Anesthetic Activity of Novel Water-Soluble 2β-Morpholinyl Steroids and Their Modulatory Effects at $GABA_A$ Receptors

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(3α,5α)-3-Hydroxypregnan-20-ones and (3α,5α)-3-hydroxypregnane-11,20-diones bearing a 2β-morpholinyl substituent were synthesized, and the utility of these steroids as anesthetic agents was evaluated through determination of their potency and duration of hypnotic activity in mice after intravenous administration. Alkylation of the morpholinyl substituent or chlorination at C-21 afforded the novel amino steroids (2β,3α,5α)-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)-pregnane-11,20-dione (**19**) and (2β,3α,5α)-21-chloro-3-hydroxy-2-(4-morpholinyl)pregnan-20-one (**37**) that were more potent and advantageously produced shorter sleep times than related compounds which were previously reported. Furthermore, salts of these and other amino steroids generally retained good aqueous solubility. In a radioligand binding assay the compounds inhibited the specific binding of [^{35}S]-*tert*-butyl bicyclophosphorothionate to rat whole brain membranes, and in an electrophysiological assay they potentiated $GABA_A$ receptor-mediated currents recorded from voltage-clamped bovine chromaffin cells. These *in vitro* results are consistent with the anesthetic activity of the amino steroids being related to their modulatory effects at $GABA_A$ receptors.

Introduction

The anesthetic effect of some hormonal steroids such as progesterone was first reported by Selye over 50 years ago.[1,2] Several attempts were made to translate this discovery into a hypnotic steroid suitable for both induction and maintenance of anesthesia.[3−5] These attempts culminated in the development of althesin (**1**) and minaxolone (**2**) (Figure 1), which were introduced to the clinic but later withdrawn because of adverse side effects.[6,7] In contrast to minaxolone, the clinical problems associated with althesin could be attributed to the vehicle, Cremophor EL, rather than either of its steroidal components.[6b] More recently the endogenous steroids (3α,5α)-3-hydroxypregnan-20-one (allopregnanolone) (**3**), (3α,5α)-3,21-dihydroxypregnan-20-one (THDOC) (**4**), and (3α,5β)-3-hydroxypregnan-20-one (pregnanolone) (**5**) (Figure 2) were identified as potent allosteric modulators of γ-aminobutyric acid A ($GABA_A$) receptors.[8] Electrophysiological studies have demonstrated that such steroids potentiate GABA-evoked currents at nanomolar concentrations,[9] while at micromolar concentrations, and in the absence of applied GABA, they can directly elicit membrane currents through activation of $GABA_A$ receptors.[10] The endogenous steroids and structurally similar synthetic steroids such as alfaxalone generally exhibit both of these concentration-dependent effects and modulate $GABA_A$ receptor complexes in like fashion,[11] though recently benz[*e*]indene derivatives which retain the potentiating effect of the steroids but lack their direct current gating effect have been reported.[12] GABA is the major inhibitory neurotransmitter within the CNS (central nervous system), and it is probable that steroids capable of potentiating the effects of GABA at $GABA_A$ receptors will induce anesthesia.[13] $GABA_A$ receptor complexes are pentameric ligand-gated ion channels, each comprising five subunits. The arrangement of these subunits around the channel is unknown, but an α subunit, a β subunit, and a γ-subunit are necessary for a fully functional $GABA_A$ receptor. As 13 different subunits (six α, three β, three γ, and one δ) have been identified in mammals to date, the CNS contains a myriad of different $GABA_A$ receptors.[14] The location of the allosteric sites through which steroids modulate $GABA_A$ receptors remains unclear, and the importance of the subunit composition for binding of steroids has also to be elucidated.[8] Intravenous administration of any of the above endogenous steroids to mice causes anesthesia, but the poor aqueous solubility of these compounds means they have to be formulated as emulsions or solubilized in water with the aid of a surfactant or complexing agent such as a cyclodextrin.[15] Pregnanolone (**5**), formulated in a lipid emulsion as eltanolone, was recently evaluated clinically as an intravenous anesthetic.[16]

A water-soluble steroid which retained the anesthetic properties of the endogenous compounds or alfaxalone would constitute a valuable addition to the current range of compounds available to an anesthetist. In this paper we wish to report syntheses of some amino steroids and their anesthetic efficacy upon intravenous administration to mice. Furthermore, the *in vitro* action of several of these compounds at $GABA_A$ receptors has been evaluated through their ability to inhibit the specific binding of [^{35}S]-*tert*-butyl bicyclophosphorothionate ([^{35}S]TBPS) to rat whole brain membranes. This

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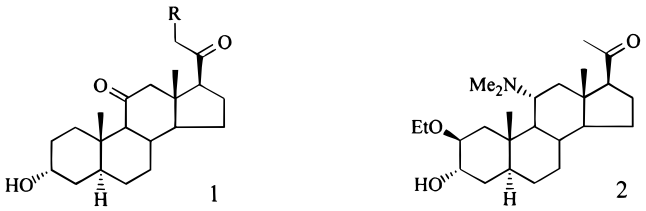
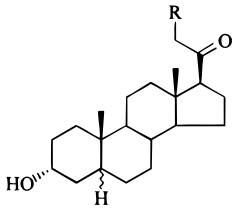


Althesin comprises alfaxalone (R = H, 3 parts) and alfadolone acetate (R = OAc, 1 part) formulated in Cremophor EL

Figure 1.

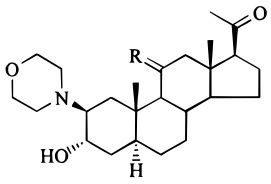


3 R = H, 5α

4 R = OH, 5α

5 R = H, 5β

Figure 2.



6 R = 2H

7 R = O

Figure 3.

radioligand binds to a site associated with the chloride channel of $GABA_A$ receptors. TBPS is inhibited by neuroactive steroids and other allosteric modulators of $GABA_A$ receptors, such as the barbiturates, and its binding is also influenced by antagonists like picrotoxin and bicuculline.[11a,17] In addition, the ability of a few of the more potent anesthetic steroids to potentiate GABA-evoked currents in bovine chromaffin cells was investigated.[10]

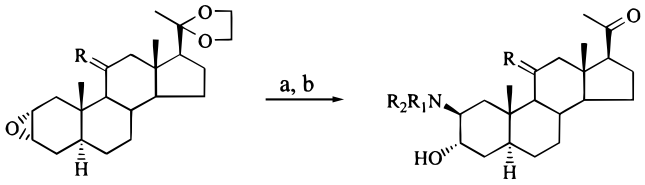
As a result of previous work in these laboratories, the amino steroids **6** and **7** (Figure 3) were known to cause a loss of righting reflex in mice upon intravenous administration of their water-soluble hydrochloride salts.[18] We sought to improve the potency and modulate the anesthetic profile of these pregnanes by modifying the 2β-amino function and introducing substituents at the 21-position.

Chemistry

Aminolysis of the known 2α,3α-epoxides,[19] followed by deprotection of the 20-ketal with aqueous methanolic methanesulfonic acid, afforded target compounds **6**−**33** (Scheme 1). Structures of all target compounds are shown in Table 1.

The 2α,3α-epoxides contained an impurity (typically about 20%) which was the corresponding 3α,4α-epoxide. This impurity was formed because, in a prior synthetic step, detosylation of a 3β-tosylate afforded an intractable Δ^2/Δ^3 mixture which was then oxidized to the mixture of 2α,3α- and 3α,4α-epoxides. To aid isolation of the desired amino steroids, the aminolysis step was optimally performed at 110−130 °C in ethylene glycol.

Scheme 1[a]



R = 2H or O

[a] Reagents: (a) R_1R_2NH, ethylene glycol, 110−130 °C; (b) aq $MeSO_3H$, MeOH.

These conditions allowed selective ring opening of the 2α,3α-epoxide while the corresponding 3α,4α-epoxide remained essentially unreacted.

Elaboration of the above amino steroids to the 21-substituted derivatives **34**−**38** and **46**−**50** was effected through introduction of either a 21-bromo substituent by treatment with bromine and acetyl chloride in methanol (Scheme 2)[20] or a 21-chloro substituent by treatment of the ethanolamine-derived ketimine with *N*-chlorosuccinimide (NCS) in diethyl ether followed by dilute aqueous hydrochloric acid (Scheme 3)[21] and then displacement of either halogen using established literature procedures.[22]

An alternative route employed to prepare 21-substituted derivatives is shown in Scheme 4. Treatment of the 21-hydroxylated steroidal epoxide mixtures **55a**,**b** (which both contained about 77% of the analogous 2α,3α-epoxides) with aqueous morpholine at reflux or with a morpholine in ethylene glycol at 110−130 °C, followed by deketalization in aqueous methanolic methanesulfonic acid at 50−55 °C, yielded target 21-hydroxy compounds **39**, **41**, and **45**.

The epoxide mixture **55a** was synthesized from the alkene mixture **54a** (which was prepared according to established methods[23]) by ketalization with triethyl orthoformate and *p*-toluenesulfonic acid in ethylene glycol at 80 °C and then epoxidation with *m*-chloroperbenzoic acid in dichloromethane.

A route to the 11-keto epoxide mixture **55b** was developed from (3β,5α)-3-hydroxypregnane-11,20-dione (**51**).[24] Established conditions for 21-bromination[20] then 21-acetoxylation[22] gave **52**. Treatment of this 3β-ol with tosyl chloride in pyridine afforded **53**. Detosylation with collidine at reflux and hydrolysis at the 21-position with potassium carbonate in methanol gave the Δ^2/Δ^3 mixture **54b**. This was epoxidized to **55b** with *m*-chloroperbenzoic acid in dichloromethane.

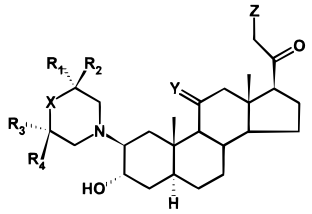
Selective mesylation of **39** and **41** at the 21-position with mesyl chloride in pyridine at −25 °C, followed by nucleophilic displacement of the mesylate, gave **40**, **42**, **43**, and **44**.

Prior to pharmacological evaluation, the target 2β-amino steroids were usually converted to water-soluble salts, often either citrates or methanesulfonates. Salts derived from mineral acids were unsatisfactory, as they resulted in epimerization of the pregnane side chain. In two cases the free base steroid was solubilized in 20% w/v aqueous (hydroxypropyl)-β-cyclodextrin[15c] or Cremophor EL for *in vivo* studies.

Results and Discussion

Pharmacology. The anesthetic potency of the steroids was determined upon their intravenous administration to mice. In each case the dose required to cause

Table 1. Hypnotic Activity (HD_{50}), Sleep Duration (SD), and $GABA_A$ Modulatory Effects of 2β-Morpholinyl Steroids

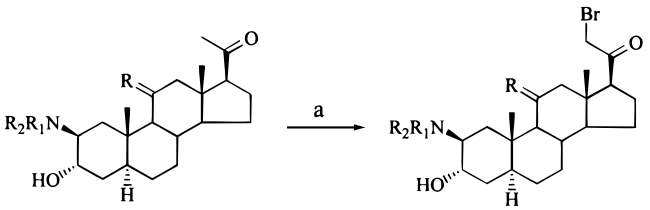


compd	R_1	R_2	R_3	R_4	X	Y	Z	salt[a]	HD_{50}[b] (μmol kg^{-1})	SD[c] (min)	TBPS[d] IC_{50} (nM) [*N*][e]	POT[f] (300%) (nM) [*N*][e]
	propofol (2,6-diisopropylphenol)[g]								68 (64−72)	3.1 ± 0.2	8300 ± 500	2000 [4]
	thiopentone[g]								83 (75−92)	3.9 ± 0.6	29500 ± 1600	30000 [4]
1	alfaxalone[g]								6.3 (5.6−7.1)	1.7 ± 0.2	538 ± 16	700 [4]
2	minaxolone[g]							cit	7.3 (6.1−8.7)	7.6 ± 0.6	71 [1]	NT[h]
3	allopregnanolone[g]								10.0 (8.2−12.2)	7.9 ± 1.1	91 ± 14	105 [6]
4	THDOC[g]								18.5[i]	13.6 ± 1.5	220 ± 70	300 [8]
5	pregnanolone[g]								9.5 (8.1−11.1)	10.6 ± 1.0	117 ± 4	115 [3]
6	H	H	H	H	O	2H	H	HCl	22.2 (21.9−24.8)	14.7 ± 0.9	341 ± 20	215 [4]
7	H	H	H	H	O	O	H	HCl	34.6[i]	NT[h]	2561 ± 61	NT[h]
8	Me	H	Me	H	O	2H	H	HCl	26.0[i]	21.3 ± 2.2	1990 [1]	NT[h]
9	Me	H	Me	H	O	O	H	cit	14.4 (13.2−15.7)	7.6 ± 0.3	3195 [1]	NT[h]
10	Me	H	H	H	O	O	H	MS	17.0 (15.5−18.6)	8.2 ± 0.8	1140 (1010,1270) [2]	NT[h]
11	Et	H	H	H	O	O	H	MS	8.7 (7.8−9.6)	5.9 ± 0.8	317 ± 133	910 [2]
12	H	Et	H	H	O	O	H	MS	15.1 (12.9−18.0)	NT[h]	1490 [1]	NT[h]
13	Pr	H	H	H	O	O	H	cit	6.4 (5.7−7.1)	13.2 ± 1.7	142 [1]	NT[h]
14	H	Pr	H	H	O	O	H	cit	10.8 (9.1−12.3)	9.3 ± 0.7	948 [1]	NT[h]
15	Bn	H	H	H	O	O	H	cit	12.6 (11.5−13.8)	18.0 ± 1.2	131 [1]	NT[h]
16	H	Bn	H	H	O	O	H	cit	12.8 (11.7−14.2)	14.3 ± 1.0	100 [1]	NT[h]
17	*i*-Pr	H	H	H	O	O	H	cit	3.4 (2.8−4.7)	1.6 ± 0.3	90 [1]	NT[h]
18	*i*-Bu	H	H	H	O	O	H	cit	6.5 (6.2−7.3)	15.6 ± 1.3	53 [1]	NT[h]
19	Me	Me	H	H	O	O	H	cit	9.8 (8.9−10.6)	3.7 ± 0.4	504 ± 73	700 [3]
20	Et	Et	H	H	O	O	H	cit	12.0 (10.7−13.1)	12.5 ± 0.1	79 (62,97) [2]	NT[h]
21	Bu	Bu	H	H	O	O	H	MS	31.1 (27.8−34.9)	NT[h]	53 ± 9	NT[h]
22	Ph	Ph	H	H	O	O	H	MS	49.9 (46.7−52.4)	NT[h]	497 [1]	NT[h]
23	Me	Me	Me	Me	O	O	H	CD	8.3 (7.6−9.6)	11.7 ± 0.9	127 [1]	NT[h]
24	Me	H	H	H	O	2H	H	MS	15.0[i]	11.7 ± 2.6	369 ± 46	250[j] [4]
25	H	Me	H	H	O	2H	H	MS	29.0[i]	NT[h]	424 [1]	NT[h]
26	Et	H	H	H	O	2H	H	cit	11.2 (10.0−13.0)	20.2 ± 1.4	910 (730,1090) [2]	NT[h]
27	H	Et	H	H	O	2H	H	cit	22.7 (20.6−24.9)	NT[h]	NT[h]	NT[h]
28	Me	Me	H	H	O	2H	H	MS	17.4 (16.6−18.3)	25.2 ± 1.1	107 ± 15	120 [2]
29	H	H	H	H	S	2H	H	MS	40.5[i]	28.9 ± 2.2	NT[h]	NT[h]
30	H	H	H	H	S	O	H	MS	20.0 (15.9−23.1)	10.7 ± 0.5	1390 ± 310	NT[h]
31	Me	Me	H	H	S	2H	H	MS	51.9 (44.1−58.8)	NT[h]	115 ± 8	NT[h]
32	Me	Me	H	H	S	O	H	cit	13.7 (11.5−15.9)	9.3 ± 0.4	209 (168,250) [2]	NT[h]
33	Me	Me	Me	Me	S	O	H	C-EL	61.8[i]	NT[h]	231 [1]	NT[h]
34	H	H	H	H	O	2H	OH	MS	31.6 (18.0−50.0)	17.3 ± 1.6	3041 ± 325	2300 [3]
35	H	H	H	H	O	2H	OAc	MS	31.0 (23.1−37.8)	17.9 ± 1.4	270 [1]	660 [3]
36	H	H	H	H	O	2H	SAc	MS	33.8[i]	NT[h]	235 [1]	NT[h]
37	H	H	H	H	O	2H	Cl	MS	8.5 (7.6−9.5)	3.6 ± 0.3	171 ± 29	150 [4]
38	H	H	H	H	O	2H	Br	MS	22.8 (21.2−27.4)	5.8 ± 1.3	148 (123,173)	NT[h]
39	H	H	H	H	O	O	OH	MS	198.2[i]	NT[h]	1160 [1]	NT[h]
40	H	H	H	H	O	O	Cl	MS	convulsant[k]		9040 ± 3314	1500 [1]
41	Me	Me	H	H	O	2H	OH	MS	18.4 (9.0−24.5)	NT[h]	267 ± 47	310 [1]
42	Me	Me	H	H	O	2H	OAc	MS	16.9 (14.2−18.6)	18.6 ± 1.1	387 (225,549) [2]	NT[h]
43	Me	Me	H	H	O	2H	SAc	MS	convulsant[k]		32 (29,35) [2]	NT[h]
44	Me	Me	H	H	O	2H	Cl	MS	17.6[i]	11.7 ± 0.7	39 ± 4	380 [3]
45	Me	Me	H	H	O	O	OH	MS	42.3[i]	NT[h]	5587 ± 886	4400 [3]
46	Me	Me	H	H	O	O	OAc	MS	21.1 (18.6−24.3)	NT[h]	951 ± 135	1300 [3]
47	Me	Me	H	H	O	O	SAc	MS	7.9 (6.6−9.7)	3.4 ± 0.3	261 ± 12	245[j] [2]
48	Me	Me	H	H	O	O	Cl	MS	13.4 (11.9−14.7)	3.7 ± 0.8	206 ± 2	450 [3]
49	Me	Me	H	H	O	O	SCN	MS	10.0 (9.4−10.5)	19.6 ± 1.9	316 ± 159	NT[h]
50	Me	Me	H	H	O	O	N_3	MS	10.7 (8.9−12.1)	NT[h]	2170 [1]	NT[h]

[a] In order to enhance aqueous solubility and assist pharmacological studies, a salt of each compound was prepared prior to evaluation: cit, citrate; HCl, hydrochloride; MS, methanesulfonate; CD, free base dissolved in 20% w/v aqueous (hydroxypropyl)-β-cyclodextrin; C-EL, free base dissolved in aqueous Cremophor EL. [b] Hypnotic dose$_{50}$ (μmol kg^{-1}): dose required to cause a loss of righting reflex for a minimum period of 30 s in 50% of treated mice after intravenous administration. [c] Sleep duration (min): determined at a dose twice that of the HD_{50}. [d] IC_{50}: concentration (nM) of steroid required to inhibit 50% of binding of [^{35}S]TBPS from rat whole brain membranes; values are means of experimental determinations ± SEM. [e] Number of experimental determinations. [f] Potentiation: concentration (nM) of steroid required to potentiate GABA-evoked currents recorded from voltage-clamped bovine chromaffin cells by 300%; values are means of experimental determinations ± SEM. [g] Compound tested as a standard. [h] Not tested. [i] Result is based on five mice (see Experimental Section). [j] Value obtained by extrapolation to 300%. [k] Compound caused convulsions and was not tested further.

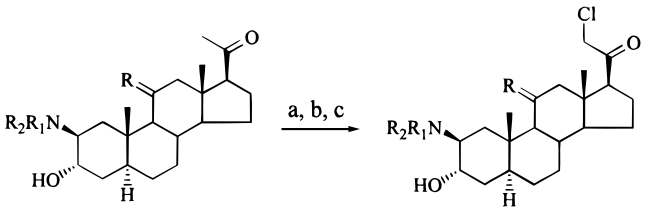
a loss of righting reflex for a minimum period of 30 s in 50% of treated mice was calculated by probit analysis. This dose is termed the HD_{50} (hypnotic dose$_{50}$). Since a probable advantage of a new steroidal anesthetic over existing agents such as barbiturates or propofol would be an improved therapeutic index (TI), this parameter was also determined for more potent compounds, as was the sleep duration (SD) at twice the HD_{50}.

Scheme 2[a]



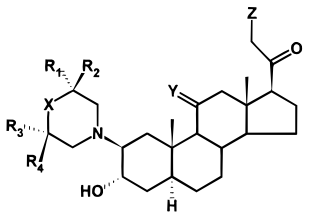
[a] Reagents: (a) AcCl, Br_2, MeOH.

Scheme 3[a]



[a] Reagents: (a) ethanolamine, toluene, Dowex 50-W resin (acid form); (b) NCS, diethyl ether; (c) dilute aq HCl.

Table 2. Therapeutic Index (TI) of Selected 2β-Morpholinyl Steroids



compd	R_1	R_2	R_3	R_4	X	Y	Z	salt[a]	TI[b]
propofol (2,6-diisopropylphenol)[c]									3.5
thiopentone[c]									3.7
1	alfaxalone[c]								24
2	minaxolone[c]						cit		4
6	H	H	H	H	O	2H	H	HCl	4.3
7	H	H	H	H	O	O	H	HCl	4.6
8	Me	H	Me	H	O	2H	H	HCl	6.9
9	Me	H	Me	H	O	O	H	cit	5.8
11	Et	H	H	H	O	O	H	MS	5.8
17	*i*-Pr	H	H	H	O	O	H	cit	9.9
19	Me	Me	H	H	O	O	H	cit	13.0
32	Me	Me	H	H	S	O	H	cit	16.3
37	H	H	H	H	O	2H	Cl	MS	6.1

[a] In order to enhance aqueous solubility and assist pharmacological studies, a salt of each compound was prepared prior to evaluation: cit, citrate; HCl, hydrochloride; MS, methanesulfonate. [b] Therapeutic index (LD_{50}/HD_{50}). [c] Compound tested as a standard.

The *in vitro* effect of several compounds at $GABA_A$ receptors was assessed through determination of their ability to inhibit [^{35}S]TBPS binding to rat whole brain membranes. In each case the concentration of drug required to inhibit 50% binding of this radioligand was determined (TBPS IC_{50}). Additionally the concentration of steroid required to potentiate GABA-evoked currents in voltage-clamped bovine chromaffin cells by 300% was determined (POT 300%). Most *in vivo* and *in vitro* results are shown in Table 1, while the TIs of selected compounds are presented in Table 2.

Structure−Activity Relationships. Previous work had shown that intravenous administration of the hydrochloride salts of the 2β-morpholinyl steroids **6** and **7** caused a loss of righting reflex in mice.[18] The potency of these water-soluble steroids was established by determination of their HD_{50} values. This showed steroids **6** and **7** were less active than previously developed steroidal anesthetics such as althesin (**1**) and minaxolone (**2**). Another disadvantage was the relatively long duration of sleep produced by steroid **6** at twice its HD_{50} (SD = 14.7 min). The duration of action of an anesthetic is critical for use in the clinic; anesthetists desire a short-acting agent (SD ≤ 10 min) with no adverse side effects and which results in rapid patient recovery.

We sought to improve the potency of the lead steroids **6** and **7** and reduce the sleep duration of compound **6** by changing the nature of the 2β-substituent and introducing groups at the synthetically accessible 21-position. It was also important that these modifications did not reduce the TI of the lead compounds (Table 2). Propofol and thiopentone, two agents used routinely in clinical practice, have low safety margins (TI = 3.5 and 3.7 respectively) in mice. In contrast, alfaxalone (**1**) has an excellent therapeutic index (TI = 24), suggesting that safer water-soluble amino steroids than compound **6** (TI = 4.3) could be developed.

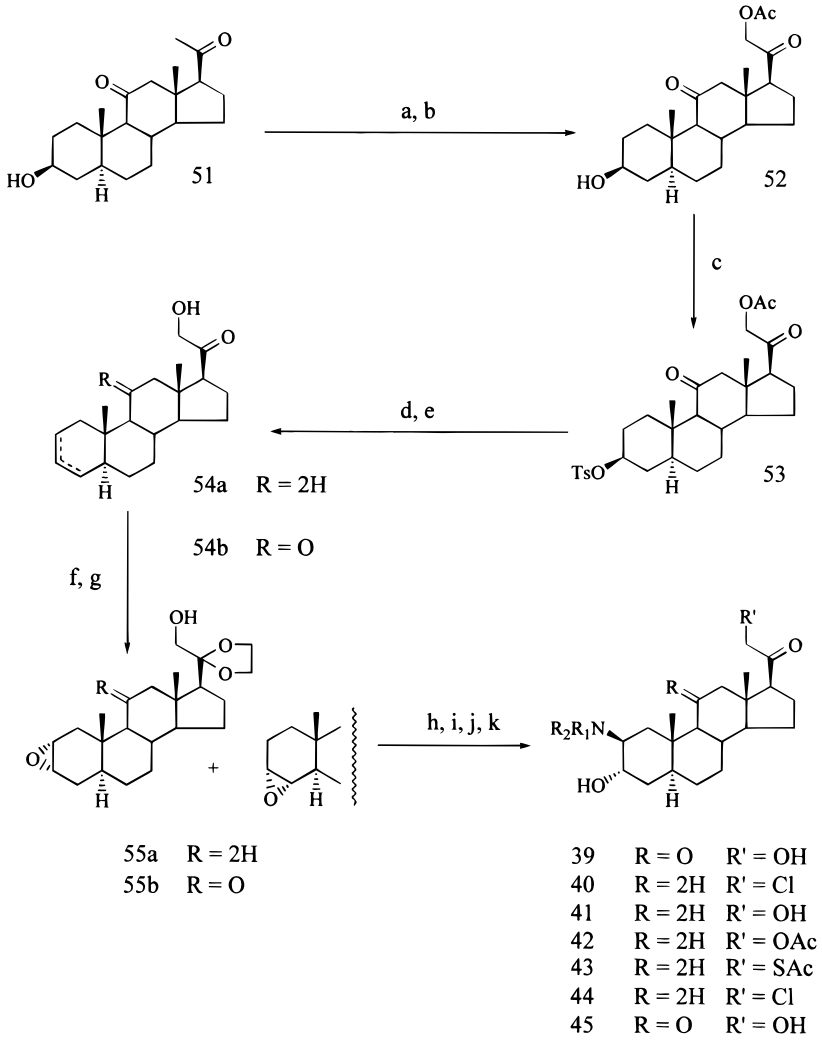
Although a wide range of commercially available amines was investigated as 2β-substituents, no advantage over the lead 2β-morpholinyl derivatives was realized until the 2β-(*cis*-2,6-dimethylmorpholinyl) steroid **9** (Figure 4) was synthesized. This was the predominant diastereomer formed after aminolysis of the 2α,3α-epoxide with 2,6-dimethylmorpholine which was used as a mixture of *cis* and *trans* isomers in approximately a 3:1 ratio.

The reduced toxicity of this alkylated 2β-morpholinyl steroid, coupled with its improved potency and shorter sleep time, led us to prepare a range of similar compounds which are shown in Table 1. In each case the alkylated morpholine was prepared according to methods already documented in the literature[25] and then coupled to the steroid. Where the alkylated morpholine was a racemate, a mixture of steroidal diastereomers resulted, which was separable via chromatography over silica. The absolute stereochemistry of each diastereomer was determined by comparison of the 2β-morpholinyl ^{13}C chemical shifts with (2β,3α,5α)-3-hydroxy-2-[(2*R*)-2-methyl-4-morpholinyl]pregnane-11,20-dione in which the alkylated morpholine had been prepared by a chiral route.[26]

Comparison of diastereomers such as compounds **11** and **12** illustrated that, for monoalkylated morpholinyl derivatives, better potency resided in isomers with *R* configuration at the 2-position of the morpholine (HD_{50} = 8.7 and 15.1 μmol kg^{-1}, respectively). Steroid **11** had a relatively short duration of action (SD = 5.9 min) and was soluble in water as its citrate salt (∼20 mg mL^{-1}, pH 4.6). This profile was sufficiently promising to warrant further testing.

In certain instances, viz. compounds **10**, **17**, and **18**, 2*R*-alkylated morpholines were synthesized from natural α-amino acids. This obviated any need for resolution of a racemic morpholine or separation of steroidal diastereomers, as had been required for the methyl, ethyl, propyl, and benzyl derivatives **11**−**16** and **24**−**27**. (*R*)-2-Isopropylmorpholine, derived from L-valine, thus afforded steroid **17**, which was more potent *in vivo* than either alfaxalone (**1**) or minaxolone (**2**) (HD_{50} = 3.4, 6.3, and 7.3 μmol kg^{-1}, respectively), had a short duration of action (SD = 1.6 min), and was soluble in water as

Scheme 4[a]



[a] Reagents: (a) AcCl, Br_2, MeOH; (b) KOAc, KI, AcOH, acetone; (c) TsCl, pyr; (d) collidine, reflux; (e) K_2CO_3, MeOH; (f) $(EtO)_3CH$, PTSA, ethylene glycol; (g) mcpba, CH_2Cl_2; (h) aq R_1R_2NH, reflux or R_1R_2NH, ethylene glycol, 110−130 °C; (i) aq $MeSO_3H$, MeOH, 50−55 °C; (j) MsCl, pyr, −25 °C; (k) various conditions (ref 21).

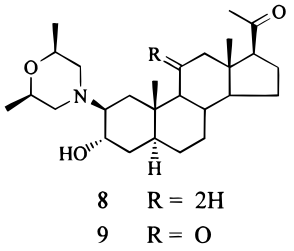


Figure 4.

its citrate salt (~20 mg mL^{-1}, pH 4.2). This compound had a markedly improved therapeutic index relative to the leads **6** and **7** (TI = 9.9, 4.3, and 4.6 respectively), and was also selected for more *in vivo* testing.

Preparation of derivatives such as compounds **19**−**23** from achiral alkylated morpholines avoided any need to separate diastereomeric steroid mixtures. Those 2β-morpholinyl steroids with small alkyl substituents such as compounds **19** and **23** retained excellent activity and short sleep duration. Compound **19** (HD_{50} = 9.8 μmol kg^{-1}; SD = 3.7 min) was soluble in water as its citrate salt (≥40 mg mL^{-1}, pH 4.2), but aqueous salt solutions of compound **23** precipitated the free base at pH values that are generally suitable for intravenous administration (pH ≥ 4). The 11-keto compound **19**, which has a shorter duration of action than its desoxy analogue **28** and an excellent therapeutic index (TI = 13.0), was selected for further evaluation. Compounds with larger substituents on the 2β-morpholine such as **21** and **22** were markedly less potent *in vivo*.

Some 2β-morpholinyl-11-desoxy steroids **24** and **26**−**28** were prepared but proved inferior to the analogous ketones **10**−**12** and **19** because of the prolonged sleeps they produced (SD ≥ 11.7 min for those determined). The 11-desoxy compounds were also less potent than the 11-ketones, an observation that has been previously reported for other steroidal anesthetics.[3]

The trend of shorter sleep duration for compounds with an 11-keto function extended to 2β-thiomorpholinyl-substituted steroids **29**−**33**. However, in contrast to 2β-morpholinyl steroids, alkylation of the 2β-thiomorpholinyl substituent with two or four methyl groups did not always improve anesthetic activity, and the tetramethyl derivative **33** was surprisingly impotent (HD_{50} = 61.8 μmol kg^{-1}).

The 2β-dimethylthiomorpholinyl steroid **32** was a reasonably potent anesthetic (HD_{50} = 13.7 μmol kg^{-1}) and had an excellent therapeutic index (TI = 16.3), but the poor solubility in water of its citrate salt (~2 mg mL^{-1}, pH 3.7), which again resulted in precipitation of

the free base at acceptable pH levels, precluded further development of this compound.

Having established that alkylation of the 2β-morpholinyl substituent could improve the potency of these anesthetic pregnanes, some 21-substituted derivatives were prepared.

The 21-position of pregnan-20-ones is a synthetically accessible position, known from previous studies[3] to tolerate quite bulky groups without abolishing anesthetic activity.

Substitution of the original lead compounds **6** and **7** at the 21-position gave steroids **34**−**40**. Of these derivatives only the 21-chloro compound **37** was more potent than the leads ($HD_{50} = 8.5\ \mu mol\ kg^{-1}$). This steroid also had a short duration of action (SD = 3.6 min) even though it lacked an 11-keto function. The short sleep duration may be due to the susceptibility of the α-chloro ketone to rapid metabolism, making the compound a "soft" drug. α-Chloro ketones are very susceptible to reaction with thiols under mildly basic conditions,[27] and this fact has been exploited for the development of affinity labels for proteins. The α-chloro ketone **37** is a soft electrophile and therefore prone to attack from soft nucleophiles such as glutathione. The metabolic deactivation of compound **37** via glutathione conjugation at the C-21 position is a plausible pathway, given that intracellular concentrations of glutathione and its associated transferases are high (3−10 mM for glutathione in most tissues). Despite the potential lability of the chloro group, the compound was stable in aqueous solution as its methanesulfonate salt. The drawback with this salt was its low solubility (~6 mg mL^{-1}, pH 3.5), but the attractive *in vivo* profile made it a candidate for further evaluation. The 11-keto analogue **40** produced convulsions in mice and was not tested further. The convulsant activity of **40** was unexpected, as 26 pairs of 11-desoxy and 11-keto compounds previously examined failed to show an 11-keto compound causing convulsions unless its 11-desoxy analogue also did.[28]

The 2β-(2,2-dimethyl)morpholinyl steroids **19** and **28** were also functionalized at the 21-position to investigate whether the effects on potency were additive to those produced by alkylation of the 2β-morpholine. The alkylated 2β-morpholinyl derivatives **41**−**50** were, with the exception of the convulsant thioacetate **43** and the 21-chloro compound **44**, more potent than analogues in which the 2β-morpholine was unsubstituted. Previous studies had demonstrated that introduction of groups such as thioacetate, thiocyanate, and azide at the 21-position afforded steroids with good anesthetic activity.[22b,c] The 21-substituted derivatives **41**−**50**, however, showed no significant increase in potency compared with the unsubstituted steroids **19** and **28**. The 21-thioacetate **47** had a similar *in vivo* profile to both **19** and the 21-chloro compound **37**, but the additional cost associated with introduction of both an 11-ketone and a 21-substituent precluded further interest in this compound.

Examination of the *in vitro* results shows that all the amino steroids tested proved to be relatively potent inhibitors of TBPS binding at $GABA_A$ receptor complexes with IC_{50} values ranging from 32 to 9040 nM. All the steroids possess a free 3α-hydroxy group which is necessary for *in vitro* activity[8a,11a] and also essential for good *in vivo* potency combined with rapid onset of anesthesia.[3] The 17β-acetyl group is also common to all the steroids; this function is important for *in vitro* and *in vivo* activity, though a variety of other substituents such as a 17β-nitrile may serve as alternatives.[11a] Structural features that have been identified as important or deleterious for steroid inhibition of TBPS binding have been reported previously.[11a] The results of this study demonstrate that (for the 13 pairs tested) 11-keto derivatives are usually less potent *in vitro* than their 11-desoxy analogues, the exceptions being compounds **11** and **39**, which were more potent than compounds **26** and **34**, respectively. It is also apparent that the putative neurosteroid binding sites on $GABA_A$ receptor complexes tolerate quite bulky substituents at the 2β-position and a range of functional groups at the 21-position.

Several compounds were tested in an electrophysiological assay and the concentration of amino steroid required to potentiate GABA-evoked currents recorded from voltage-clamped bovine chromaffin cells by 300% varied from 120 to 4400 nM. In most cases the steroids were more potent in the *in vitro* assays than the established intravenous anesthetic agents propofol and thiopentone. There is generally good agreement between the *in vitro* assays, with compounds that proved to be potent inhibitors of TBPS binding also showing marked GABA potentiating activity. There is no such correlation between the *in vivo* and *in vitro* results as evidenced, for example, by the poor anesthetic activity of the dibutyl-substituted morpholinyl derivative **21** ($HD_{50} = 31.1\ \mu mol\ kg^{-1}$), despite being one of the most potent amino steroid inhibitors of TBPS binding (IC_{50} = 53 nM). Such a discrepancy could be accounted for by pharmacokinetic factors such as the ability of a compound to penetrate the blood−brain barrier or bind to plasma proteins. Other factors such as differing selectivity of these amino steroids for $GABA_A$ receptor complexes comprising various subunit combinations and the relative importance of each of these complexes both within the CNS and for producing anesthesia, would also influence the *in vivo* profile. Potentiation of the chloride current of $GABA_A$ receptors has been shown previously to correlate with *in vivo* anesthetic potency, but the structural diversity of the compounds examined was far greater than in this study.[13a]

The relationship between anesthetic activity and the conformation of the A-ring of steroids was investigated by Phillipps, who tried to account for the fact that both 5α- and 5β-steroids can retain good *in vivo* potency even though the relative positions of a 3α-hydroxy group to the remaining steroid skeleton are quite different for such stereoisomers.[3] Although, for 5α-steroids, 2β-substitution often increased *in vivo* activity and would favor a change from the usual chair conformation to a twist−boat (due to steric interaction with the angular methyl group), no evidence for this change was found when the substituent was a methoxy group.[3] By contrast, NMR studies have revealed that 2β-amino steroids often prefer to adopt a twist−boat conformation when dissolved in apolar solvents.[29] This conformation is also favored through formation of an intramolecular hydrogen bond between the amine nitrogen and the 3α-hydroxy group. Polar solvents disrupt this bonding and favor the chair conformation which appears, for compounds **19** and **37**, to be preferred in the solid state. While such evidence lends support for steroids binding to $GABA_A$ receptor complexes in a conformation in which the A-ring is not a chair, it still fails to account

for the excellent anesthetic activity retained by **1**, **3**, and **4**, which lack a 2β-substituent and hence a driving force away from the preferred chair conformation.

The present results also demonstrate that the unusual steric situation of the 11α-(dimethylamino) substituent of minaxolone **2** is not necessary for combining water solubility with potent anesthetic activity.[5]

The most promising new compounds, **19**[30] and **37**,[31] were selected for development as potential water-soluble steroidal intravenous anesthetics. Both compounds have since been evaluated in a number of animal models and Org 21465, **19**, has progressed to the clinic, where it induced anesthesia of short duration at a dose of 2.2 μmol kg^{-1} (1.0 mg/kg), though higher doses also caused excitation.[30a] The extra cost associated with synthesizing the chiral morpholinyl substituents of compounds **11** and **17** made them less attractive development candidates than compound **19**, which has an achiral 2β-morpholinyl group. Since compounds **11** and **17** failed to show any significant advantage over compound **19** in subsequent animal models, they were eliminated from development.

The subunit specificity of **19**, **37**, and some other amino steroids at $GABA_A$ receptor complexes is currently being examined.

Conclusion

The anesthetic potency in mice of the previously reported amino steroids **6** and **7** was improved through modification of the 2β-morpholinyl function and introduction of 21-substituents. Alkylation of the 2β-morpholinyl group often resulted in more potent compounds which had better therapeutic indices than established anesthetic agents. For monoalkylated derivatives, better activity resided in diastereomers with *R* configuration at the 2-position of the 2β-morpholinyl function. Derivatives possessing an 11-keto group were generally more potent *in vivo* and produced sleeps of short duration. Among compounds lacking an 11-keto function, only the 21-chloro derivative **37** retained good potency combined with a short duration of action. The most promising new compounds, **19**[30] and **37**,[31] were selected for development as potential water-soluble steroidal intravenous anesthetics.

The *in vitro* results strongly support the hypothesis that the anesthetic effect of these steroids is due to their ability to allosterically modulate $GABA_A$ receptor complexes, causing potentiation of this inhibitory neurotransmitter system.

Experimental Section

Pharmacology. *In Vivo* Experiments. Drugs and Solutions. Stock solutions of alfaxalone, allopregnanolone, THDOC, and pregnanolone were dissolved in 20% (hydroxypropyl)-β-cyclodextrin (HPCD) (Janssen Biotech, Belgium) and diluted in saline (0.9%). Propofol was obtained formulated as the emulsion Diprivan (Zeneca) and was diluted in saline immediately prior to use. Thiopentone sodium, obtained as Intraval Sodium (May and Baker), was dissolved in saline immediately prior to use. Stock solutions of salts of amino steroids were prepared in saline and diluted as required.

Hypnotic Potency (HD_{50}). Male CFLP mice (Interfauna) (25−35 g) were used. Mice had access to food and water *ad libitum* and were kept on a 12 h light/dark cycle. All tests were performed during the light period. Groups of eight mice (unless specified otherwise) were injected intravenously (lateral tail vein) over 10 s with each dose of each compound (10 mL kg^{-1}) and were placed in separate boxes to reduce external stimuli and tested for loss of righting reflex (LRR). Dosing was performed using a micromole/kilogram scheme. A set of dose levels, e.g. 2, 4, 8, and 16 μmol kg^{-1}, was chosen, and depending on whether LRR was observed, extra doses were introduced to provide data for LRR over a narrow dose range to allow potency calculations.

Sleep Duration (SD). Immediately after the end of the injection, the mice were tested for LRR. If immediate loss did not occur, the mice were closely observed and placed on their backs repeatedly, to determine the time of LRR. Once loss was noted, the duration of sleep [the interval between LRR and the return to righting reflex (RRR)] was recorded. To compare the duration of sleep of each compound, at equipotent doses, groups of 10 mice were injected with each compound at 2 × HD_{50} over 10 s.

Therapeutic Index (TI). To determine an estimate of compound lethality (LD_{50}), groups of mice were injected in the same way, but over a higher dose range, and the numbers of mice dying at each dose was determined. The therapeutic index is the ratio LD_{50}/HD_{50}.

Statistical Analysis. From the percentage of mice in each group showing LRR for a period of 30 s or greater, a probit analysis (SAS Institute) was performed to yield an HD_{50} for each compound and 95% confidence limits. In a similar way, an LD_{50} was determined from the percentage of mice that died in each group.

***In Vitro* Experiments. [^{35}S]TBPS Assay. Tissue Preparation.** Male Sprague-Dawley rats (250−400 g) were killed by cervical dislocation and decapitated, and their whole brains were removed and rapidly homogenized in 10 volumes of ice-cold 0.32 M sucrose. Debris and nuclei together with any unbroken cells were sedimented by centrifugation of the homogenate at 1000*g* for 10 min at 4 °C. The resultant supernatant was then centrifuged at 20000*g* for 20 min at 4 °C.

The pellet produced contains synaptosomal and mitochondrial fractions; in order to remove the predominantly mitochondrial fraction, the pellet was resuspended and homogenized in 10 volumes of ice-cold deionized water, and this osmotically shocked preparation was centrifuged at 8000*g* for 20 min at 4 °C. The pellet thus produced consisted of a relatively firm brown pellet, below a lighter colored, more loosely packed "buffy coat". The buffy coat was resuspended without disturbing the predominantly mitochondrial pellet. The resulting supernatant was then centrifuged at 48000*g* for 20 min at 4 °C. The pellet was again suspended in 10 volumes of ice-cold deionized water and recentrifuged at 48000*g* for 20 min at 4 °C. Pellets were then stored at −20 °C until use.

To remove endogenous GABA, frozen pellets were allowed to thaw for 40 min, resuspended in 10 volumes of ice-cold buffer (20 mM KH_2PO_4 + 200 mM KCl pH 7.4), and centrifuged at 48000*g* for 20 min at 4 °C. The resulting pellets were then frozen at −20 °C. This procedure was repeated a total of four times.

Protein Assay. Protein was assayed using the method of Lowry *et al.*[32] using bovine serum albumin as standard. The tissue was then aliquoted and stored at −20 °C until use.

Binding Assay. The assay was performed (in triplicate) by incubating membrane protein (0.5 mg) with 2 nM [^{35}S]TBPS (61.5 Ci/mmol; New England Nuclear Dupont) in the presence or absence of various concentrations of test compounds. The incubation mixture was brought to a final volume of 500 μL with buffer (20 mM KH_2PO_4 + 200 mM KCl pH 7.4). Nonspecific binding was defined as residual binding in the presence of 200 μM picrotoxin. Experiments were performed in the presence of 0.6 μM GABA to increase the sensitivity of the assay. Incubations were performed at room temperature (21 °C) for 150 min in a shaking water bath or microtiter plate shaker and were terminated by rapid vacuum filtration through a Whatman GF/B glass-fiber filter. Filter-bound radioactivity was quantified by liquid scintillation spectrophotometry.

Potentiation of GABA-Evoked Currents to 300% of Control. Tissue Culture and Cell Preparation. Adult bovine chromaffin cells were isolated and maintained in cell culture as previously described.[33] Electrophysiological experiments were conducted from 4 h and up to 2 days after plating.

Electrical Recordings. Agonist-activated currents were recorded using the "whole-cell" recording configuration of the patch−clamp technique with a List Electronics L/M EPC-7 converter headstage and amplifier.[34] Currents were low-pass filtered (cutoff frequency 500 Hz, Bessel characteristic) and recorded on magnetic tape with an FM tape recorder (Racal Store 4DS) for subsequent analysis. In all experiments cells were continuously superfused (3−5 mL min^{-1}) with a solution comprising 140 mM NaCl, 2.8 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM HEPES−NaOH (pH 7.2). All recordings were conducted at room temperature (18−22 °C). The pipet solution which dialyzed the cell interior contained 140 mM CsCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1.1 mM EGTA, and 10 mM HEPES−NaOH (pH 7.2).

Drug Application. GABA (100 μM) was applied locally to cells by pressure ejection (1.4×10^5 Pa) for 10−30 ms using a Picrospritzer II (General Valve Corp.) from a patch pipet located close (100−200 μM) to the cell soma. Test compounds were introduced to the bath via the superfusion system.

Data Analysis. Agonist activated currents were analyzed either manually from pen recorder traces or by semiautomated programs run on a Dell personal computer (Dimension XPS P60) fitted with a Data Translator 2801 A/D converter.[35] In radioligand binding studies, concentration−effect relationships were fitted iteratively with a four-parameter logistic equation by use of Fig. P version 6.0c software (Biosoft, Cambridge, UK), which allowed the concentration of the test compound that produced half-maximal inhibition of [^{35}S]TBPS binding (IC_{50}) to be calculated. In electrophysiological experiments the concentration of the test compound that enhanced the GABA-induced response by 300% of the control was estimated by interpolation.

Reagents. All biological and synthetic media employed in cell culture were obtained from Gibco (Paisley, Scotland). Sera were heat-inactivated at 56 °C for 30 min before use. GABA and thiopentone sodium (thiopental) were obtained from Sigma. Propofol (2,6-diisopropylphenol) was obtained from Aldrich. Water-soluble steroid salts were freshly dissolved into either recording solution or double-distilled deionized water. All other steroids were prepared as concentrates in ethanol. Ethanol, at the final concentration (0.01% vol/vol) used in experiments, had no effect upon the relevant agonist-induced currents.

Chemistry. General. Melting points were taken with either a Gallenkamp capillary melting point apparatus or a Reichert hot plate apparatus and are uncorrected. Optical rotations were determined at room temperature for solutions in chloroform and *c* refers to concentrations in grams per 100 mL. ^{1}H NMR (200 or 400 MHz) spectra were obtained using a Bruker AM200 or a Bruker DRX400 instrument; chemical shifts (δ) are relative to tetramethylsilane as internal standard. Only discrete or characteristic signals are reported. Coupling constants are given in hertz. IR spectra were obtained with a Perkin-Elmer 16PC FT-IR spectrometer. Elemental analyses were determined on a (Perkin-Elmer 2400 CHN) elemental analyzer and are within 0.4% of theory unless otherwise noted. Steroids were generally characterized as free bases but pharmacological data (see Tables) were usually obtained on salts.

Materials. Reagents were used as supplied from commercial sources. Steroids **1**, **3**, **4**, and **5** are commercially available.

Syntheses. The syntheses of compounds **2**,[36] **6**,[19] and **7**[19] have been previously reported. Unless otherwise noted, diastereomeric mixtures resulting from aminolysis of a steroidal epoxide with a morpholinyl racemate were separated via chromatography over silica and each isomer purified by crystallization.

The following syntheses of **19** represents a general procedure for the synthesis of related analogues bearing a 2β-morpholinyl group. Yields were calculated using the total mass of the starting steroidal epoxide mixture rather than the mass of steroidal 2α,3α-epoxide present in the mixture.

(2*β*,3α,5α)-3-Hydroxy-2-(2,2-dimethyl-4-morpholinyl)-pregnane-11,20-dione (19). 2,2-Dimethylmorpholine hydrochloride (120 g, 801 mmol)[25a] was added to a stirred mixture (4:1) of (2α,3α,5α)-2,3-epoxypregnane-11,20-dione cyclic 20-(1,2-ethanediyl acetal) and its 3α,4α-epoxy isomer (20.0 g, 53.4 mmol)[11] in ethylene glycol (400 mL). Anhydrous sodium carbonate (42.5 g, 401 mmol) was then added cautiously and the mixture was heated at 60−70 °C until effervescence had subsided. The mixture was further heated at 120−130 °C for 4.5 h and then poured into water (4 L). The precipitated solid was filtered off, washed with water, and dissolved in methanol (270 mL). Methanesulfonic acid (10.4 mL, 160 mmol) was added and the solution was stirred at room temperature for 1 h and then poured into water (1 L). After extraction with dichloromethane, aqueous sodium carbonate (5%) was added to the aqueous phase until the pH exceeded 9.0. The resulting mixture was extracted with dichloromethane and the organic phase was washed with water. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure and the oily residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether/methanol). The purified product was crystallized from diethyl ether to give **19** as a white solid (10.73 g, 45%): mp 153−156 °C; $[\alpha]_D$ +127.5° (*c* 1.2); ^{1}H NMR ($CDCl_3$) δ 0.57 (s, 3H, 18-protons), 1.06 (s, 3H, 19-protons), 1.22 (s, 3H, 2β-morpholinyl CH_3), 1.26 (s, 3H, 2β-morpholinyl CH_3), 2.10 (s, 3H, 21-protons), 3.28 (br s, 1H, 3α-OH), 3.65−3.80 (m, 2H, 2β-morpholinyl OCH_2), 3.80−3.95 (m, 3β-H); IR (KBr) 3541, 1702 cm^{-1}. Anal. ($C_{27}H_{43}NO_4$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-(4-morpholinyl)pregnan-20-one (6):[19] crystallized from acetone (52%); mp 193−197 °C; $[\alpha]_D$ +157.8° (*c* 1.2); ^{1}H NMR ($CDCl_3$) δ 0.61 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.35−2.70 (m, 6H), 3.35 (br s, 1H, OH), 3.55−3.75 (m, 4H), 3.75−3.90 (m, 1H); IR (KBr) 3470, 1700 cm^{-1}. Anal. ($C_{25}H_{41}NO_3$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-(4-morpholinyl)pregnane-11,20-dione (7):[19] crystallized from diethyl ether (45%); mp 191−198 °C; $[\alpha]_D$ +141.2° (*c* 1.1); ^{1}H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 3.22 (br s, 1H, OH), 3.60−3.78 (m, 4H), 3.82−3.94 (m, 1H); IR (KBr) 3471, 1701 cm^{-1}. Anal. ($C_{25}H_{39}NO_3$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-(*cis*-2,6-dimethyl-4-morpholinyl)-pregnan-20-one (8): crystallized from acetone (12%); mp 190−195.5 °C; $[\alpha]_D$ +151.2° (*c* 0.6); ^{1}H NMR ($CDCl_3$) δ 0.61 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 1.15 (d, 3H, *J* ∼ 7, CH_3), 1.18 (d, 3H, *J* ∼ 7, CH_3), 2.12 (s, 3H, CH_3), 3.40−3.72 (m, 3H), 3.82−3.91 (m, 1H); IR (KBr) 3485, 1694 cm^{-1}. Anal. ($C_{27}H_{45}NO_3$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-(*cis*-2,6-dimethyl-4-morpholinyl)-pregnane-11,20-dione (9): crystallized from methanol (8%); mp 172.5−174.5 °C; $[\alpha]_D$ +142.5° (*c* 0.5); ^{1}H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 1.13 (d, 3H, *J* ∼ 7, CH_3), 1.17 (d, 3H, *J* ∼ 7, CH_3), 2.11 (s, 3H, CH_3), 3.50−3.80 (m, 3H), 3.80−3.95 (m, 1H); IR (KBr) 3520, 1708, 1689 cm^{-1}. Anal. ($C_{27}H_{43}NO_4$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-[(2*R*)-2-methyl-4-morpholinyl]-pregnane-11,20-dione (10):[25b] crystallized from diethyl ether (41%); mp 180−182 °C; $[\alpha]_D$ +136.5° (*c* 0.7); ^{1}H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.17 (d, 3H, *J* ∼ 7, CH_3), 2.11 (s, 3H, CH_3), 2.40−2.80 (m, 6H), 3.30−3.75 (m, 3H), 3.80−3.95 (m, 1H); IR (KBr) 3489, 1705, 1697 cm^{-1}. Anal. ($C_{26}H_{41}NO_4$) C, H, N.

(2*β*,3α,5α)-2-[(2*R*)-2-Ethyl-4-morpholinyl]-3-hydroxypregnane-11,20-dione (11):[25c] crystallized from diethyl ether/dichloromethane (11%); mp 188−191 °C; $[\alpha]_D$ +130.4° (*c* 0.5); ^{1}H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 0.95 (t, 3H, *J* ∼ 7, CH_3), 1.06 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.40−2.80 (m, 6H), 3.20−3.45 (m, 2H), 3.51−3.70 (m, 1H), 3.78−3.95 (m, 2H); IR (KBr) 3445, 1706 cm^{-1}. Anal. ($C_{27}H_{43}NO_4$) C, H, N.

(2*β*,3α,5α)-2-[(2*S*)-2-Ethyl-4-morpholinyl]-3-hydroxypregnane-11,20-dione (12):[25c] isolated as a foam (11%); $[\alpha]_D$ +140° (*c* 0.6); ^{1}H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 0.91 (t, 3H, *J* ∼ 7, CH_3), 1.05 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 3.25−3.65 (m, 3H), 3.75−3.95 (m, 2H); IR (CH_2Cl_2) 3605, 3430, 1703 cm^{-1}. Anal. ($C_{27}H_{43}NO_4$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-[(2*R*)-2-propyl-4-morpholinyl]-pregnane-11,20-dione (13):[25d] crystallized from methyl *tert*-butyl ether/heptane (4%); mp 121−123 °C; $[\alpha]_D$ +125.8° (*c* 0.5); ^{1}H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 0.92 (t, 3H, *J* ∼ 7, CH_3),

1.06 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.35−2.78 (m, 6H), 3.25−3.47 (m, 2H), 3.52−3.70 (m, 1H), 3.78−3.95 (m, 2H); IR (KBr) 3447, 1704 cm^{-1}. Anal. ($C_{28}H_{45}NO_4$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-[(2*S*)-2-propyl-4-morpholinyl]-pregnane-11,20-dione (14):[25d] crystallized from methanol (3%); mp 107−110 °C; $[\alpha]_D$ +131.6° (*c* 0.4); ^1H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 0.90 (t, 3H, $J \sim 7$, CH_3), 1.05 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 3.30−3.65 (m, 3H), 3.75−3.95 (m, 2H); IR (KBr) 3464, 1702 cm^{-1}. Anal. ($C_{28}H_{45}NO_4$·0.4MeOH) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-[(2*R*)-2-(phenylmethyl)-4-morpholinyl]pregnane-11,20-dione (15):[25e] crystallized from ether (11%); mp 170−171.5 °C; $[\alpha]_D$ +91.6° (*c* 0.6); ^1H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 3.32 (br s, 1H, OH), 7.10−7.35 (m, 5H); IR (KBr) 3532, 1703 cm^{-1}. Anal. ($C_{32}H_{45}NO_4$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-[(2*S*)-2-(phenylmethyl)-4-morpholinyl]pregnane-11,20-dione (16):[25e] crystallized from methanol (18%); mp 160−161.5 °C; $[\alpha]_D$ +159° (*c* 0.6); ^1H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 3.34 (br s, 1H, OH), 7.12−7.35 (m, 5H); IR (KBr) 3507, 1705, 1695 cm^{-1}. Anal. ($C_{32}H_{45}NO_4$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-[(2*R*)-2-(1-methylethyl)-4-morpholinyl]pregnane-11,20-dione (17):[25f] crystallized from diethyl ether/heptane (34%); mp 149−153 °C; $[\alpha]_D$ +121.4° (*c* 0.5); ^1H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 0.95 (br t, 6H, $J \sim 7$, 2 × CH_3), 1.07 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 3.05−3.18 (m, 1H), 3.34 (br s, 1H, OH), 3.50−3.70 (m, 1H), 3.80−3.97 (m, 2H); IR (KBr) 3451, 1702 cm^{-1}. Anal. ($C_{28}H_{45}NO_4$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-{(2*R*)-2-[(1*S*)-1-methylpropyl]-4-morpholinyl}pregnane-11,20-dione (18):[25g] crystallized from diethyl ether/heptane (51%); mp 131−135 °C; $[\alpha]_D$ +118.8° (*c* 0.6); ^1H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 0.85−0.97 (m, 6H), 1.07 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.40−2.75 (m, 7H), 3.20−3.28 (m, 1H), 3.37 (br s, 1H, OH), 3.55−3.65 (m, 1H), 3.83−3.92 (m, 2H); IR (KBr) 3490, 1703, 1700 cm^{-1}. Anal. ($C_{29}H_{47}NO_4$) C, H, N.

(2*β*,3α,5α)-2-(2,2-Diethyl-4-morpholinyl)-3-hydroxypregnane-11,20-dione (20):[25h] crystallized from diethyl ether (52%); mp 178−180 °C; $[\alpha]_D$ +123.8° (*c* 0.6); ^1H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 0.75−0.90 (m, 6H), 1.06 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 3.28 (br s, 1H, OH), 3.60−3.80 (m, 2H), 3.80−3.93 (m, 1H); IR (KBr) 3547, 1695 cm^{-1}. Anal. ($C_{29}H_{47}NO_4$) C, H, N.

(2*β*,3α,5α)-2-(2,2-Dibutyl-4-morpholinyl)-3-hydroxypregnane-11,20-dione (21):[25i] crystallized from diethyl ether (28%); mp 159.5−162.5 °C; $[\alpha]_D$ +109.2° (*c* 0.5); ^1H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 0.80−1.00 (m, 6H), 1.06 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 3.33 (br s, 1H, OH), 3.57−3.80 (m, 2H), 3.80−3.93 (m, 1H); IR (KBr) 3485, 1703, 1687 cm^{-1}. Anal. ($C_{33}H_{55}NO_4$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-(2,2-diphenyl-4-morpholinyl)-pregnane-11,20-dione (22):[25j] crystallized from methanol (11%); mp 248−250 °C; $[\alpha]_D$ +166.2° (*c* 0.7); ^1H NMR ($CDCl_3$) δ 0.55 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.90−3.30 (m, 2H), 3.35 (br s, 1H, OH), 3.55−3.75 (m, 1H), 3.75−3.95 (m, 2H), 7.15−7.45 (m, 10H); IR (KBr) 3490, 1694 cm^{-1}. Anal. ($C_{37}H_{47}NO_4$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-(2,2,6,6-tetramethyl-4-morpholinyl)pregnane-11,20-dione (23):[25k] crystallized from acetone/hexane (6%); mp 212−215 °C; $[\alpha]_D$ +126.8° (*c* 0.5); ^1H NMR ($CDCl_3$) δ 0.58 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 3.48 (br s, 1H, OH), 3.80−3.97 (m, 1H); IR (KBr) 3556, 1701 cm^{-1}. Anal. ($C_{29}H_{47}NO_4$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-[(2*R*)-2-methyl-4-morpholinyl]-pregnan-20-one (24):[25c] crystallized from ethanol (13%); mp 196−198 °C; $[\alpha]_D$ +151.5° (*c* 0.8); ^1H NMR ($CDCl_3$) δ 0.60 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 1.14 (d, 3H, $J \sim 7$, CH_3), 2.12 (s, 3H, CH_3), 2.42−2.75 (m, 5H), 3.40−3.75 (m, 3H), 3.80−3.95 (m, 2H); IR (KBr) 3470, 1693 cm^{-1}. Anal. ($C_{26}H_{43}NO_3$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-[(2*S*)-2-methyl-4-morpholinyl]-pregnan-20-one (25):[25c] crystallized from diethyl ether/hexane (3%); mp 163−167 °C; $[\alpha]_D$ +160° (*c* 1.0); ^1H NMR ($CDCl_3$) δ 0.60 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 1.15 (d, 3H, $J \sim 7$, CH_3), 2.13 (s, 3H, CH_3), 2.45−2.78 (m, 5H), 3.30−3.80 (m, 3H), 3.80−3.95 (m, 2H); IR (KBr) 3470, 1700 cm^{-1}. Anal. ($C_{26}H_{43}NO_3$) C, H, N.

(2*β*,3α,5α)-2-[(2*R*)-2-Ethyl-4-morpholinyl]-3-hydroxypregnan-20-one (26):[25c] crystallized from acetone (13%); mp 190−192 °C; $[\alpha]_D$ +151.8° (*c* 0.9); ^1H NMR ($CDCl_3$) δ 0.62 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.95 (t, 3H, $J \sim 7$, CH_3), 2.12 (s, 3H, CH_3), 2.42−2.75 (m, 5H), 3.23−3.75 (m, 3H), 3.80−3.97 (m, 2H); IR (CH_2Cl_2) 3600, 3470, 1699 cm^{-1}. Anal. ($C_{27}H_{45}NO_3$) C, H, N.

(2*β*,3α,5α)-2-[(2*S*)-2-Ethyl-4-morpholinyl]-3-hydroxypregnan-20-one (27):[25c] crystallized from acetone (8%); mp 120.5−122 °C; $[\alpha]_D$ +156.6° (*c* 0.6); ^1H NMR ($CDCl_3$) δ 0.60 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.94 (t, 3H, $J \sim 7$, CH_3), 2.12 (s, 3H, CH_3), 2.45−2.75 (m, 5H), 3.30−3.70 (m, 3H), 3.78−3.95 (m, 2H); IR (CH_2Cl_2) 3600, 3432, 1699 cm^{-1}. Anal. ($C_{27}H_{45}NO_3$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-(2,2-dimethyl-4-morpholinyl)-pregnan-20-one (28):[25a] crystallized from methanol (41%); mp 183−186 °C; $[\alpha]_D$ +153.0° (*c* 0.7); ^1H NMR ($CDCl_3$) δ 0.61 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 3.28 (br s, 1H, OH), 3.65−3.80 (m, 2H), 3.80−3.95 (m, 1H); IR (KBr) 3519, 1702 cm^{-1}. Anal. ($C_{27}H_{45}NO_3$) C, H, N.

(2*β*,3α,5α)-3-Hydroxy-2-(4-thiomorpholinyl)pregnan-20-one (29): crystallized from acetone/petroleum ether (15%); mp 198−199 °C; $[\alpha]_D$ +166.1° (*c* 0.5); ^1H NMR ($CDCl_3$) δ 0.61 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.84−3.07 (m, 2H), 3.63 (br s, 1H, OH), 3.75−3.90 (m, 1H); IR (KBr) 3495, 1705 cm^{-1}. Anal. ($C_{25}H_{41}NO_2S$) C, H, N; S, calcd 7.64; found 8.07.

Conversion of this free base to the methanesulfonate (1:1) salt in methanol gave, after removal of the solvent under reduced pressure and precipitation from dry diethyl ether, an analytically pure sample: $[\alpha]_D$ +116.4° (*c* 0.7). Anal. ($C_{26}H_{45}NO_5S_2$·0.7H_2O) C, H, N, S.

(2*β*,3α,5α)-3-Hydroxy-2-(4-thiomorpholinyl)pregnane-11,20-dione (30): crystallized from methanol (34%); mp 216.5−218 °C; $[\alpha]_D$ +156.1° (*c* 0.8); ^1H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.88−3.06 (m, 2H), 3.68 (br s, 1H, OH), 3.75−3.92 (m, 1H); IR (KBr) 3470, 1693 cm^{-1}. Anal. ($C_{25}H_{39}NO_3S$) C, H, N, S.

(2*β*,3α,5α)-3-Hydroxy-2-(2,2-dimethyl-4-thiomorpholinyl)pregnan-20-one (31):[25l] crystallized from methanol (22%); mp 184−185 °C; $[\alpha]_D$ −96.2° (*c* 0.9); ^1H NMR ($CDCl_3$) δ 0.59 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 1.34 (s, 6H, CH_3), 2.12 (s, 3H, CH_3), 3.65 (br s, 1H, OH), 3.73−3.92 (m, 1H); IR (KBr) 3454, 1700 cm^{-1}. Anal. ($C_{27}H_{45}NO_2S$) C, H, N, S.

(2*β*,3α,5α)-3-Hydroxy-2-(2,2-dimethyl-4-thiomorpholinyl)pregnane-11,20-dione (32):[25l] crystallized from methanol/dichloromethane (19%); mp 201.5−203 °C; $[\alpha]_D$ +149.7° (*c* 0.6); ^1H NMR ($CDCl_3$) δ 0.57 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 3.67 (br s, 1H, OH), 3.76−3.93 (m, 1H); IR (KBr) 3486, 1698 cm^{-1}. Anal. ($C_{27}H_{43}NO_3S$) C, H, N, S.

(2*β*,3α,5α)-3-Hydroxy-2-(2,2,6,6-tetramethyl-4-thiomorpholinyl)pregnane-11,20-dione (33):[25m] crystallized from ethanol (15%); mp 151−153 °C; $[\alpha]_D$ +142.5° (*c* 1.0); ^1H NMR ($CDCl_3$) δ 0.58 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.32 (s, 6H, CH_3), 1.36 (s, 6H, 2 × CH_3), 2.11 (s, 3H, CH_3), 2.18−2.31 (m, 1H), 2.38−2.51 (m, 3H), 2.65−2.78 (m, 5H), 3.67 (br s, 1H, OH), 3.83−3.94 (m, 1H); IR (KBr) 3470, 1705 cm^{-1}. Anal. ($C_{29}H_{47}NO_3S$) C, H, N, S.

(2*β*,3α,5α)-3,21-Dihydroxy-2-(4-morpholinyl)pregnan-20-one (34). To a stirred suspension of (2*β*,3α,5α)-21-(acetyloxy)-3-hydroxy-2-(4-morpholinyl)pregnan-20-one (**35**) (10.0 g, 21.7 mmol) in methanol (100 mL), under nitrogen, was added a saturated solution of potassium carbonate in methanol (100 mL, 0.2 M). After 20 min the reaction mixture was poured into aqueous sodium chloride (2 L). The precipitated solid was filtered off and dissolved in dichloromethane, and the solution was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the solid residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether/methanol).

Crystallization from acetone/hexane gave **34** (4.73 g, 52%): mp 190−199 °C; $[\alpha]_D$ +143.4° (*c* 0.9); ^{1}H NMR ($CDCl_3$) δ 0.63 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 3.32 (br s, 1H, OH), 3.63−3.97 (m, 5H), 4.18 (AB pattern, 2H, 21-protons); IR (KBr) 3448, 1708 cm^{-1}. Anal. ($C_{25}H_{41}NO_4$) C, H, N.

(2β,3α,5α)-21-(Acetyloxy)-3-hydroxy-2-(4-morpholinyl)-pregnan-20-one (35). A stirred mixture of (2β,3α,5α)-21-bromo-3-hydroxy-2-(4-morpholinyl)pregnan-20-one (**38**) (5.50 g, 11.4 mmol), potassium acetate (9.07 g, 92.4 mmol), and potassium iodide (0.44 g) in dimethylformamide (220 mL) and acetic acid (11.6 mL) was heated under nitrogen at 60−65 °C for 4 h. The mixture was then cooled and poured into aqueous sodium chloride (2 L), and sodium carbonate was added until the pH exceeded 9.0. The precipitated solid was filtered off and dissolved in dichloromethane, and the solution was washed with water and dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the product was crystallized from acetone/hexane.

Recrystallization from acetone/hexane gave **35** (3.99 g, 76%): mp 153−157 °C; $[\alpha]_D$ +151.6° (*c* 0.8); ^{1}H NMR ($CDCl_3$) δ 0.65 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.37−2.73 (m, 6H), 3.38 (br s, 1H, OH), 3.60−3.80 (m, 4H), 3.80−3.95 (m, 1H), 4.63 (AB pattern, 2H, 21-protons); IR (KBr) 3485, 1750, 1720 cm^{-1}. Anal. ($C_{27}H_{43}NO_5$) C, H, N.

(2β,3α,5α)-21-(Acetylthio)-3-hydroxy-2-(4-morpholinyl)pregnan-20-one (36). Anhydrous potassium thioacetate (2.95 g, 25.8 mmol) was added to a suspension of (2β,3α,5α)-21-bromo-3-hydroxy-2-(4-morpholinyl)pregnan-20-one (**38**) (5.00 g, 10.4 mmol) in ethanol (125 mL) and the mixture was heated under reflux in an atmosphere of nitrogen for 2 h. The resulting solution was poured into water (1 L), the precipitated solid was filtered off and washed with water, and the solid was dissolved in diethyl ether. After drying over anhydrous sodium sulfate the solvent was removed under reduced pressure and the residue crystallized from diethyl ether/hexane.

Recrystallization from acetone/hexane gave **36** (1.64 g, 33%): mp 127−128.5 °C; $[\alpha]_D$ +163.3° (*c* 0.38); ^{1}H NMR ($CDCl_3$) δ 0.63 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.35−2.75 (m, 6H), 3.38 (br s, 1H, OH), 3.62−3.80 (m, 6H), 3.80−3.95 (m, 1H); IR (KBr) 3520, 1695 cm^{-1}. Anal. ($C_{27}H_{43}NO_4S$) C, H, N, S.

(2β,3α,5α)-21-Chloro-3-hydroxy-2-(4-morpholinyl)pregnan-20-one (37). (2β,3α,5α)-3-Hydroxy-2-(4-morpholinyl)-pregnan-20-one (**6**) (10.0 g, 24.8 mmol), ethanolamine (50 mL), toluene (250 mL), and Dowex 50-W resin (1.0 g) were added to a flask fitted with a water separator. The resultant mixture was heated under reflux for 5 h, wherepon approximately 16 mL of a solution of ethanolamine and water had collected in the water separator. The contents of the flask were filtered while still hot, employing hot toluene (50 mL) as a rinse. After cooling, the supernatant was decanted and the residual solid was washed several times with hexane. The solid was dissolved in dichloromethane and the solution was washed with water and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and crystallization of the residue from ethanol gave (2β,3α,5α)-20-[(2-hydroxyethyl)-imino]-2-(4-morpholinyl)pregnan-3-ol (9.08 g): mp 169−173 °C; $[\alpha]_D$ +116° (*c* 0.6).

To a suspension of the above solid (5.32 g) in tetrahydrofuran (106 mL) was added *N*-chlorosuccinimide (1.61 g, 12.1 mmol). The clear solution was stirred at room temperature for 2 h, then hydrochloric acid (38 mL, 1 M) was added. Stirring was continued at room temperature for 2 h, and the reaction mixture was then poured into water (1 L). Aqueous sodium carbonate (5%) was added until the pH exceeded 9.0 and the precipitated solid was filtered off, washed with water, and dissolved in dichloromethane. The solution was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure.

Crystallization from methanol/dichloromethane gave **37** (3.88 g, 56% from **6**): mp 183−186 °C; $[\alpha]_D$ +161.9° (*c* 0.4); ^{1}H NMR ($CDCl_3$) δ 0.65 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 3.35 (br s, 1H, OH), 3.62−3.80 (m, 4H), 3.80−3.95 (m, 1H), 4.10 (s, 2H, 21-protons); IR (KBr) 3461, 1728 cm^{-1}. Anal. ($C_{25}H_{40}ClNO_3$) C, H, Cl, N.

(2β,3α,5α)-21-Bromo-3-hydroxy-2-(4-morpholinyl)pregnan-20-one (38). To a stirred suspension of (2β,3α,5α)-3-hydroxy-2-(4-morpholinyl)pregnan-20-one (**6**) (100 g, 248 mmol) in methanol (2.5 L) was added methanolic hydrogen chloride (81 mL; 3.06 M) and acetyl chloride (10.0 mL). Bromine (16.6 mL, 322 mmol) in methanol (1 L) was then added dropwise over 1.5 h. The mixture was stirred at room temperature for a further 20 min and then poured into water (18 L). Aqueous sodium carbonate (5%) was added until the pH exceeded 9.0. The precipitate was filtered off, washed with water, and dissolved in dichloromethane. The solution was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to give a solid (110.7 g).

Crystallization from acetone/hexane gave **38** (46.7 g, 39%): mp >180 °C (dec); $[\alpha]_D$ + 129.5° (*c* 0.8); ^{1}H NMR ($CDCl_3$) δ 0.64 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 2.35−2.73 (m, 6H), 2.75−2.90 (m, 1H), 3.39 (br s, 1H, OH), 3.62−3.95 (m, 5H), 3.92 (s, 2H, 21-protons); IR (KBr) 3465, 1720, 1700 cm^{-1}. Anal. ($C_{25}H_{40}BrNO_3$) C, H, Br, N.

(2β,3α,5α)-3,21-Dihydroxy-2-(4-morpholinyl)pregnane-11,20-dione (39). Morpholine (20 mL) and water (2 mL) were added to a stirred mixture (3.5:1) of (2α,3α,5α)-2,3-epoxy-21-hydroxypregnane-11,20-dione cyclic 20-(1,2-ethanediyl acetal) and its 3α,4α-epoxy isomer **55b** (2.0 g, 5.1 mmol), and the resulting mixture was heated under reflux for 24 h. The reaction mixture was poured into water (200 mL) and the precipitated product was filtered off and washed with water. The solid was dissolved in dichloromethane and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue (0.94 g) was dissolved in methanol (5.6 mL) and a solution of methanesulfonic acid (0.4 mL) in methanol (3.8 mL) and water (0.9 mL) was added. The resulting mixture was heated at 50 °C for 2 h then poured into water (940 mL). Aqueous sodium carbonate (5%) was added until the pH exceeded 9.0. The mixture was extracted with dichloromethane and the organic phase was dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave **39**, which was isolated as a foam (846 mg, 38%): $[\alpha]_D$ +112.8° (*c* 0.9); ^{1}H NMR ($CDCl_3$) δ 0.60 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 2.85 (br s, 1H, OH), 3.60−3.80 (m, 4H), 3.80−3.95 (m, 1H), 4.16 (s, 2H, 21-protons); IR (CH_2Cl_2) 3610, 1480, 1705 cm^{-1}. Anal. ($C_{25}H_{39}NO_5 \cdot 0.2CH_2Cl_2$) C, H, N.

(2β,3α,5α)-21-Chloro-3-hydroxy-2-(4-morpholinyl)pregnane-11,20-dione (40). A solution of methanesulfonyl chloride (2.0 mL, 26 mmol) in dry pyridine (9.0 mL) was added to a solution of (2β,3α,5α)-3,21-dihydroxy-2-(4-morpholinyl)pregnane-11,20-dione (**39**) (2.6 g, 6.0 mmol) in dry pyridine (26 mL) at −25 °C over 5 min. The solution was stirred at −25 °C for 1.5 h and poured into water (350 mL). Aqueous sodium carbonate (5%) was added until the pH exceeded 9.0 and the precipitated solid was filtered off and dissolved in dichloromethane. After drying over anhydrous sodium sulfate the solvent was removed under reduced pressure and the residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether/methanol) to give a gum (1.14 g).

Anhydrous lithium chloride (0.620 g, 14.6 mmol) was added to a solution of the above gum (1.08 g) in *N*-methylpyrrolidinone (22 mL) and the mixture was heated at 50 °C for 30 min. The reaction mixture was poured into water (200 mL) and the precipitated solid was filtered off and washed with water. The solid was dissolved in dichloromethane and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether).

Crystallization from diethyl ether gave **40** (356 mg, 13%): mp 160−163 °C; $[\alpha]_D$ +134.8° (*c* 0.4); ^{1}H NMR ($CDCl_3$) δ 0.60 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 2.93−3.08 (m, 1H), 3.60−3.81 (m, 4H), 3.81−3.97 (m, 1H), 4.05 (s, 2H, 21-protons); IR (CH_2Cl_2) 3605, 3430, 1728, 1705 cm^{-1}. Anal. ($C_{25}H_{38}ClNO_4$) C, H, Cl, N.

(2β,3α,5α)-3,21-Dihydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnan-20-one (41). 2,2-Dimethylmorpholine (3.0 g, 26 mmol) was added to a stirred mixture (3.5:1) of (2α,3α,5α)-2,3-epoxy-21-hydroxypregnan-20-one cyclic 1,2-ethanediyl acetal and its 3α,4α-epoxy isomer **55a** (4.00 g, 10.2 mmol) in

ethylene glycol (60 mL), and the mixture was heated under reflux in an atmosphere of nitrogen for 4 h. The mixture was poured into water (1 L) and the precipitated solid was filtered off and washed with water. The solid was dissolved in methanol (100 mL) and a solution of methanesulfonic acid (2.0 g) in methanol (50 mL) was added. This solution was stirred at 55 °C for 1 h and then poured into water (1 L). Aqueous sodium carbonate (5%) was added until the pH exceeded 9.0. The precipitated solid was filtered off and dissolved in dichloromethane, and the solution was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, the residue chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether/methanol) and the product crystallized from acetone.

Recrystallization from methanol gave **41** (1.14 g, 32%): mp 199−201 °C; $[\alpha]_D$ +133.8° (*c* 0.9); ^{1}H NMR ($CDCl_3$) δ 0.62 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 3.27 (br s, 1H, OH), 3.65−3.80 (m, 2H), 3.80−3.97 (m, 1H), 4.17 (br s, 2H, 21-protons); IR (KBr) 3512, 3470, 1720, 1695 cm^{-1}. Anal. ($C_{27}H_{45}NO_4$) C, H, N.

(2*β*,3α,5α)-21-(Acetyloxy)-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnan-20-one (42). A solution of methanesulfonyl chloride (2.5 mL, 32 mmol) in dry pyridine (9.6 mL) was added to a solution of (2*β*,3α,5α)-3,21-dihydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnan-20-one (**41**) (3.26 g, 7.28 mmol) in dry pyridine (33 mL) at −25 °C over 5 min. The solution was stirred at −25 °C for 1.5 h and poured into water (400 mL). Aqueous sodium carbonate (5%) was added until the pH exceeded 9.0, and the precipitated solid was filtered off and dissolved in dichloromethane. The solution was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to give a gum (3.97 g).

Anhydrous potassium acetate (0.47 g, 4.8 mmol) was added to a solution of the above gum (1.0 g) in ethanol (15 mL) and the solution was heated under reflux for 1.5 h. The reaction mixture was poured into water (150 mL) and the precipitated solid was filtered off and washed with water. The solid was dissolved in dichloromethane and the solution was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether).

Crystallization from methanol/dichloromethane gave **42** (218 mg, 24%): mp 174−175 °C; $[\alpha]_D$ +13.9° (*c* 0.5); ^{1}H NMR ($CDCl_3$) δ 0.65 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 3.35 (br s, 1H, OH), 3.65−3.80 (m, 2H), 3.80−3.97 (m, 1H), 4.62 (AB pattern, 2H, 21-protons); IR (CH_2Cl_2) 3600, 3400, 1749, 1723 cm^{-1}. Anal. ($C_{29}H_{47}NO_5 \cdot 0.5H_2O$) C, H, N.

(2*β*,3α,5α)-21-(Acetylthio)-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnan-20-one (43). Anhydrous potassium thioacetate (0.54 g, 4.7 mmol) was added to a solution of the intermediate 21-mesylate (1.0 g), prepared as described in the synthesis of **42**, in ethanol (15 mL). The solution was heated under reflux in an atmosphere of nitrogen for 0.5 h. The reaction mixture was poured into water (150 mL) and the precipitated solid was filtered off, washed with water, and dissolved in dichloromethane. After drying over anhydrous sodium sulfate the solvent was removed under reduced pressure and the residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether).

Crystallization from methanol/dichloromethane gave **43** (385 mg, 41%): mp 190−212 °C; $[\alpha]_D$ +159.5° (*c* 0.4); ^{1}H NMR ($CDCl_3$) δ 0.63 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 3.35 (br s, 1H, OH), 3.65−3.80 (m, 4H), 3.80−3.97 (m, 1H); IR (KBr) 3548, 1696 cm^{-1}. Anal. ($C_{29}H_{47}NO_4S$) Calcd: C, 68.87; H, 9.37; N, 2.77; S, 6.34. Found: C, 68.15; H, 9.18; N, 2.72; S, 5.52.

Conversion of this free base to the methanesulfonate (1:1) salt in methanol/dichloromethane gave, after removal of the solvent under reduced pressure, an analytically pure sample: $[\alpha]_D$ +125.6° (*c* 0.6). Anal. ($C_{30}H_{51}NO_7S_2 \cdot 0.75H_2O$) C, H, N, S.

(2*β*,3α,5α)-21-Chloro-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnan-20-one (44). A solution of methanesulfonyl chloride (0.67 mL) in dry pyridine (3.1 mL) was added to a solution of (2*β*,3α,5α)-3,21-dihydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnan-20-one (**41**) (875 mg, 1.95 mmol) in dry pyridine (9 mL) at −25 °C over 5 min. The solution was stirred at −25 °C for 45 min and poured into water (400 mL). Aqueous sodium carbonate (5%) was added until the pH exceeded 9.0, and the precipitated solid was filtered off, washed with water, and dissolved in dichloromethane. The solution was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to give a gum (1.2 g).

Anhydrous lithium chloride (575 mg, 13.6 mmol) was added to the above gum (1.2 g) in *N*-methylpyrrolidinone (24 mL) and the mixture heated at 40 °C for 40 min. The mixture was poured into water (350 mL) and the precipitated solid was filtered off and washed with water. The solid was dissolved in dichloromethane and the solution was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue (930 mg) was crystallized from ethanol/dichloromethane.

Recrystallization from ethanol/dichloromethane gave **44** (312 mg, 34%): mp 212−216 °C; $[\alpha]_D$ +133.6° (*c* 1.0); ^{1}H NMR ($CDCl_3$) δ 0.63 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 3.33 (br s, 1H, OH), 3.62−3.80 (m, 2H), 3.80−3.97 (m, 1H), 4.10 (s, 2H, 21-protons); IR (CH_2Cl_2) 3600, 3400, 1725 cm^{-1}. Anal. ($C_{27}H_{44}ClNO_3$) Calcd: C, 69.58; H, 9.52; Cl, 7.61; N, 3.01. Found: C, 68.97; H, 9.58; Cl, 9.12; N, 2.84.

Conversion of this free base to the methanesulfonate (1:1) salt in methanol/dichloromethane gave, after removal of the solvent under reduced pressure, an analytically pure sample: $[\alpha]_D$ +123.5° (*c* 0.9). Anal. ($C_{28}H_{48}ClNO_6S_2 \cdot 1.25H_2O$) C, H, Cl, N, S.

(2*β*,3α,5α)-3,21-Dihydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnane-11,20-dione (45). 2,2-Dimethylmorpholine (9.2 g, 80 mmol) was added to a stirred mixture (3.5:1) of (2α,3α,5α)-2,3-epoxy-21-hydroxypregnane-11,20-dione cyclic 20-(1,2-ethanediyl acetal) and its 3α,4α-epoxy isomer **55b** (3.0 g, 7.7 mmol) in ethylene glycol (60 mL), and the mixture was heated under reflux for 3 h. The reaction mixture was poured into water (600 mL) and the precipitated solid was filtered off and washed with water. The solid was dissolved in methanol (100 mL), and a solution of methanesulfonic acid (3.0 g) in methanol (50 mL) was added. This solution was stirred at 50 °C for 1 h, concentrated *in vacuo* to approximately 50 mL, and aqueous sodium carbonate (5%) was added until the pH exceeded 9.0. The mixture was extracted with dichloromethane and the organic phase dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (gradient elution, dichloromethane/acetone).

Crystallization from acetone gave **45** (227 mg, 6%): mp 194−200.5 °C; $[\alpha]_D$ +115.2° (*c* 0.5); ^{1}H NMR ($CDCl_3$) δ 0.60 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 3.20 (br s, 1H, OH), 3.67−3.80 (m, 2H), 3.83−3.95 (m, 1H), 4.16 (AB pattern, 2H, 21-protons); IR (KBr) 3552, 3414, 1700 cm^{-1}. Anal. ($C_{27}H_{43}NO_5$) C, H, N.

(2*β*,3α,5α)-21-(Acetyloxy)-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnane-11,20-dione (46). Potassium acetate (0.830 g, 8.46 mmol) was added to a solution of (2*β*,3α,5α)-21-chloro-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnane-11,20-dione (**48**) (0.500 g, 1.04 mmol), potassium iodide (0.04 g, 0.24 mmol), glacial acetic acid (1.0 mL), and *N*,*N*-dimethylformamide (20 mL), and the mixture was heated at 65 °C under an atmosphere of nitrogen for 1.5 h. The mixture was then poured into water (200 mL), and aqueous sodium carbonate (5%) was added until the pH exceeded 9.0. The precipitated solid was filtered off, washed with water, and dissolved in dichloromethane. After drying over anhydrous sodium sulfate the solvent was removed under reduced pressure.

Crystallization from methanol/dichloromethane gave **46** (328 mg, 63%): mp 158−160 °C; $[\alpha]_D$ +123.0° (*c* 0.7); ^{1}H NMR ($CDCl_3$) δ 0.62 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 3.25 (br s, 1H, OH), 3.62−3.80 (m, 2H), 3.80−3.95 (m, 1H), 4.57 (s, 2H, 21-protons); IR (KBr) 3545, 1754, 1738, 1702 cm^{-1}. Anal. ($C_{29}H_{45}NO_6$) C, H, N.

(2*β*,3α,5α)-21-(Acetylthio)-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnane-11,20-dione (47). Potassium thioacetate (1.32 g, 11.6 mmol) was added to a solution of

(2β,3α,5α)-21-chloro-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)-pregnane-11,20-dione (**48**) (2.22 g, 4.62 mmol) in ethanol (11 mL) and the mixture was heated under reflux in an atmosphere of nitrogen for 40 min. The mixture was poured into water (100 mL) and the precipitated solid was filtered off and washed with water. The solid was dissolved in dichloromethane and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether).

Crystallization from methanol/dichloromethane gave **47** (1.46 g, 61%): mp 181−182 °C; $[\alpha]_D$ +131.9° (*c* 0.9); ^{1}H NMR ($CDCl_3$) δ 0.61 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.21 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.27 (br s, 1H, OH), 3.60−3.95 (m, 3H), 3.66 (AB pattern, 2H, 21-protons); IR (KBr) 3517, 1698 cm^{-1}. Anal. ($C_{29}H_{45}NO_5S$) C, H, N, S.

(2β,3α,5α)-21-Chloro-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnane-11,20-dione (48). (2β,3α,5α)-3-Hydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnane-11,20-dione (**19**) (8.0 g, 18 mmol), ethanolamine (40 mL), toluene (200 mL), and Dowex 50-W resin (0.8 g) were added to a flask fitted with a water separator. The mixture was heated under reflux for 10 h, whereupon approximately 15 mL of a solution of ethanolamine and water had collected in the water separator. The contents of the flask were filtered while still hot and the residue was rinsed with hot toluene (40 mL). The filtrate was washed with water (5 × 50 mL) and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to give a gum (9.8 g).

N-Chlorosuccinimide (2.16 g, 16.2 mmol) was added to a solution of this gum (8.77 g) in tetrahydrofuran (175 mL). The clear solution was stirred at room temperature for 2 h and hydrochloric acid (53 mL, 1 M) was then added. After stirring at room temperature for 1.25 h, the reaction mixture was poured into water (1 L). Aqueous sodium carbonate (5%) was added until the pH exceeded 9.0, and the precipitated solid was filtered off, washed with water, and dissolved in dichloromethane. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure and the residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether) and crystallized from methanol/dichloromethane.

Recrystallization from diethyl ether gave **48** (3.23 g, 42%): mp 206−208 °C; $[\alpha]_D$ +130.5° (*c* 0.6); ^{1}H NMR ($CDCl_3$) δ 0.61 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 2.93−3.08 (m, 1H), 3.26 (br s, 1H, OH), 3.63−3.80 (m, 2H), 3.80−3.95 (m, 1H), 4.03 (s, 2H, 21-protons); IR (KBr) 3547, 1730, 1700 cm^{-1}. Anal. ($C_{27}H_{42}ClNO_4$) C, H, Cl, N.

(2β,3α,5α)-3-Hydroxy-2-(2,2-dimethyl-4-morpholinyl)-21-thiocyanatopregnane-11,20-dione (49). To (2β,3α,5α)-21-chloro-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnane-11,20-dione (**48**) (3.1 g, 6.5 mmol) was added a solution of methanesulfonic acid (615 mg) in methanol (63 mL) and then a solution of potassium thiocyanate (12.4 g, 128 mmol) in water (32 mL). The resulting solution was stirred at room temperature for 24 h and poured into water, and sodium carbonate (5%) was then added until the pH exceeded 9. The precipitated solid was filtered off, washed with water, and dissolved in dichloromethane. This solution was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether) and the purified product was crystallized from diethyl ether.

Recrystallization from methanol gave **49** (572 mg, 48%): mp 197−198 °C; $[\alpha]_D$ +99.3° (*c* 0.8); ^{1}H NMR ($CDCl_3$) δ 0.61 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 2.75−2.88 (m, 1H), 3.62−3.80 (m, 2H), 3.80−3.95 (m, 1H), 4.00 (AB pattern, 2H, 21-protons); IR (KBr) 3514, 1716, 1686 cm^{-1}. Anal. ($C_{28}H_{42}N_2O_4S$) C, H, N, S.

(2β,3α,5α)-21-Azido-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)pregnane-11,20-dione (50). A stirred mixture of (2β,3α,5α)-21-chloro-3-hydroxy-2-(2,2-dimethyl-4-morpholinyl)-pregnane-11,20-dione (**48**) (500 mg, 1.04 mmol), sodium azide (140 mg, 2.15 mmol), *N*,*N*-dimethylformamide (1.25 mL) in methanol (10 mL), and water (0.25 mL) was heated under reflux for 2 h and then cooled and poured into water (50 mL). The resulting precipitate was filtered off and dissolved in dichloromethane. After washing of the solution with water and drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure and the residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether).

Crystallization from methanol/dichloromethane gave **50** (176 mg, 35%): mp >200 °C (dec); $[\alpha]_D$ +147.2° (*c* 0.3); ^{1}H NMR ($CDCl_3$) δ 0.61 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 3.63−3.96 (m, 5H); IR (KBr) 3528, 2097, 1715, 1691 cm^{-1}. Anal. ($C_{27}H_{42}N_4O_4$) C, H, N.

(3β,5α)-21-(Acetyloxy)-3-hydroxypregnane-11,20-dione (52). Acetyl chloride (9.7 mL) was added to a stirred solution of (3β,5α)-3-hydroxypregnane-11,20-dione (**51**)[24] (97.1 g, 292 mmol) in methanol (2.4 L), and a solution of bromine (18.5 mL, 359 mmol) in methanol (1 L) was then added over 30 min at room temperature. The reaction mixture was poured into water (30 L) and the precipitated solid was filtered off and washed with water. The solid was dissolved in dichloromethane and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to give crude (3β,5α)-21-bromo-3-hydroxypregnane-11,20-dione (120 g): ^{1}H NMR ($CDCl_3$) δ 0.61 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 3.00−3.20 (m, 1H), 3.49−3.62 (m, 1H), 3.85 (s, 2H).

Potassium acetate (240 g, 2.45 mol), potassium iodide (6.0 g, 36 mmol), and glacial acetic acid (250 mL) were added to a solution of crude (3β,5α)-21-bromo-3-hydroxypregnane-11,20-dione (120 g) in acetone (4.8 L), and the mixture was heated under reflux for 3 h. The reaction mixture was poured into water (50 L) and the precipitated solid was filtered off and washed with water. The solid was dissolved in dichloromethane and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The resulting residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether).

Crystallization from diethyl ether gave **52** (26 g, 23% from **51**): mp 163−165 °C; $[\alpha]_D$ +103.3° (*c* 1.0); ^{1}H NMR ($CDCl_3$) δ 0.61 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 2.64−2.80 (m, 1H), 3.43−3.68 (m, 1H), 3.63−3.96 (m, 5H), 4.55 (s, 2H); IR (KBr) 3445 (br), 1755, 1720, 1703, 1685 cm^{-1}. Anal. ($C_{23}H_{34}O_5$) C, H.

(3β,5α)-21-(Acetyloxy)-3-{[(4-methylphenyl)sulfonyl]-oxy}pregnane-11,20-dione (53). (4-Methylphenyl)sulfonyl chloride (52.0 g, 273 mmol) was added to a solution of (3β,5α)-21-(acetyloxy)-3-hydroxypregnane-11,20-dione (**52**) (26.0 g, 66.6 mmol) in pyridine (260 mL), and the solution was stirred for 4 h at room temperature. The reaction mixture was poured into water (2.6 L) and the precipitated solid was filtered off and washed with water. The solid was dissolved in dichloromethane and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure.

Crystallization of the residue from methanol gave **53** (35 g, 48%): mp 155−156 °C; $[\alpha]_D$ +62.4° (*c* 1.1); ^{1}H NMR ($CDCl_3$) δ 0.59 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.63−2.79 (m, 1H), 4.32−4.55 (m, 1H), 4.55 (s, 2H), 7.27−7.38 (d, 2H), 7.72−7.83 (d, 2H); IR (KBr) 1745, 1725, 1703, 1597 cm^{-1}. Anal. ($C_{30}H_{40}O_7S\cdot 0.12CH_2Cl_2$) C, H, N, S.

(5α)-21-Hydroxypregn-2-ene-11,20-dione and Its Δ^3 Isomer 54b. A solution of (3β,5α)-21-(acetyloxy)-3-{[(4-methylphenyl)sulfonyl]oxy}pregnane-11,20-dione (**53**) (35.0 g, 64.3 mmol) in collidine (350 mL) was heated under reflux for 2 h. The reaction mixture was then poured into water (3.5 L) containing hydrochloric acid (614 mL, 5 M) and the precipitated solid was filtered off and washed with water. The solid was dissolved in dichloromethane and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Crystallization of the residue from methanol gave a mixture of (5α)-21-(acetyloxy)pregn-2-ene-11,20-dione and its Δ^3 isomer (18.8 g): ^{1}H NMR ($CDCl_3$) (major component) δ 0.63 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 4.57 (s, 2H), 5.48−5.68 (m, 2H).

A solution of potassium carbonate in methanol (188 mL; 0.19 M) was added to a suspension of (5α)-21-(acetyloxy)pregn-2-ene-11,20-dione and its Δ^3 isomer (18.8 g) in methanol (188 mL). The resulting solution was stirred at room temperature for 30 min and poured into water (3.7 L). The mixture was

extracted with dichloromethane and the organic phase was washed with water and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (gradient elution, dichloromethane/diethyl ether) to give a mixture of (5α)-21-hydroxypregn-2-ene-11,20-dione and its Δ^3 isomer **54b** (15.3 g, 72% from **53**): ^{1}H NMR ($CDCl_3$) (major component) δ 0.61 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 4.05−4.25 (m, 2H), 5.48−5.68 (m, 2H).

(2α,3α,5α)-2,3-Epoxy-21-hydroxypregnan-20-one Cyclic 1,2-Ethanediyl Acetal and Its 3α,4α-Epoxy Isomer 55a. To a stirred mixture of (5α)-21-hydroxypregn-2-en-20-one (**54a**)[23] and its Δ^3-isomer (890 g, 2.81 mol) in ethylene glycol (890 mL) and triethyl orthoformate (1.78 L) was added (4-methylphenyl)sulfonic acid (53.4 g, 280 mmol). The mixture was heated at 80 °C for 15 min and then poured into water (35 L) containing sodium carbonate (70 g). The precipitated solid was filtered off, washed with water, and dissolved in dichloromethane. The solution was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The residue (1.01 kg) was purified by chromatography on alumina (gradient elution, dichloromethane/methanol) to give (5α)-21-hydroxypregn-2-en-20-one cyclic 1,2-ethanediyl acetal and its Δ^3-isomer (393 g): ^{1}H NMR ($CDCl_3$) (major component) δ 0.70−0.80 (m, 6H, $2CH_3$), 3.43−3.68 (m, 2H), 3.84−4.18 (m, 4H), 5.48−5.68 (m, 2H).

To a stirred solution of the mixture (3.5:1) of (5α)-21-hydroxypregn-2-en-20-one cyclic 1,2-ethanediyl acetal, and its Δ^3-isomer (45.2 g, 125 mmol) in dichloromethane (180 mL) was added a solution of 3-chloroperbenzoic acid (50−60%) (47.6 g) in dichloromethane (428 mL), while the temperature was maintained below 25 °C. The solution was stirred at room temperature for 2 h and potassium hydrogen carbonate (30 g) was added. Water (500 mL) was added and the organic phase was washed with water and then dried over anhydrous sodium sulfate. The solvent was partially removed under reduced pressure. Crystallization from methanol gave (2α,3α,5α)-2,3-epoxy-21-hydroxypregnan-20-one cyclic 1,2-ethanediyl acetal and its 3α,4α-epoxy isomer **55a** (27.5 g, 23% from **54a**): ^{1}H NMR ($CDCl_3$) (major component) δ 0.70−0.80 (m, 6H, $2CH_3$), 3.05−3.20 (m, 2H), 3.40−3.58 (m, 2H), 3.85−4.17 (m, 4H). This mixture was used without further purification.

(2α,3α,5α)-2,3-Epoxy-21-hydroxypregnane-11,20-dione Cyclic 20-(1,2-Ethanediyl Acetal) and Its 3α,4α-Epoxy Isomer 55b. To (5α)-21-hydroxypregn-2-ene-11,20-dione and its Δ^3 isomer **54b** (15.22 g, 46.06 mmol) in ethylene glycol (15 mL) and triethyl orthoformate (30 mL) was added (4-methylphenyl)sulfonic acid (0.910 g, 478 mmol). The mixture was heated at 80 °C for 15 min and then poured into water (450 mL) containing sodium carbonate (0.83 g). The mixture was extracted with diethyl ether, the organic phase was washed with water and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was chromatographed on alumina (gradient elution, dichloromethane/diethyl ether/methanol) to give a mixture of (5α)-21-hydroxypregn-2-ene-11,20-dione cyclic 20-(1,2-ethanediyl acetal) and its Δ^3 isomer (12.7 g): ^{1}H NMR ($CDCl_3$) (major component) δ 0.72 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 3.40−3.65 (m, 2H), 3.85−4.20 (m, 4H), 5.48−5.68 (m, 2H).

To (5α)-21-hydroxypregn-2-ene-11,20-dione cyclic 20-(1,2-ethanediyl acetal) and its Δ^3 isomer (6.52 g) in dichloromethane (26 mL) was added a solution of 3-chloroperbenzoic acid (3.6 g) in dichloromethane (68 mL), while the temperature was maintained below 25 °C. The solution was stirred at room temperature for 1.5 h and potassium hydrogen carbonate (8.05 g) was added. Water (100 mL) was added and the organic phase was washed sequentially with water, sodium thiosulfate solution, and water and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to give (2α,3α,5α)-2,3-epoxy-21-hydroxypregnane-11,20-dione cyclic 20-(1,2-ethanediyl acetal) and its 3α,4α-epoxy isomer **55b** (6.8 g, 74% from **54b**): ^{1}H NMR ($CDCl_3$) (major component) δ 0.70 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 3.05−3.25 (m, 2H), 3.35−3.65 (m, 2H), 3.85−4.20 (m, 4H). This mixture was used without further purification.

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