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

Mingcheng Han,[†] Charles F. Zorumski,[‡,§] and Douglas F. Covey*[,†]

Departments of Molecular Biology & Pharmacology, Anatomy & Neurobiology, and Psychiatry, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110

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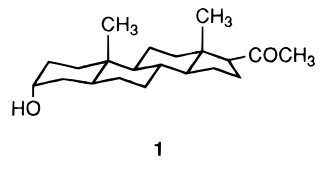
A series of analogues of the neuroactive steroids 3α-hydroxy-5α-pregnan-20-one and 3α-hydroxy-5β-pregnan-20-one were studied to elucidate the mode of binding of 5α- and 5β-reduced steroids to steroid binding sites on $GABA_A$ receptors. Analogues which were either 3α-hydroxy-20-ketosteroids or 3α-hydroxysteroid-17β-carbonitriles and which contained various methyl group substitution patterns at C-5 and C-10 were prepared. Evaluations utilized whole-cell patch clamp electrophysiological methods carried out on cultured rat hippocampal neurons, and the results obtained with the rigid 17β-carbonitrile analogs were analyzed using molecular modeling methods. The molecular modeling results provide a rationale for the observation that the configuration of the hydroxyl group at C-3 is a greater determinant of anesthetic potency than the configuration of the A,B ring fusion at C-5. The electrophysiological results identify steric restrictions for the space that can be occupied in 5α- and 5β-reduced steriod modulators of $GABA_A$ receptors in the regions of space proximate to the steroid C-5, C-10, and possibly C-4 positions. This information is useful for the development of nonsteroidal analogues that can modulate $GABA_A$ receptors via interactions at steroid binding sites.

The discovery that anesthetic steroids are potent allosteric modulators of $GABA_A$ receptor (γ-aminobutyric acid type A receptor) function[1] has led to major advances in understanding the pharmacology of anesthetic steroids.[2] However, most of the medicinal chemistry studies that established the structure−activity relationships for steroid-induced anesthesia relied on correlations of structure with anesthetic activity in mice or rats and were completed prior to the discovery that these compounds altered $GABA_A$ receptor function.[3] Hence, new opportunities for an increased understanding of the earlier derived structure−activity relationships now exist.

Among the more striking structure−activity relationships established in the earlier animal behavioral studies of anesthetic steroids was one indicating that the stereochemistry of the steroid A,B ring fusion had a relatively minor effect on anesthetic activity. For example, 3α-hydroxy-5α-pregnan-20-one (**1**) and 3α-hydroxy-5β-pregnan-20-one (**2**) have equivalent anesthetic activity in mice and rats even though the conformations of the steroids differ greatly.[3f] Explanations for how this conformational difference could be accommodated upon binding of these steroids to a common site thereby producing this behavioral result have appeared,[3g,4] but additional information regarding the molecular recognition of these steroids at their binding sites would be useful. Accordingly, we report the results of a study involving the electrophysiological evaluation of analogues of steroids **1** and **2** which contain various methyl group substitution patterns at the C-5 and C-10 positions. Additional analogues in which the 17β-subsitutent has been changed to a carbonitrile group have also been studied. The goal of the study was to determine how steric bulk in the region of the steroid A,B ring fusion altered the pharmacological actions of 5α- and 5β-reduced steroid modulators of the $GABA_A$ receptors found in cultured rat hippocampal neurons so that a better understanding of the mode of binding of these steroids to $GABA_A$ receptors can be achieved.[5]



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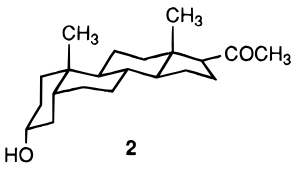

2

Chemistry

Preparation of 3α-Hydroxy-19-nor-5β-steroids. Using reaction conditions similar to those reported previously,[6] catalytic hydrogenation of 19-norsteroid **3** in alkaline 2-propanol gave steroid **5** in 60% yield after purification by column chromatography (Scheme 1). Lesser amounts of purified products **4** (7%) and **7** (16%) along with a mixture of products **5** and **6** (10%) also were obtained. In general, the ratio of 5β:5α-reduced steroids was ~9:1. The combined 5β-reduced steroids **5**−**7** were then oxidized with Jones reagent[7] in acetone to known dione **8**,[6] and the dione was reduced with K-Selectride (Aldrich) in THF at −78 °C to give the known 3β-hydroxy-5β-estran-17-one (**9a**)[6] in 93% yield. The hydroxyl group of steroid **9a** was acetylated with AcOAc/pyridine to yield known product **9b**[6] (91%), and this product was reacted with diethyl cyanophosphonate and LiCN in THF to yield a mixture of the uncharacterized intermediate cyanophosphates **10a** and **10b** (97%) which were reduced with SmI_2/THF and MeOH to give a 1:3 ratio of carbonitriles **11a** and **11b** (90%). An attempt to introduce the carbonitrile group into steroid **9a** by this method was unsatisfactory due to the reaction of

[†] Department of Molecular Biology & Pharmacology.
[‡] Department of Anatomy & Neurobiology.
[§] Department of Psychiatry.
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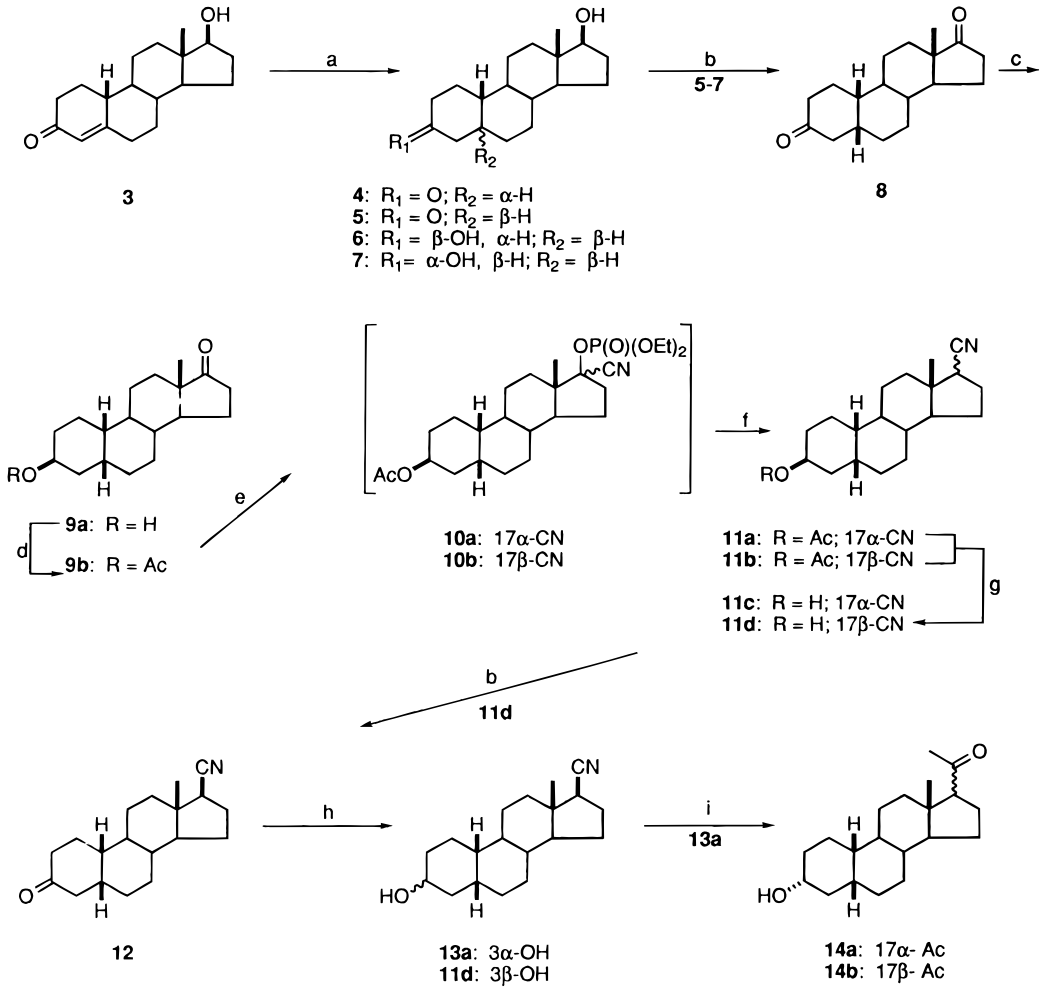
Scheme 1[a]



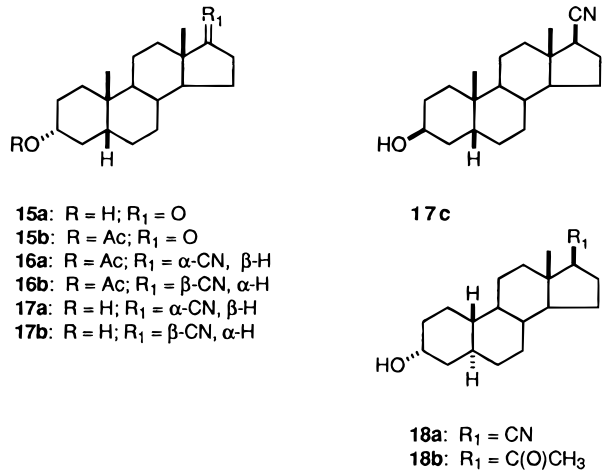
[a] (a) KOH, *i*-PrOH, Pd−C, H_2; (b) Jones reagent, acetone; (c) K-Selectride, THF, −78 °C; (d) AcOAc, pyridine, 120 °C; (e) $NCP(O)(OEt)_2$, LiCN, THF; (f) SmI_2, MeOH, THF; (g) NaOH, K_2CO_3, MeOH, H_2O, reflux; (h) $NaBH_4$, EtOH; (i) CH_3MgCl, THF, reflux.

the hydroxyl group with the diethyl cyanophosphonate. Since the originators of this method reported that the sterically hindered 17β-hydroxy group of 17β-hydroxyandrostan-3-one was unreactive,[8] it appears that the absence of steric hindrance explains the reactivity of the hydroxyl group of steroid **9a**.

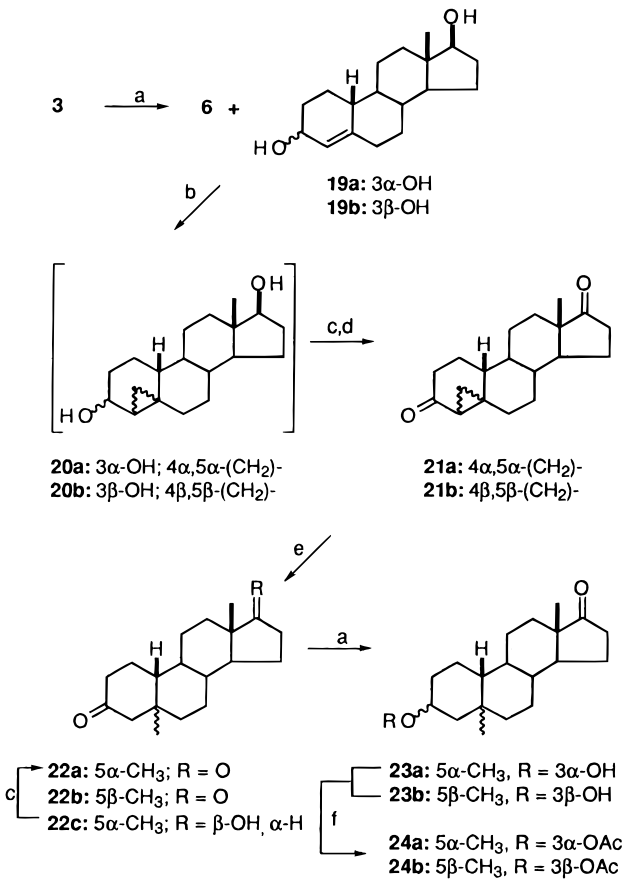
The mixture of steroids **11a** and **11b** was saponified to steroids **11c** and **11d** (84%), and the products were separated by HPLC. The 17β-carbonitrile **11d** was then oxidized with Jones reagent in acetone to steroid **12** (97%), and the 3-keto group of this product was reduced with $NaBH_4$ in EtOH to give a mixture of 17β-carbonitriles **13a** and **11d**. Purification by column chromatography gave the reduction products in isolated yields of 69% (**13a**) and 24% (**11d**). It was anticipated that steroid **13a** would be the major product because $NaBH_4$ reduction of the 3-keto group of (5β,17α)-17-hydroxy-19-norpregn-20-yn-3-one was shown previously to give (3α,5β,17α)-19-norpregn-20-yne-3,17-diol as the major product.[9] Finally, reaction of 17β-carbonitrile **13a** with CH_3MgBr in THF gave an 80% yield of an ~1:10 mixture of product **14a** and previously reported product **14b**,[3f] respectively. These products were separated by HPLC.

Preparation of 3α-Hydroxy-5*β*-steroids and 3α-Hydroxy-19-nor-5α-steroids. The same methods used to prepare the steroids described in Scheme 1 were applied for the preparation of the compounds shown in Chart 1. Commercially available steroid **15a** was acetylated to give known steroid **15b**[10] (98%), and the 17-carbonitrile group was introduced using diethyl cyanophosphonate followed by treatment with SmI_2/THF and MeOH. In this instance, partial hydrolysis of the acetyloxy group occurred during isolation of the products so that a mixture of products **16a**, **16b** and **17a**, **17b** was obtained. Column chromatogaphy was used to separate the mixture of products **16a** and **16b** (28%) from products **17a** (16%) and **17b** (20%). Pure samples of **16a** and **16b** were obtained by HPLC, and these separated products were then hydrolyzed to **17a** and

Chart 1

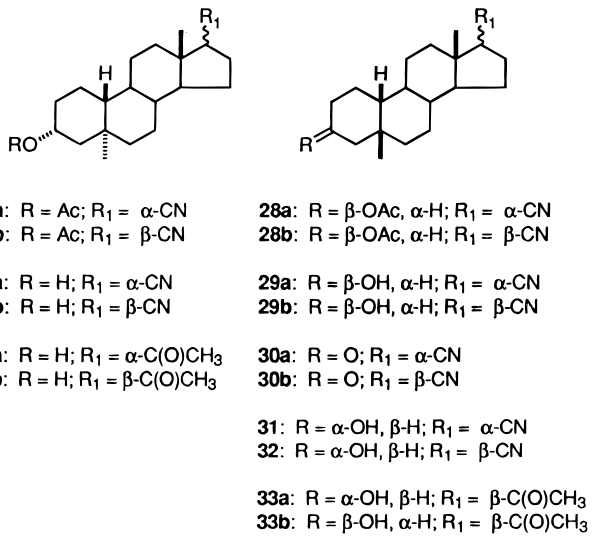


Scheme 2[a]



[a] (a) K-Selectride, THF, −78 °C; (b) CH_2I_2, Cu−Zn, I_2, EtOEt; (c) Jones reagent, acetone; (d) O_3, CH_2Cl_2, −78 °C; (e) Li, liquid NH_3, THF; (f) AcOAc, pyridine, 120 °C.

Chart 2



17b, respectively. The 3*β*-hydroxysteroid **17c** was prepared in 95% yield by carrying out a modified Mitsunobu reaction on 3α-hydroxysteroid **17b**.[11]

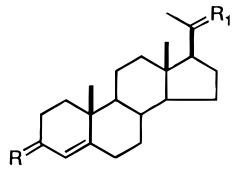
The 19-norsteroid **18a** was prepared as described previously.[12] Reaction of 19-norsteroid **18a** with CH_3-MgBr in THF gave the previously reported 19-norsteroid **18b**[13] (64%) as well as unreacted starting material (27%). No product having the C-17 side chain in the α configuration was isolated.

Preparation of 5α- and 5*β*-Methyl 3α-Hydroxy-19-norsteroids. The methodology used for the introduction of 5-methyl groups into the steroids required for this study is based on that reported by Dauben et al.[14,15] These investigators reported that the reaction of Simmons−Smith reagent[16] (CH_2I_2, Zn−Cu couple) with 3-hydroxy 4-ene steroids in the androstene and cholestene series gave 3-hydroxy-3′,4-dihydrocycloprop-[4,5]steroids (trivially referred to as 4,5-methanosteroid derivatives). The 4,5-methano group was demonstrated to form *syn* to the hydroxy group in the 3-hydroxy 4-ene steroid substrate. Oxidation of the 3-hydroxy group and reductive opening of the resultant 3-keto-4,5-methanosteroid using Li−liquid NH_3 was demonstrated to give 3-keto-5-methylsteroid products. Thus, this overall sequence yields 3-keto-5α-methylsteroids from 3α-hydroxy 4-ene steroids and 3-keto-5*β*-methylsteroids from 3*β*-hydroxy 4-ene steroids.

Steroid **3** was reduced with K-Selectride at −78 °C for 15 h to afford a mixture of known unsaturated diols **19a** and **19b**,[17] saturated alcohol **6**, and recovered steroid **3** (Scheme 2). Steroid **19b** was the major product (~60% of the total material isolated) from the reaction. Although it was possible to obtain pure samples of steroids **6** and **19b** by HPLC for product characterization, this mixture was not readily separated and it was used without purification. Reaction of the product mixture with CH_2I_2, Zn−Cu couple gave a complex mixture containing starting materials, byproducts, and the desired 4,5-methanosteroids **20a** and **20b**. Since attempts to isolate the 4,5-methanodiols by recrystallization or HPLC on a silica column were unsuccessful, this product mixture was oxidized with Jones reagent to a product mixture containing 4,5-methanodiones **21a** and **21b**. Treatment of this product mixture in CH_2Cl_2 with O_3 at −78 °C converted compounds in the mixture containing double bonds to more polar uncharacterized products that were easily removed by column chromatography. Chromatography also yielded the saturated diketone **8** (21% overall yield from steroid **3**) and an unresolved mixture of 4,5-methanodiones **21a** and **21b** (14% overall yield from steroid **3**). Recrystallization of the 4,5-methanodiones gave a pure sample of steroid **21a**. A pure sample of steroid **21b** was not obtained. A mixture of 4,5-methanodiones **21a** and **21b** was opened with Li−liquid NH_3 to give a mixture of 5-methyl steroids **22a**−**c**. Steroids **22a** and **22b** were isolated as a mixture (74% yield) by column chromatography along with pure steroid **22c** (9% yield). Similarly, the 5α-methyl steroid **22a** was obtained by reductive opening of steroid **21a** with Li−liquid NH_3. Jones oxidation of **22c** also gave 5α-methyl steroid **22a** (82% yield), thus confirming that the 5-methyl group had the same configuration in both compounds **22a** and **22c** and that both of these compounds were derived from 4,5-methanosteroid **21a**. The evidence upon which the assignments of configuration were made for the 4,5-methano groups and the 5-methyl groups in these steroids (as well as other 4,5-methano and 5-methyl steroids shown in Charts 2 and 3) is reported following the presentation of all synthetic methods.

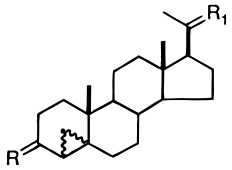
Reduction of a mixture of compounds **22a** and **22b** with K-Selectride at −78 °C for 6 h gave a mixture of 3-hydroxysteroids **23a** and **23b** in 94% yield. Although compound **23a** can be isolated from this mixture in minor amounts by recrystallization, it is more convenient to acetylate the mixture and separate the acetyloxy compounds **24a** (50% yield) and **24b** (33% yield)

Chart 3



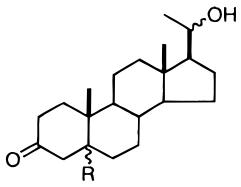
34: R = O; R_1 = O

35a: R = α-OH, β-H; R_1 = β-OH, α-H
35b: R = β-OH, α-H; R_1 = β-OH, α-H



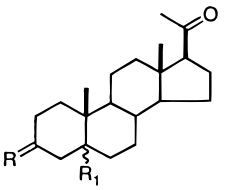
36a: R = α-OH, β-H; R_1 = β-OH, α-H; 4α,5α-(CH_2)-
36b: R = β-OH, α-H; R_1 = β-OH, α-H; 4β,5β-(CH_2)-

37a: R = R_1 = O; α-H; 4α,5α-(CH_2)-
37b: R = R_1 = O; α-H; 4β,5β-(CH_2)-



38a: R = α-CH_3; 20α-OH
38b: R = α-CH_3; 20β-OH

39a: R = β-CH_3; 20α-OH
39b: R = β-CH_3; 20β-OH



40: R = O; R_1 = α-CH_3

41: R = O; R_1 = β-CH_3

42: R = α-OH, β-H; R_1 = α-CH_3

43a: R = α-OH, β-H; R_1 = β-CH_3
43b: R = β-OH, α-H; R_1 = β-CH_3

by HPLC. Pure samples of steroids **23a** and **23b** are then readily obtained by hydrolysis after separation of acetyloxy compounds **24a** and **24b**, respectively.

Using the earlier described two-step cyanation procedure, compound **24a** was converted in 93% overall yield into a mixture of the 17α- and 17β-carbonitriles **25a** and **25b**, respectively (Chart 2). Removal of the acetyloxy group from these unresolved epimeric 17-carbonitriles by saponification gave a 1:1 mixture of the epimeric 17-carbonitriles **26a** and **26b** (93% yield), which was separated into its two pure components by HPLC. A 1:1 mixture of the epimeric 17-carbonitriles **25a** and **25b** also was reacted with CH_3MgBr in THF at reflux for 48 h to give a 1:4 mixture (90% yield) of the C-17 epimeric 3α-hydroxy-5α-methyl-19-norpregnan-20-ones **27a** and **27b**. These 20-ketosteroids were separated by HPLC.

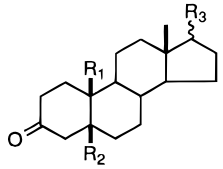
Similarly, the two-step cyanation procedure was used to convert compound **24b** in 95% overall yield into a 1:1 mixture of the 17α- and 17β-carbonitriles **28a** and **28b**, respectively (Chart 2). Saponification of these compounds (97% yield), followed by HPLC separation, gave 17α-carbonitrile **29a** and 17β-carbonitrile **29b**. Jones oxidation of compound **29a** gave 3-ketosteroid **30a** (91%) and Jones oxidation of compound **29b** gave 3-ketosteroid **30b** (95%). The $NaBH_4$ reduction of steroid **30a** gave a mixture of C-3 epimeric alcohols from which compounds **29a** and **31** were isolated by HPLC in yields of 25% and 45%, respectively. Likewise, $NaBH_4$ reduction of steroid **30b** gave a mixture of C-3 epimeric alcohols from which compounds **29b** and **32** were isolated by HPLC in yields of 46% and 50%, respectively. A 1:1 mixture of carbonitriles **29b** and **32** also was reacted with CH_3MgBr in THF at reflux for 24 h to give a mixture (64% yield) of the C-3 epimeric 3-hydroxy-5β-methyl-19-norpregnan-20-ones **33a** and **33b**. These 20-ketosteroids were separated by HPLC.

Preparation of 5α- and 5β-Methyl-3α-hydroxypregnan-20-ones. These compounds were prepared from progesterone by a route analogous to that outlined previously in Scheme 2. Reduction of progesterone (**34**, Chart 3) with K-Selectride in THF at −78 °C for 5 h gave a 98% yield of a 1:1 mixture of the known diols **35a** and **35b**.[18,19] A portion of this diol mixture was separated by HPLC for product characterization, and the remaining material was converted with CH_2I_2 and Zn−Cu couple in refluxing EtOEt for 3 d into a mixture containing 4,5-methanodiols **36a** and **36b** and other byproducts. This product mixture was not purified or characterized, but was oxidized with Jones reagent and treated with O_3 in CH_2Cl_2 to facilitate isolation of the desired 4,5-methanodiones **37a** and **37b** as an inseparable mixture in 27% yield. Reductive opening of the 4,5-methanodione mixture with Li in liquid NH_3 gave the 20-hydroxy 3-ones **38a**, **38b** and **39a**, **39b** and the 3,20-diones **40** and **41**. Separation of the 20-hydroxy 3-ones (38% yield) from the 3,20-diones (53% yield) was readily achieved by column chromatography. Pure samples of compounds **39a** and **39b** were obtained by this procedure along with additonal mixtures of 20-hydroxy 3-ones. Pure samples of compounds **40** and **41** were obtained by further purification using HPLC. The remaining mixture of 20-hydroxy 3-ones was oxidized with Jones reagent and then reduced with K-Selectride to obtain compounds **42** and **43b** in yields of 12% and 54%, respectively. Steroid **41** was reduced with $NaBH_4$ in EtOH for 1 h at room temperature to obtain a mixture of the epimeric 3-hydroxy 20-ones which was separated by HPLC to give **43a** and **43b** in yields of 22% and 57%, respectively.

Assigment of 5-Methyl and C-3, C-17, and C-20 Configurations. The assignments for the configuration at C-5 of those steroids containing a 5-methyl group are based on the NMR data summarized in Table 1. In the NMR spectra of the known 5β-reduced steroids **5** and **8**, the resonance for the C-4 axial proton is observed as an apparent triplet (J = 14.2 Hz) at δ 2.59 and 2.60, respectively. The position of the resonance is due to the deshielding effect of the C-3 carbonyl group and the multiplicity of the resonance is explained by the identical geminal and vicinal (H_{4ax}−H_5) coupling constants. Since no resonance is observed in this region of the NMR spectra for the known 5α-reduced steroid **4** or 5α-estrane-3,17-dione, the presence of this resonance in any of the 3-keto-5-methylsteroids described herein indicates that the methyl group has the 5β-configuration. As required, the presence of a 5β-methyl group in the steroid causes the C-4 axial proton resonance to appear as a doublet. The results shown in Table 1 also indicate that the 5β-methyl group shifts the C-4 axial proton downfield by ∼0.1 ppm. The presence of 5β- and 19-methyl groups results in a downfield shift for the C-4 axial proton of ∼0.4 ppm.

Deductive reasoning based on the NMR data summarized in Table 1 and the earlier established stereochemical correlations for the preparation and reductive opening of 3-keto-4,5-methanosteroids by Dauben et al. (vide supra) was used to correlate the configurations of the 5-methyl steroids with their corresponding 4,5-methanosteroid precursors. The 5α-steroids containing 3α-substituents were readily distinguished from the 5α-steroids containing 3β-substitutents by the presence of the vicinal *trans*-diaxial couplings found in the NMR

Table 1. Chemical Shifts and Multiplicities of NMR Resonances Assigned as 4-H_{ax} in 3-Keto-5β-steroids



compd	R_1	R_2	R_3	chemical shift 4-H_{ax} (δ)	multiplicity 4-H_{ax}	coupling constant 4-H_{ax}, J (Hz)
5	H	H	α-H, β-OH	2.59	triplet	14.2
8	H	H	=O	2.60	triplet	14.2
12	H	H	α-H, β-CN	2.57	triplet	14.2
22b	H	Me	=O	2.71	doublet	14.3
30a	H	Me	β-H, α-CN	2.69	doublet	14.3
30b	H	Me	α-H, β-CN	2.69	doublet	14.7
39a	Me	Me	β-(20*S*)-X[a]	3.01	doublet	14.7
39b	Me	Me	β-(20*R*)-X[a]	2.94	doublet	14.4
41	Me	Me	β-acetyl	2.95	doublet	14.9

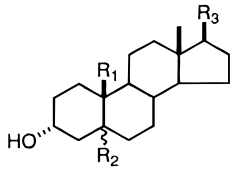
[a] X = $CH(OH)CH_3$.

resonances of the C-3 protons present in the latter class of steroids. Thus, for the 5α-steroids, the 3α-substituents have the axial configuration and the equatorial C-3 protons appear as a broadened singlet with shoulders ($W_{1/2}$ ~13 Hz). Conversely, for the 5β-steroids, the 3α-substituents have the equatorial configuration and the C-3 protons appear as complex multiplets ($W_{1/2}$ ~33 Hz). As discussed previously for other neurosteroid analogs,[12,20] the configurational assignments for the C-17 substituents were based on an NMR analysis of the coupling constants of the C-17 protons. For the purified steroid diastereomer pair **38a** and **38b**, the assignment of configuration at C-20 was based on the relative chemical shifts of the C-18 methyl groups. In accordance with literature precedents,[21] the diastereomer having the C-18 methyl group resonance at the lower field was assigned the 20*R* configuration.

Electrophysiology

Voltage clamp recordings were obtained from cultured postnatal rat hippocampal neurons using whole-cell patch clamp methods.[22] The results obtained with 3α-hydroxysteroids are summarized in Table 2. The 3β-hydroxysteroids, with the exception of compound **17c**, were not evaluated. Each compound was evaluated at 10 μM for its ability to potentiate 2 μM GABA-mediated current and also evaluated at 10 μM for its ability to initiate (gate) a current in the absence of GABA. The concentrations of GABA and test compound used in the potentiation experiments are different from those used in our previous publications. The GABA concentration was increased from 1 to 2 μM to facilitate the recording of control currents. The test compound concentration was increased from 1 to 10 μM for two reasons: (1) to increase the sensitivity for the detection of, and discrimination between, the activities of weakly potentiating compounds; and (2) because this concentration of steroid **44** gives a maximal response. No other steroids or benz[*e*]indene analogues that we have studied give responses larger than those of steroid **44** under the conditions used in this study. Recordings that are representative of the potentiation responses observed for the compounds are shown in Figure 1A−C for compounds **44**, **26b**, and **32**. A recording representative of the direct gating caused by steroid **14b** is shown in Figure 1D. Compound **17c** neither potentiated GABA-mediated current (102 ± 5% relative to control = 100%, N = 5) nor directly gated a current (0%, N = 5).

Table 2. Electrophysiological Effects of 3α-Hydroxysteroids on $GABA_A$ Receptor Function



compd	R_1	R_2	R_3	compd (10 μM) potentiation[a]	compd (10 μM) gated current[b]	N [c]
1	Me	α-H	acetyl	1111 ± 178[d]	26 ± 5	6
18b	H	α-H	acetyl	926 ± 101	46 ± 13	4
27b	H	α-Me	acetyl	110 ± 6	0	4
42	Me	α-Me	acetyl	294 ± 19	3 ± 3	6
44	Me	α-H	CN	1829 ± 272	32 ± 7	4
18a	H	α-H	CN	1721 ± 182	42 ± 9	6
26b	H	α-Me	CN	92 ± 3	0	4
2	Me	β-H	acetyl	1023 ± 192	49 ± 8	6
14b	H	β-H	acetyl	754 ± 84	110 ± 12	5
33a	H	β-Me	acetyl	652 ± 83	12 ± 2	6
43a	Me	β-Me	acetyl	498 ± 121	24 ± 5	5
17b	Me	β-H	CN	1297 ± 251	68 ± 18	4
13a	H	β-H	CN	1823 ± 360	41 ± 16	5
32	H	β-Me	CN	452 ± 75	28 ± 5	6

[a] Percent response relative to current produced by GABA (2 μM). To calculate the percentage response, the magnitude of the peak current produced by 2 μM GABA plus 10 μM compound was normalized with respect to the peak current produced by 2 μM GABA alone on the same cell. A percentage response of 100% reflects no change in the current compared to 2 μM GABA alone. 2 μM GABA is a concentration at the foot of the dose−response curve in cultured postnatal rat hippocampal neurons. These experiments were conducted at −60 mV, and compounds were applied by pressure ejection for 500 ms. [b] The compound gated current reflects the peak current directly gated by 10 μM compound in the absence of GABA compared to the response obtained from the same cell in response to 2 μM GABA alone. [c] N = Number of cells examined. [d] Values are the mean ± SEM.

Discussion

Reviews by Phillipps and co-workers on the structure−activity relationships (SARs) of anesthetic steroids indicate that the configuration of the hydroxyl group at C-3 is a greater determinant of anesthetic potency than the configuration of the A,B ring fusion at C-5.[3g,h] A recent study in which the abilities of four different diastereomers of 3-hydroxypregnan-20-one were evalu-

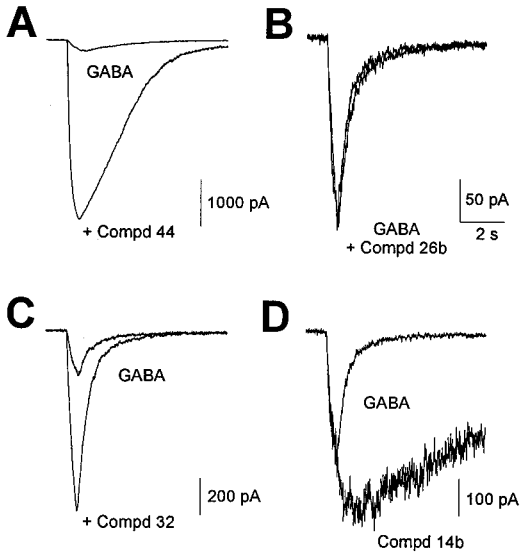


Figure 1. Effect of steroids in cultured hippocampal neurons. (A−C) The traces show currents activated by 2 μM GABA in the absence and presence of 10 μM **44** (A), **26b** (B), and **32** (C). These compds were selected to show the range of steroid effects on GABA currents. (D) The traces show currents activated by 10 μM **14b** in the absence of GABA. The response of the same neuron to 2 μM GABA is shown for comparison. Note the slow time course of the steroid activated current and the marked increase in current fluctuations. In all traces, hippocampal neurons were voltage clamped at −60 mV and drugs were applied by pressure ejection for 500 ms as described in the Experimental Section.

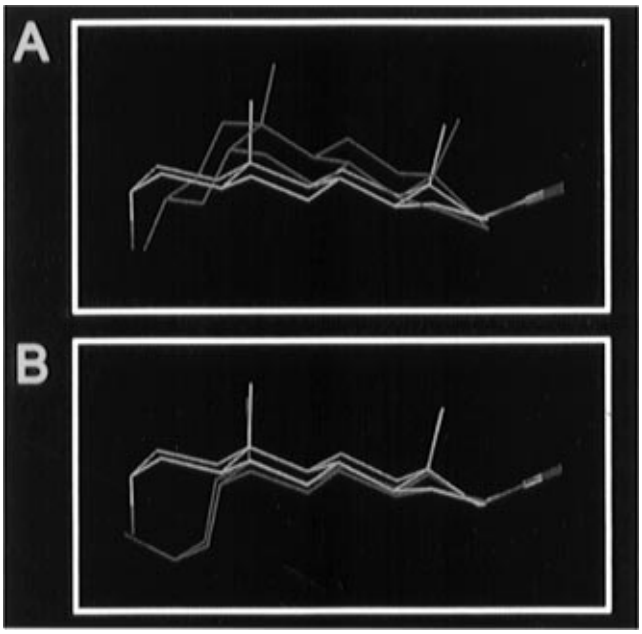


Figure 2. Molecular modeling analysis for steroids **44**, **17b**, and **17c**. A least squares fit program was used the superimpose O-3, H-17, C-17, and the carbonitrile group atoms of steroids **17b** and **17c** to steroid **44**. All five pairs of atoms for the fit were weighted equally. The hydrogen atoms have been removed from the structures for clarity. (A) Superimposition of steroids **44** (yellow) and **17b** (green). The intermolecular distance between the oxygen atoms, nitrogen atoms, and C-19 atoms are 0.29, 0.11, and 1.42 Å, respectively. (B) Superimposition of steroids **44** (yellow) and **17c** (green). The intermolecular distances between the oxygen atoms, nitrogen atoms, and C-19 atoms are 0.23, 0.06, and 0.41 Å, respectively.

ated as inhibitors of [^{35}S]TBPS binding to $GABA_A$-receptors reveals similar SARs. Thus, 3α-hydroxy-5α-steroid **1** and 3α-hydroxy-5β-steroid **2** are both potent displacers of [^{35}S]TBPS with similar IC_{50}s of 17 and 61 nM, respectively, and the corresponding 5α- and 5β-steroid diastereomers containing a 3β-hydroxy group are both weak displacers having IC_{50}s of $>10^5$ and 1734 nM, respectively.[23] Finally, similar SARs are also apparent from electrophysiological studies of steroid potentiation of GABA-mediated chloride current[24] and from studies of steroid potentiation of muscimol-stimulated radioactive chloride uptake in rat synaptoneurosomes.[4]

Explanations for the above SARs remain to be elaborated. In this regard, the results presented here have been interpreted according to the molecular modeling presented for the steroid 17β-carbonitriles shown in Figures 2 and 3. In each panel of these figures two steroids have been superimposed using a least squares fit program to superimpose O-3, H-17, C-17, and the carbonitrile group atoms of each steroid. All five pairs of atoms for each fit were weighted equally, and 3α-hydroxy-5α-steroid **44** is always the reference structure to which other steroids were fit. As shown in Figure 2A,B, superimpositions of either the 3α-hydroxy-5β-steroid **17b** or the 3β-hydroxy-5β-steroid **17c** with steroid **44** in which the oxygens and carbonitriles occupy nearly identical positions in three-dimensional space are possible. An obvious spacial difference resulting from the alignments shown in panels A and B is that the A rings of steroid **17b** and **17c** are largely located on opposite sides of the A ring of steroid **44**. This difference suggested to us that the availability of space at a steroid binding site above, but not below, the A ring of steroid **44** might explain why steroid **17b** is an active compound whereas steroid **17c** is an inactive compound. The series of steroids with the various methyl group substitution patterns at C-5 and C-10, whose electrophysiological activities at the heterogeneous $GABA_A$ receptors found in cultured rat hippocampal neurons are reported[25] in Table 2, were used to investigate this hypothesis.

Figure 3A shows the region of space occupied by the 5α-methyl group in the 5α-reduced 19-norsteroid **26b** and also shows that only minor perturbations in ring conformation are caused by the 5α-methyl group. Additionally, Figure 3B shows that the C-5 methyl group of steroid **26b** and C-4 of steroid **17c** are similarly located in three-dimensional space. Thus, the finding that both steroids **26b** and **17c** are inactive in the electrophysiological assays can be rationalized on the basis of an unfavorable interaction between the steroids and $GABA_A$ receptors in the region of space below the A ring of the active steroid **44**. Apparently, no realignment that permits this steric interaction to be relieved while maintaining an acceptable alignment of the hydrogen bonding groups at either end of the steroid with their bonding partners on the $GABA_A$ receptors of cultured rat hippocampal neurons is possible for these rigid compounds.

Steric factors in the region of space above the C-19 methyl group of 5α-steroid **44** also appear to be important determinants of electrophysiological activity. As indicated in Table 2, while 5α-steroid **44** and 5β-steroid **17b** are both active compounds, steroid **44** is substantially more active as a potentiator of GABA-mediated current. Since the superimposition of steroids **44** and **17b** shown in Figure 2A clearly shows that the C-19 methyl of steroid **17b** is located above the C-19 methyl of steroid **44**, we hypothesized that the decreased activity of steroid **17b** might result from an unfavorable

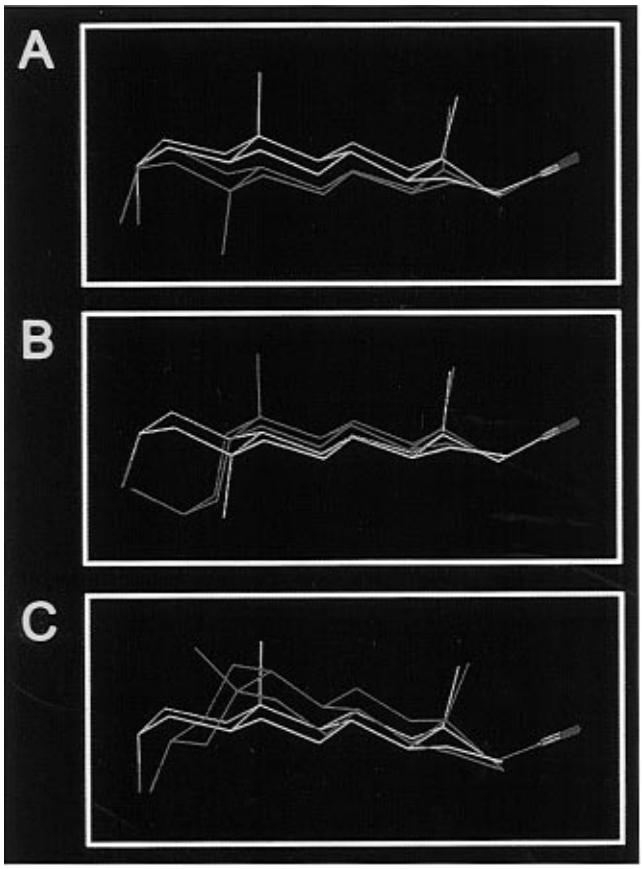


Figure 3. Molecular modeling analysis for steroids **44**, **26b**, **17c**, and **32**. The modeling parameters were as described in the legend to Figure 2. (A) Superimposition of steroids **44** (yellow) and **26b** (green). The intermolecular distances between the oxygen atoms and nitrogen atoms are 0.45 and 0.14 Å, respectively. (B) Superimposition of steroids **26b** (white) and **17c** (green) with steroid **44** (structure not shown). The intermolecular distances between the oxygen atoms and nitrogen atoms in steroids **26b** and **17c** are 0.22 and 0.09 Å, respectively. The intermolecular distance between the 5α-methyl carbon in steroid **26b** and C-4 in steroid **17c** is 0.52 Å. (C) Superimposition of steroids **32** (green) and **44** (yellow). The intermolecular distances between the oxygen atoms and nitrogen atoms are 0.24 and 0.09 Å, respectively. The intermolecular distance between the 5β-methyl carbon in steroid **32** and C-19 in steroid **44** is 2.86 Å.

steric interaction between the C-19 methyl group and the receptors. To evaluate this hypothesis the 19-nor-5β-steroid **13a** was studied. The results reported in Table 2 indicate that steroids **44** and **13a** are equipotent as potentiators of current and support the hypothesis that only limited space is available for hydrophobic groups above the region of space occupied by the C-19 methyl group of steroid **44**. Thus, accommodation of the C-19 methyl group in 5β-steroid **17b** probably results in less than optimal alignment of the 3α-hydroxy and/or 17β-carbonitrile with their receptor hydrogen bonding partners, thereby resulting in a decrease in biological activity. These results should not be further interpreted to imply that the C-19 methyl group of steroid **44** is a critical determinant of activity. Clearly, the loss of a hydrophobic contact involving this methyl group and a $GABA_A$ receptor would not be expected to have as large an effect on activity as would requiring the C-19 methyl group in steroid **17b** to be in a region of space occupied by the receptor. Consequently, it is perhaps not surprising that the 19-nor-5α-steroid **18a** is equipotent to 5α-steroid **44** as a potentiator of GABA currents.

The reduced potentiating activity reported for the 5β-methyl-19-norsteroid **32** in Table 2 indicates that an unfavorable steric interaction results from adding a methyl group in this position. Indeed, this unfavorable interaction is stronger than that caused by the presence of a 10β-methyl group in a 5β-steroid (compare the results given in Table 2 for steroids **17b** and **32**). Thus, the results obtained with steroids **17b** and **32** suggest that both C-5 and C-10 in 5β-steroids are in close contact with the receptors.

An alignment for steroids **44** and **32** is shown in Figure 3C. It is interesting to note in Figure 3C that the 5β-methyl group in steroid **32** occupies a region of space similar to that which would be occupied by a 4β-substituent on steroid **44**. Hence, we would predict that the introduction of a 4β-methyl group into 5α-steroid **44** would produce a compound with diminished activity. The previously reported result showing that the addition of a 4β-OH group into the potent anesthetic steroid alfaxalone (3α-hydroxy-5α-pregnane-11,20-dione) gave an inactive compound[3g,h] supports the conclusion that space for 4β-substituents may be restricted. However, the difference in hyrophobicities between a methyl group and a hydroxyl group, as well as the difference in the methods of evaluation used, precludes a definitive conclusion.

Comparisons similar to those made for these 17β-carbonitriles can also be made for the less potent 20-ketosteroids reported in Table 2. These comparisons are, however, complicated by the ambiguities resulting from the extra degree of freedom (rotation about the C-17, C-20 bond) present in the latter series of compounds. For example, whereas 17β-carbonitriles **44** and **17b** differ in activity as potentiators of GABA-mediated current, this activity is not significantly different for 20-ketosteroids **1** and **2**. The loss of this difference for the 20-ketosteroids could be reasonably explained by slightly different conformations of the C-17 side chain which make it possible for each steroid to achieve slightly different, but similarly optimized, interactions with the receptors.

The potentiating effects of the 5α-reduced 20-ketosteroids shown in Table 2 closely parallel those found for the corresponding 17β-carbonitriles. The presence of a methyl group at C-10 has no significant effect on the activity of 5α-steroids (compare steroids **1** and **18b**), whereas the presence of methyl group at C-5 either eliminates (steroid **27b**) or greatly diminishes (steroid **42**) this activity. A compelling reason for why compound **42** is significantly more active than compound **27b** is unclear. However, the conformational freedom of the C-17 acetyl group found in these compounds may be involved in the explanation of the results. It is possible that the effect of a weak hydrophobic contact provided by a methyl group at C-10 could be optimized if the carbonyl group in the C-17 side chain is capable of attaining a conformation that enhances this hydrophobic contact without diminishing its own hydrogen-bonding interaction with the receptors.

The effect of a methyl group substituent at C-10 on the potentiation of GABA-mediated current is opposite for 5β-reduced 17β-carbonitriles and the corresponding 20-ketosteroids (Table 2). Whereas for the 17β-carbonitriles removing this C-10 substituent increases activity, for the 20-ketosteroids, removing this substituent weakly decreases activity (compare steroids **2** and **14b**).

The reason for this result is not apparent. By contrast, the decrease in GABA potentiation caused by the presence of a 5β-methyl group is similar in both series of compounds (steroids **32** and **33a**). Additionally, in the 20-ketosteroid series, where the effect of the combined C-5 and C-10 methyl substitutents was evaluated, the C-10 methyl group did not significantly alter the effect on potentiation caused by the C-5 methyl group (compare steroids **33a** and **43a**).

The results shown in Table 2 for the direct gating effects of the steroids indicate that the effects of the methyl substituents on the potentiation of GABA-mediated currents are not always paralleled by similar effects on the direct gating actions of the steroids. We have observed a similar lack of parallel changes in these two functional assays in a previous study of benz[*e*]-indene analogues of neurosteroids, and we continue to interpret this lack of parallelism as suggestive evidence for the hypothesis that the potentiation and gating activities occur at different steroid binding sites on the same receptor.[19] Without attempting to interpret further the results from the gating assay, it is interesting to note that (1) steroid **14b** gates an amount of current equivalent to that gated by 2 μM GABA and (2) a 5α-methyl substituent either eliminates or reduces to a very low level both the potentiation and gating effects of neurosteroids.

As noted earlier in this discussion, cultured rat hippocampal neurons contain multiple subtypes of $GABA_A$ receptors. Accordingly, the responses to the compounds evaluated in this study represent average responses recorded from multiple subtypes of $GABA_A$ receptors. Previous studies carried out with heterogeneous populations of $GABA_A$ receptors have provided evidence for the existence of heterogeneity in the binding sites for anesthetic steroids.[26] In particular, the finding that 3α-hydroxy-5β-pregnan-20-one, but not 3α-hydroxy-5α-pregnan-20-one, shows two-component allosteric modulation of benzodiazepine and *tert*-butylbicyclophosphorothionate binding in some regions of bovine brain has been interpreted to indicate that the 5β-reduced steroid may show selective binding to different types of $GABA_A$ receptors.[26f] The extent to which the active anesthetic steroid analogues reported here may have selective actions on different subtypes of $GABA_A$ receptors remains to be investigated.

In summary, the results of this study provide new information about the mode of binding of 5α- and 5β-reduced steroids to $GABA_A$ receptors. The data reported clearly indicate that there are steric restrictions on the hydrophobic space that can be occupied between the hydrogen bond donating group at C-3 and the hydrogen bond accepting group at C-17. We are particularly interested in obtaining a better understanding of these steric restrictions because we believe that this information will be crucial for the development of nonsteroidal analogues that can modulate $GABA_A$ receptors via interactions at a steroid binding site.

Experimental Section

General Methods. Melting points were determined on a Kofler micro hot stage and are uncorrected. NMR spectra were recorded at ambient temperature in $CDCl_3$ with a 5 mm probe on either a Varian Gemini 300 operating at 300 MHz (^{1}H) or 75 MHz (^{13}C). For ^{1}H NMR and ^{13}C NMR spectra, the internal references were TMS (δ 0.00) and $CDCl_3$ (δ 77.00), respectively. IR spectra were recorded as films on a NaCl plate (unless noted otherwise) with a Perkin-Elmer 1710 FT-IR spectrophotometer. Elemental analyses were carried out by M-H-W Laboratories, Phoenix, AZ. Solvents were either used as purchased or dried and purified by standard methodology. Flash chromatography was performed using silica gel (32−63 μm) purchased from Scientific Adsorbents, Atlanta, GA. The 17β-hydroxyestr-4-en-3-one was purchased from Steraloids, Wilton, NH, and pregn-4-ene-3,20-dione was purchased from Sigma Chemical Co., St. Louis, MO. The Econosil HPLC column was purchased from Alltech Associates, Inc., Deerfield, IL. Steroid **44** was prepared as described previously.[12]

17β-Hydroxy-5α-estran-3-one (4), 17β-Hydroxy-5β-estran-3-one (5), and 5β-Estrane-3α,17β-diol (7). A 10% Pd−C catalyst (0.18 g) was added to a solution of 17β-hydroxyestr-4-en-3-one (**3**, 3.0 g, 10.9 mmol) and KOH (0.8 g) in 2-propanol (125 mL) in a glass bottle and hydrogenated (40−45 psi, overnight, room temperature) in a Parr hydrogenation apparatus. The catalyst was filtered off, the solvent was removed under reduced pressure, and brine (200 mL) and ether (200 mL) were added. The heterogeneous solvent mixture was neutralized with 6 N HCl while being cooled with an ice−water bath. The organic layer was separated, washed with brine (2 × 100 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give a solid which was purified by chromatography (silica gel, 30% EtOAc in hexane). In their order of elution, the fractions contained steroid **4** (200 mg, 7%), steroid **5** (1.8 g, 60%), a mixture (300 mg, 10%) of steroid **5** and 3β,17β-dihydroxy-5α-estrane (**6**), and 3α,17β-dihydroxy-5α-estrane (**7**, 500 mg, 16%). Compound **4** was obtained as colorless crystals: mp 128−130 °C (from EtOAc/hexane, lit.[6] mp 133−134 °C); IR 3292, 1713 cm^{-1}; ^{1}H NMR δ 3.65 (t, J = 8.3 Hz, 1H, C*H*OH), 0.77 (s, 3H, CH_3); ^{13}C NMR δ 211.90 (C=O), 81.73 (HOCH), 11.01 (CH_3), 49.87, 48.56, 47.72, 45.67, 43.60, 42.99, 41.23, 40.96, 36.51, 33.78, 30.47, 30.32, 30.12, 25.66, 23.13. Anal. ($C_{18}H_{28}O_2$) C, H.

Compound **5** was obtained as colorless crystals: mp 110−112 °C (from EtOAc/hexane, lit.[6] mp 108−110 °C); IR 3427, 1709 cm^{-1}; ^{1}H NMR δ 3.58−3.73 (m, 1H, C*H*OH), 2.59 (t, J = 14.2 Hz, 1H, 4-H_{ax}), 0.78 (s, 3H, CH_3); ^{13}C NMR δ 212.99 (C=O), 81.69 (HOCH), 11.03 (CH_3), 49.88, 43.10, 42.78, 41.40, 39.64, 38.32, 38.22, 36.57, 36.30, 30.41, 30.32, 27.63, 25.39, 24.92, 23.10. Anal. ($C_{18}H_{28}O_2$) C, H.

Compound **7** was obtained as colorless crystals: mp 203−204 °C (from EtOAc/hexane, lit.[6] mp 191.5−193 °C); IR 3288, cm^{-1}; ^{1}H NMR δ 3.68−3.60 (m, 2H, 2 × C*H*OH), 0.74 (s, 3H, CH_3); ^{13}C NMR δ 82.04 (CHOH), 71.72 (CHOH), 11.08 (CH_3), 50.08, 43.15, 41.84, 39.92, 38.56, 36.76, 36.37, 35.67, 31.43, 30.55, 29.70, 26.01, 25.76, 25.24, 23.25. Anal. ($C_{18}H_{30}O_2$) C, H.

5β-Estrane-3,17-dione (8). To a solution of compounds **5**−**7** (2.35 g) in acetone (100 mL) at room temperature was added Jones reagent dropwise until a yellow color persisted. After 30 min, 2-propanol was added to destroy excess Jones reagent, and brine (200 mL) was added. The reaction mixture was extracted with EtOAc (3 × 150 mL), and the combined organic solvents were dried over Na_2SO_4. Solvent removal gave compound **8** (2.24 g, 97%) as colorless crystals: mp 182−183 °C (from EtOAc/hexane, lit.[6] mp 182.5−185 °C); IR 1735, 1706 cm^{-1}; ^{1}H NMR δ 2.59 (t, 1H, J = 14.2 Hz, 4-H_{ax}), 0.92 (s, 3H, CH_3); ^{13}C NMR δ 220.62 (C=O), 212.11 (C=O), 13.59 (CH_3), 50.16, 47.66, 42.58, 40.66, 39.46, 38.24, 37.97, 36.10, 35.59, 31.30, 30.15, 27.42, 24.90, 24.10, 21.40. Anal. ($C_{18}H_{26}O_2$) C, H.

3β-Hydroxy-5β-estran-17-one (9a). K-Selectride (11.7 mL, 11.7 mmol, 1.0 M solution in THF) was added to a cooled (−78 °C), stirred solution of compound **8** (2.14 g, 7.8 mmol) in dry THF (200 mL) under nitrogen. After 6 h, the reaction was stopped by the addition of 10% aqueous NaOH (20 mL), followed by 30% H_2O_2 (30 mL). The reaction mixture was allowed to warm to room temperature, and stirring was continued for another 30 min. The mixture was extracted with EtOAc (2 × 200 mL). The combined organic solvents were washed with brine (2 × 100 mL) and dried over Na_2SO_4. Solvent removal gave compound **9a** (2.01 g, 93%) as colorless crystals: mp 166−167 °C (from EtOAc/hexane, lit.[6] mp 165−166.5 °C); IR 3471, 1725 cm^{-1}; ^{1}H NMR δ 4.11 (m, 1H, C*H*OH), 0.87 (s, 3H, CH_3); ^{13}C NMR δ 221.46 (C=O), 66.47 (HOCH),

13.56 (CH_3), 45.03, 47.74, 40.88, 40.48, 37.75, 35.65, 33.02, 31.39, 30.99, 29.61, 26.59, 24.97, 24.72, 21.43, 21.09. Anal. ($C_{18}H_{28}O_2$) C, H.

3β-(Acetyloxy)-5β-estran-17-one (9b). Acetic anhydride (1.3 g, 12.4 mmol) was added to stirred pyridine (40 mL) at 120 °C. After 30 min, steroid **9a** (1.72 g, 6.2 mmol) dissolved in pyridine (10 mL) was added, and the reaction solution was kept at 120 °C for 4 h and then allowed to cool to room temperature. Ice−water (100 mL) and 10% HCl (50 mL) were added, and the mixture was extracted with EtOAc (2 × 150 mL). The combined organic solvents were washed with brine (2 × 150 mL) and saturated $NaHCO_3$ (2 × 150 mL) and dried over Na_2SO_4. Evaporation of the combined organic solvents under reduced pressure gave a solid (1.8 g, 91%). Compound **9b** was obtained as colorless crystals: mp 128−129 °C (from EtOAc/hexane); IR 2924, 2855, 1738, 1452, 1407, 1375, 1238, 1217, 1189, 1145, 1094, 1052, 1071 cm^{-1}; ^1H NMR δ 5.09 (m, 1H, AcOCH), 2.05 (s, 3H, CH_3COO), 0.88 (s, 3H, CH_3); ^{13}C NMR δ 221.12 (C═O), 170.45 (CH_3COO), 70.39 (AcOCH), 13.68 (CH_3), 50.41, 47.81, 41.01, 40.25, 37.97, 35.75, 31.49, 30.92, 30.56, 30.28, 25.10, 24.70, 23.92, 21.97, 21.55, 21.36. Anal. ($C_{20}H_{30}O_3$) C, H.

3β-(Acetyloxy)-5β-estrane-17α-carbonitrile (11a) and 3β-(Acetyloxy)-5β-estrane-17β-carbonitrile (11b). Compound **9b** (1.77 g, 5.6 mmol) was stirred with diethyl cyanophosphonate (2.74 g, 16.8 mmol) and LiCN (554 mg, 16.8 mmol) in THF (100 mL) at room temperature overnight. After water (100 mL) was added, the mixture was extracted with EtOAc (2 × 200 mL). The combined organic solvents were washed with brine (2 × 100 mL), dried over Na_2SO_4, and evaporated under reduced pressure to yield a solid which was purified by chromatography (silica gel, 50% EtOAc in hexane) to give the desired intermediates **10a** and **10b** (2.61 g, 97%) as an oil.

A solution of SmI_2 (16.2 mmol, 162 mL of 0.1 M solution in THF) was added at room temperature to intermediates **10a** and **10b** (2.6 g, 5.4 mmol) and methanol (518 mg, 16.2 mmol) under nitrogen and stirred overnight. Then 10% HCl (20 mL) and brine (100 mL) were added, and the mixture was extracted with ethyl acetate (2 × 150 mL). The extracts were washed with brine (2 × 100 mL), 5% $Na_2S_2O_3$ (150 mL), and brine (150 mL), dried over Na_2SO_4, and evaporated under reduced pressure to yield a mixture of products **11a** and **11b** (1.6 g, 90%), which was separated by HPLC (silica gel, 11% EtOAc in hexane, 3 mL/min). The ratio of products **11a**:**11b** was 1:3, and carbonitrile **11a** eluted first from the column.

Compound **11a** was obtained as colorless crystals: mp 105−107 °C; IR 2927, 2232, 1733, 1450, 1382, 1241, 1159, 1132, 1019 cm^{-1}; ^1H NMR δ 5.10 (m, 1H, AcOCH), 2.58 (dd, J = 8.9 Hz, J = 2.0 Hz, 1H, CNCH), 2.05 (s, 3H, CH_3COO), 0.83 (s, 3H, CH_3); ^{13}C NMR δ 170.53 (CH_3COO), 122.32 (CN), 70.52 (AcOCH), 17.94 (CH_3), 51.07, 44.36, 41.85, 40.20, 40.10, 37.21, 35.04, 31.06, 30.49, 30.29, 27.16, 26.01, 25.52, 24.61, 23.95, 22.06, 21.42. Anal. ($C_{21}H_{31}NO_2$) C, H, N.

Compound **11b** was obtained as colorless crystals: mp 184−186 °C; IR 2958, 2924, 2875, 2857, 2229, 1728, 1448, 1383, 1364, 1256, 1238, 1220, 1198, 1164, 1144, 1100, 1023 cm^{-1}; ^1H NMR δ 5.08 (m, 1H, AcOCH), 2.29 (t, J = 9.5 Hz, 1H, CNCH), 2.05 (s, 3H, CH_3COO), 0.93 (s, 3H, CH_3); ^{13}C NMR δ 170.60 (CH_3COO), 121.28 (CN), 70.45 (AcOCH), 53.42 (CNCH), 14.27 (CH_3), 44.56, 41.92, 40.33, 40.18, 37.60, 37.06, 31.03, 30.49, 30.33, 26.50, 25.88, 25.49, 24.33, 23.99, 22.01, 21.44. Anal. ($C_{21}H_{31}NO_2$) C, H, N.

3β-Hydroxy-5β-estrane-17α-carbonitrile (11c) and 3β-Hydroxy-5β-estrane-17β-carbonitrile (11b). A mixture of compounds **11a** and **11b** (1.51 g) and 0.18 M aqueous methanolic K_2CO_3 (100 mL, 70% MeOH in water) and 10% NaOH (10 mL) was refluxed for 2 h. After the mixture was cooled to room temperature, brine (150 mL) and EtOAc (150 mL) were added, and the aqueous layer was again extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (2 × 100 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the products were purified by chromatography (20% EtOAc in hexane) to give carbonitriles **11c** and **11d** (1.1 g, 84%). These carbonitriles were separated by HPLC (silica gel, 25% EtOAc in hexane, 3 mL/min). Carbonitrile **11c** eluted first from the column.

Compound **11c** was obtained as colorless crystals: mp 143−144 °C (from EtOAc/hexane); IR 3286, 2923, 2232, 1448, 1386, 1342, 1294, 1226, 1160, 1131, 1093, 1070, 1008 cm^{-1}; ^1H NMR δ 4.12 (m, 1H, HOC*H*), 2.58 (d, J = 8.8 Hz, 1H, CNCH), 0.82 (s, 3H, CH_3); ^{13}C NMR δ 122.19 (CN), 66.49 (HOCH), 50.99 (*C*HCN), 17.77 (CH_3), 44.21, 41.69, 40.39, 39.91, 36.97, 34.91, 33.00, 31.10, 29.49, 26.99, 26.57, 25.99, 25.37, 24.46, 21.14. Anal. ($C_{19}H_{29}NO$) C, H, N.

Compound **11d** was obtained as colorless crystals: mp 149−150 °C; IR 3280, 2928, 2235, 1446, 1384, 1341, 1265, 1221, 1101, 1009 cm^{-1}; ^1H NMR δ 4.12 (m, 1H, HOC*H*), 2.29 (t, J = 9.5 Hz, 1H, CNCH), 0.92 (s, 3H, CH_3); ^{13}C NMR δ 121.03 (CN), 66.31 (HOCH), 53.13 (CN*C*H), 13.99 (CH_3), 44.28, 41.65, 40.27, 39.98, 37.20, 36.80, 32.89, 30.97, 29.38, 26.46, 26.21, 25.74, 25.22, 24.06, 20.98. Anal. ($C_{19}H_{29}NO$) C, H, N.

3-Oxo-5β-estrane-17β-carbonitrile (12). Jones reagent was added dropwise to a room temperature solution of compound **11d** (520 mg, 1.8 mmol) in acetone (50 mL) until a yellow color persisted. After 30 min, 2-propanol was added to destroy excess Jones reagent, brine (100 mL) was added, and the mixture was extracted with EtOAc (3 × 100 mL). The combined organic solvents were dried over Na_2SO_4. Evaporation of organic solvents gave product **12** as a solid (500 mg, 97%): mp 148−149 °C (colorless crystals from EtOAc/hexane); IR 2920, 2874, 2234, 1709, 1450, 1387, 1340, 1298, 1267, 1228, 1174, 1129, 1101 cm^{-1}; ^1H NMR δ 2.57 (t, J = 14.2 Hz, 1H, 4-H_{ax}), 2.33 (t, J = 9.5 Hz, 1H, CNCH), 0.96 (s, 3H, CH_3); ^{13}C NMR δ 212.33 (C═O), 121.07 (CN), 53.20 (CN*C*H), 14.23 (CH_3), 44.47, 42.67, 41.61, 40.20, 39.44, 37.93, 36.86, 36.21, 30.30, 27.50, 26.40, 25.32, 24.22. Anal. ($C_{19}H_{27}NO$) C, H, N.

3α-Hydroxy-5β-estrane-17β-carbonitrile (13a). $NaBH_4$ (84 mg) was added to a solution of compound **12** (420 mg, 1.5 mmol) in EtOH (20 mL). After 2 h, the excess $NaBH_4$ was destroyed by addition of HOAc, water (100 mL) was added, and the product was extracted with EtOAc (2 × 100 mL). The combined organic solvents were washed with brine (2 × 100 mL), dried over Na_2SO_4, and evaporated to give a solid which was purified by chromatography (silica gel, 30% EtOAc in hexane) to give carbonitrile **13a** (290 mg, 69%) as the first fraction and carbonitrile **11d** (100 mg, 24%) as the second fraction. Compound **13a** was obtained as colorless crystals: mp 138−139 °C (from EtOAc/hexane); IR 3397, 2926, 2860, 2235, 1451, 1387, 1374, 1300, 1266, 1247, 1223, 1160, 1090, 1060, 1039 cm^{-1}; ^1H NMR δ 3.63 (m, 1H, HOC*H*), 2.29 (t, J = 9.4 Hz, 1H, CNCH), 0.92 (s, 3H, CH_3); ^{13}C NMR δ 121.30 (CN), 71.36 (HOCH), 53.40 (CN*C*H), 14.25 (CH_3), 44.52, 42.01, 40.29, 39.68, 38.12, 37.05, 36.14, 35.36, 31.26, 29.50, 26.48, 26.12, 25.87, 25.14, 24.33. Anal. ($C_{19}H_{29}NO$) C, H, N.

(3α,5β,17α)-3-Hydroxy-19-norpregnan-20-one (14a) and (3α,5β,17β)-3-Hydroxy-19-norpregnan-20-one (14b). A solution of methylmagnesium chloride (2.7 mL, 8.0 mmol, 3.0 M solution in THF) was added at room temperature to a stirred solution of steroid **13a** (240 mg, 0.8 mmol) in THF (150 mL) under nitrogen. The mixture was refluxed overnight and cooled to 0 °C, saturated NH_4Cl (20 mL) was added, and the product was extracted with EtOAc (3 × 150 mL). The combined organic layers were washed with brine (2 × 150 mL) and dried over Na_2SO_4, and the solvent was evaporated to give the mixture of products **14a** and **14b** (203 mg, 80%), which was separated by HPLC (silica gel, 30% EtOAc and 10% $ClCH_2CH_2Cl$ in hexane, 3 mL/min). The ratio of products **14a**:**14b** was 1:10, and steroid **14b** eluted first from the column.

Compound **14a** was obtained as colorless crystals: mp 82−84 °C (from EtOAc/hexane); IR 3397, 2920, 2867, 1703, 1454, 1359, 1171, 1091, 1058, 1038 cm^{-1}; ^1H NMR δ 3.64−3.54 (m, 1H, HOC*H*), 2.81 (dd, J = 9.5 Hz, J = 2.5 Hz, 1H, C*H*COCH$_3$), 2.13 (CH_3CO), 0.91 (s, 3H, CH_3); ^{13}C NMR δ 212.97 (CH_3CO), 71.67 (HOCH), 61.39 (CH_3COCH), 20.88 (CH_3), 49.35, 45.95, 41.95, 39.83, 37.84, 36.41, 35.59, 35.31, 32.84, 31.49, 29.67, 26.45, 26.00, 25.68, 25.55, 24.29. Anal. ($C_{20}H_{32}O_2$) C, H.

Compound **14b** was obtained as colorless crystals: mp 108−109 °C (from EtOAc/hexane, lit.[3f] mp 105−106 °C); IR 3420, 2922, 2869, 1703, 1451, 1385, 1358, 1266, 1226, 1195, 1170, 1152, 1091, 1060, 1040 cm^{-1}; ^1H NMR δ 3.69−3.59 (m, 1H, HOC*H*), 2.54 (t, J = 8.7 Hz, 1H, C*H*COCH$_3$), 2.12 (s, 3H, CH_3-CO), 0.61 (s, 3H, CH_3); ^{13}C NMR δ 209.73 (CH_3CO), 71.47 (HOCH), 63.87 (CH_3COCH), 13.33 (CH_3), 55.65, 44.32, 41.65,

39.76, 38.99, 38.18, 36.22, 35.46, 31.46, 31.38, 29.53, 26.09, 25.86, 25.54, 24.18, 22.70. Anal. ($C_{20}H_{32}O_2$) C, H.

3α-(Acetyloxy)-5β-androstan-17-one (15b). Using a procedure similar to that described for the preparation of steroid **9b**, 3α-hydroxy-5β-androstan-17-one (**15a**, 500 mg, 1.72 mmol) was converted into compound **15b** (560 mg, 98%). Compound **15b** was obtained as colorless crystals: mp 90−91 °C (from EtOAc/hexane, lit.[10] mp 96−97 °C); IR 1739 cm^{-1}; ^1H NMR δ 4.73 (m, 1H, AcOCH), 2.03 (s, 3H, CH_3COO), 0.96 (s, 3H, 19-CH_3), 0.86 (s, 3H, 18-CH_3); ^{13}C NMR δ 221.28 (C=O), 170.61 (CH_3COO), 74.06 (AcOCH), 13.74 (18-CH_3), 51.44, 47.82, 41.77, 40.68, 35.88, 35.31, 34.98, 34.72, 32.13, 31.66, 26.67, 26.52, 25.23, 23.21, 21.75, 21.42, 20.05. Anal. ($C_{21}H_{32}O_3$) C, H.

3α-(Acetyloxy)-5β-androstane-17α-carbonitrile (16a), 3α-(Acetyloxy)-5β-androstane-17β-carbonitrile (16b), 3α-Hydroxy-5β-androstane-17α-carbonitrile (17a), and 3α-Hydroxy-5β-androstane-17β-carbonitrile (17b). Using a two-step cyanation procedure similar to that described for the preparation of steroids **11a** and **11b**, compound **15a** (550 mg, 1.7 mmol) was converted into a mixture (343 mg) of compounds **16a** and **16b**, along with hydrolysis products **17a** and **17b**. The partial loss of the acetyloxy group occurred after the second step of the procedure. Column chromatography (silica gel, 25% EtOAc in hexane) was performed to separate the mixture of products **16a** and **16b** (160 mg, 28%) from product **17a** (80 mg, 16%) and product **17b** (100 mg, 20%). Products **16a** (first component) and **16b** were separated by HPLC (silica gel, 7% EtOAc in hexane, 3 mL/min).

Compound **16a** was obtained as an oil: IR 2938, 2866, 2233, 1734, 1451, 1383, 1363, 1244, 1167, 1030 cm^{-1}; ^1H NMR δ 4.72 (m, 1H, AcOCH), 2.57 (dd, J = 8.9 Hz, J = 1.8 Hz, 1H, CNCH), 2.04 (s, 3H, CH_3COO), 0.94 (s, 3H, 19-CH_3), 0.81 (s, 3H, 18-CH_3); ^{13}C NMR δ 170.56 (CH_3COO), 122.21 (CN), 73.87 (AcOCH), 17.86 (18-CH_3), 51.99, 44.18, 41.52, 39.93, 39.83, 35.97, 35.13, 34.93, 34.45, 31.93, 27.18, 26.64, 26.32, 24.76, 23.07, 21.33, 20.37. Anal. ($C_{22}H_{33}NO_2$) C, H, N.

Compound **16b** was obtained as colorless crystals: mp 132−134 °C (from EtOAc/hexane); IR 2936, 2868, 2235, 1735, 1451, 1383, 1363, 1244, 1029 cm^{-1}; ^1H NMR δ 4.72 (m, 1H, AcOCH), 2.28 (t, J = 9.7 Hz, 1H, CNCH), 2.03 (s, 3H, CH_3COO), 0.95 (s, 3H, CH_3), 0.90 (s, 3H, CH_3); ^{13}C NMR δ 170.50 (CH_3COO), 121.24 (CN), 74.01 (AcOCH), 54.34 (CNCH), 14.23 (18-CH_3), 44.42, 41.59, 40.23, 37.18, 36.11, 34.95, 34.57, 32.08, 26.69, 26.53, 26.49, 26.25, 24.49, 23.17, 21.38, 20.39. Anal. ($C_{22}H_{33}NO_2$) C, H, N.

Compound **17a** was obtained as colorless crystals: mp 105−106 °C (from EtOAc/hexane); IR 3386, 2933, 2863, 2236, 1450, 1387, 1266, 1171, 1108, 1088, 1066, 1036 cm^{-1}; ^1H NMR δ 3.62 (m, 1H, HOCH), 2.58 (dd, J = 8.8 Hz, J = 1.8 Hz, 1H, CNCH), 0.93 (s, 3H, 19-CH_3), 0.80 (s, 3H, 18-CH_3); ^{13}C NMR δ 122.27 (CN), 71.79 (HOCH), 52.04, (CNCH), 17.95 (18-CH_3), 23.19 (19-CH_3), 44.26, 41.84, 40.00, 39.91, 36.31, 36.12, 35.34, 35.21, 34.53, 30.47, 27.25, 26.90, 26.50, 24.87, 20.43. Anal. ($C_{20}H_{31}NO$) C, H, N.

Compound **17b** was obtained as colorless crystals: mp 161−162 °C (from EtOAc/hexane); IR 3365, 2929, 2861, 2234, 1451, 1383, 1268, 1167, 1092, 1068, 1041 cm^{-1}; ^1H NMR δ 3.64 (m, 1H, HOCH), 2.28 (t, J = 9.5 Hz, 1H, CNCH), 0.94 (s, 3H, CH_3), 0.90 (s, 3H, CH_3); ^{13}C NMR δ 121.29 (CN), 71.46 (HOCH), 54.35 (CNCH), 14.23 (18-CH_3), 23.20 (19-CH_3), 44.43, 41.77, 40.24, 40.21, 37.21, 36.17, 35.25, 34.54, 30.35, 26.87, 26.54, 26.34, 24.52, 20.38. Anal. ($C_{20}H_{31}NO$) C, H, N.

3β-Hydroxy-5β-androstane-17β-carbonitrile (17c). Trifluoroacetic acid (78 mg, 0.68 mmol) and then solid triphenylphosphine (178 mg, 0.68 mmol) were added to diethyl azodicarboxylate (118 mg, 0.68 mmol) and compound **17b** (50 mg, 0.17 mmol) in stirred dry THF (2 mL). After 5 min, sodium benzoate (200 mg, 1.4 mmol) was added and the mixture was stirred 2 h. EtOH (5 mL) and 10% NaOH (2 mL) was added, and stirring was continued for 1 h. The reaction solution was poured into water (100 mL) and extracted with EtOAc (2 × 50 mL). The combined organic solvents were washed with brine (2 × 50 mL) and dried over Na_2SO_4. Solvent removal gave a solid that was purified by chromatography (silica gel, 30% EtOAc in hexane) to yield product **17c** (48 mg, 95%) as colorless crystals: mp 193−194 °C (from EtOAc/hexane); IR 3333, 2931, 2874, 2860, 2233, 1508, 1471, 1448, 1377, 1339, 1240, 1167, 1034, 1005 cm^{-1}; ^1H NMR δ 4.11 (m, 1H, HOCH), 2.27 (t, 1H, J = 9.6 Hz, CNCH), 0.98 (s, 3H, CH_3), 0.91 (s, 3H, CH_3); ^{13}C NMR δ 121.31 (CN), 66.83 (HOCH), 14.27 (CH_3), 54.48, 44.49, 40.26, 39.59, 37.28, 36.31, 36.00, 35.13, 33.35, 29.86, 27.76, 26.56, 26.36, 26.23, 24.52, 23.74, 20.67. Anal. ($C_{20}H_{31}NO$) C, H, N.

3α-Hydroxy-19-nor-5α-pregnan-20-one (18b). Methylmagnesium chloride (3 mL, 9.0 mmol, 3.0 M solution in THF) was reacted with previously prepared steroid **18a**[12] (59 mg, 0.2 mmol) in THF (20 mL) at reflux for 2 days, followed by a workup procedure analogous to that described for the preparation of steroids **14a** and **14b**. HPLC (silica gel, 20% EtOAc, 10% $ClCH_2CH_2Cl$ in hexane, 3 mL/min) separation of the reaction products gave the initially eluted unreacted steroid **18a** (10 mg) followed by the product **18b** (40 mg, 64%), which was recovered as colorless crystals: mp 176−177 °C (from EtOAc/hexane); IR 3307, 2918, 2859, 1705, 1442, 1353, 1200, 1160, 1099, 1001 cm^{-1}; ^1H NMR δ 4.09 (m, 1H, HOCH), 2.55 (t, J = 8.7 Hz, CHCOCH$_3$), 2.12 (s, 3H, CH_3CO), 0.62 (s, 3H, CH_3); ^{13}C NMR δ 209.81 ($COCH_3$), 66.40 (HOCH), 63.93 ($CHCOCH_3$), 13.41 (CH_3), 55.87, 47.90, 46.93, 44.31, 41.21, 40.53, 39.02, 35.92, 33.55, 32.92, 31.52, 30.91, 25.61, 24.17, 23.66, 22.70. Anal. ($C_{20}H_{32}O_2$) C, H.

3′,4β-Cycloprop[4,5]-5α-estrane-3,17-dione (21a). A solution of K-Selectride (55 mL, 55 mmol, 1.0 M solution in THF) was added to a cooled (−78 °C), stirred solution of steroid **3** (7.5 g, 27.3 mmol) in dry THF (100 mL) under nitrogen. After 15 h, the reaction mixture was allowed to warm to −10 °C, and stirring was continued for another 3 h. The reaction was stopped by the addition of 10% aqueous NaOH (15 mL) and brine (150 mL), and the mixture was extracted with EtOAc (2 × 200 mL). The combined organic layers were washed with brine (2 × 150 mL) and dried over Na_2SO_4. Evaporation of organic solvent gave a mixture of compounds **3**, **6**, **19a**, and **19b** (7.2 g). HPLC (silica gel, 50% EtOAc in hexane, 3 mL/min) separation of a portion of the product mixture gave in order of elution steroid **6**, steroid **19b**, and a mixture of steroids **3** and **19a** in the relative ratio of 14:57:29, respectively. The structures of known steroids **6**[6] and **19b**[17] were confirmed by combustion analysis and NMR and IR spectroscopic analysis.

Diiodomethane (24 g) was added to a stirred mixture of zinc−copper couple (6 g) and a small crystal of iodine in ether (50 mL). The mixture was warmed with a tungsten lamp until the reaction started, and the reaction was continued for 30 min on a water bath at 60 °C. A solution of the mixture of compounds **3**, **6**, **19a**, and **19b** (7 g) was added over 10 min, and the mixture was stirred for 3 days at reflux. The ice-cooled mixture was diluted with saturated NH_4Cl, and the precipitate was filtered and washed twice with ether. The combined ether extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give a crude mixture containing products **20a** and **20b**. This crude mixture was used without product characterization.

Jones reagent was added at room temperature to a stirred solution of the above crude mixture dissolved in acetone (100 mL) until a yellow color persisted for 10 min, and 2-propanol was added to destroy any excess Jones reagent. EtOAc (200 mL) and water (200 mL) were added, and the aqueous layer was extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with brine (3 × 200 mL) and dried over Na_2SO_4. Following solvent removal, an oil was obtained. Partial purification by chromatography (silica gel, 30% EtOAc in hexane) gave a product mixture containing steroids **21a** and **21b.** This product mixture was dissolved in dichloromethane (50 mL) and HOAc (1 mL), cooled to −78 °C, and a stream of O_3 was bubbled through the solution until a blue color persisted. The excess O_3 was removed with a stream of O_2. Additional dichloromethane (100 mL) was added, and the solution was washed with 5% Na_2CO_3, water, and brine and dried over Na_2SO_4. Solvent removal gave a solid which was purified by chromatography (silica gel, 35% EtOAc in hexane) to give steroid **8** (1.57 g) and a mixture of steroids **21a** and **21b** (1.1 g, 14% overall from compound **3**). A pure sample of product **21a** was obtained upon crystallization from EtOAc/hexane. A pure sample of product **21b** was not obtained. Compound **21a** was obtained as colorless crystals: mp 207−209 °C (from EtOAc/hexane); IR 2926, 1738, 1682, 1452, 1378,

1330, 1253, 1213, 1046 cm^{-1}; ^1H NMR δ 0.92 (s, 3H, CH_3), 0.82 (q, J = 4.5 Hz, 1H cyclopropyl-H); ^{13}C NMR δ 220.79 (C=O), 208.59 (C=O), 13.70 (CH_3), 15.10 (cyclopropyl-CH_2), 50.22, 47.75, 47.61, 40.79, 39.84, 36.10, 35.80, 35.66, 33.82, 31.30, 30.88, 28.90, 24.89, 21.68, 21.55. Anal. ($C_{19}H_{26}O_2$) C, H.

5-Methyl-5α-estrane-3,17-dione (22a) and 5-Methyl-5β-estrane-3,17-dione (22b). Lithium wire (450 mg, 64.8 mmol) was added to stirred liquid NH_3 (ca. 160 mL). After 30 min, a solution of steroids **21a** and **21b** (1.2 g, 4.2 mmol) in THF (50 mL) was added slowly, stirring was continued for 2 h, and 1,2-dibromoethane was added dropwise to discharge the blue color. A solution of HOAc (4 mL) in MeOH (16 mL) was added dropwise over 15 min, and the liquid NH_3 was allowed to evaporate at room temperature. The mixture was diluted with water (100 mL) and EtOAc (150 mL), and the organic layer was separated. The water layer was extracted with EtOAc (2 × 150 mL), and the combined organic layers were washed with brine (2 × 150 mL) and dried over Na_2SO_4. The solvent was removed to give a residue which was purified by chromatography (silica gel, 30% EtOAc in hexane) to give a first fraction that contained a mixture of products **22a** and **22b** (900 mg, 74%) and a second fraction that contained steroid **22c** (110 mg, 9%). Steroid **22c** [IR 3275, 1706 cm^{-1}; ^1H NMR δ 3.66 (t, J = 8.5 Hz, 1H, C*H*OH), 0.79 (s, 3H, CH_3), 0.77 (s, 3H, CH_3)] was oxidized with Jones reagent in acetone as described above to give a solid that was purified by chromatography (30% EtOAC in hexane) to give product **22a** (90 mg, 82%). Pure compound **22b** was obtained by Jones oxidation of steroid **23b** (vide infra).

Compound **22a** was obtained as colorless crystals: mp 176−178 °C (from EtOAc/hexane); IR 2922, 1739, 1700, 1453, 1378, 1295, 1251, 1197, 1104, 1050, 1009 cm^{-1}; ^1H NMR δ 0.91 (s, 3H, CH_3), 0.82 (s, 3H, CH_3); ^{13}C NMR δ 220.86 (C=O), 211.47 (C=O), 17.75 (CH_3), 13.73 (CH_3), 56.74, 50.20, 47.76, 47.66, 42.60, 41.40, 41.07, 40.70, 38.56, 35.70, 31.42, 25.71, 25.37, 25.19, 21.47. Anal. ($C_{19}H_{28}O_2$) C, H.

Compound **22b** was obtained as colorless crystals: mp 141−143 °C (from EtOAc/hexane); IR 2923, 2855, 1739, 1712, 1456, 1378, 1259, 1231, 1191, 1107, 1060, 1020 cm^{-1}; ^1H NMR δ 2.71 (d, J = 14.3 Hz, 4-H_{ax}), 0.97 (s, 3H, CH_3), 0.91 (s, 3H, CH_3); ^{13}C NMR δ 220.96 (C=O), 212.81 (C=O), 13.76 (18-CH_3), 50.61, 48.11, 47.67, 45.50, 40.85, 40.03, 39.46, 39.02, 36.22, 35.86, 31.55, 29.27, 25.89, 25.55, 22.79, 21.60. Anal. ($C_{19}H_{28}O_2$) C, H.

3α-Hydroxy-5-methyl-5α-estran-17-one (23a) and 3β-Hydroxy-5-methyl-5β-estran-17-one (23b). K-Selectride (6.2 mL, 6.2 mmol, 1.0 M solution in THF) was added to a cooled (−78 °C), stirred solution of compounds **22a** and **22b** (900 mg, 3.1 mmol) in dry THF (150 mL) under nitrogen. After 6 h, the reaction was stopped by the addition of 10% NaOH (10 mL), followed by 30% H_2O_2 (10 mL). The reaction mixture was allowed to warm to room temperature, and stirring was continued for another 30 min. The mixture was extracted with EtOAc (2 × 200 mL), and the combined organic solvents were washed with brine (2 × 100 mL) and dried over Na_2SO_4. Evaporation of organic solvent gave a solid (**23a** and **23b**, 850 mg, 94%), which when washed with EtOAc/methanol gave a small amount (ca. 20 mg) of pure product **23a**. The remainder of the product mixture was characterized as the acetyloxy derivatives **24a** and **24b**. The pure sample of steroid **23b** was obtained by hydrolysis of steroid **24b** (vide infra). Compound **23a** was obtained as colorless crystals: 247−255 °C (from EtOAc); IR 3488, 3278, 2918, 2868, 1720, 1451, 1401, 1371, 1328, 1259, 1228, 1198, 1110, 1020 cm^{-1}; ^1H NMR δ 4.11 (m, 1H, HOC*H*), 1.08 (s, 3H, 5-CH_3), 0.87 (s, 3H, 18-CH_3); ^{13}C NMR) δ 221.52 (C=O), 67.33 (CHOH), 13.87 (CH_3), 50.57, 49.87, 48.24, 47.94, 41.99, 41.89, 41.46, 35.85, 34.22, 33.84, 31.57, 25.28, 24.71, 21.54, 19.39, 19.10. Anal. ($C_{19}H_{30}O_2$) C, H.

Compound **23b** was obtained as colorless crystals: mp 192−194 °C (from EtOAc/hexane); IR 3503, 2921, 1728, 1452, 1377, 1323, 1268, 1155, 1101, 1055, 1021 cm^{-1}; ^1H NMR δ 4.16 (m, 1H, HOC*H*), 1.22 (s, 3H, 5-CH_3), 0.87 (s, 3H, 18-CH_3); ^{13}C NMR δ 221.62 (C=O), 67.60 (CHOH), 17.10 (5-CH_3), 13.71 (18-CH_3), 50.71, 47.71, 46.01, 41.43, 40.69, 39.58, 37.49, 35.89, 32.84, 31.61, 31.43, 26.57, 25.89, 25.44, 21.62. Anal. ($C_{19}H_{30}O_2$) C, H.

3α-(Acetyloxy)-5-methyl-5α-estran-17-one (24a) and 3β-(Acetyloxy)-5-methyl-5β-estran-17-one (24b). A solution of steroids **23a** and **23b** (830 mg, 2.9 mmol) in pyridine (10 mL) was added to a stirred solution of $(AcO)_2O$ (2.9 g, 29.6 mmol) and pyridine (40 mL) that had been preheated to 120 °C for 30 min. The reaction was continued for 2 h, the mixture was cooled to room temperature, and ice−water (100 mL) and 10% HCl (50 mL) were added. The mixture was extracted with EtOAc (2 × 150 mL). The combined organic solvents were washed with brine (2 × 150 mL) and dried over Na_2SO_4. Evaporation of organic solvent under reduced pressure gave a solid, which was purified by chromatography (silica gel, 15% EtOAc in hexane) and separated by HPLC (silica gel, 15% EtOAc in hexane, 3 mL/min) into initially eluted steroid **24a** (470 mg, 50%) and steroid **24b** (310 mg, 33%).

Compound **24a** was obtained as colorless crystals: mp 145−147 °C (from EtOAc/hexane); IR 2928, 2859, 1733, 1434, 1377, 1242, 1230, 1162, 1132, 1108, 1049, 1021 cm^{-1}; ^1H NMR δ 5.02 (m, 1H, HOC*H*), 2.03 (s, 3H, CH_3COO), 0.97 (s, 3H, 5-CH_3), 0.87 (s, 3H, 18-CH_3); ^{13}C NMR δ 220.68 (C=O), 170.06 (CH_3*C*OO), 69.65 (CHOAc), 13.54 (18-CH_3), 50.22, 49.08, 47.53, 44.52, 41.60, 41.49, 40.81, 35.47, 33.46, 31.27, 30.79, 24.95, 24.47, 21.21, 19.39, 18.32. Anal. ($C_{21}H_{32}O_3$) C, H.

Compound **24b** was obtained as colorless crystals: mp 163−164 °C (from EtOAc/hexane); IR 2925, 1737, 1454, 1376, 1241, 1153, 1113, 1019 cm^{-1}; ^1H NMR δ 5.08 (m, 1H, HOC*H*), 2.04 (s, 3H, CH_3COO), 1.13 (s, 3H, 5-CH_3), 0.87 (s, 3H, 18-CH_3; ^{13}C NMR δ 221.09 (C=O), 170.26 (CH_3*C*OO), 70.44 (CHOAc), 17.65 (5-CH_3), 13.55 (18-CH_3), 50.45, 47.48, 45.43, 40.97, 40.53, 39.39, 35.71, 34.08, 32.67, 31.41, 30.45, 25.65, 25.33, 23.49, 21.46. Anal. ($C_{21}H_{32}O_3$) C, H.

3α-Hydroxy-5-methyl-5α-estrane-17α-carbonitrile (26a) and 3α-Hydroxy-5-methyl-5α-estrane-17β-carbonitrile (26b). Using a two-step cyanation procedure similar to that described for the preparation of steroids **11a** and **11b**, compound **24a** (460 mg, 1.38 mmol) was converted into a mixture of compounds **25a** and **25b** (440 mg, 98%) which was saponified in stirred THF (5 mL) with 0.18 M aqueous methanolic K_2CO_3 (20 mL, 70% MeOH in water) and 10% NaOH (0.5 mL) at reflux for 2 d. After the mixture was cooled to room temperature, brine (150 mL) and EtOAc (150 mL) were added and the aqueous layer was extracted further with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (2 × 100 mL) and dried over Na_2SO_4. Solvent removal gave a mixture of products **26a** and **26b** (360 mg, 93%), which was separated by HPLC (silica gel, 20% EtOAc in hexane, 3 mL/min). The product ratio was 1:1 and steroid **26a** eluted first from the column.

Compound **26a** was obtained as colorless crystals: mp 220−221 °C (from EtOAc/hexane); IR 3278, 3133, 2919, 2858, 2232, 1448, 1380, 1335, 1259, 1229, 1163, 1121, 1071, 1046 cm^{-1}; ^1H NMR δ 4.10 (m, 1H, HOC*H*), 2.57 (dd, J = 8.8 Hz, J = 2.1 Hz, 1H, CNCH), 1.08 (s, 3H, 5-CH_3), 0.82 (s, 3H, 18-CH_3); ^{13}C NMR δ 122.37 (CNCH), 67.30 (AcOCH), 51.22, 49.80, 48.24, 44.37, 42.73, 41.58, 41.20, 40.05, 35.12, 34.19, 33.66, 27.24, 25.95, 25.66, 24.54, 19.37, 19.12, 18.12. Anal. ($C_{20}H_{31}NO$) C, H, N.

Compound **26b** was obtained as colorless crystals: mp 229−230 °C (from EtOAc/hexane); IR 3333, 2917, 2239, 1449, 1362, 1328, 1273, 1232, 1195, 1104, 1041 cm^{-1}; ^1H NMR δ 4.11 (m, 1H, HOC*H*), 2.28 (t, J = 9.7 Hz, 1H, CNCH), 1.06 (s, 3H, 5-CH_3), 0.92 (s, 3H, 18-CH_3); ^{13}C NMR δ 121.39 (CNCH), 67.29 (AcOCH), 14.39 (18-CH_3), 53.57, 49.75, 48.20, 44.54, 42.76, 41.60, 41.51, 40.29, 37.13, 34.19, 33.69, 26.54, 25.78, 25.60, 24.21, 19.35, 19.10. Anal. ($C_{20}H_{31}NO$) C, H, N.

(3α,5α,17α)-3-Hydroxy-5-methyl-19-norpregnan-20-one (27a) and 3α-Hydroxy-5-methyl-19-nor-5α-pregnan-20-one (27b). As described earlier for the preparation of compounds **14a** and **14b**, a mixture of steroids **26a** and **26b** (80 mg, 0.26 mmol) was reacted with CH_3MgBr (8 mL, 24.0 mmol, 3.0 M solution in THF) in THF (40 mL) to give a mixture of products **27a** and **27b** (76 mg, 90%), which was separated by HPLC (silica gel, 10% EtOAc, 10% $ClCH_2CH_2Cl$ in hexane, 3 mL/min). The product ratio was 1:1 and steroid **27b** eluted first from the column.

Compound **27a** was obtained as colorless crystals: mp 198−200 °C (from EtOAc/hexane); IR 3340, 2964, 2912, 2868, 1698,

1439, 1360, 1278, 1232, 1171, 1086, 1045 cm^{-1}; ^1H NMR δ 4.09 (m, 1H, HOC*H*), 2.80 (dd, J = 8.5 Hz, J = 2.3 Hz, 1H, C*H*COCH$_3$), 2.12 (s, 3H, CH_3CO), 1.03 (s, 3H, 5-CH_3), 0.91 (s, 3H, 18-CH_3); ^{13}C NMR δ 212.93 (*C*OCH$_3$), 67.38 (HOCH), 61.32 (*C*HCOCH$_3$), 49.84, 49.43, 48.35, 45.94, 42.64, 41.72, 41.20, 35.37, 34.17, 33.67, 32.82, 26.05, 25.93, 25.50, 24.21, 20.99, 19.36, 19.09. Anal. ($C_{21}H_{34}O_2$) C, H.

Compound **27b** was obtained as colorless crystals: mp 262−263 °C (from EtOAc/hexane); IR 3325, 2919, 2869, 1704, 1471, 1447, 1379, 1360, 1267, 1226, 1206, 1194, 1173, 1104, 1085, 1045, 1005 cm^{-1}; ^1H NMR δ 4.10 (m, 1H, HOC*H*), 2.54 (t, J = 8.4 Hz, 1H, C*H*COCH$_3$), 2.12 (s, 3H, CH_3CO), 1.07 (s, 3H, 5-CH_3), 0.60 (s, 3H, 18-CH_3); ^{13}C NMR δ 209.71 (*C*OCH$_3$), 67.15 (AcOCH), 63.80 (*C*HCOCH$_3$), 13.40 (18-CH_3), 55.73, 49.73, 48.17, 44.26, 42.31, 41.58, 38.98, 34.08, 33.61, 31.40, 25.92, 25.68, 23.98, 22.61, 19.31, 19.02. Anal. ($C_{21}H_{34}O_2$) C, H.

3β-(Acetyloxy)-5-methyl-5β-estrane-17α-carbonitrile (28a) and 3β-(Acetyloxy)-5-methyl-5β-estrane-17β-carbonitrile (28b). Using a two-step cyanation procedure similar to that described for the preparation of steroids **11a** and **11b**, compound **24b** (240 mg, 0.72 mmol) was converted into a mixture of compounds **28a** and **28b** (235 mg, 95%) which was separated by HPLC (silica gel, 15% EtOAc in hexane, 3 mL/min). The product ratio was 1:1, and steroid **28a** eluted first from the column.

Compound **28a** was obtained as colorless crystals: mp 94−96 °C; IR 2924, 2233, 1733, 1451, 1380, 1243, 1182, 1156, 1114, 1020 cm^{-1}; ^1H NMR δ 5.09 (m, 1H, AcOCH), 2.58 (d, J = 8.7 Hz, 1H, CNCH), 2.04 (s, 3H, CH_3CO), 1.11 (s, 3H, 5-CH_3), 0.82 (s, 3H, 18-CH_3); ^{13}C NMR δ 170.49 (CH_3CO), 122.38 (CN), 70.70 (AcOCH), 17.92 (CH_3), 17.85 (CH_3), 51.28, 45.52, 44.16, 41.52, 41.24, 40.12, 38.75, 35.11, 34.21, 32.67, 30.59, 27.24, 27.09, 25.89, 24.67, 23.65, 21.67. Anal. ($C_{22}H_{33}NO_2$) C, H, N.

Compound **28b** was obtained as colorless crystals: mp 117−119 °C (from EtOAc/hexane); IR 2921, 2235, 1733, 1450, 1381, 1243, 1151, 1114, 1019 cm^{-1}; ^1H NMR δ 5.07 (m, 1H, AcOC*H*), 2.28 (t, J = 9.8 Hz, 1H, CNCH), 2.04 (s, 3H, CH_3CO), 1.11 (s, 3H, 5-CH_3), 0.92 (s, 3H, 18-CH_3); ^{13}C NMR δ 170.54 (CH_3CO), 121.33 (CN), 70.60 (AcOCH), 17.79 (CH_3), 14.22 (CH_3), 53.61, 45.48, 44.35, 41.55, 41.18, 40.34, 39.13, 37.12, 34.24, 32.67, 30.56, 26.96, 26.55, 25.83, 24.35, 23.66, 21.66. Anal. ($C_{22}H_{33}NO_2$) C, H, N.

3β-Hydroxy-5-methyl-5β-estrane-17α-carbonitrile (29a) and 3β-Hydroxy-5-methyl-5β-estrane-17β-carbonitrile (29b). Using a hydrolysis procedure similar to that described for the preparation of steroids **26a** and **26b**, a mixture of steroids **28a** and **28b** (230 mg, 0.7 mmol) was converted into a mixture of products **29a** and **29b** (195 mg, 97%), which was separated by HPLC (silica gel, 30% EtOAc in hexane, 3 mL/min). Steroid **29a** eluted first from the column.

Compound **29a** was obtained as colorless crystals: mp 179−181 °C (from EtOAc/hexane); IR 3358, 2920, 2693, 2233, 1474, 1451, 1383, 1337, 1268, 1224, 1174, 1103, 1068, 1005 cm^{-1}; ^1H NMR δ 4.17 (m, 1H, HOC*H*), 2.58 (dd, J = 8.9 Hz, J = 2.0 Hz, 1H, CNCH), 1.21 (s, 3H, 5-CH_3), 0.82 (s, 3H, 18-CH_3); ^{13}C NMR δ 122.42 (CN), 67.61 (HOCH), 17.92 (CH_3), 17.85 (CH_3), 51.37, 45.94, 44.18, 41.55, 41.52, 40.14, 38.79, 37.48, 35.16, 32.67, 31.41, 27.25, 27.18, 26.57, 25.85, 24.67. Anal. ($C_{20}H_{31}NO$) C, H, N.

Compound **29b** was obtained as colorless crystals: mp 175−177 °C (from EtOAc/hexane); IR 3367, 2920, 2233, 1736, 1452, 1383, 1268, 1173, 1103, 1006 cm^{-1}; ^1H NMR δ 4.15 (m, 1H, HOC*H*), 2.27 (t, J = 9.6 Hz, 1H, CNCH), 1.21 (s, 3H, 5-CH_3), 0.92 (s, 3H, 18-CH_3); ^{13}C NMR δ 121.32 (CN), 67.56 (HOC*H*), 17.07 (5-CH_3), 14.19 (18-CH_3), 53.69, 45.88, 44.34, 41.53, 41.48, 40.34, 39.13, 37.48, 37.17, 32.69, 31.38, 27.03, 26.55, 25.78, 24.34. Anal. ($C_{20}H_{31}NO$) C, H, N.

5-Methyl-3-oxo-5β-estrane-17α-carbonitrile (30a). Using the earlier described Jones oxidation procedure, steroid **29a** (40 mg, 0.13 mmol) was oxidized into product **30a** (36 mg, 91%), which was obtained as colorless crystals: mp 140−142 °C (from EtOAc/hexane); IR 2921, 2233, 1710, 1476, 1451, 1383, 1347, 1314, 1272, 1233, 1212, 1180, 1126, 1077 cm^{-1}; ^1H NMR δ 2.72 (d, J = 14.3 Hz, 1H, 4-H_{ax}), 2.62 (dd, J = 8.9 Hz, J = 2.0 Hz, 1H, CNCH), 0.95 (s, 3H, 5-CH_3), 0.86 (s, 3H, 18-CH_3); ^{13}C NMR δ 212.60 (C=O), 122.10 (CN), 17.82 (18-CH_3), 51.13, 47.96, 45.26, 44.02, 41.48, 39.93, 39.43, 39.10, 38.77, 36.10, 34.92, 29.11, 27.11, 27.01, 25.81, 24.48, 22.75. Anal. ($C_{20}H_{29}NO$) C, H, N.

5-Methyl-3-oxo-5β-estrane-17β-carbonitrile (30b). Using the earlier described Jones oxidation procedure, steroid **29b** (85 mg, 0.28 mmol) was oxidized into product **30b** (80 mg, 95%), which was obtained as colorless crystals: mp 158−160 °C (from EtOAc/hexane); IR 2920, 2235, 1709, 1450, 1384, 1348, 1316, 1274, 1233, 1175, 1127 cm^{-1}; ^1H NMR δ 2.69 (d, J = 14.7 Hz, 1H, 4-H_{ax}), 2.32 (t, J = 9.3 Hz, 1H, CNCH), 0.95 (s, 6H, 2 × CH_3); ^{13}C NMR δ 212.54 (C=O), 121.00 (CN), 14.12 (18-CH_3), 53.32, 47.91, 45.16, 44.15, 41.48, 40.09, 39.37, 38.69, 36.84, 36.04, 29.05, 26.80, 26.37, 25.69, 24.12, 22.61. Anal. ($C_{20}H_{29}NO$) C, H, N.

3α-Hydroxy-5-methyl-5β-estrane-17α-carbonitrile (31). $NaBH_4$ (25 mg) was added at room temperature to a solution of compound **30a** (30 mg, 0.1 mmol) in THF (10 mL) and EtOH (5 mL). After 1 h, the excess $NaBH_4$ was destroyed by addition of HOAc, and then water (50 mL) was added. The mixture was extracted with EtOAc (2 × 100 mL), and the combined organic solvents were washed with brine (2 × 100 mL) and dried over Na_2SO_4. Evaporation of organic solvent gave an oil, which was separated by HPLC (silica gel, 40% EtOAc in hexane, 3 mL/min) into products and **29a** (first fraction, 10 mg, 25%) and **31** (second fraction, 18 mg, 45%). Compound **31** was obtained as an amorphous solidified foam: IR 3423, 2922, 2233, 1450, 1382, 1267, 1035, 1007 cm^{-1}; ^1H NMR δ 3.91 (m, 1H, HOC*H*), 2.58 (dd, J = 8.8 Hz, J = 1.8 Hz, 1H, CNCH), 0.98 (s, 3H, 5-CH_3), 0.81 (s, 3H, 18-CH_3); ^{13}C NMR δ 122.25 (CN), 68.17 (HOCH), 17.90 (18-CH_3), 51.28, 45.13, 44.10, 41.75, 40.93, 40.61, 40.05, 38.92, 35.27, 35.10, 29.63, 29.09, 27.22, 26.77, 25.71, 24.65, 21.28. Anal. ($C_{20}H_{31}NO$) C, H, N.

3α-Hydroxy-5-methyl-5β-estrane-17β-carbonitrile (32). Using the $NaBH_4$ reduction procedure described immediately above, compound **30b** (50 mg, 0.17 mmol) was converted into an oil which was separated by column chromatography (silica gel, 35% EtOAc in hexane) into products **29b** (first fraction, 23 mg, 46%) and **32** (second fraction, 25 mg, 50%). Compound **32** was obtained as colorless crystals: mp 161−163 °C (from EtOAc/hexane); IR 3376, 2923, 2236, 1452, 1383, 1269, 1168, 1040 cm^{-1}; ^1H NMR δ 3.91 (m, 1H, HOC*H*), 2.28 (t, J = 9.5 Hz, 1H, CNCH), 0.98 (s, 3H, CH_3), 0.91 (s, 3H, CH_3); ^{13}C NMR δ 121.32 (CN), 14.20 (18-CH_3), 68.16 (HOCH), 53.63, 45.10, 44.31, 41.80, 40.89, 40.52, 40.30, 39.26, 37.13, 35.30, 29.54, 29.09, 26.64, 26.54, 25.64, 24.34, 21.21. Anal. ($C_{20}H_{31}NO$) C, H, N.

3α-Hydroxy-5-methyl-19-nor-5β-pregnan-20-one (33a) and 3β-Hydroxy-5-methyl-19-nor-5β-pregnan-20-one (33b). As described earlier for the preparation of compounds **14a** and **14b**, a 1:1 mixture of steroids **29b** and **32** (40 mg, 0.13 mmol) was reacted (24 h at reflux) with CH_3MgBr (4 mL, 12.0 mmol, 3.0 M solution in THF) in THF (20 mL) to give a mixture of products **33a** and **33b** (40 mg, 64%), which was separated by HPLC (silica gel, 35% EtOAc in hexane, 3 mL/min). Steroid **33b** eluted first from the column.

Compound **33a** was obtained as colorless crystals: mp 140−142 °C (from EtOAc/hexane); IR 3322, 2921, 1702, 1450, 1383, 1360, 1288, 1207, 1170, 1106, 1028, 1004 cm^{-1}; ^1H NMR δ 3.92 (m, 1H, HOC*H*), 2.54 (t, J = 8.7 Hz, 1H, C*H*COCH$_3$), 2.12 (s, 3H, CH_3CO), 0.97 (s, 3H, 5-CH_3), 0.60 (s, 3H, 18-CH_3); ^{13}C NMR δ 209.76 (*C*OCH$_3$), 68.34 (HOCH), 63.93 (*C*HCOCH$_3$), 13.34 (18-CH_3), 55.92, 45.19, 44.13, 41.47, 41.05, 40.65, 39.35, 39.11, 35.37, 31.52, 29.62, 29.16, 26.65, 26.08, 24.22, 22.77, 21.25. Anal. ($C_{21}H_{34}O_2$) C, H.

Compound **33b** was obtained as colorless crystals: mp 159−160 °C (from EtOAc/hexane); IR 3347, 2921, 2870, 1698, 1449, 1383, 1359, 1272, 1205, 1165, 1105, 1028, 1005 cm^{-1}; ^1H NMR δ 4.16 (m, 1H, HOC*H*), 2.53 (t, J = 8.9 Hz, 1H, C*H*COCH$_3$), 2.12 (s, 3H, CH_3CO), 1.20 (s, 3H, 5-CH_3), 0.61 (s, 3H, 18-CH_3); ^{13}C NMR δ 209.69 (*C*OCH$_3$), 67.69 (HOCH), 63.96 (*C*HCOCH$_3$), 17.07 (5-CH_3), 13.31 (18-CH_3), 55.97, 45.94, 44.14, 41.63, 41.17, 39.21, 39.14, 37.59, 32.70, 31.45, 27.01, 26.59, 26.20, 24.20, 22.75. Anal. ($C_{21}H_{34}O_2$) C, H.

4-Pregnene-3α,20(*R*)-diol (35a) and 4-pregnene-3β,20-(*R*)-diol (35b). K-Selectride (1.0 M solution in THF, 41.9 mL, 41.9 mmol) was added to a cooled (−78 °C), stirred solution of progesterone **34** (7.5 g, 20.9 mmol) in dry THF (50 mL) under nitrogen. After 5 h, the reaction mixture was allowed to warm

to room temperature, and stirring was continued for another 4 h. The reaction was stopped by the addition of 10% NaOH (100 mL). The mixture was extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with brine (2 × 150 mL) and dried over Na_2SO_4. Evaporation of organic solvent gave a white solid, which was purified by chromatography (silica gel, 25% EtOAc in hexane) to yield a mixture of products **35a** and **35b** (7.4 g, 98%). A small portion of the product mixture was separated by HPLC (silica gel, 50% EtOAc in hexane, 3 mL/min). The product ratio was 1:1, and steroid **35b** eluted first from the column.

Compound **35a** was obtained as colorless crystals: mp 172−173 °C (lit.[18] mp 155−158 °C). The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical to those reported.[18] Anal. ($C_{21}H_{34}O_2$) C, H.

Compound **35b** was obtained as colorless crystals: mp 178−180 °C (lit.[19] mp 163−172 °C); IR 3373, 1658 cm^{-1}; ^{1}H NMR δ 5.28 (s, 1H, CH=C), 4.18−4.11 (m, 1H, 3-C*H*OH), 3.77 −3.68 (m, 1H, 20-C*H*OH), 1.13 (d, J = 6.0 Hz, 1H, 21-CH_3), 1.11 (s, 3H, CH_3), 0.77 (s, 3H, CH_3); ^{13}C NMR δ 147.57, 123.32 (C=C), 70.54 (HOCH), 67.94 (HOCH), 18.89 (CH_3), 12.47 (CH_3), 58.43, 55.58, 54.40, 42.37, 39.90, 37.33, 35.74, 35.34, 33.08, 32.15, 29.48, 25.57, 24.43, 23.62, 20.86. Anal. ($C_{21}H_{34}O_2$) C, H.

3′,4*β*-Cycloprop[4,5]-5α-pregnane-3,20-dione (37a) Containing 3′,4α-Cycloprop[4,5]-5*β*-pregnane-3,20-dione (37b). The procedure used for the preparation of these compounds was analogous to that described earlier for the preparation of steroid **21a**. In brief, diiodomethane (8.8 g) was added to a stirred mixture of zinc−copper couple (3 g) and a small crystal of iodine in ether (50 mL). The mixture was warmed with a tungsten lamp until the reaction started, and the reaction was continued for 30 min on a water bath at 60 °C. A solution of the mixture of compounds **35a** and **35b** (3.0 g, 9.4 mmol) was added over 10 min, and the mixture was stirred for 3 days at 50 °C. The crude product that was obtained was purified by chromatography (silica gel, 30% EtOAc in hexane) to give a mixture of unreacted steroids **35a** and **35b** and products **36a** and **36b** (1.87 g), which was oxidized immediately with Jones reagent in acetone (100 mL) at room temperature. The oil that was obtained from the oxidation was purified by chromatography (silica gel, 30% EtOAc in hexane) to yield an uncharacterized solid (1.72 g). This solid was treated with O_3, and the crude product that was obtained was purified by chromatography (silica gel, 30% EtOAc in hexane) to give an inseparable isomeric mixture of steroids **37a** and **37b** (820 mg, 27% overall from **35a** and **35b**) as a white solid: mp 108−110 °C; IR 1701, 1684, 1477, 1447, 1383, 1357, 1266, 1226, 1157, 1116, 1080 cm^{-1}; ^{1}H NMR δ 2.51−2.44 (m, 1H, C*H*$COCH_3$), 2.06 (s, 3H, 21-CH_3), 2.05 (s, 3H, 21-CH_3), 1.02 (s, 3H, CH_3), 0.99 (s, 3H, CH_3); ^{13}C NMR δ 209.82 (C=O), 209.35 (C=O), 209.22 (C=O), 17.79 (CH_3), 17.66 (CH_3), 13.29 (CH_3), 13.20 (CH_3), 63.65, 63.44, 56.13, 56.04, 51.70, 45.66, 44.03, 43.83, 38.76, 38.62, 38.13, 36.73, 35.72, 35.31, 34.95, 34.73, 34.31, 34.20, 32.52, 32.16, 32.11, 32.02, 31.48, 31.38, 30.62, 30.50, 29.92, 27.47, 24.26, 24.22, 22.64, 21.51, 20.48, 18.41. Anal. ($C_{22}H_{32}O_2$) C, H.

20(*S*)-Hydroxy-5-methyl-5*β*-pregnan-3-one (39a), 20(*R*)-Hydroxy-5-methyl-5*β*-pregnan-3-one (39b), 5-Methyl-5α-pregnane-3,20-dione (40), and 5-Methyl-5*β*-pregnane-3,20-dione (41). The procedure used was analogous to that described earlier for the preparation of steroids **22a** and **22b**. Briefly, Li wire (180 mg, 26 mmol) was added to stirred liquid NH_3 (ca. 70 mL). After 30 min, a solution of steroids **37a** and **37b** (400 mg, 1.2 mmol) in THF (10 mL) was added slowly, stirring was continued for 2 h, and 1,2-dibromoethane was added dropwise to discharge the blue color. After workup, the solid that was obtained was separated into two fractions by chromatography (silica gel, 30% EtOAc in hexane). The first fraction contained a mixture (150 mg, 38%) of products **40** and **41**. The second fraction contained a mixture (210 mg, 53%) of steroids **38a**, **38b**, **39a**, and **39b**. The mixture of steroids **40** and **41** was separated by HPLC (silica gel, 40% EtOAc in hexane, 3 mL/min). A portion (110 mg) of the second fraction was purified further by HPLC (40% EtOAc, 10% $ClCH_2CH_2Cl$ in hexane, 5 mL/min) to give unseparated steroids **38a** and **38b** along with separated steroids **39a** and **39b**. Steroids **38a** and **38b** were oxidized (Jones reagent), without characterization, to steroid **40**.

Compound **39a** was obtained as colorless crystals: mp 152−154 °C (from EtOAc/hexane); IR 3448, 2939, 1713, 1458, 1376, 1301, 1267, 1218, 1174, 1122, 1098, 1011 cm^{-1}; ^{1}H NMR δ 3.74 (m, 1H, C*H*OH), 3.01 (d, 1H, J = 14.7 Hz, 4-H_{ax}), 1.15 (d, 3H, J = 6.0 Hz, 21-CH_3), 0.93 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.77 (s, 3H, CH_3); ^{13}C NMR δ 213.48 (C=O), 70.52 (CHOH), 17.10 (CH_3), 12.46 (CH_3), 58.44, 56.27, 49.06, 42.26, 41.91, 41.82, 40.07, 37.81, 37.59, 35.34, 35.01, 32.09, 27.58, 25.66, 24.67, 24.43, 23.69, 21.38. Anal. ($C_{22}H_{36}O_2$) C, H.

Compound **39b** was obtained as colorless crystals: mp 153−154 °C (from EtOAc/hexane); IR 3466, 2938, 1708, 1446, 1383, 1263, 1219, 1157, 1118, 1010 cm^{-1}; ^{1}H NMR δ 3.65 (m, 1H, C*H*OH), 2.94 (d, 1H, J = 14.4 Hz, 4-H_{ax}), 2.30 (tt, 1H, J = 14.5 Hz, J = 6.2 Hz, 17-CH), 1.17 (d, 3H, J = 6.3 Hz, 21-CH_3), 0.84 (s, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.61 (s, 3H, CH_3); ^{13}C NMR δ 213.36 (C=O), 70.25 (CHOH), 17.07 (CH_3), 12.53 (CH_3), 58.37, 56.61, 49.01, 41.84, 41.79, 41.55, 39.01, 37.77, 37.53, 35.31, 34.77, 32.08, 27.49, 25.73, 24.64, 24.05, 23.46, 21.20. Anal. ($C_{22}H_{36}O_2$) C, H.

Compound **40** was obtained as colorless crystals: mp 158−160 °C; IR 2938, 1709, 1447, 1382, 1350, 1193, 1160 cm^{-1}; ^{1}H NMR δ 2.55 (t, 1H, J = 8.9 Hz, C*H*$COCH_3$), 2.13 (s, 3H, 21-CH_3), 1.16 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.63 (s, 3H, 18-CH_3); ^{13}C NMR δ 212.37 (C=O), 209.58 (C=O), 14.21 (CH_3), 13.53 (CH_3), 63.66, 56.59, 52.14, 46.12, 44.27, 39.85, 39.02, 37.77, 37.42, 35.30, 34.45, 32.66, 31.51, 26.45, 24.28, 22.76, 21.66, 20.87. Anal. ($C_{22}H_{34}O_2$) C, H.

Compound **41** was obtained as colorless crystals: mp 174−175 °C (from EtOAc/hexane); IR 2943, 1707, 1450, 1386, 1358, 1160 cm^{-1}; ^{1}H NMR δ 2.95 (d, 1H, J = 14.9 Hz, 4-H_{ax}), 2.51 (t, 1H, J = 8.8 Hz, C*H*$COCH_3$), 2.08 (s, 3H, 21-CH_3), 0.87 (s, 3H, CH_3), 0.83 (s, 3H CH_3), 0.58 (s, 3H, 18-CH_3); ^{13}C NMR 213.07 (C=O), 209.46 (C=O), 17.04 (CH_3), 13.30 (CH_3), 63.60, 56.92, 48.94, 43.88, 41.76, 41.72, 39.01, 37.76, 37.47, 35.18, 35.07, 32.06, 31.50, 27.41, 24.60, 24.31, 22.79, 21.46. Anal. ($C_{22}H_{34}O_2$) C, H.

3α-Hydroxy-5-methyl-5α-pregnan-20-one (42) and 3*β*-Hydroxy-5-methyl-5*β*-pregnan-20-one (43b). The procedure used was analogous to that used for the preparation of steroids **23a** and **23b**. Briefly, K-Selectride (1.4 mL, 1.5 mmol, 1.0 M solution in THF) was added to a cooled (−78 °C), stirred solution of compounds **40** and **41** (240 mg, 73 mmol) in dry THF (20 mL) under nitrogen. The reaction time was 4 h. The solid obtained was purified by chromatography (silica gel, 30% EtOAc in hexane) to give a mixture (230 mg) of steroids **40**, **42**, and **43b**. Separation of this mixture by HPLC (silica gel, 23% EtOAc in hexane, 3 mL/min) gave pure steroids **40** (60 mg, 24%), **42** (30 mg, 12%), and **43b** (130 mg, 54%).

Compound **42** was obtained as colorless crystals: mp 173−175 °C (from EtOAc/hexane); IR 3419, 2934, 1698, 1449, 1382, 1357, 1260, 1203, 1156, 1046, 1006 cm^{-1}; ^{1}H NMR δ 4.10 (m, 1H, C*H*OH), 2.55 (t, 1H, J = 8.5 Hz, C*H*$COCH_3$), 2.12 (s, 3H, 21-CH_3), 1.24 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.60 (s, 3H, 18-CH_3); ^{13}C NMR δ 209.58 (C=O), 14.08 (CH_3), 13.67 (CH_3), 66.93, 63.77, 56.97, 45.68, 44.50, 43.07, 39.24, 37.69, 35.50, 35.16, 34.37, 31.53, 29.23, 27.07, 25.31, 24.17, 22.74, 21.72, 21.33. Anal. ($C_{22}H_{36}O_2$) C, H.

Compound **43b** was obtained as colorless crystals: mp 195−196 °C (from EtOAc/hexane); IR 3378, 3322, 2937, 2874, 1704, 1450, 1383, 1357, 1182, 1152, 1104 cm^{-1}; ^{1}H NMR δ 4.15 (m, 1H, C*H*OH), 2.50 (t, 1H, J = 8.8 Hz, C*H*$COCH_3$), 2.10 (s, 3H, 21-CH_3), 1.08 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.57 (s, 3H, 18-CH_3); ^{13}C NMR δ 209.70 (C=O), 67.85 (CHOH), 17.65 (CH_3), 13.18 (CH_3), 63.76, 57.06, 43.92, 40.95, 39.12, 38.94, 37.71, 36.48, 35.45, 35.01, 31.46, 27.73, 27.52, 27.43, 25.80, 24.38, 22.74, 20.97. Anal. ($C_{22}H_{36}O_2$) C, H.

3α-Hydroxy-5-methyl-5*β*-pregnan-20-one (43a). The procedure used was analogous to that described earlier for the preparation of steroid **31**. Briefly, $NaBH_4$ (16 mg) was added at room temperature to a solution of compound **41** (120 mg, 0.36 mmol) in ethanol (20 mL). The reaction time was 1 h. The solid obtained was purified by HPLC (silica gel, 30% EtOAc in hexane, 4 mL/min) to give compound **43b** (first fraction, 80 mg, 59%) and compound **43a** (second fraction, 30 mg, 22%).

Compound **43a** was obtained as colorless crystals: mp 176−178 °C (from EtOAc/hexane); IR 3389, 2932, 1702, 1451, 1383, 1360, 1236, 1211, 1185, 1157, 1123, 1043 cm^{-1}; ^1H NMR δ 3.87−3.94 (m, 1H, C*H*OH), 2.53 (t, 1H, $J = 8.9$ Hz, C*H*COCH$_3$), 2.12 (s, 3H, 21-CH$_3$), 0.91 (s, 3H, CH$_3$), 0.80 (s, 3H, CH$_3$), 0.58 (s, 3H, 18-CH$_3$); ^{13}C NMR δ 209.72 (C=O), 68.30 (CHOH), 17.15 (CH$_3$), 13.23 (CH$_3$), 63.77, 56.97, 43.92, 42.04, 41.21, 39.12, 37.76, 37.20, 36.33, 35.40, 31.52, 30.40, 30.37, 27.13, 25.09, 24.42, 22.80, 20.94. Anal. ($C_{22}H_{36}O_2$) C, H.

Electrophysiology. Hippocampal cultures were prepared from 1−2-day-old albino rat pups and maintained as described previously.[27] Experiments were carried out at room temperature (~22 °C) using cultures that had been maintained in vitro for 3−10 days. At the time of an experiment the growth media was exchanged for a solution containing the following (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose, 10 hydroxyethylpiperazine ethanesulfonic acid (HEPES), and 0.001 tetrodotoxin (TTX) with pH adjusted to 7.3. TTX was included to block voltage-gated Na^+ currents and to diminish spontaneous synaptic currents. Voltage clamp recordings were obtained using whole-cell patch clamp methods.[22] Recording electrodes were fashioned from 1.2 mm borosilicate glass capillaries (World Precision Instruments) using a Flaming-Brown P-87 horizontal pipet puller (Sutter Instruments) and had resistances of 5−8 MΩ when fire-polished and filled with a solution containing the following (in mM): 140 CsCl, 4 NaCl, 4 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, and 5 ethyleneglycol−bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) with pH adjusted to 7.3 using CsOH. Currents were filtered at 1.5 kHz and were digitized at 0.125 kHz using pCLAMP V 5.5 (Axon Instruments). Data were analyzed using pCLAMP V 5.5, Sigmaplot for Windows V 2.0, and routines written in Axobasic. The data in this paper are presented as the mean ± SEM.

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

Molecular Modeling. Molecular modeling was performed on a SiliconGraphics Iris Indigo Elan 4000 computer using the Sybyl Molecular Modeling Software, Version 6.2 from Tripos Associates, Inc., St. Louis, MO. Minimizations were performed using the Powell minimization option (without charges) in the Sybyl Program.

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