SYNTHESIS OF PREGNANE 3-CARBOXYLIC ACIDS via Pd-CATALYZED ALKOXYCARBONYLATION AND THEIR EFFECT ON NMDA RECEPTOR ACTIVITY

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Received February 1, 2011
Accepted May 30, 2011
Published online August 31, 2011

Dedicated to Professor Pavel Kočovský on the occasion of his 60th birthday.

We have prepared 20-oxo-5α- and 20-oxo-5β-pregnane-3-carboxylic acids by palladium catalyzed alkoxycarbonylation using triflate and nonaflate as the leaving groups in this substitution reaction. The activity of the synthesized compounds was studied in cultured rat hippocampal neurons under voltage-clamp conditions. The 5β-carboxylic acid derivatives were found to diminish NMDA-induced responses, whereas the 5α-derivative potentiated the response.

Keywords: Neurosteroids; Carboxylic acid; Alkoxycarbonylation; NMDA receptor activity; Steroids.

1. INTRODUCTION

Currently, the majority of scientific interest in the field of neurosteroid synthesis is focused on the structure-activity relationship of neurosteroids on either γ-aminobutyric acid (GABA) or *N*-methyl-D-aspartate (NMDA) receptors. These receptors attract attention because of their importance in maintaining normal brain function, as well as their role in pathological processes[1–3] (e.g. neurodegenerative diseases).

L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system, activating three families of ionotropic receptors: the NMDA receptor, the kainic acid receptor, and the α-amino-3-hydroxy-

Collect. Czech. Chem. Commun. 2011, Vol. 76, No. 9, pp. 1141–1161

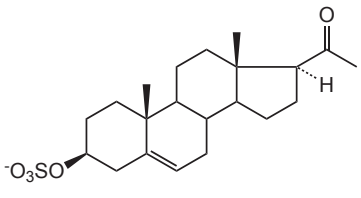
doi:10.1135/cccc2011031

5-methyl-4-isoxazole propionic acid (AMPA) receptor. Under normal conditions, glutamate is released from the presynaptic site, whereupon its extracellular concentration transiently reaches ~1 mM (ref.[4]). This periodic release of glutamate is crucial for various brain functions, including learning and memory. However, under many pathological conditions the extracellular glutamate concentrations may be increased in order to tonically activate specific receptors. This results in over-excitation, which can induce irreversible processes in neurons, including cell death via excitotoxicity[5–8].

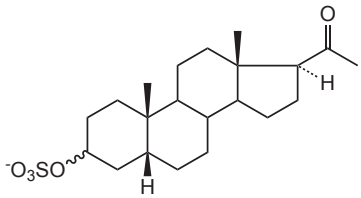
Due to the critical roles of NMDA receptors – in excitatory synaptic transmission, synaptic plasticity, and excitotoxicity – we decided to concentrate our research interests on the relationship between neurosteroids and NDMA receptors.

Early studies on the modulation of NMDA receptors were focused mainly on the effect of sulfated steroids. It has been known since the early 90s that pregnenolone sulfate (PS) potentiates NMDA-induced currents, whereas 20-oxo-5β-pregnan-3α-yl sulfate (pregnanolone sulfate; 3α5βS) and 20-oxo-5β-pregnan-3β-yl sulfate (3β5βS) inhibit NMDA-induced currents[9,10].

Subsequently, published studies began attempting to characterize the hallmarks of the steroid structure that are required for action at NMDA receptors[11–13]. Structure activity studies[13] on sulfated, saturated pregnane-20-ones (Chart 1), revealed that the A/B-ring junction predetermined the action of the neurosteroid; both 3α- and 3β-isomers which contain the hook-shaped, 5β-configuration, favor receptor inhibition almost identically, whereas the more planar 5α-configuration favors the potentiation of NMDA-induced [Ca^{2+}] increases and neuronal cell death. It is believed that the planar 5α-stereochemistry is similar to the stereochemistry of the C5-C6 double bond of PS, which is reflected in the significant decrease in receptor inhibition and increased tendency to potentiate the NMDA-



PS



3α5βS = 3α

3β5βS = 3β

CHART 1

induced currents. In general, the structure-activity studies indicate that these "bent" and "flat" structures are required for the inhibitory and potentiating actions, respectively.

In summarizing current electrophysiological experiments, naturally occurring neurosteroids can have either positive, negative, and/or combined modulatory effects on NMDA receptors, presumably mediated by two distinct binding sites[9,10,14]. Their action, which results in the observed inhibitory or potentiating effect, is influenced by the receptor subunit composition[15–17]. It is interesting to add that the action of steroids having a negative allosteric effect at NMDA receptors (e.g. pregnanolone sulfate) is use-dependent, however, voltage-independent (in contrast to ion channel blockers[17]); and those with a positive allosteric effect (e.g. pregnenolone sulfate) are disuse-dependent[14].

However, it is also important to note that the sulfate group is not essential for potentiation of the NMDA response; in fact, any negatively charged group at C-3 is sufficient for activity[18]. For instance, the activity can be retained if the sulfate group is substituted by dicarboxylic acid hemiesters of various lengths, such as hemioxalate or hemiglutarate[12,13].

Unfortunately, the aforementioned anionic compounds (when joined to a steroid core through an ester linkage), display a relatively low stability in neutral and acidic conditions, and their fast metabolic deactivation precludes any pharmacological utilization. Thus, attention was turned toward non-hydrolyzable analogs, from which, 3-carboxy steroids were chosen as more stable candidates.

The positive modulation effect on GABA receptors and negative modulation effect on NMDA receptors of 5β-pregnane 3α-carboxylic acids has been already described[19], although without any synthetic detail. Herein, we report a novel synthetic approach using a previously unpublished palladium catalyzed alkoxycarbonylation, which could also be used in further synthetic applications.

2. RESULTS AND DISCUSSION

2.1. Synthesis of Steroid 3-Carboxylic Acids

A palladium catalyzed alkoxycarbonylation of steroid triflates and nonaflates was chosen from a variety of synthetic approaches for the synthesizing the 3-carboxylic acids of steroids. Over the last few years, it has been demonstrated that alkenyl nonaflates are excellent substrates in a variety of palladium catalyzed coupling reactions such as the Heck or Sonogashira re-

actions. Also, current literature has already highlighted the advantages of using a nonaflate leaving group instead of triflate, in palladium catalyzed cross-coupling reactions[20,21]. Triflating reagents, such as triflic anhydride (Tf_2O) or triflimides like *N*-phenyltrifluoromethanesulfonimide (Tf_2NPh), are considerably more expensive than nonafluorobutanesulfonyl fluoride (NfF), which is the most common reagent for the synthesis of nonaflates. Furthermore, NfF is air-stable, non-toxic and can be stored over a long period of time. However, the triflate group is an excellent leaving group, and is often used in nucleophilic substitution reactions, and was therefore included as an alternative to the nonaflate leaving group used in our synthesis of steroid 3-carboxylic acids.

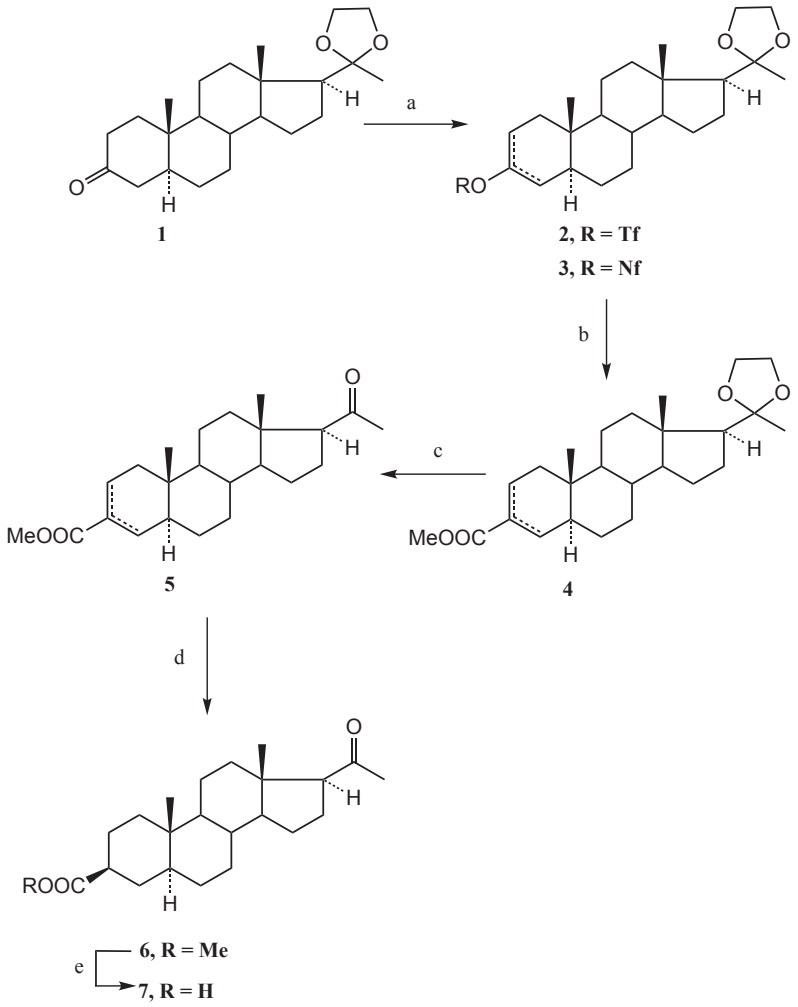
3,20-Dioxo-pregnane derivatives (**1** and **8**) were chosen as starting compounds. Their 20-keto groups were first protected as acetals, with the knowledge that an acid deprotection of a 20-keto group could be easily included into the reaction sequence. Lithium diisopropylamide (LDA) and lithium hexamethyldisilazane (LiHMDS) were chosen as the bases for deprotonation. It is known that deprotonation with LDA proceeds under kinetic control, whereas deprotonation with LiHMDS proceeds under thermodynamic control. As the 3-keto group of steroid is not especially sterically hindered, it was assumed that the ratio of isomeric enolate formation could be affected, particularly when using LiHMDS.

As expected from the literature[22], the deprotonation of the 5α-steroid afforded a mixture of Δ^2 and Δ^3 enolates, wherein the Δ^2 predominated. The results were comparable for both triflates and for nonaflates (Scheme 1). In the case where LDA was used as the base, the Δ^2 isomers were preferentially formed in a 3:1 ratio; with the mixture of triflates **2** and nonaflates **3** forming in total yield of 57 and 47%, respectively. In the case of LiHMDS was used, the Δ^2 isomers prevailed in a ratio 6:1; with the mixture of triflates **2** and nonaflates **3** forming in total yield of 29 and 20%, respectively. The low yields when using LiHMDS (and recovery of starting material) could be explained by the slow deprotonation of the ketone with the highly hindered base, and the subsequent trapping of the enolate with bulky Tf_2NPh or NfF.

The palladium catalyzed alkoxycarbonylation afforded the desired mixture of methyl esters **4** from the mixture of triflates **2**, as well as nonaflates **3** in respective yields of 52 and 69%. However, the ratio of Δ^2 and Δ^3 isomers changed to 2:1 in both cases. This can be rationalized by the isomerization of Δ^3 to the more thermodynamically favorable Δ^2 isomer.

Deprotection of the acetal protecting group by treatment with *p*-toluenesulfonic acid monohydrate in water-acetone, followed by hydrogenation on

palladium catalyst, afforded only 3β-methyl ester **6**. Its structure was confirmed by the methyl ester singlet in the ^{1}H NMR spectrum (3.69 ppm), and also in the IR spectrum by the carbonyl group bands (the 20-keto group at 1699 cm^{-1} and the ester group at 1726 cm^{-1}). The exclusive formation of



SCHEME 1

a) LDA or LiHMDS, *N*-phenyltrifluoromethanesulfonimide, THF (for **2**), LDA or LiHMDS, nonafluorobutanesulfonyl fluoride, THF (for **3**); b) CO, Et_3N, $Pd(OAc)_2$, $(C_6H_5)_3P$, MeOH, DMF; c) TsOH, H_2O, acetone; d) H_2, Pd/C, AcOEt, EtOH; e) KOH in MeOH, EtOH, H_2O

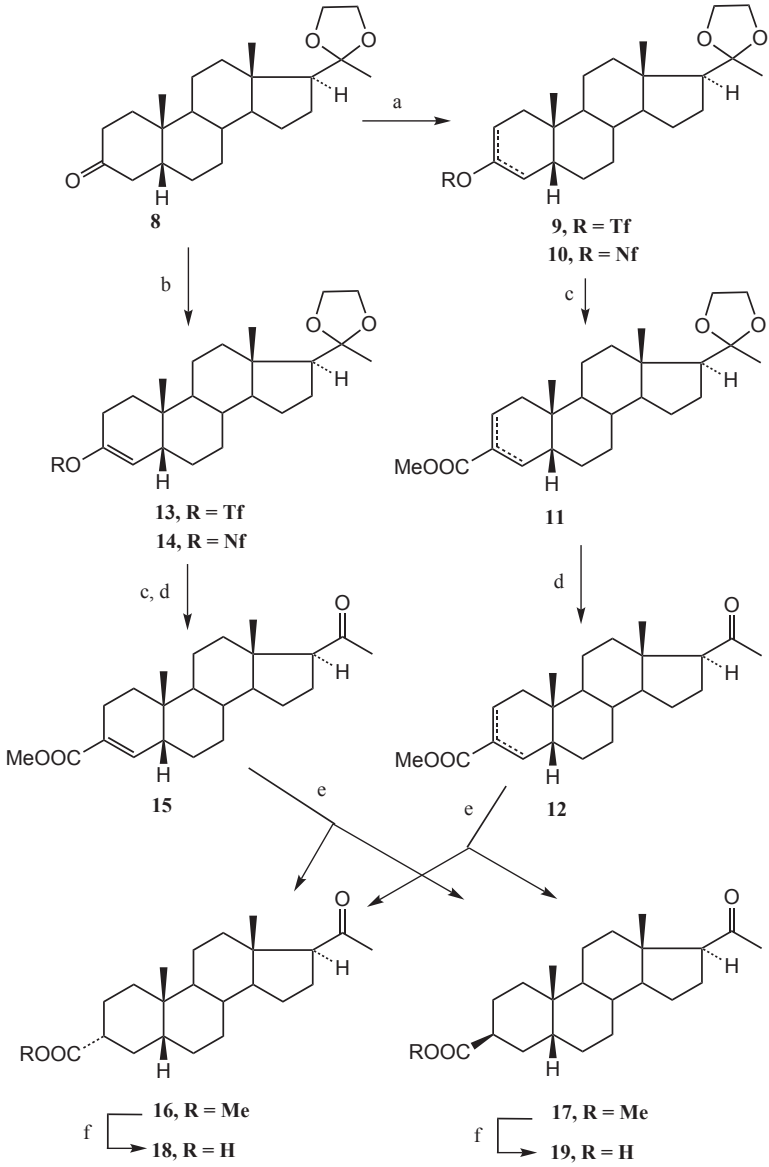
3β-isomer can be explained by a preferential attack from the less-hindered alpha side. The formation of the axial 3α-derivative was not observed after the hydrogenation. This isomer was probably created during the hydrogenation in the amount that is not detectable by used analytical methods.

Contrary to the planar geometry of the 5α-steroid, the 5β-steroid is hooked shape, and therefore more sterically hindered. Thus, deprotonation of the 5β-steroidal 3-ketone with the more hindered base (LiHMDS) confirmed our expectations, as the ketone was selectively converted to only one enolate.

When LDA was used, a mixture of triflates **9** and nonaflates **10** were formed; yielding mixtures of Δ^2 and Δ^3 isomers, in which the Δ^3 isomer prevailed in the ratios 2.5:1 and 2:1, respectively (Scheme 2). The mixture of triflates **9** was formed in 30% yield and the mixture of nonaflates **10** in 58% yield. Alkoxycarbonylation and immediate acetal deprotection of **11**, due to its protecting group instability, afforded a mixture of methyl esters **12** in 66% yield via triflate and 28% yield via nonaflate (over two steps). This was also the case in the 5α-series, wherein isomerization to the more thermodynamically stable enolate occurred during alkoxycarbonylation, shifting Δ^3/Δ^2 ratio shifted in favor of Δ^3 isomer.

As previously stated, in the case where LiHMDS was used as the base, only the Δ^3 isomer was selectively formed (Scheme 2). Further alkoxycarbonylation and deprotection of the acetal afforded methyl ester **15**, from triflate **13** in 14% and from nonaflate **14** in 6% yield (over three steps). Again, the slow deprotonation of the ketone with the highly hindered base, and subsequent trapping of enolate with bulky Tf_2NPh or NfF at low temperatures, are probable causes of the low yields and starting material recovery. The structure of methyl ester **15** was confirmed by a characteristic singlet of the methyl ester group at 3.74 ppm and H-4 at 6.71 ppm in the ^{1}H NMR spectrum. The characteristic carbonyl group's band from the ester group was observed at 1701 cm^{-1} in the IR spectrum.

During the catalytic hydrogenation of Δ^3/Δ^2 methyl esters mixtures of 3α- and 3β-derivatives (**16** and **17**) were formed (Scheme 2), in which the equatorial 3α-methyl ester **16** predominated. Epimers **16** and **17** were prepared from methyl esters **12** and **15** in total yields of 47–68% after HPLC-separation, with the ratio of epimers varying from 4.2:1 to 7:1. The structures of compounds **16** and **17** were confirmed by the characteristic singlet of the methyl ester group in the ^{1}H NMR spectrum at 3.68 and 3.69 ppm, respectively; and also by the carbonyl group bands of the 20-keto group at 1699 cm^{-1} and of the ester group at 1725 cm^{-1} for compound **16** and 1726 cm^{-1} for compound **17**.



Scheme 2

a) LDA, *N*-phenyltrifluoromethanesulfonimide, THF (for **9**), nonafluorobutanesulfonyl fluoride, THF (for **10**); b) LiHMDS, *N*-phenyltrifluoromethanesulfonimide, THF (for **13**), LiHMDS, nonafluorobutanesulfonyl fluoride, THF (for **14**); c) CO, Et_3N, $Pd(OAc)_2$, $(C_6H_5)_3P$, MeOH, DMF; d) TsOH, H_2O, acetone; e) H_2, Pd/C, AcOEt, EtOH; f) KOH in MeOH, EtOH, H_2O

TABLE I
^{1}H chemical shifts of compounds **6**, **16**, and **17** in $CDCl_3$

Proton	**6**	**16**	**17**
H-1α	0.97	1.86	1.62
H-1β	1.76	0.98	1.07
H-2α	1.78	1.66	1.56
H-2β	1.61	1.51	1.88
H-3α	2.32	–	2.71
H-3β	–	2.34	–
H-4α	1.54	1.93	1.96
H-4β	1.46	1.50	1.70
H-5	1.11	1.38	1.50
H-6α	1.26	1.28	1.26
H-6β	1.30	1.89	1.87
H-7α	0.92	1.13	1.10
H-7β	1.68	1.43	1.43
H-8	1.39	1.44	1.43
H-9	0.72	1.45	1.43
H-11α	1.60	1.49	1.46
H-11β	1.29	1.27	1.28
H-12α	1.39	1.44	1.43
H-12β	2.00	2.00	2.01
H-14	1.15	1.23	1.21
H-15α	1.66	1.20	1.21
H-15β	1.20	1.66	1.67
H-16α	2.15	2.15	2.16
H-16β	1.63	1.64	1.64
H-17α	2.52	2.54	2.53
H-18	0.60	0.59	0.59
H-19	0.80	0.94	0.91
H-21	2.11	2.11	2.11
OCH_3	3.65	3.67	3.68

In the last step of the reaction sequence, a basic hydrolysis was performed by refluxing methyl esters **6**, **16** and **17** in ethanol with potassium hydroxide and water (Schemes 1 and 2), respectively yielding carboxylic acids **7**, **18**, and **19** in 95, 58, and 87%. Their structures were confirmed by the characteristic bands of the carboxylic hydroxyl group at approximately 3500 cm^{-1} for monomers and around both 3090 and 2700 cm^{-1} for dimers.

TABLE II
^1H chemical shifts of compounds **6**, **16**, and **17** in $CDCl_3$

Carbon	**6**	**16**	**17**
1	37.80	36.51	33.28
2	24.61	23.84	22.03
3	43.71	44.09	39.75
4	31.23	29.63	27.45
5	46.08	42.90	39.31
6	28.57	27.11	26.90
7	31.97	26.34	26.27
8	35.51	35.88	35.79
9	54.33	40.40	40.28
10	35.81	34.93	34.97
11	20.99	20.88	21.01
12	39.12	39.20	39.36
13	44.24	44.33	44.36
14	56.78	56.64	56.92
15	24.39	24.44	24.45
16	22.86	22.94	22.96
17	63.88	63.92	63.98
18	13.44	13.40	13.42
19	12.21	23.74	23.92
20	209.51	209.51	209.45
21	31.44	31.49	31.45
C=O	176.45	176.73	175.84
OCH_3	51.44	51.47	51.44

The structural assignment of methyl esters **6**, **16**, and **17** was also confirmed by NMR spectroscopy (Tables I and II) as follows: the similarities in the substituting effects of the $COOCH_3$ groups and the steric proximity of the isomers did not allow this problem to be solved from routine ^1H NMR spectra. Therefore, a complete analysis of ^1H and ^{13}C spectra was performed. The APT ^{13}C NMR spectra were used to determine chemical shifts and to distinguish the multiplicity of individual carbon signals. Structural assignment of CH, CH_2 and CH_3 signals were derived from ^1H, ^1H 2D-COSY; 2D-ROESY and ^1H, and ^{13}C 2D-HSQC spectra, and by correlation with previously assigned proton signals. The remaining quaternary carbons were assigned on the basis of chemical shifts.

2.2. Biological Activity

Currents elicited by 100 μM NMDA were recorded in cultured hippocampal neurons voltage-clamped at a holding potential of –60 mV. In accordance with previous results, pregnanolone sulfate (3α5βS) diminished the amplitude of NMDA-induced currents. At 100 μM of 3α5βS, the mean inhibitory effect was –71.3 ± 5.0% (n = 5). 5β-Pregnane carboxylic acid analogs **18** and **19** also had a negative modulatory effect on NMDA-induced responses, with a mean inhibition of –64.7 ± 5.9% (n = 5) induced by 200 μM of **18** (3α,5β) and an inhibition of –40.1 ± 7.0% (n = 5) induced by 100 μM of **19** (3β,5β).

TABLE III
Inhibition of NMDA-induced response in cultured hippocampal neurons by pregnanolone sulfate (3α5βS) and its synthetic analogs[a]

Compound	Relative effect, %	IC_{50}, μM	n
3α5βS	–71.3 ± 5.0	47.2 ± 9.9	5
7 (3β,5α)	+31.1 ± 5.7	not determined	4
18 (3α,5β)	–64.7 ± 5.9	122.2 ± 26.7	5
19 (3β,5β)	–40.1 ± 7.0	143.9 ± 36.1	5

[a] Relative degree of steroid (100 μM for 3a5bS and **19**, 200 μM for **7** and **18**) inhibition of current responses induced in cultured hippocampal neurons by fast application of 100 μM NMDA. Calculated IC_{50} values are listed. Results are expressed as mean ± S.D. n indicates the number of cells studied.

This relative degree of steroid-induced effect was then used to estimate the IC_{50} value. The IC_{50} was calculated from the logistic equation: RI = 1 – (1/(1 + ([steroid]/IC_{50})^h); where RI is relative degree of steroid-induced effect, and *h* is the apparent Hill coefficient (1.2; see ref.[17]). The values of relative inhibition and estimated IC_{50} values of steroids **7**, **18** and **19** are listed in Table III. On the contrary, 200 μM of **7** (3β,5α) potentiated (+31.1 ± 5.7%; *n* = 4) NMDA receptor responses.

The ability of the 5β-pregnane derivatives (**18** and **19**) to inhibit NMDA-induced currents was maintained even after substitution of the sulfate group by a carboxylic acid; however, the IC_{50} value slightly increased compared to the IC_{50} of pregnanolone sulfate (3α5βS). In the case of **7**, the presence of a carboxylic acid did not change the relative effect of the neurosteroid; **7** slightly potentiated NDMA-induced currents. Therefore, it seems that the synthesis of pregnanes containing carboxylic acid residues with varying lengths and branching-patterns, could yield interesting results; providing more clarity on the structure-activity effects of pregnane 3-carboxylic acids on NMDA receptors.

3. CHEMICAL SYNTHESIS

3.1. General

Melting points were determined on a micro-melting point apparatus Hund/Wetzlar (Germany) and are uncorrected. Optical rotations were measured in chloroform using an Autopol IV (Rudolf Research Analytical, Flanders, USA), $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}, IR spectra were recorded on a Bruker IFS 88 spectrometer (wavenumbers in cm^{-1}). Proton NMR spectra were measured on a FT NMR spectrometer Varian UNITY-200 (at 200 MHz) or on a FT NMR spectrometer Bruker AVANCE-400 (at 400 MHz) or on Varian UNITY-500 (^{1}H at 500 MHz, ^{13}C at 125.7 MHz frequency) in $CDCl_3$ with tetramethylsilane as the internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) and width of multiplets (*W*) are given in Hz. Mass spectra were obtained with spectrometers ZAB-EQ (at 70 eV) or LCQ Classic (Thermo Finnigan).

Thin layer chromatography (TLC) was performed on silica gel (ICN Biochemicals), preparative TLC (prep-TLC) was carried out on 200 mm × 200 mm plates coated with a 0.4 mm thick layer of the same material. For column chromatography, neutral silica gel 60–120 μm (Merck) was used. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Anhydrous THF was prepared by distillation with $LiAlH_4$ immediately prior to use.

Whenever an aqueous solution of citric acid was used, the concentration was always 5%. An aqueous solution of potassium hydrogen carbonate was used as a saturated solution. Before evaporation on a rotary evaporator in vacuo (bath temperature 50 °C, pressure 1.5 kPa), solutions of organic solvents were dried over anhydrous sodium sulfate.

The HPLC system consisted of High Pressure Pump (model 361, Gilson), Inject Valve Rheodyne, Preparative Column (10 × 250 mm) with silica gel filling (Biospher PSI 200,

7 μm; Labio), and preparative ELSD Detector (Gilson) connected with PC (software Trilution LC, Gilson).

The synthesis and full characterization on compound pregnanolone sulfate (3α5βS) has been described in literature[12].

Jones reagent has been prepared from chromium trioxide (67 g, 0.67 mmol) and a solution of sulfuric acid (58 ml of H_2SO_4 in 100 ml of water) and the mixture was diluted to 250 ml with water.

Lithium diisopropylamide was prepared by the following procedure. A solution of diisopropylamine (0.45 ml, 3.2 mmol) in dry THF (7.5 ml) was stirred under argon and then cooled to –78 °C. A solution of butyllithium (1.6 M in hexanes, 2.0 ml, 3.2 mmol) was added dropwise and the stirring was continued for next 30 min.

3.2. Starting Compounds (Ketones)

3.2.1. 20,20-(Ethylenedioxy)-5α-pregnan-3-one (**1**)

This compound was prepared from commercially available pregnenolone acetate according to the literature[23].

3.2.2. 20,20-(Ethylenedioxy)-5β-pregnan-3-one (**8**)

This compound was prepared from commercially available progesterone according to the literature[24].

3.3. General Procedures

3.3.1. General Procedure for Synthesis of Triflates Using Lithium Diisopropylamide

A solution of the desired ketone in dry THF (2.5 ml per 100 mg) was added during 10 min at –78 °C under argon to a freshly prepared solution of lithium diisopropylamide (see Section 3.1.) and the mixture was stirred at –78 °C for 1.5 h. Then, the solution of *N*-phenyltrifluoromethanesulfonimide in dry THF (2 ml per 500 mg) was added dropwise and the mixture was stirred at –78 °C for 1 h. After an additional 8 h at room temperature under argon, the mixture was poured into water and extracted with ethyl acetate (2×). Subsequently, the combined organic extracts were washed with an aqueous solution of citric acid, water, saturated solution of potassium hydrogen carbonate, water, and dried. Solvents were evaporated in vacuo.

3.3.2. General Procedure for Synthesis of Triflates Using Lithium Bis(trimethylsilyl)amide

A solution of lithium bis(trimethylsilyl)amide (1.0 M in hexanes) was added during 10 min at –78 °C under an inert atmosphere to a solution of the desired ketone in dry THF (2.5 ml per 100 mg) and the mixture was stirred at –78 °C for 3.5 h. Then, the solution of *N*-phenyltrifluoromethanesulfonimide in dry THF (2 ml per 500 mg) was added dropwise and the mixture was allowed to attain room temperature. After an additional 8 h at room temperature under inert atmosphere, the mixture was poured into water and extracted with ethyl acetate (2×). Subsequently, the combined organic extracts were washed with an aqueous solution of citric acid, water, saturated solution of potassium hydrogen carbonate, water, and dried. Solvents were evaporated in vacuo.

3.3.3. General Procedure for Synthesis of Nonaflates Using Lithium Diisopropylamide

A solution of the desired ketone in dry THF (2.5 ml per 100 mg) was added during 10 min at –78 °C under argon to a freshly prepared solution of lithium diisopropylamide in dry THF and the mixture was stirred at –78 °C for 1.5 h. Then, nonafluorobutanesulfonyl fluoride was added dropwise and the mixture was stirred at –78 °C for 2 h. After an additional 8 h at

room temperature under argon, the mixture was poured into water and extracted with ethyl acetate (2×). The combined organic extracts were washed with an aqueous solution of citric acid, water, saturated solution of potassium hydrogen carbonate, water, and dried. Solvents were evaporated in vacuo.

3.3.4. General Procedure for Synthesis of Nonaflates Using Lithium Bis(trimethylsilyl)amide

To a solution of lithium bis(trimethylsilyl) (1.0 M in hexanes) was added during 10 min at –78 °C under argon to a solution of the desired ketone in dry THF (2.5 ml per 100 mg) and the mixture was stirred at –78 °C for 3.5 h. Then, nonafluorobutanesulfonyl fluoride was added dropwise and the mixture was allowed to attain room temperature. After an additional 8 h at room temperature under argon, the mixture was poured into water and extracted with ethyl acetate (2×). The combined organic extracts were washed with an aqueous solution of citric acid, water, saturated solution of potassium hydrogen carbonate, water, and dried. Solvents were evaporated in vacuo.

3.3.5. General Procedure for Alkoxycarbonylation

A mixture of nonaflate or triflate (0.4 mmol), triethylamine (1.0 mmol), palladium acetate (0.04 mmol), triphenylphosphine (0.09 mmol) in methanol (7 ml), and DMF (14 ml) was stirred under a CO balloon at room temperature. The reaction mixture was monitored by TLC. After completion of the reaction, the reaction mixture was poured into water and extracted with ethyl acetate (2×). The combined organic extracts were washed with a solution of citric acid, water, saturated solution of potassium hydrogen carbonate, water, and dried. Solvents were evaporated in vacuo.

3.3.6. General Procedure for Alkoxycarbonylation and Acetal Deprotection

A mixture of nonaflate or triflate (0.5 mmol), triethylamine (1.2 mmol), palladium acetate (0.04 mmol), triphenylphosphine (0.1 mmol) in methanol (7 ml), and DMF (14 ml) was stirred under a CO balloon at room temperature. The reaction mixture was monitored by TLC. After the completion of reaction, the reaction mixture was poured into water and extracted with ethyl acetate (2×). After the usual work-up, the residue was dissolved in acetone and a solution of *p*-toluenesulfonic acid monohydrate (0.04 mmol) in water (1 ml) was added. The mixture was allowed to stay overnight at room temperature, then poured into solution of potassium hydrogen carbonate and extracted with ethyl acetate (2×). The combined organic extracts were washed with water, and dried. Solvents were evaporated in vacuo.

3.3.7. General Procedure for Acetal Deprotection

The acetal (0.2 mmol) was dissolved in acetone (4 ml) and a solution of *p*-toluenesulfonic acid monohydrate (0.04 mmol) in water (1 ml) was added. The mixture was allowed to stay overnight at room temperature, then poured into solution of potassium hydrogen carbonate and extracted with ethyl acetate (2×). The combined organic extracts were washed with water, and dried. Solvents were evaporated in vacuo.

3.3.8. General Procedure for Hydrogenation

A solution of unsaturated ester (0.1 mmol) in ethyl acetate (3 ml) and ethanol (0.7 ml) was stirred in the presence of palladium on calcium carbonate (10%, 10 mg) under slight hydrogen overpressure at room temperature. After 5 h, the catalyst was filtered off on a short column of silica gel and washed with ethyl acetate. The combined organic extracts were evaporated in vacuo.

3.3.9. General Procedure for Basic Hydrolysis of Esters

A solution of potassium hydroxide (1.6 mmol) in water (0.56 ml) and ethanol (0.56 ml) was added to a solution of ester (0.25 mmol) in methanol (5 ml). The reaction mixture was

refluxed. After completion of the reaction, the reaction mixture was poured into ice-water and acidified to pH 1, with a HCl/H_2O (1:2) mixture. The mixture was extracted with chloroform (2 × 30 ml), the combined organic extracts were washed with water, dried, and evaporated. The residue was crystallized from the mixture of acetone-water.

3.4. 5α-Pregnane Derivatives

3.4.1. Triflate Procedure

3.4.1.1. 20,20-(Ethylenedioxy)-5α-pregn-2-en-3-yl 3-Triflate and
20,20-(Ethylenedioxy)-5α-pregn-3-en-3-yl 3-Triflate, Mixture of Isomers (**2**)

Procedure A. Compound **1** (300 mg, 0.8 mmol), lithium diisopropylamide (2.5 mmol), and *N*-phenyltrifluoromethanesulfonimide (500 mg, 1.4 mmol) were converted to a mixture of triflates **2** according to General Procedure 3.3.1. Purification by prep-TLC (7 plates) developed with petroleum ether/ether (4:1) afforded a mixture of Δ^2 and Δ^3 isomers **2** (233 mg, 57%) and starting material **1** (56 mg, 19%). The Δ^2 isomer prevailed in the ratio 3:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.76 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 1.29 s, 3 H (3 × H-21); 3.83–4.02 m, 4 H (OCH_2CH_2O); 5.38 s, 0.25 H (H-4); 5.63 m, 0.75 H (H-2). IR ($CHCl_3$): 1472, 1374, 1296 (CH_2, acetal); 1415, 1200 (SO_2, triflate); 1245, 1142 (CF_3, triflate); 1035, 1007, 873 (C–O–S, triflate). FAB MS: 491 (0.5%, M – 1); 443 (1%, M – 1 × F, CH_2O); 475 (0.5%, M – 3 × F, $C_2H_4O_2$); 347 (0.5%, M – 3 × F, CH_4O_2, 1 × O); 331 (1%, M – 3 × F, $C_2H_4O_2$, 2 × O). For $C_{24}H_{35}F_3O_5S$ (492.6) calculated: 58.52% C, 7.16% H; found: 58.56% C, 7.38% H.

Procedure B. Compound **1** (300 mg, 0.8 mmol), lithium bis(trimethylsilyl)amide (1.0 M in hexanes, 2.5 ml, 2.5 mmol), and *N*-phenyltrifluoromethanesulfonimide (893 mg, 2.5 mmol) were converted to a mixture of triflates **2** according to the General Procedure 3.3.2. Purification by prep-TLC (10 plates) developed with petroleum ether/ether (4:1) afforded mixture of Δ^2 and Δ^3 isomers **2** (194 mg, 47%), identical with the sample prepared above according to Procedure *A* and starting material **1** (48 mg, 16%). The Δ^2 isomer prevailed in the ratio 6:1 according to ^{1}H NMR spectrum.

3.4.1.2. Methyl 20,20-(Ethylenedioxy)-5α-pregn-2-ene-3-carboxylate and
Methyl 20,20-(Ethylenedioxy)-5α-pregn-3-ene-3-carboxylate, Mixture of Isomers (**4**)

Mixture of triflates **2** (299 mg, 0.6 mmol), which was prepared according Procedure *A* was converted to a mixture of methyl esters **4** according to General Procedure 3.3.5. Purification by prep-TLC (5 plates) developed with petroleum ether/ether (9:1) afforded mixture of Δ^2 and Δ^3 isomers **4** (128 mg, 52%). The Δ^2 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.72 s, 3 H (3 × H-19); 0.76 s, 3 H (3 × H-18); 1.29 s, 3 H (3 × H-21); 3.85–4.02 m, 4 H (OCH_2CH_2O); 3.72 s, 3 H ($COOCH_3$); 6.61 dt, 0.33 H, J_1 = 3.6, J_2 = 1.5 (H-4); 6.89 m, 0.66 H (H-2). IR ($CHCl_3$): 1705 (C=O, $COOCH_3$); 1650 (C=C); 1471, 1375, 1295 (CH_2, acetal); 1437 (CH_3, $COOCH_3$); 1262, 1087 (C–O, $COOCH_3$). EI MS: 387 (4%, M – CH_3); 371 (1.5%, M – CH_3O); 312 (1.5%, M – $COOCH_3$, CH_2O). For $C_{25}H_{38}O_4$ (402.6) calculated: 74.59% C, 9.51% H; found: 74.40% C, 9.51% H.

3.4.1.3. Methyl 20-Oxo-5α-pregn-2-ene-3-carboxylate and
Methyl 20-Oxo-5α-pregn-3-ene-3-carboxylate, Mixture of Isomers (**5**)

Mixture of protected methyl esters **4** (128 mg, 0.3 mmol) was converted to compound **5** according to General Procedure 3.3.7. Purification by prep-TLC (2 plates) developed with petroleum ether/ether (4:1) afforded mixture of Δ^2 and Δ^3 isomers **5** (98 mg, 86%). The Δ^2 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.61 s,

3 H (3 × H-18); 0.71 s, 3 H (3 × H-19); 1.11 s, 3 H (3 × H-21); 2.52 t, 1 H, J = 8.8 (H-17); 3.72 s, 3 H ($COOCH_3$); 6.61 dt, 0.33 H, J_1 = 3.6, J_2 = 1.5 (H-4); 6.88–6.90 m, 0.66 H (H-2). IR ($CHCl_3$): 1702 (C=O, $COOCH_3$, ketone); 1651 (C=C); 1437 (CH_3, $COOCH_3$); 1358 (CH_3, ketone); 1264, 1086 (C–O, $COOCH_3$, ketone). FAB MS: 359 (79%, M + 1); 343 (6%, M – CH_3); 327 (16%, M – CH_3O). For $C_{23}H_{34}O_3$ (358.5) calculated: 77.05% C, 9.56% H; found: 77.27% C, 9.64% H.

3.4.1.4. Methyl 20-Oxo-5α-pregnane-3β-carboxylate (**6**)

Mixture of methyl esters **5** (129 mg, 0.36 mmol) was converted into methyl ester **6** according to General Procedure 3.3.8. Purification by prep-TLC (2 plates) developed with petroleum ether/ether (9:1) afforded 3β-isomer **6** (95 mg, 73%): m.p. 78–80 °C (ethyl acetate), $[\alpha]_D$ +128.1 (*c* 0.188, $CHCl_3$). ^{1}H NMR (500 MHz): 0.60 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.32 tt, 1 H, J_1 = 4.3, J_2 = 12.4 (H-3); 2.52 t, 1 H, J = 9.0 (H-17); 3.69 s, 3 H ($COOCH_3$). IR ($CHCl_3$): 1726 (C=O, $COOCH_3$); 1699 (C=O, ketone); 1358 (C–O, ketone); 1165 (C–O, $COOCH_3$). FAB MS: 360 (53%, M); 345 (15%, M – CH_3); 300 (5%, M – 1 – $COOCH_3$); 317 (15%, M – CH_3CO). For $C_{23}H_{36}O_3$ (360.5) calculated: 76.62% C, 10.06% H; found: 76.75% C, 10.00% H.

3.4.2. Nonaflate Procedure

3.4.2.1. 20,20-(Ethylenedioxy)-5α-pregn-2-en-3-yl 3-Nonaflate and

20,20-(Ethylenedioxy)-5α-pregn-3-en-3-yl 3-Nonaflate, Mixture of Isomers (**3**)

Procedure A: Ketone **1** (300 mg, 0.8 mmol), lithium diisopropylamide (2.5 mmol), and nonafluorobutanesulfonyl fluoride (0.7 ml, 3.9 mmol) were converted to mixture of nonaflates **3** according to General Procedure 3.3.3. Purification by prep-TLC (7 plates) developed with petroleum ether/ether (4:1) afforded mixture of Δ^2 and Δ^3 isomers **3** (210 mg, 29%) and starting material **1** (53 mg, 13%). The Δ^2 isomer prevailed in the ratio 3:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.76 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 1.29 s, 3 H (3 × H-21); 3.83–4.02 m, 4 H (OCH_2CH_2O); 5.46 s, 0.25 H (H-4); 5.63 d, 0.75 H, J = 6.3 (H-2). IR ($CHCl_3$): 1472 (CH_2, acetal); 1415 (SO_2, nonaflate); 1352 (CF_3, nonaflate); 1291 (CH_2, acetal); 1242 (CF_2, nonaflate); 1203 (SO_2, nonaflate). FAB MS: 643 (3%, M + 1); 599 (5%); 553 (1%, M – $C_4H_7O_2$); 413 (1.5%, M – $C_4F_9SO_3$). For $C_{27}H_{35}F_9O_5S$ (642.6) calculated: 50.46% C, 5.49% H; found: 50.55% C, 5.30% H.

Procedure B: Ketone **1** (500 mg, 1.38 mmol), lithium bis(trimethylsilyl)amide (1.0 M in hexanes, 3.45 ml, 3.45 mmol), and nonafluorobutanesulfonyl fluoride (1.16 ml, 6.6 mmol) were converted to mixture of nonaflates **3** according to General Procedure 3.3.4. Column chromatography on silica gel (25 g, 20% ether in petroleum ether) afforded mixture of Δ^2 and Δ^3 isomers **3** (308 mg, 20%) and starting material **1** (443 mg, 49%). The Δ^2 isomer prevailed in the ratio 6:1 according to ^{1}H NMR spectrum.

3.4.2.2. Methyl 20,20-(Ethylenedioxy)-5α-pregn-2-ene-3-carboxylate and

Methyl 20,20-(Ethylenedioxy)-5α-pregn-3-ene-3-carboxylate, Mixture of Isomers (**4**)

Mixture of nonaflates **3** (270 mg, 0.4 mmol), which was prepared according Procedure *A* was converted to mixture of esters **4** according to General Procedure 3.3.5. Purification by prep-TLC (4 plates) developed with petroleum ether/ether (9:1) afforded mixture of Δ^2 and Δ^3 isomers **4** (69 mg, 40%), identical with the sample prepared above by Triflate Procedure 3.4.1.2. The Δ^2 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum.

3.4.2.3. Methyl 20-Oxo-5α-pregn-2-ene-3-carboxylate and

Methyl 20-Oxo-5α-pregn-3-ene-3-carboxylate, Mixture of Isomers (**5**)

Mixture of methyl esters **4** (88 mg, 0.2 mmol) was converted to mixture of esters **5** according to General Procedure 3.3.7. Purification by prep-TLC (2 plates) developed with petro-

leum ether/ether (4:1) afforded mixture of Δ^2 and Δ^3 isomers **5** (61 mg, 78%), identical with sample **5** prepared by Triflate Procedure 3.4.1.3. The Δ^2 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum.

3.4.2.4. Methyl 20-Oxo-5α-pregnane-3β-carboxylate (**6**)

Mixture of methyl esters **5** (25 mg, 0.07 mmol) was converted to methyl ester **6** according to General Procedure 3.3.8. Crystallization from hot ethyl acetate afforded 3β-isomer **6** (20 mg, 80%), identical with the sample prepared by Triflate Procedure 3.4.1.4.

3.4.3. 5α-Pregnane-3-carboxylic Acid

3.4.3.1. 20-Oxo-5α-pregnane-3β-carboxylic Acid (**7**)

Ester **6** (20 mg, 0.05 mmol) was converted to acid **7** according to General Procedure 3.3.9. Crystallization afforded acid **7** (18 mg, 95%): m.p. 184–187 °C, $[\alpha]_D$ +118.8 (*c* 0.17, $CHCl_3$). ^{1}H NMR (400 MHz): 0.60 s, 3 H (3 × H-18); 0.81 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.36 tt, 1 H, J_1 = 12.4, J_2 = 4.0 (H-3); 2.52 t, 1 H (H-17). IR ($CHCl_3$): 3516 (O–H, COOH, monomer); 3086, 2669 (O–H, COOH, dimer); 1734 (C=O, COOH, monomer); 1701 (C=O, COOH, dimer and C=O, ketone). EI MS: 346 (61%, M); 328 (33%, M – OH); 313 (12%, M – H_2O); 301 (10%, M – COOH). For $C_{22}H_{34}O_3$ (346.5) calculated: 76.26% C, 9.89% H; found: 76.02% C, 9.95% H.

3.5. 5β-Pregnane Derivatives

3.5.1. Triflate Procedure

3.5.1.1. 20,20-(Ethylenedioxy)-5β-pregn-2-en-3-yl 3-Triflate and
20,20-(Ethylendioxy)-5β-pregn-3-en-3-yl 3-Triflate, Mixture of Isomers (**9**)

Ketone **8** (200 mg, 0.5 mmol), lithium diisopropylamide (2.9 mmol), and *N*-phenyltrifluoromethanesulfonimide (400 mg, 1.2 mmol) were converted to mixture of triflates **9** according to General Procedure 3.3.1. Purification by prep-TLC (4 plates) developed with petroleum ether/ether (8:2) afforded mixture of Δ^2 and Δ^3 isomers **9** (167 mg, 30%) and starting material **8** (33 mg, 12%). The Δ^3 isomer prevailed in the ratio 2.5:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.76 s, 3 H (3 × H-18); 0.99 s, 3 H (3 × H-19); 1.28 s, 3 H (3 × H-21); 3.85–3.98 m, 4 H (OCH_2CH_2O); 5.45 s, 0.7 H (H-4); 5.60 d, 0.3 H, *J* = 6.0 (H-2). IR ($CHCl_3$): 1470, 1374, 1055 (CH_2, acetal); 1415, 1246, 1223 (SO_2, triflate); 1142 (CF_3, triflate). FAB MS: 493 (5%, M + 1); 447 (21%, M – OCH_2CH_2); 431 (15%, M – $C_2H_4O_2$). For $C_{24}H_{35}F_3O_5S$ (492.6) calculated: 58.52% C, 7.16% H; found: 58.35% C, 7.12% H.

3.5.1.2. Methyl 20-Oxo-5β-pregn-2-ene-3-carboxylate and
Methyl 20-Oxo-5β-pregn-3-ene-3-carboxylate, Mixture of Isomers (**12**)

Mixture of triflates **9** (130 mg, 0.3 mmol) was converted into mixture of esters **12** according to General Procedure 3.3.6. Purification by prep-TLC (2 plates) developed with petroleum ether/ether (4:1) afforded mixture of Δ^2 and Δ^3 isomers **12** (62 mg, 66%). The Δ^3 isomer prevailed in the ratio 3:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.6 s, 3 H (3 × H-18); 0.98 s, 3 H (3 × H-19); 2.10 s, 3 H (3 × H-21); 2.49 t, 1 H (H-17); 3.74 s, 3 H ($COOCH_3$); 6.71 s, 0.75 H (H-4); 6.85 m, 0.25 H (H-2). IR ($CHCl_3$): 1701 (C=O, $COOCH_3$ and ketone); 1648, 1661 (C=C); 1438 (CH_3, ketone); 1358 (CH_3, $COOCH_3$); 1257 (C–O, $COOCH_3$). FAB MS: 381 (10%, M + Na); 359 (62%, M + 1); 341 (12%, M – 1 – CH_3); 327 (32%, M – OCH_3); 315 (9%, M – $COCH_3$); 297 (6%, M – 1 – $COOCH_3$). For $C_{23}H_{34}O_3$ (358.5) calculated: 77.05% C, 9.56% H; found: 76.81% C, 9.62% H.

3.5.1.3. Methyl 20-Oxo-5β-pregnane-3α-carboxylate (**16**) and
Methyl 20-Oxo-5β-pregnane-3β-carboxylate (**17**)

Mixture of methyl esters **12** (60 mg, 0.1 mmol) was converted into 3α- and 3β-carboxy derivatives **16** and **17** according to General Procedure 3.3.8. 3α-Carboxy derivative **16** prevailed over 3β-carboxy derivative **17** in the ratio 6:1 according to the ^{1}H NMR spectrum. Preparative HPLC chromatography (9% ethyl acetate in hexanes) gave 25 mg (41%) of 3α-carboxy derivative **16** and 4 mg (6%) of 3β-carboxy derivative **17**. The ratio of desired products was 5:1.

3α-Carboxy derivative (**16**): m.p. 95–97 °C (ethyl acetate), $[\alpha]_D$ +129.5 (*c* 0.18, $CHCl_3$). ^{1}H NMR (400 MHz): 0.59 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.34 tt, 1 H, J_1 = 16.2, J_2 = 3.8 (H-3); 2.54 t, 1 H, *J* = 9 (H-17); 3.67 s, 3 H ($COOCH_3$). IR ($CHCl_3$): 1726 (C=O, ester); 1699 (C=O, ketone); 1437 (CH_3, ketone); 1358 (CH_3, ester). EI MS: 360 (45%, M); 342 (69%, M – H_2O); 329 (7%, M – CH_3O); 316 (15%, M – CH_3CO, H); 301 (11%, M – $COOCH_3$). For $C_{23}H_{36}O_3$ (360.5) calculated: 76.62% C, 10.06% H; found: 76.58% C, 10.33% H.

3β-Carboxy derivative (**17**): m.p. 86–88 °C (ethyl acetate), $[\alpha]_D$ +87.4 (*c* 0.232, $CHCl_3$). ^{1}H NMR (400 MHz): 0.59 s, 3 H (3 × H-18); 0.91 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.71 t, 1 H, *J* = 5.3 (H-3); 2.53 t, 1 H, *J* = 9 (H-17); 3.68 s, 3 H ($COOCH_3$). IR ($CHCl_3$): 1725 (C=O, ester); 1699 (C=O, ketone); 1436 (CH_3, ketone); 1358 (CH_3, ester). EI MS: 360 (65%, M); 342 (31%, M – H_2O); 329 (2%, M – CH_3O); 317 (16%, M – CH_3CO); 300 (25%, M – $COOCH_3$, H). For $C_{23}H_{36}O_3$ (360.5) calculated: 76.62% C, 10.06% H; found: 76.53% C, 10.11% H.

3.5.1.4. Methyl 20-Oxo-5β-pregn-3-ene-3-carboxylate (**15**)

Ketone **8** (200 mg, 0.25 mmol), lithium bis(trimethylsilyl)amide (1.0 M in hexanes, 0.8 ml, 0.8 mmol) and *N*-phenyltrifluoromethanesulfonimide (785 mg, 2.2 mmol) were converted to triflate according to General Procedure 3.3.2. Purification by prep-TLC (10 plates) developed in petroleum ether/ether (4:1) afforded mixture of protected **13** and deprotected triflates (160 mg) and starting material **8** (84 mg, 21%). ^{1}H NMR spectrum confirmed occurrence of only Δ^3 isomer. This mixture (160 mg) was converted to ester **15** according to General Procedure 3.3.6. Purification by prep-TLC (2 plates) developed in petroleum ether/ether (4:1) afforded Δ^3 isomer **15** (55 mg, 14%): m.p. 120–122 °C (petroleum ether/ether), $[\alpha]_D$ +212.0 (*c* 0.16, $CHCl_3$). ^{1}H NMR (400 MHz): 0.6 s, 3 H (3 × H-18); 0.98 s, 3 H (3 × H-19); 2.10 s, 3 H (3 × H-21); 2.47 t, 1 H, *J* = 8.8 (H-17); 3.74 s, 3 H ($COOCH_3$); 6.71 s, 1 H (H-4). IR ($CHCl_3$): 1701 (C=O, ketone, $COOCH_3$); 1647 (C=C); 1438 (CH_3, ketone); 1358 (CH_3, $COOCH_3$); 1257 (C–O, $COOCH_3$). FAB MS: 381 (12%, M + Na); 359 (73%, M + 1); 341 (4%, M – 1 – CH_3); 327 (16%, M – OCH_3). For $C_{23}H_{34}O_3$ (358.5) calculated: 77.05% C, 9.56% H; found: 76.85% C, 9.58% H.

3.5.1.5. Methyl 20-Oxo-5β-pregnane-3α-carboxylate (**16**) and
Methyl 20-Oxo-5β-pregnane-3β-carboxylate (**17**)

Methyl ester **15** (55 mg, 0.2 mmol) was converted to 3α- and 3β-carboxy derivatives **16** and **17** according to General Procedure 3.3.8. 3α-Carboxy derivative **16** prevailed over 3β-carboxy derivative **17** in the ratio 2.5:1 according to the ^{1}H NMR spectrum. Preparative HPLC chromatography (9% ethyl acetate in hexanes) gave 22 mg (40%) of 3α-carboxy derivative **16** and 9 mg (16%) of 3β-carboxy derivative **17**, identical with the samples prepared by Procedure 3.5.1.3.

3.5.2. Nonaflate Procedure

3.5.2.1. 20,20-(Ethylendioxy)-5β-pregn-2-en-3-yl 3-Nonaflate and
20,20-(Ethylendioxy)-5β-pregn-3-en-3-yl 3-Nonaflate, Mixture of Isomers (**10**)

Ketone **8** (260 mg, 0.7 mmol), lithium diisopropylamide (2.1 mmol), and nonafluorobutanesulfonyl fluoride (0.6 ml, 3.4 mmol) were converted to mixture of nonaflates **10** according to General Procedure 3.3.3. Purification by prep-TLC (6 plates) developed with petroleum ether/ether (9:1) afforded mixture of Δ^2 and Δ^3 isomers **10** (269 mg, 58%) and starting ketone **8** (35 mg, 13%). The Δ^3 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.75 s, 3 H (3 × H-18); 1.00 s, 3 H (3 × H-19); 1.29 s, 3 H (3 × H-21); 3.85–4.0 m, 4 H (OCH_2CH_2O); 5.46 s, 0.66 H (H-4); 5.62 d, 0.33 H, J = 6.0 (H-2). IR ($CHCl_3$): 1471, 1373 (CH_2, acetal); 1415, 1353, 1242, 1202 (SO_2, nonaflate); 1245, 1145 (CF_3, nonaflate). FAB MS: 643 (1%, M + 1); 413 (1.5%, M – $C_4F_9SO_3$). For $C_{27}H_{35}F_9O_5S$ (642.6) calculated: 50.46% C, 5.49% H; found: 50.59% C, 5.59% H.

3.5.2.2. Methyl 20-Oxo-5β-pregn-2-ene-3-carboxylate and
Methyl 20-Oxo-5β-pregn-3-ene-3-carboxylate, Mixture of Isomers (**12**)

Mixture of nonaflates **10** (270 mg, 0.4 mmol) was converted to mixture of esters **12** according to General Procedure 3.3.6. Purification by prep-TLC (3 plates) developed with petroleum ether/ether (4:1) afforded mixture of Δ^2 and Δ^3 isomers **12** (50 mg, 28%). The Δ^3 isomer, identical with the sample prepared by Procedure 3.5.1.2, prevailed in the ratio 6:1 according to ^{1}H NMR spectrum.

3.5.2.3. Methyl 20-Oxo-5β-pregnane-3α-carboxylate (**16**) and
Methyl 20-Oxo-5β-pregnane-3β-carboxylate (**17**)

Mixture of methyl esters **12** (38 mg, 0.1 mmol) was converted to 3α- and 3β-carboxy derivatives **16** and **17** according to General Procedure 3.3.8. 3α-Carboxy derivative **16** prevailed over 3β-carboxy derivative **17** in the ratio 5:1 according to the ^{1}H NMR spectrum. Preparative HPLC chromatography (9% ethyl acetate/hexanes) gave 21 mg (55%) of 3α-carboxy derivative **16** and 5 mg (13%) of 3β-carboxy derivative **17**, identical with the samples prepared in Procedure 3.5.1.3.

3.5.2.4. 20,20-(Ethylenedioxy)-5β-pregn-3-en-3-yl 3-Nonaflate (**14**)

Compound **8** (200 mg, 0.6 mmol), lithium bis(trimethylsilyl)amide (1.0 M in hexanes, 1.5 ml, 1.5 mmol) and nonafluorobutanesulfonyl fluoride (0.42 ml, 2.4 mmol) were converted to **14** according to General Procedure 3.3.4. Purification by prep-TLC (2 plates) developed with petroleum ether/ether (4:1) afforded nonaflate **14** (102 mg, 29%) and starting ketone **8** (65 mg, 32%). Nonaflate **14**: m.p. 86–88 °C (acetone/heptane), $[\alpha]_D$ +28.7 (*c* 0.23, $CHCl_3$). ^{1}H NMR (400 MHz): 0.76 s, 3 H (3 × H-18); 0.99 s, 3 H (3 × H-19); 1.29 s, 3 H (3 × H-21); 3.87–4.00 m, 4 H (OCH_2CH_2O); 5.46 s, 1 H (H-4). IR ($CHCl_3$): 1470, 1373, 1055 (CH_2, acetal); 1415, 1353, 1242, 1202 (SO_2, nonaflate); 1242, 1145, 1032 (CF_3, nonaflate). FAB MS: 622 (5%, M – 1 – 1 × F); 597 (20%, M – 1 – OCH_2CH_2); 585 (13%, M – 3 × F); 553 (4%, M – 1 – CF_3, 1 × F). For $C_{27}H_{35}F_9O_5S$ (642.6) calculated: 50.46% C, 5.49% H; found: 50.33% C, 5.38% H.

3.5.2.5. Methyl 20-Oxo-5β-pregn-3-ene-3-carboxylate (**15**)

Nonaflate **14** (183 mg, 0.3 mmol) was converted to ester **15** according to General Procedure 3.3.6. Crystallization from petroleum ether/ether afforded Δ^3 isomer **15** (22 mg, 22%), identical with the sample prepared by Procedure 3.5.1.4.

3.5.2.6. Methyl 20-oxo-5β-pregnane-3α-carboxylate (**16**) and
Methyl 20-oxo-5β-pregnane-3β-carboxylate (**17**)

Methyl ester **15** (17 mg, 0.05 mmol) was converted to 3α- and 3β-carboxy derivatives **16** and **17** according to General Procedure 3.3.8. 3α-Carboxy derivative **16** prevailed over 3β-carboxy derivative **17** in the ratio 12:1 according to the ^{1}H NMR spectrum. Preparative HPLC chromatography (9% ethyl acetate in hexanes) gave 7 mg (41%) of 3α-carboxy derivative **16** and 1 mg (6%) of 3β-carboxy derivative **17**, identical with the samples prepared by Procedure 3.5.1.3.

3.5.3. 5β-Pregnane-3-carboxylic Acids

3.5.3.1. 20-Oxo-5β-pregnane-3α-carboxylic Acid (**18**)

Ester **16** (45 mg, 0.12 mmol) was converted to acid **18** according to General Procedure 3.3.9. Crystallization afforded desired acid **18** (25 mg, 58%): m.p. 133–136 °C, $[\alpha]_D$ +81.6 (*c* 0.25, $CHCl_3$). ^{1}H NMR (400 MHz): 0.60 s, 3 H (3 × H-18); 0.95 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.39 tt, J_1 = 12.5, J_2 = 3.9, 1 H (H-3); 2.54 t, 1 H, J = 9 (H-17). IR ($CHCl_3$): 1732 (C=O, COOH, monomer); 1700 (C=O, COOH, dimer and C=O, ketone). FAB MS: 347 (10%, M + 1); 329 (35%, M – OH); 301 (15%, M – COOH). For $C_{22}H_{34}O_3$ (346.5) calculated: 76.26% C, 9.89% H; found: 76.03% C, 9.68% H.

3.5.3.2. 20-Oxo-5β-pregnane-3β-carboxylic Acid (**19**)

Ester **17** (14 mg, 0.03 mmol) was converted to acid **19** according to General Procedure 3.3.9. Crystallization afforded desired acid **19** (11 mg, 87%): m.p. 212–215 °C, $[\alpha]_D$ +94.0 (*c* 0.20, $CHCl_3$). ^{1}H NMR (400 MHz): 0.60 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.77 m, 1 H (H-3); 2.53 t, J = 9, 1 H (H-17). IR ($CHCl_3$): 3517 (C–O, COOH, monomer); 3090, 2743 (C–O, COOH, dimer); 1737 (C=O, COOH, monomer); 1700 (C=O, COOH, dimer and C=O, ketone). FAB MS: 347 (21%, M + 1); 329 (22%, M – OH); 301 (20%, M – COOH). For $C_{22}H_{34}O_3$ (346.5) calculated: 76.26% C, 9.89% H; found: 76.56% C, 9.98% H.

4. BIOLOGICAL MATERIALS AND METHODS

4.1. Cell Culture

Primary dissociated hippocampal cultures were prepared from 1- to 2-day-old postnatal rats. Animals were decapitated and the hippocampi dissected. Trypsin digestion, followed by mechanical dissociation, was used to prepare cell suspension. Single cells were plated at a density of 500 000 cells/cm^2 on 31 mm or 12 mm polylysine-coated glass cover slips. Neuronal cultures were maintained in Neurobasal™-A (Invitrogen, Carlsbad, USA) medium supplemented with glutamine (0.5 mM) and B-27 Serum-Free Supplement (Invitrogen) at 37 °C and 5% CO_2.

4.2. Electrophysiology

For the electrophysiological experiments, neurons cultured for 5–10 days were used. Whole-cell voltage-clamp recordings were made with a patch-clamp amplifier (Axopatch 1D; Axon Instruments, Inc., Foster City, USA) after capacitance and series resistance (<10 MΩ) compensation of 80–90%. Agonist-induced responses were low-pass filtered at 1 kHz with an 8-pole Bessel filter (Frequency Devices, Haverhill, USA), digitally sampled at 5 kHz and analyzed using pClamp software version 9 (Axon Instruments). Patch pipettes (3–6 MΩ) pulled from borosilicate glass were filled with Cs^+-based intracellular solution containing 125 mM

gluconic acid, 15 mM CsCl, 5 mM EGTA, 10 mM HEPES, 3 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 2 mM ATP-Mg salt (pH-adjusted to 7.2 with CsOH). Extracellular solution (ECS) contained 160 mM NaCl, 2.5 mM KCl, 10 mM HEPES, 10 mM D-glucose, 0.2 mM EDTA and 0.7 mM $CaCl_2$ (pH-adjusted to 7.3 with NaOH). Glycine (10 μM) and TTX (0.5 μM) were present in the control and test solutions. Steroids were dissolved in dimethyl sulfoxide (DMSO) to make a fresh stock solution (20 mM) before each experiment. The same concentration of DMSO was maintained in all extracellular solutions. A microprocessor-controlled multibarrel fast perfusion system, with a time constant of solution exchange around cells of ~10 ms, was used to apply test and control solutions.

We are indebted to Dr. S. Vašíčková for taking IR spectra and Dr. P. Fiedler for interpreting them. Optical rotations were measured and elemental analyses were carried out in the Analytical Laboratory (Dr. S. Matějková, Head). This work was supported by the Czech Science Foundation (grants No. 203/08/1498 and No. 309/07/0271), the Academy of Sciences of the Czech Republic (research projects Z4 055 0506 and Z 5011 0509) and Ministry of Education, Youth and Sports of the Czech Republic (1M0002375201 and LC554).

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