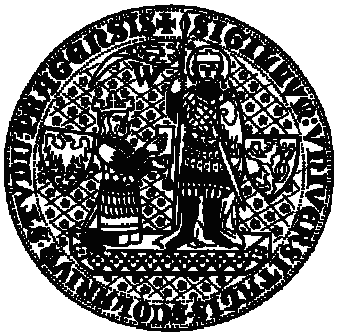
Charles University in Prague

Faculty of Science

Department of Organic and Nuclear Chemistry



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Synthesis of Neurosteroids:

Modulators of NMDA Receptor

Ph.D. Thesis

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Declaration

The Thesis was worked out at the Institute of Organic Chemistry and Biochemistry, v.v.i., Academy of Sciences of the Czech Republic, from 2004 to 2009.

"I hereby declare that I have done this Thesis independently while noting all resources used, as well as all co-authors."

Prague, 10th February 2009

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Signature

Abbreviations

Ac	acetyl
n-BuLi	butyl lithium
BBN	9-borabicyclo[3.3.1]nonane
Bu	butyl
t-BuOK	potassium *tert*-butoxide
Bz	benzoyl
d	day
dppp	1,3-bis(diphenylphosphino)propane
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
eq or equiv	equivalent
Et	ethyl
EtOH	ethanol
Et_3N	triethylamine
EtOAc	ethyl acetate
h	hour
HPLC	high-performance liquid chromatography
LDA	lithium diisopropylamide
LiHMDS	lithium hexamethyldisilazane
Me	methyl
MeOH	methanol
Nf	nonafluorobutanesulfonyl
NfF	nonafluorobutanesulfonyl fluoride ($C_4H_9SO_2F$)
NMDA	N-methyl-D-aspartic acid
PCC/Al_2O_3	pyridinium chlorochromate on alumina
$Pd(OAc)_2$	palladium(II) acetate
$Pd(PPh_3)_4$	tetrakis(triphenylphosphine)palladium(0)
Ph_3P	triphenylphosphine
PLC	preparative thin layer chromatography
rt	room temperature
TBDMS	*tert*-butyldimethylsilyl

Tf	trifluoromethanesulfonyl (CF_3SO_2)
Tf_2NPh	N-phenyltrifluoromethanesulfonimide
Tf_2O	triflic anhydride [$(CF_3SO_2)_2O$]
THF	tetrahydrofuran
TLC	thin layer chropmatography
TMS	trimethylsilyl
p-TsOH	*p*-toluenesulfonic acid monohydrate

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1. Introduction

1.1. L-Glutamic Acid and Its Role in Central Nervous System

The nerve impulse in a central nervous system is transmitted from one neuron to the next by relaying "the message" across the synaptic cleft using neurotransmitters, which diffuse across the gap. The neurotransmitters bind to receptor sites on the neighboring (postsynaptic) neuron, which results in opening of ion channels. The flow of ions subsequently results in a change in transmembrane potential and the generation of an action potential that is propagated down the processes (dendrites and axons) of the neuron to the next in line.

L-Glutamic acid (glutamate), the major excitatory neurotransmitter in the mammalian central nervous system, is released by nerve cells in the brain. It is now generally assumed that ~50% of all synapses in the central nervous system are glutamatergic. Under normal conditions glutamate is released from the presynaptic site and its extracellular concentration transiently reaches ~1 mM[1]. Specific transporting enzymes remove the glutamate from the extracellular space efficiently during several ms and under physiological conditions the steady state extracellular concentration is negligible (<0.01 μM). This periodic release of glutamate is crucial for brain functions including learning and memory. However, under many pathological conditions the extracellular glutamate concentrations may be increased to tonically activate specific receptors resulting in over-excitation, which could induce irreversible processes in neurons including cell death – excitotoxicity[2-5].

1.2. Ionotropic Receptors

Glutamate activates three families of ionotropic (ligand-gated ion channels) receptors, which all possess ion channels that are permeable to cations, although the relative permeability to K^+, Na^+ and Ca^{2+} varies according to the family and subunit composition of the receptor. Their names are based upon the pharmacological agonist that binds to the specific receptor subtype and selectively opens the associated ion channel: the *N*-methyl-D-aspartate receptor (NMDA), the kainic acid receptor (KA), and the α-amino-3-hydroxy-5-

methyl-4-isoxazole propionic acid receptor (AMPA). Those receptors are also reffered to as NMDA and non NMDA receptors[6].

1.3. Metabotropic Glutamate Receptors

Glutamate is also capable of activating a different group of receptors that are coupled via second messenger systems to enzymes and ion channels. These have been called metabotropic glutamate receptors (mGluRs), of which there are presently eight split into three different groups according to their second messenger systems, sequence homology and pharmacology. They are composed of polypeptides that have a putative 7 trans-membrane spanning domain[7]. When an agonist binds to the mGluRs, activation of a variety of G-proteins occurs, resulting in the modulation of a variety of cellular functions, including current flow through voltage-gated ion channels[8].

1.4. The N-Methyl-D-Aspartate Receptors (NMDARs)

The NMDARs are referred as heteromeric complexes comprised of NR1, NR2, and/or NR3 subunits. Multiple NR1 subunits are in combination with at least one type of NR2 (A, B, C, and D). The NR3 subunit (A, B) does not form functional receptors alone, but can co-assemble with NR1/NR2 complexes[9].

NMDARs play a critical role in excitatory synaptic transmission, synaptic plasticity, and excitotoxicity[10]. The involvement of NMDARs in diverse processes reflects their unique features, which include voltage-sensitive block by extracellular Mg^{2+}, a high permeability to Ca^{2+} and unusually slow "activation/deactivation" kinetics. NMDARs also display sensitivity to endogenous and exogenous ligands. These include binding sites for the co-agonists glycine that must be bind before the receptors can be activated by glutamate; binding sites for open channel blockers such as MK-801 (dizocilpine), and for polyamines, protons, Mg^{2+}, Zn^{2+}, and also neurosteroids[11]. ***Table I*** summarize the voltage-independent regulation of the NMDA receptor by a host of structurally unrelated compounds and ions. For details, see the original paper[12].

Modulator	*Effect*	*EC_{50}*	*Maximal Effect*
Dynorphin	Inhibition	0.3 mM[a]	100%
Osmotic pressure	Inhibition		75%
Oxidizing agents	Inhibition		70%[b]
Protons	Inhibition	50–200 nM[c]	100%
Sulfated steroids	Inhibition	150 mM[d]	100%
Zn^{2+}	Inhibition	0.2–2 mM[e]	100–80%
Arachidonic acid	Potentiation	10 mM	2-fold
PACAP[f]	Potentiation	1 mM	3-fold
Polyamines, histamine	Potentiation	100 mM[g]	2-fold
Reducing agents	Potentiation		3-fold
Sulfated steroids	Potentiation	12 mM[h]	2.5-fold

Table I – [a] Dynorphin A 1-32. [b] The response of fully reduced receptors (e.g., dithiothreitol treated) is decreased by 70% by oxidizing agents.[c] Proton inhibition depends on subunit composition. [d] 20-oxo-5β-pregn-3β-yl sulfate. [e] NR2A-containing receptors are much more sensitive to extracellular Zn^{2+} than receptors containing other subunits; NR2C and NR2D are much less sensitive to Zn^{2+}. [f] Pituitary adenylyl cyclase-activating peptide. [g] Spermine. [h] 20-oxo-5β-pregn-3α-yl sulfate.

1.5. External Mg^{2+} Block of NMDARs

As it has been described previously, one unique feature of the NMDA receptor compared to other ligand-gated ion channels is the dual dependence of function on agonist binding and membrane potential. Binding of extracellular Mg^{2+} within the pore is strongly voltage-dependent, and this property dominates the physiological role of NMDA receptors[12,13]: at resting membrane potentials, the pore of NMDA receptor channels is blocked by extracellular Mg^{2+} preventing Ca^{2+} influx as well as monovalent cation influx[14,15]. The strong voltage dependence of this block, which is relieved with membrane depolarization (*e.g.* by intense activation of postsynaptic AMPA receptors) makes the Ca^{2+} signalling mediated by NMDA receptors conditional: Ca^{2+} influx occurs only with

presynaptic release of glutamate and coincident post-synaptic depolarization[13,16]. The resulting Ca^{2+} influx can trigger under specific conditions a variety of effects resulting in a memory acquisition or cell death. The details will be discussed in a Chapter *1.7*.

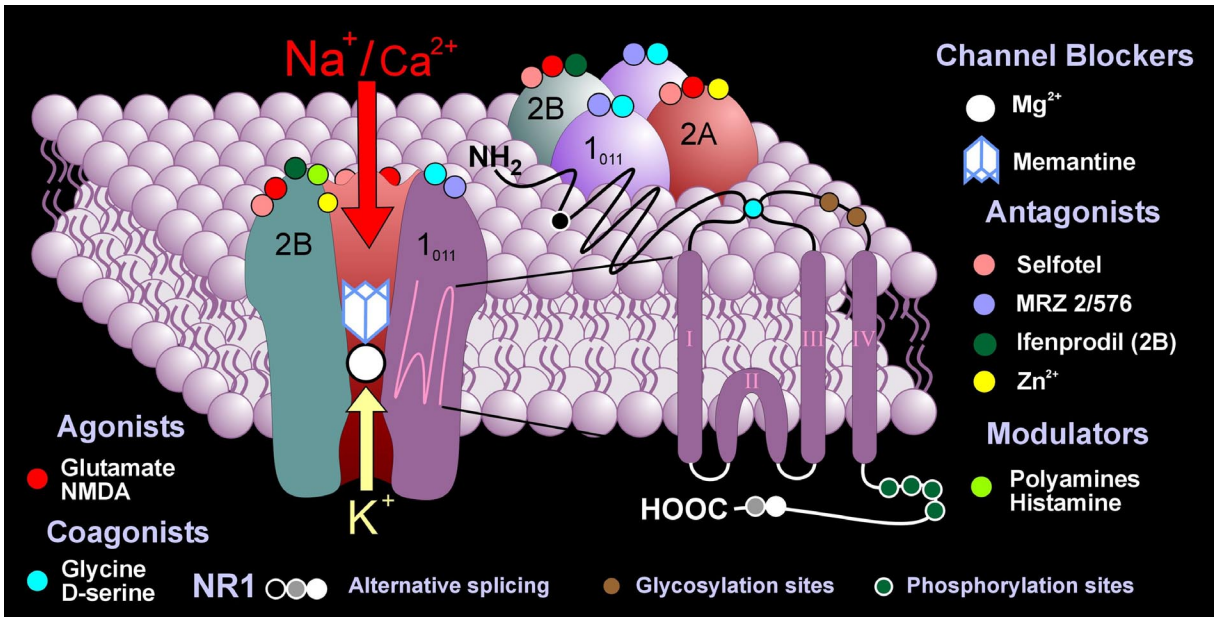


Figure I – NMDA receptor[17]

1.6. Extracellular Accumulation of Glutamate Induces Neurotoxicity [18]

For over 40 years, it has been recognized that glutamate and glutamate receptor agonists can cause toxicity in the nervous system[3,19-22]. Although acidic amino acids (*e.g.* glutamate) are not thought to readily cross the blood brain barrier, they do seem to enter the brain when the blood brain barrier is either not fully formed, such as is observed early in development, or when the blood brain barrier is compromised by injury as would occur with *e.g.* trauma. In fact, one of the first studies to demonstrate evidence for glutamate-mediated toxicity was performed in young mice with peripheral injections of glutamate[23]. During the subsequent 20 years, it became clear that several different non-endogenous excitatory amino acids cause neurotoxicity and the patterns of damage including regional and cellular specificity resembled that observed in neurodegenerative diseases, such as Huntington's, Alzheimer's, or Parkinson's disease[24,25]. In addition, several studies demonstrated that acute insults such as stroke or traumatic injury are associated with increase in the extracellular concentrations of glutamate and aspartate [26,27]. Finally, in the late 1980s, it was demonstrated

that glutamate receptor antagonists can attenuate the damage observed in animal models of these same acute insults[28]. These observations prompted several years of drug discovery efforts directed toward the development of glutamate receptor antagonists. Based on these efforts, there is very strong evidence that excessive activation of glutamate receptors can cause cell death both *in vitro* and *in vivo*.

1.7. General Mechanism of Cell Death Induced by Excess of Glutamate And Intracellular Ca^{2+} Elevation Results in Neurotoxicity [4]

When the brain suffers from an injury, some brain neurons die because of serious lack of oxygen, while other neurons die from resulting chemical reactions caused just by release of excess of glutamate onto nearby neurons. The premature unblocking of the NMDA receptor causes an increase in the entry of Ca^{2+} into the cell.

Calcium ions are ubiquitous intracellular messengers governing a large number of cellular functions such as the control of cell growth and differentiation, membrane excitability, exocytosis, and synaptic activity. Because of this, neurons must tightly regulate the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). Neurons have therefore evolved complex homeostatic mechanism to control both $[Ca^{2+}]_i$ and the intracellular location of Ca^{2+} ions. These mechanisms consist of complex interactions between four general categories of events: Ca^{2+} influx, Ca^{2+} buffering, internal Ca^{2+} storage, and Ca^{2+} efflux (***Figure II***)[4].

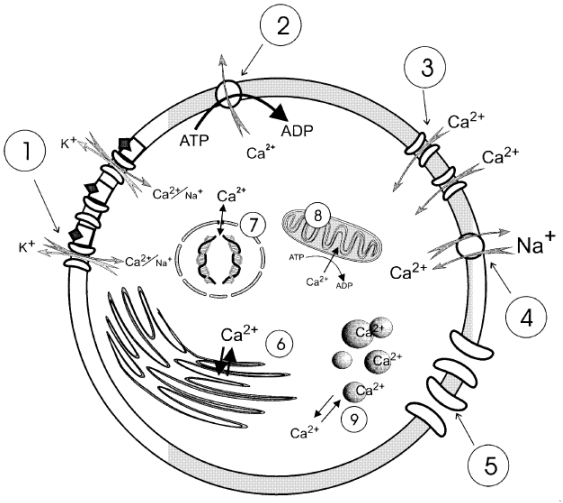


Figure II – A schematic representation of Ca^{2+} homeostasis in neurons. ***1*** Ca^{2+} and Na influx along with K efflux in receptorgated ion channels, such as glutamate receptors; ***2*** Ca^{2+} efflux

via an ATP-requiring ionic pump; ***3*** Ca^{2+} influx via voltage-gated Ca^{2+} channels; ***4*** Ca^{2+} efflux via Na/ Ca^{2+} exchanger; ***5*** additional ionic channels contributing to membrane repolarization and ionic homeostasis; ***6*** Ca^{2+} sequestration (and release) by endoplasmic reticulum; ***7*** Ca^{2+} fluxes through the nuclear membrane with potential effects on nucleic acid transcription; ***8*** Ca^{2+} sequestration by mitochondria; ***9*** intracellular Ca^{2+} buffering by Ca^{2+}-binding proteins. ***ATP*** Adenosine triphosphate; ***ADP*** adenosine diphosphate.

Under physiological conditions a delicate interplay between these processes allows multiple Ca^{2+} dependent signaling cascades to be regulated independently within the same cell. However, it is believed that excessive Ca^{2+} loading, exceeding the capacity of Ca^{2+}-regulatory mechanisms, may inappropriately activate Ca^{2+}-dependent processes which either lie dormant or normally operate at low levels. When overactivated, such processes including enzymes (*e.g.*, proteases, lipases, endonucleases) and other metabolic machinery directly damage neurons or lead to the formation of toxic reaction products which ultimately cause cell death (***Figure III***)[4].

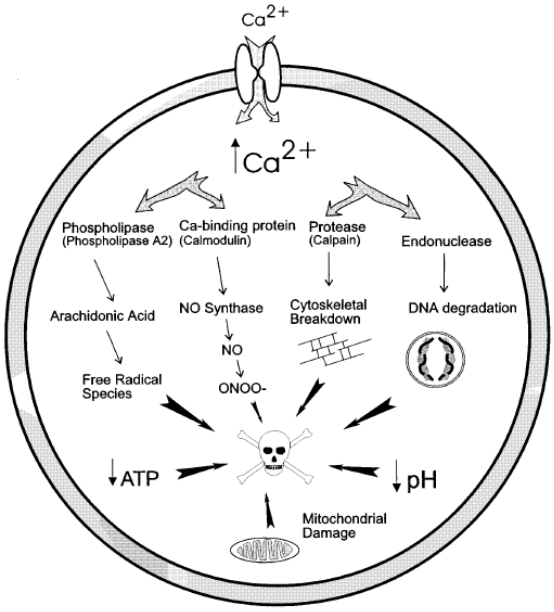


Figure III – A schematic and descriptive presentation of proposed mechanisms by which intracellular Ca^{2+} elevation (↑-*Ca*) may trigger secondary Ca^{2+}-dependent phenomena, which results in neurotoxicity. ***NO*** Nitric oxide; ***ONOO$^-$*** peroxynitrite; ***ATP*** adenosine triphosphate; ***ADP*** adenosine diphosphate; ***DNA*** deoxyribonucleic acid.

1.8. Neurosteroids as Modulators of Glutamate Receptors

As it has been mentioned previously, the neurosteroids can modulate the NMDARs. Neurosteroids are synthesized in the nervous tissue from cholesterol and/or from circulating precursors subsequently modified to neuroactive compounds[29]. In a literature, it is possible to find a term neuroactive steroids, which describes compounds having similar effects, but are not synthesized in nervous tissues, such as testosterone or alphaxalone[30]. The rapid effect of steroids has been already discovered in 1941 by Hans Seley[31] and a later development of anestheticum alphaxalone came just from Seley's discovery of anesthetic effect of steroid hormones[30]. The research relating to a mechanism of action of aphaxalone allowed revealing the influence of neurosteroids on receptors for neurotransmitters[32]. The effect of classic steroid hormones is recognized to function via intracellular receptors acting on the expression of genes with long-term effects on cell structure[33]. On contrary, neurosteroids do not act through classic steroid hormone nuclear receptors, but through neuronal membrane receptors for transmitters (*e.g.* γ-amino-butyric acid (GABA) receptors and the NMDA receptors), voltage-gated ion channels, and transporters. In addition, neurosteroids can act in central nervous system immediately[34] (within seconds or even milliseconds).

The modulatory effect of neurosteroids on the activity of ligand-gated ion channels was suggested[35,36] to play significant role in a lot of physiological processes (learning[37], aging[38], stress[39], etc.) as well as certain neurological and psychiatric disorders (Alzheimer's disease[40], epilepsy[41], etc.).

1.9. A Mechanism of Steroid Action and Steroid Binding Sites [42]

Defining a binding site of neurosteroids and the knowledge of the molecular mechanism could facilitate the creation of concept of a molecular template for design and development of novel therapeutic entities. The molecular mechanism by which neurosteroids affect NMDARs is still not clearly understood. It has been well established that neurosteroids exert a positive, negative, or combined effect on NMDA receptors[42-45].

Pregnenolone sulfate (PS), an endogenously occurring neurosteroid, acts in a subunit-dependent manner as a combined positive and negative modulator of NMDA receptors[42,46]. The mechanism of action of PS associated with a positive effect is disuse dependent

(*i.e.*, NMDA receptor affinity for PS is decreased during receptor activation) and involves an increase in the peak probability of the NMDA receptor opening (P_O).

In contrast, 20-oxo-5β-pregnan-3α-yl sulfate (pregnanolone sulfate, 3α5βS), also a naturally occurring neurosteroid that differs from PS in a single saturated bond, has an inhibitory action at NMDA receptors[45,47] is a use-dependent but voltage independent inhibitor that exerts its effect by reducing the peak P_O of NMDA receptor channels. Its inhibitory action is weaker for responses mediated by NMDA receptors activated by synaptically released glutamate than those tonically activated by the agonist.

The binding site for neurosteroids has not been identified unambiguously. Nevertheless, the results of current studies indicate that neurosteroids have their specific binding sites, independent of particular agonists or other allosteric modulators[48]. In addition it seems that steroid positive and negative modulators act through specific, extracellularly directed sites that are distinct from each other and from the sites of action of other known NMDA receptor modulators, such as spermine, redox, glycine, Mg^{2+}, MK-801, and arachidonic acid sites[49]. The experiments with chimeric receptors have shown crucial role of an extracellular loop between the third and fourth transmembrane domains of the NR2 subunit in the mechanism of potentiating and inhibitory effects of pregnenolone sulfate (PS) and 20-oxo-5β-pregnan-3α-yl sulfate (3α5βS)[10,42].

1.10. Structure-Activity Relationship for Modulation by Steroids

Without a doubt, a lot of other aspects of the receptor structure will influence the nature and extent of the neurosteroid modulation: the stereochemistry of the neurosteroids should be mentioned. The current knowledge can be summarized as follows[48-50] (***Figure IV***):

- The sulfate group of PS is not essential for potentiation of the NMDA response, but a negatively charged group at C-3 is required for activity which is retained if sulfate group is substituted by the hemisuccinate group, but not by hemisuccinate methyl ester substitution[48].
- The hook shaped steroid structure associated with 5β-stereochemistry favors receptor inhibition; whereas the more planar ring structure of the 5α-pregnanes favors potentiation of NMDA receptors: of the four possible reduced derivatives of PS, 3α5βS, 20-oxo-5β-pregnan-3β-yl sulfate (3β5βS), and 20-oxo-5α-pregnan-3α-yl

sulfate (3α5αS) are inhibitory, whereas 20-oxo-5α-pregnan-3β-yl sulfate (3β5αS) potentiates the NMDA response.

- The addition of a ketone group at C-7 or C-11 results in complete loss of activity, whereas 11β-hydroxy-PS, with a hydroxyl at C-11, is weak inhibitor.
- Compounds with modifications to the C-17 side chain, such as 20β-hydroxy-PS and 21-acetoxy-PS, still potentiate the NMDA response. Removal of the ring D side chain, as in dehydroepiandrosterone sulfate (DHEAS), markedly attenuated activity.
- 17-Hydroxy-PS, which differs from PS by the presence of a hydroxyl group at C-17, has activity similar to that of PS.

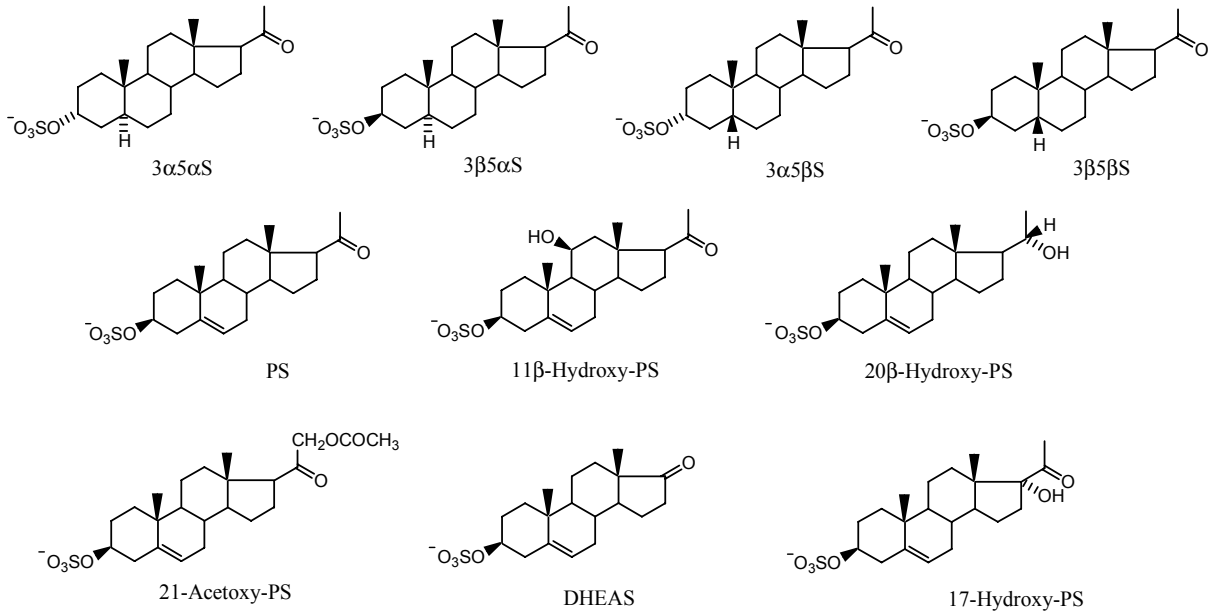


Figure IV – Structures of steroid modulators of the NMDA receptor response.

As it has been explained previously, the negatively charged group at the C-3 position in necessary for the activity. In addition, it was discovered that carboxylic acids esters of various lengths in some cases can substitute for sulfate at the C-3 position[50]: a serie of formate, hemioxalate, and hemiglutarate esters of 3α5β and pregnenolone (P) have been tested to elucidate charge and length effects on modulatory action on NMDA receptors (***Figure V***). It was revealed that the potentiating or inhibiting effect is maintained if the sulfate group is replaced by another negatively charged group, *i.e.* hemioxalate, hemisuccinate or hemiglutarate. The three negatively charged derivatives of 3α5β - pregnanolone hemisuccinate (3α5βHS), pregnanolone hemioxalate (3α5βHO), and

pregnanolone hemiglutarate (3α5βHG) are about equally effective to each other in inhibiting NMDA induced-currents, whereas the uncharged 3α5β and pregnanolone formate (3α5βF) have no significant effect. As regards derivatives of pregnenolone, the potentiation increases with chain length from pregnenolone hemioxalate (PHO) to pregnenolone hemisuccinate (PHS) and pregnenolone hemiglutarate (PHG). Similarly, the uncharged pregnenolone (P) and pregnenolone formate (PF) are ineffective.

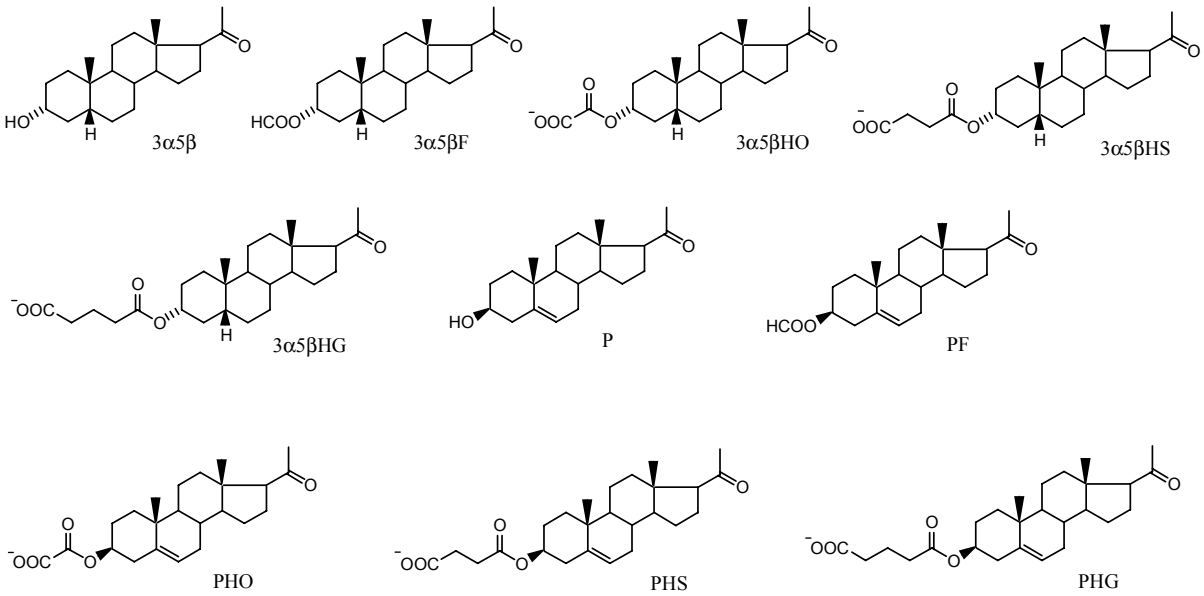


Figure V – Structures of steroid carboxylic acid esters

1.11. Conclusion

In view of universal role of glutamate in both physiological and pathological processes, it seems rational to consider glutamate receptor modulators, particularly antagonists as therapeutic agents to normalize glutamatergic function and as neuroprotective agents, respectively. The NMDA receptor seems to provide convenient target for therapeutic neuroprotective agents, as the high Ca^{2+} permeability of this receptor probably underlies its high neurotoxic potential[6,51].

From a point of view of steroid chemistry, is should be emphasize that hormonal steroid exert a variety of developmental and regulatory actions in the nervous system and are essential for adapting neural activity to modifications in the internal environment of organisms for an adequate homeostatic response. In addition, steroids produced locally in the nervous system, or neurosteroids, participate in the modulation of neural development and

function, acting as endogenous autocrine or paracrine modulators[52]. Therefore, synthesis of neurosteroids as potential modulators of NMDA receptor as well as neurosteroids structure-activity studies searching for steroid binding site may represent promising synthetic and therapeutic alternatives for the treatment of disorders of the central nervous system.

Table II – Neuroactive steroid mentioned in the text with their chemical nomenclature

3α5αS	20-Oxo-5α-pregnan-3α-yl sulfate
3β5αS	20-Oxo-5α-pregnan-3β-yl sulfate
3α5βS	20-Oxo-5β-pregnan-3α-yl sulfate
3β5βS	20-Oxo-5β-pregnan-3β-yl sulfate
PS	20-Oxo-pregn-5-en-3β-yl sulfate
11β-Hydroxy-PS	11β-Hydroxy-20-oxo-pregn-5-en-3β-yl sulfate
20β-Hydroxy-PS	(20R)-20-Hydroxy-pregn-5-en-3β-yl sulfate
21-Acetoxy-PS	20-Oxo-pregn-5-ene-3β,21-diyl 3-sulfate 21-acetate
DHEAS	17-Oxo-androst-5-en-3β-yl sulfate
17-Hydroxy-PS	17α-Hydroxy-20-oxo-pregn-5-en-3β-yl sulfate
3α5β	3α-Hydroy-5β-pregnan-20-one
3α5βF	20-Oxo-5β-pregnan-3α-yl formate
3α5βHO	20-Oxo-5β-pregnan-3α-yl hemioxalate
3α5βHS	20-Oxo-5β-pregnan-3α-yl hemisuccinate
3α5βHG	20-Oxo-5β-pregnan-3α-yl hemiglutarate
P	3β-Hydroxy-pregn-5-en-20-one
PF	20-Oxo-pregn-5-en-3β-yl formate
PHO	20-Oxo-pregn-5-en-3β-yl hemioxalate
PHS	20-Oxo-pregn-5-en-3β-yl hemisuccinate
PHG	20-Oxo-pregn-5-en-3β-yl hemiglutarate

2. Aims of the Work

The main aim of the Thesis was the structure-activity relationships study for steroid modulators on the NMDA response. Four parts of the Thesis has been focused on particular structure features that could significantly influence the biological activity of steroid on NMDA receptor. The last part of the Thesis was focused on synthetically interesting application of Luche reduction on saturated ketones of steroids.

In particular, the aims were to:

- Synthesize pregnane C3 and C7 substituted derivatives within the scope of "searching for essential substituents" of the pregnane derivatives with an anionic substituent in position C3 and particular substituent in position C7 that could be synthesized from commercially available steroids.
- Synthesize 3-carboxylic acids of steroids. The carboxyl group was chosen as it has been known that a negatively charged substituent on steroid in position C-3 is important for biological activity of neurosteroids at NMDA receptor. In addition, the C-C bond is metabolically more stable than the ester bond in case of sulfate group.
- Synthesize a pregnane derivative with C-3 substituent holding two carboxylic groups. The aim of this part of the Thesis was based on elementary question: If a negatively charged substituent is essential for the biological activity on NMDA receptor, in which way could be changed the activity by a substituent with two negatively charged groups?
- Synthesize the steroid carboxylic acids via Wadsworth-Horner-Emmons reaction. This part of the Thesis should lead to more stable ligands of NMDARs with anionic group joined to steroid in position 3 by spacer of 1-3 carbon atoms.
- Study and analyze conditions of sodium borohydride reduction of saturated ketones of steroids under the conditions of Luche reduction using cerium chloride heptahydrate, anhydrous cerium chloride, and samarium iodide, within the synthesis of steroid hydroxy derivatives, the widely used precursors for neurosteroid synthesis.

3. Known Synthetic Methodologies

3.1. Synthesis of Steroid Carboxylic Acids

Innumerable studies on the synthesis of carboxylic acids and their derivatives have been already reported. In general, carboxylic acids can be produced by oxidation of primary alcohols or aldehydes with strong oxidants such as potassium dichromate, Jones reagent, potassium permanganate, or sodium chlorite; by ozonolysis of alkenes and alkynes and they may also be produced by the oxidative cleavage of olefins by potassium permanganate, or potassium dichromate. With the addition of acid or base, they can be prepared by the hydrolysis if nitriles, esters, anhydrides, acid chlorides, or amides. Treatment of Grignard reagent or organolithium reagent with carbon dioxide followed by acidic work up is also efficient methodology for the synthesis of carboxylic acids.

Carboxylic acids may also form from the following reactions:

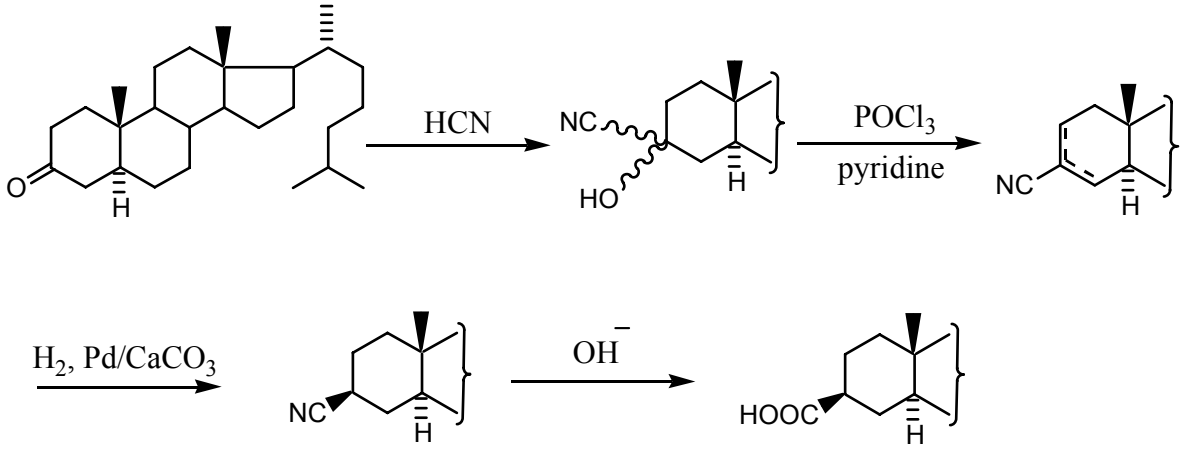
1. Disproportionation of aldehyde in the Canizzaro reaction.
2. Rearrangement of diketones in the benzilic acid rearrangement.
3. Rearrangement of cyclopropanones under basic conditions in the Favorskii reaction.
4. Halogenation followed by hydrolysis of methyl ketones in the haloform reaction.
5. Hydroformylation of an alkene followed by hydrolysis in the Koch reaction.

Section *3.1.* of the Known Synthetic Methodologies is focused only on procedures from literature those are leading to carboxylic acids that were prepared within this Thesis, their possible precursors or structural analogs.

3.1.1. Synthesis of Steroid 3-Carboxylic Acids and Its Derivatives

The second part of the Thesis was focused on the synthesis 3-carboxylic acids of 5α- and 5β-pregnanes. A reaction, which generates a new carbon-carbon bond, should proceed under mild reaction conditions that would not affect the protecting group of 20-keto group. 3-Carboxylic acids of steroids in general have been already prepared via cyanohydrin synthesis, by Grignard, or Wittig reaction and also by palladium catalyzed alkoxycarbonylation.

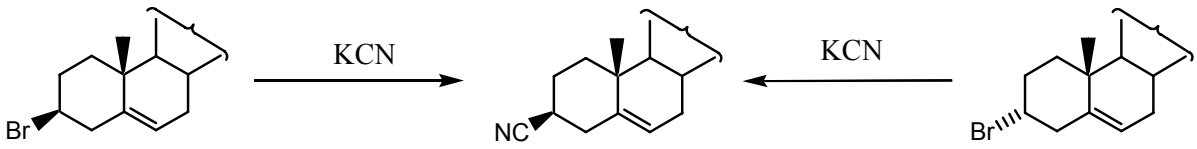
The work of Lábler *et al.*[53] describes quite easy and simple methodology via cyanohydrin reaction (***Scheme 1***); cholestanone was converted to the cyanohydrin which on dehydration with phosphorus oxychloride in pyridine yielded the dehydronitrile (3-cyanocholest-2-ene or 3-ene). Catalytic hydrogenation of this compound on palladium gave the nitrile of 5α-cholestane-3β-carboxylic acid, which on saponification yielded the desired carboxylic acid.



Scheme 1

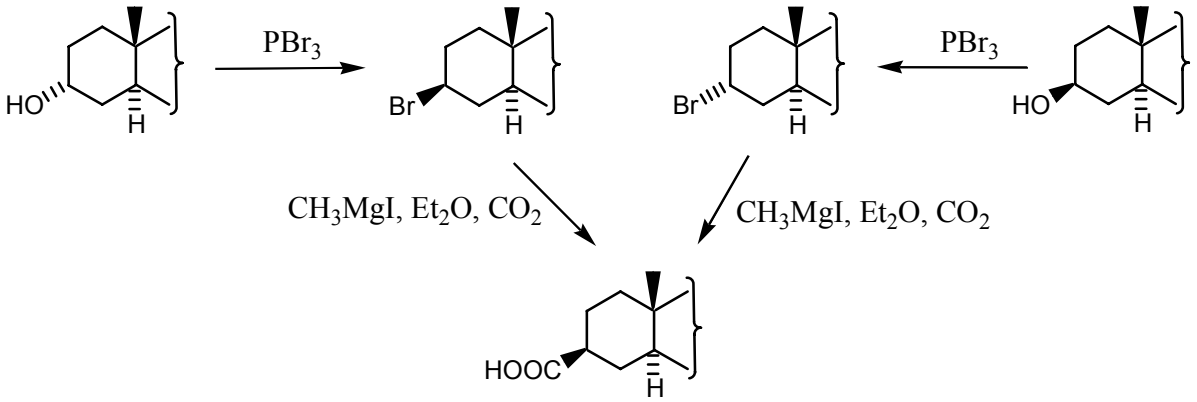
The Grignard reagents react with carbon dioxide to yield acid salts, which, upon acidification, produce carboxylic acids. This carboxylation reaction can be carried out by bubbling stream of dry CO_2 gas through a solution of Grignard reagent. Marker *et al.*[54] accomplished reactions of a cholesteryl chloride or bromide with magnesium affording Grignard reagents that undergo the reaction with carbon dioxide to proceed a mixture of 3-carboxylic acids. The configuration of the 3-carboxylic acid group of cholest-5-ene-3-carboxylic acid was not denoted. Because Marker had oxygenated the cholesteryl Grignard reagent and found the product to be a mixture of epicholesterol (3α-hydroxy group) and cholesterol, the carbon dioxide product was assumed to be

an equimolar mixture of 3α- and 3β-carboxylic acids. Nevertheless, later work indicated[55,56] so called Marker's acid to be at least 90% isomerically pure 3β-derivative. The stereochemistry of Grignard carboxylation was clarified in the work of Roberts *et al.*[57]; the preferential formation of 3β-carboxylic acid was explain on the model nucleofilic substitution of 3-bromo-cholest-5-ene epimers (***Scheme 2***) into 3-cyanoderivatives.



Scheme 2

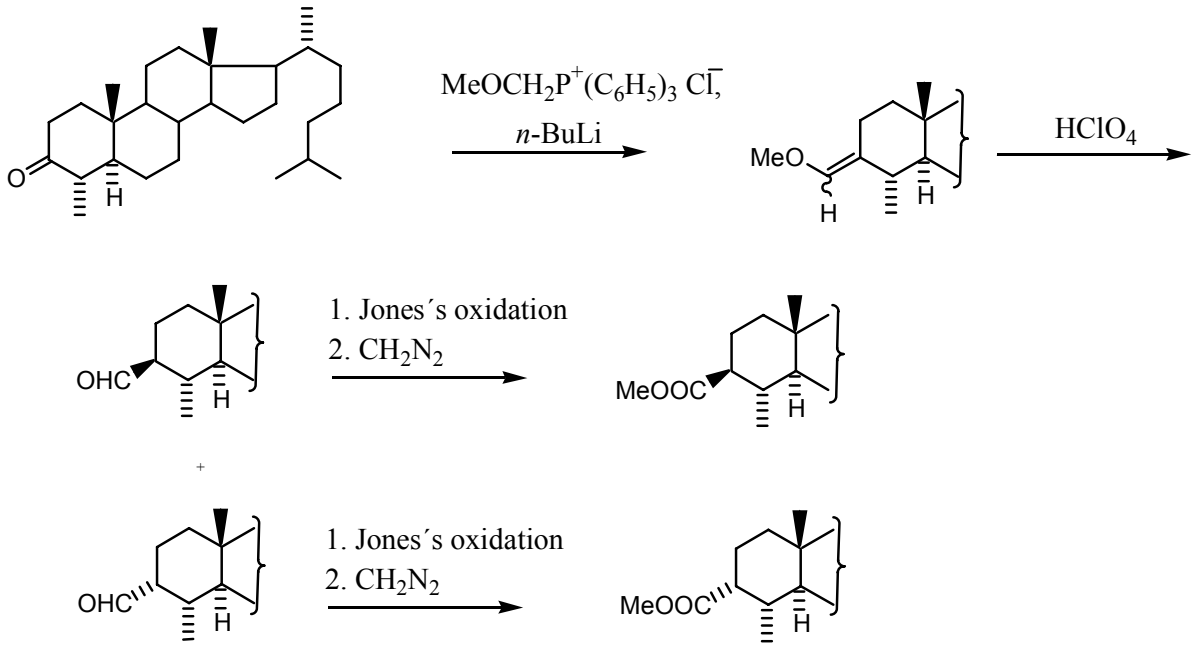
The authors regarded the reaction with retention of configuration as proceeding by an unimolecular substitution S_N1 involving the cholest-5-en-3β-yl cation (sp^3-hybridisation with a vacant π-orbital), whose stability is enhanced and whose configuration is preserved by interaction with the π-electrons of the 5(6)-double bond. Similarly, a Grignard carboxylation of the epimeric cholestanyl bromides gave one and the same 5α-cholestane-3β-carboxylic acid (***Scheme 3***), identical with the product obtained by hydrogenation of Marker's acid. The authors assumed that 3α-carboxylic acid was formed on the carboxylation of 3α-bromo derivative, but substantially undergoes complete epimerization (95% conversion) into more thermodynamically stable 3β-isomer under the conditions of the reaction.



Scheme 3

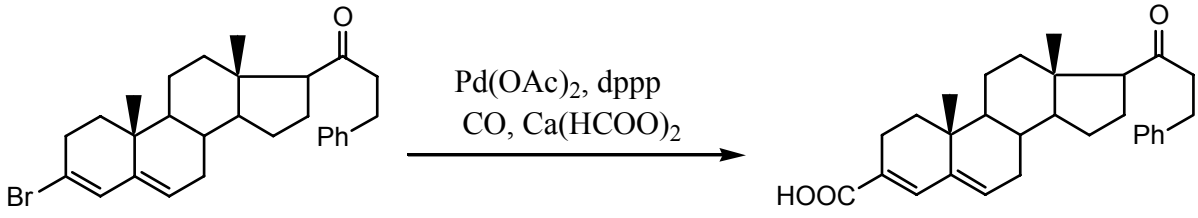
The identical Grignard methodology was used in the work of Squire[58] and Baker *et al.*[59]; steroidyl chloride was refluxed with methylmagnesium iodide to afford Grignard reagent that undergoes the reaction with carbon dioxide to obtain, after acidic work-up, the desired carboxylic acid.

An alternative methodology for synthesis of carboxylic acid is reaction of a ketone with particular Wittig reagent. In the work of Schaeffer *et al.*[60] (***Scheme 4***) methoxymethylenetriphenylphosphonium chloride was used as Wittig reagent. 4α-Methyl-5α-cholestan-3-one was converted into the mixture of two enol ethers. Acidic hydrolysis, Jones oxidation, and further esterification with diazomethane afforded methyl esters of 4α-methyl-5α-cholestane-3-carboxylic acids.

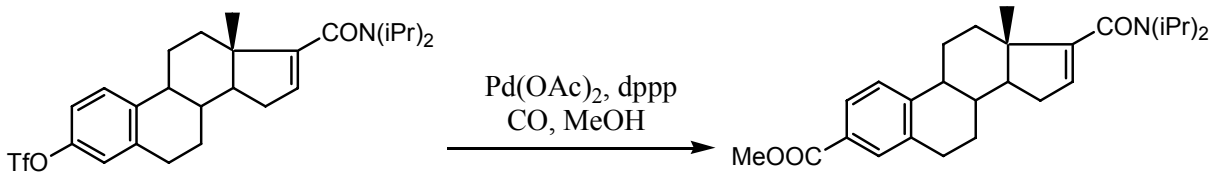


Scheme 4

Finally, palladium catalyzed alkoxycarbonylation of steroid with convenient leaving group at position C-3 could be used as an elegant and easy method for the synthesis of 3-carboxylic acids. The most frequently, vinyl or aryl chloride, bromide, or triflate are used for their excellent leaving group properties and facile synthesis. They react in the presence of a base and a catalytic amount of a Pd(II)-complex such as palladium acetate (***Scheme 5***)[61], (***Scheme 6***)[62]. Addition of phosphine ligand facilitates the reaction and might influence the regioselectivity.



Scheme 5

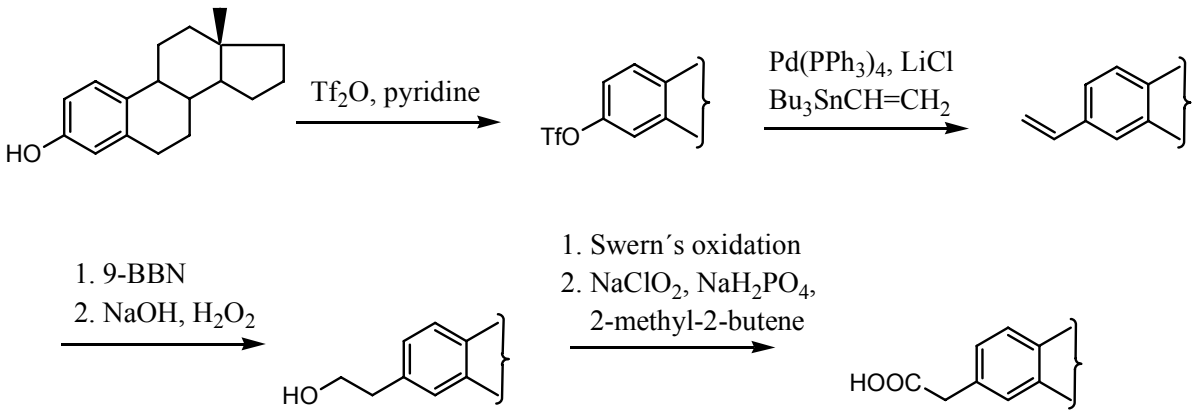


Scheme 6

3.1.2. Synthesis of 2-(Steroid-3-yl)acetic Acids and Its Derivatives

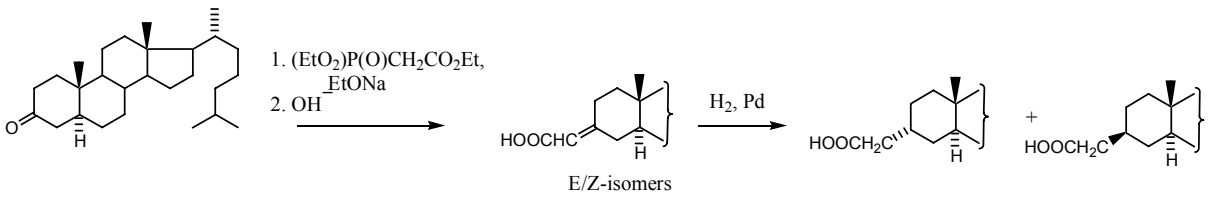
The most important step of the sequence of reactions leading to 2-(steroid-3-yl)acetic acid is formation of C-C bond. This requirement is possible to be passed by using cross-coupling reactions (*e.g.* Stille reaction) or by formation of C-C bond via Wadsworth-Horner-Emmons reagents.

Scheme 7 describes synthesis of 2-(steroid-3-yl)acetic acid[63] using Stille coupling to form C-C bond; the 3-hydroxy group of estrane was converted to triflate[64] that was subsequently coupled with tributylvinyltin under standard Stille coupling conditions[65,66]. 3-Vinylsteroid derivative undergoes hydroboration with 9-BBN and oxidative workup gave primary alcohol. Swern's procedure[67] followed by sodium chlorite oxidation[68] led to desired carboxylic acid.



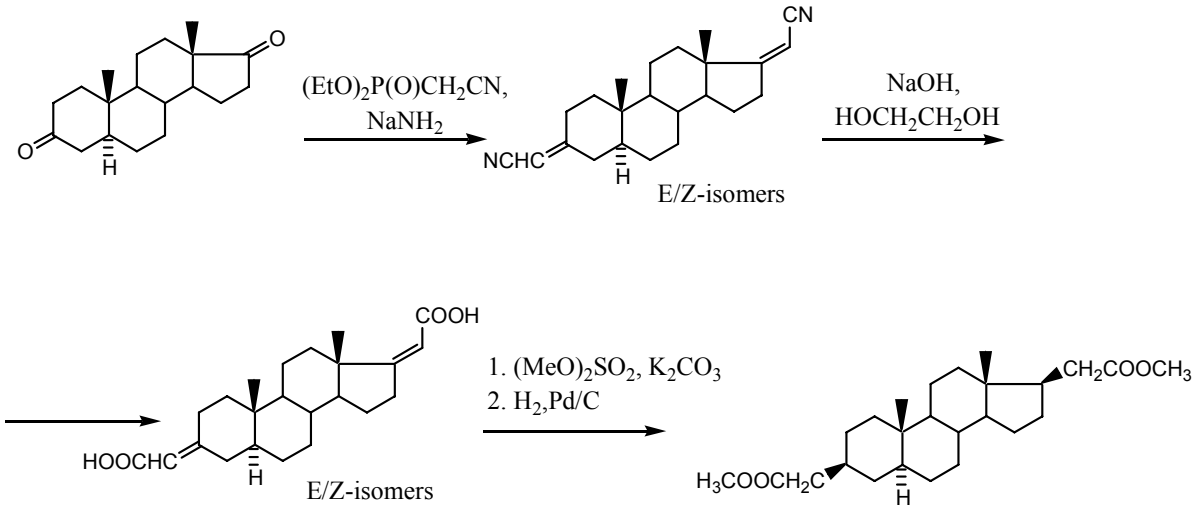
Scheme 7

Scheme 8 shows the approach of Schaeffer *et al.*[60] leading to 2-(5α-cholestane-3-yl)acetic acids. Unsaturated intermediates were obtained by a Wadsworth-Horner-Emmons reaction[69], starting from 5α-cholestan-3-one. Their catalytic hydrogenation on palladium catalyst gave a mixture of 3α- and 3β-isomers.



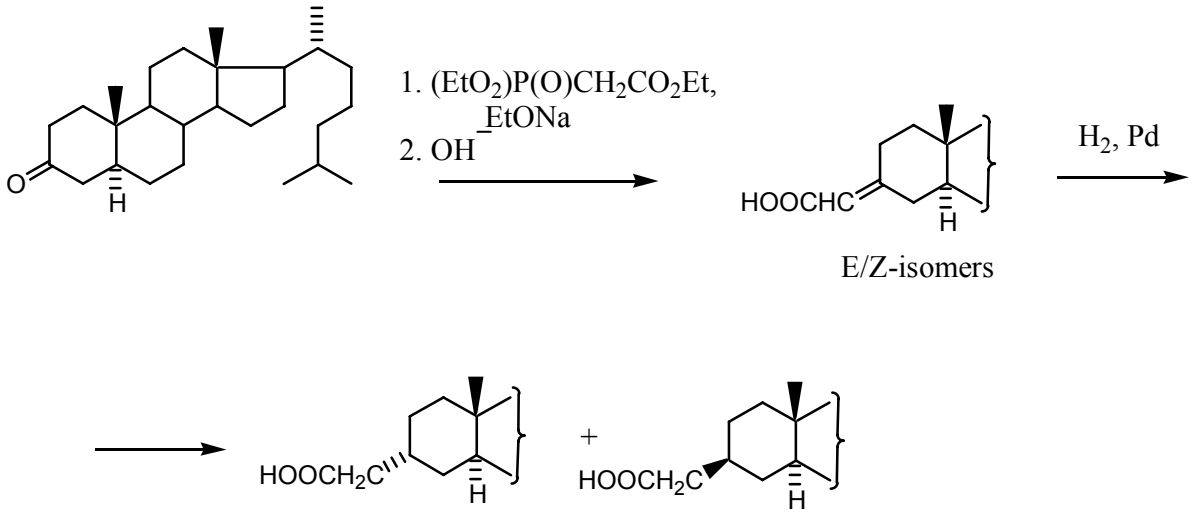
Scheme 8

Similar methodology was used in the work of Casimiro-Garcia *et al.* (***Scheme 9***)[70]; 5α-androstane-3,20-dione was converted to a mixture of nitriles followed by basic hydrolysis with potassium hydroxide in ethylene glycol. A mixture of diacids was then treated with dimethyl sulfate and potassium carbonate to afford a mixture of methyl E- and Z-unsaturated carboxylic acid esters. The last step of the synthesis, the stereoselective hydrogenation on palladium catalyst gave desired 3β,17β-isomers in high yield.



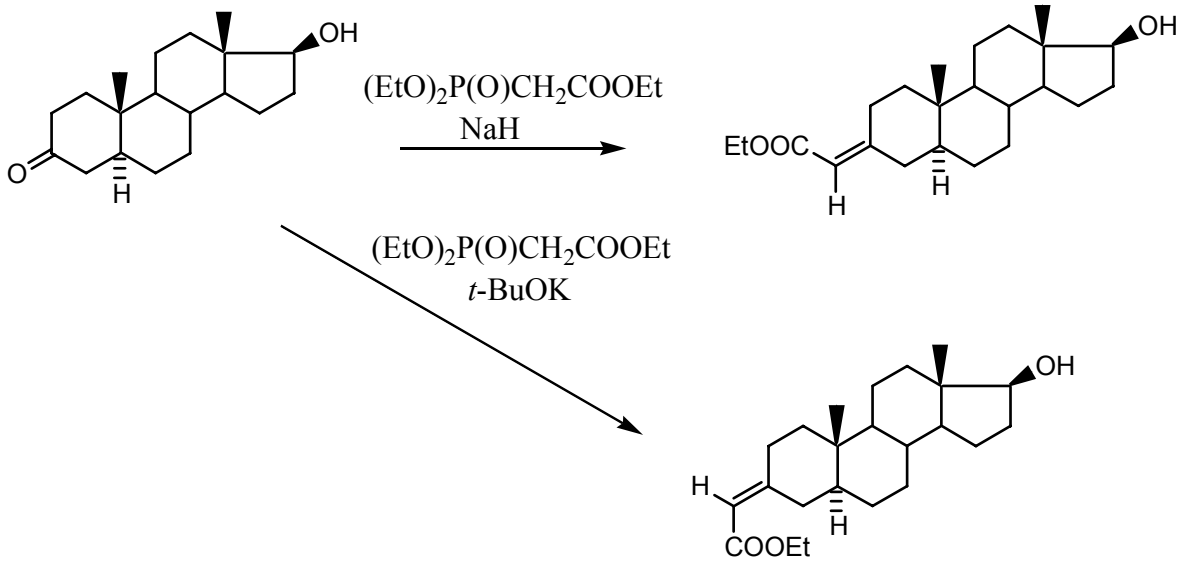
Scheme 9

In the work of Bose *et al.* (***Scheme 10***)[71], which was focused on synthesis of unsaturated steroid esters which could serve as intermediates for the introduction of the cortical side chain at different sites, the triethyl 2-phosphonoacetate was used as a Wadsworth-Horner-Emmons reagent. A mixture of unsaturated esters was firstly hydrolyzed, then hydrogenated on palladium catalyst to give a mixture of epimeric acids, which could be afforded individual 2-(5α-cholestane-3α-yl)acetic acid through crystallization.



Scheme 10

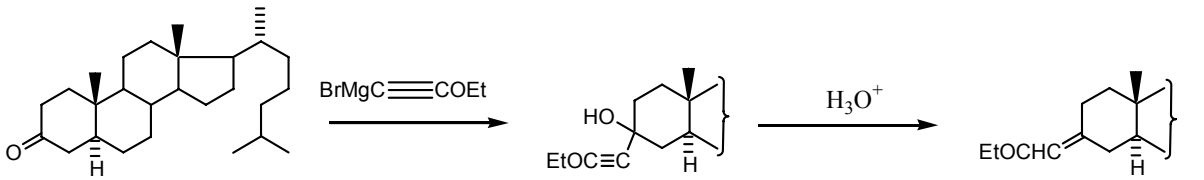
The identical Wadsworth-Horner-Emmons procedure was described in the work of Bose and Dahill[72] for 5α- and also 5β-cholestanone derivatives. Kaneko and Okazaki (***Scheme 11***)[73] found out that the stereochemistry of Wadsworth-Horner-Emmons using triethyl 2-phosphonoacetate is base-dependent in the case of 17β-hydroxy-5α-androstan-3-one. If one equivalent of sodium hydride or potassium *tert*-butoxide was used, the product was E- or Z-isomer, respectively.



Scheme 11

The previously mentioned product of Wadsworth-Horner-Emmons reaction, the ethyl ester of (E/Z)-2-(5α-cholestan-3-ylidene)acetic acid was also prepared by Milas *et al.* from 3-cholestanone and Grignard reagent from ethoxyacetylene, followed by acid rearrangement

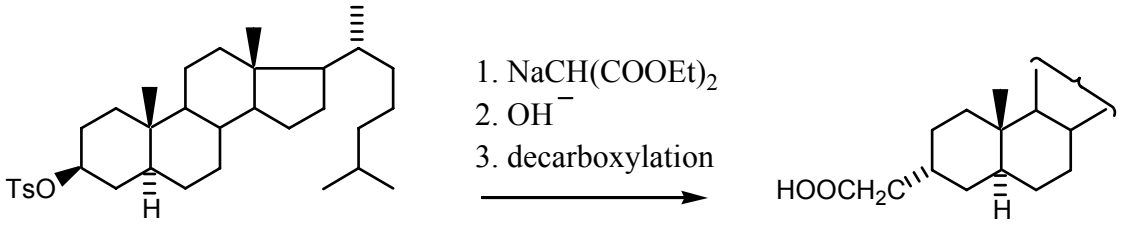
of resulting carbinol (***Scheme 12***)[74]. The acid rearrangement proceeded by treatment of steroid with tartaric acid and the stereochemistry of E/Z isomers was not specified.



E/Z-isomers

Scheme 12

In the work of Shoppee and Stephenson (***Scheme 13***)[75] the nucleophilic substitution was employed as the first step of the synthesis. 5α-Cholestane-3β-yl tosylate gave by condensation with sodium salt of dimethyl malonate, followed by alkaline hydrolysis and subsequent decarboxylation desired (5α-cholestane-3α-yl)acetic acid.

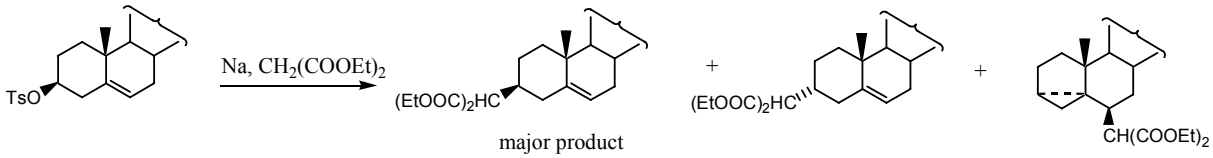


Scheme 13

3.1.3. Synthesis of 2-(Steroid-3-yl)propandioic Acids and Its Derivatives

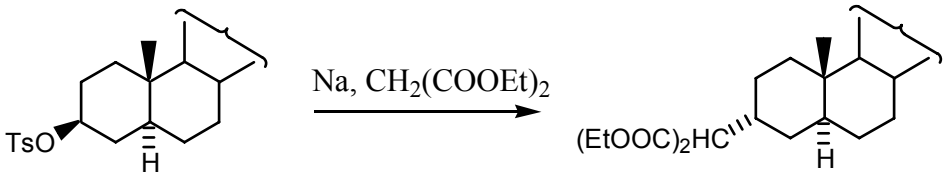
Interesting conclusions and discussion concerning condensation of cholest-5-en-3β-yl tosylate with diethyl sodiomalonate revealed a work of Shoppee and Stephenson[76] in which the authors repeated the chemistry of Kaiser and Svarz[77] and focused on the stereochemistry of previously mentioned condensation. The authors demonstrated that replacement reactions such as hydrolysis, acetolysis, etc., of cholesteryl chloride and tosylate, or more generally 3β-substituted-Δ^5-steroids, proceed with retention of configuration at C-3 or with rearrangement to 6β-substituted 3α,5α-cyclosteroids (***Scheme 14***). This was interpreted in terms of a unimolecular mechanism S_N1 leading to a carbonium ion in which configuration was maintained at C-3 by intervention of the π-electrons of 5(6)-double bond and under suitable conditions and with appropriate nucleophiles, the unimolecular substitution S_N1

leading to the product of retention (3β-isomer) or rearrangement (6β-substituted 3α,5α-cyclosteroids) may be accompanied by a bimolecular substitution S_N2 leading to inversion (3α-isomer).



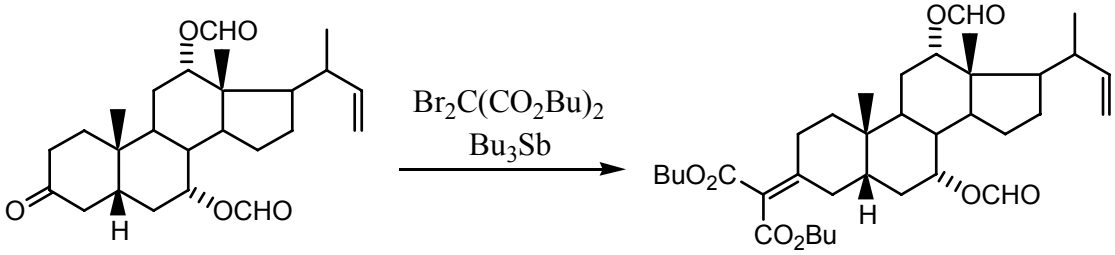
Scheme 14

In addition, Shoppee and Stephenson confirmed that condensation of saturated steroid with diethyl sodiomalonate is a substitution reaction S_N2 taking place with inversion of configuration (***Scheme 15***).



Scheme 15

Davis and Bhattarai[78] extended the procedure of Huang[79], who had accomplished the alkylidenation of carbonyl compound using combination of organoantimony or tellurium compounds with bromo- or diazo-malonate esters. While Huang focused mainly on aldehyde substrates, Davis and Bhattarai used this methodology to a broader range of ketones including steroid ketones. Due to problems of obtaining and handling organotellurium reagents, they decided to examine the antimony-based method. The authors established a set of conditions under which various forms of esters protection (including formyl ester) are not affected and the reaction proceeds smoothly in high yield. At the ***Scheme 16***, the representative procedure is depicted; the ketone was treated with dibutyl dibromomalonate and tributylstibine in THF to afford desired product.



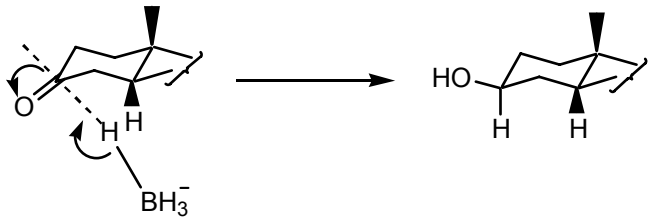
Scheme 16

The previously described procedure was also successfully used in the work of Bhattarai *et al.*[80] for a synthesis of cholaphanes's precursors (macrocyclic system of two molecules of cholic acid and convenient linkers).

3.2. Reduction of Steroid Ketones with Complex Hydrides

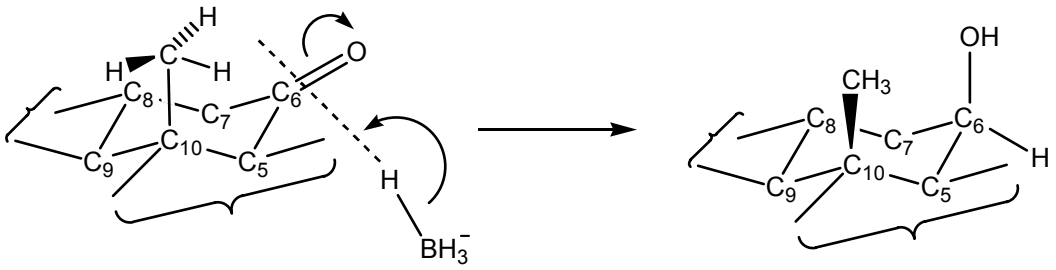
In a cyclohexane rings system, such as rings A, B, or C of the steroid nucleus, one of the substituents in sp^3 configuration of chair conformation must be axial and the relative stabilities of the two possible epimers will therefore be determined, apart from polar effects, by the relative steric requirements of the oxygen atom and the newly introduced atom or group. It can be predicted that the oxygen function will be more stable in an equatorial configuration when the other atom is small, *e.g.* H, while a bulky alkyl group, for example, is likely to enforce an axial configuration of the oxygen atom.

In the field of steroid chemistry, it has been postulated from Barton's recognition[81] that sterically unhindered ketones are reduced by complex hydrides to give products corresponding closely to thermodynamic equilibrium of the epimeric alcohols. Although later work has shown that significant deviations from the equilibrium ratio frequently occur, it remains true that unhindered ketones are reduced to give predominantly the more stable product, *i.e.* the equatorial alcohol (***Scheme 17***). Dauben[82] used the term "product development control", with implied acceptance of the view that the factors affecting the relative stabilities of the products are also able to influence the energy levels of the respective transition states.



Scheme 17

The less stable axial alcohols predominate when the approach of the reducing agent is hindered by the presence of an angular methyl group (***Scheme 18***). This effect is most pronounced in the exclusive formation of 11β-alcohols[83], as a result of shielding of the β-face of the 11-ketone by both the C-18 and the C-19 methyl groups. Similar effects involving only C-19 methyl group lead to high yields (>90%) of axial 4β- and 6β-alcohols from corresponding ketones. Dauben named this "steric approach control", considering that the bulky axial methyl groups prevent close approach of the complex hydride anion from the β-direction.



Scheme 18

The stereochemistry of reduction of 7-ketones with complex hydrides is highly dependent on the reagent used and the neighboring groups (double bond and 14-methyl group)[84]. An approach of the hydride reagent from the β-side appears to be also hindered by the C-18 and the C-19 methyl groups. Accordingly, the 7β-alcohol, which is also the thermodynamically more stable epimer, should be preferentially produced. Nevertheless, sodium borohydride reduction of 3β-hydroxy-5α-cholestan-7-one gave the 7α-ol and 7β-ol in a ratio 6:4. Reduction with lithium aluminium hydride increased the amount of the thermodynamically controlled product, *i.e.* the 7β-ol; however, only up to the ratio 1:1. Introduction of the 5(6)-double bond into the 7-ketones removes the 5α-axial hydrogen and facilitates the approach of the reagent from the α-side. Therefore, the amount of 7β-isomer in a mixture of 7α/7β-ol should be increased. The influence of the 14α-methyl group on complex hydride reduction appears to be mainly under "product development control", because 3β-hydroxylanostan-7-one gave a lower 7α/7β-ol ratio compared to 3β-hydroxy-4,4-dimethylcholestan-7-one and 3β-hydroxy-cholestan-7-one; despite the α-side attack of the reagent being highly hindered by the 14α-methyl group.

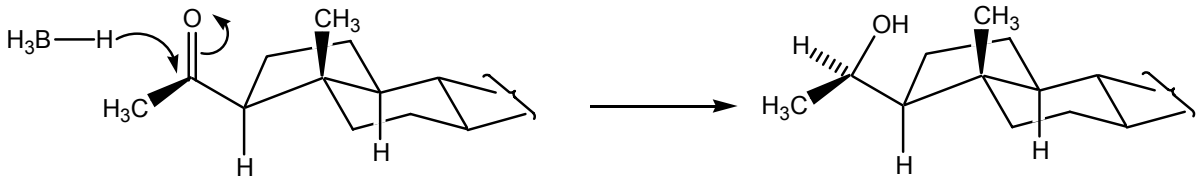
Ketones which afford mixtures of alcohols in comparable amounts may display a combination of the "steric approach" and "product development" control. Steroidal 12-ketones provide and interesting example of reactions apparently controlled mainly by product stabilities. The product ratio is determined by the nature of the side chain. A cholane or cholestane side chain in its stable conformation places the C-21-methyl group in steric opposition to a 12β-substituent with a significant increase in conformational strain energy, and the results in a preference formation of the axial 12α-alcohol by complex hydride reduction[85,86].

The pregnane side chain is somewhat less demanding in space, and may cause a slight preference for one or other of the epimeric pregnan-12-ols, according to the reagent used[87].

At C-2 it can be seen "steric approach control" and "product development control" in direct conflict, for although the axial 2β-alcohol is the predominant product when sodium borohydride is used in a polar solvent[88], this preference almost disappears when the reagent is lithium aluminium hydride in ether[82]. The anion in the later reagent is effectively quite small because of the weak anion-solvating power of ether, so the "steric approach" factor is small compared with the much more heavily solvated borohydride ion in a hydroxylic solvent. Even the sterically unhindered 3-ketones and simple analogues like 4-tert-butylcyclohexanone[89], give the equatorial alcohols in greater proportion with lithium aluminium hydride in ether than with sodium borohydride in hydroxylic solvents.

Reduction of D-ring ketones may be subjected to both steric and product development control. 17-Ketones give only 17β-alcohols with all the used reagents, as a result of nucleophilic attack from the less hindered α-direction. Steric hindrance on the β-face probably also explains the predominant formation of 16β-alcohols[90].

Reduction of 20-keto pregnanes with complex hydrides gives products which are invariably rich in the 20β-isomer (20R)[91-93]. This probably results to a large extent from "steric approach" control. The preferred conformation about C-17 – C-20 in pregnan-20-ones causes the carbonyl C=O bond to lie nearly eclipsing the C-17 – C-16 bond (***Scheme 19***). Hydride attack on the "front" face of the ketone is then restricted by the C-18 methyl group, and rear attack leading to the 20β-epimer predominates. Reduction that converts the 20-ketone exclusively into 20α-alcohol (20S) is apparently managed under "product development control"[93].



Scheme 19

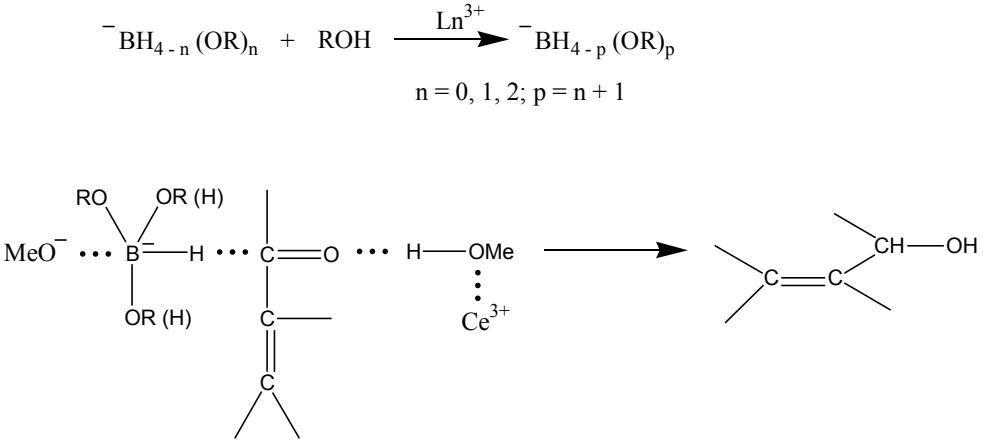
3.3. Luche Reduction

In the work from 1978[94] Jean-Louis Luche published efficient methodology for regioselective reduction of α-enones; treatment of an equimolecular amount of ketone and lanthanoid chloride in ethanol with sodium borohydride (1 molar equiv) produced a gas hydrogen evolution coupled with a quantitative yield of the corresponding alcohol in 5-10 min. Of the lanthanoids tested, samarium and cerium appeared to offer the best combination of yield and selectivity. The method evidently offers the following advantages. First, nearly exclusive selective 1,2-reduction is obtained under conditions which do not affect carboxylic acids, esters, amides, halides, cyano, and nitro groups. Further more, the reactions may be conduced at room temperature, without special exclusion of air or moisture, and excellent yields of products are obtained within 5 min.

In the work from 1981[95], the mechanistic, synthetic, and stereochemical aspects of the reaction were discussed and could be summarized as follows:

1. The best conditions found for maximum yield and regioselectivity were to employed 1 molar equiv of sodium borohydride for each mole of substrate in 0.4 M methanolic $CeCl_3 \cdot 6H_2O$. The effect of temperature on the stechiometry was not investigated.
2. The nature of the metallic ion was found to be an important factor for the regioselectivity. Among the lanthanoids tested, cerium gave the highest selectivity with most enones and was generally recommended.
3. The overall concentration in methanol is also in important factor; a decrease in the concentration of all the reacting species resulted in more selective reaction.
4. Methanol was found to provide the highest selectivity accompanied by a very high reaction rate.
5. The vigorous gas hydrogen evolution occurred as a result of formation of alkoxyborohydrides $NaBH_{4-n}(OR)_n$, those were established as the actual reducing species (***Scheme 20***).
6. The first reaction ($NaBH_4 + ROH \rightarrow NaBH_3OR + H_2$) was demonstrated to be the rate determing and the process terminates with the formation of tetraalkoxyborate $NaB(OR)_4$. The reducing specie, $NaBH(OCH_3)_3$, is stable in THF solution[96,97].
7. From the hard and soft acids and bases theory, it was deduced that the substitution of hydrides in BH_4^- by alkoxy groups increases the hardness of the reagent. The attack of the conjugate enone system is then enhanced at the hard site, *i.e.* carbon 2.

8. The previously mentioned data and the known fact[98] that the harder the reagent, the more favored the axial attack of cyclohexanone resulted in experimentally confirmed prediction that more enone reductions performed in the presence of Ce^{3+} yielded greater preference of the equatorial alcohols.
9. In parallel with α-enones, saturated ketones are also attacked on the axial side, with a selectivity enhanced by Ce^{3+}.

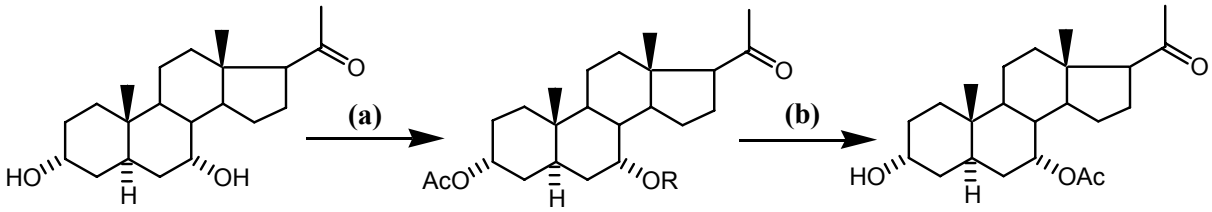


Scheme 20

4. Results and Discussion

4.1. Synthesis of C-3 and C-7 Substituted Pregnane Derivatives

The starting compound for the synthesis of 5α-derivatives, (20R)-pregn-5-ene-3β,20-dio **1**, was prepared according to literature[99]. Acetylation afforded diacetate **2** and selective hydrolysis of less stable acetate group, 7-monoacetate **4**, the starting compound for the synthesis of all derivatives of the 5α-serie was obtained in the yield of 85% (***Scheme 21***). Its structure was confirmed by comparison of 3β-H and 7β-H chemical shifts in ^{1}H NMR spectrum.

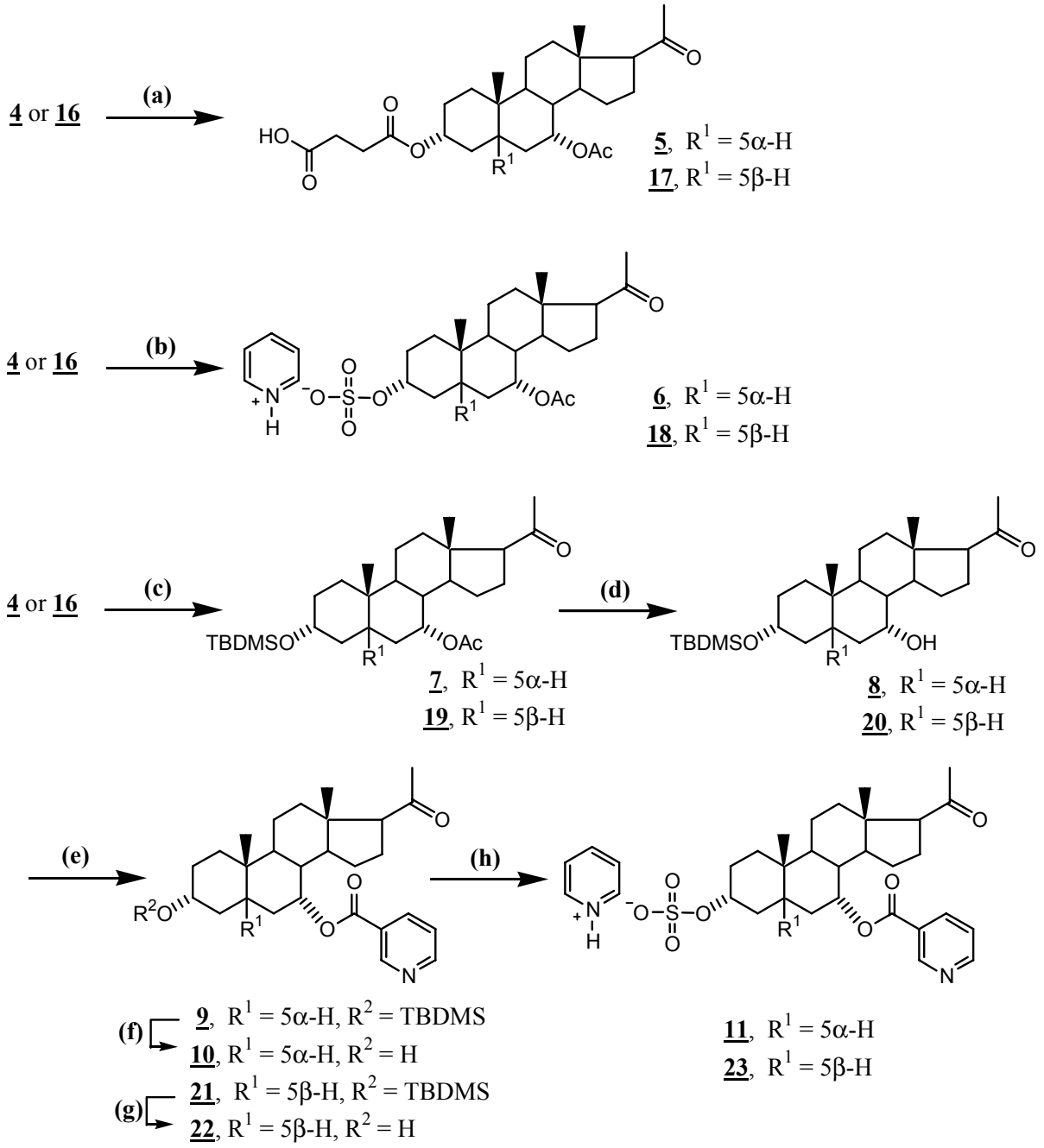


1 **2**, R = Ac; **3**, R = H **4**

Scheme 21 - Synthesis of compounds 2-4.
Reaction conditions: (a) Ac_2O, pyridine, 50 °C; (b) KOH, MeOH, benzene.

Treatment of monoacetate **4** with succinic anhydride in pyridine under catalysis by 4-dimethylaminopyridine (DMAP) gave hemisuccinate **5** in the yield of 35% (***Scheme 22***). Pyridinium salt of 3-sulfate **6,** the next derivative with a polar substituent in the position 3, was obtained by treatment of monoacetate **4** with a sulfur trioxide pyridine complex in chloroform. Preservation of 7-acetate group was confirmed by ^{1}H NMR spectrum. The synthesis of compound **10** (***Scheme 22***), derivative with a nicotinate group in the position 7, involved firstly protecting of a 3-hydroxy group as a *tert*-butyldimethylsilyl derivative by the reaction of monoacetate **4** with *tert*-butyldimethylsilyl chloride and subsequently selective hydrolysis of acetate protecting group by treating with methanolic solution of potassium hydroxide. Secondly, the free 7-hydroxy group was converted to nicotinate ester and finally, the *tert*-butyldimethylsilyl group of compound **9** was selectively cleaved by solution of *p*-toluenesulfonic acid in methanol. Using a solution of tetrabutylammonium fluoride, the reagent comfortable for cleavage of the *tert*-butyldimethylsilyl derivatives was not

successful, because lead to cleavage both *tert*-butyldimethylsilyl group and nicotinate group. On contrary, this reagent was successfully used in 5β-serie to afford desired compound **22**. The structure of compound **10** was confirmed by chemical shifts in ^{1}H NMR spectrum. Compound **10** was converted into desired sulfate pyridinium salt **11** in the total yield of 59% from compound **4** (***Scheme 22***).

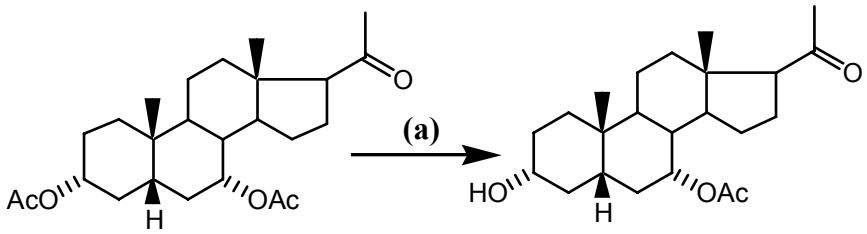


Scheme 22 - Synthesis of compounds 5-11 and 17-23.

Reaction conditions: (a) Succinic anhydride, pyridine, 4-dimethylaminopyridine, 140 °C; (b) sulfur trioxide pyridine complex, $CHCl_3$; (c) *tert*-butyldimethylsilyl chloride, imidazole, DMF; (d) KOH, MeOH, benzene, 60 °C; (e) nicotinoyl chloride hydrochloride,

4-dimethylaminopyridine, pyridine; (f) *p*-toluenesulfonic acid monohydrate, MeOH, rt; (g) tetrabutylammonium fluoride, THF, rt; (h) sulfur trioxide pyridine complex, $CHCl_3$.

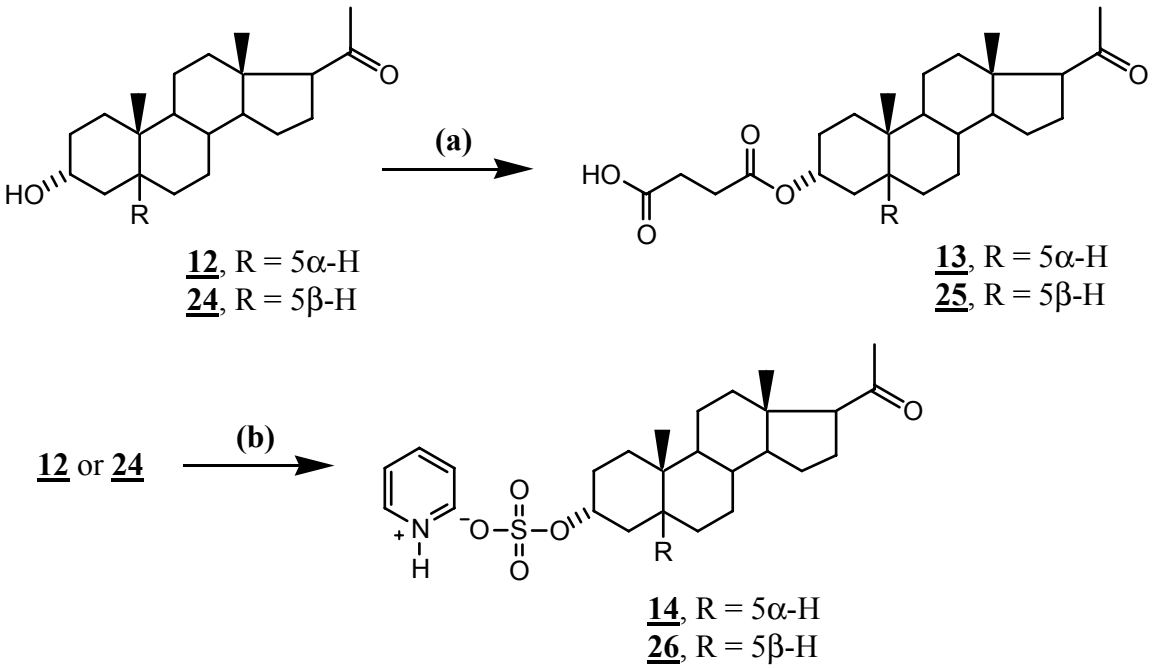
For the synthesis of 5β-derivatives, the chenodeoxycholic acid was used as starting material, because it contains the required 5β-configuration and suitable substituents in positions 3 and 7. Diacetate **15** was prepared by degradation[100] of a part (carbons 22-24) of the side chain. All target compounds of 5β-serie were prepared analogously to the 5α-serie. Partial hydrolysis of diacetate **15** gave 7-acetate **16** (***Scheme 23***). Hemisuccinate **17** and pyridinium salt of sulfate **18** were prepared in the yield of 35% and 99%, respectively. Compound **23** was prepared in the total yield of 12% from compound **16**. The structures of all derivatives in both series were confirmed by ^{1}H NMR spectra.



15 **16**

Scheme 23 - Synthesis of compound 16.
Reaction conditions: (a) $NaHCO_3$, H_2O, MeOH, 70 °C.

For the synthesis of hemisuccinates (**13**, **25**) and sulfates (**14**, **26**), the 3α-hydroxy pregnane derivatives (**12**, **24**) were used, previously prepared by selective reduction of 3-keto group[101] of particular 5α- and 5β- derivatives with $NaBH_4$ in a mixture of methanol and pyridine (derivative **12** is afforded as a by-product). The hemisuccinates were prepared by a common procedure of treating the steroid alcohol in pyridine with succinic anhydride and the sulfates by treating the steroid alcohol with sulfur trioxide pyridine complex (***Scheme 24***). Hemisuccinate **13** and **25** were prepared in the yield of 53% and 48%, respectively. Pyridinium salt of sulfate **14** and **26** were prepared in the yield of 85% and 80%, respectively.



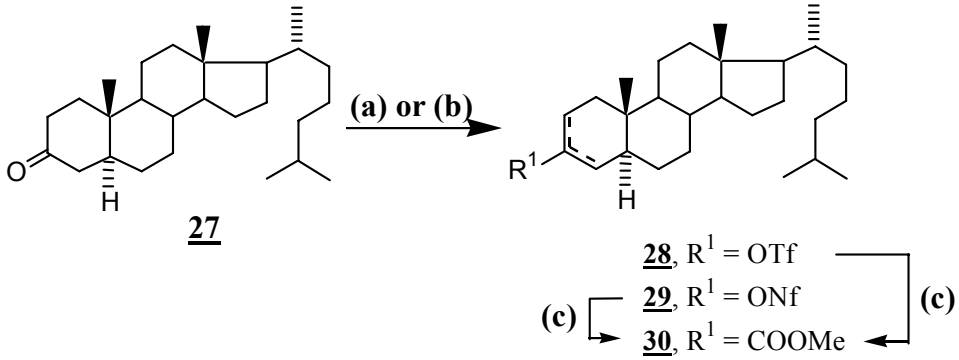
Scheme 24 - Synthesis of compounds 13, 14, 25 and 26.

Reaction conditions: (a) Succinic anhydride, pyridine, 4-dimethylaminopyridine, 140 °C; (b) sulfur trioxide pyridine complex, $CHCl_3$.

4.2. Synthesis of 3-Steroid Carboxylic Acids

The palladium catalyzed alkoxycarbonylation of steroid triflate was chosen from a variety of synthetic approaches for the synthesis of 3-carboxylic acids of steroids. Over the last years, it has been demonstrated that the alkenyl nonaflates are excellent substrates in a variety of palladium catalyzed coupling reactions (*e.g.* Heck or Sonogashira coupling reactions). The recent works have already compared the advantages and disadvantages of using nonaflate leaving group instead of triflate in palladium catalyzed cross-coupling reactions[102,103]: the use of nonaflates has several advantages: triflating reagents such as Tf_2O (triflic anhydride) or triflimides like Tf_2NPh (N-phenyltrifluoromethanesulfonimide), are considerably more expensive than NfF (nonafluorobutanesulfonyl fluoride), which is the most convenient reagent for synthesis of nonaflates. Furthermore, NfF is air-stable, non-toxic and can be stored over a long period.

The methodology of alkoxycarbonylation of triflates and nonaflates (***Scheme 25***) was firstly examined on 5α-cholestanone **27**, the steroid compound with any other functional groups except 3-keto group.



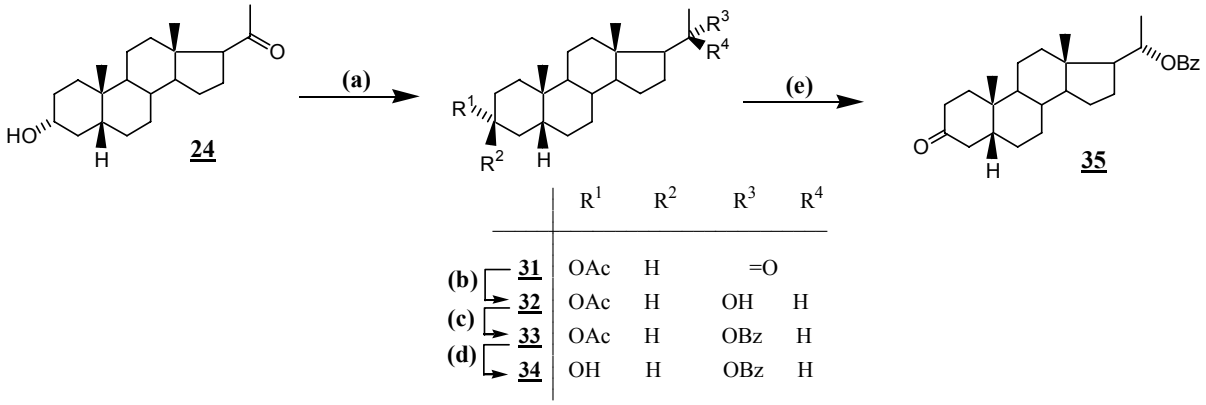
Scheme 25 – Synthesis of compounds 28-30.

Reaction conditions: (a) Triflic anhydride, 2,6-di-*tert*-butylpyridine, CH_2Cl_2; (b) LDA, NfF, from –78 °C to rt; (c) $Pd(OAc)_2$, Et_3N, Ph_3P, CO, MeOH, DMF, rt.

A mixture of triflates **28** was prepared by treatment of **27** in dichloromethane with 2,6-di-*tert*-butylpyridine and triflic anhydride (Tf_2O) in 81 % yield.
In case of a synthesis of a mixture of nonaflates **29**, lithium diisopropylamide (LDA) was used for generating enolate. The enolate was then trapped with NfF to afford **29** in 52 % yield. It has been demonstrated from ^1H NMR spectra that both enolates were generated in case of triflates **28** (5.38 ppm for H-4 and 5.65 ppm for H-2, the Δ^2 isomer prevailed in the

ratio 5:1), as well as nonaflates **29** (5.40 ppm for H-4 and 5.67 ppm for H-2, the Δ^2 isomer prevailed in the ratio 2:1). Then, **28** and **29** were converted into a mixture of methyl esters **30** under the conditions of palladium catalyzed alkoxycarbonylation; a mixture of methyl esters **30** was prepared in the yield of 60% from triflates **28** and 50% from nonaflates **29**. The ratios of Δ^2/Δ^3 isomers were preserved for both triflates **28** and nonaflates **29**.

Compound **35** was proposed as a model structure for palladium catalyzed alkoxycarbonylation of pregnane derivatives (***Scheme 26***): generally known hydrogenation of progesterone afforded 5β-pregnane-3,20-dione, which was reduced according to the literature[101] under the conditions of sodium borohydride reduction to afford 3α-hydroxy-5β-pregnan-20-one **24**. Acetylation of hydroxy group under standard conditions, reduction of 20-keto group with sodium borohydride at low temperature, and subsequent benzoylation with benzoyl chloride in pyridine afforded compound **33**. Its structure was confirmed by peaks of acetate group at 2.04 ppm and characteristic multiplets of benzoyl group at 7.43, 7.55, and 8.03 ppm in ^{1}H NMR spectrum. Then, acidic hydrolysis and Jones oxidation afforded desired 3-keto derivative with protected 20-hydroxy group **35**. Compound **35** was prepared from **24** in total yield of 46%.



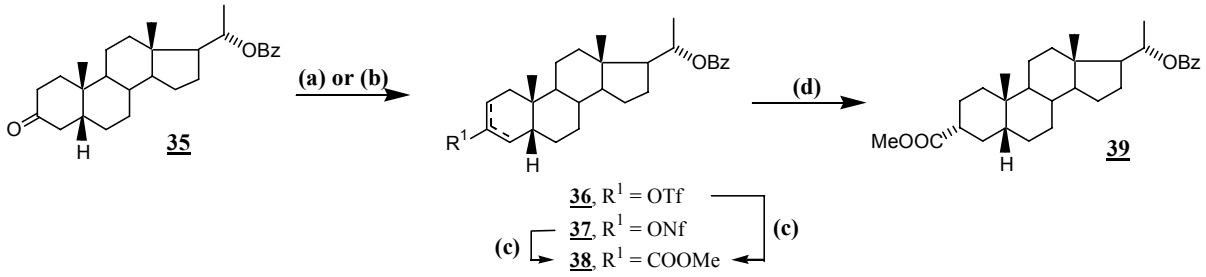
		R^1	R^2	R^3	R^4
(b)	**31**	OAc	H	=O	
	32	OAc	H	OH	H
(c)	**33**	OAc	H	OBz	H
(d)	**34**	OH	H	OBz	H

Scheme 26 – Synthesis of compounds 31-35.

Reaction conditions: (a) Ac_2O, pyridine, rt; (b) $NaBH_4$, MeOH, EtOAc, 5-10 °C; (c) BzCl, pyridine, rt; (d) $HClO_4$, MeOH, $CHCl_3$, rt; (e) Jones reagent, Me_2CO, rt.

Pregnane derivative **35** was converted to methyl ester of carboxylic acid **39** (***Scheme 27***) according to the previously mentioned methodology for cholestane derivative; *i.e.* 3-keto group was converted to a mixture of triflates or nonaflates and subsequent alkoxycarbonylation afforded a mixture of unsaturated methyl esters **38** in the yield 67% from a mixture of triflates **36** and 52% from a mixture of nonaflates **37**. The structures of

desired mixtures of triflates and nonaflates were confirmed by the peak of H-2 and H-4 in ^{1}H NMR spectra (the Δ^3 isomers prevailed in the ratio 2:1 and 2.5:1, respectively). A successful conversion of triflates or nonaflates to a mixture of methyl esters **38** was confirmed by a presence of characteristic singlet of methyl group at 3.75 ppm in ^{1}H NMR spectrum. Unfortunately, it was found out that the hydrogenation afforded a mixture of 3α- and 3β-derivatives, in which the 3α-derivative predominated in the ratio 10:1 according to ^{1}H NMR spectrum. PLC of isomers allowed separating only 3α-derivate **39** as a pure compound. 3β-Derivative was isolated in a mixture with 3α-derivative in a very small amount that was not sufficient for isolation in pure form. Compound **39** was prepared from **35** in the 12 % yield via triflate as well as via nonaflate.



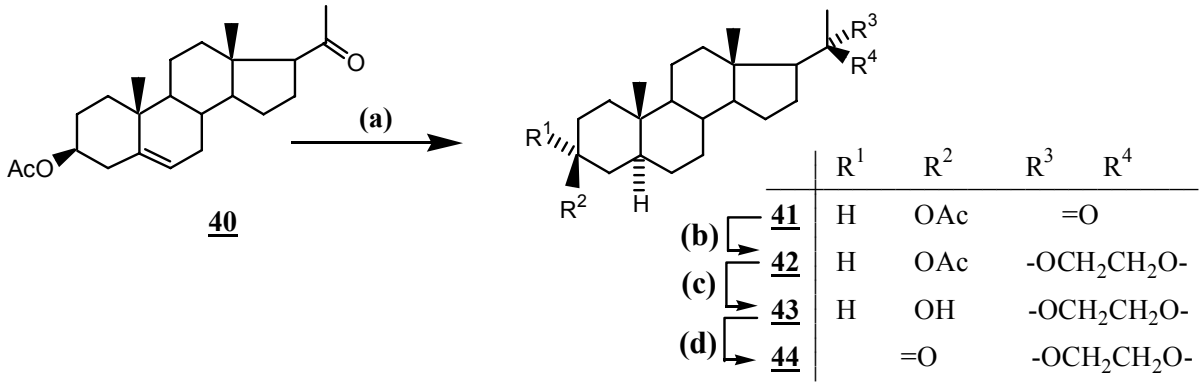
Scheme 27 – Synthesis of compounds 36-39.

Reaction conditions: (a) Triflic anhydride, 2,6-di-*tert*-butylpyridine, CH_2Cl_2; (b) LDA, NfF, from –78 °C to rt; (c) $Pd(OAc)_2$, Et_3N, Ph_3P, CO, MeOH, DMF, rt; (d) H_2, $Pd/CaCO_3$, EtOAc, EtOH, rt.

Since it was found out that the total yield of the reaction is not very high and for the synthesis of desired 3-carboxylic acid of pregnane would be even necessary to hydrolyze 20-ester group, oxidize 20-hydroxy group, and hydrolyze methyl ester, which would probably more decrease the total yield and prolong the reaction sequence compounds **44** and **46** were proposed as more convenient starting compounds. Their 20-keto group can be protected as acetal, because acid deprotection of 20-keto group could be than easily included into the reaction sequence as a part of a work up of a reaction mixture after basic hydrolysis.

Commercially available pregnenolone acetate **40** was firstly reduced by catalytic hydrogenation on palladium catalyst to afford 5α-steroid **41**. Protecting of 20-keto group by treatment with ethylene glycol, triethyl orthoformate, and *p*-toluenesulfonic acid monohydrate at room temperature, basic hydrolysis, and subsequent oxidation with PCC/Al_2O_3 of 3-hydroxy group afforded desired ketone **44**, the starting material for pregnane

3-carboxylic acids of 5α-serie (***Scheme 28***). Compound **44** was prepared from pregnenolone acetate **40** in a total yield 38%.

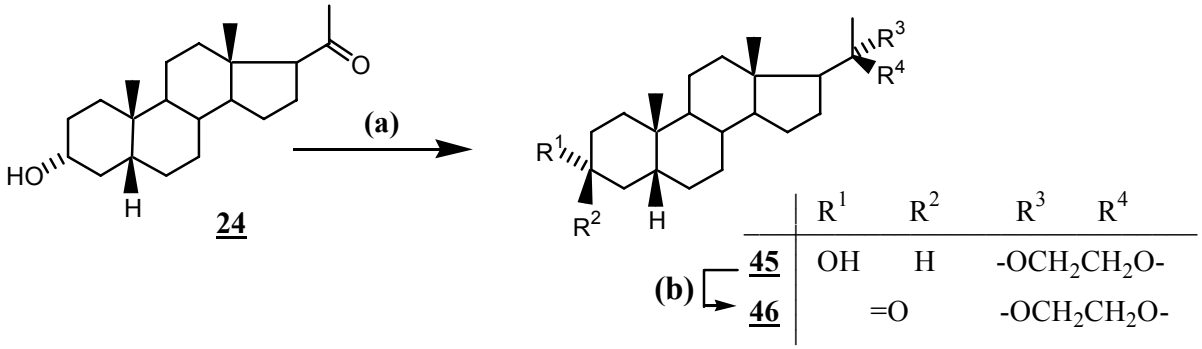


		R^1	R^2	R^3 R^4
(b)	**41**	H	OAc	=O
	42	H	OAc	$-OCH_2CH_2O-$
(c)	**43**	H	OH	$-OCH_2CH_2O-$
(d)	**44**		=O	$-OCH_2CH_2O-$

Scheme 28 – Synthesis of compounds 41-44.

Reaction conditions: (a) H_2, $Pd/CaCO_3$, EtOAc, EtOH, rt; (b) ethylene glycol, triethyl orthoformate, *p*-TsOH monohydrate, benzene, rt; (c) KOH, MeOH; (d) PCC/Al_2O_3, benzene, rt.

As regards synthesis of a starting compound for 5β-serie (***Scheme 29***), 20-keto group of a previously prepared 3α-hydroxy-5β-pregnan-20-one **24** was protected as acetal and then, its 3-hydroxy group was oxidized to afford ketone **46** in a total yield of 34% from compound **24**. The structure of compounds **44** and **46** were confirmed by characteristic multiplets of acetal protecting group in ^1H NMR spectra and by a comparison of their melting points and $[\alpha]_D$ with the literature data. The obtained values were in a good agreement with those from the literature.



		R^1	R^2	R^3 R^4
	45	OH	H	$-OCH_2CH_2O-$
(b)	**46**		=O	$-OCH_2CH_2O-$

Scheme 29 – Synthesis of compounds 45 and 46.

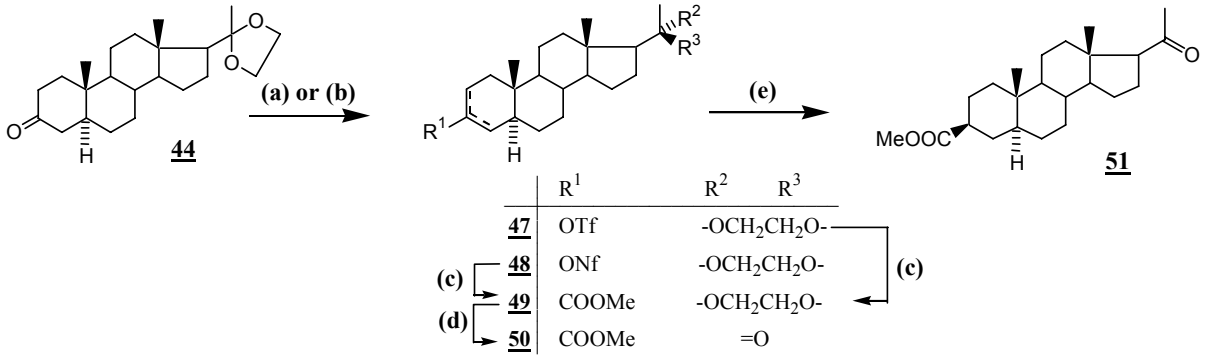
Reaction conditions: (a) Ethylene glycol, triethyl orthoformate, *p*-TsOH monohydrate, benzene, rt; (b) PCC/Al_2O_3, benzene, rt.

The conditions of synthesis were partially modified in order to influence the ratios of generated enolates. Lithium diisopropylamide (LDA) and lithium hexamethyldisilazane (LiHMDS) were chosen as bases. As it has been known that deprotonation with LDA proceeds under kinetic reaction control and deprotonation with LiHMDS proceeds under thermodynamic reaction control, it was supposed that the ratio of formation of isomeric enolate from 3-ketones could be partially affected, although the 3-keto group of steroid is not in general sterically hindered. Likewise, the air and moisture sensitive triflic anhydride was altered with Tf_2NPh.

Since the molecule of 5α-pregnane **44** is almost planar, the previously described expectations were not accomplished significantly as it was supposed. Nevertheless, the results were comparable for both triflates and for nonaflates (***Scheme 30***). If the LDA was used for generating enolate (Procedure A), the Δ^2 isomers prevailed in the ratio 3:1; a mixture of triflates **47** and nonaflates **48** were formed in the yield of 57% and 47%, respectively. If the LiHMDS was used (Procedure B), the Δ^2 isomers prevailed in the ratio 6:1; triflates **47** and nonaflates **48** were formed in the yield of 29% and 20%, respectively. Low yields in case of LiHMDS could by explained by sterical hindrance of steroid and Tf_2NPh or NfF. Furthermore, their interaction was probably decreased by a very low temperature of the reaction.

Palladium catalyzed alkoxycarbonylation afforded desired mixture of methyl esters **49** both from a mixture of triflates **47** and nonaflates **48**, in the yield of 52% and 69%, respectively; nevertheless, the ratio of Δ^2 and Δ^3 isomers changed to 2:1 in both cases that was probably caused by isomerization of Δ^2 and Δ^3 isomers. Deprotection of acetal protecting group by treatment with *p*-toluenesulfonic acid monohydrate in water and acetone mixture and hydrogenation on palladium catalyst afforded only 3β-methyl ester **51**. Its structure was confirmed by the singlet of methyl ester group in ^1H NMR spectrum at 3.69 ppm and also in IR spectrum by the carbonyl group's bands of 20-keto group at 1726 cm^{-1} and ester group at 1699 cm^{-1}.

The formation of only 3β-isomer although in the reaction mixture there were both Δ^2 and Δ^3 isomers could be explained by preferential formation of thermodynamically more stable product and isomerization of Δ^2 and Δ^3 isomers.



	R^1	R^2 R^3
47	OTf	$-OCH_2CH_2O-$
48	ONf	$-OCH_2CH_2O-$
49	COOMe	$-OCH_2CH_2O-$
50	COOMe	=O

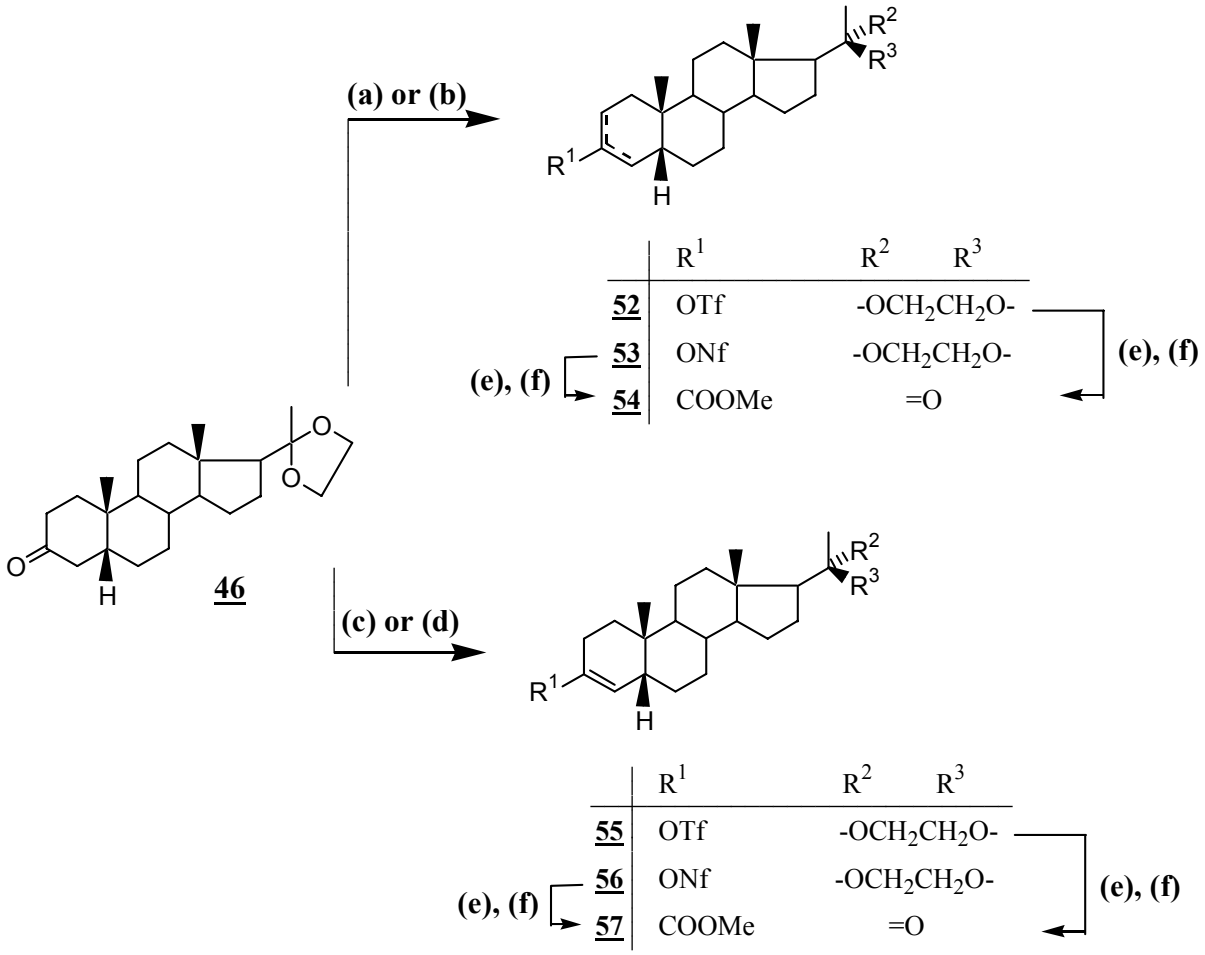
Scheme 30 – Synthesis of compounds 47-51.

Reaction conditions: (a) Procedure A: LDA, Tf_2NPh, THF, from –78 °C to rt; procedure B: LiHMDS, Tf_2NPh, THF, from –78 °C to rt; (b) Procedure A: LDA, NfF, THF, from –78 °C to rt; procedure B: LiHMDS, NfF, THF, from –78 °C to rt; (c) $Pd(OAc)_2$, Et_3N, Ph_3P, CO, MeOH, DMF, rt; (d) *p*-TsOH monohydrate, H_2O, acetone; (e) H_2, $Pd/CaCO_3$, EtOAc, EtOH, rt.

On the contrary to a planar molecule of 5α-steroid, a molecule of 5β-steroid is hooked shape. Therefore, the expectations of LiHMDS were confirmed in case of 5β-serie. If LDA was used, a mixture of triflates **52** and nonaflate **53** were formed as mixtures of Δ^2 and Δ^3 isomers, in which the Δ^3 isomer prevailed in the ratio 2.5:1 and 2:1, respectively (***Scheme 31***). A mixture of triflates **52** was formed in 30 % yield and a mixture of nonaflates **53** in 58 % yield. Alkoxycarbonylation and immediate deprotection of acetal afforded a mixture of methyl esters **54**, in a yield of 66% via triflate and 28% via nonaflate. As well as in case of 5α-serie, the isomerization of Δ^3 and Δ^2 isomers occurred and probably therefore a changes of their ratio to 3:1 were observed in both cases in ^{1}H NMR spectrum.

While in case of LDA both Δ^2 and Δ^3 isomers were formed, in case of LiHMDS only Δ^3 isomer was prepared (***Scheme 31***). Further alkoxycarbonylation and deprotection of acetal afforded methyl ester **57** from triflate **55** and nonaflate **56**, in a yield of 29% and 6%, respectively. Low yields could be again explained by sterical hindrance of LiHMDS, steroid, and Tf_2NPh or NfF. Furthermore, their interaction was probably decreased by a very low temperature of the reaction.

The structure of methyl ester **57** was confirmed by a singlet of methyl ester group at 3.74 ppm and by peak of H-4 at 6.71 ppm in ^{1}H NMR spectrum. Characteristic carbonyl group's band of ester group at 1701 cm^{-1} was observed in IR spectrum.



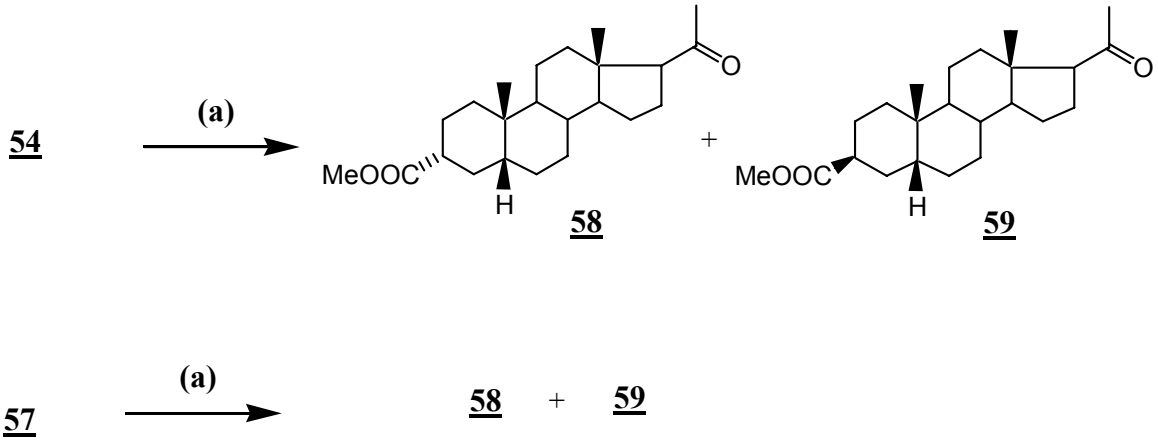
Scheme 31 – Synthesis of compound 52-57.

Reaction conditions: (a) LDA, Tf_2NPh, THF, from –78 °C to rt; (b) LDA, NfF, THF, from -78 °C to rt; (c) LiHMDS, Tf_2NPh, THF, from –78 °C to rt; (d) LiHMDS, NfF, THF, from –78 °C to rt; (e) $Pd(OAc)_2$, Et_3N, Ph_3P, CO, MeOH, DMF, rt; (f) *p*-TsOH monohydrate, H_2O, acetone.

The predictions of isomerization of Δ^2 and Δ^3 isomers and preferential formation of thermodynamically more stable product were confirmed by the results of hydrogenation of a mixture of methyl esters **54** and Δ^3-methyl ester derivative **57** (***Scheme 32***). In both cases, under the conditions of catalytic hydrogenation on palladium catalyst, 3α- and 3β-derivatives were formed. From a mixture of methyl esters **54**, 3α- and 3β-isomers **58** and **59** were formed in a ratio 6:1 in 47 % yield. From Δ^3-methyl ester derivative **57**, 3α- and 3β-isomers were formed in a ratio 2.5:1 in 56 % yield.

The structure of compounds **58** and **59** were confirmed by characteristic singlet of methyl ester group in ^1H NMR spectrum at 3.68 and 3.69 ppm, respectively; and also by the carbonyl group's bands of 20-keto group at 1725 and 1726 cm^{-1}, respectively and ester group

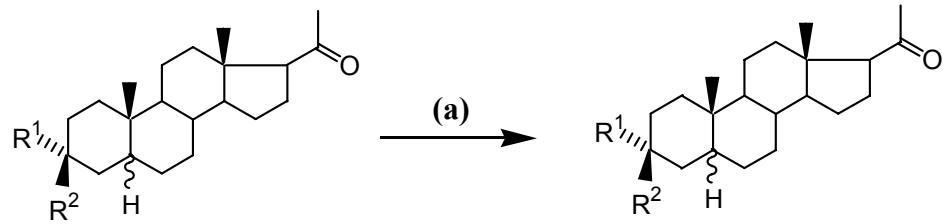
at 1699 cm^{-1}.



Scheme 32 – Synthesis of compounds 58 and 59.

Reaction conditions: (a) H_2, $Pd/CaCO_3$, EtOAc, EtOH, rt.

The last step of the reaction sequence, basic hydrolysis was performed by a refluxing of methyl esters **51**, **58** and **59** in ethanol with potassium hydroxide and water (***Scheme 33***). The carboxylic acids **60**, **61**, and **62** were obtained in a yield of 95%, 58%, and 87%, respectively. Their structure were confirmed by characteristic bands of hydroxyl group of carboxylic acids at cca 3500 cm^{-1} for monomers and cca 3090 and 2700 cm^{-1} for dimers.



		R^1	R^2
51	5α-H	H	COOMe
58	5β-H	COOMe	H
59	5β-H	H	COOMe

		R^1	R^2
60	5α-H	H	COOH
61	5β-H	COOH	H
62	5β-H	H	COOH

Scheme 33 – Synthesis of compounds 60-62.

Reaction conditions: (a) KOH, H_2O, EtOH.

The structural assignment of methylesters **39**, **51**, **58**, and **59** was also confirmed by NMR spectroscopy (***Table 1*** and ***Table 2***) as follows: similar substituting effects of COOMe group and sterical proximity of 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 carbon-^{13}C NMR spectra were used to determine chemical shifts and distinguish

the multiplicity of individual carbon signals. Structure assignment of CH, CH_2 and CH_3 signals were derived from ^1H, ^1H 2D-COSY; 2D-ROESY and ^1H, ^{13}C 2D-HSQC spectra by correlation with previously assigned proton signals. The remaining quaternary carbons were assigned on the basis of chemical shifts.

Table 1 – ^1H chemical shifts of compounds 39, 51, 58, and 59 in $CDCl_3$.

Proton	**39**	**51**	**58**	**59**
H-1α	1.79	0.97	1.86	1.62
H-1β	0.92	1.76	0.98	1.07
H-2α	1.50	1.78	1.66	1.56
H-2β	1.61	1.61	1.51	1.88
H-3α	-	2.32	-	2.71
H-3β	2.32	-	2.34	-
H-4α	1.93	1.54	1.93	1.96
H-4β	1.49	1.46	1.50	1.70
H-5	1.35	1.11	1.38	1.50
H-6α	1.26	1.26	1.28	1.26
H-6β	1.87	1.30	1.89	1.87
H-7α	1.12	0.92	1.13	1.10
H-7β	1.40	1.68	1.43	1.43
H-8	1.41	1.39	1.44	1.43
H-9	1.41	0.72	1.45	1.43
H-11α	1.31	1.60	1.49	1.46
H-11β	1.13	1.29	1.27	1.28
H-12α	1.28	1.39	1.44	1.43
H-12β	1.84	2.00	2.00	2.01
H-14	1.16	1.15	1.23	1.21
H-15α	1.67	1.66	1.20	1.21
H-15β	1.17	1.20	1.66	1.67
H-16α	1.78	2.15	2.15	2.16

H-16β	1.30	1.63	1.64	1.64
H-17α	1.78	2.52	2.54	2.53
H-18	0.64	0.60	0.59	0.59
H-19	0.89	0.80	0.94	0.91
H-20	5.12	-	-	-
H-21	1.26	2.11	2.11	2.11
OCH_3	3.75	3.65	3.67	3.68
H-2,6 (benzoate)	8.05	-	-	-
H-3,5 (benzoate)	7.44	-	-	-
H-4 (benzoate)	7.55	-	-	-

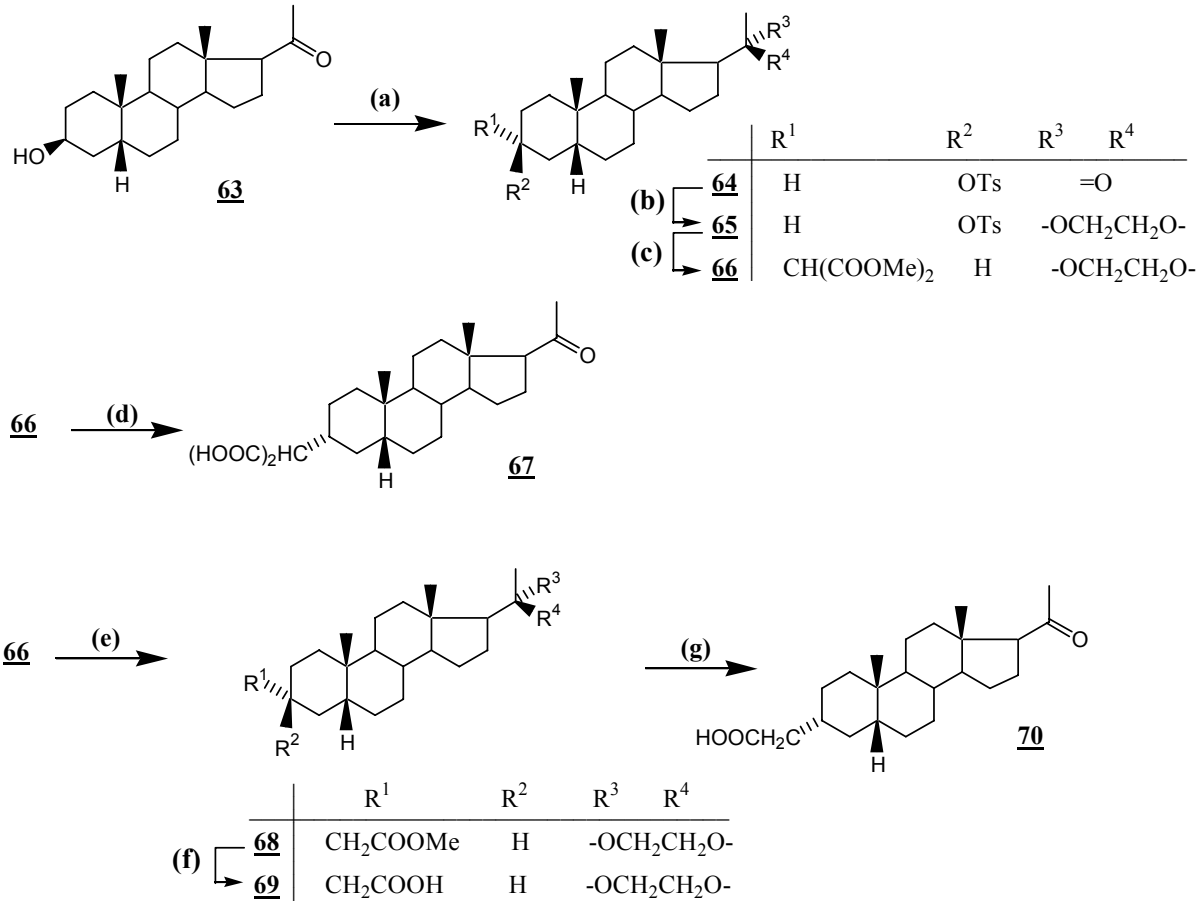
Table 2 – ^{13}C chemical shifts of compounds 39, 51, 58, and 59 in $CDCl_3$.

Carbon	**39**	**51**	**58**	**59**
1	36.41	37.80	36.51	33.28
2	23.78	24.61	23.84	22.03
3	44.05	43.71	44.09	39.75
4	29.58	31.23	29.63	27.45
5	42.84	46.08	42.90	39.31
6	27.11	28.57	27.11	26.90
7	26.37	31.97	26.34	26.27
8	35.68	35.51	35.88	35.79
9	40.39	54.33	40.40	40.28
10	34.84	35.81	34.93	34.97
11	20.72	20.99	20.88	21.01
12	39.41	39.12	39.20	39.36
13	42.55	44.24	44.33	44.36
14	55.76	56.78	56.64	56.92
15	24.27	24.39	24.44	24.45
16	25.56	22.86	22.94	22.96
17	55.36	63.88	63.92	63.98
18	12.59	13.44	13.40	13.42
19	23.73	12.21	23.74	23.92
20	73.37	209.51	209.51	209.45
21	19.98	31.44	31.49	31.45
C=O	176.87	176.45	176.73	175.84
OCH_3	51.51	51.44	51.47	51.44
C=O (benzoate)	165.74	-	-	-
C-1 (benzoate)	130.75	-	-	-
C-2,6 (benzoate)	129.63	-	-	-
C-3,5 (benzoate)	128.31	-	-	-
C-4 (benzoate)	132.67	-	-	-

4.3. Synthesis of 2-(Steroid-3-yl)propandioic Acid and 2-(Steroid-3-yl)acetic Acid

The starting compound for the synthesis of dicarboxylic acid **67** (***Scheme 34***), 3β-hydroxy-5β-pregnan-20-one **63**, was prepared according to literature[101] as a by-product of sodium borohydride reduction of 5β-pregnane-3,20-dione. Tosylation of 3β-hydroxy group and protection of 20-keto group afforded intermediate **65** for the substitution reaction in 89 % yield. The structure of **65** was confirmed by characteristic peaks of tosylate and acetal groups in ^{1}H NMR spectrum. The S_N2 reaction of protected tosylate **65** with sodium malonate that was generated in situ from sodium and dimethyl malonate afforded dimethyl ester of steroid propandioic acid **66** in 77 % yield. The structure of the dicarboxylic acid **66** was confirmed by doublet at 3.19 ppm of C**H**$(COOMe)_2$ group and characteristic singlet of methyl esters at 3.72 ppm in the ^{1}H NMR spectrum. The diester was hydrolyzed under the standard conditions of basic hydrolysis. As it was found out from the ^{1}H NMR spectrum that the acidic working up of the reaction mixture led to partially deprotection of 20-keto group, the reaction mixture was after the basic hydrolysis allowed to stay in a mixture HCl with water (1:2) for 3 h to obtain the dicarboxylic acid of pregnane **67** directly in 58 % yield, without separation of dicarboxylic acid with protected 20-keto group. The structure was confirmed by preservation of doublet at 3.17 ppm in ^{1}H NMR and by the presence of characteristic bands for monomer and dimer of carboxylic acid in IR spectrum (3500 and 3200 cm^{-1}). Compound **67** was prepared in the total yield of 40% from compound **63**.

As it is synthetically easy[104] to decarbethoxylation of derivative **66** to afford monoester, compound **66** was heated with sodium cyanide in dimethylsulfoxide at 210 °C. The monoester **68** was obtained in 57 % yield. The next step as well as in case of dicarboxylic acid was the basic hydrolysis of monoester **68** by treatment with potassium hydroxide in a mixture of water and ethanol. In contrast to hydrolysis of diester **66**, it was possible to isolate acid **69** with protected 20-keto group. The deprotection of 20-keto group was performed by treatment with *p*-toluenesulfonic acid monohydrate in a mixture of acetone and water. The desired monocarboxylic acid **70** was obtained in 85 % yield (***Scheme 34***). The structure of compound **70** was confirmed by doublet at 2.27 ppm of C$\mathbf{H}_2$COOH group and by the presence of characteristic bands for monomer and dimer of carboxylic acid in IR spectrum (3516 and 3090 cm^{-1}). Compound **70** was prepared in the total yield of 30% from compound **66**.

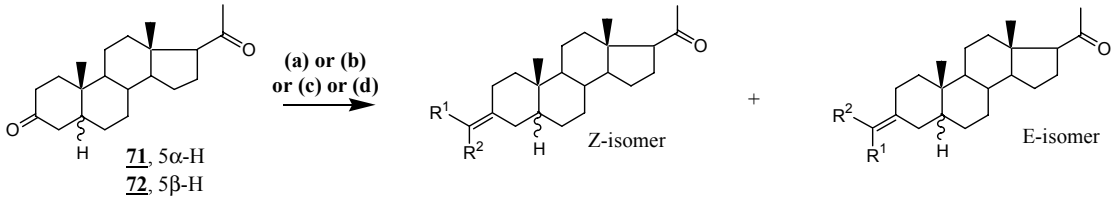


Scheme 34 - Synthesis of compounds 64-70.

Reaction conditions: (a) TsCl, pyridine, rt; (b) triethyl orthoformate, *p*-TsOH monohydrate, ethylene glycol, rt; (c) Na, dimethyl malonate, toluene, reflux; (d) KOH, EtOH, H_2O, 120 °C; (e) NaCN, DMSO, 210 °C; (f) KOH, EtOH, H_2O, 120 °C; (g) *p*-TsOH monohydrate, H_2O, acetone, rt.

4.4. Synthesis of Steroid Carboxylic Acids via Wadsworth-Horner-Emmons Reaction (WHE)

In a range of fourth part of the Thesis, a serie of carboxylic acid of 5α- and 5β-pregnane was prepared via Wadsworth-Horner-Emmons (WHE) reaction (***Scheme 35***). A starting 5α-pregnane-3,20-dione **71** is commercially available and 5β-pregnane-3,20-dione **72** can be prepared by basic hydrogenation of progesterone on palladium catalyst. From commercially available Wadsworth-Horner-Emmons reagents triethyl 2-phosphonoacetate, triethyl 2-phosphonopropionate, triethyl 2-phosphonobutyrate, and triethyl 4-phosphonocrotonate were chosen.



71, 5α-H
72, 5β-H

		R^1	R^2			R^1	R^2
73	5α-H	H	COOEt	**74**	5α-H	H	COOEt
75	5β-H	H	COOEt	**76**	5β-H	H	COOEt
77	5α-H	CH_3	COOEt	**78**	5α-H	CH_3	COOEt
79	5β-H	CH_3	COOEt	**80**	5β-H	CH_3	COOEt
81	5α-H	CH_2CH_3	COOEt	**82**	5α-H	CH_2CH_3	COOEt
83	5β-H	CH_2CH_3	COOEt	**84**	5β-H	CH_2CH_3	COOEt
85	5α-H	H	CH=CH-COOEt	**86**	5α-H	H	CH=CH-COOEt
87	5β-H	H	CH=CH-COOEt	**88**	5β-H	H	CH=CH-COOEt

Scheme 35 – Synthesis of compounds 73-88.

Reaction conditions: (a) For **73**-**76**: NaH, triethyl 2-phosphonoacetate, THF, from 0 °C to rt; (b) For **77**-**80**: NaH, triethyl 2-phosphonopropionate, THF, from 0 °C to rt; (c) For **81**-**84**: NaH, triethyl 2-phosphonobutyrate, THF, from 0 °C to rt; (d) For **85**-**88**: NaH, triethyl 4-phosphonocrotonate, THF, from 0 °C to rt.

The general procedure for the synthesis was suggested as follows: deprotonation of phosphonate (1.1 equiv) by NaH in THF at 0 °C, addition of particular ketone (1 equiv), and stirring the reaction mixture at room temperature. If triethyl 2-phosphonoacetate was used, 5α-derivatives **73** and **74** were isolated in a yield of 34% and 36%, respectively. In case of 5β-serie, derivatives **75** and **76** were isolated in a yield of 40% and 46%, respectively. Utilization of 1.1 equiv of other WHE reagents did not afford acceptable yields and therefore, the amounts of WHE reagents were modified: in case of triethyl 2-phosphonopropionate 1.75

equiv were used, in case of triethyl 2-phosphonobutyrate 3 equiv were used, and in case of triethyl 4-phosphonocrotonate 2.5 equiv were used. Even though, the conditions of reactions were modified; a period of time for deprotonation and a period of time for coupling of deprotonated species with steroid were prolonged, the yields were not significantly increased and the starting material (5-54%) were also isolated from the reaction mixtures. If triethyl 2-phosphonopropionate was used, a mixture of E/Z isomer **77**, **78** and **79**, **80** yielded 55% and 53% in case of 5α-, and 5β-serie, respectively. If triethyl 2-phospohonobutyrate was used, a mixture of E/Z isomer **81**, **82** and **83**, **84** yielded 36% and 37% in case of 5α-, and 5β-serie, respectively. If triethyl 4-phosphonocrotonate was used, a mixture of E/Z isomer **85**, **86** and **87**, **88** yielded 57% and 38% in case of 5α-, and 5β-serie, respectively.

Relatively low yields could be explained by sterical hindrance of intermediate of steroid and particular phosphonate.

The structural assignment of compounds **73-88** was also confirmed by NMR spectroscopy (***Table 3***, ***Table 4***, ***Table 5*** and ***Table 6***) as follows: similar substituents effects of E- and Z-isomers did not allow solving this problem from routine ^{1}H NMR spectra. Therefore a complete analysis of ^{1}H and ^{13}C spectra were performed utilizing ^{1}H, ^{1}H 2D-COSY; 2D-ROESY; APT carbon-^{13}C NMR; ^{1}H, ^{13}C 2D-HSQC; and ^{1}H, ^{13}C 2D-HMBC techniques.

Table 3 – Proton chemical shifts of derivatives 73-80 in $CDCl_3$.

Proton	73	74	75	76	77	78	79	80
H-1α	1.115	1.08	1.92	1.93	1.04	1.06	1.87	1.82
H-1β	1.85	1.85	1.135	1.10	1.81	1.76	1.08	1.10
H-2α	2.14	3.74	2.24	1.92	2.52	2.90	1.92	2.00
H-2β	2.34	2.00	2.03	3.60	2.00	2.06	2.38	2.78
H-4α	3.49	1.87	2.32	2.66	2.64	2.23	2.37	2.32
H-4β	1.87	2.18	3.45	1.84	1.895	1.84	2.64	2.19
H-5	1.22	1.25	1.51	1.54	1.18	1.18	1.47	1.47
H-6α	1.41	1.32*	1.37	1.27	1.31*	~1.325	1.29	1.30
H-6β	1.32	1.27*	1.88	1.90	1.27*	~1.325	1.85	1.88
H-7α	0.91	0.91	1.16	1.13	0.905	0.91	1.13	1.13
H-7β	1.69	1.695	1.47	1.48	1.67	1.70	1.44	1.46
H-8	1.38	1.39	1.45	1.46	1.37	1.385	1.43	1.45
H-9	0.715	0.715	1.53	1.55	0.70	0.705	1.53	1.46
H-11α	1.61	1.615	1.53	1.58	1.60	1.595	1.53	1.53
H-11β	1.32	1.34	1.32	1.32	1.32	1.325	1.32	1.31
H-12α	1.40	1.38	1.46	1.46	1.40	1.40	1.45	1.45
H-12β	2.00	2.00	2.03	2.04	2.00	2.00	2.03	2.03
H-14	1.14	1.14	1.22	1.23	1.13	1.15	1.21	1.21
H-15α	1.66	1.66	1.69	1.68	1.63	1.66	1.68	1.68
H-15β	1.16	1.17	1.23	1.22	1.14	1.17	1.21	1.21
H-16α	1.64	1.63	1.66	1.64	1.63	1.64	1.66	1.65
H-16β	2.16	2.16	2.17	2.17	2.15	2.16	2.16	2.19
H-17	2.52	2.525	2.54	2.55	2.52	2.53	2.54	2.55
H-18	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
H-19	0.91	0.91	0.94	0.94	0.88	0.88	0.93	0.93
H-21	2.11	2.11	2.12	2.12	2.11	2.11	2.12	2.12
=CH-	5.59	5.58	5.62	5.60	--	--	--	--
=C-CH_3	--	--	--	--	1.85	1.85	1.86	1.86
OCH_2	4.135	4.14	4.14	4.14	4.20	4.19	4.19	4.19
CH_3	1.27	1.27	1.27	1.27	1.30	1.30	1.30	1.30

A-ring: axial protons = red; equatorial protons = blue; proton with NOE to C(3)=C-**R** (**R** = H,CH_3 or CH_2) shown in a green cell unit; * structural assignment is not doubtless and can interchange with each other.

Table 4 – Proton chemical shifts of derivatives 81-88 in $CDCl_3$.

Proton	81	82	83	84	85	86	87	88
H-1α	1.14	1.08	1.90	1.83	1.07	1.02	1.92	1.88
H-1β	1.83	1.77	1.07	1.09	1.83	1.87	1.09	1.03
H-2α	2.50	2.76	1.92	1.98	2.17	2.82	2.24	1.95
H-2β	2.00	2.06	2.38	2.665	2.35	2.05	2.04	2.68
H-4α	2.50	2.21	2.38	2.34	2.54	1.91	2.38	1.94
H-4β	1.905	1.85	2.50	2.17	1.89	2.18	2.51	2.67
H-5	1.20	1.18	1.48	1.45	1.14	1.22	1.44	1.50
H-6α	~1.30*	~1.32	1.28	1.30	1.39*	~1.32*	1.35	1.28
H-6β	~1.26*	~1.32	1.85	1.89	1.32*	~1.27*	1.89	1.87
H-7α	0.91	0.92	1.12	1.14	0.91	0.91	1.15	1.14
H-7β	1.675	1.69	1.44	1.46	1.71	1.69	1.49	1.47
H-8	1.375	1.38	1.43	1.44	1.38	1.38	1.46	1.46
H-9	0.71	0.71	1.54	1.53	0.71	0.71	1.55	1.56
H-11α	1.59	1.59	1.53	1.52	1.60	1.60	1.53	1.55
H-11β	1.33	1.31	1.32	1.30	1.33	1.32	1.32	1.33
H-12α	1.39	1.39	1.45	1.44	1.40	1.40	1.46	1.47
H-12β	2.00	2.00	2.03	2.03	2.00	2.01	2.04	2.045
H-14	1.13	1.14	1.20	1.22	1.14	1.14	1.24	1.23
H-15α	1.66	1.66	1.68	1.67	1.67	1.66	1.69	1.68
H-15β	1.16	1.16	1.22	1.22	1.17	1.18	1.22	1.21
H-16α	1.64	1.63	1.66	1.65	1.64	1.64	1.66	1.65
H-16β	2.16	2.16	2.16	2.17	2.16	2.16	2.17	2.17
H-17	2.52	2.525	2.54	2.55	2.52	2.52	2.54	2.55
H-18	0.608	0.607	0.610	0.610	0.61	0.61	0.616	0.605
H-19	0.888	0.986	0.932	0.932	0.90	0.90	0.940	0.940
H-21	2.11	2.11	2.118	2.12	2.11	2.11	2.12	2.123
=CH-	--	--	--	--	5.93	5.92	5.95	5.94
=CH-	--	--	--	--	7.63	7.63	7.62	7.615
=CH-	--	--	--	--	5.78	5.78	5.79	5.78
OCH_2	4.20	4.195	4.20	4.194	4.20	4.20	4.20	4.20
CH_3	1.30	1.30	1.298	1.30	1.30	1.30	1.297	1.296
CH_2	2.295	2.295	2.30	2.30	--	--	--	--
CH_3	0.998	0.99	0.994	0.993	--	--	--	--

A-ring: axial protons = red; equatorial protons = blue; proton with NOE to C(3)=C-**R** (**R** = H,CH_3 or CH_2) shown in a green cell unit; * structural assignment is not doubtless and can interchange with each other.

Table 5 – ^{13}C chemical shifts of steroid derivatives 73-80 in $CDCl_3$.

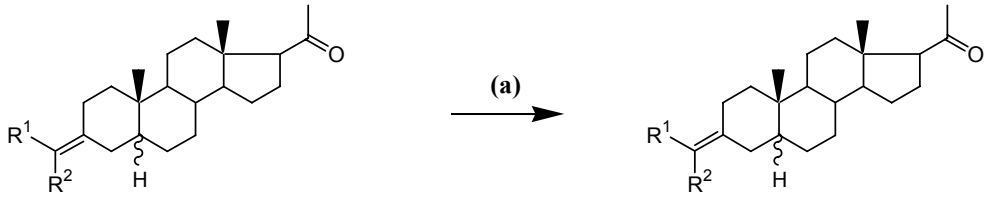
Proton	73	74	75	76	77	78	79	80
C-1	39.98	39.40	38.52	38.03	39.15	39.64	37.66	38.17
C-2	33.50	25.40	32.72	24.67	26.54	27.59	25.92	26.76
C-3	163.29	163.29	164.53	164.45	147.09	147.02	148.72	148.53
C-4	32.02	40.17	30.25	38.18	34.33	33.35	32.60	31.64
C-5	47.40	48.18	44.72	45.53	47.48	47.09	44.67	44.02
C-6	28.83	28.64	27.00	26.84	28.76	28.93	27.19	27.24
C-7	31.87	31.83	26.14	26.14	31.86	31.88	26.20	26.24
C-8	35.56	35.36	35.71	35.69	35.40	35.40	35.74	35.73
C-9	54.09	54.03	40.61	40.53	54.12	54.14	40.82	40.86
C-10	36.09	36.14	35.30	35.31	35.93	36.00	35.11	35.07
C-11	21.11	21.18	21.07	21.01	21.10	21.13	21.09	21.05
C-12	38.98	39.01	39.19	39.21	39.02	39.03	39.25	39.24
C-13	44.23	44.24	44.32	44.34	44.26	44.27	44.33	44.34
C-14	56.57	56.59	56.71	56.73	56.63	56.63	56.74	56.76
C-15	24.36	24.37	24.40	24.41	24.37	24.369	24.41	24.41
C-16	22.75	22.72	22.86	22.82	22.75	22.72	22.84	22.81
C-17	63.78	63.78	63.83	63.84	63.80	63.79	63.85	63.85
C-18	13.45	13.46	13.43	13.43	13.47	13.46	13.44	13.43
C-19	11.94	11.85	23.25	23.13	11.81	11.84	23.23	23.90
C-20	209.71	209.70	209.67	209.64	209.76	209.73	209.40	209.68
C-21	31.53	31.55	31.55	31.57	31.55	31.55	31.55	31.56
=CR-	112.60	112.87	112.78	112.72	119.63	119.69	119.56	119.70
=C-CH_3	--	--	--	--	14.28	15.17	15.12	15.13
C=O	166.87	166.93	166.96	166.81	170.67	170.72	170.56	170.50
OCH_2	59.45	59.45	59.46	59.45	60.13	60.12	60.09	60.11
CH_3	14.31	14.32	14.31	14.32	14.00	14.28	14.29	14.28

Table 6 – ^{13}C chemical shifts of steroid derivatives 81-88 in $CDCl_3$.

Proton	81	82	83	84	85	86	87	88
C-1	39.64	39.75	38.13	38.27	40.02	39.50	38.56	38.00
C-2	26.10	27.95	25.46	27.12	33.31	25.49	32.47	24.73
C-3	145.80	145.63	147.29	147.06	153.96	153.96	154.94	155.02
C-4	34.61	32.88	32.87	31.16	32.27	40.06	30.35	38.08
C-5	47.66	47.66	44.80	44.69	47.64	48.26	44.84	45.47
C-6	28.72	28.92	27.18	27.29	28.82	28.64	27.03	26.84
C-7	31.82	31.92	26.21	26.25	31.84	31.83	26.17	26.17
C-8	35.36	35.38	35.76	35.74	35.36	35.37	35.70	35.70
C-9	54.09	54.13	40.75	40.74	54.13	54.06	40.43	40.39
C-10	36.03	36.14	35.23	35.21	36.49	36.47	35.68	35.66
C-11	21.09	21.12	21.07	21.04	21.12	21.16	21.01	20.99
C-12	38.99	39.04	39.25	39.25	39.00	39.00	39.19	39.20
C-13	44.23	44.27	44.34	44.35	44.23	44.23	44.32	44.32
C-14	56.60	56.64	56.75	56.78	56.58	56.60	56.71	56.72
C-15	24.34	24.37	24.41	24.42	24.37	24.37	24.40	24.41
C-16	22.62	22.73	22.84	22.82	22.75	22.72	22.85	22.82
C-17	63.76	63.80	63.85	63.86	63.78	63.78	63.84	63.84
C-18	13.43	13.46	13.44	13.44	13.46	13.46	13.43	13.43
C-19	11.87	11.90	23.25	23.25	11.88	11.84	23.32	23.24
C-20	209.71	209.74	209.71	209.70	209.71	209.70	209.67	209.62
C-21	31.51	31.55	31.56	31.57	31.54	31.55	31.55	31.56
=CR-	126.62	126.67	126.64	126.74	120.17	120.37	120.31	120.24
=CH-	--	--	--	--	140.33	140.35	140.43	140.29
=CH-	--	--	--	--	118.61	118.66	118.61	118.51
C=O	170.62	170.73	170.57	170.54	167.84	167.81	167.85	167.83
OCH_2	60.02	60.06	60.02	60.05	60.12	60.10	60.12	60.09
CH_3	14.28	14.31	14.32	14.31	14.35	14.34	14.35	14.34
CH_2	22.62	22.64	22.67	22.64	--	--	--	--
CH_3	14.06	14.02	14.10	14.09	--	--	--	--

The previously mentioned ethyl esters (**73**-**80** and **85**-**88**) were subsequently treated with aqueous solution of potassium hydroxide in ethanol to afford desired carboxylic acid (***Scheme 36***). Nevertheless, it was not possible to isolate all carboxylic acids as pure individuals according to ^1H NMR spectra due to mainly isomerization of 17β-side chain that

can afford under basic conditions. Therefore, ester **77**, **80**, **87**, **88** were hydrolyzed as late as their 20-keto groups were protected as acetals. Acidic work up of the reaction mixture allowed easily deprotect 20-keto group to afford pure carboxylic acids **93**, **96**, **103**, and **104**.



		R^1	R^2
73	5α-H	H	COOEt
74	5α-H	COOEt	H
75	5β-H	H	COOEt
76	5β-H	COOEt	H
77	5α-H	CH_3	COOEt
78	5α-H	COOEt	CH_3
79	5β-H	CH_3	COOEt
80	5β-H	COOEt	CH_3
81	5α-H	CH_2CH_3	COOEt
82	5α-H	COOEt	CH_2CH_3
83	5β-H	CH_2CH_3	COOEt
84	5β-H	COOEt	CH_2CH_3
85	5α-H	H	CH=CH-COOEt
86	5α-H	CH=CH-COOEt	H
87	5β-H	H	CH=CH-COOEt
88	5β-H	CH=CH-COOEt	H

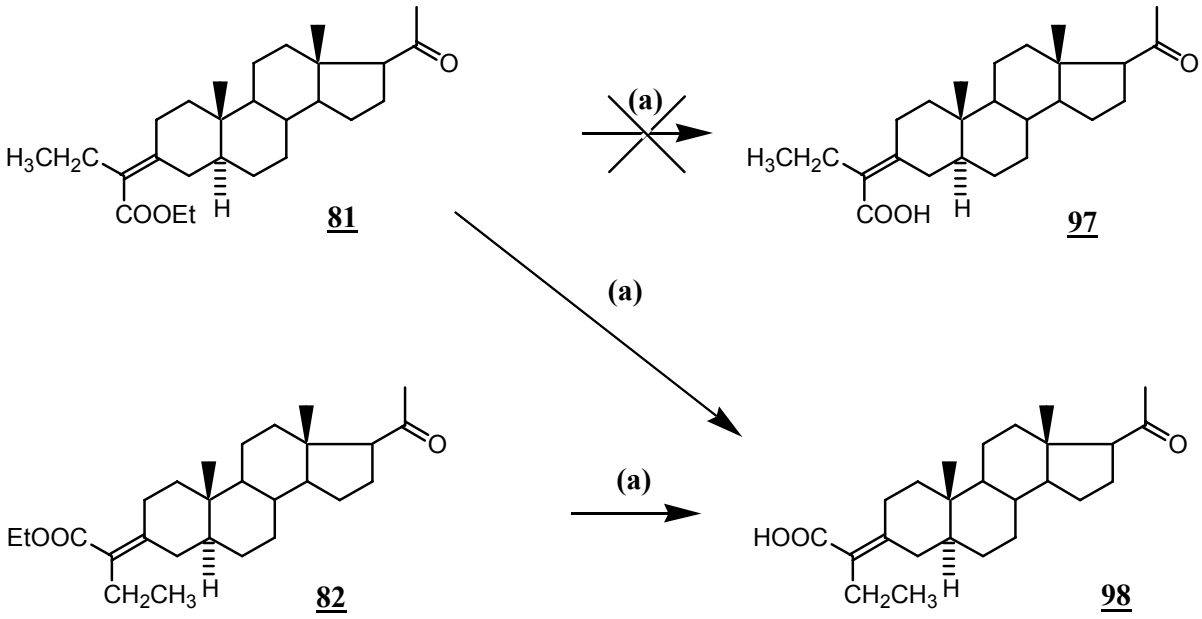
		R^1	R^2
89	5α-H	H	COOH
90	5α-H	COOH	H
91	5β-H	H	COOH
92	5β-H	COOH	H
93	5α-H	CH_3	COOH
94	5α-H	COOH	CH_3
95	5β-H	CH_3	COOH
96	5β-H	COOH	CH_3
97	5α-H	CH_2CH_3	COOH
98	5α-H	COOH	CH_2CH_3
99	5β-H	CH_2CH_3	COOH
100	5β-H	COOH	CH_2CH_3
101	5α-H	H	CH=CH-COOH
102	5α-H	CH=CH-COOH	H
103	5β-H	H	CH=CH-COOH
104	5β-H	CH=CH-COOH	H

Scheme 36 – Synthesis of compounds 89-104.

Reaction conditions: (a) KOH, H_2O, EtOH.

Basic hydrolysis of derivatives **81**, **82**, **83**, and **84** did not surprisingly give desired carboxylic acid **97-100**. Although the equivalents of potassium hydroxide were increased up to 12 and a reaction time was prolonged up to 10 h, only traces of carboxylic acids were formed. Therefore, the conditions of acidic hydrolysis (3 M $HClO_4$ in 50 % THF) were examined.

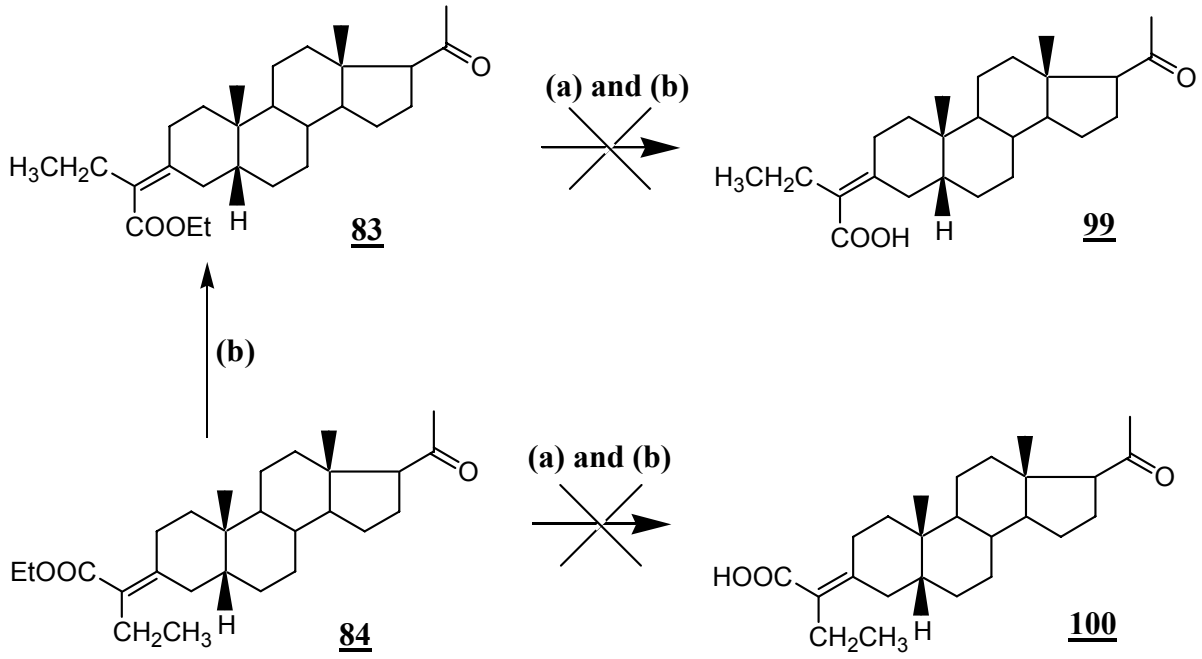
Surprisingly, in case of 5α-serie, hydrolysis of Z-isomer **81** gave carboxylic acid **98** which is in E-conformation (***Scheme 37***). This means that Z-isomer **81** is probably less stable and firstly isomerizes to more stable E-isomer **82** that can be hydrolyzed to carboxylic acid **98**. These predictions were supported by the observation that the hydrolysis of E-isomer **82** affords acid **98**.



Scheme 37 – Synthesis of compounds 97 and 98.

Reaction conditions: (a) 3 M $HClO_4$ in 50 % THF.

In case of 5β-serie, both attempts to hydrolyze esters **83** and **84** under the conditions of acidic hydrolysis failed (***Scheme 38***): in case of Z-isomer **83**, only traces of carboxylic acid were formed and any isomerization was observed. In case of E-isomer **84**, also only traces of desired carboxylic acid were formed, but the isomerization occurred in a ratio 1:1 to Z-isomer **83**.



Scheme 38 – Attempts of Synthesis of compounds 99 and 100.

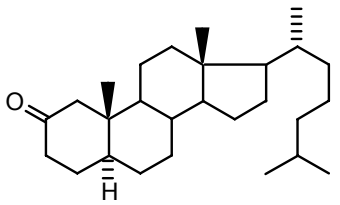
Reaction conditions: (a) KOH, H_2O, EtOH, (b) 3 M $HClO_4$ in 50 % THF.

Towards explaining previously described failures in such easy chemical reaction as a hydrolysis of ester is, which could be easily explained by sterical hindrance of ethyl group that does not allow carbon or oxygen of carbonyl group to be attacked, another experiments were carried out on derivative **81**: hydrolysis utilizing lithium hydroxide and trimethylsilyl iodide. Unfortunately, lithium hydroxide hydrolysis led only to isomerization to derivative **82** and hydrolysis with trimethylsilyl iodide gave no reaction.

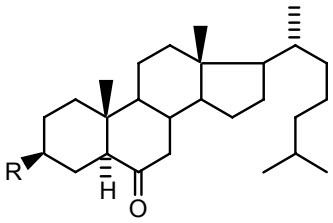
4.5. Luche Reduction of Saturated Steroid Ketones

The fifth part of the Thesis was focused on utilization of conditions of Luche reduction on saturated steroid ketones. As it was previously discovered[95], a ratio of axial and equatorial alcohols could be significantly changed, if the sodium borohydride reduction was catalyzed with cerium(III) chloride. The attack of cerium ion on oxygen of carbonyl group allows forming a complex of cerium-steroid that distinctively enhances the axial attack of sodium borohydride and subsequent formation of equatorial alcohol (for details see section Known Synthetic Methodologies, part Luche Reduction, *3.3.*). Whereas the utilization of condition of Luche reduction on saturated ketones has not been already published in the field of steroid chemistry as a complex review, it is worth of summarizing this easy synthetic application for changing the stereochemistry of steroid ketone reductions.

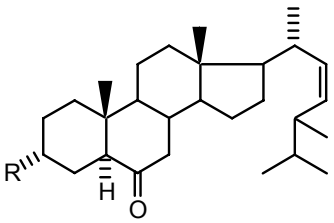
A serie of steroid ketones (**40**, **105**, **106**, and **112-116**) was chosen to examine the ratio of R/S or equatorial/axial alcohols that were formed under the conditions of sodium borohydride reduction in comparison with the conditions of Luche reduction; for chemical structures see ***Figure 1***. 3-Ketone steroid is not a part of this study, as it has been already studied in the work of Luche[95] with comparable results those were obtained within this part of Thesis.



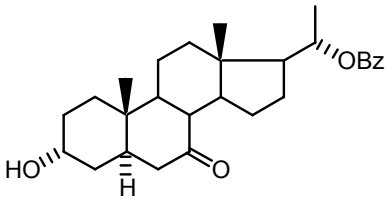
105



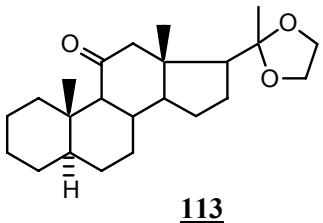
106, R = OH
107, R = OAc
108, R = OTBDMS



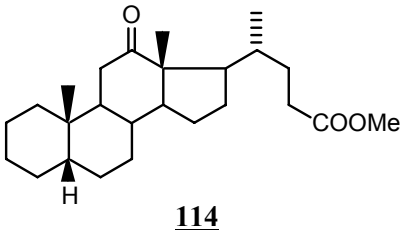
109, R = OH
110, R = OAc
111, R = OTBDMS



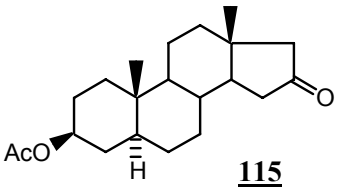
112



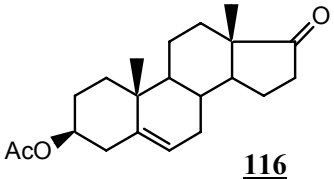
113



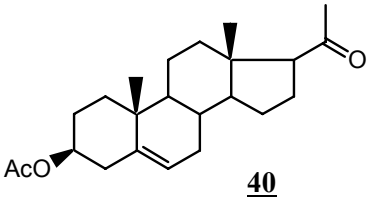
114



115



116



40

Figure 1

Ketones **40**, **105-116** were treated with one equivalent of sodium borohydride at room temperature (for details see *6.6.1.*, General procedure for $NaBH_4$ reduction). Under the conditions of Luche reduction (for details see *6.6.2.*, General procedure for Luche reduction using $CeCl_3 \cdot 7H_2O$), a mixture of steroid in THF/MeOH was firstly treated with 1.1 equiv of cerium(III) chloride heptahydrate up to entire solution and then, one equiv of sodium borohydride was added. The vigorous gas hydrogen evolution occurred, which indicated formation of alkoxyborohydrides, the actual reducing species. After a work up, all mixtures of alcohols were primarily analyzed by ^1H NMR spectra to confirm formation of desired alcohols and to establish their ratios (***Table 7***); the chemical shifts and shapes of signals of CH-O protons were compared with literature data[105].

Table 7 – The ^{1}H NMR analysis of alcohols formed from ketones 105-116 and 40.

Ketone	Chemical shifts of protons of C**H**$_2$-OH group	Coupling constants (*J*)	Eq. / axial alcohols (no Ce^{3+})[a]	Eq. / axial alcohols (with Ce^{3+})[b]
105	3.75 tt, (H-2β) 4.14 bm, (H-2α)	J_1 = 10.8, J_2 = 4.5 (H-2β)	20 / 80	50 / 50
106	3.42 m, (H-6β) 3.80 m, (H-6α)	-	0 / 100	20 / 80
107	3.40 m, (H-6β) 3.80 m, (H-6α)	-	0 / 100	20 / 80
108	3.80 bm, (H-6α)	-	0 / 100	0 / 100
109	3.39 m, (H-6β) 3.75 q, (H-6α)	J = 2.9 (H-6α)	0 / 100	20 / 80
110	3.38 m, (H-6β) 3.73 m, (H-6α)	-	0 / 100	20 / 80
111	3.38 m, (H-6β) 3.72 q, (H-6α)	J = 2.7 (H-6α)	0 / 100	0 / 100
112	3.41 ddd, (H-7α) 3.84 q, (H-7β)	J_1 = 5.2, J_2 = 9.2, J_3 = 10.9 (H-7α) J = 2.5 (H-7β)	20 / 80	80 / 20
113	No reaction [c]	- [c]	- [c]	- [c]
114	3.42 bm, (H-12α) 3.98 m, (H-12β)	-	20 / 80	70 / 30
115	4.38 tdd, (H-16α) 4.45 tdd, (H-16β)	J_1 = 2.3, J_2 = 5.6, J_3 = 7.8 (H-16α) J_1 = 1.6, J_2 = 5.8, J_3 = 7.6 (H-16β)	5 / 95 [d]	10 / 90 [d]
116	3.65 t, (H-17α)	J = 8.5	100 / 0 [d]	100 / 0 [d]
40	3.73 dq, (H-20(R)) 3.71 m, (H-20(S))	J_1 = 5.9, J_2 = 9.9 W = 36	5 / 95 [e]	20 / 80 [e]

[a] Ratio of equatorial/axial alcohols formed under the conditions of *6.6.1.*, General procedure for $NaBH_4$ reduction. Determined from ^1H NMR spectrum. [b] Ratio of equatorial/axial alcohols formed under the conditions of *6.6.2.* General procedure for Luche reduction using $CeCl_3 \cdot 7H_2O$. Determined from ^1H NMR spectrum. [c] Compound **113** did not react neither under the condition of General procedure for $NaBH_4$ reduction nor of General procedure for Luche reduction using $CeCl_3 \cdot 7H_2O$. For details see Experimental part. [d] Ratio of pseudoequatorial / pseudoaxial alcohols. [e] Ratio of S/R alcohols.

The exact ratios were determined by integration of the peaks from HPLC analysis (***Table 8***). ***Table 8*** revealed interesting influence of cerium catalyst on formation of equatorial alcohol especially in case of C-2 (**105**), C-7 (**112**), and C-12 (**114**) ketones.

Reduction of 2-ketone steroid (**105**) under the conditions of sodium borohydride reduction allowed forming only 11% of equatorial alcohol. To increase the amount of equatorial alcohol it would be necessary to use lithium aluminium hydride reduction conditions[82]. Nevertheless this change of reagent could be a disadvantage, because it is not as mild reagent as sodium borohydride and could not be used in presence of *e.g.* ester group in the molecule. If the conditions of cerium catalysis were used, the ratio of equatorial and axial alcohols changed to 55:45, in which the desired equatorial isomer prevailed.

In case of C-7 and C-12 ketone reduction, the ratio completely turned round. The addition of cerium into the reaction mixture of 7-ketone steroid (**112**) allowed changing the ratio of equatorial alcohol from 16% to almost 83%. As regards 12-ketone, according to the literature[86,106] the product ratio is determined by the nature of the side chain: if the conditions of sodium borohydride reduction were used on 12-ketone steroid (**114**), indeed the axial 12α-alcohol formed in 85 % yield. While the cerium catalysis was added to the reaction mixture, the ratio changed in favor of equatorial alcohol that afforded in 71% yield.

Steric hindrance effect of C-19 methyl group of C-6 keto group (**106**) that led to equatorial attack of sodium borohydride and subsequent formation of axial 6β-alcohol in 100 % yield was partially limited by addition of cerium(III) chloride heptahydrate and the 6α-alcohol was formed in 16 % yield.

11-Ketone steroid (**113**) did not react neither under the condition of sodium borohydride reduction or Luche reduction, not even the microwaves were used (for details

see Experimental part.). These negative results were probably caused by the steric hindrance of C-18 and C-19 methyl groups and the mild reagent used.

Reduction of D-ring ketones did not bring any unexpected results. An addition of cerium(III) chloride heptahydrate into the reaction mixture of 16-ketone (**115**) steroid did not significantly influence the ratio of pseudoaxial and pseudoequatorial alcohol and led to change only from 2.5% to 9%. The reduction of 17-ketone (**116**) afforded only 17β-alcohol.

Table 8 – The HPLC analysis of alcohols formed from ketones 105, 106, 112-116, and 40.

Ketone	Eq. / axial alcohols (%) (without lanthanoid) [a]	Eq. / axial alcohols (%) (with Ce^{3+}) [b]
40	4.5 / 95.5 [c]	15 / 85 [c]
105	11 / 89	55 / 45
106	0 / 100	16 / 84
112	16 / 84	82.5 / 17.5
113	- [d]	- [d]
114	15 / 85	71 / 29
115	2.5 / 97.5 [e]	9 / 91 [e]
116	100 / 0 [e]	100 / 0 [e]

[a] Amount (%) of equatorial/axial alcohols formed under the conditions of *6.6.1.*, General procedure for $NaBH_4$ reduction. Determined from HPLC analysis. [b] Amount (%) of equatorial/axial alcohols formed under the conditions of *6.6.2.*, General procedure for Luche reduction using $CeCl_3 \cdot 7H_2O$. Determined from HPLC analysis. [c] Amount (%) of S/R alcohols. [d] Compound **113** did not react neither under the condition of General procedure for $NaBH_4$ reduction nor of General procedure for Luche reduction using $CeCl_3 \cdot 7H_2O$. For details see Experimental part. [e] Amount (%) of pseudoequatorial/pseudoaxial alcohols.

As in the original work of Luche from 1978[94] were cerium and samarium mentioned to afford the best yields and selectivity, three steroids from the previously mentioned serie were chosen to be tested on conditions of Luche reduction with samarium iodide. 2-Ketone steroid (**105**) was chosen as its ratio of formed equatorial and axial alcohol by addition of

$CeCl_3 \cdot 7H_2O$ (55:45) allows maximum divergence of the result. 6-Ketone steroid (**106**) was chosen as the steroid that reduction can be changed by addition of lanthanoid; nevertheless, is significantly sterically hindered. And finally, 20-ketone steroid (**40**) was chosen, as an easy and simple synthesis of endogenous S-alcohol, which is not as easy to be prepared by other synthetic methodologies, would be of value in the field steroid chemistry. The results of using samarium iodide for reduction of ketones **40**, **105**, and **106** are summarized in ***Table 9***. Samarium iodide was found to provide higher yields of equatorial alcohols: reduction of 2-ketone steroid (**105**) with samarium catalysis gave almost 67.5% of equatorial alcohol and reduction of 6-ketone steroid (**106**) gave 32.5%. Really promising was 35 % yield of S-alcohol formed by reduction of 20-ketone steroid (**40**) under samarium catalysis. The disadvantage of samarium(III) iodide catalysis is as its air and moisture sensitivity compared to cerium(III) chloride heptahydrate.

Table 9 – The HPLC analysis of alcohols formed from ketones 40, 105, and 106 under catalysis of samarium(III) iodide.

Entry	Eq. / axial alcohols (%) (without lanthanoid) [a]	Eq. / axial alcohols (%) (with Ce^{3+}) [b]	Eq. / axial alcohols (with Sm^{3+}) [c]
40	4.5 / 95.5 [d]	15 / 85 [d]	35 / 65 [d]
105	11 / 89	55 / 45	67.5 / 32.5
106	0 / 100	16 / 84	33 / 67

[a] Amount (%) of equatorial/axial alcohols formed under the conditions of *6.6.1.*, General procedure for $NaBH_4$ reduction. Determined from HPLC analysis. [b] Amount (%) of equatorial/axial alcohols formed under the conditions of *6.6.2.*, General procedure for Luche reduction using $CeCl_3 \cdot 7H_2O$. Determined from HPLC analysis. [c] Amount (%) of equatorial/axial alcohols formed under the conditions of *6.6.3.*, General procedure for Luche reduction using SmI_3. Determined from HPLC analysis. [d] Amount (%) of S/R alcohols.

Since the conditions of treatment of 1.1 equiv of cerium(III) chloride heptahydrate were used within this study, it should be noted that in case of heptahydrate seven molecules of water compose 34% of reagent. This raises a question of an effect of water on selectivity of the reaction. Therefore, a serie of 2-ketone steroid (**105**), 6-ketone steroid (**106**), and

20-ketone steroid (**40**) was treated with anhydrous cerium(III) chloride in anhydrous solvents under the conditions of Luche reduction (for details see *6.6.4.*, General procedure for Luche reduction using anhydrous $CeCl_3$). Anhydrous $CeCl_3$ was prepared by dehydration of $CeCl_3 \cdot 7H_2O$ in a vacuum dryer at 50 °C and 2 kPa for 24 h. It was discovered that 7 molecules of water in a molecule of cerium(III) chloride heptahydrate indeed have significant effect on selectivity of reaction (***Table 10***). More preciously, in case of 2-ketone (**105**) and 6-ketone (**106**) steroid catalysis of anhydrous cerium chloride led to decrease of equatorial alcohol formation from 55% to 42% and from 16% to 6%, respectively. An increase in the concentration of cerium(III) chloride resulted in a more selectivity of reaction on behalf of axial alcohol. Nevertheless, in case of 20-ketone steroid (**40**) the amount of S-alcohol doubled, from 15% up to 30.5%, which is almost identical results comparing with result of samarium iodide catalysis.

Table 10 – The HPLC analysis of alcohols formed from ketones 40, 105, and 106 under catalysis of anhydrous cerium(III) chloride.

Entry	Eq. /axial alcohols (%) (without lanthanoid) [a]	Eq. /axial alcohols (%) (with Ce^{3+}) [b]	Eq. / axial alcohols (%) (with anhydrous Ce^{3+}) [c]
40	4.5 / 95.5 [d]	15 / 85 [d]	30.5 / 69.5 [d]
105	11 / 89	55 / 45	42 / 58
106	0 / 100	16 / 84	6 / 94

[a] Amount (%) of equatorial/axial alcohols formed under the conditions of *6.6.1.*, General procedure for $NaBH_4$ reduction. Determined from HPLC analysis. [b] Amount (%) of equatorial/axial alcohols formed under the conditions of *6.6.2.*, General procedure for Luche reduction using $CeCl_3 \cdot 7H_2O$. Determined from HPLC analysis. [c] Amount (%) of equatorial/axial alcohols formed under the conditions of *6.6.4.*, General procedure for Luche reduction using anhydrous $CeCl_3$. Determined from HPLC analysis. [d] Amount (%) of S/R alcohol.

All the previously described results for utilization of Luche reduction on saturated steroid ketones seem to be very promising in the field of preparative steroid chemistry. Therefore an important question considering the result on particular ketone steroid reduction

should be raised. Or more preciously, whether the substituents can influence the ratios of equatorial and axial alcohols.

6-Ketone steroid (**106**) was chosen from the serie of primarily tested steroids as a model structure. Steroid **106** was then converted to 3-acetoxy and 3-*tert*-butyldimethylsilyl (TBDMS) derivatives to obtain steroids with synthetically frequently used protecting group and sterically heavily space demanding group. Nevertheless, from the stereochemical point of view, 3β-substituent is in an equatorial position which means that will not sterically hinder the attack of alkoxyborohydride from axial side. Therefore, a serie of 6-ketone steroids (**109**, **110**, and **111**) with identical 3α-substituents was included. The results are summarized in ***Table 11***. It was discovered that the results are comparable for both 3α- and 3β-serie. In case of 3-hydroxy (**106** and **109**) and 3-acetate group (**107** and **110**), the equatorial 6α-alcohols were formed in a yield of 16-20%. On the contrary, if 3-TBDMS groups (**108** and **111**) were presented in a molecule, their sterically hindrance disallowed formation of equatorial alcohols.

Results from ***Table 11*** can be summarized as follows: a sterically space demanding substituent close to a carbonyl group can significantly influence the ratio of formed equatorial and axial alcohols.

Table 11 – The HPLC analysis of alcohols formed from ketones 106-111 under catalysis of cerium(III) chloride heptahydrate.

Entry	Eq. / axial alcohols (without lanthanoid) [a]	Eq. / axial alcohols (with Ce^{3+}) [b]
106	0 / 100	16 / 84
107	1 / 99	20 / 80
108	0 / 100	0 / 100
109	0 / 100	16.5 / 83.5
110	0.5 / 99.5	17 / 83
111	0 / 100	0 / 100

[a] Amount (%) of equatorial/axial alcohols formed under the conditions of *6.6.1.*, General procedure for $NaBH_4$ reduction. Determined from HPLC analysis. [b] Amount (%) of

equatorial/axial alcohols formed under the conditions of *6.6.2.*, General procedure for Luche reduction using $CeCl_3 \cdot 7H_2O$. Determined from HPLC analysis.

5. Summary of the Scientific Achievements

- The serie of C-3 and C-7 substituted derivatives of 5α- and 5β-pregnan-20-one has been prepared.

- The methodology for the synthesis of 3-carboxylic acids of 5α- and 5β-pregnan-20-one has been developed.

- 2-(20-Oxo-5β-pregnan-3α-yl)propandioic acid (**67**) and 2-(20-Oxo-5β-pregnan-3α-yl)acetic acid (**70**) have been prepared.

- The serie of carboxylic acids of 5α- and 5β-pregnan-20-one having carboxylic group joined directly to steroid in position 3 or by spacer of 1-3 carbon atoms has been prepared.

- The analysis of steroid alcohols prepared by sodium borohydride reduction of saturated steroids under the conditions of Luche reduction using $CeCl_3 \cdot 7H_2O$, SmI_3, and anhydrous $CeCl_3$ has been done.

6. Experimental Section

6.1. 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 (^1H 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 (PLC) was carried out on 200 mm x 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 ^oC/100 Pa.

Pyridine was dried by distillation over potassium hydroxide and chloroform by distillation over phosphorus pentoxide. Anhydrous methanol was obtained by treatment with magnesium and distillation and anhydrous THF by distillation with $LiAlH_4$ immediately prior to use.

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

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 completed into 250 mL with water.

Sodium borohydride (≥96%, Fluka), $CeCl_3 \cdot 7H_2O$ (99%, Janssen Chimica, Belgium) and anhydrous samarium iodide (Sigma Aldrich) were used without further purification.

Anhydrous $CeCl_3$ was prepared by dehydration of $CeCl_3 \cdot 7H_2O$ in a vacuum dryer at 50 °C and 2 kPa for 24 h. Other reagents were purchased from commercial sources and used without further purification.

The acetate (**107** and **110**) and TBDMS (**108** and **111**) derivatives were prepared by generally known methodologies with standard work up and purification. Steroid ketones utilized in this study (**105**, **106**, **109**, **112**, **113**, **114**, **115**, **116**, **40**) were obtained from the internal archive of the Department of Medicinal Steroids. The purity of steroid ketones (**40, 105-116**) were examined by ^1H NMR spectrum and by GC/MS with electron impact ionization of 70 eV on a 6890N network GC System and 5975B inert XL MSD instruments (Agilent technologies) fitted with a capillary column (J and W 122-0132 DB-1ms) of nominal length 30 m and diameter 250 μm. The purity of all used steroid was >98%.

Used 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), preparative ELSD Detector (Gilson) connected with PC (software Trilution LC, Gilson).

6.2. Synthesis of C-3 and C-7 Substituted Pregnane Derivatives

6.2.1. Synthesis of 5α-Pregnane Derivatives

*6.2.1.1. 20-Oxo-5α-pregnane-3α,7α-diol (**1**)*

This compound was prepared according to the literature[99].

*6.2.1.2. 20-Oxo-5α-pregnane-3α,7α-diyl Diacetate (**2**) and 7α-Hydroxy-20-oxo-5α-pregnan-3α-yl Acetate (**3**)*

To a solution of dihydroxy derivative **1** (100 mg, 0.3 mmol) in pyridine (5 mL), acetic anhydride (0.42 mL, 4.4 mmol) was added and the mixture was heated for 6 h at 50 °C. After standing at room temperature for 2 days, the reaction mixture was poured into ice-water and extracted with ethyl acetate (50 mL). Extract was washed with solution of potassium hydrogen carbonate, water, and dried. Solvent was evaporated and the oily residue purified by PLC (3 plates) in a mixture of acetone/petroleum ether (1:1) to give diacetate **2** (67 mg, 54%) and monoacetate **3** (9 mg, 8%) as a side product.

Diacetate **2**: m.p. 112–114 °C, $[\alpha]_D$ +21.8 (*c* 0.36, $CHCl_3$). IR spectrum ($CHCl_3$): 1726 (C=O, acetate); 1702 (C=O, ketone); 1259, 1241, 1030 (C-O). ^{1}H NMR (200 MHz): 0.60 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 2.05 s, 3 H (C(3)-OAc); 2.08 s, 3 H (C(7)-OAc); 2.12 s, 3 H (3 × H-21); 2.54 t, 1 H, J = 8.8 (H-17); 4.92 q, 1 H, J = 2.9 (H-7); 5.03 m, 1 H (H-3). FAB MS: 441 (73 %, M + Na), 419 (8 %, M + H), 299 (42 %, M + 1 – 2 × AcOH). For $C_{25}H_{38}O_5$ (418.5) calculated. C, 71.74; H, 9.15. Found. C, 71.94; H, 9.22.

Monoacetate **3**: m.p. 174–178 °C, $[\alpha]_D$ +30.6 (*c* 0.51). IR spectrum ($CHCl_3$): 3617 (O-H); 1726 (C=O, acetate); 1702 (C=O, ketone); 1263, 1220 (C-O). ^{1}H NMR (200 MHz): 0.60 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 2.05 s, 3 H (OAc); 2.12 s, 3 H (3 × H-21); 2.56 t, 1 H, J = 8.8 (H-17); 3.87 m, 1 H (H-7); 5.03 quintet, 1 H, J = 2.9 (H-3). FAB MS: 399 (12%, M + Na), 359 (8%, M + 1 – H_2O), 299 (80%, M + 1 – H_2O, AcOH). For $C_{23}H_{36}O_4$ (376.5) calculated. C, 73.37; H, 9.64. Found. C, 73.28; H, 9.67.

*6.2.1.3. 3α-Hydroxy-20-oxo-5α-pregnan-7α-yl Acetate (**4**)*

A solution of diacetate **2** (90 mg, 0.22 mmol) in benzene (10 mL) was treated with a solution of potassium hydroxide (15.4 mg, 0.27 mmol) in methanol (1 mL). After standing overnight

at room temperature, the mixture was poured into water and extracted with ethyl acetate (50 mL). The extract was washed with water, dried and the solvent was evaporated. The residue was purified by PLC (2 plates) in a mixture of acetone/petroleum ether (1:1) to give compound **4** (69 mg, 85%): m.p. 148–150 °C (ether/petroleum ether), $[\alpha]_D$ +64.5 (*c* 0.35, $CHCl_3$). IR spectrum ($CHCl_3$): 3616 (O-H); 1717 (C=O, acetate); 1701 (C=O, ketone); 1256 (C-O). ^{1}H NMR (200 MHz): 0.59 s, 3 H (3 × H-18); 0.78 s, 3 H (3 × H-19); 2.07 s, 3 H (OAc); 2.11 s, 3 H (3 × H-21); 2.55 t, 1 H, J = 8.8 (H-17); 4.06 quintet, 1 H, J = 2.4 (H-3); 4.91 q, 1 H, J = 2.9 (H-7). FAB MS: 399 (17%, M + Na), 317 (8%, M + 1 – AcOH), 299 (9%, M + 1 – AcOH, H_2O). For $C_{23}H_{36}O_4$ (376.5) calculated. C, 73.37; H, 9.64. Found. C, 73.12; H, 9.87.

*6.2.1.4. 20-Oxo-5α-pregnane-3α,7α-diyl 3-Hemisuccinate 7-Acetate (**5**)*

A mixture of compound **4** (100 mg, 0.27 mmol) and succinic anhydride (100 mg, 1 mmol) was dried in vacuo (25 °C, 100 Pa) for 30 min. Dry pyridine (10 mL) and 4-dimethylaminopyridine (20 mg, 0.17 mmol) were added. The mixture was heated for 6 h at 140 °C. Additional succinic anhydride (200 mg, 2 mmol) and 4-dimethylaminopyridine (20 mg, 0.17 mmol) were added and the mixture was heated for 10 h at 140 °C. The reaction mixture was poured into water and extracted with ethyl acetate (50 mL). Aqueous phase was extracted again with ethyl acetate (50 mL), and the collected extracts dried. The solvent was evaporated and the residue crystallized from hot ethyl acetate to give hemisuccinate **5** (71 mg, 35%): m.p. 193–195 °C, $[\alpha]_D$ +43.3 (*c* 0.25, $CHCl_3$). IR spectrum ($CHCl_3$): 3516, 3100 broad (COOH); 1717 (C=O, acetate); 1701 (C=O, ketone); 1379 (CH_3); 1257, 1248 (C-O, acetate). ^{1}H NMR (200 MHz): 0.60 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 2.08 s, 3 H (OAc); 2.12 s, 3 H (3 × H-21); 2.54 t, 3 H, J = 8.8 (H-17); 2.60–2.71 m, 4 H ($OOCCH_2CH_2COO$); 4.92 m, 1 H (H-3); 5.06 q, 1 H, J = 2.4 (H-7). FAB MS: 499 (100%, M + Na), 417 (13%, M + 1 – AcOH), 299 (55%, M – AcOH, $C_4H_5O_4$). For $C_{27}H_{40}O_7$ (476.6) calculated. C, 68.04; H, 8.46. Found. C, 68.13; H, 8.46.

*6.2.1.5. 20-Oxo-5α-pregnane-3α,7α-diyl 3-Sulfate 7-Acetate Pyridinium Salt (**6**)*

The mixture of compound **4** (200 mg, 0.53 mmol) and a sulfur trioxide pyridine complex (400 mg, 2.5 mmol), dried in vacuo (25 °C, 100 Pa) for 1 h, was dissolved in dry chloroform (10 mL). The reaction mixture was stirred for 4 h at room temperature under argon. After standing overnight at -20 °C, the undissolved sulfur trioxide pyridine complex was filtered

off. The solvent was evaporated and the residue was dissolved in absolute methanol (1 mL). The absolute ether (15 mL) was added and the mixture was concentrated to almost one-half. After standing overnight at –20 °C, the crystals were collected and dried in a desiccator (over potassium hydroxide) to afford compound **6** (230 mg, 82%): m.p. 156–160 °C, $[\alpha]_D$ +29.3 (*c* 0.29, $CHCl_3$). IR spectrum ($CHCl_3$): 3072 (pyridinium); 1716 (C=O, acetate); 1702 (C=O, ketone); 1258, 1220 (C-O, acetate); 1258 (S-O). ^{1}H NMR (400 MHz): 0.59 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 2.06 s, 3 H (OAc); 2.12 s, 3 H (3 × H-21); 2.56 t, 1 H, J = 9.2 (H-17); 4.78 quintet, 1 H, J = 2.6 (H-3); 4.89 q, 1 H, J = 2.9 (H-7); 7.98 m, 2 H (H-3 and H-5, pyridinium); 8.48 tt, 1 H, J_1 = 7.8, J_2 =1.5 (H-4, pyridinium); 8.94 m, 2 H (H-2 and H-6, pyridinium). ESI MS: 574 (17%, M + K), 494 (14%, M + K – pyridinium), 478 (21%, M + Na – pyridinium), 412 (15%, M – CH_3CO, pyridinium), 341 (47%, M + Na – $OSO_3C_5H_6N$, CH_3CO). For $C_{28}H_{41}NO_7S$ (535.7) calculated. C, 62.78; H, 7.71; N, 2.61; S, 5.99. Found. C, 62.68; H, 7.62; N, 2.70; S, 6.23.

6.2.1.6. 3α-(tert-Butyldimethylsilyloxy)-20-oxo-5α-pregnan-7α-yl Acetate (7)

Alcohol **4** (528 mg, 1.4 mmol) and imidazole (570 mg, 8.37 mmol) were dissolved in *N*,*N*-dimethylformamide (11 mL) and the mixture was cooled to 0 °C. Then, *tert*-butyldimethylsilyl chloride was added (528 mg, 3.5 mmol). The reaction mixture was allowed to attain room temperature and stirred. After 2 h, the reaction was diluted with ethyl acetate (150 mL) and washed with solution of citric acid, potassium hydrogen carbonate, and water. The organic layer was dried and evaporated in vacuo. Crystallization from ethyl acetate gave 687 mg (99%) of compound **7**: m.p. 120–121 °C, $[\alpha]_D$ +29.0 (*c* 0.37, $CHCl_3$). IR spectrum ($CHCl_3$): 2897 ($(CH_3)_2Si$); 1715 (C=O, acetate); 1701 (C=O, ketone); 1472, 1463 ($(CH_3)_3C$); 1258 (C-O, acetate); 1053 (C-OSi). ^{1}H NMR (200 MHz): 0.02 s, 6 H ($(CH_3)_2Si$); 0.59 s, 3 H (3 × H-18); 0.76 s, 3 H (3 × H-19); 0.89 s, 9 H ($(CH_3)_3C$); 2.03 s, 3 H (OAc); 2.12 s, 3 H (3 × H-21); 2.54 t, 1 H, J = 8.7 (H-17); 3.97 quintet, 1 H, J = 2.9 (H-3); 4.89 q, 1 H, J = 2.4 (H-7). ESI MS: 513 (83%, M + Na), 457 (20%, M + Na – $(CH_3)_3C$), 353 (42%, M + Na – $(CH_3)_3C(CH_3)_2SiO$, CH_3CO, AcOH). For $C_{29}H_{50}O_4Si$ (490.8) calculated. C, 70.97; H, 10.27. Found. C, 70.89; H, 10.39.

*6.2.1.7. 3α-(tert-Butyldimethylsilyloxy)-7α-hydroxy-5α-pregnan-20-one (**8**)*

The method followed that described for compound **4** (*6.2.1.3.*) but using acetate **7** (650 mg, 1.32 mmol) in benzene (50 mL) and potassium hydroxide in ethanol (0.89 M, 60 mL) at

60 °C for 16 h. Compound **8** (527 mg, 88%): m.p. 135–140 °C (ethyl acetate), $[\alpha]_D$ +63.3 (*c* 0.23, $CHCl_3$). IR spectrum ($CHCl_3$): 3615 (O-H); 1699 (C=O, ketone); 1472, 1463 ($(CH_3)_3C$); 1253 ($(CH_3)_2Si$); 1052 (C-OSi). ^{1}H NMR (200 MHz): 0.02 s, 6 H ($(CH_3)_2Si$); 0.59 s, 3 H (3 × H-18); 0.88 s, 12 H (3 × H-19 and $(CH_3)_3C$); 2.11 s, 3 H (3 × H-21); 2.56 t, 1 H, J = 8.8 (H-17); 3.83 m, 1 H (H-7); 3.98 m, 1 H (H-3). ESI MS: 919 (100%, 2M + Na), 471 (50%, M + Na). For $C_{27}H_{48}O_3Si$ (448.8) calculated. C, 72.26; H, 10.70. Found. C, 72.25; H, 11.11.

*6.2.1.8. 3α-(tert-Butyldimethylsilyloxy)-20-oxo-5α-pregnan-7α-yl Nicotinate (**9**)*

Compound **8** (450 mg, 1.0 mmol) and 4-dimethylaminopyridine (10 mg, 0.09 mmol) were dissolved in pyridine (15 mL) and the solution was cooled to 0 °C. Nicotinoyl chloride hydrochloride (900 mg, 5.0 mmol) was slowly added to a stirred mixture in small portions. The reaction mixture was stirred at room temperature for 3 h. Then, it was poured into water (50 mL) and, after standing overnight at –20 °C, the precipitate was separated by suction and subsequently dried in a desiccator (over potassium hydroxide) overnight to yield compound **9** (498 mg, 89%): m.p. 147–151 °C, $[\alpha]_D$ +14.5 (*c* 0.39, $CHCl_3$). IR spectrum ($CHCl_3$): 2897 ($(CH_3)_2Si$); 1714 (C=O, nicotinate); 1703 (C=O, ketone); 1286 (C-O, nicotinate); 1053 (C-OSi). ^{1}H NMR (400 MHz): 0.05 s, 6 H ($(CH_3)_2Si$); 0.64 s, 3 H (3 × H-18); 0.72 s, 9 H ($(CH_3)_3C$); 0.83 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.51 t, 1 H, J = 8.8 (H-17); 3.96 quintet, J = 2.4, 1 H (H-3); 5.22 q, 1 H, J = 2.6 (H-7); 7.40 ddd, 1 H, J_1 = 7.8, J_2 = 4.8, J_3 = 0.7, H-5 (nicotinate); 8.29 dt, 1 H, J_1 = 8, J_2 = 2 (H-4, nicotinate); 8.79 dd, 1 H, J_1 = 4.8, J_2 = 1.8 (H-6, nicotinate); 9.25 m, 1 H (H-2, nicotinate). FAB MS: 554 (36%, M + 1), 496 (8%, M – $(CH_3)_3C$), 299 (4%, M – $(CH_3)_3C(CH_3)_2SiO$, $OCOC_5H_4N$), 255 (79%, M – $(CH_3)_3(CH_3)_2SiO$, $OCOC_5H_4N$, CH_3CO). For $C_{33}H_{51}NO_4Si$ (553.2) calculated. C, 71.56; H, 9.28; N, 2.53. Found. C, 71.40; H, 9.41; N, 2.45.

*6.2.1.9. 3α-Hydroxy-20-oxo-5α-pregnan-7α-yl Nicotinate (**10**)*

Compound **9** (120 mg, 0.21 mmol) was treated with a methanolic solution of *p*-toluenesulfonic acid monohydrate (0.005 M, 72 mL). After standing at room temperature for 10 days, the mixture was neutralized with 10% potassium carbonate solution, extracted with ethyl acetate (100 mL), organic layer was washed with water and dried. The solvent was evaporated and the crude product purified by PLC (3 plates) in a mixture of petroleum ether/acetone (8:2) to give **10** (83 mg, 87%): m.p. 183–187°C (acetone/heptane), $[\alpha]_D$ +15.0

(*c* 0.17, $CHCl_3$). IR spectrum ($CHCl_3$): 3615 (O-H); 1715 (C=O, nicotinate); 1703 (C=O, ketone); 1287 (C-O, nicotinate); 1002 (C-OH). ^{1}H NMR (200 MHz): 0.72 s, 3 H (3 × H-18); 0.86 s, 3 H (3 × H-19); 2.25 s, 3 H (3 × H-21); 2.53 t, 1 H, J = 8.8 (H-17); 4.05 quintet, 1 H, J = 2.4 (H-3); 5.25 q, 1 H, J = 2.8 (H-7); 7.44 m, 1 H (H-5, nicotinate); 8.30 dt, 1 H, J_1 = 7.8, J_2 = 1.9 (H-4, nicotinate); 8.78 dd, 1 H, J_1 = 4.8, J_2 = 1.4 (H-6, nicotinate); 9.26 m, 1 H (H-2, nicotinate). ESI MS: 901 (100%, 2M + Na), 462 (93%, M + Na), 440 (24%). For $C_{27}H_{37}NO_4$ (439.6) calculated. C, 73.77; H, 8.48; N, 3.19. Found. C, 73.75; H, 8.64; N, 2.93.

*6.2.1.10. 20-Oxo-5α-pregnane-3α,7α-diyl 3-Sulfate 7-Nicotinate Pyridinium Salt (**11**)*

The method followed that described for compound **6** (*6.2.1.5.*) but using alcohol **10** (60 mg, 0.13 mmol) and a sulfur trioxide pyridine complex (120 mg, 0.75 mmol) in dry chloroform (10 mL) afforded compound **11** (71 mg, 87%) as a foam: $[\alpha]_D$ +6.0 (*c* 0.2, $CHCl_3$). IR spectrum ($CHCl_3$): 3457, 3139 (pyridinium); 1715 (C=O, nicotinate); 1702 (C=O, ketone); 1289 (C-O, nicotinate); 1243, 1046 (SO_3). ^{1}H NMR (400 MHz): 0.63 s, 3 H (3 × H-18); 0.87 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.54 t, 1 H, J = 8.7 (H-17); 4.79 quintet, J = 2.7, 1 H (H-3); 5.25 q, 1 H, J = 2.5 (H-7); 7.74 dd, 1 H, J_1 = 7.8, J_2 = 5.5 (H-5, nicotinate); 7.83 m, 2 H (H-3 and H-5, pyridinium); 8.30 tt, 1 H, J_1 = 7.8, J_2 = 1.5 (H-4, pyridinium); 8.62 dt, 1 H, J_1 = 7.8, J_2 = 1.5 (H-4, nicotinate); 8.84 m, 2 H (H-2 and H-6, pyridinium); 8.93 m, 1 H (H-6, nicotinate); 9.29 m, 1 H (H-2, nicotinate). FAB MS: 554 (3%, M – CH_3CO), 440 (0.5%, M – 2 x C_5H_6N), 422 (10%, M + 1 – $OSO_3C_5H_6N$). For $C_{32}H_{42}N_2O_7S$ (598.7) calcd. C, 64.19; H, 7.07; N, 4.68; S, 5.36. Found. C, 64.27; H, 7.33; N, 4.55; S, 5.37.

*6.2.1.11. 3α-Hydroxy-5α-pregnan-20-one (**12**)*

This compound was prepared according to the literature[101].

*6.2.1.12. 20-Oxo-5α-pregnan-3α-yl Hemisuccinate (**13**)*

The method followed that described for compound **5** (*6.2.1.4.*) but using alcohol **12** (150 mg, 0.47 mmol) and succinic anhydride (150 mg, 1.5 mmol) in dry pyridine (15 mL) at reflux. After 7 h the reaction was completed. Compound **13** (105 mg, 53%): m.p. 76–79 °C (toluene), $[\alpha]_D$ +77.7 (*c* 0.23, $CHCl_3$). IR spectrum ($CHCl_3$): 1727 (C=O, ester); 1716 (C=O, COOH dimer); 1700 (C=O, ketone); 1188 (C-O, ester). ^{1}H NMR (400 MHz): 0.60 s,

3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.54 t, 3 H, J = 9 (H-17); 2.64–2.69 m, 4 H ($OOCCH_2CH_2COO$); 5.05 m, 1 H (H-3). ESI MS: 457 (3.5% M + 39), 441 (100% M + 23), 418 (7.78%, M). For $C_{25}H_{38}O_5$ (418.2) calculated. C, 71.74; H, 9.51. Found. C, 71.55; H, 9.32.

*6.2.1.13. 20-Oxo-5α-pregnan-3α-yl Sulfate Pyridinium Salt (**14**)*

The method followed that described for compound **6** (*6.2.1.5.*) but using alcohol **12** (200 mg, 0.6 mmol) and a sulfur trioxide pyridine complex (400 mg, 2.5 mmol) in dry chloroform (5 mL). Compound **14** (254 mg, 85%): m.p. 181–183 °C (methanol/ether), literature[107] gives 184 °C $[\alpha]_D$ +69.0 (*c* 0.21), literature[107] gives $[\alpha]_D$ +70.0. IR spectrum ($CHCl_3$): 3139 (pyridinium); 1699 (C=O); 1261, 1253, 1237, 1194, 1171 (SO_3 and pyridinium). ^{1}H NMR (400 MHz): 0.58 s, 3 H (3 × H-18); 0.91 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.52 t, 1 H, J = 8.7 (H-17); 4.45 m, W = 30 Hz (H-3); 8.02 m, 2 H (H-3 and H-5, pyridinium); 8.50 tt, 1 H, J_1 = 7.8, J_2 = 1.5 (H-4, pyridinium); 9.00 m, 2 H (H-2 and H-6, pyridinium). FAB MS: 478 (11%, M + 1). For $C_{26}H_{39}NO_5S$ (477.6) calculated. C, 65.38; H, 8.32; N, 2.93; S, 6.71. Found: C, 65.11; H, 8.02; N, 2.99; S, 6.85.

6.2.2. Synthesis of 5β-Pregnane Derivatives

*6.2.2.1. 20-Oxo-5β-pregnane-3α,7α-diyl Diacetate (**15**)*

This compound was prepared according to the literature[100].

*6.2.2.2. 3α-Hydroxy-20-oxo-5β-pregnan-7α-yl Acetate (**16**)*

A solution of diacetate **15** (100 mg, 0.24 mmol) in methanol (8 mL) was treated with a solution of potassium hydrogen carbonate in water (0.2 M, 0.7 mL) at 70 °C. After 5 h, the mixture was poured into water and extracted with ethyl acetate (70 mL). The organic layer was washed with water, dried and the solvents were evaporated in vacuo. Crystallization from hot ether gave compound **16** (40 mg, 45%): m.p. 143–145 °C, $[\alpha]_D$ +40.3 (*c* 0.3, $CHCl_3$). IR spectrum ($CHCl_3$): 3610, 3527 (O-H); 1726 (C=O, acetate); 1703 (C=O, ketone); 1252 (C-O, acetate); 1047 (C-O). ^{1}H NMR (200 MHz): 0.61 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 2.06 s, 3 H (OAc); 2.12 s, 3 H (3 × H-21); 2.55 t, 1 H, J = 8.8 (H-17); 3.52 m, 1 H, W = 32.7 (H-3); 4.89 q, 1 H, J = 2.4 (H-7). FAB MS: 377 (5%, M + 1), 317 (12%, M + 1 – AcOH), 299 (40%, M + 1 – AcOH, H_2O), 283 (32%, M – AcOH, H_2O, CH_3), 255 (17%, M – AcOH, H_2O, CH_3CO), 159 (30%), 145 (34%), 131 (33%), 119 (37%). For $C_{23}H_{36}O_4$ (376.5) calculated. C, 73.37; H, 9.64. Found. C, 73.49; H, 9.93.

*6.2.2.3. 20-Oxo-5β-pregnane-3α,7α-diyl 3-Hemisuccinate 7-Acetate (**17**)*

The method followed that described for compound **5** (*6.2.1.4.*) but using alcohol **16** (100 mg, 0.27 mmol), succinic anhydride (200 mg, 2 mmol) and 4-dimethylaminopyridine (20 mg, 0.17 mmol) in dry pyridine (20 mL) at reflux. After 4 h the reaction was completed. The residue purified by PLC (3 plates) in a mixture of petroleum ether/acetone (9:1) to give compound **17** (44 mg, 35%): m.p. 131–134 °C, $[\alpha]_D$ +50.3 (*c* 0.22, $CHCl_3$). IR spectrum ($CHCl_3$): 3517, 2676 broad (COOH); 1756 (C=O, COOH); 1726 (C=O, acetate); 1720 (C=O, hemisuccinate); 1703 (C=O, ketone); 1252 (C-O, acetate); 1173 (C-O, hemisuccinate). ^{1}H NMR (200 MHz): 0.61 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 2.06 s, 3 H (OAc); 2.12 s, 3 H (3 × H-21); 2.54–2.69 m, 5 H (H-17 and $OOCCH_2CH_2COO$); 4.62 m, 1 H, W = 31.8 (H-3); 4.89 q, 1 H, J = 2.7 (H-7). FAB MS: 499 (69%, M + Na), 417 (18%, M + 1 – AcOH), 299 (28%, M – AcOH, $C_4H_5O_4$). For $C_{27}H_{40}O_7$ (476.6) calculated. C, 68.04; H, 8.46. Found. C, 68.10; H, 8.59.

*6.2.2.4. 20-Oxo-5β-pregnan-3α,7α-diyl 3-Sulfate 7-Acetate Pyridinium Salt (**18**)*

The method followed that described for compound **6** (*6.2.1.5.*) but using alcohol **16** (110 mg, 0.3 mmol) and a sulfur trioxide pyridine complex (220 mg, 1.4 mmol) in dry chloroform (4 mL). The crystals of **18** were collected and dried in a desiccator (over potassium hydroxide) for 5 days (155 mg, 99%): m.p. 155–158 °C, $[\alpha]_D$ + 45.7 (*c* 0.28, $CHCl_3$). IR spectrum ($CHCl_3$): 3140 (pyridinium); 1725 (C=O, acetate); 1702 (C=O, ketone); 1253 (C-O); 1363, 1171, 960 (O-SO_3). ^{1}H NMR (200 MHz): 0.60 s, 3 H (3 × H-18); 0.92 s, 3 H (3 × H-19); 2.04 s, 3 H (OAc); 2.56 t, 1 H, J = 8.7 (H-17); 4.34 m, 1 H, W = 31 (H-3); 4.88 q, 1 H, J = 2.9 (H-7); 7.97 m, 2 H (H-3 and H-5, pyridinium); 8.48 tt, 1 H, J_1 = 7.8, J_2 = 1.5 (H-4, pyridinium); 8.95 m, 2 H (H-2 and H-6, pyridinium). EI MS: 558 (0.5 %, M + Na), 455 (0.5%, M – pyridinium), 256 (0.5%, M – pyridinium, OSO_3, CH_3CO, AcOH), 230 (3%), 80 (100%). For $C_{28}H_{41}NO_7S$ (535.7) calculated. C, 62.78; H, 7.71; N, 2.61; S, 5.99. Found. C, 62.59; H, 7.71; N, 2.41; S, 6.25.

*6.2.2.5. 3α-(tert-Butyldimethylsilyloxy)-5β-pregnan-7α-yl Acetate (**19**)*

The method followed that described for compound **7** (*6.2.1.6.*) but using hydroxy derivative **16** (70 mg, 0.18 mmol), imidazole (76 mg, 1.1 mmol) and *tert*-butyldimethylsilyl chloride (84 mg, 0.56 mmol) in *N*,*N*-dimethylformamide (1.4 mL). After 1 h the reaction was completed. Compound **19** (50 mg, 55%): m.p. 110–112 °C (ethyl acetate), $[\alpha]_D$ +30.5 (*c* 0.33, $CHCl_3$). IR spectrum ($CHCl_3$): 2955, 2906 ($(CH_3)_3C$); 1725 (C=O, acetate); 1702 (C=O, ketone); 1254, 1021 (C-O, acetate); 1090, 1076 (C-OSi); 853, 837 ($(CH_3)_2Si$). ^{1}H NMR (200 MHz): 0.05 s, 6 H ($(CH_3)_2Si$); 0.6 s, 3 H (3 × H-18); 0.88 s, 9 H ($(CH_3)_3C$); 0.91 s, 3 H (3 × H-19); 2.04 s, 3 H (OAc); 2.12 s, 3 H (3 × H-21); 2.54 t, 1 H, J = 8.7 (H-17); 3.45 m, 1 H, W = 32 (H-3); 3.87 q, 1 H, J = 2.4 (H-7). FAB MS: 433 (2%, M – $(CH_3)_3C$), 373 (18%, M – $(CH_3)_3C$, AcOH), 299 (100%, M – $(CH_3)_3(CH_3)_2Si$, AcOH), 255 (12%, M – $(CH_3)_3(CH_3)_2SiO$, AcOH, CH_3CO). For $C_{29}H_{50}O_4Si$ (490.8) calculated. C, 70.97; H, 10.27. Found. C, 70.63; H, 10.44.

*6.2.2.6. 3α-(tert-Butyldimethylsilyloxy)-7α-hydroxy-5β-pregnan-20-one (**20**)*

The method followed that described for compound **4** (*6.2.1.3.*) but using acetate **19** (40 mg, 0.08 mmol) and solution of potassium hydroxide in ethanol (0.89 M, 4 mL) in benzene (5 mL) at reflux. After 3 h the reaction was completed. The residue was purified by plate thin layer chromatography (2 plates) in a mixture of petroleum ether/acetone (8:2) to give alcohol

20 (20 mg, 55%): m.p. 131–136 °C, $[\alpha]_D$ +47.6 (*c* 0.29, $CHCl_3$). IR spectrum ($CHCl_3$): 3621 (O-H); 1699 (C=O); 1472, 1463, 1385 ($(CH_3)_3C$); 1361 (Ac, $(CH_3)_3C$); 1254 ($(CH_3)_2Si$); 1098, 1082 (C-OSi). ^1H NMR (200 MHz): 0.05 s, 6 H ($(CH_3)_2Si$); 0.60 s, 3 H (3 × H-18); 0.88 s, 12 H (3 × H-19 and $(CH_3)_3C$); 2.12 s, 3 H (3 × H-21); 2.53 t, 1 H, J = 9 (H-17); 3.41 m, 1 H, W = 32.7 (H-3); 3.86 m, 1 H (H-7). FAB MS: 449 (11%, M + 1), 317 (10%, M – $(CH_3)_3(CH_3)_2SiO$), 299 (26%, M + 1 – $(CH_3)_3(CH_3)_2SiO$, H_2O), 283 (13%, M + 1 – $(CH_3)_3(CH_3)_2SiO$, H_2O, O), 255 (14%, M – $(CH_3)_3(CH_3)_2SiO$, H_2O, CH_3CO). For $C_{27}H_{48}O_3Si$ (448.8) calculated. C, 72.26; H, 10.70. Found. C, 72.20; H, 10.84.

*6.2.2.7. 3α-(tert-Butyldimethylsilyloxy)-20-oxo-5β-pregnan-7α-yl Nicotinate (**21**)*

The method followed that described for compound **9** (*6.2.1.8.*) but using alcohol **20** (240 mg, 0.53 mmol), 4-dimethylaminopyridine (10 mg, 0.09 mmol) and nicotinoyl chloride hydrochloride (720 mg, 4.0 mmol) in pyridine (10 mL). After 10 h the reaction was completed. The crude product was purified by PLC (6 plates) in a mixture of petroleum ether/acetone (9:1) to give compound **21** (208 mg, 70%): m.p. 57–59 °C, $[\alpha]_D$ +54.9 (*c* 0.39, $CHCl_3$). IR spectrum ($CHCl_3$): 2907 (CH_3, $(CH_3)_3(CH_3)_2SiO$); 1714 (C=O, nicotinate); 1703 (C=O, ketone); 1286, 1108 (C-O, nicotinate); 1092 (C-OSi). ^1H NMR (400 MHz): 0.05 s, 6 H ($(CH_3)_2Si$); 0.63 s, 3 H (3 × H-18); 0.77 s, 9 H ($(CH_3)_3C$); 0.96 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.52 t, 1 H, J = 9.1 (H-17); 3.43 m, 1 H, W = 32 (H-3); 5.21 q, 1 H, J = 2.8 (H-7); 7.44 ddd, 1 H, J_1 = 7.8, J_2 = 7.8, J_3 = 0.7 (H-5, nicotinate); 8.30 dt, 1 H, J_1 = 8.3, J_2 = 2 (H-4, nicotinate); 8.78 dd, 1 H, J_1 = 4.8, J_2 = 1.7 (H-6, nicotinate); 9.27 dd, 1 H, J_1 = 2, J_2 = 0.7 (H-2, nicotinate). FAB MS: 579 (6%, M + Na), 554 (22%, M + 1), 496 (7%, M – $(CH_3)_3C$), 299 (4%, M – $(CH_3)_3(CH_3)_2SiO$, $OCOC_6H_4N$), 255 (79%, M – $(CH_3)_3C(CH_3)_2SiO$, $OCOC_6H_4N$, CH_3CO). For $C_{33}H_{51}O_4Si$ (553.2) calculated. C, 71.56; H, 9.28; N, 2.53. Found. C, 71.47; H, 9.32; N, 2.23.

*6.2.2.8. 3α-Hydroxy-20-oxo-5β-pregnan-7α-yl Nicotinate (**22**)*

A solution of protected compound **21** (100 mg, 0.18 mmol) in freshly distilled THF (10 mL) was cooled to 0 °C and solution of tetrabutylammonium fluoride (1 M in THF, 0.3 mL, 0.3 mmol) was added. The mixture was stirred at room temperature. After 2 days, further tetrabutylammonium fluoride solution (1 M in THF, 0.1 mL, 0.1 mmol) was added and the mixture was stirred for another 3 days. The mixture was diluted with ethyl acetate (100 mL), organic layer was washed with solution of citric acid, potassium hydrogen carbonate, water,

and dried. The solvent was evaporated and the crude product purified by PLC (2 plates) in a mixture of petroleum ether/acetone (9:1) to give **22** (58 mg, 74%): m.p. 70–73 °C (acetone/heptane), $[\alpha]_D$ +29.2 (*c* 0.28, $CHCl_3$). IR spectrum ($CHCl_3$): 3613; 3451 (O-H); 1715 (C=O, nicotinate); 1705 (C=O, ketone); 1287 (C-O, nicotinate); 1034, 1026 (C-OH); 1592, 1421 (nicotinate). ^{1}H NMR (400 MHz): 0.64 s, 3 H (3 × H-18); 0.98 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.54 t, 1 H, J = 9.2 (H-17); 3.49 m, 1 H, W = 31 (H-3); 5.20 q, 1 H, J = 2.7 (H-7); 7.45 ddd, 1 H, J_1 = 7.8, J_2 = 4.8, J_3 = 0.7 (H-5, nicotinate); 8.31 dt, 1 H, J_1 = 7.8, J_2 = 1.7 (H-4, nicotinate); 8.80 dd, 1 H, J_1 = 4.8, J_2 = 1.7 (H-6, nicotinate); 9.26 dd, 1 H, J_1 = 2, J_2 = 0.7 (H-2, nicotinate). FAB MS: 440 (12%, M + 1), 145 (7%), 124 (100%), 105 (35%). For $C_{27}H_{37}NO_4$ (439.6) calculated. C, 73.77; H, 8.48; N, 3.19. Found. C, 73.71; H, 8.95; N, 2.97.

*6.2.2.9. 20-Oxo-5β-pregnane-3α,7α-diyl 3-Sulfate 7-Nicotinate Pyridinium Salt (**23**)*

The method followed that described for compound **6** (*6.2.1.5.*) but using alcohol **22** (45 mg, 0.1 mmol) and a sulfur trioxide pyridine complex (90 mg, 0.56 mmol) in freshly distilled chloroform (5 mL). After 4 h the reaction was completed to afford **23** (40 mg, 74%) as a white foam: $[\alpha]_D$ +42.0 (*c* 0.32, $CHCl_3$). IR spectrum ($CHCl_3$): 3139, 2652 (pyridinium); 1714 (C=O, nicotinate); 1703 (C=O, ketone); 1287 (C-O, nicotinate); 1175, 978, 958 (O-SO_3). ^{1}H NMR (400 MHz): 0.63 s, 3 H (3 × H-18); 0.98 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.56 t, 1 H, J = 8.7 (H-17); 4.32 m, 1 H, W = 32 (H-3); 5.26 m, 1 H (H-7); 7.74 dd, 1 H, J_1 = 7.6, J_2 = 5.3 (H-5, nicotinate); 7.82 m, 2 H (H-3 and H-5, pyridinium); 8.33 t, 2 H, J =7.8 (H-4, pyridinium); 8.60 d, 1 H, J = 7.6 (H-4, nicotinate); 8.88 m, 2 H (H-2 and H-6, pyridinium); 8.93 d, 1 H, J = 5 (H-6, nicotinate); 9.33 m, 1 H (H-2, nicotinate). FAB MS: 564 (10%), 542 (20%), 519 (12%, M + 1 – pyridinium), 444 (18%), 422 (10%, M + 1 – $OSO_3C_5H_6N$). For $C_{32}H_{42}N_2O_7S$ (598.7) calculated. C, 64.19; H, 7.07; N, 4.68; S, 5.36. Found. C, 64.09; H, 6.98; N, 4.70; S, 5.51.

*6.2.2.10. 3α-Hydroxy-5β-pregnan-20-one (**24**)*

This compound was prepared according to the literature[101].

*6.2.2.11. 20-Oxo-5β-pregnan-3α-yl Hemisuccinate (**25**)*

The method followed that described for compound **5** (*6.2.1.4.*) but using alcohol **24** (100 mg, 0.27 mmol), succinic anhydride (250 mg, 2.5 mmol) and 4-dimethylaminopyridine (25 mg,

0.21 mmol) in dry pyridine (10 mL) at reflux. After 4 h the reaction was completed. Compound **25** (63 mg, 48%): m.p. 156–159 °C (ether), $[\alpha]_D$ +101.6 (*c* 0.3, $CHCl_3$). IR spectrum ($CHCl_3$): 1726 (C=O, ester); 1717 (C=O, COOH-dimer); 1701 (C=O, ketone); 1176 (C-O, ester); 1175, 978, 958 (O-SO_3). ^{1}H NMR (400 MHz): 0.60 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.53 t, 3 H, J = 9 (H-17); 2.56–2.69 m, 4 H ($OOCCH_2CH_2COO$); 4.76 m, 1 H, W = 32 (H-3). EI MS: 418 (1.5%, M), 400 (12%, M – H_2O), 300 (100%, M + 1 – $HOOCCH_2CH_2COO$), 285 (19%, M – $HOOCCH_2CH_2COO$, O), 267 (15%, M – $HOOCCH_2CH_2COO$, $COCH_3$). For $C_{25}H_{38}O_5$ (418.2) calculated. C, 71.74; H, 9.51. Found. C, 71.65; H, 9.61.

*6.2.2.12. 20-Oxo-5β-pregnan-3α-yl Sulfate Pyridinium Salt (**26**)*

The method followed that described for compound **6** (*6.2.1.5.*) but using alcohol **24** (200 mg, 0.6 mmol) and a sulfur trioxide pyridine complex (400 mg, 2.5 mmol) in dry chloroform (5 mL). After 4 h the reaction was completed. Compound **26** (240 mg, 80%): m.p. 173–175 °C (methanol/ether), literature[107] gives 170 °C. $[\alpha]_D$ +100.0 (*c* 0.28), literature[107] gives $[\alpha]_D$ +103.0. IR spectrum ($CHCl_3$): 3139 (pyridinium); 1698 (C=O, ketone); 1270, 1257, 1236, 1179, 1166 (SO_3 and pyridinium). ^{1}H NMR (400 MHz): 0.60 s, 3 H (3 × H-18); 0.78 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.52 t, 1 H, J = 8.7 (H-17); 4.45 m, 1 H, W = 30 (H-3); 8.03 m, 2 H (H-3 and H-5, pyridinium); 8.50 tt, 1 H, J_1 = 7.8, J_2 = 1.5 (H-4, pyridinium); 9.00 m, 2 H (H-2 and H-6, pyridinium). FAB MS: 478 (22%, M + 1), 440 (22%, M + 1 – C_5H_6N), 301 (17%, M + 1 – $OSO_3C_5H_6N$). For $C_{26}H_{39}NO_5S$ (477.6) calculated. C, 65.38; H, 8.32; N, 2.93; S, 6.71. Found: C, 65.27; H, 8.20; N, 3.05; S, 6.92.

6.3. Synthesis of 3-Steroid Carboxylic Acids

6.3.1. General Procedure for Synthesis of Triflates Using 2,6-Di-tert-butylpyridine

Triflic anhydride (0.2 mL, 1.1 mmol) was added dropwise to a stirred mixture of ketone (0.2 mmol) and 2,6-di-*tert*-butylpyridine (246 mg, 1.2 mmol) in dichloromethane (2 mL) under argon at 0 °C. The course of the reaction was followed by TLC. After completion of reaction, the reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic phase was washed with aqueous potassium hydrogen carbonate and water, dried and evaporated *in vacuo*.

6.3.2. General Procedure for Synthesis of Triflates Using LDA

A solution of diisopropylamine (0.3 mL, 2.1 mmol) in dry THF (5 mL) was stirred under argon and then cooled to –78 °C. A solution of *n*-BuLi (1.6 M in hexanes, 1.3 mL, 2.1 mmol) was added dropwise and the stirring was continued for next 30 min. To a freshly prepared solution of LDA in dry THF a solution of particular ketone in dry THF (2.5 mL per 100 mg) was added during 10 min at –78 °C under argon and the mixture was stirred at –78 °C for 1.5 hours. Then, N-phenyltrifluoromethanesulfonimide in dry THF (2 mL per 500 mg) was added dropwise and the mixture was stirred at –78 °C for 1 hours. After additional 8 h at room temperature under argon, the mixture was poured into water, diluted with ethyl acetate, washed with an aqueous solution of citric acid, water, saturated solution of potassium hydrogen carbonate, water and dried over anhydrous sodium sulfate. Solvents were evaporated *in vacuo*.

6.3.3. General Procedure for Synthesis of Triflates Using LiHMDS

To a solution of LiHMDS (1.0 M in hexanes) a solution of particular ketone in dry THF (2.5 mL per 100 mg) was added during 10 min at –78 °C under argon and the mixture was stirred at –78 °C for 3.5 hours. Then, N-phenyltrifluoromethanesulfonimide in dry THF (2 mL per 500 mg) was added dropwise and the mixture was allowed to attain the room temperature. After additional 8 h at room temperature under argon, the mixture was poured into water and extracted with ethyl acetate. Organic phase was washed with an aqueous solution of citric acid, water, saturated solution of potassium hydrogen carbonate, water and dried over anhydrous sodium sulfate. Solvents were evaporated *in vacuo*.

6.3.4. General Procedure for Synthesis of Nonaflates Using LDA

A solution of diisopropylamine (0.3 mL, 2.1 mmol) in dry THF (5 mL) was stirred under argon and then cooled to –78 °C. A solution of *n*-BuLi (1.6 M in hexanes, 1.3 mL, 2.1 mmol) was added dropwise and the stirring was continued for next 30 min. To a freshly prepared solution of LDA in dry THF a solution of particular ketone in dry THF (2.5 mL per 100 mg) was added during 10 min at –78 °C under argon and the mixture was stirred at –78 °C for 1.5 hours. Then, nonafluorobutanesulfonyl fluoride was added dropwise and the mixture was stirred at –78 °C for 2 hours. After additional 8 h at room temperature under argon, the mixture was poured into water and extracted with ethyl acetate. Organic phase was washed with an aqueous solution of citric acid, water, saturated solution of potassium hydrogen carbonate, water and dried over anhydrous sodium sulfate. Solvents were evaporated *in vacuo*.

6.3.5. General Procedure for Synthesis of Nonaflates Using LiHMDS

To a solution of LiHMDS (1.0 M in hexanes) a solution of particular ketone in dry THF (2.5 mL per 100 mg) was added during 10 min at –78 °C under argon and the mixture was stirred at –78 °C for 3.5 hours. Then, nonafluorobutanesulfonyl fluoride was added dropwise and the mixture was allowed to attain the room temperature. After additional 8 h at room temperature under argon, the mixture was poured into water, diluted with ethyl acetate, washed with an aqueous solution of citric acid, water, saturated solution of potassium hydrogen carbonate, water and dried over anhydrous sodium sulfate. Solvents were evaporated *in vacuo*.

6.3.6. General Procedure of Alkoxycarbonylation

A mixture of nonaflate or triflate (0.4 mmol), triethylamine (0.14 mL, 1.0 mmol), palladium acetate (9 mg, 0.04 mmol), triphenylphosphine (23.5 mg, 0.09 mmol), and methanol (7 mL) in DMF (14 mL) were stirred under slight CO overpressure at room temperature. The reaction mixture was monitored by TLC on silica gel. After the observed completion of reaction, the reaction mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with a solution of citric acid (5%), water, saturated solution of potassium hydrogen carbonate, water, and dried over anhydrous sodium sulfate. Solvents were evaporated *in vacuo*.

6.3.7. General Procedure for Deprotection of 20-Keto Group

Acetal (0.2 mmol) was dissolved in acetone (4 mL) and a solution of *p*-toluenesulfonic acid monohydrate (7.5 mg, 0.04 mmol) in water (1 mL) was added. The mixture was allowed to stay overnight at room temperature, then poured into a solution of potassium hydrogen carbonate and extracted with ethyl acetate. The organic phase was diluted with water, dried over anhydrous sodium sulfate and solvents were evaporated *in vacuo*.

6.3.8. General Procedure of Hydrogenation

A solution of olefine (0.1 mmol) in ethyl acetate (3 mL) and ethanol (0.7 mL) was stirred in the presence of palladium on calcium carbonate (5%, 6 mg) under slight hydrogen overpressure at room temperature. After 5 h, the catalyst was filtered off and the filtrate was evaporated *in vacuo*.

6.3.9. General Procedure of Alkoxycarbonylation and Deprotection of 20-Keto Group

A mixture of nonaflate or triflate (0.5 mmol), triethylamine (0.167 mL, 1.2 mmol), palladium acetate (9 mg, 0.04 mmol), triphenylphosphine (26 mg, 0.1 mmol), and methanol (7 mL) in DMF (14 mL) were stirred under slight CO overpressure at room temperature. The reaction mixture was monitored by TLC on silica gel. After the observed completion of reaction, the reaction mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with a solution of citric acid (5%), water, saturated solution of potassium hydrogen carbonate, water, and dried over anhydrous sodium sulfate. Solvents were evaporated *in vacuo*. The residue was dissolved in acetone (5 mL) and a solution of *p*-toluenesulfonic acid monohydrate (7.5 mg, 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. The organic phase was washed with water, dried over anhydrous sodium sulfate, and solvent was evaporated *in vacuo*.

6.3.10. General Procedure for Basic Hydrolysis of Ester

A solution of potassium hydroxide (9 mg, 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 heated at 120 °C and the progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured into ice-water and acidified by a mixture HCl/H_2O (1:2) to pH 1. The mixture was extracted with chloroform (2 × 30 mL),

the collected organic phases were diluted with water, dried and evaporated. The residue was crystallized from the mixture of acetone–water.

*6.3.11. 5α-Cholest-2-en-3-yl Triflate and 5α-Cholest-3-en-3-yl Triflate, a Mixture of Isomers (**28**)*

Ketone **27** (100 mg, 0.25 mmol) was converted to triflate **28** according to the *General Procedure 6.3.1*. Purification by PLC (2 plates) in petroleum ether afforded the mixture of Δ^2 and Δ^3 isomers **28** (109 mg, 81%). The Δ^2 isomer prevailed in the ratio 5:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.66 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 0.85 d, 3 H, J = 6.6 (3 × H-27); 0.87 d, 3 H, J = 6.6 (3 × H-26); 0.90 d, 3 H, J = 6.5 (3 × H-21); 5.38 m, 0.16 H (H-4); 5.65 m, 0.84 H (H-2). IR spectrum ($CHCl_3$): 1650 (C=C); 1414, 1245, 1227 (SO_2, triflate); 1142 (CF_3, triflate). APCI MS: 517 (100%, M – 1). For $C_{28}H_{45}F_3O_3S$ (518.7) calculated. C, 64.83; H, 8.74. Found. C, 64.87; H, 8.72.

*6.3.12. 5α-Cholest-2-en-3-yl Nonaflate and 5α-Cholest-3-en-3-yl Nonaflate, a Mixture of Isomers (**29**)*

Compound **27** (100 mg, 0.25 mmol), LDA (0.7 mmol), and nonafluorobutanesulfonyl fluoride (0.13 mL, 0.77 mmol) were converted to **29** according to the *General Procedure 6.3.4*. Purification by PLC (3 plates) in petroleum ether afforded the mixture of Δ^2 and Δ^3 isomers, **29** (90 mg, 52%). The Δ^2 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.66 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 0.86 d, 3 H, J = 6.6 (3 × H-27); 0.87 d, 3 H, J = 6.6 (3 × H-26); 0.90 d, 3 H, J = 6.6 (3 × H-21); 5.40 s, 0.34 H (H-4); 5.67 m, 0.66 H (H-2). IR spectrum ($CHCl_3$): 1415, 1242 (SO_2, nonaflate); 1202, 1145 (CF_3, nonaflate). APCI MS: 667 (100%, M – 1). For $C_{31}H_{45}F_9O_3S$ (668.7) calculated. C, 55.68; H, 6.78. Found. C, 55.54; H, 6.94.

*6.3.13. Methyl 1-(5α-Cholest-2-en-3-yl)acetate and Methyl 1-(5α-Cholest-3-en-3-yl)acetate, a Mixture of Isomers (**30**)*

Procedure A:

A mixture of triflates **28** (65 mg, 0.13 mmol) was converted to **30** according to the *General Procedure 6.3.6*. Purification by PLC (1 plate) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers, **30** (33 mg, 60%). The Δ^2 isomer prevailed in the ratio 5:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.66 s, 3 H (3 × H-18); 0.72

s, 3 H (3 × H-19); 0.85 d, 3 H, J = 6.6 (3 × H-27); 0.87 d, 3 H, J = 6.6 (3 × H-26); 0.91 d, 3 H, J = 6.5 (3 × H-21); 6.62 m, 0.16 H (H-4); 6.90 dt, J_1 = 3.6, J_2 = 1.7, 0.84 H (H-2). IR spectrum ($CHCl_3$): 1706 (C=O, $COOCH_3$); 1650 (C=C); 1264 (C-O, $COOCH_3$). FAB MS: 466 (20%, M + K), 6429 (47%, M + 1), 397 (9%, M – CH_3O), 315 (5%, M – C_8H_{17}). For $C_{29}H_{48}O_2$ (428.7) calculated. C, 81.25; H, 11.29. Found. C, 81.04; H, 11.47.

Procedure B:

A mixture of nonaflates **29** (70 mg, 0.1 mmol) was converted to **30** according to the *General Procedure 6.3.6*. Purification by PLC (1 plate) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers, **30** (22 mg, 50%) identical with the sample prepared above according to Procedure A. The Δ^2 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum.

*6.3.14. 20-Oxo-5β-pregnan-3α-yl Acetate (**31**)*

To a solution of hydroxy derivative **24** (2.3 g, 7.2 mmol) in pyridine (76 mL), acetic anhydride (6 mL, 63 mmol) was added and the mixture was allowed to stay at 50 °C for 3 days. Then, the reaction mixture was poured into ice-water and extracted with ethyl acetate (200 mL). Organic phase was washed with a solution of hydrochlorid acid (5 %, 70 mL), a solution of potassium hydrogen carbonate (70 mL), water (70 mL), and dried. Solvent was evaporated and the oily residue was purified by chromatography on a column of silica gel (80 g) in a mixture of petroleum ether/ether (9:1) to give acetate **31** (2 g, 77%): m.p. 89–91 °C (petroleum ether/ether)), literature[108] gives 100–101 °C (ether). $[\alpha]_D$ +112.5 (*c* 0.41, $CHCl_3$), literature[108] gives $[\alpha]_D$ +123. IR spectrum ($CHCl_3$): 1720 (C=O, ketone); 1700 (C=O, acetate); 1253, 1029 (C-O). ^{1}H NMR (200 MHz): 0.6 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 2.04 s, 3 H (3 × OAc); 2.12 s, 3 H (3 × H-21); 2.53 t, 1 H, J = 9 (H-17); 4.73 m, 1 H, W = 32 (H-3). FAB MS: 361 (6%, M + 1), 360 (15%, M – AcOH), 360 (21%, M – AcOH, CH_3CO). For $C_{23}H_{36}O_3$ (360.5) calculated. C, 76.62; H, 10.06. Found. C 76.76; H, 10.21.

*6.3.15. (20R)-20-Hydroxy-5β-pregnan-3α-yl Acetate (**32**)*

Sodium borohydride (156 mg, 4.1 mmol) was added during 5 min to a cooled solution (10 °C) of derivative **31** (1.2 g, 3.3 mmol) in methanol (18 mL) and ethyl acetate (7.2 mL). The reaction mixture was stirred and the course of the reaction was followed by TLC. After the observed completion of reaction, a solution of acetic acid (0.72 mL, 12.5 mmol) in water

(7.2 mL) was added. The reaction mixture was poured into ice-water and allowed to attain room temperature. The precipitate was filtered off and subsequently purified by chromatography on a column of silica gel (40 g) in a mixture of petroleum ether/ether (9:1) to give **32** (1 g, 83%): 120–121 °C (petroleum ether/ether), literature[109] gives 131–132 °C (hexane). $[\alpha]_D$ +32.6 (*c* 0.23, $CHCl_3$), literature[110] gives $[\alpha]_D$ +28.8. IR spectrum ($CHCl_3$): 3611, 1039 (O-H); 1721 (C=O, acetate); 1260, 1252 (C-O, acetate). ^{1}H NMR (200 MHz): 0.65 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 1.22 d, 3 H, J = 6.1 (3 × H-21); 2.03 s, 3 H (3 × OAc); 3.70 dq, 1 H, J_1 = 6.1, J_2 = 12 (H-20); 4.73 m, 1 H, W = 32 (H-3). FAB MS: 363 (2%, M + 1), 302 (10%, M – AcOH), 284 (15%, M – AcOH, H_2O). For $C_{23}H_{38}O_3$ (362.5) calculated. C, 76.20; H, 10.56. Found. C 76.46; H, 10.79.

*6.3.16. (20R)-5β-pregnane-3α,20-diyl 3-Acetate 20-Benzoate (**33**)*

Benzoyl chloride (2 mL, 17.2 mmol) was added dropwise to a solution of **32** (900 mg, 2.5 mmol) in pyridine (40 mL) cooled to 0°C. The reaction mixture was allowed to attain a room temperature and stirred for 4 hours. Then, the reaction mixture was poured into a hot water (90 °C) and extracted with ethyl acetate (2 × 100 mL). Organic phase was washed with a solution of potassium hydrogen carbonate (150 mL), water (100 mL), dried and the solvent was evaporated. The residue was crystallized from methanol to give compound **33** (1.1 g, 95%): m.p. 76–78 °C (petroleum ether/ether), literature[110] gives 89–90 °C (MeOH), $[\alpha]_D$ +34.8 (*c* 0.21, $CHCl_3$). ^{1}H NMR (400 MHz): 0.65 s, 3 H (3 × H-18); 0.88 s, 3 H (3 × H-19); 1.26 d, 3 H, J = 6.0 (3 × H-21); 2.04 s, 3 H (3 × OAc); 4.72 m, 1 H, W = 32 (H-3); 5.12 dq, 1 H, J_1 = 10.1, J_2 = 6.0 (H-20); 7.43 m, 2 H (H-3 and H-5, benzoate); 7.55 m, 1 H, (H-4, benzoate); 8.03 m, 2 H (H-2 and H-6, benzoate). IR spectrum ($CHCl_3$): 3021 (=CH); 1724 (C=O, acetate); 1708 (C=O, benzoate); 1278 (C-O, benzoate); 1259, 1252 (C-O, acetate); 1291, 1120, 1071, 1027 (ring, benzoate). FAB MS: 489 (6%, M + Na), 406 (33%, M – AcOH), 345 (62%, M – $C_7H_5O_2$). For $C_{30}H_{42}O_4$ (466.6) calculated. C, 77.21; H, 9.07. Found. C, 77.04; H, 9.08.

*6.3.17. (20R)-3α-Hydroxy-5β-pregnan-20-yl Benzoate (**34**)*

A solution of compound **33** (500 mg, 1.0 mmol) in methanol (8 mL) and chloroform (1 mL) was treated with a solution of perchloric acid (37%, 0.625 mL) at 40 °C for 4 hours. Then, the reaction mixture was poured into ice-water (25 mL), neutralized with a solution of potassium hydrogen carbonate to pH 7, and evaporated to one-half of volume. The

precipitate was filtered off and dried in a desiccator to give compound **34** (435 mg, 95%): m.p. 69–72 °C ($CHCl_3$), $[\alpha]_D$ +1.08 (*c* 0.19, $CHCl_3$). ^1H NMR (200 MHz): 0.65 s, 3 H (3 × H-18); 0.88 s, 3 H (3 × H-19); 1.26 d, 3 H, J = 6.0 (3 × H-21); 3.63 m, 1 H, W = 31 (H-3); 5.13 dq, 1 H, J_1 = 10.8, J_2 = 6.0 (H-20); 7.45 m, 2 H (H-3 and H-5, benzoate); 7.55 m, 1 H, (H-4, benzoate); 8.06 m, 2 H (H-2 and H-6, benzoate). IR spectrum ($CHCl_3$): 3610 (OH); 3028 (=CH); 1706 (C=O, benzoate); 1278 (C-O, benzoate); 1036 (C-OH); 1291, 1176 (ring, benzoate). FAB MS: 447 (10%, M + Na), 406 (22%, M – H_2O), 303 (23%, M – $C_7H_5O_2$). For $C_{28}H_{40}O_3$ (424.6) calculated. C, 79.20; H, 9.50. Found. C, 79.38; H, 9.61.

*6.3.18. (20R)-3-Oxo-5β-pregnan-20-yl Benzoate (**35**)*

A solution of hydroxy derivative **34** (450 mg, 1.05 mmol) in acetone (10 mL) was treated with Jones reagent (0.410 mL) at room temperature for 5 min. The course of the reaction was followed by TLC. Then, propan-2-ol was added dropwise up to the reaction mixture get green. After 5 min, it was poured into water (50 mL), extracted with ether (100 mL). Extract was washed with a solution of potassium hydrogen carbonate (40 mL), water (40 mL), dried, and evaporated. Crystallization from ether afforded ketone **35** (370 mg, 83%): m.p. 141–143 °C ($CHCl_3$), $[\alpha]_D$ +13.0 (*c* 0.45, $CHCl_3$). ^1H NMR (200 MHz): 0.68 s, 3 H (3 × H-18); 0.98 s, 3 H (3 × H-19); 1.27 d, 3 H, J = 6.0 (3 × H-21); 5.14 dq, 1 H, J_1 = 10.1, J_2 = 6.0 (H-20); 7.45 m, 2 H (H-3 and H-5, benzoate); 7.56 m, 1 H, (H-4, benzoate); 8.06 m, 2 H (H-2 and H-6, benzoate). IR spectrum ($CHCl_3$): 3026 (=CH); 1706 (C=O, benzoate and ketone); 1278 (C-O, benzoate); 1292, 1175, 1071 (ring, benzoate). FAB MS: 445 (33%, M + Na), 406 (5%, M – O), 301 (45%, M – $C_7H_5O_2$). For $C_{28}H_{38}O_3$ (422.6) calculated. C, 79.58; H, 9.06. Found. C, 79.55; H, 9.13.

*6.3.19. (20R)-5β-Pregn-2-ene-3,20-diyl 3-Triflate 20-Benzoate and (20R)-5β-Pregn-3-ene-3,20-diyl 3-Triflate 20-Benzoate, a Mixture of Isomers (**36**)*

Ketone **35** (100 mg, 0.2 mmol) was converted to **36** according to the *General Procedure 6.3.1*. Purification by PLC (2 plates) in the mixture of petroleum ether/ether (9:1) afforded the mixture of Δ^2 and Δ^3 isomers as yellow oily product **36** (68 mg, 52%) and the starting material **35** (8 mg, 8%). The Δ^3 isomer prevailed in the ratio 2:1 according to ^1H NMR spectrum. ^1H NMR ($CDCl_3$): 0.66 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 1.25 d, 3 H, J = 6.06 (3 × H-21); 5.12 dq, 1 H, J_1 = 10.1, J_2 = 6.1 (H-20); 5.45 s, 0.66 H (H-4); 5.59 d, 0.34 H, J = 8.08 (H-2); 7.43 m, 2 H (H-3 and H-5, benzoate); 7.54 m, 1 H, (H-4, benzoate);

8.05 m, 2 H (H-2 and H-6, benzoate). IR spectrum ($CHCl_3$): 1706 (C=O, benzoate); 1661 (C=C); 1414 (SO_2, triflate); 1279 (C-O, benzoate); 1246, 1142, 614 (CF_3, triflate); 1121, 1071, 1027, 1027 (ring, benzoate). FAB MS: 577 (5%, M + Na), 433 (10%, M – $C_7H_5O_2$), 403 (2%, M – 1 – F_3CSO_3), 283 (4%, M – $C_7H_5O_2$, F_3CSO_3). For $C_{29}H_{37}F_3O_5S$ (554.6) calculated. C, 62.80; H, 6.72. Found. C, 62.79; H, 6.85.

*6.3.20. (20R)-5β-Pregn-2-ene-3,20-diyl 3-Nonaflate 20-Benzoate and (20R)-5β-Pregn-3-ene-3,20-diyl 3-Nonaflate 20-Benzoate,a Mixture of Isomers (**37**)*

Compound **35** (100 mg, 0.5 mmol), LDA (2.5 mmol), and nonafluorobutanesulfonyl fluoride (0.130 mL, 1.6 mmol) was converted to **37** according to the *General Procedure 6.3.4*. Purification by PLC (4 plates) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers, **37** (82 mg, 49%) and starting material **36** (7 mg, 7%). The Δ^3 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.66 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 1.25 d, 3 H, J = 6.5 (3 × H-21); 5.12 dq, 1 H, J_1 = 10.2, J_2 = 6.1 (H-20); 5.46 s, 0.66 H (H-4); 5.61 d, 0.34 H, J = 6.1 (H-2); 7.45 m, 2 H (H-3 and H-5, benzoate); 7.55 m, 1 H, (H-4, benzoate); 8.04 m, 2 H (H-2 and H-6, benzoate). IR spectrum ($CHCl_3$): 1706, 714 (C=O, benzoate); 1652 (C=C); 1415, 590 (SO_2, nonaflate); 1279 (C-O, benzoate); 1242, 1145 (CF_3, nonaflate). FAB MS: 727 (9%, M + Na), 583 (5%, M + $C_7H_60_2$), 406 (1%, M + 1 – 299, $C_4H_9SO_3$). For $C_{32}H_{37}F_9O_3S$ (704.6) calculated. C, 54.54; H, 5.29. Found. C, 54.70; H, 5.20.

*6.3.21. Methyl (20R)-20-Benzoyloxy-5β-pregn-2-ene-3-carboxylate and Methyl (20R)-20-Benzoyloxy-5β-pregn-3-ene-3-carboxylate, a Mixture of Isomers (**38**)*

Procedure A:

A mixture of triflates **36** (260 mg, 0.5 mmol) was converted to **38** according to the *General Procedure 6.3.6*. Purification by PLC (6 plates) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers, **38** (146 mg, 67%). The Δ^3 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.66 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 1.25 d, 3 H, J = 6.0 (3 × H-21); 3.75 s, 3 H (COOMe); 5.12 dq, 1 H, J_1 = 10.3, J_2 = 6.2 (H-20); 6.72 s, 0.66 H (H-4); 6.84 m, 0.34 H (H-2); 7.44 m, 2 H (H-3 and H-5, benzoate); 7.55 m, 1 H, (H-4, benzoate); 8.03 m, 2 H (H-2 and H-6, benzoate). IR spectrum ($CHCl_3$): 1706 (C=O, $COOCH_3$); 1706 (C=O, benzoate); 1657, 1647 (C=C); 1438 (CH_3, $COOCH_3$); 1277 (C-O, benzoate); 1258 (C-O, $COOCH_3$). FAB MS: 487 (6%, M

+ Na), 343 (5%, M – $C_7H_5O_2$). For $C_{30}H_{40}O_4$ (464.6) calculated. C, 77.55; H, 8.68. Found. C, 77.45; H, 8.65.

Procedure B:

A mixture of nonaflates **37** (299 mg, 0.6 mmol) was converted to a mixture of esters **38** according to the *General Procedure 6.3.6*. Purification by PLC (1 plate) in the mixture of petroleum ether/ether (9:1) afforded the mixture of Δ^2 and Δ^3 isomers **38** (128 mg, 52%) identical with the sample prepared above according to Procedure A. The Δ^2 isomer prevailed in the ratio 2.5:1 according to ^{1}H NMR spectrum.

*6.3.22. Methyl (20R)-20-Benzoyloxy-5β-pregnane-3α-carboxylate (**39**)*

Methyl ester **38** (146 mg, 0.3 mmol) was converted to 3α-carboxy derivatives **39** according to the *General Procedure 6.3.8*. PLC of the residue (92 mg) in the mixture of petroleum ether/ether (97:3) (4 plates, 15 times) and further crystallization from ethyl acetate gave 51 mg (35%) of 3α-carboxy derivative **39** and 10 mg of a mixture of 3α-carboxy, 3β-carboxy derivative, and starting compound **38**. Compound **39**: m.p. 59–61 °C (ethyl acetate), $[\alpha]_D$ +22.7 (*c* 0.39, $CHCl_3$). ^{1}H NMR (400 MHz): 0.67 s, 3 H (3 × H-18); 0.91 s, 3 H (3 × H-19); 1.28 d, 3 H, J = 6.1 (3 × H-21); 2.35 tt, 1 H, J_1 = 12.4, J_2 = 3.8 (H-3); 3.70 s, 3 H (COOMe); 5.14 dq, 1 H, J_1 = 10.2, J_2 = 6.3 (H-20); 7.46 m, 2 H (H-3 and H-5, benzoate); 7.56 m, 1 H, (H-4, benzoate); 8.06 m, 2 H (H-2 and H-6, benzoate). IR spectrum ($CHCl_3$): 3092, 3064, 3027 (=C-H, benzoate); 1727 (C=O, COOMe); 1707 (C=O, benzoate); 1437 (CH_3, $COOCH_3$); 1278 (C-O, benzoate), 1163 (C-O, $COOCH_3$). FAB MS: 665 (10%, M – 1), 489 (28%, M + Na). For $C_{30}H_{42}O_4$ (466.6) calculated. C, 77.21; H, 9.07. Found. C, 77.41; H, 9.20.

*6.3.23. 20-Oxo-5α-pregnan-3β-yl Acetate (**41**)*

Pregnenolone acetate **40** (1 g, 2.78 mmol) was converted to saturated 5α-derivative **41** according to the *General Procedure 6.3.8*. Crystallization from acetone/heptane afforded **41** (790 mg, 79%): m.p. 139–140 °C (ethanol), literature[111] gives 135–141 °C. $[\alpha]_D$ +73.8 (*c* 0.27, $CHCl_3$), literature[112] gives $[\alpha]_D$ +72.0. ^{1}H NMR (400 MHz): 0.60 s, 3 H (3 x H-18); 0.82 s, 3 H (3 x H-19); 2.02 s, 3 H (3 x OAc); 2.11 s, 3 H (3 x H-21); 2.52 t, 1 H, J = 9 (H-17); 4.68 m, 1 H, W = 32.5 (H-3).

*6.3.24. 20,20-(Ethylenedioxy)-5α-pregnan-3β-yl 3-Acetate (**42**)*

A mixture of compound **41** (750 mg, 2.08 mmol) in dry benzene (7.5 mL), ethylene glycol (1.13 mL, 20 mmol), triethyl orthoformate (1.43 mL, 8.5 mmol), and *p*-toluenesulfonic acid monohydrate (7.5 mg, 0.04 mmol) was stirred overnight at room temperature. The reaction mixture was poured into saturated solution of potassium hydrogen carbonate (20 mL), extracted with ethyl acetate (50 mL). Extract was washed with water, dried and evaporated *in vacuo*. Crystallization from hot methanol afforded 638 mg (76%) of **42**: m.p. 167–168 °C (methanol), literature[113] gives 171–173 °C, $[\alpha]_D$ +4.2 (*c* 0.23, $CHCl_3$), literature[113] gives $[\alpha]_D$ –5.0. ^{1}H NMR (400 MHz): 0.65 s, 3 H (3 x H-18); 0.72 s, 3 H (3 x H-19); 1.19 s, 3 H (3 x H-21); 1.92 s, 3 H (3 x OAc); 3.74-3.92 m, 4 H (OCH_2CH_2O); 4.56 m, 1 H, W = 32.6 (H-3).

*6.3.25. 20,20-(Ethylenedioxy)-5α-pregnan-3β-ol (**43**)*

To a hot solution of compound **42** (538 mg, 1.33 mmol) in methanol (3 mL) a solution of potassium hydroxide (72 mg, 1.29 mmol) in water (1 mL) was added and a mixture was allowed to attain room temperature. After 1 hour, the reaction mixture was poured into water (200 mL), the precipitate was filtered off, and dried over potassium hydroxide in desiccator for 24 hours to yield hydroxy derivative **43** (420 mg, 63%): m.p. 164–166 °C (methanol/dichloromethane), literature[114] gives 172–175 °C, $[\alpha]_D$ +7.8 (*c* 0.3, $CHCl_3$), literature[114] gives $[\alpha]_D$ +10.2). ^{1}H NMR (400 MHz): 0.75 s, 3 H (3 x H-18); 0.80 s, 3 H (3 x H-19); 1.29 s, 3 H (3 x H-21); 3.84-4.02 m, 4 H (OCH_2CH_2O); 3.58 m, 1 H, W = 35.1 (H-3).

*6.3.26. 20,20-(Ethylenedioxy)-5α-pregnan-3-one (**44**)*

A solution of hydroxy derivative **43** (3.66 g, 10 mmol) in benzene (300 mL) was stirred with pyridinium chlorochromate on aluminium oxide[115] (13 g) at room temperature. After 72 hours, the solids were filtered off and the filtrate was evaporated. Crystallization from petroleum ether/ether afforded ketone **44** (2.66 g, 72%): m.p. 185–187 °C, literature[116] gives 184–186 °C, $[\alpha]_D$ +39.4 (*c* 0.65, $CHCl_3$), literature[116] gives $[\alpha]_D$ +33.0. ^{1}H NMR (400 MHz): 0.78 s, 3 H (3 x H-18); 1.01 s, 3 H (3 x H-19); 1.29 s, 3 H (3 x H-21); 3.85-4.03 m, 4 H (OCH_2CH_2O).

*6.3.27. 20,20-(Ethylenedioxy)-5β-pregnan-3α-ol (**45**)*

A mixture of compound (1 g, 3.13 mmol) in dry benzene (20 mL), ethylene glycol (1.4 mL, 25 mmol), triethyl orthoformate (2 mL, 12 mmol), and *p*-toluenesulfonic acid monohydrate (10 mg, 0.05 mmol) was stirred overnight at room temperature. The reaction mixture was poured into saturated solution of potassium hydrogen carbonate (100 mL) and extracted with ethyl acetate (200 mL). Extract was washed with water, dried and evaporated *in vacuo*. Crystallization from hot methanol afforded 743 mg (66 %) of **45**: m.p. 134–136 °C (petroleum ether/ether), literature[117] gives 146–147 °C, $[\alpha]_D$ +34.4 (*c* 0.29, $CHCl_3$), literature[117] gives $[\alpha]_D$ +27.0. ^{1}H NMR (400 MHz): 0.74 s, 3 H (3 x H-18); 0.92 s, 3 H (3 x H-19); 1.29 s, 3 H (3 x H-21); 3.63 m, 1 H (H-3); 3.83-4.02 m, 4 H (OCH_2CH_2O).

*6.3.28. 20,20-(Ethylenedioxy)-5β-pregnan-3-one (**46**)*

A solution of hydroxy derivative **45** (300 mg, 0.82 mmol) in benzene (30 mL) was stirred with pyridinium chlorochromate on aluminium oxide[115] (600 mg) at room temperature. After 48 h, solids were filtered off and the filtrate was evaporated. Crystallization from methanol afforded ketone **46** (137 mg, 47%): m.p. 166–168 °C (methanol), literature[117] gives 168–173 °C, $[\alpha]_D$ +50.7 (*c* 0.18, $CHCl_3$), literature[117] gives $[\alpha]_D$ +48.1. ^{1}H NMR (400 MHz): 0.78 s, 3 H (3 x H-18); 1.02 s, 3 H (3 x H-19); 1.30 s, 3 H (3 x H-21); 3.82-4.03 m, 4 H (OCH_2CH_2O).

*6.3.29. 20,20-(Ethylenedioxy)-5α-pregn-2-en-3-yl Triflate and 20,20-(Ethylenedioxy)-5α-pregn-3-en-3-yl Triflate, a Mixture of Isomers (**47**)*

Procedure A:

Compound **44** (300 mg, 0.8 mmol), LDA (2.5 mmol), and N-phenyltrifluoromethanesulfonimide (500 mg, 1.4 mmol) were converted to a mixture of triflates **47** according to the *General Procedure 6.3.2*. Purification by PLC (7 plates) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers, **47** (233 mg, 57%) and starting material **44** (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 spectrum ($CHCl_3$): 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, $C_2H_4O_2$, 1 × O); 331 (1%, M – 3 × F, $C_2H_4O_2$, 2 × O). For $C_{24}H_{35}F_3O_5S$ (492.6) calculated. C, 58.52; H, 7.16. Found. H, 58.56; H, 7.38.

Procedure B:

Compound **44** (300 mg, 0.8 mmol), LiHMDS (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 **47** according to the *General Procedure 6.3.3*. Purification by PLC (10 plates) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers, **47** (194 mg, 47%), identical with the sample prepared above according to Procedure A and starting material **44** (48 mg, 16%). The Δ^2 isomer prevailed in the ratio 6:1 according to ^{1}H NMR spectrum.

*6.3.30. 20,20-(Ethylenedioxy)-5α-pregn-2-en-3-yl Nonaflate and 20,20-(Ethylenedioxy)-5α-pregn-3-en-3-yl Nonaflate, a Mixture of Isomers (**48**)*

Procedure A:

Compound **44** (300 mg, 0.8 mmol), LDA (2.5 mmol), and nonafluorobutanesulfonyl fluoride (0.7 mL, 3.9 mmol) were converted to a mixture of nonaflates **48** according to the *General Procedure 6.3.4*. Purification by PLC (7 plates) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers **48** (210 mg, 29%) and starting material **44** (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 spectrum ($CHCl_3$): 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. C, 50.46; H, 5.49. Found. C, 50.55; H, 5.30.

Procedure B:

Compound **44** (500 mg, 1.38 mmol), LiHMDS (1.0 M in hexanes, 3.45 mL, 3.45 mmol), and nonafluorobutanesulfonyl fluoride (1.16 mL, 6.6 mmol) were converted to a mixture of nonaflates **48** according to the *General Procedure 6.3.5*. Purification by chromatography on column of silica gel (25 g) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers **48** (308 mg, 20%), identical with the sample prepared above according to Procedure A and starting material **44** (443 mg, 49%). The Δ^2 isomer prevailed in the ratio 6:1 according to ^{1}H NMR spectrum.

*6.3.31. Methyl 20,20-(Ethylenedioxy)-5α-pregn-2-en-3-carboxylate and Methyl 20,20-(Ethylenedioxy)-5α-pregn-3-en-3-carboxylate, a Mixture of Isomers (**49**)*

Procedure A:

A mixture of triflates **47** (299 mg, 0.6 mmol), which was prepared according Procedure A was converted to a mixture of esters **49** according to the *General Procedure 6.3.6*. Purification by PLC (5 plates) in the mixture of petroleum ether/ether (9:1) afforded the mixture of Δ^2 and Δ^3 isomers **49** (128 mg, 52%). The Δ^2 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum. ^{1}H NMR (400 MHz): 0.76 s, 3 H (3 × H-18); 0.72 s, 3 H (3 × H-19); 1.29 s, 3 H (3 × H-21); 3.85-4.02 m, 4 H (OCH_2CH_2O); 3.72 s, 3 H (COOMe); 6.61 dt, J_1 = 3.6, J_2 = 1.5, 0.34 H (H-4); 6.89 m, 0.66 H (H-2). IR spectrum ($CHCl_3$): 1705 (C=O, COOMe); 1650 (C=C); 1471, 1375, 1295 (CH_2, acetal); 1437 (CH_3, COOMe); 1262, 1087 (C-O, COOMe). 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. C, 74.59; H, 9.51. Found. C, 74.40; H, 9.48.

Procedure B:

A mixture of nonaflates **48** (270 mg, 0.4 mmol), which was prepared according Procedure A was converted to a mixture of esters **49** according to the *General Procedure 6.3.6*. Purification by PLC (4 plates) in the mixture of petroleum ether/ether (9:1) afforded the mixture of Δ^2 and Δ^3 isomers **49** (69 mg, 40%), identical with the sample prepared above according to Procedure A. The Δ^2 isomer prevailed in the ratio 2:1 according to ^{1}H NMR spectrum.

*6.3.32. Methyl 20-Oxo-5α-pregn-2-ene-3-carboxylate and Methyl 20-Oxo-5α-pregn-3-ene-3-carboxylate, a Mixture of Isomers (**50**)*

A mixture of protected methyl esters **49** (128 mg, 0.3 mmol) was converted to **50** according to the *General Procedure 6.3.7*. Purification by PLC (2 plates) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers **50** (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, J_1 = 3.6, J_2 = 1.5, 0.34 H (H-4); 6.88–6.90 m, 0.66 H (H-2). IR spectrum ($CHCl_3$): 1702 (C=O, $COOCH_3$, ketone); 1651 (C=C); 1437 (CH_3, $COOCH_3$); 1358 (CH_3, ketone); 1264, 1086 (C-O, COOMe, 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. C, 77.05; H, 9.56.

Found. C, 77.27; H, 9.64.

*6.3.33. Methyl 20-Oxo-5α-pregnane-3β-carboxylate (**51**)*

A mixture of unsaturated derivatives **50** (129 mg, 0.36 mmol) was converted to methyl ester **51** according to the *General Procedure 6.3.8*. Purification by PLC (2 plates) in the mixture of petroleum ether/ether (9:1) afforded 3β-isomer, **51** (95 mg, 73%): m.p. 78–80 °C (ethyl acetate), $[\alpha]_D$ +128.1 (*c* 0.19, $CHCl_3$). ^1H 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 (COOMe). IR spectrum ($CHCl_3$): 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. C, 76.62; H, 10.06. Found. C, 76.75; H, 10.00.

*6.3.34. 20,20-(Ethylenedioxy)-5β-pregn-2-en-3-yl Triflate and 20,20-(Ethylenedioxy)-5β-pregn-3-en-3-yl Triflate, a Mixture of Isomers (**52**)*

Compound **46** (200 mg, 0.5 mmol), LDA (2.9 mmol), and N-phenyltrifluoromethanesulfonimide (400 mg, 1.2 mmol) were converted to a mixture of triflates **52** according to the *General Procedure 6.3.2*. Purification by PLC (4 plates) in the mixture of petroleum ether/ether (8:2) afforded the mixture of Δ^2 and Δ^3 isomers **52** (167 mg, 30%) and starting material **46** (33 mg, 12%). The Δ^3 isomer prevailed in the ratio 2.5:1 according to ^1H NMR spectrum. ^1H 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, J = 6.0, 0.3 H (H-2). IR spectrum ($CHCl_3$): 1470, 1374, 1055 (CH_2, acetal); 1415, 1246, 1223 (SO_2, triflate); 1142 (CF_3, nonaflate). 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. C, 58.52; H, 7.16. Found. H, 58.35; H, 7.12.

*6.3.35. 20,20-(Ethylenedioxy)-5β-pregn-2-en-3-yl Nonaflate and 20,20-(Ethylenedioxy)-5β-pregn-3-en-3-yl Nonaflate, a Mixture of Isomers (**53**)*

Compound **46** (260 mg, 0.7 mmol), LDA (2.1 mmol), and nonafluorobutanesulfonyl fluoride (0.6 mL, 3.4 mmol) were converted to a mixture of nonaflates **53** according to the *General Procedure 6.3.4*. Purification by PLC (6 plates) in the mixture of petroleum ether/ether (9:1) afforded the mixture of Δ^2 and Δ^3 isomers **53** (269 mg, 58%) and starting material **46** (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, J = 6.0, 0.34 H (H-2). IR spectrum ($CHCl_3$): 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. C, 50.46; H, 5.49. Found. H, 50.59; H, 5.59.

*6.3.36. Methyl 20-Oxo-5β-pregn-2-ene-3-carboxylate and Methyl 20-Oxo-5β-pregn-3-ene-3-carboxylate, a Mixture of Isomers (**54**)*

Procedure A:

A mixture of triflates **52** (130 mg, 0.3 mmol) was converted to a mixture of esters **54** according to the *General Procedure 6.3.9*. Purification by PLC (2 plates) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers **54** (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 (COOMe); 6.71 s, 0.75 H (H-4); 6.85 m, 0.25 H (H-2). IR spectrum ($CHCl_3$): 1701 (C=O, $COOCH_3$); 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. C, 77.05; H, 9.56. Found. C, 76.91; H, 9.62.

Procedure B:

A mixture of nonaflate **53** (270 mg, 0.4 mmol) was converted to a mixture of esters **54** according to the *General Procedure 6.3.9*. Purification by PLC (3 plates) in the mixture of petroleum ether/ether (4:1) afforded the mixture of Δ^2 and Δ^3 isomers **54** (50 mg, 28%), identical with the sample prepared above according to Procedure A. The Δ^3 isomer prevailed in the ratio 3:1 according to ^{1}H NMR spectrum.

*6.3.37. 20,20-(Ethylenedioxy)-5β-pregn-3-en-3-yl Triflate (**55**)*

It was not possible to separate this compound as a pure identity, only as a mixture with its derivative with unprotected 20-keto group and thus the material was transformed into **57** directly.

*6.3.38. 20,20-(Ethylenedioxy)-5β-pregn-3-en-3-yl Nonaflate (**56**)*

Compound **46** (200 mg, 0.5 mmol), LiHMDS (1.0 M in hexanes, 1.5 mL, 1.5 mmol) and nonafluorobutanesulfonyl fluoride (0.42 mL, 2.4 mmol) were converted to **56** according to the *General Procedure 6.3.5*. Purification by PLC (2 plates) in the mixture of petroleum ether/ether (4:1) afforded Δ^3 isomer **56** (102 mg, 28%) and starting material **46** (65 mg, 32%): 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); 3.87-4.0 m, 4 H (OCH_2CH_2O); 5.46 s, 1 H (H-4). IR spectrum ($CHCl_3$): 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 – F), 597 (20%, M – 1 – OCH_2CH_2), 585 (13%, M – 3 × F), 553 (4%, M – 1 – CF_3, F). For $C_{27}H_{35}F_9O_5S$ (642.6) calculated. C, 50.46; H, 5.49. Found. H, 50.33; H, 5.38.

*6.3.39. Methyl 20-Oxo-5β-pregn-3-ene-3-carboxylate (**57**)*

Procedure A:

Compound **46** (200 mg, 0.25 mmol), LiHMDS (1.0 M in hexanes, 0.8 mL, 0.8 mmol) and N-phenyltrifluoromethanesulfonimide (785 mg, 2.2 mmol) were converted to a mixture of triflates according to the *General Procedure 6.3.3*. Purification by PLC (10 plates) in the mixture of petroleum ether/ether (4:1) afforded mixture of protected and deprotected triflates (160 mg) and starting material **46** (84 mg, 21%). ^{1}H NMR spectrum confirmed occurrence of only Δ^3 isomer**.** The mixture of protected and deprotected triflates (160 mg) was converted to a mixture of esters **57** according to the *General Procedure 6.3.9*. Purification by PLC (2 plates) in the mixture of petroleum ether/ether (4:1) afforded the Δ^3 isomers, **57** (55 mg, 14%): m.p. 120–122 °C (petroleum ether/ether), $[\alpha]_D$ +212.0 (*c* 0.15, $CHCl_3$). ^{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 (COOMe); 6.71 s, 1 H (H-4). IR spectrum ($CHCl_3$): 1701 (C=O, ketone and $COOCH_3$); 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. C, 77.05; H, 9.56. Found. C, 76.95; H, 9.58.

Procedure B:

Nonaflate **56** (183 mg, 0.3 mmol) was converted to an ester **57** according to the *General Procedure 6.3.9*. Crystallization from petroleum ether/ether afforded the Δ^3 isomers **57** (22 mg, 21%), identical with the sample prepared above according to Procedure A.

*6.3.40. Methyl 20-Oxo-5β-pregnane-3α-carboxylate (**58**) and Methyl 20-oxo-5β-pregnane-3β-carboxylate (**59**)*

Procedure A:

A mixture of methyl esters **54** (60 mg, 0.1 mmol) was converted to 3α- and 3β-carboxy derivatives **58** and **59** according to the *General Procedure 6.3.8*. 3α-Carboxy derivative prevailed above 3β-carboxy derivative in the ratio 6:1 according to the ^{1}H NMR spectrum. HPLC chromatography in the mixture of ethyl acetate/hexanes (9:91) gave 25 mg (41%) of 3α-carboxy derivative **58** and 4 mg (6%) of 3β-carboxy derivative **59**. The ratio of desired products was 5:1.

3α-Carboxy derivative (**58**): 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 (COOMe). IR spectrum ($CHCl_3$): 1726 (C=O, ketone); 1699 (C=O, ester); 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. C, 76.62; H, 10.06. Found. C, 76.58; H, 10.33.

3β-Carboxy derivative (**59**): m.p. 86–88 °C (ethyl acetate), $[\alpha]_D$ +87.4 (*c* 0.23, $CHCl_3$). ^{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 (COOMe). IR spectrum ($CHCl_3$): 1725 (C=O, ketone); 1699 (C=O, ester); 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. C, 76.62; H, 10.06. Found. C, 76.53; H, 10.11.

Procedure B:

The methyl ester **57** (55 mg, 0.2 mmol) was converted to 3α- and 3β-carboxy derivatives **58** and **59** according to the *General Procedure 6.3.8*. 3α-Carboxy derivative prevailed above 3β-carboxy derivative in the ratio 2.5:1 according to the ^{1}H NMR spectrum. HPLC chromatography in the mixture of ethyl acetate/hexanes (9:91) gave 22 mg (40%) of 3α-carboxy derivative **58** and 9 mg (16%) of 3β-carboxy derivative **59**, identical with the samples prepared above according to Procedure A. The ratio of desired products was 2.5:1.

*6.3.41. 20-Oxo-5α-pregnane-3β-carboxylic Acid (**60**)*

Ester **51** (20 mg, 0.05 mmol) was converted to acid **60** according to the *General Procedure*

6.3.10. Crystallization afforded desired acid **60** (18 mg, 95%): m.p. 184–187 °C, $[\alpha]_D$ +118.8 (*c* 0.17, $CHCl_3$). ^1H NMR (400 MHz): 0.60 s, 3 H (3 x H-18); 0.81 s, 3 H (3 x H-19); 2.11 s, 3 H (3 x 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 spectrum: 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. C, 76.26; H, 9.89. Found. C, 76.02; H, 9.95.

*6.3.42. 20-Oxo-5β-pregnane-3α-carboxylic Acid (**61**)*

Ester **58** (45 mg, 0.12 mmol) was converted to acid **61** according to the *General Procedure 6.3.10*. Crystallization afforded desired acid **61** (25 mg, 58%): m.p. 133–136 °C, $[\alpha]_D$ +81.6 (*c* 0.25, $CHCl_3$). ^1H NMR (400 MHz): 0.60 s, 3 H (3 x H-18); 0.95 s, 3 H (3 x H-19); 2.11 s, 3 H (3 x 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 spectrum: 3515 (O-H, COOH, monomer); 2672 (O-H, COOH, dimer); 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. C, 76.26; H, 9.89. Found. C, 76.03; H, 9.68.

*6.3.43. 20-Oxo-5β-pregnane-3β-carboxylic Acid (**62**)*

Ester **59** (14 mg, 0.03 mmol) was converted to acid **62** according to the *General Procedure 6.3.10*. Crystallization afforded desired acid **62** (11 mg, 87%): m.p. 212–215 °C, $[\alpha]_D$ +94.0 (*c* 0.2, $CHCl_3$). ^1H NMR (400 MHz): 0.60 s, 3 H (3 x H-18); 0.93 s, 3 H (3 x H-19); 2.12 s, 3 H (3 x H-21); 2.77 m, 1 H (H-3); 2.53 t, 1 H (H-17). IR spectrum: 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. C, 76.26; H, 9.89. Found. C, 76.56; H, 9.98.

6.4. Synthesis of 2-(Steroid-3-yl)propandioic Acid and 2-(Steroid-3-yl)acetic Acid

*6.4.1. 3β-Hydroxy-5β-pregnan-20-one (**63**)*

This compound was prepared according to the literature[101].

*6.4.2. 20-Oxo-5β-pregnan-3β-yl 3-Tosylate (**64**)*

This compound was prepared according to the literature[118].

*6.4.3. 20,20-(Ethylenedioxy)-5β-pregnan-3β-yl Tosylate (**65**)*

To a stirred mixture of steroid **64** (200 mg, 0.42 mmol) in dry benzene (2 mL), triethyl orthoformate (0.38 mL, 2.3 mmol), ethylene glycol (0.3 mL, 5.8 mmol), and *p*-toluenesulfonic acid monohydrate (2 mg, 0.01 mmol) were added. The reaction mixture was stirred for 2 days at room temperature, and then poured into an aqueous solution of sodium hydrogen carbonate (30 mL), steroid was extracted with ethyl acetate (2 × 60 mL). The extract was washed with water, dried and the solvents were evaporated. The residue was purified by PLC (4 plates) in a mixture of ether/petroleum ether (1:1) and two drops of pyridine to give acetal **65** (194 mg, 89%): m.p. 146–148 °C (petroleum ether/ether), $[\alpha]_D$ +11.7 (*c* 0.37, $CHCl_3$). IR spectrum ($CHCl_3$): 1358 (SO_2, tosylate); 1189 (SO_2, tosylate); 1053 (OCH_2CH_2O); 901 (C-O, tosylate). ^1H NMR (200 MHz): 0.73 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 1.27 s, 3 H (3 × H-21); 2.44 s, 3 H (CH_3, tosylate); 3.81-4.01 m, 4 H (OCH_2CH_2O); 4.83 m, 1 H (H-3); 7.32 d, 2 H, J = 7.8 (H-3 and H-5, tosylate); 7.78 d, 2 H, J = 8.3 (H-2 and H-6, tosylate). FAB MS: 517 (6%, M + 1), 345 (2.5%, M – tosylate). For $C_{30}H_{44}O_5S$ (516.7) calculated. C, 69.73; H, 8.58; S, 6.21. Found. C 69.92; H, 8.79; S, 6.41.

*6.4.4. Dimethyl 2-[20,20-(Ethylenedioxy)-5β-pregnan-3α-yl]propandioate (**66**)*

To a stirred solution of sodium salt of dimethyl malonate, prepared by refluxing toluene (30 mL), sodium (80 mg, 3.5 mmol) and dimethyl malonate (0.707 mL, 6.2 mmol) until all the sodium had been dissolved, was added a solution of steroid **65** (600 mg, 1.16 mmol) in toluene (20 mL) dropwise during vigorous stirring. After refluxing for additional 12 hours, the solution was cooled to room temperature, the precipitated sodium *p*-toluenesulfonate was filtered off, washed with small amount of toluene, and the combined toluene extracts were evaporated. The oil obtained was dissolved in ether (200 mL) and washed with water, and the

solution was dried and evaporated. The residue was purified by chromatography on a column of silica gel (20 g) in a mixture of petroleum ether/ether (9:1) to afforded ester **66** (430 mg, 77%): m.p. 98–102 °C (ethyl acetate), $[\alpha]_D$ +55.4 (*c* 0.25, $CHCl_3$). IR spectrum: 1753 (C=O, COOMe), 1731 (C=O, COOMe); 1436 (CH_3, COOMe); 1472, 1295 (CH_2, acetal); 1149 (C-O). ^{1}H NMR (400 MHz): 0.73 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 1.29 s, 3 H (3 × H-21); 3.19 d, 1 H, J = 9.2 (C**H**$(COOMe)_2$); 3.72 s, 6 H (2 × COOMe); 3.80-4.02 m, 4 H (OCH_2CH_2O). ESI MS: 976 (47%, M + 1 + Na), 975 (100%, 2M + Na), 499 (15%, M + Na). For $C_{28}H_{44}O_6$ (476.6) calculated. C, 70.56; H, 9.30. Found. C, 70.61; H, 9.53.

*6.4.5. 2-(20-Oxo-5β-pregnan-3α-yl)propandioic Acid (**67**)*

To a stirred solution of ester **66** (55 mg, 0.11 mmol) in methanol (4 mL) a solution of potassium hydroxide (140 mg, 2.5 mmol) in ethanol (0.35 mL) and water (0.35 mL) was added. The mixture was heated for 6 hours at 120 °C. After cooling, it was poured into water and extracted with ether (50 mL). A mixture of HCl/H_2O (1:2) was added to the aqueous phase up to pH 1 and it was allowed to stay at room temperature for 3 hours. Subsequently, it was extracted with chloroform (2 × 30 mL), collected organic phases were washed with water, dried and evaporated in vacuo. The residue was crystallized from petroleum ether/ether to give acid **67** (27 mg, 58%): m.p. 214–215 °C, $[\alpha]_D$ +82.7 (*c* 0.25, $CHCl_3$). IR spectrum ($CHCl_3$): 3500 (O-H, COOH, monomer); 3200 (O-H, COOH, dimer); 1741 (C=O, COOH, monomer); 1702 (C=O, ketone). ^{1}H NMR (400 MHz): 0.58 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.15 m, 1 H (H-3β); 2.54 t, 1 H, J = 8.6 (H-17); 3.17 d, 1 H, J = 8.5 (C**H**$(COOH)_2$). ESI MS: 808 (4%, 2M), 404 (22%, M), 403 (100%, M – 1). For $C_{24}H_{36}O_5$ (404.5) calculated. C, 71.26; H, 8.97. Found: C, 71.08; H, 8.80.

*6.4.6. Methyl 2-[20,20-(Ethylenedioxy)-5β-pregnan-3α-yl]acetate (**68**)*

A mixture of ester **66** (187 mg, 0.39 mmol), sodium cyanide (37.5 mg, 0.76 mmol), and dimethylsulfoxide (11 mL) was heated in an argon atmosphere for 3 hours at 210 °C. After cooling, the mixture was partitioned between ether (100 mL) and water (100 mL), the aqueous layer was extracted with ether (50 mL), and combined organic phases were washed with water and taken down. The residue was purified by PLC (4 plates) in a mixture of petroleum ether/ether (8:2), affording 90 mg (57%) of monoester **68**: m.p. 75–76 °C (ethyl acetate), $[\alpha]_D$ +68.0 (*c* 0.25, $CHCl_3$). IR spectrum ($CHCl_3$): 1729 (C=O); 1295, 1073, 1054 (CH_2, acetal); 1438 (CH_3, $COOCH_3$); 1142 (C-O). ^{1}H NMR (400 MHz): 0.73 s, 3 H

(3 × H-18); 0.93 s, 3 H (3 × H-19); 1.29 s, 3 H (3 × H-21); 2.01 m, 1 H (H-3β); 2.22 d, 2 H, J = 7.3 (C**H**$_2$COOCH$_3$); 3.66 s, 3 H (COOCH$_3$); 3.85-4.00 m, 4 H (OCH$_2$CH$_2$O). ESI MS: 419 (61%, M + 1), 357 (26%, M – CH$_2$COOCH$_3$), 230 (100%). For $C_{26}H_{42}O_4$ (418.6) calculated. C, 74.60; H, 10.11. Found. 74.42; H, 10.30.

*6.4.7. 2-[20,20-(Ethylenedioxy)-5β-pregnan-3α-yl]acetic Acid (**69**)*

To a stirred mixture of methyl ester **68** (75 mg, 0.18 mmol) in methanol (2 mL) a solution of potassium hydroxide (55 mg, 0.98 mmol) in ethanol (0.125 mL) and water (0.125 mL) was added. The mixture was heated for 1.5 hour at 120 °C. After cooling, it was poured into water (50 mL) and extracted with ether (50 mL). A mixture of HCl/H$_2$O (1:2) was added to the aqueous phase to reach pH 1. Immediately, it was extracted with chloroform (2 × 30 mL), organic phase was washed with water, dried and evaporated *in vacuo*. The residue was crystallized from petroleum ether/ether to give acid **69** (45 mg, 63%): m.p. 164–167 °C (petroleum ether/ether), $[\alpha]_D$ +41.0 (*c* 0.19, CHCl$_3$). IR spectrum (CHCl$_3$): 3516 (O-H, COOH, monomer); 3088 (O-H, COOH, dimer); 1739 (C=O, COOH, monomer); 1705 (C=O, COOH, dimer); 1469, 1375, 1054 (CH$_2$, acetal). ^{1}H NMR (400 MHz): 0.74 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 1.29 s, 3 H (3 × H-21); 2.02 m, 1 H (H-3β); 2.26 d, 2 H, J = 6.6 (C**H**$_2$COOH); 3.86-3.98 m, 4 H (OCH$_2$CH$_2$O). FAB MS: 405 (4%, M + 1), 261 (1%, M – CH$_2$COOH, $C_4H_7O_2$), 231 (2%, M – CH$_2$COOH, $C_4H_7O_2$, 2 × CH$_3$). For $C_{25}H_{40}O_4$ (404.2) calculated. C, 74.22; H, 9.97. Found. C 73.99; H, 10.16.

*6.4.8. 2-(20-Oxo-5β-pregnan-3α-yl)acetic acid (**70**)*

To a stirred solution of compound **69** (55 mg, 0.13 mmol) in acetone (5 mL), *p*-toluenesulfonic acid monohydrate (20 mg, 0.12 mmol) in water (0.6 mL) was added. The reaction mixture was stirred for 24 hours at room temperature, poured into water and extracted with ether. A mixture of HCl/H$_2$O (1:2) was added to the aqueous phase to reach pH 1 and then extracted with chloroform (2 × 30 mL). Organic phase was washed with water, dried and evaporated in vacuo to afford **70** (42 mg, 85%): m.p. 189–192 °C (acetone), $[\alpha]_D$ +127.6 (*c* 0.2, CHCl$_3$). IR spectrum (CHCl$_3$): 3516 (O-H, COOH, monomer), 3090 (O-H, COOH, dimer), 1739 (C=O, COOH, monomer); 1702 (C=O, COOH, dimer and C=O, ketone), 1702 (C=O, COCH$_3$). ^{1}H NMR (400 MHz): 0.59 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.16 m, 1 H (H-3β); 2.27 d, 2 H, J = 6.8 (C**H**$_2$COOH); 2.536 t, 1 H, J = 8.8 (H-17). ESI MS: 742 (15%), 720 (42%, 2M), 719 (100%, 2M – 1), 359

(21%, M – 1). For $C_{23}H_{36}O_3$ (360.26) calculated. C, 76.62; H, 10.06. Found. C, 76.54; H, 10.25.

6.5. Synthesis of Steroid Carboxylic Acids via Wadsworth-Horner-Emmons Reaction (WHE)

6.5.1. General Procedure for Wadsworth-Horner-Emmons (WHE) Synthesis Using Triethyl 2-Phosphonoacetate

Triethyl 2-phosphonoacetate (0.28 mL, 1.6 mmol) was added dropwise to a suspension of NaH (50% dispersion in paraphine oil; 72 mg, 1.5 mmol) in THF (10 mL) at 0 °C under argon atmosphere. A hydrogen gas evolution occurred, a heterogeneous reaction mixture became transparent, and it was allowed to warm to room temperature with stirring for 30 min. Then, ketone (1.42 mmol) in dry THF (10 mL) was added dropwise. After stirring for 2 hours at room temperature under argon atmosphere, it was poured into saturated solution of ammonium chloride (200 mL) and diluted twice with ethyl acetate (150 mL). The organic layer was diluted with saturated solution of ammonium chloride (100 mL), water (2 × 100 mL), dried over Na_2SO_4 and the solvent was evaporated *in vacuo*. The residue was purified by chromatography.

6.5.2. General Procedure for WHE Synthesis Using Triethyl 2-Phosphonopropionate

Triethyl 2-phosphonopropionate (0.52 mL, 2.5 mmol) was added dropwise to a suspension of NaH (50% dispersion in paraphine oil; 105 mg, 2.15 mmol) in THF (10 mL) at 0 °C under argon atmosphere. A hydrogen gas evolution occurred, a heterogeneous reaction mixture became transparent, and it was allowed to warm to room temperature with stirring for 30 min. Then, ketone (1.42 mmol) in dry THF (10 mL) was added dropwise during 20 min at 0 °C and the mixture was stirred at 0 °C for 2 hours. After standing overnight at room temperature under argon atmosphere, it was worked-up. The work up of the reaction mixture was identical as for *6.5.1*.

6.5.3. General Procedure for WHE Synthesis Using Triethyl 2-Phosphonobutyrate

Triethyl 2-phosphonobutyrate (0.52 mL, 2.2 mmol), NaH (50% dispersion in paraphine oil; 87 mg, 1.8 mmol) in THF (5 mL), and ketone (0.71 mmol) in dry THF (5 mL) were converted to a mixture of E/Z-isomers according to *6.5.2*.

6.5.4. General Procedure for WHE Synthesis Using Triethyl 4-Phosphonocrotonate

Triethyl 4-phosphonocrotonate (0.39 mL, 1.8 mmol), NaH (50% dispersion in paraphine oil; 72 mg, 1.5 mmol) in THF (5 mL), and ketone (0.71 mmol) in dry THF (5 mL) were converted to a mixture of E/Z-isomers according to *6.5.2*. The deprotonation of phosphonoacetate was indicated by hydrogen gas evolution and the reaction mixture turned red and homogeneous.

6.5.5. General Procedure for Basic Hydrolysis of Esters

A solution of potassium hydroxide (165 mg, 2.95 mmol) in water (0.375 mL) and ethanol (0.375 mL) was added to a solution of ester (0.15 mmol) in methanol (3 mL). The reaction mixture was refluxed and the progress of the reaction was monitored by TLC. After the observed completion of reaction, the reaction mixture was poured into ice-water and acidified by a mixture HCl/H_2O (1:2) to pH 1. The precipitate was filtered off, washed with water (2 ×) and dried overnight in a thermo regulator (40 °C). The solid was crystallized from a mixture of acetone–water.

6.5.6. General Procedure for Basic Hydrolysis of Esters from WHE Reaction Using Protection of 20-Oxo group

A mixture of an ester (0.15 mmol) in dry benzene (3 mL) was treated with ethylene glycol (0.11 mL, 1.97 mmol), triethyl orthoformate (0.135 mL, 1.27 mmol) and *p*-toluenesulfonic acid monohydrate (1 mg, 0.006 mmol) at room temperature. After 12 hours, the mixture was poured into water and extracted with ethyl acetate (60 mL). The organic phase was washed with saturated solution of sodium hydrogen carbonate, water, dried and concentrated. The residue was purified by plate PLC (1 plate) in the mixture of petroleum ether/ether (4:1) and two drops of pyridine to give white solid. A solution of potassium hydroxide (1.87 mmol) in water (0.24 mL) and ethanol (0.24 mL) was added to a solution of protected ester (0.09 mmol) in methanol (5 mL). The reaction mixture was refluxed and the progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured into ice-water, acidified by a mixture HCl/H_2O (1:2) to pH 1 and allowed to stay at room temperature for 3 hours. The precipitate was filtered off, washed with water (2 ×) and dried overnight at 40 °C. The solid was crystallized from a mixture of acetone–water.

*6.5.7. Ethyl (Z)-(20-Oxo-5α-pregnan-3-ylidene)acetate (**73**) and Ethyl (E)-(20-Oxo-5α-pregnan-3-ylidene)acetate (**74**)*

5α-Pregnane-3,20-dione **71** (340 mg, 1.07 mmol) was converted to **73** and **74** according to *General Procedure 6.5.1*. Purification by PLC (8 plates) in petroleum ether/ether (4:1) afforded **73** (141 mg, 34%) and **74** (151 mg, 36%).

Z-isomer **73**: m.p. 105–108 °C (acetone/heptane), $[\alpha]_D$ +151.9 (*c* 0.28, $CHCl_3$). ^1H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.91 s, 3 H (3 × H-19); 1.27 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 2.12 s, 3 H (3 × H-21); 2.34 m, 1 H (H-2β); 2.52 t, 1 H, J = 9.0 (H-17); 3.49 ddd, 1 H, J_1 = 14.4, J_2 = 3.4, J_3 = 1.9 (H-4α); 4.14 q, 2 H, J = 7.1 ($OC\mathbf{H}_2CH_3$); 5.59 t, 1 H, J = 1.9 (=CH). IR spectrum ($CHCl_3$): 1700 (C=O, ester and ketone); 1644 (C=C); 1473 (CH_2, OCH_2CH_3); 1387 (CH_3, OCH_2CH_3); 1330 (=CH). EI MS: 386 (100%, M), 341 (13%, M – OCH_2CH_3), 298 (5%, M – $COCH_3$, OCH_2CH_3). For $C_{25}H_{38}O_3$ (386.3) calculated. C, 77.68; H, 9.91. Found. C, 77.55; H, 10.09.

E-isomer **74**: m.p. 102–104 °C (acetone/heptane), $[\alpha]_D$ +25.5 (*c* 0.21, $CHCl_3$). ^1H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.91 s, 3 H (3 × H-19); 1.27 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 2.12 s, 3 H (3 × H-21); 2.53 t, 1 H, J = 9.0 (H-17); 3.74 dp, 1 H, J_1 = 14.9, J_2 = 2.1 (H-2α); 4.14 q, 2 H, J = 7.0 ($OC\mathbf{H}_2CH_3$); 5.58 t, 1 H, J = 1.9 (=CH). IR spectrum ($CHCl_3$): 1699 (C=O, ester and ketone); 1645 (C=C); 1473 (CH_2, OCH_2CH_3); 1388 (CH_3, OCH_2CH_3); 1327 (=CH); 1164, 1150 (C-O). EI MS: 386 (100%, M), 341 (8%, M – OCH_2CH_3). For $C_{25}H_{38}O_3$ (386.3) calculated. C, 77.68; H, 9.91. Found. C, 77.57; H, 10.03.

*6.5.8. Ethyl (Z)-(20-Oxo-5β-pregnan-3-ylidene)acetate (**75**) and Ethyl (E)-(20-Oxo-5β-pregnan-3-ylidene)acetate (**76**)*

5β-Pregnane-3,20-dione **72** (450 mg, 1.42 mmol) was converted to **75** and **76** according to *General Procedure 6.5.1*. Purification by chromatography on column of silica gel (25 g) in petroleum ether/ether (95:5) afforded **75** (223 mg, 40%) and **76** (251 mg, 46%).

Z-isomer **75**: m.p. 64–65 °C (acetone/heptane), $[\alpha]_D$ +118.9 (*c* 0.3, $CHCl_3$). ^1H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 1.27 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 2.12 s, 3 H (3 × H-21); 2.32 t, 1 H, J = 14.2 (H-4α); 2.54 t, 1 H, J = 8.8 (H-17); 3.45 ddd, 1 H, J_1 = 14.6, J_2 = 4.3, J_3 = 1.76 (H-4β); 4.14 q, 2 H, J = 7.0 ($OC\mathbf{H}_2CH_3$); 5.62 t, 1 H, J = 1.8 (=CH). IR spectrum ($CHCl_3$): 1700 (C=O, ester and ketone); 1645 (C=C); 1247, 1040 (C-O, ester); 2980 (CH_2, OCH_2CH_3). EI MS: 386 (100%, M), 371 (7%, M – CH_3), 341 (20%,

M – OCH_2CH_3), 297 (12%, M –$COCH_3$, OCH_2CH_3). For $C_{25}H_{38}O_3$ (386.3) calculated. C, 77.68; H, 9.91. Found. C, 77.47; H, 10.04.

E-isomer **76**: m.p. 76–77 °C (acetone/heptane), $[\alpha]_D$ +134.9 (*c* 0.53, $CHCl_3$). ^{1}H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 1.27 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 2.12 s, 3 H (3 × H-21); 2.55 t, 1 H, J = 8.8 (H-17); 2.66 t, 1 H, J = 13.4 (H-4α); 3.60 d, 1 H, J = 12.6 (H-2β); 4.14 q, 2 H, J = 7.0 ($OC\mathbf{H}_2CH_3$); 5.60 t, 1 H, J = 1.8 (=CH). IR spectrum ($CHCl_3$): 2979, 2961 (CH_2, OCH_2CH_3); 1700 (C=O, ester and ketone); 1646 (C=C); 1395 (CH_3, OCH_2CH_3); 1255, 1039 (C-O). EI MS: 386 (100%, M), 371 (10%, M – CH_3), 341 (26%, M – OCH_2CH_3), 297 (18%, M – $COCH_3$, OCH_2CH_3). For $C_{25}H_{38}O_3$ (386.3) calculated. C, 77.68; H, 9.91. Found. C, 77.54; H, 10.06.

*6.5.9. Ethyl (Z)-2-(20-Oxo-5α-pregnan-3-ylidene)propanoate (**77**) and Ethyl (E)-2-(20-Oxo-5α-pregnan-3-ylidene)propanoate (**78**)*

5α-Pregnane-3,20-dione **71** (225 mg, 0.71 mmol) was converted to **77** and **78** according to *General Procedure 6.5.2*. Purification by PLC in petroleum ether/ether (4:1) afforded **77** (73 mg, 26%) and **78** (83 mg, 29%) and the starting material **71** (23 mg, 10%).

Z-isomer **77**: m.p. 90–93 °C (petroleum ether/ether), $[\alpha]_D$ +100.0 (*c* 0.27, $CHCl_3$). ^{1}H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.88 s, 3 H (3 × H-19); 1.30 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 1.85 t, 1 H, J_1 = 1.3, J_2 = 2.0 (CH_3, propanoate moiety); 2.11 s, 3 H (3 × H-21); 2.52 m, 2 H (H-17 and H-2α); 2.64 ddd, 1 H, J_1 = 13.6, J_2 = 2.5, J_3 = 1.3 (H-4α); 4.20 q, 2 H, J = 7.0 ($OC\mathbf{H}_2CH_3$). IR spectrum ($CHCl_3$): 1699 (C=O, ester and ketone; 1636 (C=C); 1473, 1366 (CH_2, OCH_2CH_3); 1387 (CH_3, OCH_2CH_3); 1230, 1180 (C-O). EI MS: 400 (100%, M), 355 (16%, M – OCH_2CH_3), 311 (6%, M – $COCH_3$, OCH_2CH_3), 256 (3%, M – $C_5H_8O_2$, $COCH_3$). For $C_{26}H_{40}O_3$ (400.6) calculated. C, 77.95; H, 10.06. Found. C, 77.84; H, 10.30.

E-isomer **78**: m.p. 111–114 °C (methanol/chloroform), $[\alpha]_D$ +56.2 (*c* 0.22, $CHCl_3$). ^{1}H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.88 s, 3 H (3 × H-19); 1.30 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 1.85 dd, 1 H, J_1 = 2.3, J_2 = 1.2 (CH_3, propanoate moiety); 2.11 s, 3 H (3 × H-21); 2.53 t, 1 H, J = 9.0 (H-17); 2.90 dp, 1 H, J_1 = 14.9, J_2 = 2 (H-2α); 4.20 q, 2 H, J = 7.0 ($OC\mathbf{H}_2CH_3$). IR spectrum ($CHCl_3$): 1699 (C=O, ester and ketone); 1639 (C=C); 1473, 1365 (CH_2, OCH_2CH_3), 1387 (CH_3, OCH_2CH_3); 1239, 1182 (C-O). EI MS: 400 (100%, M), 355 (17%, M – OCH_2CH_3), 311 (6%, M – $COCH_3$, OCH_2CH_3), 256 (2%, M – $C_5H_8O_2$, $COCH_3$). For $C_{26}H_{40}O_3$ (400.6) calculated. C, 77.95; H, 10.06. Found. C, 77.92; H, 10.12.

*6.5.10. Ethyl (Z)-2-(20-Oxo-5β-pregnan-3-ylidene)propanoate (**79**) and Ethyl (E)-2-(20-Oxo-5β-pregnan-3-ylidene)propanoate (**80**)*

5β-Pregnane-3,20-dione **72** (450 mg, 1.42 mmol) was converted to **79** and **80** according to *General Procedure 6.5.2*. Purification by PLC in petroleum ether/ether (4:1) afforded **79** (152 mg, 27%) and **80** (150 mg, 26%) and the starting material **72** (24 mg, 5%).

Z-isomer **79**: m.p. 66–67 °C (acetone/heptane), $[\alpha]_D$ +106.3 (*c* 0.23, $CHCl_3$). ^1H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 1.30 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 1.85 dd, 1 H, J_1 = 1.0, J_2 = 2.0 (CH_3, propanoate moiety); 2.11 s, 3 H (3 × H-21); 2.38 doublet of sextets, 1 H, J_1 = 14.2, J_2 = 2.3 (H-4α); 2.54 t, 1 H, J = 9.0 (H-17); 2.64 dq, 1 H, J_1 = 14.4, J_2 = 2.3 (H-4β); 4.19 m, 2 H ($OC\mathbf{H}_2CH_3$). IR spectrum ($CHCl_3$): 1699 (C=O, ester and ketone); 1640 (C=C); 1473 (CH_2, OCH_2CH_3); 1386 (CH_3, OCH_2CH_3); 1228, 1191 (C-O). EI MS: 400 (77%, M), 385 (5%, M – CH_3), 354 (35%, M – OCH_2CH_3), 339 (6%, M – O, OCH_2CH_3), 311 (8%, M – $COCH_3$, OCH_2CH_3), 256 (2 %, M – $C_5H_8O_2$, $COCH_3$). For $C_{26}H_{40}O_3$ (400.6) calculated. C, 77.95; H, 10.06. Found. C, 77.88; H, 10.30.

E-isomer **80**: m.p. oily product, $[\alpha]_D$ +101.1 (*c* 0.37, $CHCl_3$). ^1H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 1.30 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 1.86 dd, 1 H, J_1 = 1.7, J_2 = 1.5 (CH_3, propanoate moiety); 2.11 s, 3 H (3 × H-21); 2.32 t, 1 H, J = 14.4 (H-2α); 2.55 t, 1 H, J = 9.0 (H-17); 2.78 doublet of sextets, 1 H, J_1 = 14.3, J_2 = 2.3 (H-2β); 4.19 q, 2 H, J = 7.0 ($OC\mathbf{H}_2CH_3$). IR spectrum ($CHCl_3$): 1699 (C=O, ester and ketone); 1637, 1103 (C=C); 1232, 1163 (C-O, OCH_2CH_3). EI MS: 401 (77%, M + 1), 355 (35%, M – OCH_2CH_3). For $C_{26}H_{40}O_3$ (400.6) calculated. C, 77.95; H, 10.06. Found. C, 78.11; H, 10.16.

*6.5.11. Ethyl (Z)-2-(20-Oxo-5α-pregnan-3-ylidene)butanoate (**81**) and Ethyl (E)-2-(20-Oxo-5α-pregnan-3-ylidene)butanoate (**82**)*

5α-Pregnane-3,20-dione **71** (225 mg, 0.71 mmol) was converted to **81** and **82** according to *General Procedure 6.5.3*. Purification by PLC in petroleum ether/ether (4:1) afforded **81** (68 mg, 23%) and **82** (38 mg, 13%) and the starting material **71** (121 mg, 54%).

Z-isomer **81**: m.p. 77–79 °C (acetone/heptane), $[\alpha]_D$ +45.6 (*c* 0.33, $CHCl_3$). ^1H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 0.99 t, 3 H, J = 7.6 ($=CCH_2C\mathbf{H}_3$); 1.30 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 2.11 s, 3 H (3 × H-21); 2.30 bq, 2 H, J = 7.6 ($=CC\mathbf{H}_2CH_3$); 2.35-2.40 m, 2 H (H-4α and H-2β); 2.48-2.56 m, 2 H (H-17 and H-4β); 4.20 q, 2 H, J = 7

(OC**H**$_2$CH$_3$). IR spectrum ($CHCl_3$): 1711 (C=O, ester and ketone); 1638, 1108 (C=C); 1473 (CH_2, OCH_2CH_3); 1245, 1181, 1155, 1025 (C-O, OCH_2CH_3). EI MS: 414 (63%, M), 369 (13%, M – OCH_2CH_3). For $C_{27}H_{42}O_3$ (414.6) calculated. C, 78.21; H, 10.21. Found. C, 78.26; H, 10.14.

E-isomer **82**: m.p. 130–132 °C (methanol/chloroform), $[\alpha]_D$ +52.0 (*c* 0.25, $CHCl_3$). ^{1}H NMR (600MHz): 0.61 s, 3 H (3 × H-18); 0.98 s, 3 H (3 × H-19); 0.99 t, 3 H, J = 7.6 (=CCH$_2$C**H**$_3$); 1.30 t, 3 H, J = 7.0 (OCH$_2$C**H**$_3$); 2.11 s, 3 H (3 × H-21); 2.21 m, 1 H (H-4α); 2.30 bq, 2 H, J = 7.6 (=CC**H**$_2$CH$_3$); 2.52 t, J = 9 (H-17); 2.76 doublet of sextets, 1 H, J_1 = 14.6, J_2 = 2.1 (H-2α); 4.20 q, 2 H, J = 7.0 (OC**H**$_2$CH$_3$). IR spectrum ($CHCl_3$): 1699 (C=O, ester and ketone); 1631, 1108 (C=C); 1472 (CH_2, OCH_2CH_3); 1237, 1030 (C-O, OCH_2CH_3). EI MS: 414 (54%, M), 369 (10%, M – OCH_2CH_3). For $C_{27}H_{42}O_3$ (414.6) calculated. C, 78.21; H, 10.21. Found. C, 78.07; H, 10.28.

*6.5.12. Ethyl (Z)-2-(20-Oxo-5β-pregnan-3-ylidene)butanoate (**83**) and Ethyl (E)-2-(20-Oxo-5β-pregnan-3-ylidene)butanoate (**84**)*

5β-Pregnane-3,20-dione **72** (450 mg, 1.42 mmol) was converted to **83** and **84** according to *General Procedure 6.5.3*. Purification by PLC in petroleum ether/ether (4:1) afforded **83** (123 mg, 21%) and **84** (98 mg, 16%) and the starting material **72** (185 mg, 41%).

Z-isomer **83**: m.p. 75–77 °C (acetone/heptane), $[\alpha]_D$ +114.4 (*c* 0.38, $CHCl_3$). ^{1}H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 0.99 t, 3 H, J = 7.6 (=CCH$_2$C**H**$_3$); 1.30 t, 3 H, J = 7.0 (OC**H**$_2$CH$_3$); 2.11 s, 3 H (3 × H-21); 2.30 bq, 2 H, J = 7.6 (=CC**H**$_2$CH$_3$); 2.35-2.40 m, 2 H (H-4α and H-2β); 2.48-2.56 m, 2 H (H-17 and H-4β); 4.20 m, 2 H (OC**H**$_2$CH$_3$). IR spectrum ($CHCl_3$): 1699 (C=O, ester and ketone); 1637 (C=C); 1228, 1163, 1025 (C-O, OCH_2CH_3). FAB MS: 437 (23%, M + Na), 415 (57%, M + 1), 369 (90%, M – OCH_2CH_3). For $C_{27}H_{42}O_3$ (414.6) calculated. C, 78.21; H, 10.21. Found. C, 78.29; H, 10.32.

E-isomer **84**: m.p. 84–86 °C (acetone/heptane), $[\alpha]_D$ +112.6 (*c* 0.24, $CHCl_3$). ^{1}H NMR (600MHz): 0.61 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 0.99 t, 3 H, J = 7.6 (=CCH$_2$C**H**$_3$); 1.30 t, 3 H, J = 7.0 (OCH$_2$C**H**$_3$); 2.12 s, 3 H (3 × H-21); 2.30 bq, 2 H, J = 7.6 (=CC**H**$_2$CH$_3$); 2.34 m, 1 H (H-4α); 2.55 t, 1 H, J = 9.0 (H-17); 2.65 doublet of sextets, 1 H, J_1 = 14, J_2 = 2.3 (H-2β); 4.20 q, 2 H, J = 7.0 (OC**H**$_2$CH$_3$). IR spectrum ($CHCl_3$): 1699 (C=O, ester and ketone); 1633 (C=C); 1233, 1026 (C-O, OCH_2CH_3). FAB MS: 437 (13%, M + Na), 415

(18%, M + 1), 369 (56%, M – OCH_2CH_3). For $C_{27}H_{42}O_3$ (414.6) calculated. C, 78.21; H, 10.21. Found. C, 78.10; H, 10.28.

*6.5.13. Ethyl (2E,4Z)-4-(20-Oxo-5α-pregnan-3-ylidene)but-2-enoate (**85**) and Ethyl (2E,4E)-4-(20-Oxo-5α-pregnan-3-ylidene)but-2-enoate (**86**)*

5α-Pregnane-3,20-dione **71** (225 mg, 0.71 mmol) was converted to **85** and **86** according to *General Procedure 6.5.4*. Purification by PLC in petroleum ether/ether (4:1) afforded **85** (85 mg, 29%) and **86** (83 mg, 28%) and the starting material **71** (21 mg, 9%).

Z-isomer **85**: m.p. 116–118 °C (petroleum ether/ether), $[\alpha]_D$ +182.4 (*c* 0.24, $CHCl_3$). ^{1}H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.90 s, 3 H (3 × H-19); 1.30 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 2.11 s, 3 H (3 × H-21); 2.35 m, 1 H (H-2β); 2.50-2.56 m, 2 H (H-17 and H-4α); 4.20 m, 2 H ($OC\mathbf{H}_2CH_3$); 5.78 dq, 1 H, J_1 = 15.1, J_2 = 0.8 (H-α, butenoate moiety); 5.93 dt, 1 H, J_1 = 11.7, J_2 = 1.8 (H-γ, butenoate moiety); 7.63 dd, 1 H, J_1 = 15.1, J_2 = 11.7 (H-β, butenoate moiety). IR spectrum ($CHCl_3$): 3060 (=CH); 1699 (C=O, ester and ketone); 1632, 1609 (C=C); 1472 (CH_2, OCH_2CH_3), 1386 (CH_3, OCH_2CH_3); 1230, 1153 (C-O). EI MS: 412 (66%, M), 367 (12%, M – OCH_2CH_3), 339 (12%, M – $COOCH_2CH_3$), 323 (7%, M – $COCH_3$, OCH_2CH_3), 299 (19%, M – $C_6H_8O_2$), 281 (8%, M – $C_4H_7O_2$, $COCH_3$). For $C_{27}H_{40}O_3$ (412.6) calculated. C, 78.60; H, 9.77. Found. C, 78.65; H, 10.06.

E-isomer **86**: m.p. 105–107 °C (petroleum ether/ether), $[\alpha]_D$ +11.4 (*c* 0.21, $CHCl_3$). ^{1}H NMR (600MHz): 0.61 s, 3 H (3 × H-18); 0.90 s, 3 H (3 × H-19); 1.30 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 2.11 s, 3 H (3 × H-21); 2.52 t, 1 H, J = 9.0 (H-17); 2.82 dp, 1 H, J_1 = 14.5, J_2 = 2.3 (H-2α); 4.20 q, 2 H , J = 7 ($OC\mathbf{H}_2CH_3$); 5.78 dq, 1 H, J_1 = 14.9, J_2 = 0.8 (H-γ, butenoate moiety); 5.92 dt, 1 H, J_1 = 11.8, J_2 = 1.8 (H-α, butenoate moiety); 7.63 dd, 1 H, J_1 = 15.1, J_2 = 11.6 (H-β, butenoate moiety). IR spectrum ($CHCl_3$): 3058 (=CH); 1699 (C=O, ester and ketone); 1633, 1611 (C=C); 1472, 1367 (CH_2, OCH_2CH_3); 1386 (CH_3, OCH_2CH_3); 1231, 1151 (C-O). EI MS: 412 (63%, M), 367 (13%, M – OCH_2CH_3), 339 (13%, M – $COOCH_2CH_3$), 323 (8%, M – $COCH_3$, OCH_2CH_3), 299 (17%, M – $C_6H_8O_2$), 281 (8 %, M – $C_4H_7O_2$, $COCH_3$). For $C_{27}H_{40}O_3$ (412.6) calculated. C, 78.60; H, 9.77. Found. C, 78.52; H, 9.83.

*6.5.14. Ethyl (2E,4Z)-4-(20-Oxo-5β-pregnan-3-ylidene)but-2-enoate (**87**) and Ethyl (2E,4E)-4-(20-Oxo-5β-pregnan-3-ylidene)but-2-enoate (**88**)*

5β-Pregnane-3,20-dione **72** (225 mg, 0.71 mmol) was converted to **87** and **88** according to *General Procedure 6.5.4*. Purification by PLC in petroleum ether/ether (4:1) afforded **87** (63 mg, 22 %) and **88** (48 mg, 16%) and starting material **72** (13.5 mg, 6%).

Z-isomer **87**: m.p. 74–77 °C (petroleum ether/ether), $[\alpha]_D$ +122.2 (*c* 0.25, $CHCl_3$). ^{1}H NMR (600 MHz): 0.61 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 1.30 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 2.12 s, 3 H (3 × H-21); 2.38 t, 1 H (H-4α); 2.48-2.56 m, 2 H (H-17 and H-4β); 4.20 q, 2 H, J = 7.0 ($OC\mathbf{H}_2CH_3$); 5.79 dq, 1 H, J_1 = 15.2, J_2 = 0.8 (H-α, butenoate moiety); 5.95 dt, 1 H, J_1 = 11.6, J_2 = 1.8 (H-γ, butenoate moiety); 7.62 dd, 1 H, J_1 = 15.2, J_2 = 11.5 (H-β, butenoate moiety). IR spectrum ($CHCl_3$): 3060, 981 (=CH); 1699 (C=O, ester and ketone); 1632, 1610 (C=C); 1152, 1036 (CH_3, OCH_2CH_3). EI MS: 435 (5%, M + Na), 413 (15%, M + 1), 367 (22%, M – OCH_2CH_3). For $C_{27}H_{40}O_3$ (412.6) calculated. C, 78.60; H, 9.77. Found. C, 78.56; H, 10.06.

E-isomer **88**: m.p. 127–129 °C (petroleum ether/ether), $[\alpha]_D$ +124.7 (*c* 0.26, $CHCl_3$). ^{1}H NMR (600MHz): 0.60 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 1.30 t, 3 H, J = 7.0 ($OCH_2C\mathbf{H}_3$); 2.12 s, 3 H (3 × H-21); 2.52 t, 1 H, J = 9.0 (H-17); 2.61-2.71 m, 2 H (H-2β and H-4β); 4.20 q, 2 H, J = 7.0 ($OC\mathbf{H}_2CH_3$); 5.78 bd, 1 H, J = 15.2 (H-γ, butenoate moiety); 5.94 dt, 1 H, J_1 = 11.6, J_2 = 1.8 (H-α, butenoate moiety); 7.61 dd, 1 H, J_1 = 15.2, J_2 = 11.6 (H-β, butenoate moiety). IR spectrum ($CHCl_3$): 3060, 981 (=CH); 1699 (C=O, COOH and ketone); 1633, 1613 (C=C); 1150, 1043 (CH_3, OCH_2CH_3). EI MS: 435 (75%, M + Na), 413 (23%, M + 1), 367 (38%, M – OCH_2CH_3). For $C_{27}H_{40}O_3$ (412.6) calculated. C, 78.60; H, 9.77. Found. C, 78.58; H, 9.82.

*6.5.15. (Z)-(20-Oxo-5α-pregnan-3-ylidene)acetic Acid (**89**)*

An ester **73** (60 mg, 0.15 mmol) and potassium hydroxide (79 mg, 1.42 mmol) were converted after 2.5 hours into **89** according to *General Procedure 6.5.5*. The crystallization afforded acid **89** (39 mg, 71%): m.p. 248–251 °C, $[\alpha]_D$ +160.0 (*c* 0.22, $CHCl_3$). ^{1}H NMR (400 MHz): 0.61 s, 3 H (3 × H-18); 0.91 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.36 m, 1 H (H-2β); 2.52 t, 1 H, J = 9.0 (H-17); 3.47 doublet of multiplets, 1 H, J = 13.9 (H-4α); 5.62 t, 1 H, J = 1.5 (=CH). IR spectrum ($CHCl_3$): 3523 (OH, COOH, monomer); 2682, 2588 (OH, COOH, dimer); 1727 (C=O, COOH, monomer); 1696 (C=O, COOH and ketone); 1638

(C=C); 1419, 1292 (C-O, COOH, dimer). ESI MS: 716 (44%, 2M), 715 (100%, 2M – 1), 358 (13%, M), 357 (61%, M – 1). For $C_{23}H_{34}O_3$ (358.5) calculated. C, 77.05; H, 9.56. Found. C, 76.97; H, 9.76.

*6.5.16. (E)-(20-Oxo-5α-pregnan-3-ylidene)acetic Acid (**90**)*

An ester **74** (60 mg, 0.15 mmol) and potassium hydroxide (79 mg, 1.42 mmol) were converted after 2.5 hours into **90** according to *General Procedure 6.5.5*. The crystallization afforded acid **90** (15 mg, 27%): m.p. 239–240 °C, $[\alpha]_D$ +29.2 (*c* 0.27, $CHCl_3$). ^{1}H NMR (400 MHz): 0.61 s, 3 H (3 × H-18); 0.92 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.53 t, 1 H, J = 9.0 (H-17); 3.74 doublet of multiplets, 1 H, J = 14.9 (H-2α); 5.62 t, 1 H, J = 1.9 (=C-H). IR spectrum ($CHCl_3$): 3525 (OH, COOH, monomer); 2687, 2584 (OH, COOH, dimer); 1725 (C=O, COOH, monomer); 1694 (C=O, COOH and ketone); 1639 (C=C); 1419, 1295 (C-O, COOH, dimer). ESI MS: 716 (43%, 2M), 715 (100%, 2M – 1), 358 (12%, M), 357 (53%, M – 1). For $C_{23}H_{34}O_3$ (358.5) calculated. C, 77.05; H, 9.56. Found. C, 77.01; H, 9.63.

*6.5.17. (Z)-(20-Oxo-5β-pregnan-3-ylidene)acetic Acid (**91**)*

An ester **75** (60 mg, 0.15 mmol) and potassium hydroxide (159 mg, 2.85 mmol) were converted after 2.5 hours into **91** according to *General Procedure 6.5.5*. The crystallization afforded acid **91** (30 mg, 56%): m.p. 178-180 °C, $[\alpha]_D$ +71.9 (*c* 0.18, $CHCl_3$). ^{1}H NMR (400 MHz): 0.61 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 2.16 s, 3 H (3 × H-21); 2.34 t, 1 H, J = 13.38 (H-4α); 2.54 t, 1 H, J = 8.8 (H-17); 3.43 d, 1 H, J = 13.89 (H-4β); 5.64 s, 1 H (=CH). IR spectrum ($CHCl_3$): 3525 (OH, COOH, monomer), 3090 (OH, COOH, dimer), 1726 (C=O, COOH, monomer), 1696 (C=O, COOH, dimer), 1643 (C=C). EI MS: 358 (30%, M), 340 (45%, M – H_2O), 312 (18%, M – COOH), 255 (15%, M – CHCOOH, $COCH_3$), 227 (13%, M + 1 – CHCOOH, $COCH_3$, CH_3, CH_3). For $C_{23}H_{34}O_3$ (358.5) calculated. C, 77.05; H, 9.56. Found. C, 76.93; H, 9.61.

*6.5.18. (E)-(20-Oxo-5β-pregnan-3-ylidene)acetic Acid (**92**)*

An ester **76** (60 mg, 0.15 mmol) and potassium hydroxide (159 mg, 2.85 mmol) were converted after 6 hours into **92** according to *General Procedure 6.5.5*. The crystallization afforded acid **92** (22 mg, 41%): m.p. 210-212 °C, $[\alpha]_D$ +130.4 (*c* 0.24, $CHCl_3$). ^{1}H NMR (400 MHz): 0.61 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.54 t,

1 H, J = 8.8 (H-17); 2.68 t, 1 H, J = 13.38 (H-4α); 3.59 d, 1 H, J = 12.6 (H-2β); 5.64 s, 1 H (=CH). IR spectrum ($CHCl_3$): 3525 (OH, COOH, monomer), 3090 (OH, COOH, dimer), 1725 (C=O, COOH, monomer), 1694 (C=O, COOH, dimer), 1639 (C=C). EI MS: 358 (100%, M), 340 (20%, M – H_2O), 256 (6%, M + 1 – CHCOOH, $COCH_3$). For $C_{23}H_{34}O_3$ (358.5) calculated. C, 77.05; H, 9.56. Found. C, 77.03; H, 9.59.

*6.5.19. (Z)-2-(20-Oxo-5α-pregnan-3-ylidene)propanoic Acid (**93**)*

An ester **77** (74 mg, 0.18 mmol) was converted into **93** according to *General Procedure 6.5.6*. The crystallization afforded acid **93** (36 mg, 65%): m.p. 190–192 °C, $[\alpha]_D$ +106.4 (*c* 0.25, $CHCl_3$). ^{1}H NMR (400 MHz): 0.61 s, 3 H (3 × H-18); 0.88 s, 3 H (3 × H-19); 1.90 t, 3 H, J = 1.5 (CH_3, propanoate moiety); 2.11 s, 3 H (3 × H-21); 2.49-2.61 m, 2 H (H-17 and H-2α); 2.97 doublet of multiplets, 1 H, J = 14.1 (H-4α). IR spectrum ($CHCl_3$): 3516 (OH, COOH, monomer); 2706, 2631, 2538 (OH, COOH, dimer); 1397 (C=O, ketone); 1678 (C=O, COOH and ketone); 1625, 1154 (C=C); 1407, 1292 (C-O, COOH, dimer). ESI MS: 744 (33%, 2M), 743 (100%, 2M – 1), 372 (7%, M), 371 (30%, M – 1). For $C_{24}H_{36}O_3$ (372.5) calculated. C, 77.38; H, 9.74. Found. C, 77.22; H, 9.72.

*6.5.20. (E)-2-(20-Oxo-5α-pregnan-3-ylidene)propanoic Acid (**94**)*

An ester **78** (60 mg, 0.15 mmol) and potassium hydroxide (159 mg, 2.85 mmol) were converted after 6 hours into **94** according to *General Procedure 6.5.5*. The crystallization afforded acid **94** (23.5 mg, 42%): m.p. 168–171 °C, $[\alpha]_D$ +47.4 (*c* 0.22, $CHCl_3$). ^{1}H NMR (400 MHz): 0.61 s, 3 H (3 × H-18); 0.89 s, 3 H (3 × H-19); 1.90 t, 3 H, J = 1.6 (CH_3, propanoate moiety); 2.11 s, 3 H (3 × H-21); 2.51 t, 1 H, J = 9.0 (H-17); 3.24 dp, 1 H, J_1 = 14.9, J_2 = 2 (H-2α). IR spectrum ($CHCl_3$): 3517 (OH, COOH, monomer); 2690, 2619 (OH, COOH, dimer); 1697 (C=O, ketone); 1682 (C=O, COOH, dimer); 1625, 1154 (C=C); 1405, 1292 (C-O, COOH, dimer). ESI MS: 744 (33%, 2M), 743 (100%, 2M – 1), 372 (7%, M), 371 (30%, M – 1). For $C_{24}H_{36}O_3$ (372.5) calculated. C, 77.38; H, 9.74. Found. C, 77.16; H, 9.68.

*6.5.21. (Z)-2-(20-Oxo-5β-pregnan-3-ylidene)propanoic Acid (**95**)*

An ester **79** (32 mg, 0.08 mmol) and potassium hydroxide (80 mg, 1.44 mmol) were converted after 6.5 hours into **95** according to *General Procedure 6.5.5*. The crystallization afforded acid **95** (14 mg, 48%): m.p. 219-222°C, $[\alpha]_D$ +102.5 (*c* 0.16, $CHCl_3$). ^1H NMR (400 MHz): 0.61 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 1.90 dd, 3 H, J_1 = 1.7, J_2 = 1.0 (CH_3, propanoate moiety); 2.11 s, 3 H (3 × H-21); 2.39 m, 1 H (H-4α); 2.54 t, 1 H, J = 9.0 (H-17); 2.95 dq, 1 H, J_1 = 14.6, J_2 = 2.02 (H-4β). IR spectrum ($CHCl_3$): 3516 (OH, COOH, monomer); 2706, 2648, 2536 (OH, COOH, dimer); 1724 (C=O, COOH, monomer), 1697 (C=O, ketone); 1681 (C=O, COOH, dimer); 1626, 1164 (C=C); 1406, 1297 (C-O, COOH, dimer). ESI MS: 744 (36%, 2M), 743 (100%, 2M – 1), 372 (8%, M), 371 (35%, M – 1). For $C_{24}H_{36}O_3$ (372.5) calculated. C, 77.38; H, 9.74. Found. C, 77.35; H, 9.66.

*6.5.22. (E)-2-(20-Oxo-5β-pregnan-3-ylidene)propanoic Acid (**96**)*

An ester **80** (60 mg, 0.08 mmol) was converted into **96** according to *General Procedure 6.5.6*. The crystallization afforded acid **96** (17 mg, 31%): m.p. 123–125°C, $[\alpha]_D$ +135.6 (*c* 0.16, $CHCl_3$). ^1H NMR (400 MHz): 0.61 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 1.90 t, 3 H, J = 1.3 (CH_3, propanoate moiety); 2.11 s, 3 H (3 × H-21); 2.36 m, 1 H (H-2α); 2.54 t, 1 H, J = 9.0 (H-17); 3.09 doublet of multiplets, 1 H, J = 14.4 (H-2β). IR spectrum ($CHCl_3$): 3520 (OH, COOH, monomer); 2687 (OH, COOH, dimer); 1698 (C=O, ketone); 1680 (C=O, COOH, dimer); 1624, 1164 (C=C); 1406, 1297 (C-O, COOH, dimer). ESI MS: 744 (44%, 2M), 743 (100%, 2M – 1), 372 (14%, M), 371 (58%, M – 1). For $C_{24}H_{36}O_3$ (372.5) calculated. C, 77.38; H, 9.74. Found. C, 77.30; H, 9.77.

*6.5.23. (E)-2-(20-Oxo-5α-pregnan-3-ylidene)butanoic Acid (**98**)*

Procedure A:

A solution of ester **81** (70 mg, 0.19 mmol) in 3 M $HClO_4$ in 50 % THF (40 mL) was heated for 20 h at 120 °C. Then, 20 mL of 3 M $HClO_4$ in 50 % THF were added. After heating for 27 h at 120 °C, the reaction mixture was poured into water (50 mL), extracted with $CHCl_3$ (2 × 50 mL), dried and evaporated. The residue was purified by PLC (2 plates) in a mixture of petroleum ether/ether and then twice crystallized from a mixture of acetone/water to afford 22 mg (44%) of **98**.

Procedure B:

A solution of ester **82** (127 mg, 0.3 mmol) in 3 M $HClO_4$ in 50 % THF (36 mL) was heated for 12 h at 120 °C. Then, 20 mL of 3 M $HClO_4$ in 50 % THF were added. After heating for 10 h at 120 °C, the reaction mixture was poured into water (50 mL), extracted with $CHCl_3$ (2 × 50 mL), dried and evaporated. The residue was purified by PLC (2 plates) in a mixture of petroleum ether/ether and then twice crystallized from a mixture of acetone/water to afford 20 mg (17%) of **98**.

E-isomer **98**: m.p. 110–115 °C, $[\alpha]_D$ +91.8 (*c* 0.22, $CHCl_3$). ^{1}H NMR (400MHz): 0.62 s, 3 H (3 × H-18); 0.94 s, 3 H (3 × H-19); 1.03 t, 3 H, J = 7.4 (=$CCH_2C\mathbf{H}_3$); 2.12 s, 3 H (3 × H-21); 2.34 q, 2 H, J = 7.6 (=$CC\mathbf{H}_2CH_3$); 2.54 t, J = 8.9 (H-17); 2.76 doublet of sextets, J_1 = 14.4, J_2 = 2.1 (H-2α). IR spectrum ($CHCl_3$): 3515 (OH, COOH, momoner); 2704, 2610 (OH, COOH, dimmer); 1697 (C=O, ketone); 1680 (C=O, COOH, dimer); 1621 (C=C). ESI MS: 772 (27%, 2M), 771 (100%, 2M – 1), 386 (8%, M), 385 (44%, M – 1). For $C_{25}H_{38}O_3$ (386.6) calculated. C, 77.68; H, 9.91. Found. C, 77.37; H, 10.08.

*6.5.24. (2E,4Z)-4-(20-Oxo-5α-pregnan-3-ylidene)but-2-enoic Acid (**101**)*

An ester **85** (60 mg, 0.14 mmol) and potassium hydroxide (70 mg, 1.26 mmol) were converted after 4 hours into **101** according to *General Procedure 6.5.5*. The crystallization afforded acid **101** (29 mg, 52%): m.p. 178–181°C, $[\alpha]_D$ +161.6 (*c* 0.18, $CHCl_3$). ^{1}H NMR (400 MHz): 0.61 s, 3 H (3 × H-18); 0.90 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.35 m, 1 H (H-2β); 2.50-2.55 m, 2 H (H-17 and H-4α); 5.79 d, 1 H, J = 14.9 (H-α, butenoic acid moiety); 5.96 d, 1 H, J = 11.6 (H-γ, butenoic acid moiety); 7.72 dd, 1 H, J_1 = 14.9, J_2 = 11.6 (H-β, butenoic acid moiety). IR spectrum ($CHCl_3$): 3526 (OH, COOH, monomer); 2668, 2579 (OH, COOH, dimer); 1694 (C=O, ketone); 1684 (C=O, COOH, dimer); 1629, 1607, 983 (C=C); 1416, 1286 (C-O, COOH, dimer). ESI MS: 768 (50%, 2M), 767 (100%, 2M – 1), 384 (17%, M), 383 (64%, M – 1). For $C_{25}H_{36}O_3$ (384.5) calculated. C, 78.08; H, 9.44. Found. C, 77.99; H, 9.53.

*6.5.25. (2E,4E)-4-(20-Oxo-5α-pregnan-3-ylidene)but-2-enoic Acid (**102**)*

An ester **86** (60 mg, 0.14 mmol) and potassium hydroxide (70 mg, 1.26 mmol) were converted after 8 hours into **102** according to *General Procedure 6.5.5*. The crystallization afforded acid **102** (27 mg, 48%): m.p. 190–193 °C, $[\alpha]_D$ –13.0 (*c* 0.25, $CHCl_3$). ^{1}H NMR (400 MHz): 0.61 s, 3 H (3 × H-18); 0.91 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.52 t, 1 H, J = 9.0 (H-17); 2.80 m, 1 H (H-2α); 5.79 d, 1 H, J = 15.1 (H-α, butenoic acid moiety);

5.95 dt, 1 H, J = 11.8 (H-γ, butenoic acid moiety); 7.72 dd, 1 H, J_1 = 15.1, J_2 = 11.8 (H-β, butenoic acid moiety). IR spectrum ($CHCl_3$): 3526 (OH, COOH, monomer); 2661, 2579 (OH, COOH, dimer); 1698 (C=O, ketone); 1684 (C=O, COOH, dimer); 1630, 1608, 983 (C=C); 1406, 1297 (C-O, COOH, dimer). ESI MS: 768 (22%, 2M), 767 (44%, 2M – 1), 384 (26%, M), 383 (100%, M – 1). For $C_{25}H_{36}O_3$ (384.5) calculated. C, 78.08; H, 9.44. Found. C, 77.91; H, 9.60.

*6.5.26. (2E,4Z)-4-(20-Oxo-5β-pregnan-3-ylidene)but-2-enoic Acid (**103**)*

An ester **87** (65 mg, 0.16 mmol) was converted into **103** according to *General Procedure 6.5.6*. The crystallization afforded acid **103** (18 mg, 30%): m.p. 204–206 °C, $[\alpha]_D$ +147.4 (*c* 0.19, $CHCl_3$). ^{1}H NMR (400 MHz): 0.62 s, 3 H (3 × H-18); 0.95 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.40 t, 1 H, J = 13.5 (H-4α), 2.48-2.58 m, 2 H (H-17 and H-4β), 5.79 d, 1 H, J = 15.1 (H-α, butenoic acid moiety), 6.05 d, 1 H, J = 11.7 (H-γ, butenoic acid moiety), 7.7 dd, 1 H, J_1 = 15, J_2 = 11.7 (H-β, butenoic acid moiety). IR spectrum ($CHCl_3$): 3527 (OH, COOH, monomer); 3092 (OH, COOH, dimer); 1696 (C=O, ketone); 1684 (C=O, COOH, dimer); 1629, 1608 (C=C); 1286 (C-O, COOH, dimer). EI MS: 384 (52%, M), 366 (15%, M – H_2O), 341 (7%, M – CH_3CO), 323 (10%, M – COOH, O), 299 (13%, M – CH=CH=CH-COOH, H), 257 (7%, M – CH=CH=CH-COOH, CH_3CO). For $C_{25}H_{36}O_3$ (384.5) calculated. C, 78.08; H, 9.44. Found. C, 78.04; H, 9.49.

*6.5.27. (2E,4E)-4-(20-Oxo-5β-pregnan-3-ylidene)but-2-enoic Acid (**104**)*

An ester **88** (67 mg, 0.16 mmol) was converted into **104** according to *General Procedure 6.5.6*. The crystallization afforded acid **104** (34 mg, 55%): m.p. 170–172 °C, $[\alpha]_D$ +123.7 (*c* 0.24, $CHCl_3$). ^{1}H NMR (400 MHz): 0.62 s, 3 H (3 × H-18); 0.95 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.55 t, 1 H, J = 9 (H-17), 2.64-2.76 m, 2 H (H-2β and H-2β), 5.79 d, 1 H, J = 15.1 (H-α, butenoic acid moiety), 5.98 d, 1 H, J = 11.7 (H-γ, butenoic acid moiety), 7.7 dd, 1 H, J_1 = 15, J_2 = 11.7 (H-β, butenoic acid moiety). IR spectrum ($CHCl_3$): 3527 (OH, COOH, monomer); 3090 (OH, COOH, dimer); 1695 (C=O, ketone); 1686 (C=O, COOH, dimer); 1631, 1610 (C=C) 1285 (C-O, COOH, dimer). EI MS: 384 (53%, M), 366 (16%, M – H_2O), 341 (8%, M – CH_3CO), 323 (10%, M – COOH, O), 299 (14%, M – CH=CH=CH-COOH, H), 257 (7%, M – CH=CH=CH-COOH, CH_3CO). For $C_{25}H_{36}O_3$ (384.5) calculated. C, 78.08; H, 9.44. Found. C, 77.96; H, 9.61.

*6.5.28. Attempted Hydrolysis of Compounds **81**, **82**, **83**, and **84***

Procedure A:

A mixture of ester **81** (22 mg, 0.05 mmol) in methanol (3 mL) was treated with potassium hydroxide (26 mg, 0.47 mmol) in H_2O/EtOH (0.25 mL, 1:1). After refluxing for 8 h potassium hydroxide (78 mg, 1.39 mmol) in H_2O/EtOH (0.75 mL, 1:1) were added. After refluxing for 6 h, the reaction mixture was worked up. Unchanged starting material and carboxylic acid a very small amount that was not sufficient for isolation in pure form were recovered.

A mixture of ester **82** (20 mg, 0.05 mmol) in methanol (3 mL) was treated with potassium hydroxide (26 mg, 0.47 mmol) in H_2O/EtOH (0.25 mL, 1:1). After refluxing for 8 h potassium hydroxide (78 mg, 1.39 mmol) in H_2O/EtOH (0.75 mL, 1:1) were added. After refluxing for 6 h, the reaction mixture was worked up. Unchanged starting material and carboxylic acid a very small amount that was not sufficient for isolation in pure form were recovered.

A mixture of ester **83** (12 mg, 0.03 mmol) in methanol (3 mL) was treated with potassium hydroxide (14 mg, 0.25 mmol) in H_2O/EtOH (0.14 mL, 1:1). After refluxing for 8 h potassium hydroxide (42 mg, 0.75 mmol) in H_2O/EtOH (0.41 mL, 1:1) were added. After refluxing for 6 h, the reaction mixture was worked up. Unchanged starting material and carboxylic acid a very small amount that was not sufficient for isolation in pure form were recovered.

A mixture of ester **84** (23 mg, 0.05 mmol) in methanol (3 mL) was treated with potassium hydroxide (26 mg, 0.47 mmol) in H_2O/EtOH (0.25 mL, 1:1). After refluxing for 8 h potassium hydroxide (78 mg, 1.39 mmol) in H_2O/EtOH (0.75 mL, 1:1) were added. After refluxing for 6 h, the reaction mixture was worked up. Unchanged starting material and carboxylic acid a very small amount that was not sufficient for isolation in pure form were recovered.

Procedure B:

A solution of ester **83** (79 mg, 0.19 mmol) in 3 M $HClO_4$ in 50 % THF (40 mL) was heated for 24 h at 120 °C. Then, the reaction mixture was partially evaporated, poured into water (50 mL, extracted with $CHCl_3$ (50 mL), dried and evaporated. Unchanged starting material and carboxylic acid a very small amount that was not sufficient for isolation in pure form were recovered.

A solution of ester **84** (80 mg, 0.19 mmol) in 3 M $HClO_4$ in 50 % THF (40 mL) was heated for 24 h at 120 °C. Then, the reaction mixture was partially evaporated, poured into water (50

mL, extracted with $CHCl_3$ (50 mL), dried and evaporated. A mixture of isomer **83** and **84** in a ratio 1:1 and carboxylic acid a very small amount that was not sufficient for isolation in pure form were recovered.

Procedure C:

A mixture of ester **81** (20 mg, 0.05 mmol) in methanol (3 mL) was treated with lithium hydroxide (20 mg, 0.83 mmol) in H_2O/EtOH (0.25 mL, 1:1). After refluxing for 5 h, the reaction mixture was worked up. A mixture of isomer **81** and **82** in a ratio 1:1 was recovered.

Procedure D:

A mixture of compound **81** (50 mg, 0.12 mmol) and sodium iodide (62 mg, 0.41 mmol) was dried in vacuo (25 °C, 100 Pa) for 30 min. Then, dry acetonitril (5 mL) and trimethylsilyl chloride were added under argon atmosphere via cannula. A yellow reaction mixture was stirred at 60 °C for 8 h. Then, it was poured into water and extracted with ether (50 mL). Organic phase was diluted with an aqueous solution of $Na_2S_2O_3$, water, and dried. Starting compound was recovered unchanged.

6.6. Luche Reduction of Saturated Steroid Ketones

6.6.1. General Procedure for $NaBH_4$ Reduction

Sodium borohydride (1 equiv) was added to a stirred solution of a ketone (1 equiv) in MeOH/THF (2:1, 2.5 mL per 50 mg). The progress of the reaction was controlled by TLC. Then, the pH was adjusted to neutrality with a hydrochlorid acid and the mixture was extracted with EtOAc. Extract was washed with a solution of potassium hydrogen carbonate, water, dried and evaporated *in vacuo*. For details see ***Table 12***. For results see ***Table 8*** and ***Table 11***.

Table 12 – Details of sodium borohydride reduction of compounds 40 and 105-116.

Compound	*Amount*	*MeOH/THF*	*$NaBH_4$*	*HPLC*
40	100 mg (0.28 mmol)	5 mL	10.5 mg (0.28 mmol)	EtOAc/hexanes (16:84)
105	50 mg (0.12 mmol)	2.5 mL	4.5 mg (0.12 mmol)	EtOAc/hexanes (7:93)
106	50 mg (0.12 mmol)	2.5 mL	4.5 mg (0.12 mmol)	EtOAc/hexanes (60:40)
107	50 mg (0.11 mmol)	2.5 mL	4 mg (0.11 mmol)	EtOAc/hexanes (15:75)
108	50 mg (0.1 mmol)	2.5 mL	4 mg (0.11 mmol)	EtOAc/hexanes (10:90)
109	40 mg (0.1 mmol)	2 mL	4 mg (0.11 mmol)	EtOAc/hexanes (60:40)
110	10 mg (0.02 mmol)	0.5 mL	1 mg (0.022 mmol)	EtOAc/hexanes (30:70)
111	10 mg (0.02 mmol)	0.5 mL	1 mg (0.022 mmol)	EtOAc/hexanes (5:95)
112	100 mg (0.23 mmol)	5 mL	8.7 mg (0.23 mmol)	THF/benzene (20:80)
113[a]	50 mg (0.13 mmol)	2.5 mL	7 mg (0.19 mmol)	-
114	50 mg (0.13 mmol)	2.5 mL	5 mg (0.13 mmol)	EtOAc/hexanes (5:95)
115	50 mg (0.15 mmol)	2.5 mL	5.6 mg (0.15 mmol)	ether/petroleum ether (30:70)
116	50 mg (0.15 mmol)	2.5 mL	5.6 mg (0.15 mmol)	ether/petroleum ether (30:70)

[a] 1.5 Equiv of $NaBH_4$ was used. The progress of the reaction was controlled by TLC and no reduction was observed. Neither under the conditions of microwave irradiation at elevated temperature 100 °C for 20 min nor 140°C for 30 min was any reduction observed. Only deprotection of acetal occurred according to ^{1}H NMR spectrum.

6.6.2. General Procedure for Luche Reduction Using $CeCl_3 \cdot 7H_2O$

Cerium(III) chloride heptahydrate (1.1 equiv) was added to a stirred solution of a ketone (1 equiv) in MeOH/THF (2:1, 2.5 mL per 50 mg). The mixture was allowed to stir at room temperature as long as all chloride had been dissolved. Then, sodium borohydride (1 equiv) was added in small portions. The vigorous gas hydrogen evolution occurred and the progress of the reaction was controlled by TLC. The work up of the reaction mixture was identical as for *General Procedure 6.6.1*. For details see ***Table 13***. For results see ***Table 8*** and ***Table 11***.

Table 13 – Details of Luche reduction using $CeCl_3 \cdot 7H_2O$ of compounds 40 and 105-116.

Compound	*Amount*	*MeOH/THF*	*$CeCl_3 \cdot 7H_2O$*	*$NaBH_4$*	*HPLC*
40	100 mg, (0.28 mmol)	5 mL	115 mg (0.31 mmol)	10.5 mg (0.28 mmol)	EtOAc/hexanes (16:84)
105	50 mg (0.12 mmol)	2.5 mL	48 mg (0.13 mmol)	4.5 mg (0.12 mmol)	EtOAc/hexanes (7:93)
106	50 mg (0.12 mmol)	2.5 mL	48 mg (0.13 mmol)	4.5 mg (0.12 mmol)	EtOAc/hexanes (60:40)
107	50 mg (0.11 mmol)	2.5 mL	45 mg (0.12 mmol)	4 mg (0.11 mmol)	EtOAc/hexanes (15:75)
108	50 mg (0.1 mmol)	2.5 mL	41 mg (0.11 mmol)	4 mg (0.11 mmol)	EtOAc/hexanes (10:90)
109	40 mg (0.1 mmol)	2 mL	41 mg (0.11 mmol)	4 mg (0.11 mmol)	EtOAc/hexanes (60:40)
110	10 mg (0.02 mmol)	0.5 mL	8 mg (0.02 mmol)	1 mg (0.02 mmol)	EtOAc/hexanes (30:70)
111	10 mg (0.02 mmol)	0.5 mL	8 mg (0.02 mmol)	1 mg (0.02 mmol)	EtOAc/hexanes (5:95)
112	100 mg (0.23 mmol)	5 mL	93 mg (0.25 mmol)	8.7 mg (0.23 mmol)	THF/benzene (20:80)
113[a]	45 mg (0.12 mmol)	3 mL	48 mg (0.13 mmol)	7 mg (0.18 mmol)	-
114	50 mg (0.13 mmol)	2.5 mL	52 mg (0.14 mmol)	5 mg (0.13 mmol)	EtOAc/hexanes (5:95)
115	50 mg (0.15 mmol)	2.5 mL	61 mg (0.16 mmol)	5.6 mg (0.15 mmol)	ether/petroleum ether (30:70)
116	50 mg (0.15 mmol)	2.5 mL	61 mg (0.16 mmol)	5.6 mg (0.15 mmol)	ether/petroleum ether (30:70)

[a] 1.5 Equiv of $NaBH_4$ was used. The progress of the reaction was controlled by TLC and no reduction was observed. Neither under the conditions of microwave irradiation at elevated temperature 140 °C for 30 min nor 140°C for 1.5 h was any reduction observed. According to ^{1}H NMR spectrum only deprotection of acetal occurred.

6.6.3. General Procedure for Luche Reduction Using SmI_3

Anhydrous samarium iodide (1.1 equiv), particular ketone (1 equiv), MeOH/THF (2:1, 2.5 mL per 50 mg), and sodium borohydride (1 equiv) under argon were converted to a mixture of equatorial and axial alcohols according to *General Procedure for 6.6.2*. For details see ***Table14***. For results see ***Table 9***.

Table 14 – Details of Luche reduction using SmI_3 of compounds 40, 105, and 106.

Compound	*Amount*	*MeOH/THF*	*SmI_3*	*$NaBH_4$*	*HPLC*
40	50 mg (0.14 mmol)	2.5 mL	82 mg (0.15 mmol)	5.3 mg (0.14 mmol)	EtOAc/hexanes (16:84)
105	50 mg (0.12 mmol)	2.5 mL	69 mg (0.13 mmol)	4.5 mg (0.12 mmol)	EtOAc/hexanes (7:93)
106	50 mg (0.12 mmol)	2.5 mL	69 mg (0.13 mmol)	4.5 mg (0.12 mmol)	EtOAc/hexanes (60:40)

6.6.4. General procedure for Luche reduction using anhydrous $CeCl_3$

Anhydrous cerium(III) chloride (1.1 eq), particular ketone (1 equiv), and anhydrous MeOH/THF (2:1, 2.5 mL per 50 mg), and sodium borohydride (1 equiv) under argon were converted to a mixture of equatorial and axial alcohols according to *General Procedure 6.6.2*. For details see ***Table 15***. For results see ***Table 10***.

Table 15 – Details of Luche reduction using $CeCl_3$ of compounds 40, 105, and 106.

Compound	*Amount*	*MeOH/THF*	*$CeCl_3$*	*$NaBH_4$*	*HPLC*
40	100 mg (0.28 mmol)	5 mL	76 mg (0.31 mmol)	10.5 mg (0.28 mmol)	EtOAc/hexanes (16:84)
105	100 mg (0.24 mmol)	5 mL	64 mg (0.26 mmol)	9 mg (0.24 mmol)	EtOAc/hexanes (7:93)
106	100 mg (0.24 mmol)	5 mL	64 mg (0.26 mmol)	9 mg (0.24 mmol)	EtOAc/hexanes (60:40)

7. Apendix - Biological Activities

7.1. General

Biological activities of steroids (**5**, **6**, **11**, **13**, **14**, **17**, **18**, **23**, **25**, **26**, **60-62**, **67**, **70**, **89-96**, **98**, and **101-104**) has been determined for native NMDA receptors in cultured hippocampal neurons and recombinant NR1/NR2B receptors expressed in HEK293 cells. An electrophysiological technique has been used to estimate the relative potency of each neurosteroid (see[119] for technical details). The experiments were carried out in the laboratory of Dr. Vyklický (Department of Cellular Neurophysiology at the Institute of Physiology, Academy of Sciences of the Czech Rebublic, v.v.i.) by Mgr. Jiřina Borovská and Ing. Ondřej Cais. There was no significant difference in the degree of neurosteroid-induced inhibition or potentiation of native and recombinant receptors and therefore the results were pooled.

7.2. Results

7.2.1. Biological Activities of C-3 and C-7 Substituted Pregnane Derivatives (5, 6, 11, 13, 14, 17, 18, 23, 25, 26)

The results of structural-functional analysis of C-3 and C-7 substituted 5α- and 5β-pregnanes could be summarized as follows (***Table 16***): (i.) 5β-neurosteroids are more potent inhibitors than those with 5α-configuration; (ii.) succinate substitution for the C-3-sulfate of the 3α5βS has no effect for the steroid affinity at NMDA receptors, however, similar substitution of 3α5αS reduces its affinity; (iii.) binding site for the inhibitory neurosteroids at NMDA receptors can adopt rather large substituents at C-3 (-hemisuccinate) and at C-7 (-acetate, -nicotinate); (iv.) substituents at C-3 and C-7 affect steroid binding to the receptor; and (v.) C-7 substituents inhibit NMDA receptor channel activity.

Table 16 – Inhibition of NMDA-induced response by compounds 5, 6, 11, 13, 14, 17, 18, 23, 25, 26.

Compound	Inhibition (%)	*n*
5	- 17.4 ± 4.3	5
6	- 35.2 ± 2.7	5
11	- 37.8 ± 4.7	6
13	- 35.1 ± 2.6	5
14	- 56.8 ± 2.3	6
17	- 21.4 ± 2.7	6
18	- 34.1 ± 10.2	5
23	- 46.9 ± 9.7	5
25	- 69.2 ± 7.0	6
26	- 71.3 ± 5.0	5

The second column shows relative degree of steroid (100 μM) inhibition of responses mediated by native and recombinant NMDA receptors. Results are expressed as mean ± SD, with *n* indicating the number of cells studied.

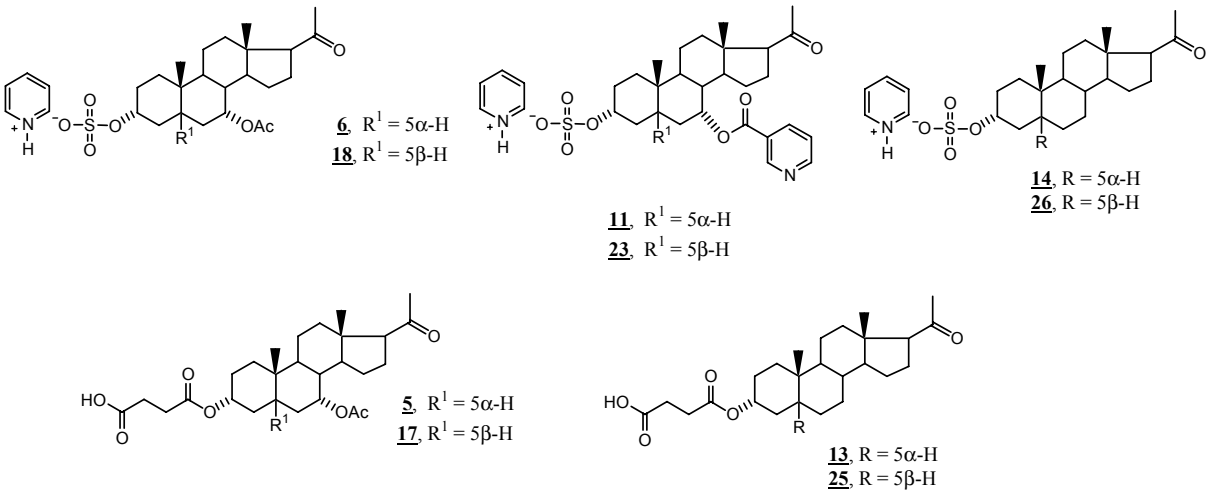


Figure 2 – C-3 and C-7 Substituted Pregnane Derivatives (5, 6, 11, 13, 14, 17, 18, 23, 25, 26).

7.2.2. Biological Activities of 3-Steroid Carboxylic Acids (60-62)

The results of structural-functional analysis of 3-steroid carboxylic acids could be summarized as follows (***Table 17***): (i.) 5β-neurosteroids (**61**,**62**) are inhibitors, whereas 5α-neurosteroid (**60**) potentiate and; (ii.) carboxyl substitution of the 3-sulfate group (**61**, **62**) did not significantly change the inhibition activity as compared with 3α5βS and 3β5βS.

Table 17 – Inhibition and potentiation of NMDA-induced response by compounds 60-62.

Compound	Relative effect (%)	*n*
60	+ 31.1 ± 5.7	4
61	- 64.7 ± 5.9	5
62	- 40.1 ± 7.0	5

The second column shows relative degree of steroid (100 μM in case of compound 62 and 200 μM in case of compounds 60 and 61) inhibition or potentiation of current responses mediated by native and recombinant NMDA receptors. Results are expressed as mean ± SD, with *n* indicating the number of cells studied.

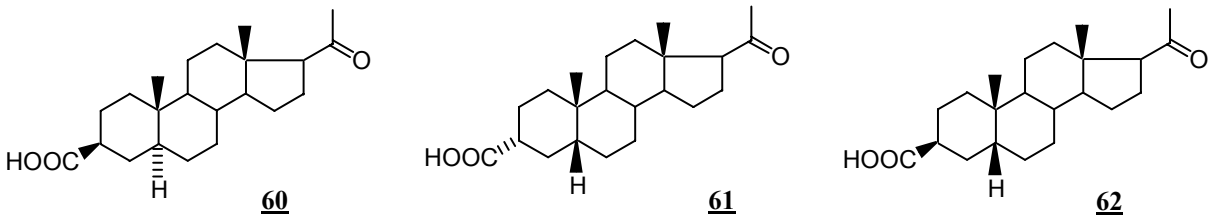


Figure 3 – Steroid 3-carboxylic acids 60-62.

7.2.3. Biological Activities of 2-(20-Oxo-5β-pregnan-3α-yl)propandioic Acid (67) and 2-(20-Oxo-5β-pregnan-3α-yl)acetic Acid (70)

The results of structural-functional analysis of steroid carboxylic acids could be summarized as follows (***Table 18***): (i.) both derivatives inhibit NMDA receptor channel activity; (ii.) bigger space demands of branched substituent may result in slight decrease of the activity.

Table 18 – Inhibition of NMDA-induced response by compounds 67 and 70.

Compound	Inhibition (%)	*n*
67	- 43.4 ± 3.4	4
70	- 63.6 ± 21.0	5

The second column shows relative degree of steroid (200 μM) inhibition or potentiation of current responses mediated by native and recombinant NMDA receptors. Results are expressed as mean ± SD, with *n* indicating the number of cells studied.

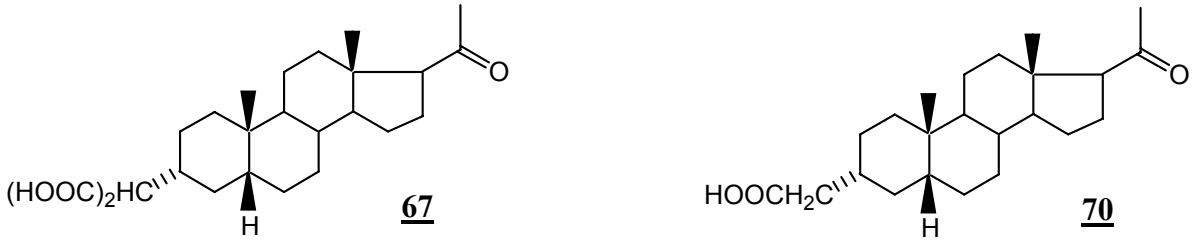


Figure 4 – 2-(20-Oxo-5β-pregnan-3α-yl)propandioic acid (67) and 2-(20-Oxo-5β-pregnan-3α-yl)acetic acid (70).

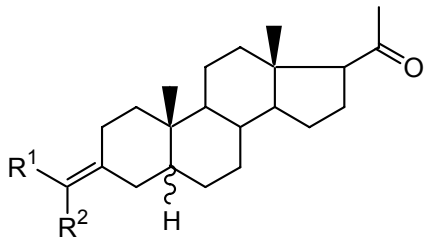
7.2.4. Biological Activities of Steroid Carboxylic Acids prepared via Wadsworth-Horner-Emmons reaction (WHE) (89-96, 98, and 101-104)

The results of structural-functional analysis of steroid carboxylic acids prepared via WHE reaction could be summarized as follows (***Table 19***): (i.) in general, steroid carboxylic derivatives with 3-exo double bond(s) do not inhibit, but potentiate NMDA-induced response; the exceptions are compounds **95**, **96**, and **98**. We hypothesize that the intriguing effect of unsaturated carboxylic derivatives is due to neurosteroid interaction with two different binding sites at the NMDA receptor – while interaction with the first site has a positive allosteric effect, interaction with the second site has a negative allosteric effect. Relative neurosteroid effect on responses mediated by NMDA receptors could be explained by summation of both – the potentiating and inhibitory effects. These interactions are characterized by *e.g.* affinity of compound for the receptor, or kinetic properties, efficacy and receptor activation. Further insight will be possible after detailed analysis of the steroid structural – functional characteristics and detail knowledge of the molecular mechanism of neurosteriod interaction with the NMDA receptor. Known data suggest that neurosteroids activity is strongly dependent on the geometry of substituents in the position 3. The presence of double bond close to the carbons 3-5 could be an important factor, too.

Table 19 – An effect of compounds 89-96, 98, and 101-104 on NMDA-induced responses.

Compound	Relative effect (%)	*n*
89	+ 28.7 ± 27.6	6
90	+ 23.6 ± 26.1	8
91	+ 58.4 ± 6.2	2
92	+ 58.4 ± 14.0	3
93	+ 69.3 ± 26.0	6
94	+ 35.4 ± 8.0	5
95	- 58.4 ± 22.4	7
96	- 59.4 ± 18.5	5
98	- 67.1 ± 13.4	6
101	+ 14.4 ± 12.0	6
102	+ 30.7 ± 12.9	6
103	+ 149.0 ± 60.1	6
104	- 26.4 ± 18.2	3
	+ 135.1 ± 74.4	3

The second column shows relative degree of steroid (200 μM) inhibition or potentiation of current responses mediated by native and recombinant NMDA receptors. Results are expressed as mean ± SD, with *n* indicating the number of cells studied.



		R^1	R^2
89	5α-H	H	COOH
90	5α-H	COOH	H
91	5β-H	H	COOH
92	5β-H	COOH	H
93	5α-H	CH_3	COOH
94	5α-H	COOH	CH_3
95	5β-H	CH_3	COOH
96	5β-H	COOH	CH_3
98	5α-H	COOH	CH_2CH_3
101	5α-H	H	CH=CH-COOH
102	5α-H	CH=CH-COOH	H
103	5β-H	H	CH=CH-COOH
104	5β-H	CH=CH-COOH	H

Figure 5 – Steroid Carboxylic Acids prepared via WHE reaction (89-96, 98, and 101-104).

8. Scientific Presentations and Posters

Papers

- Šťastná E.: Diazomethane (CH_2N_2). Synlett, 2007,15,2454.
- Stastna E., Chodounska H., Pouzar V., Kapras V., Borovska J, Cais O., L Vyklicky L.: Synthesis of C3, C5, and C7 pregnane derivatives and their effect on NMDA receptor responses in cultured rat hippocampal neurons. Steroids 2009, 74, 256-263.
- Kapras V., Šťastná E., Chodounská H., Pouzar V., Krištofíková Z.: Preparation of steroid sulfamates and their interaction with $GABA_A$ receptor. Coll. Czech. Chem. Comm., submitted, manuscript number CCCC/2008/000187.
- Eignerová B., Slavíková B., Buděšínský M., Stastna E., Kotora M.: Synthesis of Fluorinated Brassinosteroids Based on Alkane Cross-Metathesis and Preliminary Biological Assessment. J. Org. Chem., under revision, manuscript number jo-2009-002079.

Patents

- Stastna E., Chodounska H., Cais O., Vyklicky L., Kapras V., Pouzar V., Kohout L.: Steroidní anionické sloučeniny, způsob jejich výroby a jejich použití. Pregnane anionic compounds, the methods of production and their use. Filed 10th July 2008. CZ PV 2008-434.

Presentations

- Šťastná Eva, Pouzar Vladimír, Chodounská Hana: Stability of 7-OH epimers of 3β-hydroxy-5-en steroids. Conference of the Czech Chemical Society 2003. Nymburk, Czech Republic. Chemické listy 2003,97,1128. Awarded „One of the best student lecture of the conference“.
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