# Allopregnanolone (3α-Hydroxy-5α-pregnan-20-one) Derivatives with a Polar Chain in Position 16α: Synthesis and Activity

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The lipophilic nature of allopregnanolone prevents its user-friendly application in human medicine. On inspiration by previously prepared allopregnanolone with a 16α-bound tetraethylammonium salt, an attempt was made to produce allopregnanolone analogues with polar groups introduced into position 16α with the goal of increasing water solubility, brain accessibility, and potency of neuroactive steroids. The Michael addition to derivatives of pregn-16-en-20-one was the key reaction step. The link between the steroid skeleton and the new side chain was either a methylene group (when diethyl malonate was added) or an oxygen atom (when a hydroxy derivative was added). [$^{35}$S]TBPS displacement was used to evaluate the products. Several carbamates (but not their parent alcohols) displaced TBPS from the picrotoxin binding site on $GABA_A$ receptors. Although none of them was more potent than the above ammonium salt, which stimulated this study, their nonionic nature should not prevent their passage into the brain.

## 1. Introduction

Of all the bioactive steroids, which have been studied for almost a century, two groups continue to keep scientists busy: neuroactive steroids and brassinosteroids. The renewed interest in neurosteroids and their synthetic analogues, neuroactive steroids, is inspired both by pure desire to establish the mechanism of their action and by their potential use in medicine. Many reviews prove that this group of steroids does not act at cell nuclei but at receptors contained within cell membranes; their targets are receptors of some neurotransmitters.[1−8]

One class of neurosteroids activates receptors of γ-aminobutyric acid (GABA[a]), thus slowing the transmission of neural signals. The binding sites for this type of compounds at the GABA receptor are distinct from the binding sites for benzodiazepines, barbiturates, and picrotoxin. Specific binding sites for neurosteroids have not yet been fully identified. Multiple binding sites for these compounds were suggested to explain their electrophysiological properties.[9−12] Recent results show that one site spans the M1 and M4 transmembrane domains of the α subunit and account for the potentiating actions of some steroids. Another site, between the M1 transmembrane domain of the α subunit and the M3 domain of the β subunit, is responsible for the gating of the channel by steroids.[13] The proportion of the subunits was found to be affected by other steroids in the body.[14,15]

Neuroactive steroids can be useful as anesthetics, sedatives, anticonvulsants, and anxiolytics.[15,16] These conditions are presently treated with other potentiators of the $GABA_A$ receptor (e.g., diazepam, carbamazepine, valproate, felbamate). In spite of the large therapeutic arsenal, about 30% of epileptic patients are still not seizure-free; sometimes unwanted side effects are observed, and thus, there is a need to develop new types of drugs.[17,18]

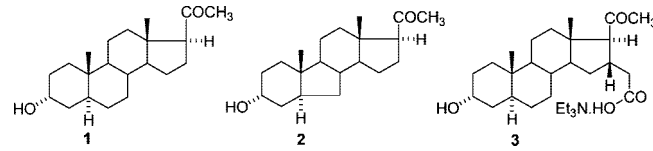


**Figure 1.** Allopregnanolone and some of its analogues.

Neuroactive steroids are presently studied by organic chemists who try to recognize the structure−activity relationship of the compounds.[5,19−23] The 3α-hydroxyl is considered essential for the neuronal[24] but also for other activities.[25] We have published several papers on the synthesis and activity of various analogues of 3α-hydroxy-5α-pregnan-20-one (**1**, "allopregnanolone"; see Figure 1).[26,27] Some of the analogues were found active (e.g., **2**) when in vitro tests[28] were used; many more modifications turned out to be inactive.

Presently, there are three hurdles against the use of neuroactive steroids in medicine: one is the blood−brain barrier,[29,30] which prevents their action in the brain. The other is their low solubility in aqueous media, which makes the use of injections necessary. The last is their fast metabolism.[31] The solubility problem prompted us to prepare some more polar analogues with additional oxo, hydroxy, and carboxy groups. A useful option was the introduction of a polar substituent situated far from the sites, which are essential for the receptor binding.[1] Such a suitable position seemed to be position 16 not only because it was used many times in other venues of medicinal steroids (e.g., refs 32−35) but also because a side chain in position 16 already led to compounds as active as allopregnanolone (e.g., **3**). However, though the 16-susbtituted steroid **3** was active in an in vitro test,[36,37] it was inactive in an in vivo test (the test of epilepsy) probably because of its inability to cross the hematoencephalic barrier.[38,39]

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[a] Abbreviations: GABA, γ-aminobutyric acid; TBAF, tetrabutylammonium fluoride; TBPS, *tert*-butyl bicyclo[2.2.2]phosphorothionate.

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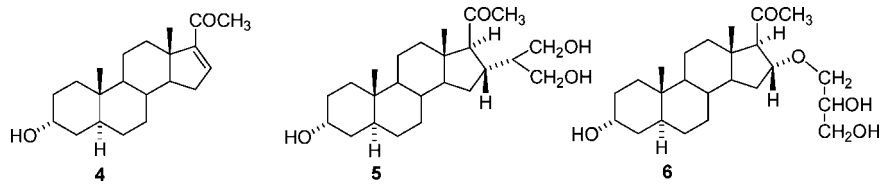
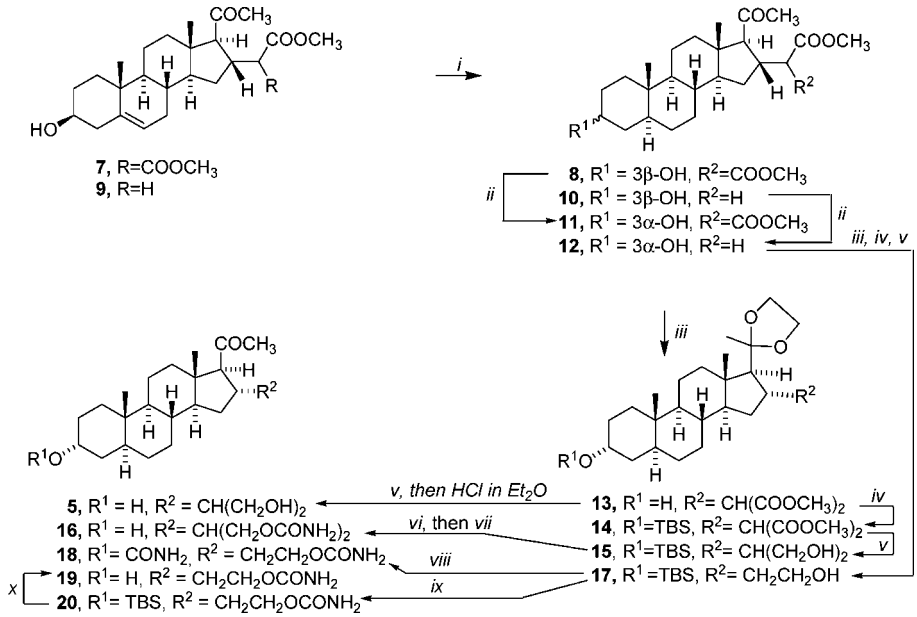



**Figure 2.** 3α-Hydroxy-5α-pregn-16-en-20-one and some Michael-type adducts.

**Scheme 1**[a]




[a] (i) $H_2$ +Pd/C in MeOH; (ii) *p*-TsCl in py, then $NaNO_2$ in HMPA, 90 °C; (iii) $(CH_2OH)_2$, $HC(OEt)_3$, *p*-TsOH; (iv) TBSCl, imidazol; (v) LAH, THF; (vi) $COCl_2$ in toluene, then $NH_3$; (vii) *p*-TsOH, acetone; (viii) excess of chlorosulfonyl isocyanate, $CHCl_3$; (ix) 1 equiv of chlorosulfonyl isocyanate, $CHCl_3$; (x) TBAF, THF.

The goal of the present paper was to synthesize and test new allopregnanolone analogues of increased polarity without abandoning the fully covalent nature of the products. Hydroxyl-containing side chains in the 16α-position were considered the compounds of choice. Their synthesis was based on the Michael addition to suitable derivatives of pregn-16-en-20-one (**4**; see Figure 2). Diethyl malonate would yield compounds with a carbon link to the steroid skeleton (e.g., **5**), while the oxy Michael reaction with suitable primary alcohols would produce compounds with an oxygen link (e.g., **6**). Carbamate derivatives have also shown antiepileptic activity;[17] thus, the introduction of this moiety to the alcohol groups of the additional side chains appeared as an interesting alternative.

## 2. Results

**Chemistry.** The Michael reaction between diethyl malonate and 20-oxopregna-5,16-dien-3$\beta$-yl acetate was recently performed by us.[36,37] Acetic acid and its derivatives were then introduced to position 16α. This time, the primary product, the steroid substituted dimethyl malonate **7** (see Scheme 1), and the product of decarboxylation **9** were hydrogenated to a 5α-pregnane derivatives **8** and **10**, respectively The 3$\beta$-hydroxyl configuration was inverted to the desired 3α. The inversion was carried out using solvolysis of corresponding 3$\beta$-tosyloxy intermediates, leading to diester and monoester **11** and **12**.

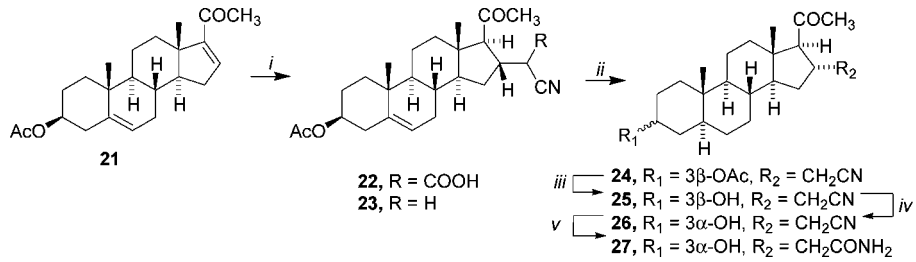
The diester **11** was protected at carbon 20 (by ketalization) to give **13**, which was reduced with LAH and deprotected to afford the target triol **5**. The same diester **13** was also silylated to a TBS derivative **14**, which was reduced and deprotected to give compound **15**. Treatment of **15** with fosgene and ammonia yielded compound **16**. The monoester **12** was also protected at carbon 20 (by ketalization) and 3 (by silylation), before the LAH reduction to yield **17**. When the 16α-hydroxyethyl derivative **17** was treated with an excess of chlorosulfonyl isocyanate (4.25 equiv), the TBS protecting group at position 3 was cleaved, giving the dicarbamate **18**. When only 1.2 equiv of the reagent was used, the silylated carbamate **20** was obtained; deprotection of the 3-hydroxyl gave the monocarbamate **19**.

Another carbanion source used for the Michael reaction was ethyl cyanoacetate, which converted 20-oxopregna-5,16-dien-3$\beta$-yl acetate **21** (see Scheme 2) into a substituted cyanomalonic acid **22**.[40] Decarboxylation led to nitrile **23**, which was hydrogenated (compound **24**), hydrolyzed to 3$\beta$-alcohol **25**, inverted at carbon 3 to 3α-alcohol **26**, and hydrolyzed to yield the allopregnanolone analogue **27**, with an acetamide group in position 16α.

Many examples show the synthetic potential of the hetero Michael addition.[41] The oxy Michael reaction on this type of steroidal substrate was first observed on the attempted alkaline hydrolysis of 20-oxopregna-5,16-dien-3$\beta$-yl acetate in MeOH:[42] 3$\beta$-hydroxy-16α-methoxypregn-5-en-20-one was inadvertently formed in a quantitative yield, although the structure of this product had to be corrected.[43] Hydrolysis without side reactions therefore has to be done differently, e.g., in propan-2-ol. Higher alcohols, however, are much less prone[44] to react in this way.

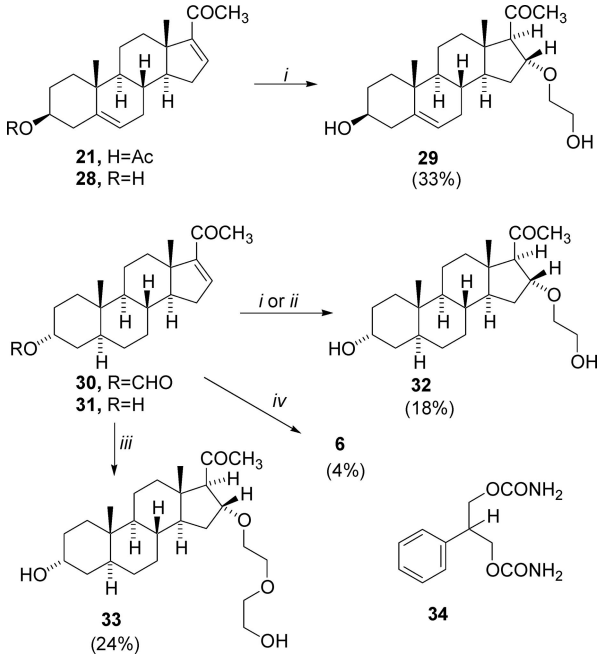
Alkaline conditions are routinely employed for the conjugate addition of alcohols to unsaturated ketones,[45,46] though acids

**Scheme 2**[a]




[a] (i) NC-$CH_2$COOEt and *t*-BuOK in *t*-BuOH; (ii) $H_2$ + Pd/C in MeOH; (iii) KOH in MeOH; (iv) *p*-TsCl in py, then $NaNO_2$ in HMPA; (v) NaOH and $H_2O_2$ in MeOH.

**Scheme 3**[a]




[a] (i) $(CH_2CH_2OH)_2$+$H_2SO_4$ in THF; (ii) $(CH_2CH_2OH)_2$ + *t*-BuOK; (iii) diethyleneglycol + *t*-BuOK; (iv) glycerol + *t*-BuOK.

can catalyze the reaction by activation of the enone reaction component.[47] Other catalysts (e.g., strong Bronsted acids) were also used to promote the addition of higher alcohols to a diene system.[48−51] High pressure was recommended to increase the yields of the addition in sterically congested systems.[52] Using dehydropregnenolone acetate (**21**)[53] as a model, we found that ethylene glycol adds in a 1,4-manner under the formation of compound **29** in the presence of sulfuric acid (see Scheme 3). In this case, acid catalysis gave a better yield (33%, or 63% when the recovered compound **28** was accounted for) than alkaline conditions. The outcome of the transformation was evident in the $^1$H NMR spectrum of the major product, with substantial changes in the signals of the enone system: the 16-proton signal lost its vinylic nature, the 21-proton was shifted downfield, and multiplets of four new protons appeared in the region of H-C-OR signals.

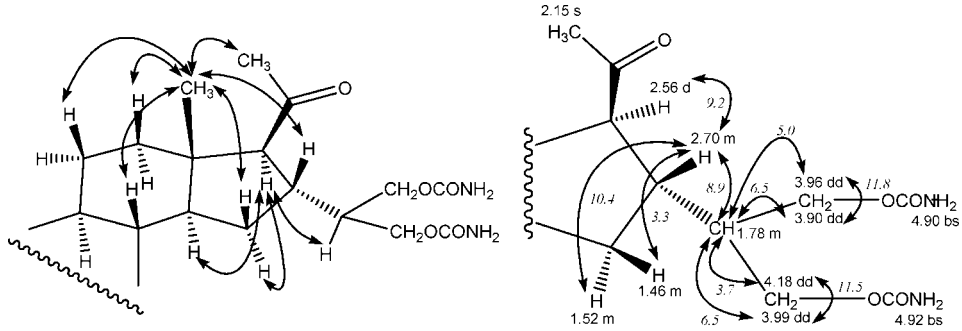
Under the same conditions, however, the overall recovery of the steroid material was much lower with a 3α-hydroxy analogue **30**. Eventually, potassium *tert*-butoxide catalyzed the addition of ethylene glycol, diethylene glycol, and glycerin to give the desired 16α-alkoxy products **6**, **32**, and **33**.

All the above products were characterized by their $^1$H NMR spectra. For selected compounds **5**, **11**, **13**, **16**−**20**, the detailed analysis was done using $^1$H and $^{13}$C NMR, 2D homonuclear (H,H COSY and H,H ROESY) and 2D heteronuclear (H,C HSQC and H,C HMBC) spectra. The proton and carbon-13 chemical shifts are given in Tables 1 and 2 in Supporting Information. The configuration at individual centers was controlled by the reaction mechanism involved in each of the transformations. However, additional proof was required to establish the configuration at carbons 16 and 17 in the products of the Michael addition, as these compounds were exposed to strongly alkaline conditions. The above suggested configurations were based on circular dichroism of diol **32** ($\Delta\varepsilon_{287nm} = +4.1$), which speaks for the natural configuration of the pregnane skeleton.[54,55] For stereochemical assignment of methylene protons and determination of the configuration of substituents at positions 16 and 17, we used 2D H,H ROESY spectra. Chemical shifts and coupling constants for compound **16** are shown in Figure 3 (see also Tables 1 and 2 in Supporting Information). The observed NOE contacts of 18-methyl protons revealed the stereochemical assignment of β-oriented H-11, H-12, and H-15 methylene protons and additional contacts of 18-Me protons to 17-CO-$CH_3$ and H-16, which indicated the 17β and 16α orientation of the substituents. This is further supported by observed NOEs of H-17 with H-14, H-15α, and methine protons of C(16)-substituent. The typical observed coupling constants pattern of protons in ring D and its substituents is in agreement with the configurations at C-16 and C-17.

**Biological Evaluation.** All the allopregnanolone analogues described above (compounds **5**, **6**, **16**, **18**, **19**, **26**, **27**, **32**, and **33**) were subjected to preliminary screening using a γ-aminobutyric acid ($GABA_A$) receptor test. $GABA_A$ receptor activity was evaluated by assaying the effect of the synthetic analogues on the binding of [$^{35}$S]-*tert*-butylbicyclo[2.2.2]phosphorothionate. The binding of this convulsant in the presence of GABA closely reflects the functional state of $GABA_A$ receptors and may be useful for characterization of allosteric interactions between various sites on the receptor. Allopregnanolone (**1**) was used as a positive control to check the viability of the method. As shown in Table 1, the analogues with a carbamoyloxyethyl moiety in position 16α (**16**, **18**, and **19**) were positive modulators of the $GABA_A$ receptor. Removal of the carbamoyl moieties in dicarbamate **16** resulted in an inactive compound (**5**). Other structurally related alcohols, e.g., adducts of ethylene glycol, diethylene glycol, or glycerin, were also inactive.

## 3. Discussion

Tailoring allopregnanolone analogues to $GABA_A$ receptors with all their complexity and variability is an elusive task.[56] In vitro testing using corresponding receptors is generally useful for preliminary screening, although its results may be corrected in later stages by in vivo experiments. The good binding properties of the 16α-substituted allopregnanolone **3** suggested a pocket in the receptor that could allow further structural




**16** (NOEs) **16** (δ and *J*-values)

**Figure 3.** NOE contacts observed in compound **16** and chemical shifts and coupling patterns of protons of ring D and its substituents.

**Table 1.** Modulatory Effect of the above Allopregnanolone Analogues on $GABA_A$ Receptors

| | compd | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **3** | **5** | **6** | **16** | **18** | **19** | **26** | **27** | **32** | **33** | **34** |
| $I_{max}$(%)[a] | 79 | 19 | *c* | *c* | 26 | 59 | 61 | *c* | *c* | *c* | *c* | 18 |
| $IC_{50}$ (nM)[b] | 80 | 40 | *c* | *c* | 86 | 300 | 750 | *c* | *c* | *c* | *c* | 95 |

[a] The maximal suppression of the binding. [b] The steroid concentration producing a half-maximal inhibition. [c] Not determined (preliminary screening at 100 nM yielded less than 15% inhibition).

modifications in this part of the molecule, without a detrimental effect on activity. The TBPS binding data of the above analogues to the receptor (see Table 1) showed that carbamates **16**, **18**, and **19** inhibited the binding to the $GABA_A$ receptor while neither the corresponding alcohol **5** nor related alcohols **6**, **32**, and **33** were active. The comparison shows that there is more to a carbamoyloxy group in a molecule than its polarity.[57]

The position 16 was confirmed now as a suitable region for structural modifications of allopregnanolone, although the presence of mere hydroxy groups in the side chain is not a sufficient precondition. The dicarbamate moiety in compound **16** is also present in commercial felbamate (**34**), one of antiepileptic drugs used since its approval in 1993.[17,58−60] Although no in vivo studies have been carried out yet, we may speculate that compound **16** could be an alternative to felbamate and related drugs whose metabolites have shown some undesired side effects.

## 4. Experimental Section

**Chemistry. General Methods.** Melting points were determined on a Boetius micromelting point apparatus (Germany) and a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured at 25 °C in $CHCl_3$ solution using an Autopol IV (Rudolf Research Analytical, Flanders). $[\alpha]_D$ values are given in $10^{-1}$ deg $cm^2$ $g^{-1}$. Infrared spectra (wavenumbers in $cm^{-1}$) were recorded on a Bruker IFS 88 spectrometer or on a Nicolet Magna 550 FT-IR spectrophotometer. $^1H$ NMR spectra were taken on Bruker AVANCE-400 (at 400 MHz) in $CDCl_3$ at 23 °C. For selected compounds **5**, **11**, **13**, **16**−**20** a detailed NMR analysis was done using 1D and 2D NMR spectra measured on a Bruker AVANCE-500 instrument ($^1H$ at 500.13 MHz, $^{13}C$ at 125.77 MHz). Proton and carbon chemical shifts are referenced to TMS and $CHCl_3$, respectively, and are given in ppm ($\delta$ scale); coupling constants ($J$) and width of multiplets ($W$) are given in Hz. Multiplicity determinations and 2D spectra were obtained using standard Bruker software. The homogeneity of all compounds was confirmed by thin-layer chromatography (TLC), which was performed on silica gel G (ICN Biochemicals, detection by spraying with concentrated sulfuric acid was followed by heating). Preparative thin-layer chromatograms (PLC) were detected by inspection in a UV light after spraying with a methanolic solution of morin (0.02%). For column chromatography, silica gel 60 (Merck, 63−100 $\mu$m) was used. When solutions were washed with aqueous solutions of hydrochloric acid or potassium hydrogencarbonate, their concentration was always 5%; brine was a saturated sodium chloride solution in water. Prior to evaporation on a rotary evaporator in a vacuum (0.25 kPa, bath temperature of 40 °C), solutions in organic solvents were dried over anhydrous $MgSO_4$ or $Na_2SO_4$. High resolution mass spectra (HRMS) were measured on a VG-ZAB mass spectrometer and on an Agilent LCTOF (2006). The preparation of compounds **8**, **9**, **21**−**27** is given in Supporting Information.

**3α-Hydroxy-16α-bis(methoxycarbonyl)methyl-5α-pregnan-20-one (11).** 4-Toluenesulfonyl chloride (6.0 g, 31.2 mmol) was added to a solution of 3$\beta$-alcohol **8** (6.0 g, 13.37 mmol) in pyridine (60.0 mL).[61,62] After 48 h, the mixture was diluted with brine (150 mL). The precipitate was extracted with $CHCl_3$, and the extract was washed with water and a solution of hydrochloric acid and potassium hydrogencarbonate and dried over anhydrous sodium sulfate. Evaporation of the solvent in a vacuum to dryness afforded tosylate, which was solvolyzed with sodium nitrite (22 g, 32.10 mmol) in HMPA (115.0 mL) at 90 °C h under nitrogen for 2 h. The mixture was poured into water, and the product was extracted with ethyl acetate. The extract was subsequently washed with water, dilute hydrochloric acid, water, a solution of potassium hydrogen carbonate, and water. After evaporation of the solvent, the product was purified using flash chromatography with a column of silica gel (400.0 mL). Elution with toluene/ethyl acetate (85:15) yielded white crystals of compound **11** (3.15 g, 52%). Mp 149−151 °C (acetone/heptane), $[\alpha]_D$ +67 (*c* 0.25). IR ($CHCl_3$): 3617, 1000 (OH); 1751, 1729, 1232, 1161, 1029 ($COOCH_3$); 1702 (C═O). For $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2 in Supporting Information. Anal. ($C_{26}H_{40}O_6$) C, H.

**3α-Hydroxy-16α-methoxycarbonylmethyl-5α-pregnan-20-one (12).** Analogously, alcohol **10** was isomerized into the 3α-alcohol **12**. Mp 112.115 °C. $[\alpha]_D$ +62 (*c* 0.30). IR ($CHCl_3$): 1729, 1700 (C═O); 1438 ($OCH_3$); 1232, 1161 (C−O). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.63 (s, 3H, H-18); 0.78 (s, 3H, H-19); 2.12 (s, 3H, H-21); 2.40 (d, $J$ = 8.6, 1H, H 17); 2.97 (m, $W$ = 46, 1H, H-16); 3.62 (s, 3H, OCH3); 4.05 (m, $W$ = 16, 1H, H-3). Anal. ($C_{24}H_{38}O_4$) C, H.

**20,20-Ethylenedioxy-16α-bis(methoxycarbonyl)methyl-5α-pregnan-3α-ol (13).** A mixture of ketone **11** (700 mg, 1.56 mmol), ethylene glycol (13.5 mL), *p*-toluenesulfonic acid monohydrate (1.13 mg, mmol), ethyl *O*-formate (2.7 mL), and benzene (4.5 mL) was allowed to stand 2 days at 50 °C. The mixture was poured into ethyl acetate, washed with a solution of potassium hydrogen carbonate, water, and dried over magnesium sulfate. Evaporation of the solvent afforded a colorless oil of compound **13**. Yield 292

mg (38%). $[\alpha]_D$ −2 (*c* 0.24). IR ($CHCl_3$): 3616, 1000 (OH); 1749, 1728, 1437, 1244, 1234, 1162 ($COOCH_3$). HRMS (FAB), $M^+$ calcd, 493.316 529; found, 493.317 259. For $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2 in Supporting Information. Anal. ($C_{28}H_{44}O_7$) C, H.

**3α-Hydroxy-16α-bis(hydroxymethyl)methyl-5α-pregnan-20-one (5).** Lithium aluminum hydride (50 mg) was added to a solution of compound **13** (70 mg, 0.14 mmol) in THF (2.0 mL). The mixture was heated to reflux under nitrogen for 2 h. The mixture was cooled, and excess reagent was decomposed with brine. Inorganic material was filtered off and washed with ether. The filtrate was washed with a solution of hydrochloric acid, water, and a solution of potassium hydrogen carbonate. After evaporation of the solvent in vacuo, the residue was purified by PLC on silica gel ($CHCl_3$/2-propanol, 9:1). White crystals of compound **5** (25 mg, 45%) were crystallized from MeOH/acetone. Mp 181−183 °C. $[\alpha]_D$ +60 (*c* 0.14). IR (KBr): 3434, 1027, 1002 (OH); 1694 (C═O). For $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2 in Supporting Information. Anal. ($C_{24}H_{40}O_4$) C, H.

**3α-*tert*-Butyldimethylsilyloxy-20,20-ethylenedioxy-16α-bis-(methoxycarbonyl)methyl-5α-pregnane (14).** *tert*-Butyldimethylsilyl chloride (723 mg, 4.80 mmol) was added to a solution of 3α-alcohol **13** (790 mg, 1.60 mmol) and imidazole (653 mg, 9.60 mmol) in *N*,*N*-dimethylformamide (13.0 mL). The reaction mixture was allowed to stand at room temperature for 72 h, then diluted with ether (200.0 mL) and washed successively with 5% aqueous citric acid (3×), water (3×), a solution of potassium hydrogen carbonate (2×) and water. The solvent was evaporated, and the residue was purified by PLC on silica gel (toluene/ether, 7/3). Compound **14** (618 mg, 63%) failed to crystallize from common solvents. $[\alpha]_D$ −5 (*c* 0.14). IR ($CHCl_3$): 1746, 1728, 1244, 1164, 1026 ($COOCH_3$); 1462, 1361 ($(CH_3)_3C-Si$); 1407, 1250 ($CH_3Si$). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.05 (s, 6H, $(CH_3)_2Si$); 0.74 (s, 3H, H-18); 0.80 (s, 3H, H-19); 0.90 (s, 9H, $(CH_3)_3CSi$); 1.33 (s, 3H, H-21); 1.91 (d, $J$ = 8.0, 1H, H-17); 2.76 (m, $W$ = 36, 1H, H-16); 3.71 (s, 3H, $OCH_3$) and 3.75 (s, 3H, $OCH_3$); 3.92 (m, 4H, $OCH_2CH_2O$); 3.96 (d, $J$ = 4, 1H, H-16a); 4.03 (m, $W$ = 16, 1H, H-3). HRMS (FAB), $M^+$ calcd, 607.403 008; found, 607.402 002.

**3α-*tert*-Butyldimethylsilyloxy-20,20-ethylenedioxy-16α-bis(hydroxymethyl)methyl-5α-pregnane (15).** Lithium aluminum hydride (200 mg) was added to the solution of compound **14** (760 mg, 1.25 mmol) in THF (25.0 mL). The mixture was allowed to stand at room temperature for 4 h. The reaction mixture was poured into a saturated aqueous solution of ammonium chloride (75.0 mL), and the product was extracted with ether. Evaporation of the solvent afforded 560 mg (81%) of diol **15** as a colorless oil. $[\alpha]_D$ −4 (*c* 0.25). IR ($CHCl_3$): 3626, 3463, 1031 (OH); 1463, 1362 ($(CH_3)_3C-Si$); 1406, 1253 ($CH_3Si$). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.05 (s, 6H, $(CH_3)_2Si$); 0.74 (s, 3H, H-18); 0.79 (s, 3H, H-19); 0.90 (s, 9H, $(CH_3)_3CSi$); 1.33 (s, 3H, H-21); 1.82 (d, $J$ = 8.0, 1H, H-17); 2.76 (m, $W$ = 36, 1H, H-16); 3.77 (m, 4H, $2xCH_2O$); 3.92 (m, 4H, $OCH_2CH_2O$); 4.05 (m, $W$ = 16, 1H, H-3). HRMS (FAB), $M^+$ calcd, 573.395 124; found, 573.393 566.

**16α-Bis(carbamoyloxymethyl)methyl-3α-hydroxy-5α-pregnan-20-one (16).** A solution of diol **15** (250 mg, 0.45 mmol), dimethylaniline (0.552 mL), and toluene (1.8 mL) was cooled to −10 °C, and a solution of $COCl_2$ in toluene (1.09 M, 5.1 mL) was added. The mixture then was kept at −5 to +5 °C for 20 h. The mixture was washed with a solution of hydrochloric acid, dried over sodium sulfate, and saturated with ammonia. Heating at reflux for 7 min, filtration, and evaporation of the solvent gave a yellow oil residue, which was treated with *p*-toluenesulfonic acid (200 mg, 1.05 mmol) in acetone (20.0 mL). After standing for 18 h at laboratory temperature, the acid was neutralized with a solution of potassium hydrogen carbonate (105 mg, 1.05 mmol) in water (2.0 mL). The reaction mixture was concentrated to a quarter of its volume in a vacuum. The product was extracted with $CHCl_3$ and washed with water, and the solvent was evaporated. The residue was chromatographed on three preparative plates ($CHCl_3$/MeOH, 9:1). The major zone was eluted to afford dicarbamate **16** (37 mg, 17%) as white crystals. Mp 181−183 °C ($CHCl_3$/MeOH). $[\alpha]_D$ +61 (*c* 0.26). IR ($CHCl_3$): 3617, 999 (OH); 3547, 3433 ($NH_2$); 1727, 1703 (C═O). For $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2 in Supporting Information. Anal. ($C_{26}H_{42}O_6N_2$) C, H, N.

**3α-*tert*-Butyldimethylsilyloxy-16α-ethyl-20,20-ethylenedioxy-5α-pregnan-16b-ol (17).** Functional groups in compound **12** (800 mg, 2.05 mmol) were protected by ketalization and silylation as above, and the product was reduced with LAH in THF. Alcohol **17** (434 mg, 41%) was obtained. Mp 112−115 °C (ethyl acetate). $[\alpha]_D$ +2 (*c* 0.30). IR ($CHCl_3$): 3622, 3471 (OH); 1472 ($(CH_3)_3C-Si$); 1254 ($(CH_3)_2Si$); 1373 ($CH_3$). For $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2 in Supporting Information.

**16α-Ethyl-20-oxo-5α-pregnane-3α,16b-diyl Dicarbamate (18).** To a solution of the silyloxy derivative **17** (43 mg, 0.08 mmol) in 1.0 mL of $CHCl_3$, chlorosulfonyl isocyanate (48.8 mg, 0.34 mmol) was added.[63] The reaction mixture was stirred at room temperature under nitrogen. After 90 min, THF (0.5 mL) was added, followed by a saturated aqueous solution of sodium hydrogen carbonate (1.0 mL). After being stirred for an additional 1 h at room temperature, the mixture was concentrated to a third of its volume. Water was added to the residue and then extracted with dichloromethane and ethyl acetate. The organic layer was washed with water and dried with sodium sulfate, and the solvent was evaporated under vacuum. The resulting solid was purified by flash chromatography on silica gel (ethyl acetate/hexane, 1:1) to give dicarbamate **18** (19 mg, 57%) as a white solid. Mp 170−172 °C (ethyl acetate). IR ($CHCl_3$): 3353, 3205, 2930, 1701, 1603, 1401, 1356, 1333, 1261, 1042, 750. For $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2 in Supporting Information. HRMS (ESI), $[M + 1]^+$ calcd for $C_{25}H_{41}N_2O_5$, 449.2937; found, 449.3015.

**16α-Carbamoyloxyethyl-3α-hydroxy-5α-pregnan-20-one (19).** Alcohol **17** (40.0 mg, 0.076 mmol) was treated with chlorosulfonyl isocyanate (13.0 mg, 0.092 mmol) as above. The resulting solid was purified by flash chromatography on silica gel (ethyl acetate/hexane, 1:1) to give carbamate **20** (24.0 mg, 60%) as an amorphous solid. For $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2 in Supporting Information.

Deprotection of the silyloxy derivative was carried out by treatment with TBAF (36 mg, 0.126 mmol) in THF (2.0 mL) at room temperature. After 18 h, the solvent was evaporated. The residue was purified by flash chromatography on silica gel ($CH_2Cl_2$) and PLC (ethyl acetate/hexane, 8:2) to give **19** (10.0 mg, 33%) as a white solid. Mp 164−166 °C (ethyl acetate). IR ($CHCl_3$): 3470, 3204, 2931, 2852, 1706, 1602, 1399, 1260, 1037, 750. For $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2 in Supporting Information. HRMS (ESI), $[M + 1]^+$ calcd for $C_{24}H_{40}NO_4$, 406.2879; found, 406.2885.

**3β-Hydroxy-16α-(2′-hydroxyethoxy)-pregn-5-en-20-one (29).** Sulfuric acid (5 drops, i.e., 122 mg, 1.24 mmol) was mixed with ethylene glycol (30 drops, 670 mg, 10.7 mmol), and a solution of 20-oxopregna-5,16-dien-3β-yl acetate (**21**, 34 mg, 0.095 mmol) in warm THF was added. The mixture was briefly shaken to ease the dissolution of the solid and then kept at 50 °C for 72 h. Sulfuric acid was partly neutralized with potassium hydrogen carbonate (300 mg, 2.17 mmol), and THF was removed on a rotary evaporator. Steroid material was precipitated with brine (4.0 mL), and the vessel was put in a refrigerator. The precipitate was filtered off using a paper filter. The product was dissolved in $CHCl_3$, and the solution was concentrated in a vacuum and applied on a preparative thin layer of silica gel. PLC plates were developed with benzene/ether/2-propanol, 3:3:0.1. The lipophilic zone was eluted to yield 3β-hydroxypregna-5,16-dien-20-one (**28**, 16 mg, 53%) as a colorless oil. Mp 209−212 °C (MeOH−water) (ref 64 gives 210−212 °C). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.92 (s, 3H, H-18); 1.05 (s, 3H, H-19); 2.26 (s, 3H, H-21); 3.53 (m, $W$ = 35, 1H, H-3); 5.36 (m, $W$ = 21, H-6); 6.71 (s, $W$ = 17, 1H, H-16).

The polar zone yielded the title compound **29** (12 mg, 33%, or 63% when the recovered compound **28** was calculated for) as white crystals. Mp 167−168 °C (acetone). $[\alpha]_D$ −15 (*c* 0.20). IR ($CHCl_3$): 3608, 3474, 1048 (OH); 1702, 1359, 595 ($COCH_3$); 1667, 809 (C═C). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.64 (s, 3H, H-18); 1.00 (s, 3H, H-19); 2.19 (s, 3H, H-21); 2.58 (d, $J$ = 6.6, 1H, H-17); 3.39 (m, $W$ = 20, 1H), 3.50 (m, $W$ = 20, 1H) and 3.68 (m, $W$ =

36, 2H, -O-$CH_2CH_2OH$); 3.75 (m, $W = 36$, 1H, H-3); 4.54 (t, $J = 6.5$, 1H, H-16); 5.36 (m, $W = 21$, H-6). Anal. ($C_{23}H_{36}O_4$) C, H.

**3α-Hydroxy-16α-(2-hydroxyethoxy)-5α-pregnan-20-one (32). (a) Using Alkaline Catalysis.** Formate **30** (93 mg, 0.27 mmol) and potassium *tert*-butoxide (177 mg, 1.58 mmol) were placed into a test tube. *tert*-Butanol (2.0 mL) and ethylene glycol (1.0 mL) were added, air was flushed out with argon, and the tube was sealed. The tube was kept at 100 °C for 48 h. After cooling, the mixture was transferred into a flask using 2-propanol (6.0 mL), neutralized with citric acid (290 mg, 1.51 mmol), and concentrated on a rotary evaporator. The mixture was diluted with brine (5.0 mL) and set aside in a refrigerator. A steroid precipitate was filtered off, washed with brine, dissolved in $CHCl_3$, and purified using PLC (toluene/ether/2-propanol, 5:8:0.2). The most polar zone yielded the title compound **32** as white crystals (18 mg, 18%). Mp 171−172 °C (acetone). $[\alpha]_D$ +42 (*c* 0.2). CD: $\Delta\varepsilon_{287nm} = +4.1$ (MeOH, $c = 1.32 \times 10^{-3}$ mol/L). IR ($CHCl_3$): 3616, 3469, 1052, 1002 (OH); 1701, 1359, 589 ($COCH_3$) $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.61 (s, 3H, H-18); 0.78 (s, 3H, H-19); 1.96 (dt, $J = 12.2$ and 3.4, 1H, H-12$\beta$); 2.17 (s, 3H, H-21); 2.57 (d, $J = 6.6$, 1H, H-17); 3.38 (m, $W = 20$, 1H), 3.48 (m, $W = 23$, 1H), and 3.68 (m, $W = 38$, 2H, -O-$CH_2$-$CH_2OH$); 4.05 (m, $W = 17$, 1H, 3-H); 4.51 (bt, $J = 7.2$, 1H, H-16). Anal. ($C_{23}H_{38}O_4$) C, H.

The least polar product was identified (TLC, IR) as 3α-hydroxy-5α-pregn-16-en-20-one (**31**, 51 mg, 60%). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.81 (s, 3H, H-18); 0.89 (s, 3H, H-19); 2.25 (s, 3H, H-21); 4.05 (m, $W = 18$, 1H, H-3); 4.51(t, $J = 6.4$, 1H, H-16).

**(b) Using Acid Catalysis.** Formate **30** (31 mg, 0.09 mmol) was treated with sulfuric acid (5 drops) in ethylene glycol (10 drops), THF (0.24 mL), and *tert*-butanol (0.4 mL) as in the preceding paragraph. The polar product **32** (1 mg, 9%) had its NMR spectrum identical with the above sample.

**3α-Hydroxy-16α-[2-hydroxy-(2-ethoxyethoxy)]-5α-pregnan-20-one (33).** Analogously, a solution of formate **30** (95 mg, 0.26 mmol) in *tert*-butanol (2.0 mL) was treated with diethylene glycol (1.0 mL) in the presence of potassium *tert*-butoxide (200 mg). After the usual workup, the target compound **33** was obtained as white crystals (28 mg, 24%, or 50% when 40 mg of the unsaturated ketone **31** was accounted for). Mp 157−158 °C (acetone); $[\alpha]_D$ +93 (*c* 0.08). IR ($CHCl_3$): 3615, 3459, 1075, 1057, 1002 (OH); 1701, 1354, 596 ($COCH_3$). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.60 (s, 3H, H-18); 0.77 (s, 3H, H-19); 2.17 (s, 3H, H-21); 2.61 (d, $J = 6.6$, 1H, H-17); 4.05 (m, $W = 18$, 1H, H-3); 4.49 (bt, $J = 6.6$, 1H, H-16). Anal. ($C_{25}H_{42}O_5$) C, H.

**3α-Hydroxy-16α-(2,3-dihydroxypropoxy)-5α-pregnan-20-one (6).** Formate **30** (93 mg, 0.27 mmol) was treated with glycerol (1.0 mL) in *tert*-butanol (2.0 mL) in the presence of potassium *tert*-butoxide (175 mg, 1.56 mmol) as above. The product was not precipitated but extracted with $CHCl_3$. The extract was washed with a solution of citric acid (295 mg, 1.53 mmol), dried by filtration over sodium sulfate, and evaporated. PLC in the above solvent system yielded compound **31** (76.9 mg, 90.0%) and the target compound **6** (4.7 mg, 4.3%) as white crystals. Mp 174−175 °C (acetone/heptane); $[\alpha]_D$ +56 (*c* 0.11). IR ($CHCl_3$): 3616, 3570, 3448, 1089, 1038, 1002 (OH); 1700, 1358 ($COCH_3$). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.60 (s, 3H, H-18); 0.77 (s, 3H, H-19); 2.17 (s, 3H, H-21); 2.55 (d, $J = 6.6$, 1H, H-17); 4.05 (m, $W = 16$, 1H, H-3); 4.49 (bt, $J = 6.6$, 1H, H-16). Anal. ($C_{24}H_{40}O_5$) C, H.

**Biological Evaluation.** An in vitro test, based on binding [$^{35}$S]-*tert*-butyl bicyclo[2.2.2]phosphorothionate to receptor of $\gamma$-aminobutyric acid ($GABA_A$), was used. Membranes were isolated from whole brains of adult male Wistar rats in accordance with our previous study.[37] The membranes were resuspended in a buffer (20 mM $KH_2PO_4$, 200 mM KCl, pH 7.4). Aliquots were incubated with 2 nM [$^{35}$S]-*tert*-butyl bicyclo[2.2.2]phosphorothionate (TBPS, Perkin-Elmer), 1 $\mu$M GABA, and 1 nM to 10 $\mu$M steroids for 60 min at 37 °C. The nonspecific binding was estimated using 200 $\mu$M picrotoxinin. The results were related to the control samples containing DMSO and expressed in %.

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**Supporting Information Available:** General experimental methods, additional NMR data, and combustion analysis of products. This material is available free of charge via the Internet at http://pubs.acs.org.

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