Neurosteroid Analogues. 9. Conformationally Constrained Pregnanes: Structure−Activity Studies of 13,24-Cyclo-18,21-dinorcholane Analogues of the GABA Modulatory and Anesthetic Steroids (3α,5α)- and (3α,5*β*)-3-Hydroxypregnan-20-one

Xin Jiang,[†] Brad D. Manion,[‡] Ann Benz,[§] Nigam P. Rath,[∥] Alex S. Evers,[†,‡] Charles F. Zorumski,[§,⊥] Steven Mennerick,[§,⊥] and Douglas F. Covey*[,†]

Departments of Molecular Biology and Pharmacology, Anesthesiology, Psychiatry, and Anatomy and Neurobiology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110, and Department of Chemistry, University of Missouri−St. Louis, St. Louis, Missouri 63121

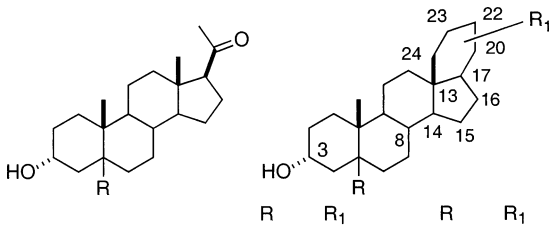
Received June 20, 2003

The hydrogen-bond-acceptor properties of the carbonyl moiety in the 17*β*-acetyl group on the D-ring of the anesthetic steroids (3α,5α)- and (3α,5*β*)-3-hydroxypregan-20-one form an important part of the anesthetic steroid pharmacophore. 13,24-Cyclo-18,21-dinorcholanes containing a ketone or conjugated ketone group at C-20, C-22, C-23, or C-24 were prepared as conformationally constrained analogues of these anesthetic steroids and were used to probe for alternate locations for the D-ring hydrogen-bond-accepting carbonyl group. The analogues were evaluated (1) in [^{35}S]-*tert*-butylbicyclophosphorothionate binding experiments, (2) in electrophysiological experiments using rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes, and (3) as tadpole anesthetics. In the binding assay, the relative order of potencies for the analogues in the 5α- and 5*β*-series is identical. For the ketones, the order is 24-one ≥ 23-one > 20-one > 22-one. Likewise, for the enones, the order is Δ^{22}-24-one > $\Delta^{20(22)}$-23-one > Δ^{22}-20-one > Δ^{23}-22-one. Similar relative orders of potencies are also found in the other two bioassays. The activities of the 24-one and Δ^{22}-24-one compounds were expected to be very low, because the carbonyl group in these compounds is located over the steroid C-ring and oriented toward C-8. Instead, these compounds have the highest activities in their respective series, with the Δ^{22}-24-one compounds having activities comparable to those of the reference anesthetic steroids. The electrophysiology results obtained with the 24-oxo-cyclosteroids suggest that rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors contain more than one donor for the hydrogen-bond-acceptor group of anesthetic steroids. The family of cyclosteroids should be useful for future structure−activity relationship studies of steroid modulation of other $GABA_A$ receptor subtypes.

The endogenous steroids (3α,5α)- and (3α,5*β*)-3-hydroxypregnan-20-one (**1a**, **1b**; Chart 1) are positive allosteric modulators of $GABA_A$ receptors composed of α, *β*, and *γ* subunits.[1] The physiological and pharmacological actions of these and structurally related steroids are a topic of widespread interest. At pharmacological doses, these steroids have anxiolytic, anticonvulsant, sedative hypnotic, and anesthetic activities.[2]

Along with a critical role for the hydrogen-bond-donating 3α-hydroxyl group, previous structure−activity relationship (SAR) studies have indicated that a hydrogen-bond-acceptor group located at C-17 and positioned to accept a hydrogen bond from above the plane of the steroid C,D-rings is important for high pharmacological activity. Structural modifications that position this hydrogen-bond-accepting group in or below the plane of the steroid C,D-rings yield analogues having no anesthetic activity and little, if any, actions at $GABA_A$ receptors.[3−5]

Chart 1



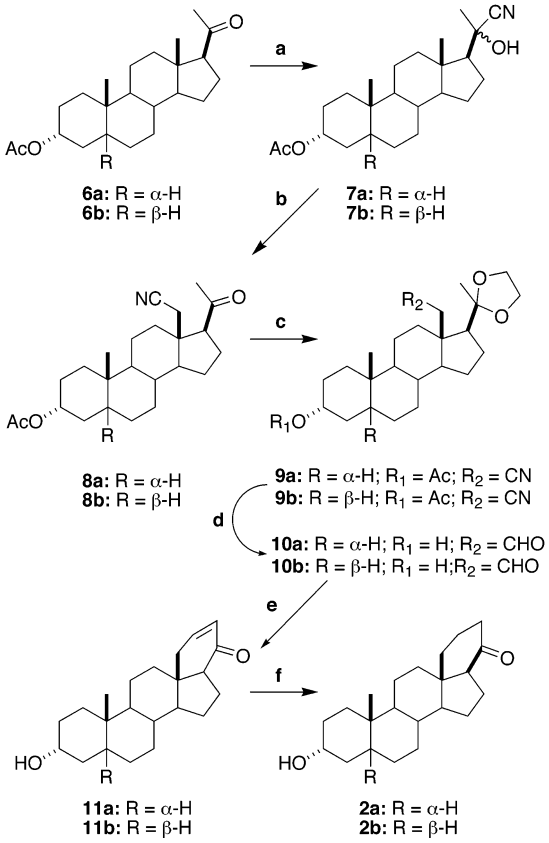
	R	R1		R	R1
1a: R = α-H	**2a:** α-H	20-oxo		**4a:** α-H	23-oxo
1b: R = β-H	**2b:** β-H	20-oxo		**4b:** β-H	23-oxo
	3a: α-H	22-oxo		**5a:** α-H	24-oxo
	3b: β-H	22-oxo		**5b:** β-H	24-oxo

In a recent study, conformationally constrained steroids (D-ring epoxides and oxetanes) were examined to define further the optimal location of the hydrogen-bond-accepting group. From that study it was concluded that this group should be near perpendicular to the plane of the D-ring above C-17.[6] In the study reported herein, we have prepared and evaluated a series of 13,24-cyclo-18,21-dinorcholane analogues of steroids **1a** and **1b** to probe for alternate locations for the D-ring

* Corresponding author. Telephone: 314-362-1726. Fax: 314-362-7058. E-mail: dcovey@molecool.wustl.edu.
[†] Department of Molecular Biology and Pharmacology, Washington University School of Medicine.
[‡] Department of Anesthesiology, Washington University School of Medicine.
[§] Department of Psychiatry, Washington University School of Medicine.
[∥] Department of Chemistry, University of Missouri−St. Louis.
[⊥] Department of Anatomy and Neurobiology, Washington University School of Medicine.

10.1021/jm030302m CCC: $25.00
Published on Web 10/30/2003

Scheme 1[a]



[a] Reagents and conditions: (a) acetone cyanohydrin, Et_3N, 50−25 °C; (b) $Pb(OAc)_4$, $CaCO_3$, I_2, cyclohexane, reflux, *hν*; (c) ethylene glycol, pyridinium *p*-toluenesulfonate, toluene, reflux; (d) 1 M DIBALH in toluene, THF, −78 to 25 °C; (e) 4 N HCl, THF, 25 °C; (f) 5% Pd/$BaSO_4$, H_2, EtOAc, 40 psi, 25 °C.

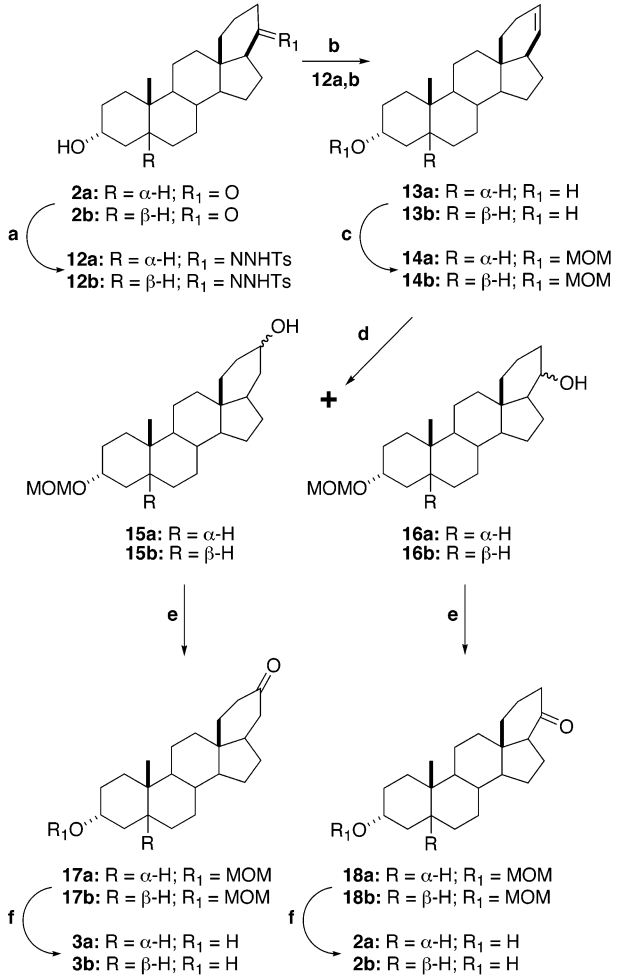
hydrogen-bond-accepting group (Chart 1). The fifth ring present in these analogues provides an invariant framework upon which hydrogen-bond-accepting groups can be positioned to probe for receptor hydrogen-bond interactions from many different directions and at varying distances in or above the plane of the steroid C,D-rings.

The analogues have been evaluated by three different methods. These methods are (1) noncompetitive displacement of [^{35}S]TBPS ([^{35}S]-*tert*-butylbicyclophosphorothionate) from the picrotoxin site found on the heterogeneous population of $GABA_A$ receptors of rat brain membranes, (2) electrophysiological actions on rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes, and (3) loss of righting and swimming reflex in *X. laevis* tadpoles. We found that 13,24-cyclo-18,21-dinorcholane analogues containing a C-24 carbonyl group (e.g., **35a**, **35b**) have activities comparable to those of steroids **1a** and **1b**. Notably, the C-24 carbonyl group in these analogues is positioned to accept a hydrogen bond from above the C-8, C-14, C-15 edge of the steroid. The possibility that a hydrogen-bond interaction between a steroid and a $GABA_A$ receptor could occur in this three-dimensional arrangement could not have been anticipated from previous SAR studies.

Chemistry

Schemes 1−5 show the synthetic routes to the target cyclosteroids in both the 5α- and 5*β*-series. However,

Scheme 2[a]

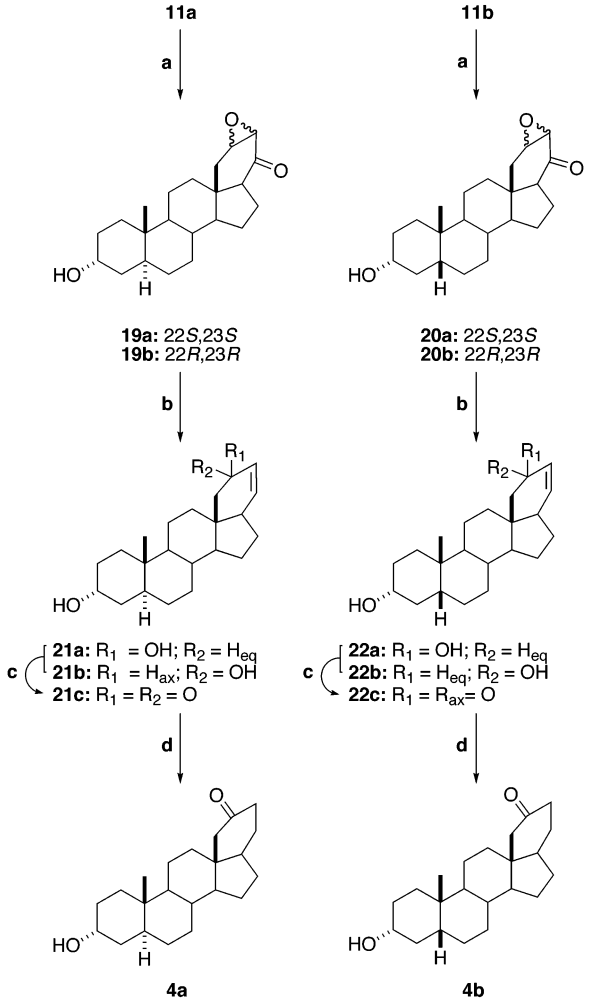


[a] Reagents and conditions: (a) *p*-toluenesulfonhydrazide, MeOH, concentrated H_2SO_4, 25 °C; (b) 2.5 N *n*-BuLi in hexanes, THF, 0−25 °C; (c) MOMCl, (*i*-Pr)$_2$NEt, CH_2Cl_2, 25 °C; (d) (i) 1 M BH_3 in THF, 0 °C to 25 °C; (ii) aqueous NaOH, 30% H_2O_2, 0−25 °C; (e) pyridinium chlorochromate, NaOAc, CH_2Cl_2, 25 °C; (f) 37% aqueous HCl, MeOH, 25 °C.

since the synthetic routes for the two series are identical, only a description for the 5α-series is summarized here. Using reaction conditions similar to those reported previously,[7,8] cyano ketone **8a** was prepared from the 20-ketosteroid **6a** (Scheme 1) in two steps (40%). The 20-keto group was then protected with ethylene glycol to give compound **9a** (83%), and this compound was reacted with DIBALH in toluene at room temperature to yield aldehyde **10a**. As described for the preparation of a different 13,24-cyclo-18,21-dinorchol-22-en-20-one, treatment of aldehyde **10a** with aqueous HCl removed the ketal protecting group and affected a Robinson annulation to yield the cyclosteroid **11a** (57% from steroid **9a**).[9] Hydrogenation of the 22-en-20-one **11a** gave the 20-one **2a** (91%).

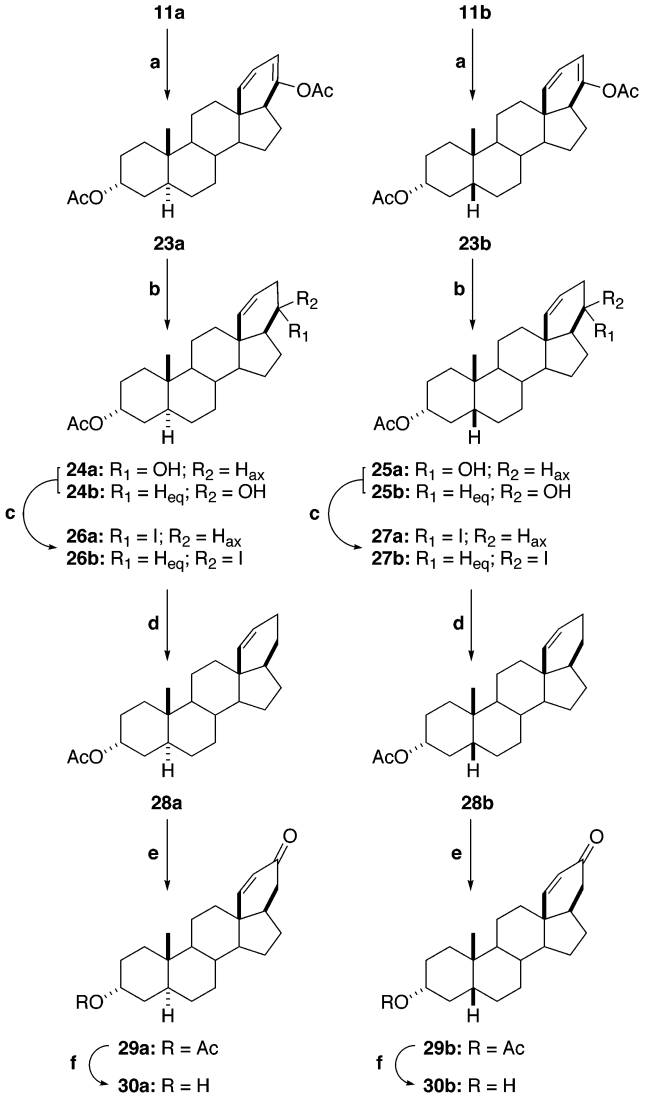
The 22-ones were obtained from the 20-ones via the intermediate 20(22)-olefins (Scheme 2). For the 5α-series, cyclosteroid **2a** was first converted to tosylhydrazone **12a** and then treated with excess *n*-BuLi in THF at room temperature to produce selectively the 20(22)-olefin **13a** (64% from steroid **2a**).[10] After protecting the 3α-hydroxyl group as the MOM ether (compound **14a**, 95%), hydroboration gave a mixture of the two pairs of diastereomeric alcohols **15a** and **16a**. Without

Scheme 3[a]



[a] Reagents and conditions: (a) 30% H_2O_2, aqueous NaOH, MeOH/1,4-dioxane, 0 °C; (b) $NH_2NH_2{\cdot}xH_2O$ ($x \sim 1.5$), AcOH, MeOH, reflux; (c) MnO_2, $CHCl_3$, 25 °C; (d) 5% Pd/$BaSO_4$, H_2, MeOH, 40 psi, 25 °C.

Scheme 4[a]



[a] Reagents and conditions: (a) NaI, Ac_2O, Me_3SiCl, 0−25 °C; (b) $NaBH_4$, EtOH, 25 °C; (c) Ph_3P, I_2, imidazole, toluene, 95 °C; (d) SmI_2, HMPA, *i*-PrOH, THF, 25 °C; (e) CrO_3, 3,5-dimethylpyrazole, CH_2Cl_2, 25 °C; (f) aqueous NaOH, MeOH, reflux.

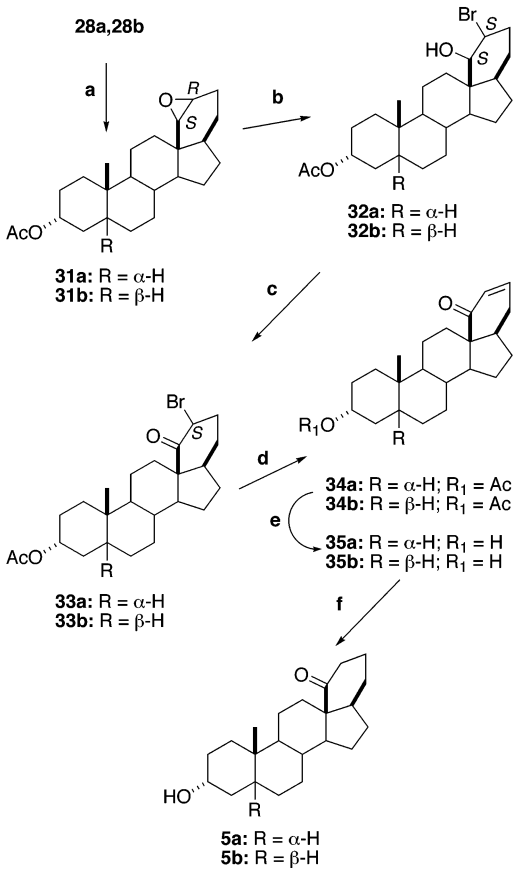
further separation, these alcohols were oxidized with PCC in CH_2Cl_2 at room temperature and the MOM group was removed by hydrolysis to give the 22-one **3a** (51% from compound **14a**) and recovered 20-one **2a** (33% from compound **14a**).

The 23-ones were obtained from the 22-en-20-ones (Scheme 3). For the 5α-series, epoxidation of enone **11a** with alkaline H_2O_2 in MeOH and 1,4-dioxane at 0 °C gave a mixture (86%) of (22*S*,23*S*)-epoxide **19a** and (22*R*,23*R*)-epoxide **19b** in the ratio of 1.8:1. The epoxides underwent a Wharton rearrangement[11] upon treatment with hydrazine hydrate and acetic acid in refluxing MeOH for 2h to give allylic alcohols **21a** and **21b** (62%). Selective oxidation of the allylic hydroxyl groups with activated MnO_2 in $CHCl_3$ for 48 h at room temperature led to the 20(22)-en-23-one **21c** (47%), and hydrogenation of this enone gave the 23-one **4a** (94%).

As reported below, we evaluated both the 20-, 22-, 23-, 24-ones and an enone analogue of each of these compounds for their GABA-ergic and anesthetic effects. The synthetic routes to the 22-en-20-ones and 20(22)-en-23-ones are included in Schemes 1 and 3, respectively. Syntheses of the 23-en-22-ones are shown in Scheme 4. Syntheses of the 22-en-24-ones, along with the 24-ones, are illustrated in Scheme 5.

The 23-en-22-ones were obtained from the 22-en-20-ones via the intermediate 20(22),23-dienol-20-acetates (Scheme 4). For the 5α-series, enone **11a** was reacted with Me_3SiCl/NaI in Ac_2O at room temperature for 24 h to give dienol acetate **23a** (69%).[12] Compound **23a** was then reduced with $NaBH_4$ in ethanol at room temperature to give the β,γ-unsaturated alcohols **24a** and **24b** (92%).[12] These alcohols were then reacted with Ph_3P/I_2 and imidazole in toluene at 95 °C for 1.5 h to give, in quantitative yield, the iodides **26a** and **26b**. Several methods were tried for the conversion of these iodides to compound **28a**. The attempted hydride displacement of the iodide using either $LiB(Et)_3H$ in THF (Super-Hydride) or Bu_3SnH/AIBN[13] and an attempted iodide removal using Zn reduction were unsatisfactory. The reactions gave an elimination product, the 20(22),23-diene, as the main product. Ultimately, we found that when iodides **26a** and **26b** were reduced with SmI_2/HMPA in THF,[14] the desired 23-olefin **28a** (66% from compounds **24a** and **24b**) was obtained along with the 20(22),23-diene in the ratio of 4:1. The 20(22),23-diene was readily removed by chromatography after adduct

Scheme 5[a]



[a] Reagents and conditions: (a) *m*-chloroperbenzoic acid, $NaHCO_3$, CH_2Cl_2, 25 °C; (b) 48% aqueous HBr, MeCN, −40 °C to 25 °C; (c) Jones reagent, acetone, 5 °C; (d) Li_2CO_3, LiBr, DMF, 125 °C; (e) aqueous NaOH, MeOH, reflux; (f) 5% $Pd/BaSO_4$, EtOAc, 40 psi, 25 °C.

formation with 4-phenyl-1,2,4-triazoline-3,5-dione.[15] Allylic oxidation of compound **28a** with 3,5-dimethylpyrazole/CrO_3 complex[16] in CH_2Cl_2 at −15 °C for 3 h, followed by hydrolysis of the 3-acetyloxy group, gave the 23-en-22-one **30a** (55% from compound **28a**).

The 22-en-24-ones and 24-ones were obtained from 23-olefin precursors (Scheme 5). For the 5α-series, olefin **28a** was oxidized with *m*-CPBA in CH_2Cl_2 at room temperature for 5 h to form epoxide **31a** in 90% yield. Cleavage of this epoxide with aqueous HBr (48%) in acetonitrile at room temperature led to bromohydrin **32a** (70%).[17] Oxidation of compound **32a** with Jones reagent in acetone at 5 °C for 1 h afforded α-bromo ketone **33a** in quantitative yield. Conversion of compound **33a** to the enone **34a** was accomplished using Li_2CO_3/LiBr in DMF at 125 °C (70%). Hydrolysis of the 3-acetyloxy group gave the 22-en-24-one **35a** (80%). Hydrogenation of compound **35a** gave the 24-one **5a** (92%).

Molecular Modeling

Figure 1 shows the superimposition of steroid **1a** with a cyclosteroid that contains a carbonyl group at each of the four possible positions on the fifth ring. Regardless of the position of the carbonyl group in the cyclosteroid analogues, the most stable conformation of the fifth ring is a chair. Boat or twist-boat conformations for the fifth ring are higher in steric energy by ∼2.5−3.5 kcal/mol

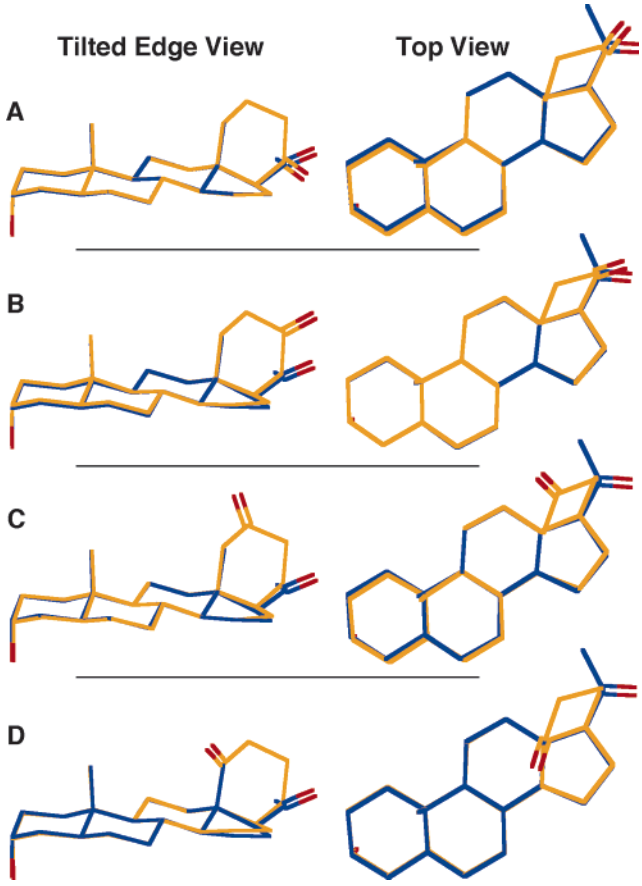


Figure 1. Superimpositions of the cyclosteroids **2a**−**5a** (orange) with anesthetic steroid **1a** (blue). The hydrogen atoms are not displayed. In each panel, the two structures were superimposed using the six carbon atoms of the steroid C-ring for alignment: (A) 20-oxo-cyclosteroid **2a**/steroid **1a**, (B) 22-oxo-cyclosteroid **3a**/steroid **1a**, (C) 23-oxo-cyclosteroid **4a**/steroid **1a**, and (D) 24-oxo-cyclosteroid **5a**/steroid **1a**.

and would be populated in solution at 25 °C to only a minor extent (<2%). A crystal structure determination for cyclosteroid **5a** confirms that the conformation of the fifth ring for this cyclosteroid in the solid state is a chair (see Supporting Information). Although not shown, molecular modeling for the analogous superimpositions of steroid **1b** and its corresponding cyclosteroid analogues yields equivalent results for both the conformation of the cyclosteroid fifth ring and its alignment with the pregnane side chain of steroid **1b**.

Whereas Figure 1 demonstrates a reasonable alignment for each pair of steroids when bound to a $GABA_A$ receptor, the purpose of the figure is to provide a visual context for comparing the relative positions of the carbonyl groups in the aligned molecules. Additionally, a difference of 2.5−3.5 kcal/mol between the chair and boat or twist-boat conformations of each cyclosteroid is too small a difference to exclude a boat conformation for the receptor bound conformation of any of the cyclosteroids. An additional figure showing C-ring alignments for the chair and boat or twist-boat conformations of the fifth ring of each cyclosteroid shown in Figure 1 is included in the Supporting Information. The spacial orientation of the carbonyl group in cyclosteroid **4a** is dramatically altered by the chair to boat interconversion, since the carbonyl group in this molecule is at the "bow" of the boat. The spacial orientation of the carbonyl

Table 1. Displacement of [^{35}S]TBPS Binding by Cyclosteroids

compd	IC_{50} (nM)[a]	n_{Hill}	O−O distance (Å)[b]
	5α-Steroids		
1a; 20-oxo	74 ± 7	0.89 ± 0.06	11.17
5a; 24-oxo	301 ± 20	1.01 ± 0.06	8.31
4a; 23-oxo	334 ± 17	1.14 ± 0.05	10.02
2a; 20-oxo	514 ± 64	0.84 ± 0.08	10.93
3a; 22-oxo	1440 ± 100	0.95 ± 0.06	11.77
35a; Δ^{22}-24-one	197 ± 23	1.03 ± 0.10	8.16
21c; $\Delta^{20(22)}$-23-one	243 ± 11	0.89 ± 0.03	10.19
11a; Δ^{22}-20-one	373 ± 20	1.17 ± 0.06	11.05
30a; Δ^{23}-22-one	1260 ± 100	1.00 ± 0.07	11.76
	5β-Steroids		
1b; 20-oxo	71 ± 18	0.57 ± 0.06	10.74
5b; 24-oxo	329 ± 35	0.95 ± 0.08	8.56
4b; 23-oxo	899 ± 150	0.89 ± 0.11	10.15
2b; 20-oxo	1780 ± 180	1.36 ± 0.16	10.32
3b; 22-oxo	3230 ± 640	0.73 ± 0.11	11.57
35b; Δ^{22}-24-one	105 ± 12	0.90 ± 0.07	8.42
22c; $\Delta^{20(22)}$-23-one	268 ± 43	0.64 ± 0.05	10.35
11b; Δ^{22}-20-one	1570 ± 140	1.15 ± 0.10	10.44
30b; Δ^{23}-22-one	4750 ± 970	0.95 ± 0.18	11.52

[a] Results presented are from duplicate experiments performed in triplicate. Error limits are calculated as standard error of the means. [b] Intramolecular oxygen−oxygen (O−O) distances. For the cyclosteroids, the fifth ring has the chair conformation.

groups in cyclosteroids **2a**, **3a**, and **5a** are only modestly altered by the chair to boat interconversion. Future molecular modeling/QSAR studies with additional analogues will be required to provide information regarding the receptor bound alignment and conformation of the compounds.

As displayed in Figure 1A, cyclosteroid **2a** mimics the high-energy conformer of steroid **1a** in which its C-21 methyl group is aligned vertically in a 1,3-diaxial arrangement with that steroid's C-18 methyl group. The carbonyl group of cyclosteroid **2a** is aligned along the axis of the C-17, C-20 bond and, unlike the carbonyl group of superimposed steroid **1a** (Figure 1A), is projecting toward the α-face of the molecule. Cyclosteroids **3a**, **4a**, and **5a** do not mimic any conformations of steroid **1a**. In cyclosteroid **3a**, the carbonyl group is elevated above the corresponding carbonyl group of steroid **1a** and both carbonyl groups project in the same general direction (Figure 1B). In cyclosteroid **4a**, the carbonyl group is located above the plane of the cyclosteroid C,D-rings, is aligned with the axis of the C-16, C-17 bond in the D-ring, and is projecting away from the direction of the carbonyl group in superimposed steroid **1a** by ∼130° (Figure 1C). In cyclosteroid **5a**, the carbonyl group is as elevated above the plane of the C,D-rings, as it is in cyclosteroid **3a**, but it now projects toward C-8. Relative to the position of the carbonyl group in superimposed steroid **1a** (Figure 1D), the carbonyl group of cyclosteroid **5a** is higher above the plane of the C,D-ring plane and projecting in a different direction by ∼110°.

[^{35}S]TBPS Displacement

The results for the noncompetitive displacement of [^{35}S]TBPS from the picrotoxin binding site found on the heterogeneous $GABA_A$ receptors present in rat brain membranes by the 13,24-cycloketones and their corresponding enones are reported in Table 1. Within the 5α-series, none of the cyclosteroids is as potent a displacer of [^{35}S]TBPS as the prototype 5α-reduced steroid **1a**. The order of potency for [^{35}S]TBPS displacement by the 13,24-cycloketones is: **5a** ≈ **4a** > **2a** > **3a**. The addition of a conjugated double bond in the 13,24-cyclo ring causes a minor enhancement of displacement potency for each compound in the enone series. For the enones, the order of potency is **35a** > **21c** > **11a** > **30a**.

Similar displacement potencies were found for the 5β-series. Once again, none of the cyclosteroids is as potent a displacer of [^{35}S]TBPS as the prototype 5β-reduced steroid **1b**. The order of potency for displacement by the 13,24-cycloketones is **5b** > **4b** > **2b** > **3b**. For the enones, the order of potency is **35b** > **22c** > **11b** > **30b**. Thus, the relative order of potencies is identical for the ketones (24-one ≥ 23-one > 20-one > 22-one) and the enones (Δ^{22}-24-one > $\Delta^{20(22)}$-23-one > Δ^{22}-20-one > Δ^{23}-22-one) in both the 5α- and 5β-series of congeners. The Hill coefficients for [^{35}S]TBPS displacement are near unity (0.84−1.17) in the 5α-series, but have a wider range of values (0.57−1.15) in the 5β series.

Also shown in Table 1 are the intramolecular oxygen−oxygen (O−O) distances for each compound. Although within each cyclosteroid series [^{35}S]TBPS displacement potency correlates with decreasing intramolecular O−O distance, the reference steroids have intramolecular O−O distances similar to those of the least potent cyclosteroid analogues. Hence, intramolecular O−O distance alone is not a sufficient parameter to account for displacement potency. Not surprisingly, the spacial relationship between the 3α-hydroxy group and the D-ring hydrogen-bond-acceptor group is also an important parameter.

Electrophysiology

The ability of the compounds to affect $GABA_A$ receptor function was determined by electrophysiological techniques using *X. laevis* oocytes expressing rat $\alpha_1\beta_2\gamma_{2L}$-type $GABA_A$ receptors (Table 2). For screening purposes, each cyclosteroid was evaluated at three different concentrations for its ability to enhance currents mediated by 2 μM GABA. Direct gating effects of each compound (10 μM) also were determined. This screening method is useful for making a qualitative distinction between active and inactive modulators. However, because all compounds were not screened on the same oocyte and because oocytes vary within and between batches in their potentiation, the screening results do not permit meaningful quantitative comparisons among compounds.

Quantitative comparisons were made for compounds in subsequent experiments wherein both the analogue (500 nM) and corresponding 5α- or 5β-reduced reference steroid (500 nM) were applied to the same oocyte (Figure 2). This concentration was chosen for comparison because it was well above the threshold for potentiation by reference steroids (∼0.1 μM, Table 2) but did not evoke detectable desensitization, which can confound quantitation. All test steroids showed significantly less potentiation than the corresponding reference steroids ($p < 0.05$, $n = 5$ oocytes for each compound). At this concentration, only enone **35a** in the 5α-series was quantitatively similar as a potentiator to reference steroid **1a**, and was notably better than other test compounds. In the 5β-series, enone **35b** was quantitatively most similar to the reference steroid **1b**. Most compounds with little activity at 500 nM

Table 2. Modulation of Rat $\alpha_1\beta_2\gamma_{2L}$ $Gaba_A$ Receptor Function by Cyclosteroids

	oocyte electrophysiology[a]			
compd	0.1 μM	1 μM	10 μM	gating (10 μM)
		5α-Steroids		
1a; 20-oxo[b]	1.26 ± 0.14	3.89 ± 1.34	9.65 ± 3.87	0.37 ± 0.07
5a; 24-oxo	1.22 ± 0.23	3.05 ± 0.79	10.73 ± 1.87	0.39 ± 0.69
4a; 23-oxo	1.39 ± 0.38	1.64 ± 0.15	6.49 ± 0.59	0.32 ± 0.01
2a; 20-oxo	1.01 ± 0.03	1.44 ± 0.03	2.28 ± 0.05	0 ± 0.1
3a; 22-oxo	1.30 ± 0.15	1.61 ± 0.22	2.93 ± 0.68	0.06 ± 0.06
35a; Δ^{22}-24-one	1.98 ± 0.25	8.33 ± 0.75	9.45 ± 4.19	0.07 ± 0.10
21c; $\Delta^{20(22)}$-23-one	1.59 ± 0.14	5.53 ± 0.66	16.58 ± 2.45	0.35 ± 0.12
11a; Δ^{22}-20-one	1.49 ± 0.20	5.87 ± 1.52	9.07 ± 1.86	0.72 ± 0.03
30a; Δ^{23}-22-one	1.12 ± 0.08	1.54 ± 0.30	5.07 ± 0.98	−0.04 ± 0.05
		5β-Steroids		
1b; 20-oxo	1.20 ± 0.10	2.82 ± 0.51	9.77 ± 2.15	0.06 ± 0.03
5b; 24-oxo	1.26 ± 0.05	3.45 ± 0.29	10.42 ± 1.65	0.04 ± 0.02
4b; 23-oxo	0.97 ± 0.03	1.03 ± 0.08	3.17 ± 0.42	0.01 ± 0.01
2b; 20-oxo	0.93 ± 0.06	1.12 ± 0.14	1.51 ± 0.13	−0.25 ± 0.21
3b; 22-oxo	0.97 ± 0.01	1.11 ± 0.14	2.65 ± 0.33	−0.10 ± 0.06
35b; Δ^{22}-24-one	1.36 ± 0.60	3.15 ± 1.19	21.38 ± 11.08	0.20 ± 0.19
22c; $\Delta^{20(22)}$-23-one	1.07 ± 0.10	1.83 ± 0.25	4.50 ± 0.46	−0.02 ± 0.01
11b; Δ^{22}-20-one	1.07 ± 0.17	1.17 ± 0.11	2.34 ± 0.37	0.11 ± 0.04
30b; Δ^{23}-22-one	1.87 ± 0.24	1.80 ± 0.25	4.44 ± 0.87	0.26 ± 0.31

[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 the 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 means. [b] Values reported are from ref 21.

(Figure 2C, D) were able to potentiate $GABA_A$ receptor activity in a concentration-dependent manner (Table 2). This suggests that activity is not completely lost in any of the cyclosteroids in this series.

Overall, the pattern of potentiation was strikingly similar for the 5α- and 5β-series (Figure 2C, D) and matched well the rank order of steroids assayed in the TBPS binding assay (Figure 3A, B). In the case of both assays (along both axes of the plots in Figure 3A,B), the rank order for the 5α-reduced compounds closely paralleled the rank order of the corresponding 5β-reduced compounds. Although the single-point electrophysiology assay reflects a mixture of potency and efficacy and the TBPS EC_{50} reflects a purer potency measure, the results suggest that SAR for the cyclosteroids obey similar constraints, regardless of whether the steroids are 5α- or 5β-reduced. The results of Figure 3A,B also suggest that there is a reasonably positive correlation between cyclosteroids that potently displace TBPS binding and those that potentiate GABA-gated currents at a low concentration. An IC_{50} in the TBPS assay of greater than ∼250 nM for 5α-reduced steroids or ∼500 nM for 5β-reduced steroids is associated with little potentiation, except in the case of cyclosteroids **30a** and **30b** (gray symbols in Figure 3A,B). These compounds were among the weakest displacers of TBPS binding but yielded measurable potentiation of GABA currents. An explanation for the actions of these outliers was not ascertained.

The ability of 10 μM steroids to directly gate $GABA_A$ receptor currents was also measured. In the 5α-series, compounds **1a**, **5a**, **4a**,and **21c** all had similar gating actions. Compound **11a** appeared to gate about twice as much current as these compounds. Both the reference steroid **1b** and the entire 5β-series of cyclosteroids were found to have nonsubstantial direct gating actions. Without exception, no compound with weak potentiation effects was found to have substantial gating effects.

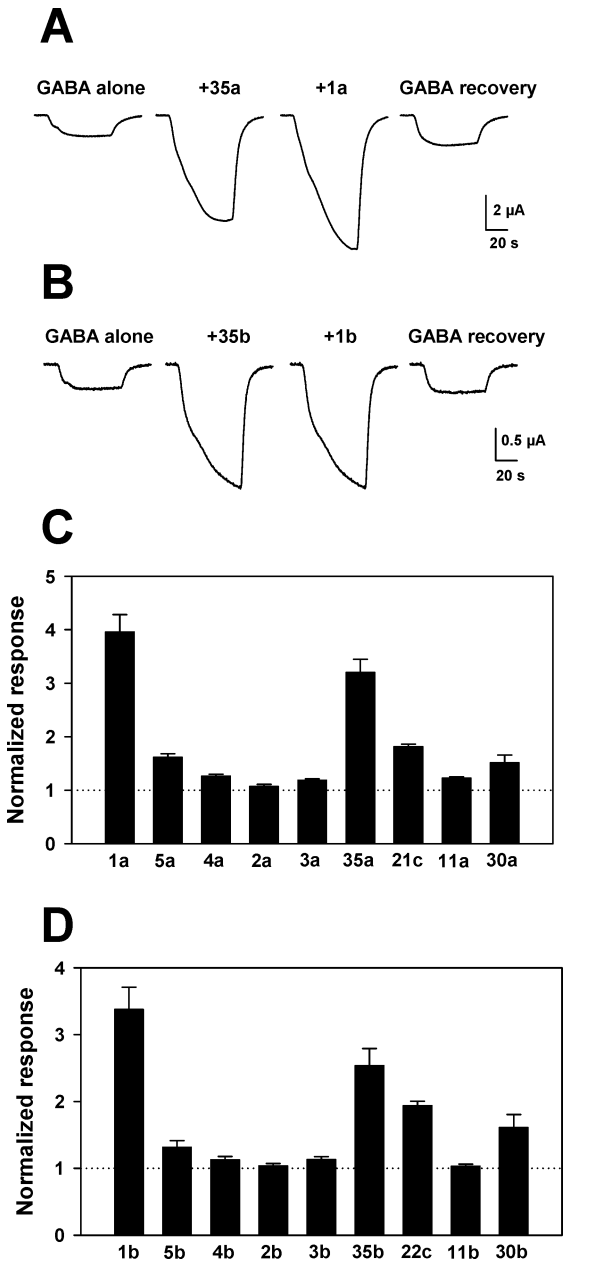


Figure 2. Electrophysiological responses to GABA are potentiated by cyclosteroids. (A) GABA currents potentiated by 5α-reduced cyclosteroids. Two-electrode voltage-clamp recording (−70 mV) from an oocyte showing the response to 2 μM GABA alone and in the presence of 500 nM test compound **35a** and in the presence of reference compound **1a**. At this concentration, effects of steroids were readily reversible (right panel). (B) Analogous experiment in another oocyte showing effects of 5β-reduced compounds. (C) Summary of the effects of the 5α-cyclosteroid series tested at 500 nM against the response to 2 μM GABA alone (dotted line denotes the normalized response to GABA alone). (D) Similar summary for the 5β-reduced series.

Tadpole Behavior

The anesthetic actions of the cyclosteroids were evaluated using tadpoles. The EC_{50}s for loss of righting response (LRR), a widely used index for comparing potency of anesthetics,[18] are shown in Table 3. Also

Table 3. Cyclosteroid Effects on Tadpole Righthing and Swimming Reflexes

	LRR[a]		LSR[b]	
compd	ED_{50} (μM)	n_{Hill}	EC_{50} (μM)	n_{Hill}
		5α-Steroids		
1a; 20-one	0.42 ± 0.04	−1.83 ± 0.32	5.50 ± 0.48	−7.5 ± 1.1
5a; 24-one	0.32 ± 0.02	−1.75 ± 0.17	1.73± 0.03	−36.5 ± 0.07
4a; 23-one	1.12 ± 0.36	−1.75 ± 0.83	3.58 ± 1.08	−16.7 ± 28.3
2a; 20-one	1.36 ± 0.20	−2.51 ± 0.76	5.45 ± 0.07	−33.5 ± 0.1
3a; 22-one	2.37 ± 0.47	−2.02 ± 0.64	none @ 10 μM	
35a; Δ^{23}-24-one	1.02 ± 0.0	−17.1 ± 0.91	2.66 ± 0.0	−24.2 ± 0.0
21c; $\Delta^{20(22)}$-23-one	1.28 ± 0.38	−1.16 ± 0.26	10.0 ± 0.0	−17.0 ± 0.0
11a; Δ^{22}-20-one	3.38 ± 0.67	−2.79 ± 1.89	none @ 10 μM	
30a; Δ^{23}-22-one	0.73 ± 0.16	−1.57 ± 0.43	5.48 ± 0.09	−33.5 ± 0.1
		5β-Steroids		
1b; 20-one	0.063 ± 0.003	−1.54 ± 0.12	0.30 ± 0.0	−6.93 ± 0.47
5b; 24-one	0.40 ± 0.12	−4.97 ± 5.29	0.89 ± 0.0	−18.2 ± 0.4
4b; 23-one	1.44 ± 0.02	−3.02 ± 0.09	3.55 ± 1.15	−17.5 ± 33.6
2b; 20-one	0.94 ± 0.55	−1.23 ± 0.64	5.48 ± 0.08	−33.5 ± 0.1
3b; 22-one	2.47 ± 0.53	−1.86 ± 057	9.43 ± 0.06	−18.7 ± 0.6
35b; Δ^{23}-24-one	0.40 ± 0.13	−4.97 ± 5.75	1.00 ± 0.0	−18.6 ± 0.1
22c; $\Delta^{20(22)}$-23-one	1.05 ± 0.00	−16.6 ± 0.9	2.72 ± 0.01	−22.1 ± 0.7
11b; Δ^{22}-20-one	2.56 ± 1.39	−1.59 ± 0.95	8.95 ± 0.01	−19.8 ± 0.01
30b; Δ^{23}-22-one	0.56 ± 0.9	−1.50 ± 0.27	5.48 ± 0.01	−33.5 ± 0.1

[a] LRR = loss of righting response. Error limits are calculated as standard error of the means. [b] LSR = loss of swimming response. Error limits are calculated as standard error of the means.

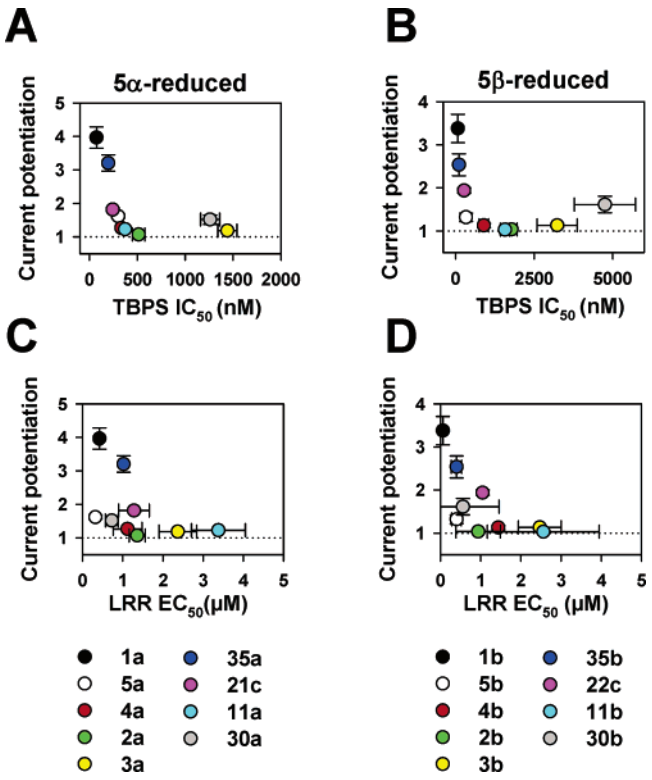


Figure 3. Correlations between assays. (A and B) Correlation between the IC_{50} values of the [^{35}S]TBPS binding assay with the degree of potentiation from the electrophysiology assay. The data are a plot of values from Table 1 and a replot of potentiation values from Figure 2C,D. The dotted line indicates no potentiation in the electrophysiology assay. (C and D) Correlation between the potency (EC_{50}) of the compounds in the anesthesia LRR assay and potentiation in the electrophysiological assay. The data are a plot of values from Table 3 and a replot of potentiation values from Figure 2C,D. The legend below panel C applies to panels A and C (the 5α-reduced series), and the legend below panel D applies to panels B and D (the 5β-reduced series).

shown in Table 3 are the EC_{50}s for loss of swimming reflex (LSR). This behavior is generally observed at higher concentrations than LRR and can be used to quantitate the steroid concentrations required for total impairment of tactile stimulation.

For the ketones in the 5α-series, the most potent compounds for causing tadpole LRR were reference steroids **1a** and the 24-ketone **5a**. The remaining ketones were about 3−7-fold less potent. The LRR Hill slope values for the five compounds in the series were all around −2. Compound **5a** was about 3-fold more potent in causing LSR than reference steroid **1a**. Compounds **4a** and **2a** and reference steroid **1a** all had comparable LSR EC_{50} values. Compound **3a**, the least potent compound in the series for causing LRR, did not induce LSR in any tadpoles at the highest dose tested (10 μM). As is generally the case, the Hill values for all compounds causing LSR are very steep.

The enones in the 5α-series had LRR and LSR potencies that were similar to those found for the ketones of the series. The LRR Hill slope for enone **35a** was uncharacteristically steep (∼ −17) and the slope for compound **21c** was somewhat shallow (∼ −1). Otherwise, the LRR Hill slopes were similar to those found for the ketones (i.e., ∼ −2). When LSR was observed, the LSR Hill slopes were very steep.

For the ketones in the 5β-series, none of the analogues was as potent in causing LRR as reference steroid **1b**. Among the analogues, compound **5b** had the lowest LRR EC_{50} and also produced a steep concentration−response curve. The Hill value for compound **4b** was similarly steep. Compound **5b** was the only analogue in the series to have an LSR value similar to that found for reference steroid **1b**. The LSR Hill slopes for all the ketone analogues were steeper than the slope value recorded for reference steroid **1b**. The enones in the 5β-series had LRR and LSR potencies that were similar to those found for the ketones of the series. The LRR Hill slope for compound **22c** was very steep.

Because potentiation of $GABA_A$ receptor mediated currents likely underlies anesthetic properties of neuroactive steroids,[19] we examined the correlation between LRR EC_{50} and the current potentiation observed for recombinant $GABA_A$ receptors (Figure 3C,D). While the

relationship was not linear, it is clear from the plot that no compound with poor anesthetic potency yielded strong GABA current potentiation. Possible reasons for the lack of a stronger correlation between these assays include limited dynamic range of the electrophysiological assay, different receptor subunit combinations responsible for the behavioral outcome, species differences, additional in vivo targets of the compounds, and differential in vivo metabolism. Interestingly, compounds **30a** and **30b**, which appeared to be outliers in the TBPS vs electrophysiology correlation (Figure 3A,B), did not stand out as outliers in the LRR vs electrophysiology relationship (gray symbols, Figure 3C,D).

Discussion

The goal of this study was to develop a series of conformationally restricted analogues that would provide new information about the SAR of 5α- and 5*β*-reduced anesthetic steroids. In particular, we were interested in gaining new information concerning the optimal location(s) for a hydrogen-bond-acceptor group on the steroid D-ring. To achieve our goal we chose the 13,24-cyclo-18,21-dinorcholane ring system as the invariant framework for the analogues. The systematic rules of nomenclature tend to obscure the fact that this ring system arises from the formation of a one-carbon bridge between C-18 and C-21 of pregnanes. Hence, for these cyclosteroids the additional fifth ring introduces little new hydrophobic bulk into the core structure of the pregnane ring system. At the same time, the new ring mimics the conformation of the pregnane side chain at C-21 in which there is a sterically unfavorable 1,3-diaxial interaction between C-18 and C-21.

Compounds in the study that mimic a high-energy conformation of 5α-reduced anesthetic steroid **1a** are the 20-oxo-cyclosteroids **2a** and **11a**. As shown in Figure 1A, the carbonyl group in the analogues projects toward the steroid α-face. It is known from previous SAR studies that having the D-ring hydrogen-bond-acceptor group in either the plane of the steroid rings or oriented toward the steroid α-face yields compounds with no anesthetic and minimal GABAergic actions.[3−5] Hence, it is not surprising that cyclosteroids **2a** and **11a** were found to have low potencies in all bioassays used in this study. Similar results were expected, and found, for cyclosteroids **2b** and **11b**, the mimics of the analogous high-energy conformer of 5*β*-reduced anesthetic steroid **1b**.

For the 22-oxo-cyclosteroids, the hydrogen-bond-accepting carbonyl group is located above the plane of the steroid D-ring (Figure 1B). This group projects in the same general direction as it does in the global minimum energy conformations of anesthetic steroids **1a** and **1b**.[5] Previous SAR studies carried out with a variety of D-ring-constrained analogues have shown that maximal activity occurs when the hydrogen-bond-acceptor group is near perpendicular to the plane of the D-ring above the *β*-face of the steroid.[6] The most potent displacer of [^{35}S]TBPS in that study was the C-17 exocyclic epoxide, (3α,5α,17*β*)-spiro[androstane-17,2′-oxiran]-3-ol. Using the C-ring carbon atoms to superimpose this epoxide and cyclosteroid **3a**, we found that the epoxide oxygen and C-20 of cyclosteroid **3a** are separated in space by only 0.17 Å. The carbonyl oxygen of cyclosteroid **3a** is 2.42 Å away from the epoxide oxygen. Accordingly, we anticipated that 22-oxo-cyclosteroids **3a**, **30a** (5α-series) and **3b**, **30b** (5*β*-series) would be more potent in the bioassays than the corresponding 20-oxo-cyclosteroids because of the more favorable location of the hydrogen-bond-acceptor group above the steroid D-ring. This was not the case since the 22-oxo analogues usually had lower activity in all three bioassays. The reason for the poor activity of the 22-oxo analogues is currently uncertain. We speculate that either the 22-ketone group is elevated too high over the D-ring or that the carbonyl group projects in the wrong direction. Further studies with additional cyclosteroids having different types of hydrogen-bond-acceptor groups at this position will be investigated to provide additional insight into the possible reasons for the poor activity of the 22-oxo-cyclosteroids.

The carbonyl group of the 23-oxo-cyclosteroids is located in a region of space that has not been probed in previous SAR studies. Hence, predictions as to the actions of these cyclosteroid positional isomers were not possible. We found that the 23-oxo-cyclosteroids **4a**, **21c** (5α-series) and **4b**, **22c** (5*β*-series) were more active than either the 20-oxo- or 22-oxo-cyclosteroids. When the fifth ring is in a chair conformation (Figure 1C), the carbonyl group of the 23-oxo-cyclosteroids projects far above and to the left of the D-ring. However, as noted earlier, the 23-oxo-cyclosteroids are unique among the group of cyclosteroid positional isomers in that changing the conformation of the fifth ring for the 23-oxo-cyclosteroids to a twist-boat causes a major change in the orientation of the C-23 carbonyl group (see modeling figure in Supporting Information). In the twist-boat conformation, while the carbonyl group still projects far above the steroid, it is now angled toward the C-15, C-16 edge of the steroid D-ring. Until more is known about the receptor-bound conformation of the 23-oxo-cyclosteroids, it is not possible to decide which conformation best explains the actions of these compounds. Additional studies with other 23-substituted analogues will be needed to refine the SAR of this group of compounds.

The most surprising results in the study were obtained with the 24-oxo-cyclosteroids **5a**, **35a** (5α-series) and **5b**, **35b** (5*β*-series). These compounds were the most potent analogues identified in this study. As shown in Figure 1D, the carbonyl group in these analogues is as far above the D-ring as the carbonyl group of the 22-oxo-cyclosteroids. However, the carbonyl groups in the 22-oxo- and 24-oxo-cyclosteroids project toward opposite edges of the steroid rings. Clearly, on the basis of previous SAR studies, one would have predicted that the 22-oxo group was located in a more favorable position to interact with a $GABA_A$ receptor hydrogen-bond donor than the 24-oxo group. The fact that this was not the case indicates that much remains to be learned about the SAR of the D-ring hydrogen-bond-acceptor group of anesthetic steroids. Perhaps there are multiple receptor hydrogen-bond-donor groups arranged in a band over the steroid D-ring that have the potential to interact with steroid hydrogen-bond-acceptor groups located in different locations. Alternatively, it may be that the same receptor hydrogen-bond donor interacts

through an intervening water molecule with steroid hydrogen-bond-acceptor groups positioned in different locations.

There were two reasons for preparing ketone and enone analogues for the four families of 13,24-cyclo-18,21-dinorcholanes included in this study. For the ketones, the fifth ring can adopt a higher energy boat conformation as well as the lower energy chair conformation. The molecular modeling and analysis of our results was based on the lower energy chair conformation for the fifth ring. Adding a double bond in conjugation with the carbonyl group flattens the fifth ring and essentially restrains it to an envelope conformation. Flattening of the ring (chair conformation) has a minor effect on the orientation of the carbonyl group. Additionally, adding the conjugated double bond improves its hydrogen-bond-acceptor properties.[20] We speculate that this electronic effect contributes to the nearly uniform increase in activity observed with the enone derivatives of the ketones.

The finding that the SAR for the 5α- and 5*β*-cyclosteroid analogues are closely similar deserves additional comment. We have been interested in preparing anesthetic steroid analogues for which the SAR of 5α- and 5*β*-reduced congeners are different. Our goal has been to attain a better understanding of the structural features of the binding sites for the two families of anesthetic steroids. Previously, we found some modest SAR differences for a series of 5α- and 5*β*-reduced *N*-acylated 17a-aza-D-homosteroid analogues.[21] We had suspected that by constraining the D-ring hydrogen-bond-acceptor group to several different locations in three-dimensional space, new differences in the SAR for the two families of compounds would become apparent. Unfortunately, this was not the case, and our cyclosteroid results provide no new insights into any possible differences in structural features of the binding sites for 5α- and 5*β*-reduced anesthetic steroids. We remain interested in the possibility that such differences exist and we are preparing other types of analogues to explore this issue further.

In conclusion, we have presented results obtained with a new family of cyclosteroids indicating that there are multiple, and heretofore unexpected, locations possible for the D-ring hydrogen-bond-acceptor group of anesthetic steroids. We plan to expand on this study by changing the hydrogen-bond-acceptor group present in this series of cyclosteroids. For example, we have previously used the cyano group as a hydrogen-bond-acceptor group in our analogue studies of anesthetic steroids.[22] Since a cyano group can occupy either an axial or equatorial position at each of the four positions on the fifth ring of the 13,24-cyclo-18,21-dinorcholane ring system, it can be used to provide additional information on the directionality of hydrogen-bond formation. Additionally, we plan to prepare the series of steroids in which the fifth ring is a five-membered ring instead of a six-membered ring. These analogues are 18,21-cyclopregnanes (formally named as cyclopenta[13,17]-18-norandrostanes), and analogues in this new series will provide additional opportunities to refine the directionality of steroid D-ring/receptor hydrogen-bond interactions. Last, the potential that unique pharmacological activities for the relatively potent cyclosteroids such as **35a** and **35b** exist needs to be explored. Because of the novel location of the hydrogen-bond-acceptor group in these molecules it may be that there will be $GABA_A$ receptor subtype specificity associated with these congeners. There are modest differences in the actions of steroid **1a** at some subtypes of $GABA_A$ receptors.[1] However, thus far, steroids with markedly selective $GABA_A$ receptor subtype specificity have not been identified. The identification of such steroids might affect the pharmacological uses for neuroactive steroids. By analogy, the identification of $GABA_A$ receptor subtype specific benzodiazepines has had a major impact on the pharmacology and clinical use of benzodiazepines.[23]

Experimental Section

General Methods. Melting points were determined on a Kofler micro hot stage and are uncorrected. NMR spectra were recorded in $CDCl_3$ at 300 MHz (^1H) or 75 MHz (^{13}C). IR spectra were recorded as films on a NaCl plate. Elemental analyses were carried out by M-H-W Laboratories, Phoenix, AZ. Solvents were used either as purchased or dried and purified by standard methodology. Flash chromatography was performed using silica gel (32−63 *μ*m) purchased from Scientific Adsorbents, Atlanta, GA.

(3α,5α)-3-(Acetyloxy)-20-hydroxypregnane-20-carbonitrile (7a). Compound **7a** was prepared using a reported procedure.[7] Et_3N (0.2 mL) was added to (3α,5α)-3-(acetyloxy)-pregnan-20-one (**6a**, 1.0 g, 2.77 mmol) in acetone cyanohydrin (2 mL) at 50 °C. The reaction mixture was slowly cooled to room temperature with stirring. After 3 h, water (5 mL) was added to the reaction mixture, and the white solid precipitate (mixture of compound **6a** and **7a**) was filtered and washed thoroughly with water, then dried under high vacuum at room temperature for 24 h. The precipitate was used without further purification.

An analytical sample of **7a** was purified by column chromatography (silica gel; hexanes/EtOAc, 7:1) and obtained as white crystals: mp 192−195 °C (EtOAc−hexanes); ^1H NMR *δ* 0.81 (s, 3H, 19-CH_3), 1.00 (s, 3H, 18-CH_3), 1.62 (s, 3H, 21-CH_3), 2.05 (s, 3H, CH_3CO_2), 5.01 (m, 1H, CHOAc); ^{13}C NMR *δ* 11.3, 13.0, 20.5, 21.5, 24.0, 25.0, 26.1, 28.2, 30.6, 31.8, 32.8 (2 × C), 34.9, 35.8, 40.0, 40.2, 43.5, 54.1, 55.9, 59.1, 70.1, 71.7, 122.0 (CN), 170.7 (CO_2); IR ν_{max} 3389, 2924, 2231, 1701, 1281 cm^{-1}. Anal. ($C_{24}H_{37}NO_3$) C, H, N.

(3α,5α)-3-(Acetyloxy)-20-oxopregnane-18-carbonitrile (8a). Compound **8a** was prepared using a reported procedure.[8] I_2 (1.6 g, 6.30 mmol) was added to a refluxing mixture of $Pb(OAc)_4$ (6.0 g, 13.5 mmol) and $CaCO_3$ (2 g, 20.0 mmol) in cyclohexane (200 mL). The purple solution was refluxed for 1 h and the mixture of compound **6a** and **7a** obtained in the last step [prepared from **6a** (1.0 g, 2.77 mmol)] was added. The reaction mixture was refluxed while being irradiated with a 300 W tungsten lamp for 3 h and then cooled to room temperature. After filtration, the precipitate was washed thoroughly with ether. The combined filtrate was washed successively with 10% $Na_2S_2O_3$ and brine and dried over Na_2SO_4. Removal of solvent under reduced pressure gave a residue which was purified by column chromatography (silica gel; hexanes/EtOAc, 4:1) to give compound **8a** (400 mg, 40% from **6a**) and recovered compound **6a (**400 mg).

Compound **8a** was obtained as a colorless oil: ^1H NMR *δ* 0.80 (s, 3H, 19-CH_3), 2.06 (s, 3H, CH_3CO_2), 2.29 (s, 3H, 21-CH_3), 2.70 (t, J = 9.0 Hz, 1H, C*H*COCH$_3$), 5.02 (m, 1H, CHOAc); ^{13}C NMR *δ* 11.3, 16.4, 20.6, 21.5, 23.1, 24.0, 26.0, 28.0, 31.6, 32.5, 32.7, 32.7, 35.5, 35.7, 36.0, 39.8, 46.1, 53.7, 56.5, 62.0, 69.8, 118.1 (CN), 170.5 (CO_2), 208.7 (CO); IR ν_{max} 2933, 2249, 1732, 1704, 1245 cm^{-1}. Anal. ($C_{24}H_{35}NO_3$) C, H, N.

(3α,5α)-3-(Acetyloxy)-20,20-[1,2-ethanediylbis(oxy)]pregnane-18-carbonitrile (9a). A mixture of compound **8a** (1.93 g, 5.00 mmol), ethylene glycol (3.1 g, 50 mmol), and PPTS (0.64

g, 2.55 mmol, 30% W/W) in toluene (50 mL) was refluxed using a Dean−Stark apparatus under N_2 for 2 h. The reaction mixture was cooled to room temperature, washed with 10% $NaHCO_3$ and brine, and dried over Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 9:1) to give compound **9a** (1.78 g, 83%).

Compound **9a** was obtained as white crystals: mp 176−177 °C (EtOAc−hexanes); ^{1}H NMR δ 0.80 (s, 3H, 19-CH_3), 1.29 (s, 3H, 21-CH_3), 2.05 (s, 3H, CH_3CO_2), 2.27 (d, J = 16.8 Hz, 1H, CH_2CN), 2.52 (d, J = 16.8 Hz, 1H, CH_2CN), 4.04 (m, 4H, OCH_2-CH_2O), 5.01 (m, 1H, CHOAc); ^{13}C NMR δ 11.2, 16.7, 20.3, 21.4, 23.0, 23.2, 23.4, 25.9, 28.0, 31.4, 32.6 (2 × C), 35.0, 35.6, 36.6, 39.7, 43.7, 53.7, 56.0, 56.3, 63.0, 63.7, 69.8, 110.8 (20-C), 119.8 (CN), 170.4 (CO_2); IR ν_{max} 2937, 2241, 1730, 1237 cm^{-1}. Anal. ($C_{26}H_{39}NO_4$) C, H, N.

(3α,5α)-20,20-[1,2-Ethanediylbis(oxy)]-3-hydroxypregnane-18-carboxaldehyde (10a). Compound **9a** (2.0 g, 4.66 mmol) in THF (120 mL) was cooled to −78 °C and DIBALH (1.0 M in toluene, 23.3 mL, 23.3 mmol) was added. The colorless solution was stirred at ambient temperature for 26 h and then cooled to 0 °C. After H_2O (5 mL) was added dropwise to quench the reaction, the solvent was removed at room temperature. EtOAc (50 mL) and H_2O (20 mL) were added to the residue. Insoluble $Al(OH)_3$ was filtered through a pad of Celite 545 and washed thoroughly with EtOAc. The combined filtrate was washed with water and dried over Na_2SO_4. Solvent removal under reduced pressure gave aldehyde **10a** as a white solid that was partially characterized and immediately converted into cyclosteroid **11a**. Aldehyde **10a**: ^{1}H NMR δ 0.77 (s, 3H, 19-CH_3), 1.29 (s, 3H, 21-CH_3), 3.85 (m, 4H, OCH_2CH_2O), 4.03 (m, 1H, C*H*OH), 9.82 (t, J = 2.4 Hz, 1H, CHO); ^{13}C NMR δ 11.1, 20.2, 22.9, 23.4, 23.5, 28.3, 28.9, 31.7, 32.0, 34.8, 35.7, 36.0, 36.3, 39.0, 40.2, 44.1, 54.1, 57.6, 57.7, 62.6, 64.0, 66.2, 111.1 (20-C), 204.9 (CHO).

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-22-en-20-one (11a). A solution of aldehyde **10a** in THF (100 mL) and aqueous HCl (4 N, 32 mL) was stirred under N_2 at room temperature for 16 h. THF was removed under reduced pressure. The residue was extracted with EtOAc. The combined EtOAc extracts were washed with 10% $NaHCO_3$ and brine and dried over Na_2SO_4. After solvent removal under reduced pressure, the residue was purified by column chromatography (silica gel; CH_2Cl_2/EtOAc, 8:1) to give enone **11a** (0.86 g, 57% from **9a**).

Compound **11a** was obtained as white crystals: mp 230−232 °C (EtOAc−hexanes); ^{1}H NMR δ 0.79 (s, 3H, 19-CH_3), 4.05 (m, 1H, C*H*OH), 5.96 (dd, J = 3.0 Hz, J = 9.9 Hz, 1H, CH═C*H*CO), 6.83 (m, 1H, C*H*═CHO); ^{13}C NMR δ 11.2, 20.3, 25.8, 26.2, 27.2, 28.3, 28.9, 32.0, 32.1, 33.9, 35.3, 35.8, 36.1, 39.0, 47.5, 53.7, 56.3, 57.7, 66.3, 127.4 (CH═*C*HCO), 148.3 (*C*H═CHCO), 202.3 (CO); IR ν_{max} 3429, 2924, 1666, 1444 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-20-one (2a). Enone **11a** (328 mg, 1.0 mmol) was dissolved in EtOAc (50 mL) and hydrogenated (40 psi, H_2, 5% Pd/$BaSO_4$, 100 mg) for 1.5 h. The reaction mixture was filtered through a pad of Celite 545 to remove catalyst and the solvent was removed under reduced pressure. The product was purified by column chromatography (silica gel; hexanes/EtOAc/CH_2Cl_2, 6:1:0.3) to give compound **2a** (300 mg, 91%) as white crystals: mp 200−202 °C (EtOAc−hexanes); ^{1}H NMR δ 0.79 (s, 3H, 19-CH_3), 2.50 (m, 1H, CHCO), 4.04 (m, 1H, C*H*OH); ^{13}C NMR δ 11.2, 20.3, 22.1 (2 × C), 25.1, 27.0, 28.4, 29.0, 32.1, 32.2, 33.5, 35.2, 35.8, 36.1, 37.3, 39.0, 49.6, 53.9, 56.7, 61.5, 66.4, 215.6 (CO); IR ν_{max} 3548, 2928, 1701, 1253 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5α)-13,24-Cyclo-18,21-dinorchol-20(22)-en-3-ol (13a). To the solution of compound **2a** (330 mg, 1.0 mmol) and *p*-toluenesulfonhydrazide (186 mg, 1.0 mmol) in MeOH (40 mL) was added three drops of 96% H_2SO_4. The reaction mixture was stirred at room temperature for 3−4 h and the MeOH was removed under reduced pressure. The residue was dissolved in EtOAc (50 mL), washed with 10% $NaHCO_3$ and brine, and dried over Na_2SO_4. After solvent removal, hydrazone **12a** was obtained and used without further purification or characterization.

n-BuLi (2.5 M in hexanes, 1.6 mL, 4 mmol) was added dropwise to the solution of hydrazone **12a** (obtained from 1.0 mmol ketone **2a**) in anhydrous THF (10 mL) under N_2 at 0 °C. The orange solution was stirred overnight (14 h) at ambient temperature and quenched with water (0.4 mL) at 0 °C. EtOAc (50 mL) was added and the mixture was washed with saturated aqueous NH_4Cl and brine and dried over Na_2SO_4. After solvent removal under reduced pressure, the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give compound **13a** (201 mg, 64% from ketone **2a**).

Compound **13a** was obtained as white crystals: mp 169−170 °C (hexanes); ^{1}H NMR δ 0.79 (s, 3H, 19-CH_3), 4.04 (m, 1H, C*H*OH), 5.55 (m, 1H, CH═), 5.76 (m, 1H, CH═); ^{13}C NMR δ 11.2, 19.8, 20.2, 21.6, 24.7, 28.6, 29.1, 30.6, 32.3 (2 × C), 33.2, 34.7, 35.9, 36.2, 39.3, 40.9, 46.6, 54.7, 56.1, 66.6, 124.4 (CH═), 130.9 (CH═); IR ν_{max} 3307, 3019, 2927, 1430, 1002 cm^{-1}. Anal. ($C_{22}H_{34}O$) C, H.

(3α,5α)-3-Methoxymethoxy-13,24-cyclo-18,21-dinorchol-20(22)-ene (14a). Methoxy methyl chloride (0.11 mL, 1.5 mmol) was added to compound **13a** (158 mg, 0.5 mmol) and *N*,*N*-diisopropylethylamine (0.44 mL, 2.5 mmol) in CH_2Cl_2 (20 mL). The resultant solution was stirred at room temperature for 24 h. The solvent was partially removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give compound **14a (**170 mg, 95%) as white crystals: mp 77−78 °C (hexanes); ^{1}H NMR δ 0.80 (s, 3H, 19-CH_3), 3.37 (s, 3H, CH_3O), 3.83 (m, 1H, C*H*OCH_2), 4.66 (m, 2H, OCH_2O), 5.56 (m, 1H, CH═), 5.76 (m, 1H, CH═); ^{13}C NMR δ 11.4, 19.8, 20.2, 21.6, 24.7, 26.4, 28.7, 30.6, 32.3, 32.9, 33.3, 33.7, 34.7, 36.0, 39.9, 40.9, 46.6, 54.7, 55.1, 56.2, 71.7, 94.6 (OCH_2O), 124.4 (CH═), 130.9 (CH═); IR ν_{max} 3016, 2927, 1455, 1043 cm^{-1}. Anal. ($C_{24}H_{38}O_2$) C, H.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-22-one (3a). BH_3 (1.0 M in THF, 1.59 mL, 1.59 mmol) was added to compound **14a** (190 mg, 0.53 mmol) in anhydrous THF (20 mL) under N_2 at 0 °C. The resultant solution was stirred at room temperature for 3.5 h and cooled to 0 °C. Water (0.1 mL) was added to quench the reaction followed by aqueous NaOH (3 N, 3.0 mL) and 30% H_2O_2 (3.0 mL). The reaction mixture was stirred at ambient temperature for 1.5 h and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with brine until the pH was neutral and then dried over Na_2SO_4. After solvent removal under reduced pressure, the residue (a mixture of two pairs of diastereomers of **15a** and **16a**) was used without further purification or characterization.

NaOAc (130 mg, 1.59 mmol) and PCC (228 mg, 1.06 mmol) were added to the solution of compounds **15a** and **16a** in CH_2Cl_2 (30 mL). The reaction mixture was stirred at room temperature for 2 h and Et_2O (50 mL) was added. The mixture was filtered through a pad of Celite 545 and washed thoroughly with ether. Solvent was removed from the combined filtrate to give a mixture of ketones **17a** and **18a** which was dissolved in CH_3OH (12 mL). Then, 37% aqueous HCl (4 mL) was added. The solution was stirred at room temperature for 1 h and MeOH was removed under reduced pressure. EtOAc (30 mL) was added to the residue and the solution was washed with water, 10% $NaHCO_3$, and brine and dried over Na_2SO_4. Solvent removal under reduced pressure gave ketones **3a** and **2a** which were separated by column chromatography (silica gel; EtOAc/CH_2Cl_2, 1:15) to give ketone **3a** (90 mg, 51% from **14a**) and recovered compound **2a** (58 mg, 33% from **14a**).

Compound **3a** was obtained as white crystals: mp 195−197 °C (EtOAc−hexanes); ^{1}H NMR δ 0.79 (s, 3H, 19-CH_3), 2.39 (m, 1H, CH_2CO), 2.54 (dd, J = 6.0 Hz, J = 14.4 Hz, 1H, CH_2-CO), 4.05 (m, 1H, C*H*OH); ^{13}C NMR δ 11.2, 20.4, 23.3, 23.6, 27.7, 28.4, 29.0, 32.0, 32.2, 32.7, 35.6, 35.8, 36.2, 36.9, 39.1, 41.2, 41.5, 48.9, 54.4, 55.6, 66.4, 213.4 (CO); IR ν_{max} 3308, 2918, 1712, 1449, 1007 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5α,22*S*,23*S*)-22,23-Epoxy-3-hydroxy-13,24-cyclo-18,21-dinorcholan-20-one (19a) and (3α,5α,22*R*,23*R*)-22,23-Epoxy-3-hydroxy-13,24-cyclo-18,21-dinorcholan-20-one (19b). A mixture of MeOH (5 mL), 30% H_2O_2 (1.5 mL),

and aqueous NaOH (4 N, 0.25 mL) was added to a solution of enone **11a** (100 mg, 0.30 mmol) in 1:1 MeOH and 1,4-dioxane (20 mL) at 0 °C. The resultant solution was stirred at 0 °C for 3−4 h. EtOAc (30 mL) was added and the organic phase was washed with water and brine and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which was purified by column chromatography (silica gel; hexanes/EtOAc/CH_2Cl_2, 4:1:0.2) to give a mixture of compound **19a** and **19b** (1.8:1) 90 mg (86%). Analytical samples were obtained by HPLC separation (silica gel; hexanes/EtOAc, 6:1).

Compound **19a** was obtained as white crystals: mp 238−240 °C (EtOAc−hexanes); ^{1}H NMR δ 0.81 (s, 3H, 19-CH_3), 3.15 (d, J = 3.3 Hz, 1H, epoxide H-22), 3.55 (bs, 1H, epoxide H-23), 4.05 (m, 1H, C*H*OH); ^{13}C NMR δ 11.2, 20.1, 20.9, 24.7, 27.6, 28.3, 29.0, 32.0, 32.1, 35.1, 35.7, 36.1, 36.4, 39.0, 42.0, 51.8, 53.7, 54.8, 56.4, 57.0, 66.4, 207.1 (CO); IR ν_{max} 3551, 2925, 1691 cm^{-1}. Anal. ($C_{22}H_{32}O_3$) C, H.

Compound **19b** was obtained as white crystals: mp 194−195 °C (EtOAc−hexanes); ^{1}H NMR δ 0.79 (s, 3H, 19-CH_3), 3.23 (d, J = 3.6 Hz, 1H, epoxide H-22), 3.55 (t, J = 3.6 Hz, 1H, epoxide H-23), 4.05 (m, 1H, C*H*OH); ^{13}C NMR δ 11.3, 20.2, 24.2, 25.5, 26.0, 28.3, 29.0, 32.0, 32.2, 35.1, 35.8, 36.1, 36.4, 39.0, 51.5, 53.6, 54.5, 56.7, 57.9, 59.2, 66.4, 209.2 (CO); IR ν_{max} 3435, 2926, 1699 cm^{-1}. Anal. ($C_{22}H_{32}O_3$) C, H.

(3α,5α,23*S*)-13,24-Cyclo-18,21-dinorchol-20(22)-ene-3,23-diol (21a) and (3α,5α,23*R*)-13,24-Cyclo-18,21-dinorchol-20(22)-en-3,23-diol (21b). The mixture of epoxides **19a** and **19b** (68 mg, 0.20 mmol), $NH_2NH_2{\cdot}xH_2O$ ($x \sim 1.5$, 136 μL, 2.4 mmol), and AcOH (2.35 μL) in MeOH (4 mL) was refluxed for 2 h. Water (20 mL) was added and the product was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with water until the pH was neutural and then dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which was purified by column chromatography (silica gel; CH_2Cl_2/hexanes/EtOAc, 1:1:0.2) to give compound **21a** (26 mg, 40%) and compound **21b** (14 mg, 22%).

Compound **21a** was obtained as white crystls: mp 94−96 °C (EtOAc−hexanes); ^{1}H NMR δ 0.82 (s, 3H, 19-CH_3), 4.05 (m, 1H, C*H*OH), 4.22 (m, 1H, CH=CHC*H*OH), 5.72 (m, 1H, C*H*=C*H*CHOH), 5.97 (m, 1H, CH=CHCHOH); ^{13}C NMR δ 11.3, 20.4, 25.3, 28.1, 28.6, 29.1, 30.4, 32.2, 32.3, 34.8, 35.7, 35.9, 36.3, 39.3, 40.0, 46.6, 54.7, 57.0, 64.8, 66.6, 125.6 (CH=), 133.9 (CH=); IR ν_{max} 3359, 3018, 2927, 1446, 1003 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

Compound **21b** was obtained as white crystals: mp 215−217 °C (EtOAc−hexanes); ^{1}H NMR δ 0.81 (s, 3H, 19-CH_3), 4.05 (m, 1H, C*H*OH), 4.28 (m, 1H, CH=CHC*H*OH), 5.57 (d, J = 10.2, 1H, CH=C*H*CHOH), 5.85 (m, 1H, C*H*=CHCHOH); ^{13}C NMR δ 11.3, 20.2, 24.9, 28.5, 29.0, 29.8, 31.0, 32.2, 32.3, 34.2, 34.7, 35.9, 36.2, 39.2, 44.2, 46.4, 54.6, 56.4, 65.9, 66.6, 128.4 (CH=), 132.6 (CH=); IR ν_{max} 3328, 3019, 2926, 1446, 1003 cm^{-1}.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-20(22)-en-23-one (21c). Activated MnO_2 (420 mg, 4.83 mmol) was added to a mixture of compounds **21a** and **21b** (60 mg, 0.18 mmol) in $CHCl_3$ (20 mL). The reaction mixture was stirred at room temperature for 48 h. The MnO_2 was removed by filtration and the $CHCl_3$ was removed under reduced pressure. The residue was purified by column chromatography (silica gel; CH_2Cl_2/EtOAc, 20:1) to give compound **21c** (28 mg, 47%) and a recovered mixture of compounds **21a** and **21b** (27 mg).

Compound **21c** was obtained as white crystals: mp 209−210 °C (EtOAc−hexanes); ^{1}H NMR δ 0.78 (s, 3H, 19-CH_3), 2.37 (d, J = 16.2 Hz, 1H, CH_2CO), 4.05 (m, 1H, C*H*OH), 5.90 (d, J = 9.9 Hz, 1H, C(O)C*H*=CH), 6.96 (dd, J = 5.4, J = 9.9 Hz, 1H, C(O)CH=C*H*); ^{13}C NMR δ 11.3, 19.7, 26.3, 28.3, 29.0, 29.4, 32.0, 32.2, 34.2, 34.8, 35.8, 36.2, 38.0, 39.1, 47.3, 47.4, 54.2, 56.1, 66.5, 127.2 (C(O)*C*H=CH), 152.1 (C(O)CH=*C*H), 200.3 (CO); IR ν_{max} 3467, 2926, 1664, 1451, 1004 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-23-one (4a). Compound **21c** (28 mg, 0.085 mmol) was dissolved in MeOH (10 mL) and hydrogenated (40 psi, H_2, 5% Pd/$BaSO_4$, 9 mg) for 2 h. The reaction mixture was filtered through a pad of Celite 545 to remove catalyst and the solvent was removed under reduced pressure. The product was purified by column chromatography (silica gel; hexanes/EtOAc, 4:1) to give compound **4a** (26 mg, 94%).

Compound **4a** was obtained as white crystals: mp 185−186 °C (EtOAc−hexanes); ^{1}H NMR δ 0.76 (s, 3H, 19-CH_3), 4.04 (m, 1H, C*H*OH); ^{13}C NMR δ 11.2, 19.8, 24.3, 24.9, 25.2, 28.3, 29.0, 31.9, 32.2, 34.2, 34.9, 35.8, 36.2, 36.5, 39.1, 40.5, 44.7, 48.8, 54.3, 56.3, 66.5, 213.7 (CO); IR ν_{max} 3435, 2921, 1704, 1005 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5α)-13,24-Cyclo-18,21-dinorchola-20(22),23-diene-3,20-diol, 3,20-Diacetate (23a). A mixture of compound **11a** (200 mg, 0.61 mmol), NaI (366 mg, 2.44 mmol), and Ac_2O (9 mL) was cooled to 0 °C under N_2, and Me_3SiCl (0.31 mL, 2.44 mmol) was added dropwise. The reaction mixture was stirred at ambient temperature for 24 h and then poured into 10% $NaHCO_3$ (50 mL). After the mixture was stirred for 10 min, the product was extracted using hexanes (4 × 50 mL). The combined extracts were washed with 10% $NaHCO_3$ (5 mL), 10% $Na_2S_2O_3$ (2 × 10 mL), and water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 30:1) to give compound **23a** (173 mg, 69%).

Compound **23a** was obtained as white crystals: mp 135−136 °C (hexanes); ^{1}H NMR δ 0.82 (s, 3H, 19-CH_3), 2.06 (s, 3H, CHOC(O)C*H*$_3$), 2.15 (s, 3H, =COC(O)CH_3), 5.02 (m, 1H, CHOAc), 5.56 (d, J = 5.7 Hz, 1H, CH=COAc), 5.65 (d, J = 9.6 Hz, 1H, C*H*=CHCH=), 5.84 (dd, J = 5.7 Hz, J = 9.6 Hz, 1H, CH=C*H*CH=); ^{13}C NMR δ 11.4, 20.1, 21.2, 21.5, 26.1, 28.4, 29.7, 32.3 (2 × C), 32.9, 33.0, 35.9, 36.0, 37.5, 40.1, 47.9, 48.4, 54.2, 56.2, 70.1, 107.9 (*C*H=COAc), 122.4 (=*C*HCH=COAc), 127.7 (C*C*H=CH), 154.6 (=COAc), 169.1 (O*C*(O)CH_3), 170.7 (O*C*(O)CH_3); IR ν_{max} 3039, 2932, 1763, 1734, 1204 cm^{-1}. Anal. ($C_{26}H_{36}O_4$) C, H.

(3α,5α,20*R*)-13,24-Cyclo-18,21-dinorchol-23-en-3,20-diol, 3-Acetate (24a) and (3α,5α,20*S*)-13,24-Cyclo-18,21-dinorchol-23-en-3,20-diol, 3-Acetate (24b). Compound **23a** (170 mg, 0.41 mmol) was dissolved in EtOH (15 mL), and $NaBH_4$ (78 mg, 2.05 mmol) was added. The solution was stirred under N_2 for 6 h. Most of the EtOH was removed under reduced pressure and the residue was dissolved in EtOAc (50 mL), washed with 5% HCl (10 mL), aqueous $NaHCO_3$ (10 mL), and brine (10 mL), and dried over Na_2SO_4. The solvent was removed and the residue obtained was filtered through a short column (silica gel; hexanes/EtOAc, 8:1) to give a mixture of compounds **24a** and **24b** (140 mg, 92%). Analytical samples of compounds **24a** and **24b** were obtained by column chromatography (silica gel; hexanes/EtOAc, 20:1).

Compound **24a** was obtained as white crystals: mp 159−161 °C; ^{1}H NMR δ 0.81 (s, 3H, 19-CH_3), 2.06 (s, 3H, CH_3C(O)O), 4.10 (m, 1H, C*H*OH), 5.01 (m, 1H, CHOAc), 5.54 (m, 1H, =C*H*CH_2), 5.66 (d, 1H, J = 10.5 Hz, C*H*=CHCH_2); ^{13}C NMR δ 11.3, 20.6, 20.7, 21.5, 25.8, 26.0, 28.3, 30.7, 32.2, 32.8, 32.9, 35.3, 35.9, 38.6, 40.0, 47.9, 51.0, 54.5, 55.1, 66.8, 70.0, 124.7 (CH=), 129.6 (CH=), 170.6 (C(O)O); IR ν_{max} 3399, 3023, 2929, 1734, 1245 cm^{-1}. Anal. ($C_{24}H_{36}O_3$) C, H.

Compound **24b** was obtained as white crystals: mp 168−169 °C; ^{1}H NMR δ 0.83 (s, 3H, 19-CH_3), 2.06 (s, 3H, CH_3C(O)O), 4.06 (m, 1H, C*H*OH), 5.02 (m, 1H, CHOAc), 5.65 (m, 1H, CH_2C*H*=), 5.86 (d, J = 10.5 Hz, 1H, CCH=); ^{13}C NMR δ 11.3, 20.7, 21.5, 25.8, 26.1 (2 × C), 28.3, 30.0, 32.2, 32.8 (2 × C), 34.9, 35.9, 39.7, 40.0, 42.8, 50.1, 54.2, 55.7, 68.4, 70.1, 124.0 (CH=), 129.7 (CH=), 170.7 (C(O)O); IR ν_{max} 3442, 3021, 2931, 1735, 1249 cm^{-1}. Anal. ($C_{24}H_{36}O_3$) C, H.

(3α,5α)-13,24-Cyclo-18,21-dinorchol-23-en-3-ol, Acetate (28a). A mixture of compounds **24a** and **24b** (150 mg, 0.40 mmol), Ph_3P (195 mg, 0.74 mmol), imidazole (101 mg, 1.48 mmol), and I_2 (150 mg, 0.59 mmol) in toluene (15 mL) was heated at 95 °C for 1.5 h. The toluene was removed under reduced pressure and the residue was purified by column chromatography (silica gel, CH_2Cl_2) to give a mixture of compounds **26a** and **26b** in quantitative yield. This product mixture was used without further purification or characterization.

The mixture of compounds **26a** and **26b**, *i*-PrOH (0.20 mL), and HMPA (0.50 mL) in THF (1 mL) was added to a freshly made SmI_2-THF solution (0.1 M, 10 mL) under Ar. The purple solution was stirred at room temperature for 30 min and quenched with saturated aqueous NH_4Cl (1 mL). The solution was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with saturated aqueous NH_4Cl and water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 30:1) to give compound **28a** contaminated with about 20% of the 20(22),23-diene. The mixture was dissolved in acetone (15 mL) and a 2% solution of 4-phenyl-1,2,4-triazoline-3,5-dione in acetone was added dropwise at 0 °C until the pink color persisted. After all of the diene was reacted with 4-phenyl-1,2,4-triazoline-3,5-dione (monitored by TLC), the solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 50:1) to give compound **28a** (94 mg, 66% from compound **24a** and **24b**) as a single spot on TLC (silica gel plate). NMR analysis showed that product **28a** contained ~10% of another inseparable and unidentified isomeric olefin product.

Compound **28a** was obtained as a white solid: mp 117−119 °C; ^{1}H NMR δ 0.82 (s, 3H, 19-CH_3), 2.05 (s, 3H, CH_3C(O)O), 5.01 (m, 1H, CHOAc), 5.67 (bs, 2H, CH=CH); ^{13}C NMR δ 11.4, 20.5, 20.9, 21.2, 21.5, 24.5, 26.1, 26.1, 28.4, 32.3, 32.9 (2 × C), 35.0, 35.9, 38.5, 40.1, 43.3, 43.8, 54.5, 55.1, 70.1, 128.0 (CH=), 129.7 (CH=), 170.7 (C(O)O); IR ν_{max} 3019, 2932, 1736, 1446 cm^{-1}. Anal. ($C_{24}H_{36}O_2$) C, H.

(3α,5α)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorchol-23-en-22-one (29a). A solution of CrO_3 (280 mg, 2.8 mmol) in CH_2Cl_2 (4 mL) was cooled to −15 °C and 3,5-dimethylpyrazole (274 mg, 2.85 mmol) was added in one portion. The resultant brown slurry was stirred for 30 min followed by addition of compound **28a** (40 mg, 0.112 mmol) in CH_2Cl_2 (4 mL). The mixture was stirred at −15 °C for 3 h and a 1:1 mixture of hexanes and ethyl acetate (20 mL) was added. The reaction mixture was filtered through a pad of silica gel. Solvent was removed under reduced pressure to give a residue which was purified by column chromatography (silica gel; hexanes/EtOAc, 10:1) to give compound **29a** (28 mg, 67%). This product was contaminated with ~5% of an unidentified impurity which was removed after hydrolysis of the acetate group.

Partially purified compound **29a** was obtained as a white solid: mp 133−138 °C; ^{1}H NMR δ 0.86 (s, 3H, 19-CH_3), 2.07 (s, 3H, CH_3C(O)O), 2.41 (dm, J = 17.4 Hz, 1H, CH_2CO), 2.62 (dd, J = 4.5 Hz, J = 17.4 Hz, 1H, CH_2CO), 5.03 (m, 1H, CHOAc), 6.01 (d, J = 10.5 Hz, 1H, =CHCO), 6.97 (dd, J = 1.5 Hz, J = 10.5 Hz, 1H, C*H*=CHCO); ^{13}C NMR δ 11.4, 20.7, 21.5, 26.0, 26.7, 27.5, 28.1, 32.0, 32.8, 32.9, 35.3, 35.9, 36.4, 38.2, 40.0, 43.9, 45.5, 54.2, 56.9, 69.9, 130.1 (=*C*HCO), 152.3 (*C*H=CHCO), 170.6 (C(O)O), 199.7 (CO).

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-23-en-22-one (30a). Compound **29a** (30 mg, 0.08 mmol) was dissolved in MeOH (2 mL) and 15% aqueous NaOH (0.2 mL) was added. The solution was refluxed for 20 min and cooled to room temperature. EtOAc (20 mL) was added and the organic layer was washed with water and dried over Na_2SO_4. Solvent removal under reduced pressure gave a residue which was purified by column chromatography (silica gel; CH_2Cl_2/EtOAc, 20:1) to give compound **30a** (22 mg, 83%).

Compound **30a** was obtained as white crystals: mp 168−170 °C (Et_2O-EtOAc); ^{1}H NMR δ 0.84 (s, 3H, 19-CH_3), 2.41 (dm, J = 17.1 Hz, 1H, CH_2CO), 2.62 (dd, J = 4.5 Hz, J = 17.1 Hz, 1H, CH_2CO), 4.07 (m, 1H, C*H*OH), 6.00 (d, J = 10.2 Hz, 1H, =CHCO), 6.97 (dd, J = 1.8 Hz, J = 10.2 Hz, 1H, C*H*=CHCO); ^{13}C NMR δ 11.3, 20.7, 26.7, 27.6, 28.4, 29.0, 32.1, 32.2, 35.4, 35.8, 36.2, 36.5, 38.2, 39.1, 43.9, 45.6, 54.3, 56.9, 66.4, 130.1 (=CHCO), 152.5 (*C*H=CHCO), 199.9 (CO); IR ν_{max} 3320, 3028, 2926, 1679, 1445 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5α,23*R*,24*S*)-23,24-Epoxy-13,24-cyclo-18,21-dinorcholan-3-ol, Acetate (31a). A mixture of $NaHCO_3$ (33 mg, 0.40 mmol), *m*-CPBA (103 mg, 0.60 mmol), and compound **28a** (71 mg, 0.20 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 5 h. It was washed successively with 5% $Na_2S_2O_3$, 10% $NaHCO_3$, and brine and dried over Na_2SO_4. After solvent removal under reduced pressure, the residue obtained was purified by column chromatography (silica gel; hexanes/EtOAc, 50:1) to give compound **31a** (67 mg, 90%).

Compound **31a** was obtained as a white solid: mp 126−128 °C [containing ~10% (23*S*,24*R*)-epoxide]; ^{1}H NMR δ 0.81 (s, 3H, 19-CH_3), 2.06 (s, 3H, CH_3C(O)O), 2.96 (d, J = 3.9 Hz, 1H, epoxide 24-H), 3.16 (m, 1H, epoxide 23-H), 5.02 (m, 1H, CHOAc); ^{13}C NMR δ 11.4, 18.3, 19.4, 21.1, 21.5, 24.5, 26.0, 26.1, 28.3, 32.3, 32.8, 32.9, 34.5, 34.7, 35.9, 40.0, 40.7, 41.3, 54.3, 54.4, 54.6, 55.8, 70.0, 170.6 (C(O)O); IR ν_{max} 2930, 1735, 1235 cm^{-1}.

(3α,5α,23*S*,24*S*)-23-Bromo-13,24-cyclo-18,21-dinorcholan-3,24-diol, 3-Acetate (32a). Compound **31a** (71 mg, 0.19 mmol) in MeCN (10 mL) was cooled to −40 °C and hydrobromic acid (48%, 0.25 mL) was added dropwise. The solution was stirred at 0 °C for 2 h and then at room temperature for 1 h. EtOAc (50 mL) was added and the organic layer was washed with water to neutral pH and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 50:1) to give compound **32a** (60 mg, 70%).

Compound **32a** was obtained as a white solid: mp 161.5−163.5 °C; ^{1}H NMR δ 0.77 (s, 3H, 19-CH_3), 2.05 (s, 3H, CH_3C(O)O), 3.65 (dd, J = 1.8 Hz, J = 10.5 Hz, 1H, C*H*OH), 4.32 (m, 1H, CHBr), 5.00 (m, 1H, CHOAc); ^{13}C NMR δ 11.2, 21.6, 22.6, 23.3, 24.8, 24.9, 26.1, 28.4, 31.9, 32.5, 32.9 (2 × C), 33.0, 35.3, 36.1, 40.3, 47.7, 48.2, 54.6, 58.3, 63.7, 70.3, 74.4, 170.7 (C(O)O); IR ν_{max} 3554, 2931, 1733, 1261 cm^{-1}. Anal. ($C_{24}H_{37}BrO_3$) C, H.

(3α,5α,23*S*)-3-(Acetyloxy)-23-bromo-13,24-cyclo-18,21-dinorcholan-24-one (33a). Jones reagent was added dropwise to a solution of compound **32a** (58 mg, 0.13 mmol) in acetone (15 mL) at 5 °C until an orange color persisted. The resultant mixture was stirred at 0−5 °C for 1 h. 2-Propanol was added to consume excess oxidant and the acetone was removed under reduced pressure. The residue obtained was dissolved in EtOAc (30 mL), washed with water to neutral pH, and dried over Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 20:1) to give compound **33a** (57 mg, 98%).

Compound **33a** was obtained as a white solid: mp 207−209 °C; ^{1}H NMR δ 0.76 (s, 3H, 19-CH_3), 2.04 (s, 3H, CH_3C(O)O), 4.92 (dd, J = 6.0 Hz, J = 12.9 Hz, 1H, CHBr), 5.00 (m, 1H, CHOAc); ^{13}C NMR δ 11.5, 21.5, 21.6, 23.6, 25.2, 25.4, 26.0, 28.2, 32.3, 32.8, 32.9, 35.2 (2 × C), 35.8, 36.3, 40.0, 49.3, 54.6, 56.3, 57.0, 59.8, 70.0, 170.6 (C(O)O), 205.0 (CO); IR ν_{max} 2942, 1733, 1240 cm^{-1}. Anal. ($C_{24}H_{35}BrO_3$) C, H.

(3α,5α)-3-(Acetyloxy)-13,24-cyclo-18,21-dinorchol-22-en-24-one (34a). A mixture of compound **33a** (54 mg, 0.12 mmol), Li_2CO_3 (89 mg, 1.2 mmol), and LiBr (42 mg, 0.48 mmol) in DMF was heated at 125 °C under N_2 for 24 h. After cooling to room temperature, EtOAc (30 mL) was added and the organic layer was washed with water until neutral pH and dried over Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (silica gel; hexanes/EtOAc, 45:1) to give compound **34a** (31 mg, 70%).

Compound **34a** was obtained as white crystals: mp 122−124 °C; ^{1}H NMR δ 0.87 (s, 3H, 19-CH_3), 2.05 (s, 3H, CH_3C(O)O), 5.02 (m, 1H, CHOAc), 5.80 (m, 1H, =CHCO), 6.62 (m, 1H, =C*H*CH_2); ^{13}C NMR δ 11.5, 20.3, 21.5, 24.3, 26.1, 26.7, 27.7, 28.3, 32.7 (2 × C), 32.9, 33.1, 35.4, 36.0, 40.3, 45.1, 53.5, 54.3, 57.2, 70.2, 129.4 (=*C*HCO), 144.4 (=*C*HCH_2), 170.6 (C(O)O), 204.0 (CO); IR ν_{max} 3031, 2938, 1734, 1666, 1247 cm^{-1}. Anal. ($C_{24}H_{34}O_3$) C, H.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-22-en-24-one (35a). Using the same procedure described for the preparation of compound **30a** from compound **29a**, compound **35a** (21 mg, 80%) was prepared from compound **34a** (30 mg, 0.08 mmol).

Compound **35a** was obtained as white crystals: mp 242−243 °C (Et_2O−hexanes); ^{1}H NMR δ 0.86 (s, 3H, 19-CH_3), 4.05 (m, 1H, C*H*OH), 5.80 (m, 1H, =CHCO), 6.62 (m, 1H, =C*H*CH_2); ^{13}C NMR δ 11.3, 20.3, 24.3, 26.8, 27.7, 28.5, 29.1, 32.4, 32.8 (2 × C), 35.5, 36.0, 36.3, 39.4, 45.1, 53.5, 54.4, 57.2,

66.6, 129.4 (=*C*HCO), 144.5 (=*C*HCH$_2$), 204.1 (CO); IR ν_{max} 3439, 3031, 2908, 1647 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5α)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-24-one (5a). Using the same procedure described for the preparation of compound **2a** from compound **11a**, compound **5a** (37 mg, 92%) was prepared from compound **35a** (40 mg, 0.12 mmol).

Compound **5a** was obtained as white crystals: mp 203−204 °C (Et$_2$O−hexanes); ^{1}H NMR δ 0.75 (s, 3H, 19-CH$_3$), 4.04 (m, 1H, C*H*OH); ^{13}C NMR δ 11.3, 21.6, 23.9, 23.9, 24.0, 25.4, 28.5, 29.0, 32.3, 32.6, 35.1, 35.3, 35.9, 36.2, 39.3, 40.2, 49.7, 54.9, 56.4, 58.5, 66.6, 216.7 (CO); IR ν_{max} 3317, 2923, 1708, 1003 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

Physical Properties and Spectroscopic Data for Evaluated Compounds in the 5β-Series. (3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-20-one (2b). Compound **2b** was obtained as white crystals: mp 220−222 °C (EtOAc−hexanes); ^{1}H NMR δ 0.93 (s, 3H, 19-CH$_3$), 2.50 (m, 1H, CHCO), 3.63 (m, 1H, C*H*OH); ^{13}C NMR δ 20.3, 22.1 (2 × C), 23.3, 25.1, 26.7, 27.0, 27.1, 30.5, 33.7, 34.6, 35.4, 35.5, 36.3, 37.3, 40.2, 42.0, 49.6, 56.7, 61.5, 71.6, 215.5 (CO); IR ν_{max} 3449, 2921, 1698, 1460, 1037 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-22-one (3b). Compound **3b** was obtained as white crystals: mp 175−177 °C (EtOAc−hexanes); ^{1}H NMR δ 0.93 (s, 3H, 19-CH$_3$), 2.38 (m, 1H, CH$_2$CO), 2.54 (dd, J = 6.0 Hz, J = 14.4 Hz, 1H, CH$_2$CO), 3.65 (m, 1H, C*H*OH); ^{13}C NMR δ 20.4, 23.3 (2 × C), 23.6, 26.4, 27.0, 27.7, 30.4, 32.8, 34.6, 35.4, 35.9, 36.3, 36.9, 40.6, 41.2, 41.5, 42.0, 49.0, 55.5, 71.6, 213.4 (CO); IR ν_{max} 3418, 2936, 1714, 1447 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-23-one (4b). Compound **4b** was obtained as white crystals: mp 197−198 °C (ether−hexanes); ^{1}H NMR δ 0.91 (s, 3H, 19-CH$_3$), 2.50 (m, 1H, CH$_2$CO), 3.64 (m, 1H, C*H*OH); ^{13}C NMR δ 19.8, 23.4, 24.4, 24.9, 25.2, 26.4, 26.9, 30.5, 34.4, 34.7, 35.2, 35.3, 36.3, 36.5, 40.5 (2 × C), 42.0, 44.7, 48.9, 56.3, 71.7, 213.7 (CO); IR ν_{max} 3398, 2927, 1709, 1448, 1039 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorcholan-24-one (5b). Compound **5b** was obtained as white crystals: mp 177−178 °C (Et$_2$O−hexanes); ^{1}H NMR δ 0.89 (s, 3H, 19-CH$_3$), 3.64 (m, 1H, C*H*OH); ^{13}C NMR δ 21.7, 23.6, 23.9 (2 × C), 24.0, 25.5, 27.0, 27.1, 30.5, 34.7, 35.3, 35.4, 35.6, 36.5, 40.2, 41.1, 42.2, 49.8, 56.4, 58.5, 71.8, 216.6 (CO); IR ν_{max} 3334, 2937, 1701, 1450, 1037 cm^{-1}. Anal. ($C_{22}H_{34}O_2$) C, H.

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-22-en-20-one (11b). Compound **11b** was obtained as white crystals: mp 253−254 °C (EtOAc); ^{1}H NMR δ 0.93 (s, 3H, 19-CH$_3$), 3.63 (m, 1H, C*H*OH), 5.96 (dd, J = 3.0 Hz, J = 9.9 Hz, 1H, =CHCO), 6.83 (m, 1H, =C*H*CH$_2$); ^{13}C NMR δ 20.3, 23.3, 25.8, 26.3, 26.5, 26.9, 27.3, 30.5, 34.1, 34.6, 35.3, 35.6, 36.3, 40.1, 42.0, 47.6, 56.2, 57.8, 71.6, 127.4 (=*C*HCO), 148.3 (=*C*HCH$_2$), 202.2 (CO); IR ν_{max} 3430, 2921, 1663, 1038 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-20(22)-en-23-one (22c). Compound **22c** was obtained as white crystals: mp 209−211 °C (EtOAc−hexanes); ^{1}H NMR δ 0.92 (s, 3H, 19-CH$_3$), 2.35 (d, J = 15.9 Hz, 1H, CCH$_2$CO), 3.64 (m, 1H, C*H*OH), 5.90 (d, J = 9.6 Hz, 1H, =CHCO), 6.96 (m, 1H, =C*H*CH);^{13}C NMR δ 19.7, 23.3, 26.3, 26.4, 26.9, 29.4, 30.4, 34.4, 34.6, 35.1, 35.3, 36.3, 37.9, 40.4, 42.0, 47.3, 47.4, 56.0, 71.6, 127.1 (=*C*HCO), 152.0 (=*C*HCH), 200.2 (CO); IR ν_{max} 3446, 2932, 1677, 1451, 1042 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-23-en-22-one (30b). Compound **30b** was obtained as white crystals: mp 196.5−199 °C; ^{1}H NMR δ 0.99 (s, 3H, 19-CH$_3$), 2.41 (dm, J = 17.1 Hz, 1H, CH$_2$CO), 2.64 (dd, J = 4.2 Hz, J = 17.1 Hz, 1H, CH$_2$CO), 3.67 (m, 1H, C*H*OH), 6.00 (d, J = 10.5 Hz, 1H, =CHCO), 6.95 (dd, J = 2.1 Hz, J = 10.5 Hz, 1H, =CHC); ^{13}C NMR δ 20.8, 23.3, 26.5, 26.8, 27.0, 27.7, 30.5, 34.7, 35.4, 35.7, 36.4, 36.6, 38.2, 40.7, 42.0, 44.0, 45.6, 56.9, 71.6, 130.1 (=*C*HCO), 152.3 (=*C*HC), 199.8 (CO); IR ν_{max} 3412, 2933, 1677, 1038 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

(3α,5β)-3-Hydroxy-13,24-cyclo-18,21-dinorchol-22-en-24-one (35b). Compound **35b** was obtained as white crystals mp 222−223 °C (EtOAc−hexanes); ^{1}H NMR δ 1.00 (s, 3H, 19-CH$_3$), 3.65 (m, 1H, C*H*OH), 5.80 (dd, 1H, J = 3.0 Hz, J = 10.2 Hz, =CHCO), 6.62 (m, 1H, =C*H*CH$_2$); ^{13}C NMR δ 20.3, 23.6, 24.4, 26.7, 27.1 (2 × C), 27.8, 30.6, 32.9, 34.8, 35.5, 35.7, 36.5, 40.5, 42.2, 45.2, 53.5, 57.2, 71.8, 129.4 (=*C*HCO), 144.5 (=*C*HCH$_2$), 204.0 (CO); IR ν_{max} 3418, 2861, 1661, 1038 cm^{-1}. Anal. ($C_{22}H_{32}O_2$) C, H.

[^{35}S]TBPS Binding Methods. Rat brain cortical membranes were prepared with minor modifications of the method previously reported.[24] Briefly, frozen rat cerebral cortices (Pelfreez, Rogers, AK) were thawed and homogenized in 10 volumes of ice-cold 0.32 M sucrose using a glass/Teflon pestle. The homogenate was centrifuged at 1500*g* for 10 min at 4 °C. The resultant supernatant was centrifuged at 10 000*g* for 30 min at 4 °C. The pellet (P2) from this centrifugation was resuspended in 200 mM NaCl, 50 mM potassium phosphate buffer, pH 7.4, and centrifuged at 10 000*g* for 20 min at 4 °C. This washing procedure was done a total of three times, and then pellets were resuspended in buffer (~4 mL/brain) using a glass/Teflon pestle. The membrane suspension was aliquoted, frozen in liquid nitrogen, and stored at −80 °C prior to use.

[^{35}S]TBPS binding assays were done according to the procedure described previously[24] with modifications. Briefly, aliquots of membrane solution (0.5 mg/mL final protein concentration in assay) were incubated with 5 μM GABA, 2 nM [^{35}S]TBPS (45−120 Ci/mmol), and 5 μL aliquots of steroid in DMSO solution (final assay concentrations ranged from 1 nM to 10 μM), and brought to a final volume of 1 mL with 200 mM NaCl, 50 mM potassium phosphate buffer, pH 7.4. Control binding was defined as binding observed in the presence of 0.5% DMSO and the absence of steroid. Nonspecific binding was defined as binding observed in the presence of 200 μM picrotoxinin and ranged from 6.1 to 14.3% of total binding. Assay tubes were incubated for 2 h at room temperature. A Brandel (Gaithersburg, MD) cell harvester was used for filtration of the assay tubes through Whatman/GF/C glass filter paper. The filter paper was rinsed with 4 mL of ice-cold buffer three times. Radioactivity bound to the filters was read by liquid scintillation counter and data was fit using Sigma Plot version 3.0 to the Hill equation

$$f = R_{max}/\{1 + ([conc]/IC_{50})^n\}$$

where R_{max} is the maximal effect, [conc] is steroid concentration, IC_{50} is the half-maximal inhibitor concentration, and n is the Hill coefficient. Each data point was determined in triplicate and two or three full concentration−response curves were generated for each steroid.

Xenopus Oocyte Electrophysiological Methods. Stage V−VI oocytes were harvested from sexually mature female *X. laevis* (Xenopus One, Northland, MI) under 0.1% tricaine (3-aminobenzoic acid ethyl ester) anesthesia. Oocytes were defolliculated by shaking for 20 min at 37 °C in collagenase (2 mg/mL) dissolved in calcium-free solution containing (in mM) 96 NaCl, 2 KCl, 1 MgCl$_2$, and 5 HEPES at pH 7.4. Capped mRNA, encoding rat GABA$_A$ receptor α_1, β_2 and γ_{2L} subunits was transcribed in vitro using the mMESSAGE mMachine Kit (Ambion, Austin, TX) from linearized pBluescript vectors containing receptor coding regions. Subunit transcripts were injected in equal parts (20−40 ng total RNA) 8−24 h following defolliculation. Oocytes were incubated up to 5 days at 18 °C in ND96 medium containing (in mM) 96 NaCl, 1 KCl, 1 MgCl$_2$, 2 CaCl$_2$,and 5 HEPES at pH 7.4, supplemented with pyruvate (5 mM), penicillin (100 U/ml), streptomycin (100 μg/mL), and gentamycin (50 μg/mL). The cDNAs for the rat GABA$_A$-receptor subunits were originally provided by A. Tobin, University of California, Los Angeles (α_1); P. Malherbe, Hoffman-La Roche, Switzerland (β_2); and C. Fraser, National Institute on Alcohol Abuse and Alcoholism (γ_{2L}).

Two-electrode voltage-clamp experiments were performed with a Warner OC725 amplifier 2−5 days following RNA injection. The extracellular recording solution was ND96

medium with no supplements. Intracellular recording pipets were filled with 3 M KCl and had open tip resistances of ~1 MΩ. Drugs were applied from a common tip via a gravity-driven multibarrel drug-delivery system. Steroids were simultaneously coapplied with GABA. Cells were clamped at −70 mV for all experiments, and peak current during 20 s drug applications was measured. Data acquisition and analysis were performed with pCLAMP software (Axon Instruments, CA). Statistical differences were determined using a two-tailed Student's *t*-test.

Tadpole Behavioral Methods. Tadpole LRR was measured as described previously.[18] Briefly, groups of 10 early prelimb-bud stage *X. laevis* tadpoles (Nasco, Fort Atkinson, WI) were placed in 100 mL of oxygenated Ringer's stock solution containing various concentrations of compound. Compounds were added from a 10 mM DMSO stock (final concentration of DMSO in test solutions ≈0.1%). After equilibrating at room temperature for 3 h, tadpoles were evaluated using the LRR and LSR behavioral endpoints. LRR was defined as failure of the tadpole to right itself within 5 s after being flipped by a smooth glass rod. LSR was defined as the failure of the tadpole to move the tip of its tail in a purposeful manner (swimming movement) within 10 seconds of gently sliding the tadpole around the beaker with a glass stirring rod for 5 s. In general, the tadpoles regained their righting and swimming reflexes when placed in fresh oxygenated Ringer's solution. Control beakers containing up to 0.6% DMSO produced no LRR or LSR in tadpoles.

Tadpole LRR and LSR concentration−response curves were fit using Sigma Plot version 3.0 to the Hill equation

$$f = R_{max}/\{1 + ([conc]/EC_{50})^n\}$$

where R_{max} is the maximum effect, [conc] is the steroid concentration, EC_{50} is the half-maximal effective concentration, and n is the Hill coefficient.

Acknowledgment. We thank Amanda Shute for technical help with the oocyte experiments. This work was supported by NIH Grant GM 47969 (D.F.C., A.S.E., C.F.Z.), NIH Grant AA 12952 (S.M.), NIH Grant MH 45493 (C.F.Z.), Research Scientist Development Award MH 00964 (C.F.Z.), the Klingenstein Foundation (S.M.), and the Bantly Foundation (C.F.Z.). Funding for the X-ray diffractometer by NSF and DOE is also gratefully acknowledged.

Supporting Information Available: Spectroscopic data for compounds **7b**, **8b**, **9b**, **13b**, **14b**, **23b**, **25a**, **25b**, **28b**, **29b**, **31b**, **32b**, **33b**, and **34b**; X-ray crystallographic data for (3α,5α)-3-hydroxy-13,24-cyclo-18,21-dinorcholan-24-one (**5a**); molecular modeling data comparing the chair and boat conformations for the fifth ring of cyclosteroids **2a**, **3a**, **4a**, and **5a**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Belelli, D.; Casula, A.; Ling, A.; Lambert, J. J. The Influence of Subunit Composition on the Interaction of Neurosteroids with $GABA_A$ Receptors. *Neuropharmacol.* **2002**, *43*, 651−661. (b) Lambert, J. J.; Belelli, D.; Harney, S. C.; Peters, J. A.; Frenguelli, B. G. Modulation of Native and Recombinant $GABA_A$ Receptors by Endogenous and Synthetic Neuroactive Steroids. *Brain Res. Rev.* **2001**, *37*, 68−80 2001. (c) Lambert, J. J.; Belelli, D.; Pistis, M.; Hill-Venning, C.; Peters, J. A.; The Interaction of Intravenous Anesthetic Agents with Native and Recombinant $GABA_A$ Receptors: An Electrophysiological Study. In *GABA Receptors*, 2nd ed.; Enna, S. A., Bowery, N. G., Eds.; Humana Press: Totowa, 1997; pp 121−156.

(2) Mellon, S. H.; Griffin, L. D. Neurosteroids: Biochemistry and Clinical Significance. *Trends Endocrinol. Metab.* **2002**, *13*, 35−43. (b) Zorumski, C. F.; Mennerick, S.; Isenberg, K. E.; Covey, D. F. Potential Clinical Uses of Neuroactive Steroids. *Curr. Opin. Investig. Drugs* **2000**, *1*, 360−369.

(3) Phillipps, G. H. Structure−Activity Relationships in Steroidal Anesthetics. In *Molecular Mechanisms in General Anaesthesia*; Halsey, M. J., Millar, R. A.. Sutton, J. A., Eds.; Churchill Livingston: New York, 1974; pp 32−47. (b) Phillips, G. H. Structure−Activity Relationships in Steroidal Anesthetics. *J. Steroid Biochem.* **1975**, *6*, 607−613. (c) Purdy, R. H.; Morrow, A. L.; Blinn, J. R.; Paul, S. M. Synthesis, Metabolism, and Pharmacological Activity of 3α-Hydroxy Steroids Which Potentiate GABA-Receptor-Mediated Chloride Ion Uptake in Rat Cerebral Cortical Synaptoneurosomes.

(4) Fesik, S. W.; Makriyannis, A. Geometric Requirements for Membrane Perturbation and Anesthetic Activity. Conformational Analysis of Alphaxalone and Δ^{16}-Alphaxalone and ^2H NMR Studies on Their Interactions with Model Membranes. *Mol. Pharmacol.* **1985**, *27*, 624−629.

(5) Bolger, M. B.; Wieland, S.; Hawkinson, J. E.; Xia, H.; Upasani, R.; Lan, N. C. *In Vitro* and In Vivo Activity of 16,17-Dehydroepipregnanolones: 17,20-Bond Tortional Energy Analysis and D-Ring Conformation. *Pharm. Res.* **1996**, *13*, 1488−1494.

(6) Anderson, A.; Boyd, A. C.; Clark, J. K.; Fielding, L.; Gemmell, D. K.; Hamilton, N. M.; Maidment, M. S.; May, V.; McGuire, R.; McPhail, P.; Sansbury, F. H.; Sundaram, H.; Taylor, R. Conformationally Constrained Anesthetic Steroids That Modulate $GABA_A$ Receptors. *J. Med. Chem.* **2000**, *43*, 4118−4125.

(7) Mickova, R.; Hutlova, H.; Pihera, P.; Protiva, J.; Schwarz, V. Isolation and the Structure of the Product of Cholesterol Biodegradation by the Mutant Mycobacterium sp. CCM 3529. *Collect. Czech. Chem. Commun.* **1985**, *50*, 1110−1113.

(8) Kalvoda, J.; Meystre, Ch.; Anner, G. Reactions of Steroid Hypoiodites. VIII. 1,4-Shift of the Nitrile Group (18-Cyanopregnanes). *Helv. Chim. Acta* **1966**, *49*, 424−436.

(9) Perard, S.; Viger, A.; Marquet, A. 18-Functionalized Steroids: Synthesis of Thio Derivatives of Progesterone. *Steroids* **1990**, *55*, 271−275.

(10) Bose, A. K.; Steinberg, N. G. Steroids. VII. Olefins from Some Steroid Hydroxyketones. *Synthesis* **1970**, 595−596.

(11) Djerassi, C.; Williams, D. H.; Berkoz, B. A New Route to 1-Oxygenated Steroids. *J. Org. Chem.* **1962**, *27*, 2205−2207.

(12) Jiang, X.; Covey, D. F. Total Synthesis of *ent*-Cholesterol via a Steroid C, D.-Ring Side-Chain Synthon. *J. Org. Chem.* **2002**, *67*, 4893−4900.

(13) Raw, A. S.; Jang, E. B. Enantioselective Synthesis of Ceralure B1, Ethyl *cis*-5-Iodo-*trans*-2-methylcyclohexane-1-carboxylate. *Tetrahedron* **2000**, *56*, 3285−3290.

(14) Molander, G. A. Reductions with Samarium (II) Iodide. *Org. React.* **1994**, *46*, 211−367.

(15) Gilani, S. H.; Triggle, D. J. Synthesis of Nitrogen-Containing Steroids. I. Diels−Alder Adducts of Steroids and 4-Phenyl-1,2,4-triazoline-3,5-dione. *J. Org. Chem.* **1966**, *31*, 2397−2398.

(16) Salmond, W. G.; Barta, M. A.; Havens, J. L. Allylic Oxidation with 3,5-Dimethylpyrazole. Chromium Trioxide Complex Steroidal Δ^5-7-Ketones. *J. Org. Chem.* **1978**, *43*, 2057−2059.

(17) Wei, C.-Q.; Zhao, G.; Jiang, X.-R.; Yu, D. New Ring Construction Strategy in Taxane Synthesis: Stereocontrolled Synthesis of Taxane CB−Ring System. *J. Chem. Soc., Perkin 1* **1999**, 3531−3536.

(18) Wittmer, L. L.; Hu, Y.; Kalkbrenner, M.; Evers, A. S.; Zorumski, C. F.; Covey, D. F. Enantioselectivity of steroid-induced γ-aminobutyric $acid_A$ receptor modulation and anesthesia. *Mol. Pharm.* **1996**, *50*, 1581−1586.

(19) Krasowski, M. D.; Harrison, N. L. General anaesthetic actions on ligand-gated ion channels. *Cell. Mol. Life Sci.* **1999**, *55*, 1278−1303.

(20) Abraham, M. H.; Platts, J. A. Hydrogen bond structural group constants. *J. Org. Chem.* **2001**, *66*, 3484−391. (b) Tonge, P. J.; Fausto, R.; Carey, P. R. FTIR studies of hydrogen bonding between α,β-unsaturated esters and alcohols. *J. Mol. Struct.* **1996**, *379*, 135−142.

(21) Covey, D. F.; Han, M.; Kumar, A. S.; de la Cruz, A. M.; Meadows, E. S.; Hu, Y.; Tonnies, A.; Nathan, D.; Coleman, M.; Benz, A.; Evers, A. S.; Zorumski, C. F.; Mennerick, S. Neurosteroid Analogues. 8. Structure−Activity Studies of *N*-Acylated 17a-Aza-D-homosteroid Analogues of the Anesthetic Steroids (3α,5α)- and (3α,5β)-3-Hydroxypregnan-20-one. *J. Med. Chem.* **2000**, *43*, 3201−3204.

(22) Hu, Y.; Zorumski, C. F.; and Covey, D. F. Neurosteroid Analogues: Structure−Activity Studies of Benz[*e*]indene Modulators of $GABA_A$ Receptor Function. 1. The Effect of 6-Methyl-substitution on the Electrophysiological Activity of 7-Substituted Benz-[*e*]indene-3-carbonitriles. *J. Med. Chem.* **1993**, *36*, 3956−3967. (b) Han, M.; Hu, Y.; Zorumski, C. F.; and Covey, D. F. Neurosteroid Analogues. 3. The Synthesis and Electrophysiological Evaluation of Benz[*e*]indene Congeners of Neuroactive Steroids Having the 5β-Configuration. *J. Med. Chem.* **1995**, *38*, 4548−4556. (c) Han, M.; Zorumski, C. F.; and Covey, D. F. Neurosteroid Analogues. 4. The Effect of Methyl Substitution at the C-5 and

C-10 Positions of Neurosteroids on Electrophysiological Activity at $GABA_A$ Receptors. *J. Med. Chem.* **1996**, *39*, 4218−4232.

(23) Mohler, H.; Fritsckhy, J. J.; Rudolph, U. A New Benzodiazepine Pharmacology. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 2−8. (b) Stahl, S. M. Selective Actions on Sleep or Anxiety by Exploiting GABA-A/ Benzodiazepine Receptor Subtypes. *J. Clin. Psychiatry.* **2002**, *63*, 179−180.

(24) Hawkinson, J. E.; Kimbrogh, C. L.; Belelli, D.; Lambert, J. J.; Purdy, R. H.; Lan, N. C. Correlation of Neuroactive Steroid Modulation of [^{35}S]*tert*-Butylbicyclophosphorothionate and [^{3}H]-Flunitrazepan Binding and γ-Aminobutyric $Acid_A$ Receptor Function. *Mol. Pharmacol.* **1994**, *46*, 977−985.

JM030302M