16%). In the presence of GABA + **ent-12**, the open times were 0.29 ms (29%), 2.1 ms (23%), and 13.2 ms (48%), and the closed times were 0.25 ms (54%), 2.0 ms (24%), and 27.8 ms (22%). The summary of averaged data from multiple patches is given in Table 4.
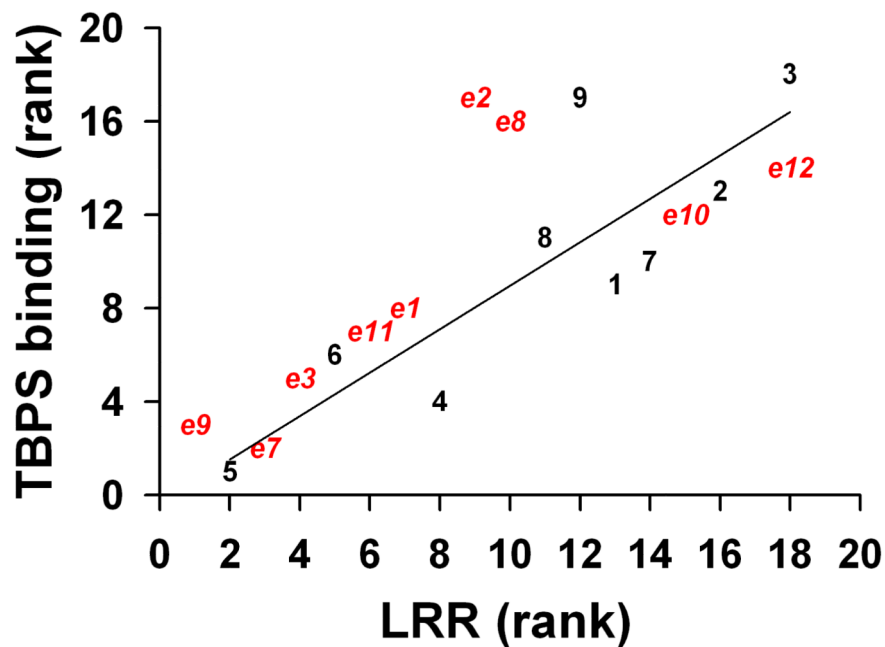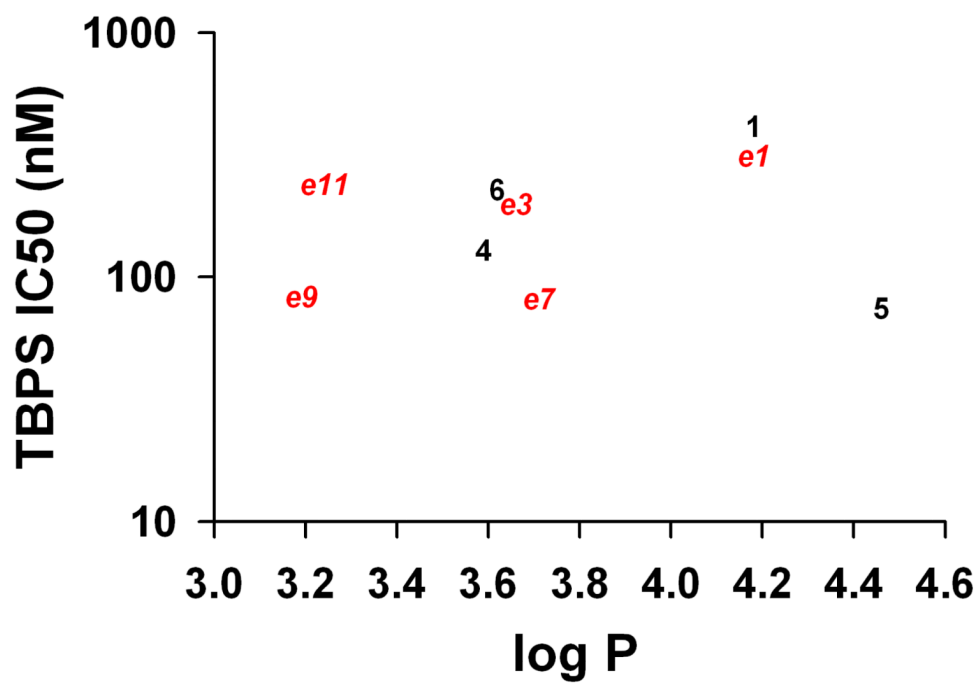
Figure 4.
Correlations between rank order LRR anesthetic effects in tadpoles and TBPS displacement potency. The highest potency compound was assigned the rank of 1 and the least potent compound was assigned the rank of 18. For the least potent anesthetic compounds **3** and **ent-12**, their weak activity did not allow determination of the LLR EC_{50} value. These compounds were arbitrarily assigned the two highest LRR rankings of 18 and 17, respectively. Red symbols represent enantiomers ("*e*"). Black symbols represent natural compounds. Pearson r value of a linear regression (solid line) through all points was 0.81 (p<0.05).
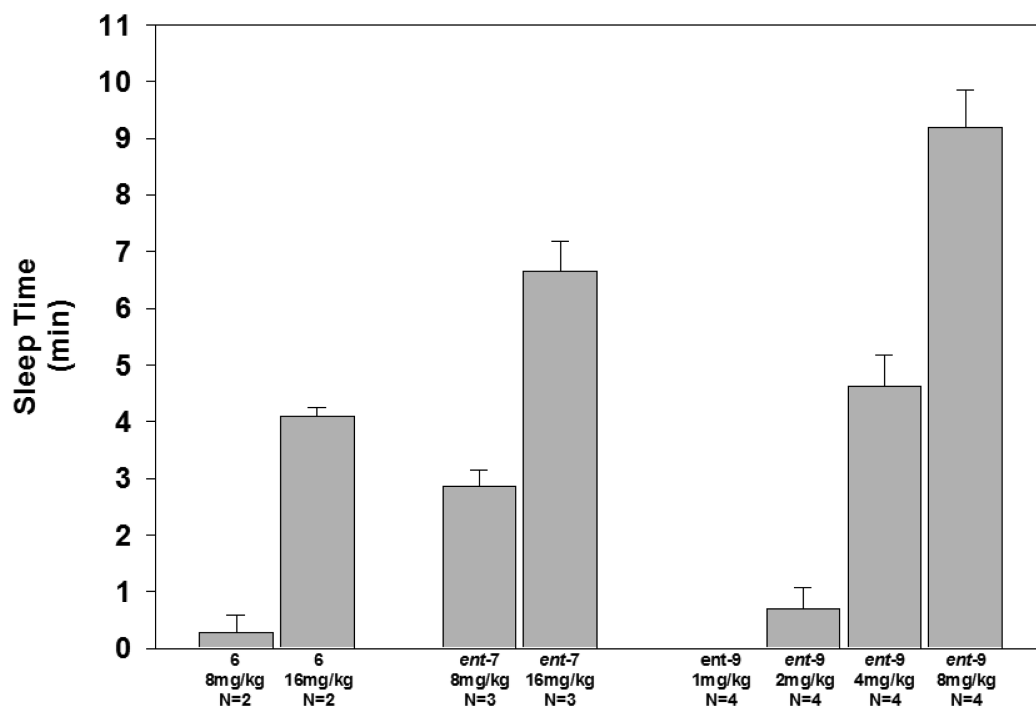
Figure 5.
Duration of anesthesia (LRR) induced by tail vein injection of alphaxalone (**6**), and *ent*-**7** and *ent*-**9**. The compounds were dissolved in 22.5% aqueous 2-(hydroxypropyl)-β-cyclodextrin. For compound *ent*-**9**, serial dilutions of the 8 mg/kg stock solution with 0.9% saline were made to obtain the lower doses tested.

Figure 6.
Correlation between compound logP and log TBPS IC_{50} values for compounds with reported IC_{50} values below 500 nM. Red symbols represent enantiomers ("*e*"). Black symbols represent natural compounds. There was no correlation between logP and log TBPS IC_{50} (or between logP and TBPS IC_{50}, Supplemental Material Figure S3).
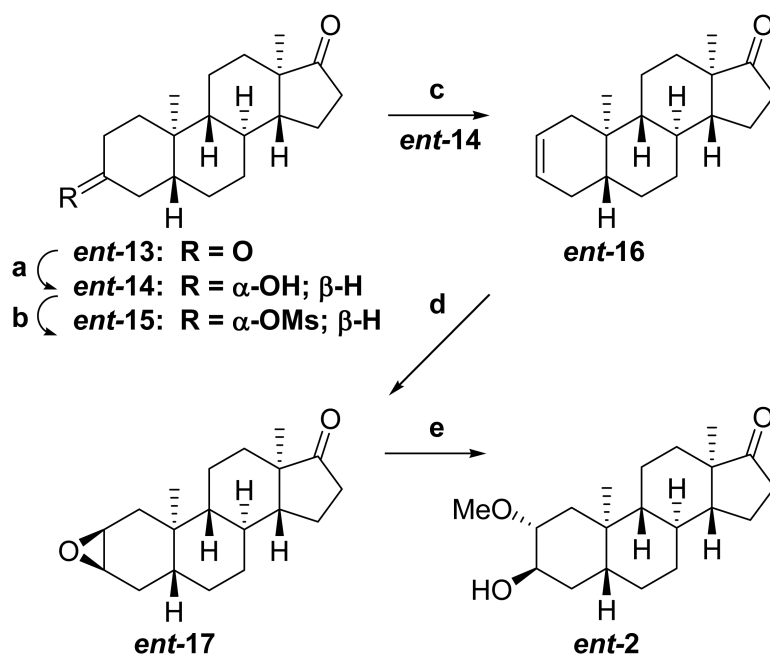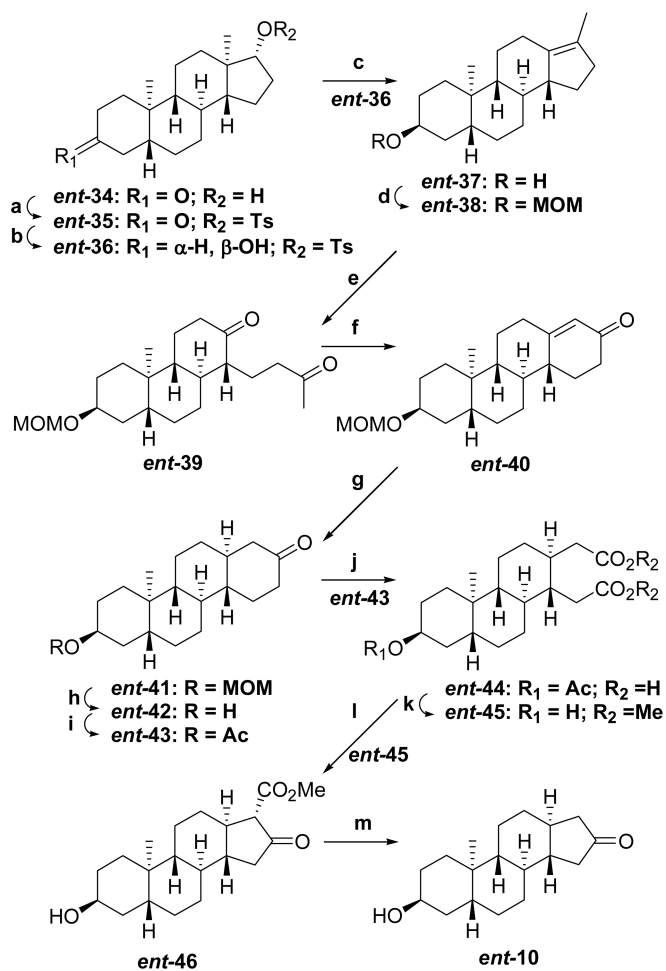
Scheme 1.
[a]Reagents: (a) Li(*t*-OBu)$_3$AlH, THF, –40 °C (82%); (b) MeSO$_2$Cl, Et$_3$N, CH$_2$Cl$_2$ (100%); (c) LiBr, DMF, 125 °C (87%, Δ^2 & Δ^3, 5:1); (d) 30% H$_2$O$_2$, HCO$_2$H, CH$_2$Cl$_2$ (100%, 2,3 & 3,4 epoxides); (e) H$_2$SO$_4$, MeOH (62%).
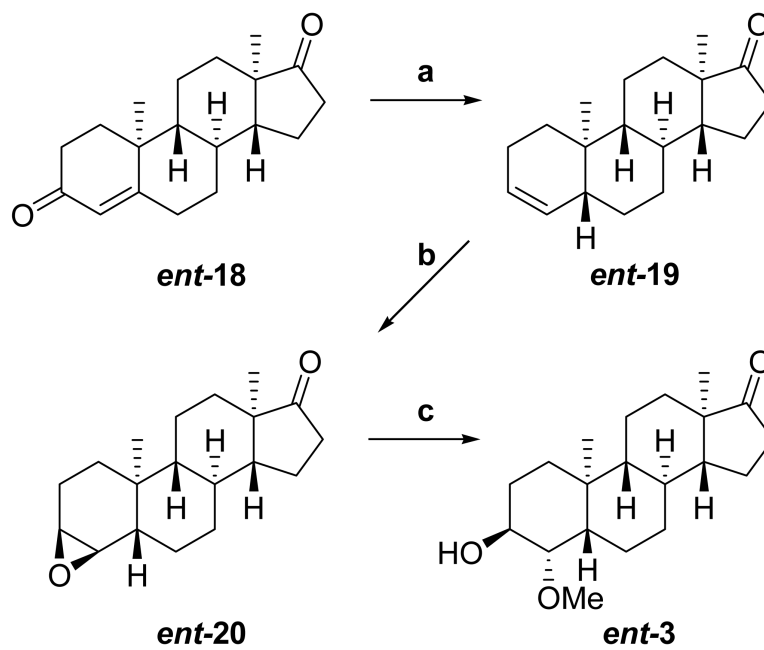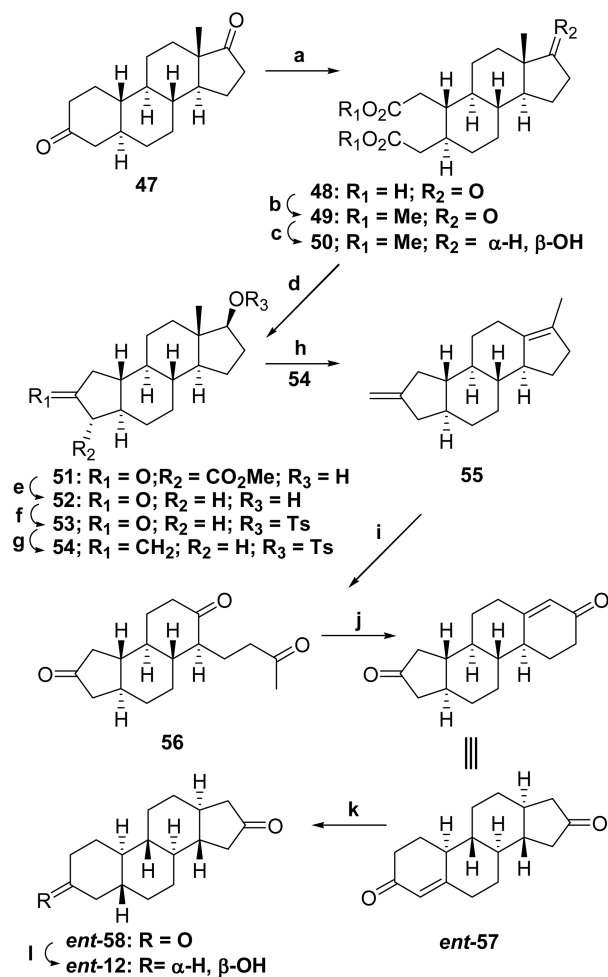
Scheme 2.
[a]Reagents: (a) Zn dust, AcOH (43%); (b) 30% H_2O_2, HCO_2H, CH_2Cl_2 (84%); (c) H_2SO_4, MeOH (78%).
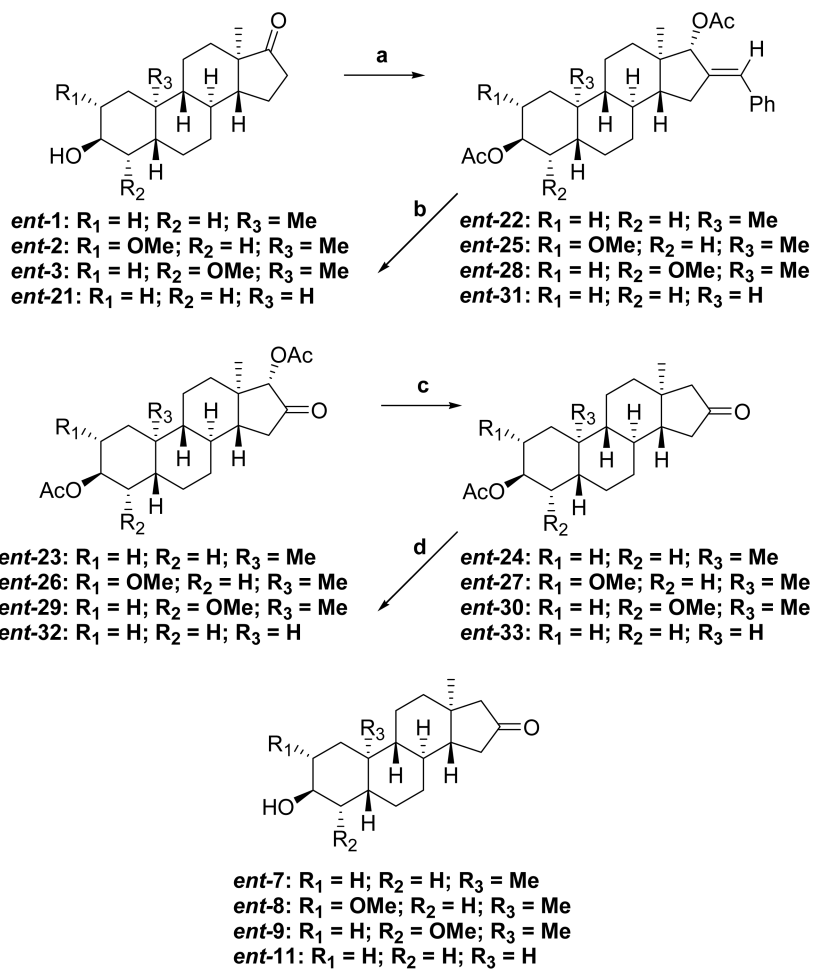
Scheme 3.

[a]Reagents: (a) i) C6H5CHO, EtOH, KOH; ii) NaBH4, CeCl3, EtOH, 0 °C; iii) Ac2O, Et3N, DMAP (91–97% overall); (b) i) O3, MeOH/EtOAc, −78 °C, ii) Me2S (82–98% overall); (c) i) Jones reagent; ii) SmI2,THF (under N2) (70–94%); (d) K2CO3, MeOH/H2O (93-98%).

Scheme 4.

[a]Reagents: (a) *p*-TsCl, pyr, 40 °C (91%); (b) i) K(*s*-Bu)$_3$BH, THF, −78 °C; ii) NaOH, 30% H$_2$O$_2$ (71% overall); (c) MeMgBr, toluene, reflux (91%); (d) ClCH$_2$OMe, (*i*-Pr)$_2$EtN, CH$_2$Cl$_2$, 0 °C (90%); (e) O$_3$, CH$_2$Cl$_2$, −78 °C; ii) Zn dust, AcOH (65% overall); (f) NaOH, MeOH/H$_2$O (75%); (g) Li/liq. NH$_3$, THF (72%); (h) 6 N HCl, MeOH (92%); (i) Ac$_2$O, Et$_3$N, DMAP (95%); (j) CrO$_3$, AcOH, 60 °C; (k) AcCl, MeOH (51%, steps j & k); (l) NaOMe, THF, reflux (71%); (m) LiCl, DMF, 160 °C (86%).

Scheme 5.

^{a}Reagents: (a) CrO$_3$, AcOH, 60 °C; (b) AcCl, MeOH (61%, steps a & b); (c) NaBH$_4$, EtOH, 0 °C (74%); (d) NaOMe, THF under N$_2$, 100 °C (81%); (e) LiCl, DMF, 160 °C (85%); (f) p-TsCl, pyr, DMAP, 65 °C (91%); (g) n-BuLi, MeP(Ph)$_3$Br, C$_6$H$_6$/THF under N$_2$ (80%); (h) MeMgBr, toluene under N$_2$, 100–115 °C (90%); (i) O$_3$, CH$_2$Cl$_2$, −78 °C; ii) Zn dust, AcOH (51% overall); (j) NaOH, MeOH/H$_2$O (82%); (k) Li/liq. NH$_3$, THF (63%); (l) K(s-Bu)$_3$BH, THF, −78 °C; ii) NaOH, 30% H$_2$O$_2$ (70%).
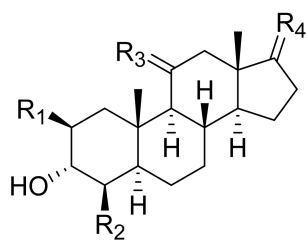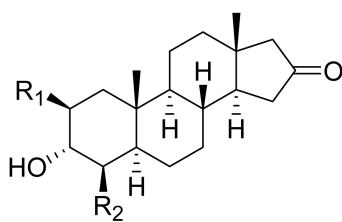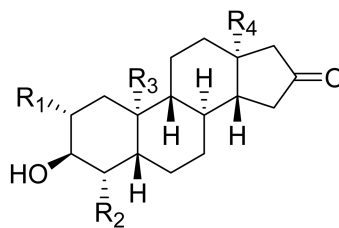
	R₁	R₂	R₃	R₄
1:	H	H	H,H	O
2:	OMe	H	H,H	O
3:	H	OMe	H,H	O
4:	H	H	O	(Z)-C(CN)H
5:	H	H	H,H	α-H, β-C(C=O)Me
6:	H	H	O	α-H, β-C(C=O)Me
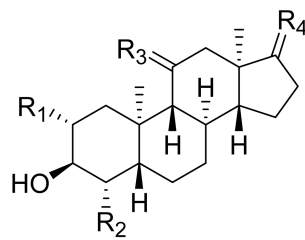
	R₁	R₂	R₃	R₄
ent-1:	H	H	H,H	O
ent-2:	OMe	H	H,H	O
ent-3:	H	OMe	H,H	O

	R₁	R₂
7:	H	H
8:	OMe	H
9:	H	OMe

	R₁	R₂	R₃	R₄
ent-7:	H	H	Me	Me
ent-8:	OMe	H	Me	Me
ent-9:	H	OMe	Me	Me
ent-10:	H	H	Me	H
ent-11:	H	H	H	Me
ent-12:	H	H	H	H

Chart 1.

Table 1

Inhibition of [^{35}S]TBPS Binding by Steroids 1-9 and Steroid Enantiomers *ent*-1-*ent*-3 and *ent*-7-*ent*-12[a]

compd	IC$_{50}$(nM)	*n*Hill
1 [b]	414 ± 127	0.89 ± 0.20
***ent*-1** [b]	311 ± 36	1.00 ± 0.10
2	2,190 ± 420	1.26 ± 0.24
***ent*-2**	6,490 ± 3,330	1.10 ± 0.33
3	>30,000[c]	–
***ent*-3**	198 ± 25	0.98 ± 0.11
4 [d]	128 ± 11	1.44 ± 0.15
5 [e]	74 ± 7	0.89 ± 0.06
6[f]	226 ± 24	1.10 ± 0.11
7	895 ± 49	1.03 ± 0.05
***ent*-7**	81 ± 8	0.92 ± 0.07
8	1,270 ± 162	1.13 ± 0.14
***ent*-8**	3,030 ± 430	1.12 ± 0.13
9 [g]	6,680 ± 2,180	1.56 ± 0.51
***ent*-9**	83 ± 11	1.15 ± 0.15
***ent*-10**	1,520 ± 230	1.06 ± 0.14
***ent*-11**	238 ± 21	0.86 ± 0.06
***ent*-12** [h]	2,020 ± 550	1.05 ± 0.23

[a]The results presented are from duplicate experiments performed in triplicate. Error limits are calculated as the standard error of the mean. Unless noted otherwise, compounds inhibited binding of [^{35}S]TBPS by 95%.

[b]Literature values.[9]

[c]A binding curve could not be calculated. Maximum displacement at 30 μM was 45%.

[d]Literature values.[15]

[e]Literature values.[46]

[f]Literature values.[48]

[g]Inhibition was partial at 30 μM. Maximal displacement for the calculated binding curve was 65%.

[h]Inhibition was partial at 30 μM. Maximal displacement for the calculated binding curve was 88%.

Table 2

Modulation of Rat $\alpha_1\beta_2\gamma_2L$ GABA$_a$ Receptor Function by Steroids 1-9 and Steroid Enantiomers *ent*-1–*ent*-3 and *ent*-1–*ent*-12

compd	oocyte electrophysiology[a]			
	0.1 μM	**1 μM**	**10 μM**	**(gating) 10 μM**
1 [b]	0.97 ± 0.02	1.41. ± 0.01	5.44 ± 0.19	0.02 ± 0.01
ent-1 [b]	1.27 ± 0.29	3.66 ± 0.89	18.87 ± 2.38	0.03 ± 0.21
2	0.84 ± 0.01	1.04 ± 0.04	3.39 ± 0.32	0.08 ± 0.07
ent-2	0.93 ± 0.04	0.93 ± 0.10	1.53 ± 0.11	−0.01 ±0.01
3	1.08 ± 0.21	1.01 ± 0.14	0.91 ± 0.07	0.12 ± 0.17
ent-3	1.21 ± 0.02	3.67 ± 0.33	12.21 ± 1.26	0.05 ± 0.03
4 [c]	1.17 ± 0.04	5.06 ± 0.60	21.51 ± 7.07	0.17 ± 0.00
5 [d]	1.26 ± 0.14	3.89 ± 1.34	9.65 ± 3.87	0.37 ± 0.07
6 [e]	2.91 ± 0.57	4.70 ± 1.11	19.64 ± 4.04	0.11 ± 0.02
7	1.01 ± 0.16	1.38 ± 0.13	9.98 ± 3.09	−0.02 ±0.02
ent-7	1.42 ± 0.02	7.72 ± 0.39	34.70 ± 1.69	0.15 ± 0.04
8	0.92 ± 0.04	1.23 ± 0.09	4.48 ± 0.90	0.01 ± 0.00
ent-8	0.91 ± 0.01	1.03 ± 0.03	2.95 ± 0.24	0.01 ± 0.01
9	0.88 ± 0.08	0.72 ± 0.02	0.69 ± 0.01	0.02 ± 0.01
ent-9	1.41 ± 0.07	6.48 ± 0.40	16.00 ± 2.21	0.08 ± 0.03
ent-10	0.93 ± 0.02	1.13 ± 0.02	3.56 ± 0.15	0.01 ± 0.02
ent-11	0.97 ± 0.02	2.43 ± 0.02	10.52 ± 0.61	0.02 ± 0.01
ent-12	0.80 ± 0.02	0.76 ± 0.06	1.65 ± 0.13	0 ± 0.02

[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/2 μM GABA current. Error limits are calculated as standard error of the mean (*N* ≥ 4).

[b]Literature values. [9]

[c]Literature values. [15]

[d]Literature values. [46]

[e]Literature values. [48]

Table 3

Modulation of Rat Wild-Type and Mutant $\alpha_1\beta_2\gamma_2$L GABA$_A$ Receptor Function by Steroid Enantiomers *ent*-9 and *ent*-7[a]

Receptor	*ent*-9	*ent*-7
$\alpha_1\beta_2\gamma_{2L}$	9.2 ± 5.2[**]	7.8 ± 3.9[**]
α_1(Q241L)$\beta_2\gamma_{2L}$	1.3 ± 0.1[**, **]	1.3 ± 0.3[*, **]
α_1(S240L)$\beta_2\gamma_{2L}$	9.4 ± 2.9[**, #]	7.6 ± 3.8[*, #]
α_1(W245L)$\beta_2\gamma_{2L}$	0.9 ± 0.1[#, **]	1.0 ± 0.1[#, **]

[a]The data give the mean ($\pm$S.D.) effect of 20 µM ***ent*-9** or ***ent*-7** on peak currents elicited by GABA at a concentration (5 µM for wild-type; 10 µM for mutants) producing 11-17% of maximal current. Number of cells was 4-7 at each receptor-compound combination. Receptors were expressed in HEK 293 cells. Statistical analysis applies to comparison with no effect (using paired two-sample t-test) and comparison with potentiation observed at the wild-type receptor (ANOVA with Bonferroni post-hoc correction). (

[#], not significant;

[*], $p < 0.05$;

[**], $p < 0.01$)

Table 4

Summary of Results on the Effects of *ent*-9, *ent*-7, *ent*-11, *ent*-10, *ent*-12 on Single-Channel Currents from Rat $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$ Receptors[a]

compd	OT long (ms)	Fraction OT long	Fraction CT long
Control[b] (no steroid)	6.3 ± 2.6	0.13 ± 0.05	0.29 ± 0.06
ent-9	17.7 ± 6.6[***]	0.53 ± 0.03[***]	0.06 ± 0.03[***]
ent-7	12.4 ± 3.4	0.57 ± 0.05[***]	0.05 ± 0.01[***]
ent-11	9.8 ± 3.0	0.20 ± 0.08	0.11 ± 0.05[***]
ent-10	16.1 ± 3.9[*]	0.53 ± 0.06[***]	0.21 ± 0.04
ent-12	11.5 ± 3.3	0.49 ± 0.04[***]	0.27 ± 0.04

Non-significant changes are not marked.

[a]The rat wild-type $\alpha_1\beta_2\gamma_{2L}$ GABA$_A$ receptor was activated by 50 μM GABA in the absence (control) or presence of 20 μM *ent*-9, *ent*-7, *ent*-11, *ent*-10, or *ent*-12. Number of patches was 4-7. The intracluster open time histograms were best-fitted to the sums of three (control, *ent*-11, *ent*-10, and *ent*-12) or two exponentials (*ent*-9 and *ent*-7). The intracluster closed time histograms were fitted to the sums of three exponentials. The data give the mean duration and relative frequency (fraction) of the longest-lived intracluster open time component and the relative frequency (fraction) of the longest-lived intracluster closed-time component. Previous work has shown that potentiation by steroids is mediated by changes in was conducted using ANOVA these three parameters. [16, 30] Statistical analysis was conducted using ANOVA with Bonferroni post-hoc correction. The significance level applies to comparison to control condition.

[*], $P < 0.05$;

[***], $P < 0.001$.

[b]The control data are from our previous publication. [49]

Table 5

Effects of Steroids 1–9 and Steroid Enantiomers *ent*-1–*ent*-3 and *ent*-1–*ent*-12 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]	3.38 ± 0.90	−2.83 ±2.66	None[d]	–
***ent*-1** [c]	1.42 ± 0.18	−2.17 ±0.48	5.48 ± 0.12	−33 ± 0
2	5.09 ± 2.43	−1.29 ± 1.22	>10	–
***ent*-2**	2.39 ± 0.28	−2.10 ±0.42	>10	–
3	None	–	None	–
***ent*-3**	0.59 ± 0.07	−1.84 ± 0.32	2.94 ± 0	−20 ± 1
4 [e]	1.44 ± 0.20	−2.84 ± 0.77	5.48 ± 0.20	−33 ± 0
5 [f]	0.42 ±0.04	−1.83 ± 0.32	5.5 ± 0.5	−7.5 ± 1.1
6 [g]	1.12 ± 0.14	−3.38 ± 2.28	5.48 ± 0.11	−34 ± 0
7	3.60 ± 1.52	−2.74 ± 3.25	7.54 ± 0	−30 ± 0
***ent*-7**	0.55 ± 0.09	−2.90 ± 0.07	1.73 ± 0.04	−37
8	3.15 ± 0.30	−2.55 ± 0.78	9.29 ± 0.03	−15 ± 1
***ent*-8**	2.91 ± 0.36	−2.37 ± 0.80	10.3 ± 0	−21 ± 0
9	3.15 ± 0.25	−2.60 ± 0.70	13.5 ± 0	−74 ± 0
***ent*-9**	0.34 ± 0.04	−2.75 ± 1.13	1.73 ± 0.03	−36 ± 0
***ent*-10**	3.64 ± 2.17	−4.32 ± 11.8	None	–
***ent*-11**	1.16 ± 0.21	−4.03 ± 4.06	5.48 ± 0.12	−33 ± 0
***ent*-12**	None	–	None	–

[a]The methods are as reported previously.[46] Error limits are calculated as the standard error of the mean ($N = 10$ or more animals at each of five or more different concentrations).

[b]LSR typically has a very steep concentration–response curve. The reported ^{n}Hill values vary widely depending on how many animals had LSR at the concentration (typically, 3 µM) preceding the concentration (typically, 10 µM) that produced LSR in all animals. No SAR conclusions were based on these slope values.

[c]Literature values.[9]

[d]"None" indicates that all animals had a swimming response at 10 µM test compound.

[e]Literature values. [15]

[f]Literature values. [46]

[g]Literature values. [48]