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Side chain azasteroids and thiasteroids as sterol methyltransferase inhibitors in ergosterol biosynthesis

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ABSTRACT

The synthesis of some novel azasteroids and thiasteroids based on a pregnan nucleus with a Δ7 double bond in two to five steps from the key aldehyde (3*S*,20*S*)-20-formylpregn-7-en-3-yl acetate has been disclosed herein. These compounds were evaluated as potential inhibitors of the enzyme Δ24-sterol methyltransferase (24-SMT), which is a key enzyme in the biosynthesis of ergosterol, and for their effects on the growth of the yeast *Yarrowia lipolytica*. Most of the side chain modified analogues were recognized as 24-SMT inhibitors, and in particular the 23-azasteroids **5f–5i** and the 24-azasteroid **11** showed potent antifungal activity. The target enzyme could be identified unambiguously using an improved whole-cell assay combined with GC–MS analysis of the sterol pattern resulting upon incubation with the inhibitors.

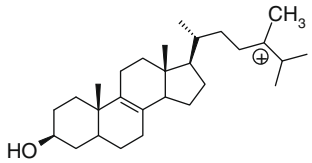
1. Introduction

The production of selective enzyme inhibitors represents a major strategy in the development of novel drugs, and almost one third of the current top 50 drugs by sales are enzyme inhibitors. The inhibition of an enzyme-catalyzed reaction can enable the selective modulation of a variety of biochemical processes such as making cell growth, division and viability untenable or interrupting major metabolic pathways by blocking formation of essential metabolites.[1] Thus, specific inhibitors of the ergosterol biosynthesis are under development as novel antifungal drugs.[2,3]

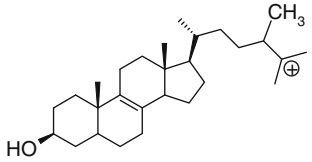
Biosynthesis of ergosterol, the major sterol component in fungi, requires methylation at the 24-position of the side chain using *S*-adenosyl-L-methionine as the methyl donor. This step is catalyzed by the enzyme Δ24-sterol methyltransferase (24-SMT, EC 2.1.1.41).[4] Since this step is not to be found in the cholesterol biosynthesis in man,[5] 24-SMT is an ideal target for the development of specific antifungal chemotherapies.

Studies on Δ24-sterol methyltransferase mechanisms reviewed by Nes et al.[3,6–8] have shown the participation of carbocationic high energy intermediates (HEIs) at C-24 and C-25 (Fig. 1) which are used to design inhibitors of 24-SMT. From these studies, the following requirements appeared necessary to design a 24-SMT inhibitor: a free 3β-hydroxy function, a flat tetracyclic nucleus, (*R*)-configuration at C-20 and a functional group in the side chain that is able to mimic the cationic HEIs.

Several groups[3,9–11] have shown that sterols (pregnanes, Δ5-pregnenes, Δ8(9)-pregnenes, cycloartenol derivatives) containing a functional group such as sulfonium, ammonium and arsonium ions in position 24 or 25 of the sterol are effective inhibitors of 24-SMT in fungi. Only one example reports a cholestan derivative with a *N*-isobutyl group at C-23 (Fig. 2) and its activity on Δ24-sterol methyltransferase from *Saccharomyces cerevisiae*.[3,12]



C-24 carbocationic HEI



C-25-carbocationic HEI

Figure 1. Carbocationic high energy intermediates of the reaction catalyzed by 24-SMT.

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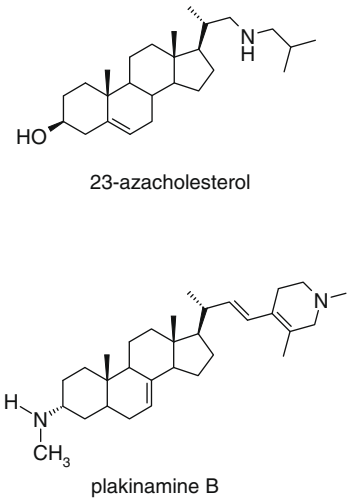


Figure 2. Structures of the cholestan-derived 24-SMT inhibitor and plakinamine B.

Taking in account previous work done in this field, and inspired by azasteroids[13] with antifungal activities, for example, the marine Δ7-pregnene-derived alkaloid plakinamine B[14] (Fig. 2), the development of a series of sterols with the Δ7-pregnene core and positively charged or protonable heteroatoms at positions 23 and 24 as new antifungals was envisaged. Suitable functional groups are amines, as well as ammonium, thionium and pyridinium salts. Herein, we report the synthesis and the evaluation of activities of novel azasteroids and thiasteroids on the growth and on the sterol metabolism of the yeast *Yarrowia lipolytica*. Moreover, we describe an improved whole-cell assay for the identification of the target enzyme in ergosterol biosynthesis.

2. Chemistry

Reaction of ergosterol with acetic anhydride and pyridine gave ergosteryl acetate (**1**). This ester **1** was subjected to selective hydrogenation of the Δ5,6 double bond using Raney nickel W2[15] poisoned with 4-dimethylaminobenzaldehyde to give 5α,6-dihydroergosteryl acetate (**2**) in good yield (83%).[16,17] Selective ozonolysis of the Δ22(23) double bond of **2** in dichloromethane/pyridine followed by reductive workup using dimethyl sulfide gave (3*S*,20*S*)-20-formylpregn-7-en-3-yl acetate (**3**) in yields ranging from 32% to 40%[18] (Scheme 1).

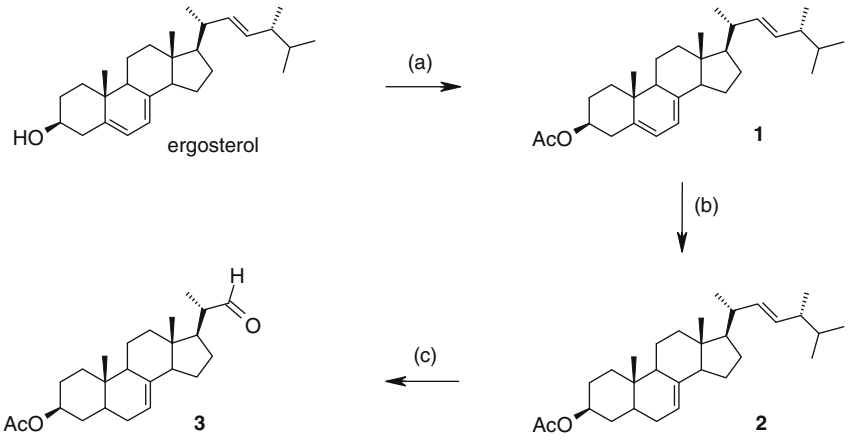
This aldehyde was used as the key intermediate for the synthesis of the target azasteroid and thiasteroid derivatives.

The series of 23-azasterols **5a**–**5k** were easily prepared in two steps from aldehyde **3** by reductive amination with primary amines. This reaction was performed using the zinc chloride-modified cyanoborohydride reagent[19] in anhydrous tetrahydrofuran and applied to diamines, *n*-alkylamines and branched alkylamines in order to obtain the intermediates **4a**–**4k**. These latter compounds were then deprotected by cleavage of the 3-acetoxy group with potassium carbonate in a mixture of chloroform and methanol to give the azasterols **5a**–**5k** in yields ranging from 55% to 98% (Scheme 2).

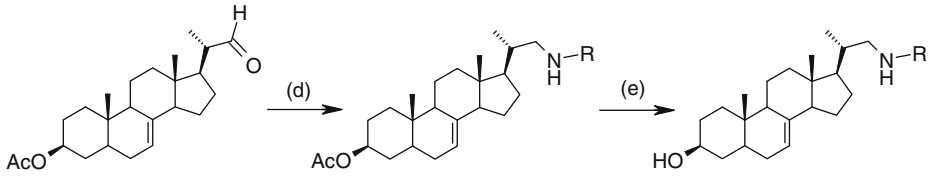
As the *N*-butyl derivative **5g** was identified as a potent antifungal compound in the first screenings, we also prepared its tertiary *N*-methyl derivative **7**. The treatment with formic acid and formaldehyde, using the Eschweiler–Clarke procedure,[20] of **4g** afforded the tertiary amine **6** (94% yield), which was easily hydrolyzed with potassium carbonate in chloroform/methanol to give the tertiary aminosterol **7** (Scheme 3).

In order to obtain mimics of the C-24 carbocationic high energy intermediate, an amino function was to be introduced in the side chain at that position. For this purpose a homologization reaction of the aldehyde **3** was performed with the ylide, prepared in situ from *n*-butyllithium and triphenyl(methoxymethyl)phosphonium chloride,[21] to afford a mixture of four enol ethers: (*E*)- and (*Z*)-isomers of **8a** and (*E*)- and (*Z*)-isomers of the deprotected sterol **8b** in a ratio of about 2:1 and with an overall yield of 52%. Both *E*/*Z*-mixtures could not be separated on a preparative scale. Under acidic conditions, the enol ethers **8a** were converted into the aldehyde **9a** and to the deprotected aldehyde **9b** (ratio: 2:1, 86% overall yield). The homologous aldehyde **9a** was subjected to reductive amination with *n*-propylamine and the zinc chloride-modified cyanoborohydride reagent, in order to end up with the same length of the side chain as present in the active *N*-butylamino compound **5g**. Subsequent deprotection by ester hydrolysis with potassium carbonate in chloroform/methanol afforded **11** in 52% yield over two steps (Scheme 4).

A sole thiasteroid model was also prepared, in analogy to the highly active *N*-butylamino derivative **5g** we selected the *S*-butyl residue. The selective reduction of the aldehyde **3** to the corresponding primary alcohol **12** was performed with sodium borohydride in chloroform/methanol as previously reported.[22] Using methyltriphenoxy-phosphonium iodide,[23] the alcohol **12** was converted into the corresponding iodide derivative **13** in 88% yield. Subsequent treatment with lithium *n*-butanethiolate, formed in situ from *n*-butanethiol and *n*-butyllithium, to give the thioether **14** and hydrolysis of the acetate group with potassium carbonate

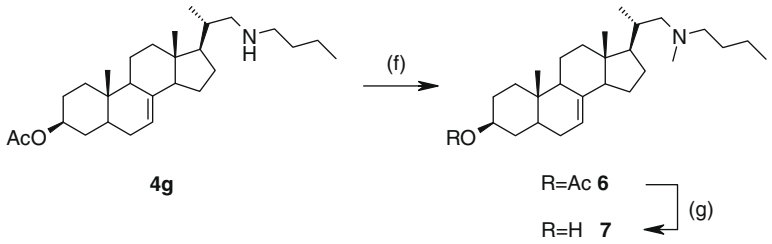


Scheme 1. Reagents and conditions: (a) acetic anhydride, pyridine, reflux, 2 h (83%); (b) H_2, Raney nickel, 4-dimethylaminobenzaldehyde, THF/ethyl acetate, rt, 6 h (83%); (c) O_3, dichloromethane, pyridine, −70 °C, 10 min, then methanol/dimethyl sulfide, −70 °C to 20 °C (32–40%).

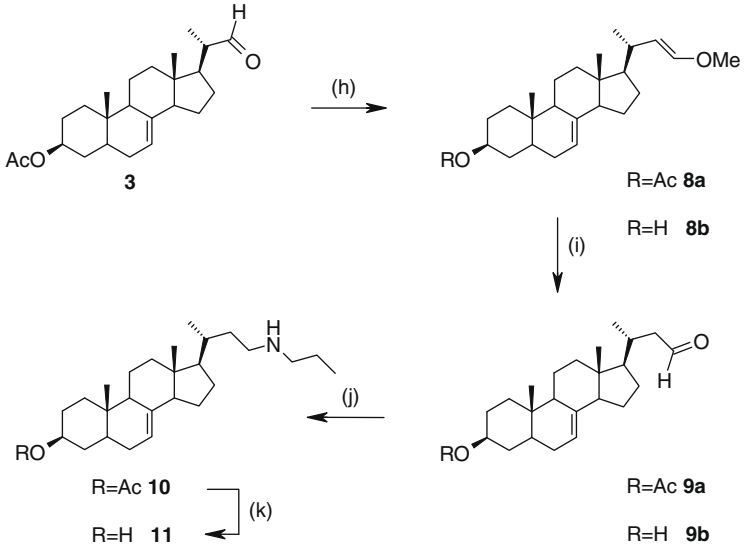


a. R= $CH_2CH_2N(CH_3)_2$	77%	55%
b. R= $CH_2CH_2CH_2N(CH_3)_2$	63%	85%
c. R= $CH_2CH_2N(CH_2CH_2CH_2CH_2)$	68%	94%
d. R= CH_3	60%	68%
e. R= CH_2CH_3	74%	85%
f. R= $CH_2CH_2CH_3$	82%	87%
g. R= $CH_2CH_2CH_2CH_3$	78%	85%
h. R= $CH_2CH_2CH_2CH_2CH_3$	74%	78%
i. R= iso-butyl	80%	79%
j. R= 3-methyl-2-butyl	84%	82%
k. R= dodecyl	62%	75%

Scheme 2. Reagents and conditions: (d) R–NH_2, $NaBH_3CN$, $ZnCl_2$, THF, rt, 16 h; (e) K_2CO_3, chloroform/methanol (1:1), rt, 16 h.



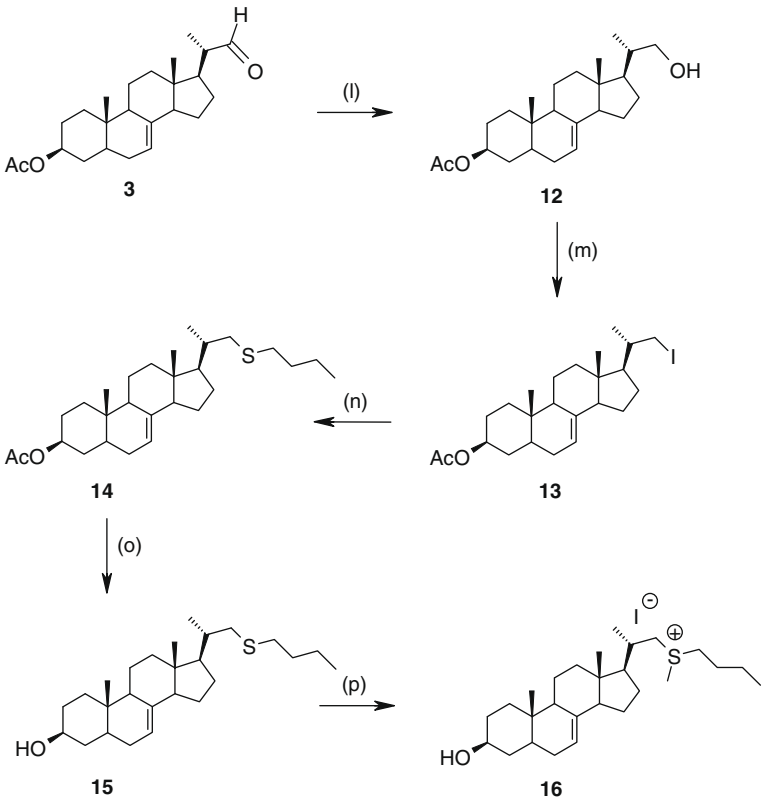
Scheme 3. Reagents and conditions: (f) acetic acid, formaldehyde, 80 °C, 2 h (94%); (g) K_2CO_3, chloroform/methanol, rt, 16 h, (91%).



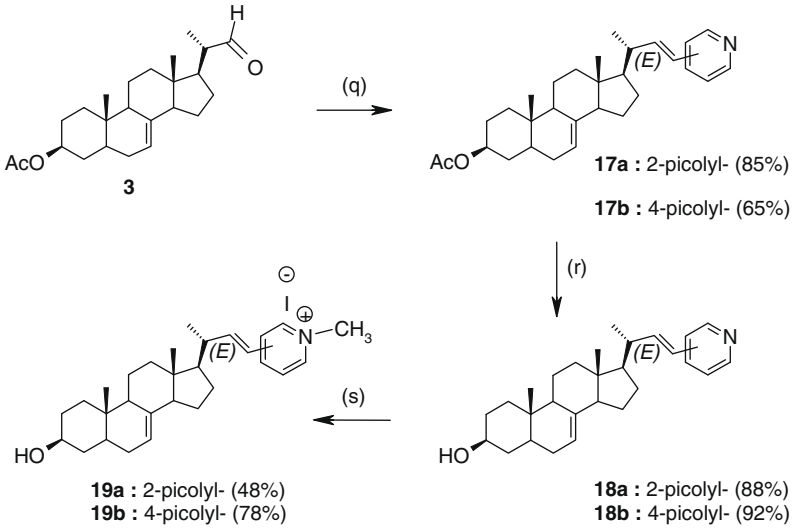
Scheme 4. Reagents and conditions: (h) $(C_6H_5)_3P{=}CHOMe$, THF, rt, 16 h (34% of **8a**); (i) **8a**, sulfuric acid, THF, 70 °C, 5 h (58% of **9a**); (j) **9a**, *n*-propylamine, $NaBH_3CN$, $ZnCl_2$, THF, rt, 16 h (72% of **10**); (k) K_2CO_3, chloroform/methanol, rt, 16 h (81%).

in chloroform/methanol afforded the thioether **15** in 71% overall yield. Finally, S-alkylation of **15** with iodomethane[24] gave the thionium iodide **16** in 31% yield (Scheme 5).

Pyridine and pyridinium substituents were likewise introduced into the side chain. At first, 2- and 4-picolyltriphenylphosphonium ylides were prepared in situ from the corresponding picolyltriphenylphosphonium chlorides[25] and *n*-butyllithium, and then reacted with the aldehyde **3** to afford the (*E*)-configurated vinylpyridines **17a** and **17b**. The acetate protecting groups were removed under standard conditions with potassium carbonate, and finally the pyridines were N-methylated using iodomethane to obtain the corresponding pyridinium salts **19a** and **19b** (Scheme 6).



Scheme 5. Reagents and conditions: (l) $NaBH_4$, chloroform/methanol, rt, 10 min (87%); (m) $(PhO)_3PMeI$, THF, in the dark, rt, 16 h (88%); (n) *n*-butyllithium, *n*-butanethiol, THF, rt, 16 h (87%); (o) K_2CO_3, chloroform/methanol, rt, 16 h (82%); (p) iodomethane, rt, 3 d (31%).



Scheme 6. Reagents and conditions: (q) *n*-butyllithium, 2- or 4-picolyltriphenylphosphonium chloride, THF, rt; (r) K_2CO_3, chloroform/methanol, rt, 16 h; (s) iodomethane, appropriate solvent, rt, 4–6 d.

3. Biological assays

All substances were subjected to a biological test assay defining their MIC (Minimal Inhibitory Concentration; this value is defined as the lowest concentration that leads to a visibly detectable complete inhibition of growth) according to DIN 58940-84,[26] and the composition of their unsaponifiable matter after work up was determined by GC–MS analysis of the sterol trimethylsilyl ethers. For all experiments we used the non-pathogenic yeast *Y. lipolytica* as a model organism.[27] The inhibited enzyme could be deduced from the relative composition of the sterol pattern obtained after cell lysis and micro extraction (Table 1). All sterols were identified by comparison with authentical reference material and/or on basis of their relative retention times and mass spectra. As reference

Table 1
Relative sterol composition (determined as their trimethylsilyl ethers) after treatment of *Yarrowia lipolytica* with the substances

Sterol	Zymosterol-3-TMS (%)	Ergosta-5,8,22,24(28)-tetraen-3-TMS (%)	Ergosterol-3-TMS (%)	Cholesta-5,7,24-trien-3-TMS (%)	Cholesta-7,24-dien-3-TMS (%)	Dehydroergosterol-3-TMS (%)	5-Dehydroepisterol-3-TMS (%)	14α-Methylergosta-8,24(28)-dien-3β,6α-di-TMS (%)	Lanosterol-3-TMS (%)	Sum (%)
Control[a]		0,5	17,6			81,3	0,6			100
Ket[b] (0.2 μg/mL)[c]			9,6			59,1		2,4	28,9	100
5a (1 μg/mL)				68,0	4,5	24,3	3,1			100
5b (2 μg/mL)			2,7	25,6	2,0	66,4	3,3			100
5b (8 μg/mL)				65,4	5,1	27,2	2,3			100
5c (1 μg/mL)			1,7	24,3	3,6	65,4	4,8			100
5c (2 μg/mL)	0,4		3,3	46,6	6,0	40,1	3,6			100
5d (2 μg/mL)	1,6			40,4		55,4	2,6			100
5e (0.2 μg/mL)			3,4	25,5	3,6	64,7	2,8			100
5f (0.1 μg/mL)	0,5			48,5		47,3	3,7			100
5g (0.06 μg/mL)				47,9	5,0	42,4	4,7			100
5h (0.06 μg/mL)	0,4		4,9	20,4	1,6	68,9	3,8			100
5i (0.1 μg/mL)	1,2			43,3	3,1	47,6	4,4		0,3	100
5j (0.2 μg/mL)	1,4			18,0	2,4	73,3	4,6		0,3	100
7 (0.2 μg/mL)	4,2			48,2	5,6	37,4	4,2		0,4	100
11 (0.06 μg/mL)			5,0	60,8	5,4	25,9	2,9			100
16 (2 μg/mL)				87,5	3,5	7,8	1,0		0,2	100
19a (0.06 μg/mL)			15,0	2,2		82,1	0,7			100
19a (0.2 μg/mL)			4,1	83,4	4,3	7,0	1,0		0,3	100
19b (0.06 μg/mL)			11,8	18,3		67,9	1,8		0,2	100

[a] Negative control.
[b] Ketoconazole.
[c] The values in brackets refer to the concentrations of the inhibitors used in the experiments.

substance for the determined MIC values and as positive control we used ketoconazole, an inhibitor of the enzyme sterol C14-demethylase.

4. Results and discussion

A series of azasterols and a thiasterol derivative were prepared and fully characterized. The in vitro antifungal activities of these derivatives were assessed against the yeast *Y. lipolytica*. The MIC values of these compounds are listed in Table 2 with ketoconazole as reference. The 23-azasterols **5f**–**5i** containing C_3–C_5 *N*-alkyl groups and the *N*-propylamino 24-azasterol **11** showed the highest activities with MIC values of 0.25 μg/mL. These values are better than the one of the reference ketoconazole (MIC 0.5 μg/mL).

Several informations may be drawn from the data of the in vitro screening for antifungal activities. The compounds with small to medium sized (C_3–C_5) alkylamines at positions 23 and 24 and a free 3β-hydroxy function are the most potent antifungal derivatives. Reducing the length of the *N*-alkyl chain at C-23 further (compounds **5d** and **5e**) results in lower activity. The presence of bulky groups in the side chain (compound **5j**) also reduces the activity. It also has to be noted that analogues with a very long *N*-substituent such as the dodecylamine **5k** show no activity at all. In addition, the introduction of a second amino group in the side chain (compounds **5a**–**5c**), or the replacement of the amino group with a sulfonium group (compound **16**) lead to almost complete loss of activity. Furthermore, the replacement of the aliphatic amine with a pyridinium ring in the side chain (compounds **19a**–**b**) or conversion of the secondary amino group at C-23 into a tertiary amine (compound **7**) resulted in minor activity. Finally, it is to be noted that the pyridine derivatives **18a**–**b** did not show any antimicrobial activity in an agar diffusion pretest.

Table 2
Minimal inhibitory concentrations (MIC values) against *Yarrowia lipolytica*

Compound	MIC value (μg/mL)
5a	>2
5b	>2
5c	>2
5d	>2
5e	2
5f	0.25
5g	0.25
5h	0.25
5i	0.25
5j	0.5
5k	[a]
7	0.5
11	0.25
16	>2
19a	1
19b	0.5
ketoconazole	0.5

[a] Compound was inactive in the agar diffusion pretest.

In order to provide a fast and reliable test methodology for the identification of the target enzyme[28–30] of our substances we decided to combine the procedures provided by the fully validated DIN 58940-84[26] protocol for MIC determination and the commonly used analysis of the unsaponifiable matter of a test organism (*Y. lipolytica*). This provides an opportunity to a simultaneous determination of the MIC value and identification of the inhibited enzyme (in the post-squalene-pathway) in a single test procedure. This investigation can be carried out in a 24 well format, thus reducing effort and material requirements. As the MIC value of ketoconazole was determined every testing day, an easy comparison between the inhibitory strengths of the test substances and the reference inhibitor ketoconazole can be drawn. This procedure allowed deducing the efficiency of the herein described substances without the need for the determination of K_i values in an isolated enzyme assay.

The quantitative analysis of the sterol composition of the yeast *Y. lipolytica* treated with the aforementioned compounds for 48 h (at which time complete growth arrest takes place at the respective MIC) is presented in Table 1. The relative sterol composition clearly reveals changes in the overall occurring sterols with or without inhibitor treatment. These differences allow deducing

the inhibited enzyme. The appearance of C_{27} sterols is associated with an inhibition or lack of the enzyme 24-SMT (ERG 6 gene),[30] what had particularly been shown by the identification of cholesta-5,7,24-trien-3β-ol and cholesta-7,24-dien-3β-ol in a ERG 6 mutant of *S. cerevisiae*.[31] It is well kown from literature that a strong accumulation of cholesta-5,7,24-trien-3β-ol (**22**) is indicative for an inhibition of 24-SMT (Fig. 3).[30] Inhibition of 24-SMT does not simply end up with an accumulation of zymosterol, the substrate of this enzyme, but methyl transfer to C-24 is omitted, and further biosynthetic steps take place, giving an unphysiological sterol, which cannot properly fulfill the biological functions of ergosterol. The predominant sterols found in the untreated control cultures were (in decreasing order): dehydroergosterol (the direct precursor of ergosterol in its biosynthesis), ergosterol, 5-dehydroepisterol and ergosta-5,8,22,24(28)-tetraen-3-ol.

Azasterols and the thiasterol **16** at about MIC concentrations significantly increased the relative content of the C_{27}-Δ24-sterols cholesta-5,7,24-trien-3β-ol (**22**) (Fig. 3), and (to a lesser extent) cholesta-7,24-trien-3β-ol, thus leading to the same effects as previously described for a ERG 6 mutant of the yeast *S. cerevisiae*. This clearly demonstrates that the compounds described here are inhibitors of the enzyme 24-SMT.[30,31] Furthermore the sterol pattern for ketoconazole treated samples shows a strong accumulation of lanosterol, as expected for a sterol C14-demethylase inhibitor.

Most of the side chain modified sterols synthesised herein can mimic the HEIs involved in the mechanism of action of 24-SMT[3], since they contain a functional group which is either already positively charged or becomes charged as a consequence of protonation under physiological conditions. All the compounds except the pyridinium salts **19a** and **19b** bear this functional group at positions resembling C-23 or C-24 of the sterol side chain, and contain a linear side chain similar in shape to those of the carbocationic intermediates. These compounds are structural and electronic mimics of the HEIs and were identified as specific inhibitors of 24-SMT. So the experimental results obtained are consistent with our hypothesis.

The pyridinium salts are only electronic mimics of the HEIs: the positive charges in the side chains are located at positions resembling C-25 and C-27. Nevertheless, these compounds have also been found to be 24-SMT inhibitors. Consequently this result demonstrates that the positive charge may be located at different positions in the side chain and also be located in a ring system.

This study shows that even 23-azasterols are able to mimic the carbocationic C-24 and C-25-HEIs of the 24-SMT reaction. In particular compounds **5f**–**5i**, but also the 24-azasterol **11** exhibit potent antifungal activity and were shown to be inhibitors of 24-SMT in a whole-cell assay, with activity being strongly influenced by the length of the alkylamino residue. Since the parasites that cause trypanosoma and leishmaniasis diseases also have ergosterol and related 24-alkylated sterols in their cells,[32,33] the compounds shown here will be evaluated for their antiprotozoal activities in the near future.

5. Experimental section

5.1. Analysis and materials

All solvents were of HPLC or p.a. grade, otherwise they were distilled before use over appropriate drying agents. All chemicals were purchased from Sigma–Aldrich (Schnelldorf, Germany), Fluka and Acros and were used without further purification; except: sodium hydroxide solution was from VWR (Darmstadt, Germany), MSTFA and TSIM were from Machery & Nagel (Düren, Germany), Bondesil PSA was from Varian (Darmstadt, Germany), 24 well plates were from Peske (Aindling-Arnhofen, Germany). The culture medium consisted of yeast extract 10 g/L, peptone 20 g/L and glucose 20 g/L. The yeast culture was splitted once a week to keep the culture in the log phase. The yeast culture was maintained at 28 °C. The 0.5 Mc Farland standard was prepared from 0.05 mL solution A (0.117 g $BaCl_2 \times 2\ H_2O$ in 10 mL of water) and 9.95 mL 10% (w/v) H_2SO_4. The *Y. lipolytica* test culture was purchased from DSMZ (Braunschweig, Germany).

All anhydrous reactions were carried out under an inert atmosphere using Schlenk techniques. Reactions were monitored by TLC using pre-coated plastic sheets POLYGRAM® SIL G/UV_{254} from Macherey-Nagel. Merck Silica Gel 60 (particle size 0.040–0.063 mm) was used for silica column chromatography (SCC). Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Structure assignment for all prepared compounds was done using ^{1}H, ^{13}C NMR, as well as DEPT, HMQC, and HMBC techniques. NMR spectra were recorded on JEOL Eclipse plus NMR workstations (Jeol GSX 400 or JNMR GX 500 instrument), respectively at 500.1599 MHz or 399.7820 MHz for ^{1}H and 125.7653 MHz or 100.5253 MHz for

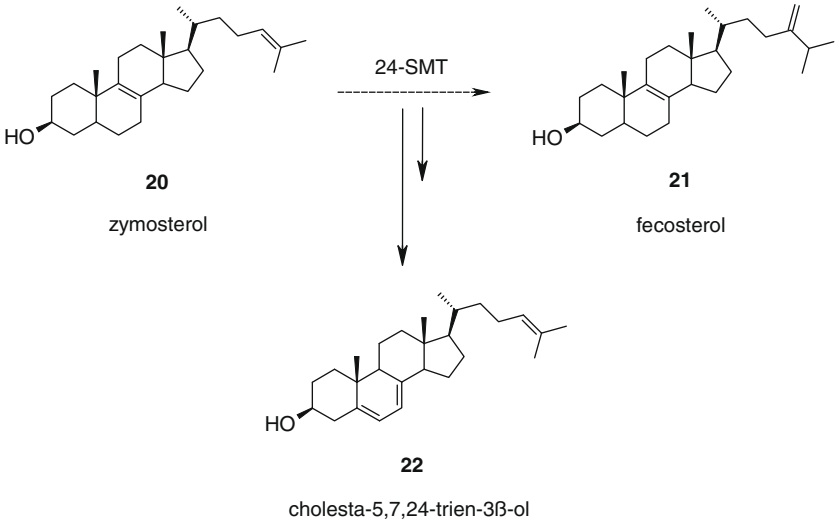


Figure 3. Accumulation of cholesta-5,7,24-trien-3β-ol (**22**) upon incubation of *Yarrowia lipolytica* with an inhibitor of the enzyme Δ24-sterol methyltransferase (24-SMT).

^{13}C. Spectra were calibrated by using residual undeuterated solvent as an internal reference ($CHCl_3$ with ^1H at 7.26 ppm, ^{13}C at 77.00 ppm). Mass spectra were recorded on Hewlett–Packard 5989A, using electron impact ionization (EI) at 70 eV and chemical ionization with methane (CI). Electron impact high resolution mass spectra (EI-HRMS) were recorded on GC Mate II Jeol. Fast atom bombardement (FAB)-HRMS spectra were recorded on Jeol Mstation JMS-700. The measurement conditions are Xenon for gas, at 6 kV, matrix 3-nitrobenzyl alcohol. Electrospray ionization (ESI) spectra were recorded on PE-Biosystems API 2000. ESI-HRMS spectra were recorded on Thermo Finnigan LTQ FT (Thermo Finnigan, Bremen, Germany). The measurement conditions are nitrogen for gas, at 3 kV, with a flow rate of 200 μL/min. The substances were directly injected. Elemental analyses were performed on a CHN-Analyser Rapid (Heraeus), results are given in percentages. IR spectra were obtained on Perkin–Elmer Paragon 1000 spectrometer. The UV spectrophotometer Hitachi U 1100 was from Hitachi (Krefeld, Germany). Melting points were determined on a Büchi 540 apparatus.

For biological screening all substances were dissolved in absolute ethanol to give standard solutions with a concentration of 0.8 mg/mL. These solutions were stepwise diluted 1:1 with ethanol to give the final test concentrations of 2, 1, 0.5, 0.25, 0.125, 0.06125 μg/mL if not otherwise stated. The dilutions were added to the 24 well plate. Each well plate contained a control row consisting of two wells to which only 2.0 mL of medium had been added (sterility control), two wells to which only 10 μL of ethanol had been added (negative control) and two wells containing a concentration of 0.5 μg/mL clotrimazole (positive control). Each testing day additionally one row on a single plate was treated with ketoconazole (4, 2, 1, 0.5, 0.25, 0.125 μg/mL) as 'MIC control'.

5.2. Determination of the MIC values

Two hundred microliters of the test culture were diluted 1:10 with distilled water under sterile conditions. The UV absorption was measured at a wavelength of 570 nm against the absorption of the 0.5 Mc Farland standard. The number of CFU (colony forming units) per mL of the test culture solution was determined taking into account that the Mc Farland standard refers to about 5×10^6 CFU/mL. The test culture was diluted 1:100 with culture medium and the volume referring to 100,000 CFU was transferred into each well of a 24 well plate resulting in a final CFU concentration of 50,000 CFU/mL. Each well was filled up with medium to 1990 μL and finally 10 μL of the substance solution were added. The plates were gently shaken for 30 s and incubated for 48 ± 2 h at 28 °C. The MIC values were determined visually by making use of the lowest substance concentration at which no visible growth could be detected.[26]

5.3. Analysis of the unsaponifiable matter and identification of the inhibited enzyme

5.3.1. Sterol extraction

The content of the wells representing MIC/2 and/or MIC/4 were transferred into a 2 mL plastic vial and were centrifuged for 5 min at 15,000*g*. The residual yeast pellet was taken up in 1 mL of PBS and centrifuged again under the same conditions. To the pellet were added 1.0 mL of 1 M NaOH, the vial was vortexed for 30 s, and the whole content was transferred into a 4 mL glass vial with Teflon septum. The vial was flooded with nitrogen and closed tightly. The suspension was ultrasonicated for 5 min and heated to 70 °C for 1 h. After cooling to room temperature 650 μL of *tert*-butylmethylether (TBME) and 100 μL of an internal standard solution (cholesterol in TBME; 10 μg/mL) were added and the vial was shaken for 30 s. The whole content of the glass vial was transferred back into the plastic vial and the plastic vial was shaken for an additional 30 s. The upper organic layer was transferred into a 2 mL plastic vial containing 40 mg of a mixture of dry sodium sulfate/BondElut PSA 7:1. The extraction was repeated with another 750 μL of TBME. The combined organic extracts were shaken vigorously for 30 s and centrifuged for 3 min at 10,000*g*. One milliliter of the purified organic extract was transferred into a brown glass vial and the extract was brought to dryness under a gentle stream of nitrogen at 50 °C. The residue was taken up in 950 μL of TBME and 50 μL of silylation reagent (MSTFA/TSIM 9:1) were added. The samples were stored for at least 30 min at room temperature before 1 μL was injected into the GC–MS system.

5.3.2. GC–MS analysis

We used a Varian GC 3800 equipped with a CTC Combi Pal autosampler coupled to a Varian Saturn 2000 ion trap. The column used was a Varian Factor Four EZ Guard VF 5 MS 30 m × 0.25 mm × 0.25 μm (+10 m methyl-deactivated guard column). The 1177 injector was held at 250 °C and operated in splitless mode for 1 min. The transfer line was held at 270 °C. The oven program started at 50 °C held for 1 min, and ramped to 260 °C with 50 °C per min, followed by a gradient of 4 °C per min to 310 °C held for 0.5 min. The MS was operated in full scan mode from 9 min to 12 min at a mass range from 50 to 450 *m*/*z* and from 12 min to 18 min at a mass range from 100 to 650 *m*/*z*. For substance identification three qualifier ions were chosen (Table 3). The relative sterol composition for each sterol is expressed in percentage of the overall found area after integration with the given quantifier ions.

5.4. Syntheses

5.4.1. General procedure A (reductive amination)

To a stirred solution of 0.5–1.0 mmol (3*S*,20*S*)-20-formylpregn-7-en-3-yl acetate (**3**) and the required primary amine (4.0 equiv) in anhydrous THF, a solution of sodium cyanoborohydride (1.0 equiv) and zinc chloride (0.5 equiv) in THF (2 mL/mmol) was added at ambient temperature under nitrogen atmosphere. The reaction mixture was stirred at ambient temperature for 16 h and then quenched with aqueous NaOH [2 N]. The biphasic mixture was extracted with dichloromethane (3 × 10 mL) and the combined organic extracts were washed with water (10 mL), brine (10 mL), dried over sodium sulfate, and evaporated to dryness under reduced pressure. The residue was subjected to silica column chromatography (SCC) as specified below to afford pure compounds **4a**–**4k** as white solids.

5.4.1.1. (3*S*,20*S*)-20-[(2-Dimethylamino-ethylamino)-methyl]-pregn-7-en-3-yl acetate (4a). Aldehyde **3** (200 mg, 0.54 mmol) and 2-dimethylaminoethylamine (0.30 mL, 2.20 mmol) in anhydrous THF (3 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 7:3, followed by pentane/diethyl ether/EDMA (*N*-ethyl-*N*,*N*-dimethylamine) 6:4:0.5) (184 mg, 77%). Mp 91 °C. ^1H NMR ($CDCl_3$, 500 MHz): δ 5.13 (m, 1H, H-7), 4.68 (m, 1H, H-3), 2.63 (m, 3H, H-1″, H-1′a), 2.38 (m, 2H, H-2″), 2.28 (dd, 1H, H-1′b, J = 11.8 Hz, J = 8.8 Hz), 2.21 (s, 6H, $N(CH_3)_2$), 2.02 (s, 3H, CH_3CO, with underneath m, 1H, H-12a), 1.82–1.20 (m, 20H), 1.10 (ddd, 1H, H-1b, J = J = 13.5 Hz, J = 3.3 Hz), 0.99 (d, 3H, H-21, J = 6.7 Hz), 0.80 (s, 3H, H-19), 0.53 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6 (CO), 139.4 (8-CH), 117.4 (7-CH), 73.4 (3-CH), 59.3 (2″-CH_2), 55.7 (1′-CH_2), 54.8 (CH), 54.2 (CH), 49.2 (CH), 47.8 (1″-CH_2), 45.6 ($N(CH_3)_2$), 43.5 (13-C), 40.0 (CH), 39.4 (CH_2), 36.9 (CH), 36.8 (CH_2), 34.2 (10-C), 33.8 (CH_2), 29.5 (CH_2), 27.7 (CH_2), 27.5 (CH_2), 23.0 (CH_2), 21.4 (CH_3CO, CH_2), 17.9 (21-CH_3), 12.9 (19-CH_3), 11.9 (18-CH_3). HRMS Found 444.3716 (Calcd 444.3716). Anal. Calcd: C, 75.63; H, 10.88; N, 6.30. Found: C, 75.52; H, 10.77; N, 6.23. IR

Table 3
Identification of the sterols accumulating upon incubation of *Yarrowia lipolytica* with the inhibitors by GC–MS

Sterol	RRT[a]	Qual/quant Ions (*m*/*z*)	Reference
Cholesterol-3-TMS[b] (I.S.)[c]	1.00	458[1], 368[2], 329[3]	RS[d]
Zymosterol-3-TMS	1.05	456[1], 441[4], 351[5]	RS—isolated from *Yarrowia lipolytica*, [34] mass spectral data[35]
Ergosta-5,8,22,24(28)-tetraen-3-TMS	1.06	466[1], 361[5], 251[*]	Interpretation of the mass spectra, free 3β-hydroxy sterol[34]
Cholesta-5,7,24-trien-3-TMS	1.07	454[1], 349[5], 323[6]	[35,28]
Ergosterol-3-TMS	1.07	378[2], 363[5], 337[6]	RS
Cholesta-7,24-dien-3-TMS	1.09	441[4], 343[7], 255[8]	[35,36]
Dehydroergosterol-3-TMS	1.09	376[2], 361[5], 251[*]	NIST[e], free 3β-hydroxy sterol[29]
5-Dehydroepisterol	1.12	363[5], 337[6], 294[8]	[37]
14α-Methylergosta-8,24(28)-dien-3β,6α-di-TMS	1.15	557[4], 468[*], 467[5]	[38]
Lanosterol	1.16	Qualifier: 393[5], 351[*], 241[*] Quantifier: 394[*], 393[5], 241[*]	RS

[a] Relative retention time referring to cholesterol (RRT = 1.00).
[b] TMS = trimethylsilyl derivative.
[c] Internal standard.
[d] Authentic reference substance.
[e] NIST mass spectral library 2005, [1][M]$^+$, [2][M−TMSOH]$^+$ (TMSOH = trimethylsilanol), [3][M−($C_6H_{13}OSi$)]$^+$, [4][M−CH_3]$^+$, [5][M−(TMSOH+CH_3)]$^+$, [6][M−($C_6H_{15}OSi$)]$^+$, [7][M−(SC+2H)]$^+$ (SC = side chain), [8][M−(SC+TMSOH)]$^+$, [*]not defined.

(KBr, cm^{-1}): 2947, 2847, 2814, 2764, 1733, 1468, 1367, 1248, 1032, 896.

5.4.1.2. (3*S*,20*S*)-20-[(3-Dimethylamino-propylamino)-methyl]-pregn-7-en-3-yl acetate (4b). Aldehyde **3** (210 mg, 0.56 mmol) and 3-dimethylaminopropylamine (300 μL, 2.26 mmol) in anhydrous THF (6 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 5:5 followed by pentane/diethyl ether/EDMA 2:8:0.5) (161 mg, 63%). Mp 77 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.15 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.63 (m, 3H), 2.30 (m, 3H), 2.22 (s, 6H, N(CH_3)$_2$), 2.03 (s, 3H, CH_3CO), 2.00 (m, 1H), 1.85–1.21 (m, 22H), 1.13 (ddd, 1H, H-1b, J = J = 13.0 Hz, J = 4.0 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7 (CO), 139.3 (8-CH), 117.4 (7-CH), 73.4 (3-CH), 58.1 (3″-CH_2), 55.6 (1′-CH_2), 54.8 (CH), 54.2 (CH), 49.2 (CH), 48.8 (1″-CH_2), 45.6 (N(CH_3)$_2$), 43.5 (13-C), 40.0 (CH), 39.4 (CH_2), 36.81 (CH), 36.79 (CH_2), 34.2 (10-C), 33.8 (CH_2), 29.5 (CH_2), 28.2 (2″-CH_2), 27.7 (CH_2), 27.5 (CH_2), 22.9 (CH_2), 21.4 (CH_2 and *C*H_3CO), 17.8 (21-CH_3), 12.9 (19-CH_3), 11.9 (18-CH_3). HRMS Found 458.3873 (Calcd 458.3872). IR (KBr, cm^{-1}): 3440, 2946, 1734, 1467, 1367, 1249, 1032.

5.4.1.3. (3*S*,20*S*)-20-[(2-Pyrrolidin-1-yl-ethylamino)-methyl]-pregn-7-en-3-yl acetate (4c). Aldehyde **3** (200 mg, 0.54 mmol) and *N*-(2-aminoethyl)-pyrrolidine (200 μL, 2.15 mmol) in anhydrous THF (4 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 7:3 followed by pentane/diethyl ether/EDMA 7:3:0.5) (173 mg, 68%). Mp 76 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.14 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.80 (m, 2H), 2.71 (m, 1H), 2.65 (m, 2H), 2.57 (m, 4H), 2.33 (dd, 1H, H-1′b, J = 11.7 Hz, J = 8.8 Hz), 2.02 (s, 3H, CH_3CO), 1.99 (m, 1H), 1.84–1.19 (m, 24H), 1.12 (ddd, 1H, H-1b, J = J = 13.0 Hz, J = 3.4 Hz), 1.00 (d, 3H, H-21, J = 6.6 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6 (CO), 139.3 (8-CH), 117.4 (7-CH), 73.4 (3-CH), 56.1 (2″-CH_2), 55.7 (1′-CH_2), 54.8 (CH), 54.2 (CH, 2xCH_2), 49.1 (CH), 49.0 (1″-CH_2), 43.4 (13-C), 39.9 (CH), 39.3 (CH_2), 36.8 (CH, CH_2), 34.1 (10-C), 33.8 (CH_2), 29.5 (CH_2), 27.6 (CH_2), 27.4 (CH_2), 23.4 (2 CH_2), 22.9 (CH_2), 21.4 (CH_2, *C*H_3CO), 18.8 (21-CH_3), 12.1 (19-CH_3), 11.9 (18-CH_3). HRMS Found 470.3872 (Calcd 470.3872). IR (KBr, cm^{-1}): 2943, 1731, 1444, 1238, 1029, 972, 896.

5.4.1.4. (3*S*,20*S*)-20-(Methylamino-methyl)-pregn-7-en-3-yl acetate (4d). Aldehyde **3** (200 mg, 0.54 mmol) and methylamine (2 M solution in methanol, 1.10 mL, 2.20 mmol) in anhydrous THF (3 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 7:3 followed by pentane/diethyl ether/EDMA 5:5:0.5) (110 mg, 60%). Mp 140 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.14 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.56 (dd, 1H, H-1′a, J = 11.7 Hz, J = 3.2 Hz), 2.41 (s, 3H, N-CH_3), 2.31 (dd, 1H, H-1′b, J = 11.7 Hz, J = 8.0 Hz), 2.02 (s, 3H, CH_3CO), 2.00 (m, 1H), 1.91–1.20 (m, 20H), 1.12 (ddd, 1H, H-1b, J = J = 13.1 Hz, J = 3.2 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.7 (CO), 139.4 (8-CH), 117.4 (7-CH), 73.4 (3-CH), 58.0 (1′-CH_2), 54.8 (CH), 54.0 (CH), 49.3(CH), 43.4 (13-C), 40.0 (CH), 39.4 (CH_2), 37.0 (N-CH_3), 36.8 (CH_2), 36.7 (CH), 34.2 (10-C), 33.8 (CH_2), 29.5 (CH_2), 27.7 (CH_2), 27.4 (CH_2), 23.0 (CH_2), 21.4 (CH_2 and *C*H_3CO), 18.0 (21-CH_3), 12.9 (19-CH_3), 11.9 (18-CH_3). HRMS Found 387.3130 (Calcd 387.3137). Anal. Calcd: C, 77.47; H, 10.66; N, 3.61. Found: C, 76.72; H, 10.57; N, 3.57. IR (KBr, cm^{-1}): 3347, 3268, 2948, 2872, 2847, 1734, 1473, 1443, 1378, 1366, 1237, 1032, 1032, 896, 726.

5.4.1.5. (3*S*,20*S*)-20-(Ethylamino-methyl)-pregn-7-en-3-yl acetate (4e). Aldehyde **3** (300 mg, 0.80 mmol), ethylamine hydrochloride (261 mg, 3.20 mmol) and triethylamine (0.5 mL, 3.2 mmol) in anhydrous THF (5 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 7:3 followed by pentane/diethyl ether/EDMA 9:1:0.4) (237 mg, 74%). Mp 140 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.63 (m, 3H), 2.31 (dd, 1H, H-1′b, J = 11.6 Hz, J = 8.6 Hz), 2.02 (s, 3H, CH_3CO, with underneath m, 1H, H-12a), 1.90–1.24 (m, 20H), 1.13 (m, 1H), 1.10 (t, 3H, H-2″, J = 7.3 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.81 (s, 3H, H-19), 0.55 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6 (CO), 139.3 (8-CH), 117.4 (7-CH), 73.4 (3-CH), 55.4 (1′-CH_2), 54.8 (CH), 54.2 (CH), 49.2 (CH), 44.4 (1″-CH_2), 43.5 (13-C), 40.0 (CH), 39.4 (CH_2), 36.9 (CH), 36.8 (CH_2), 34.2 (10-C), 33.8 (CH_2), 29.5 (CH_2), 27.7 (CH_2), 27.5 (CH_2), 23.0 (CH_2), 21.4 (*C*H_3CO, CH_2), 17.9 (21-CH_3), 15.4 (2″-CH_3), 12.9 (19-CH_3), 11.9 (18-CH_3). HRMS Found 401.3295 (Calcd 401.3294). Anal. Calcd: C, 77.75; H, 10.79; N, 3.49. Found: C, 77.55; H, 10.82; N, 3.42. IR (KBr, cm^{-1}): 2948,

2899, 2868, 2845, 1729, 1469, 1455, 1443, 1374, 1364, 1237, 1029, 971, 895, 743, 723.

5.4.1.6. (3*S*,20*S*)-20-(Propylamino-methyl)-pregn-7-en-3-yl acetate (4f). Aldehyde **3** (330 mg, 0.880 mmol) and *n*-propylamine (290 μL, 3.55 mmol) in anhydrous THF (5 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 7:3 followed by pentane/diethyl ether/EDMA 9:1:0.2) (299 mg, 82%). Mp 134 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.62 (dd, 1H, H-1′a, J = 11.5 Hz, J = 3.1 Hz), 2.52 (m, 2H, H-1″), 2.28 (dd, 1H, H-1′b, J = 11.5 Hz, J = 8.9 Hz), 2.02 (s, 3H, CH_3CO, with underneath m, 1H, H-12a), 1.90–1.24 (m, 22H), 1.13 (ddd, 1H, H-1b, J = J = 13.0 Hz, J = 3.7 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.91 (t, 3H, H-3″, J = 7.3 Hz), 0.81 (s, 3H, H-19), 0.55 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7 (CO), 139.4 (8-CH), 117.4 (7-CH), 73.4 (3-CH), 55.4 (1′-CH_2), 54.8 (CH), 54.2 (CH), 52.2 (1″-CH_2), 49.1 (CH), 45.5 (13-C), 40.0 (CH), 39.4 (CH_2), 36.9 (CH), 36.8 (CH_2), 34.1 (10-C), 33.7 (CH_2), 29.5 (CH_2), 27.7 (CH_2), 27.4 (CH_2), 23.2 (2″-CH_2), 23.1 (CH_2), 21.5 (*C*H_3CO), 21.4 (CH_2), 17.9 (21-CH_3), 12.9 (19-CH_3), 11.9 (18-CH_3), 11.8 (3″-CH_3). HRMS Found 415.3446 (Calcd 415.3450). Anal. Calcd: C, 78.02; H, 10.91; N, 3.37. Found: C, 77.93; H, 10.77; N, 3.35. IR (KBr, cm^{-1}): 2943, 2879, 2821, 1729, 1480, 1468, 1455, 1374, 1366, 1251, 1034, 784.

5.4.1.7. (3*S*,20*S*)-20-(Butylamino-methyl)-pregn-7-en-3-yl acetate (4g). Aldehyde **3** (200 mg, 0.54 mmol) and *n*-butylamine (200 μL, 2.15 mmol) in anhydrous THF (3 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 7:3 followed by pentane/diethyl ether/EDMA 9:1:0.1) (180 mg, 78%). Mp 121 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.14 (m, 1H, H-7), 4.68 (m, 1H, H-3), 2.58 (m, 3H), 2.29 (dd, 1H, H-1′b, J = 11.5 Hz, J = 8.8 Hz), 2.02 (s, 3H, CH_3CO, with underneath m, 1H, H-12a), 1.89–1.20 (m, 24H), 1.12 (ddd, 1H, H-1b, J = J = 13.2 Hz, J = 3.3 Hz), 0.99 (d, 3H, H-21, J = 6.3 Hz), 0.91 (t, 3H, H-4″, J = 7.3 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.7 (CO), 139.4 (8-CH), 117.5 (7-CH), 73.5 (3-CH), 55.6 (1′-CH_2), 54.9 (CH), 54.3 (CH), 50.1 (1″-CH_2), 49.3 (CH), 43.6 (13-C), 40.1 (CH), 39.5 (CH_2), 36.91 (CH_2), 36.89 (CH), 34.3 (10-C), 33.9 (CH_2), 32.2 (2″-CH_2), 29.6 (CH_2), 27.8 (CH_2), 27.6 (CH_2), 23.1 (CH_2), 21.5 (CH_2, *C*H_3CO), 20.6 (3″-CH_2), 18.0 (21-CH_3), 14.1 (4″-CH_3), 13.0 (19-CH_3), 12.0 (18-CH_3). HRMS Found 429.3609 (Calcd 429.3607). Anal. Calcd: C, 78.27; H, 11.03; N, 3.26. Found: C, 78.19; H, 10.96; N, 3.22. IR (KBr, cm^{-1}): 2951, 2876, 2855, 1729, 1480, 1455, 1380, 1365, 1249, 1031, 898, 779.

5.4.1.8. (3*S*,20*S*)-20-(Pentylamino-methyl)-pregn-7-en-3-yl acetate (4h). Aldehyde **3** (300 mg, 0.800 mmol) and *n*-pentylamine (0.36 mL, 3.1 mmol) in anhydrous THF (5 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 7:3 followed by pentane/diethyl ether/EDMA 9:1:0.1) (263 mg, 74%). Mp 118 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.14 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.62 (dd, 1H, H-1′a, J = 11.9 Hz, J = 2.7 Hz), 2.55 (m, 2H), 2.28 (dd, 1H, H-1′b, J = 11.9 Hz, J = 8.7 Hz), 2.02 (s, 3H, CH_3CO, with underneath m, 1H, H-12a), 1.90–1.21 (m, 26H), 1.12 (ddd, 1H, H-1b, J = J = 13.5 Hz, J = 4.0 Hz), 1.00 (d, 3H, H-21, J = 6.6 Hz), 0.89 (t, 3H, H-5″, J = 7.1 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6 (CO), 139.4 (8-CH), 117.4 (7-CH), 73.4 (3-CH), 55.6 (1′-CH_2), 54.8 (CH), 54.3 (CH), 50.4 (1″-CH_2), 49.2 (CH), 43.5 (13-C), 40.0 (CH), 39.4 (CH_2), 37.0 (CH), 36.8 (CH_2), 34.2 (10-C), 33.8 (CH_2), 29.9 (CH_2), 29.6 (C-CH_2), 29.5 (CH_2), 27.7 (CH_2), 27.5 (CH_2), 23.0 (CH_2), 22.6 (CH_2), 21.4 (CH_2, *C*H_3CO), 17.9 (21-CH_3), 14.0 (5″-CH_3), 12.9 (19-CH_3), 11.9 (18-CH_3). HRMS Found 443.3784 (Calcd 443.3763). Anal. Calcd: C, 78.50; H, 11.13; N, 3.16. Found: C, 78.23; H, 11.25; N, 3.08. IR (KBr, cm^{-1}): 2955, 2936, 2869, 2800, 1730, 1471, 1382, 1365, 1250, 1029, 897, 800.

5.4.1.9. (3*S*,20*S*)-20-[(2-Methylpropyl)amino-methyl]-pregn-7-en-3-yl acetate (4i). Aldehyde **3** (300 mg, 0.81 mmol) and 2-methyl-propylamine (300 μL, 3.23 mmol) in anhydrous THF (5 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 1:1 followed by pentane/diethyl ether/EDMA 9:1:0.5) (277 mg, 80%). Mp 112 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.14 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.62 (dd, 1H, H-1′a, J = 11.6 Hz, J = 3.1 Hz), 2.42 (dd, 1H, H-1″a, J = 11.6 Hz, J = 7.0 Hz), 2.33 (dd, 1H, H-1″b, J = 11.6 Hz, J = 6.8 Hz), 2.27 (dd, 1H, H-1′b, J = 11.6 Hz, J = 8.8 Hz), 2.03 (s, 3H, CH_3CO, with underneath m, 1H, H-12a), 1.89–1.20 (m, 21H), 1.12 (ddd, 1H, H-1b, J = J = 13.2 Hz, J = 3.3 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.89 (d, 6H, 2 CH_3, J = 6.4 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7 (CO), 139.3 (8-CH), 117.4 (7-CH), 73.4 (3-CH), 58.3 (1″-CH_2), 55.5 (1′-CH_2), 54.8 (CH), 54.2 (CH), 49.2 (CH), 43.6 (13-C), 40.0 (CH), 39.4 (CH_2), 36.81 (CH), 36.76 (CH_2), 34.1 (10-C), 33.7 (CH_2), 29.5 (CH_2), 28.1 (2″-CH), 27.7 (CH_2), 27.4 (CH_2), 23.0 (CH_2), 21.5 (*C*H_3CO), 21.4 (CH_2), 20.68 (CH_3), 20.63 (CH_3), 17.8 (21-CH_3), 12.9 (19-CH_3), 11.9 (18-CH_3). HRMS Found 429.3608 (Calcd 429.3607). Anal. Calcd: C, 78.27; H, 11.03; N, 3.26. Found: C, 78.17; H, 10.82; N, 3.22. IR (KBr, cm^{-1}): 3435, 2953, 2873, 1733, 1469, 1364, 1252, 1032, 766.

5.4.1.10. (3*S*,20*S*)-20-[(1*R*,*S*)-(1,2-Dimethyl-propylamino)-methyl]-pregn-7-en-3-yl acetate (4j) (mixture of diastereomers). Aldehyde **3** (300 mg, 0.81 mmol) and 3-methyl-2-butylamine (400 μL, 3.24 mmol) in anhydrous THF (5 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 1:1 followed by pentane/diethyl ether/EDMA 9:1:0.5) (300 mg, 84%). Mp 147 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.14 (m, 2H, H-7), 4.69 (m, 2H, H-3), 2.68 (dd, 1H, H-1′a, J = 11.2 Hz, J = 2.7 Hz), 2.62 (dd, 1H, H-1′a, J = 11.6 Hz, J = 2.9 Hz), 2.38 (m, 2H, H-1″), 2.28 (dd, 1H, H-1′b, J = 11.6 Hz, J = 8.5 Hz), 2.17 (dd, 1H, H-1′b, J = 11.2 Hz, J = 8.7 Hz), 2.02 (s, 8H, H-12a and CH_3CO), 1.85–1.21 (m, 42H), 1.12 (ddd, 2H, H-1b, J = J = 13.2 Hz, J = 3.2 Hz), 1.00 (d, 3H, H-21, J = 6.4 Hz), 0.99 (d, 3H, H-21, J = 6.4 Hz), 0.95 (d, 3H, H-1‴, J = 6.6 Hz), 0.94 (d, 3H, H-1‴, J = 6.6 Hz), 0.89 and 0.88 (2 d, 6H, CH_3, J = 6.8 Hz), 0.86 and 0.84 (2 d, 6H, CH_3, J = 6.8 Hz), 0.81 (s, 6H, H-19), 0.55 (s, 6H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6 (CO), 139.3 (8-CH), 117.4 (7-CH), 73.4 (3-CH), 58.9 and 58.1 (C-1″-CH), 54.83 and 54.81 (CH), 54.33 and 54.31 (CH), 53.2 and 52.7 (1′-CH_2), 49.2 (CH), 43.49 and 43.47 (13-C), 40.0 (CH), 39.4 (CH_2), 37.4 (CH), 36.8 (CH_2), 34.2 (10-C), 33.8 (CH_2), 32.6 and 31.7 (2″-CH), 29.5 (CH_2), 27.73 and 27.66 (CH_2), 27.5 (CH_2), 23.0 (CH_2), 21.5 (*C*H_3CO, CH_2), 19.51 and 19.48 (CH_3), 18.04 and 17.92 (21-CH_3), 17.5 and 17.0 (CH_3), 16.3 and 16.0 (1‴-CH_3), 12.9 (19-CH_3), 11.9 (18-CH_3). HRMS Found 400.3170 (Calcd 400.3216 for $C_{26}H_{42}NO_2$). Anal. Calcd: C, 78.30; H, 11.13; N, 3.16. Found: C, 78.28; H, 11.32; N, 3.00. IR (KBr, cm^{-1}): 3443, 2952, 2902, 2869, 1734, 1447, 1368, 1247, 1032, 896, 719.

5.4.1.11. (3*S*,20*S*)-20-(Dodecylamino-methyl)-pregn-7-en-3-yl acetate (4k). Aldehyde **3** (300 mg, 0.81 mmol) and *n*-dodecylamine (300 mg, 1.62 mmol) in anhydrous THF (3 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether 7:3 followed by pentane/diethyl ether/EDMA 9:1:0.1) (180 mg, 62%). Mp 165 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.14 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.64–2.49 (m, 3H, H-1″, H-1′a), 2.29 (dd, 1H, H-1′b, J = 11.5 Hz, J = 8.5 Hz), 2.02 (s, 3H, CH_3CO), 2.01 (m, 1H, H-12a), 1.89–1.20 (m, 40H), 1.13 (ddd, 1H, H-1b, J = J = 13.2 Hz, J = 3.6 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.88 (t, 3H, H-12″, J = 6.9 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.7 (CO), 139.4 (8-

CH), 117.5 (7-CH), 73.4 (3-CH), 55.6 (1′-CH_2), 54.8 (14-CH), 54.3 (17-CH), 50.4 (1″-CH_2), 49.2 (9-CH), 43.5 (13-C), 40.0 (5-CH), 39.4 (12-CH_2), 36.9 (20-CH), 36.8 (1-CH_2), 34.2 (10-C), 33.8 (4-CH_2), 31.9, 30.2, 29.65, 29.62, 29.60, 29.58 (CH_2 side chain), 29.5 (6-CH_2), 29.3 (CH_2 side chain), 27.7 (16-CH_2), 27.5 (2-CH_2), 27.4 (CH_2 side chain), 23.0 (15-CH_2), 22.7 (CH_2 side chain), 21.44 (11-CH_2), 21.42 (*C*H_3CO), 17.6 (21-CH_3), 14.1 (12″-CH_3), 12.9 (19-CH_3), 11.9 (18-CH_3). HRMS Found 541.4856 (Calcd 541.4859) Anal. Calcd: C, 79.79; H, 11.72; N, 2.58. Found: C, 79,61; H, 11.79; N, 2.58. IR (KBr, cm^{-1}): 2919, 2870, 2850, 1729, 1472, 1447, 1383, 1366, 1263, 1038, 898, 722.

5.4.2. General procedure B (deprotection of the sterol acetates)

To a stirred solution of the 3-acetoxy compound **4a**–**4k** in chloroform/methanol (1:1; 10 mL/mmol ester) was added potassium carbonate (3 equiv) at ambient temperature. After stirring for 16 h the mixture was evaporated to dryness under reduced pressure, and the resulting residue was subjected to SCC as specified below to afford pure compounds **5a**–**5k** as white solids.

5.4.2.1. (3*S*,20*S*)-20-[(2-Dimethylamino-ethylamino)-methyl]-pregn-7-en-3-ol (5a). Ester **4a** (100 mg, 0.230 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 4:6:1) (50 mg, 55%). Mp 120 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.16 (m, 1H, H-7), 3.59 (m, 1H, H-3), 2.68 (m, 3H), 2.45 (m, 2H), 2.34 (dd, 1H, H-1′b, J = 11.7 Hz, J = 8.7 Hz), 2.24 (s, 6H, N(CH_3)$_2$), 2.02 (d, 2H, H-12, J = 12.4 Hz), 1.90–1.20 (m, 20H), 1.09 (ddd, 1H, H-1b, J = J = 13.1 Hz, J = 3.4 Hz), 1.02 (d, 3H, H-21, J = 6.7 Hz), 0.79 (s, 3H, H-19), 0.55 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 139.3 (8-CH), 117.6 (7-CH), 71.0 (3-CH), 58.6 (2″-CH_2), 55.4 (1′-CH_2), 54.8 (CH), 54.2 (CH), 49.4 (CH), 47.4 (1″-CH_2), 45.5 (N(CH_3)$_2$), 43.5 (13-C), 40.2 (CH), 39.5 (CH_2), 38.0 (CH_2), 37.1 (CH_2), 36.6 (CH), 34.2 (CH_2), 31.5 (CH_2), 29.6 (CH_2), 27.7 (CH_2), 23.0 (CH_2), 21.5 (CH_2), 17.9 (21-CH_3), 13.0 (19-CH_3), 12.0 (18-CH_3). HRMS Found 402.3594 (Calcd 402.3610). Anal. Calcd: C, 77.55; H, 11.51; N, 6.96. Found: C, 77.36; H, 11.88; N, 6.96. IR (KBr, cm^{-1}): 3298, 3162, 2944, 2913, 2878, 2827, 1463, 1370, 1259, 1124, 1055, 792.

5.4.2.2. (3*S*,20*S*)-20-[(3-Dimethylamino-propylamino)-methyl]-pregn-7-en-3-ol (5b). Ester **4b** (89 mg, 0.19 mmol) was treated as described in procedure B. The crude product was subjected to SCC (diethyl ether/EDMA 10:0.5) (65 mg, 85%). Mp 114 °C. ^{1}H NMR ($CDCl_3$, 100 MHz): δ 5.14 (m, 1H, H-7), 3.58 (m, 1H, H-3), 2.61 (m, 2H), 2.57 (m, 1H), 2.29 (m, 3H), 2.20 (s, 6H, N(CH_3)$_2$), 2.00 (ddd, 1H, H-12a, J = 12.7 Hz, J = 3.5 Hz, J = 3.2 Hz), 1.88–1.19 (m, 23H), 1.06 (ddd, 1H, H-1b, J = J = 13.5 Hz, J = 3.7 Hz), 0.98 (d, 3H, H-21, J = 6.6 Hz), 0.78 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 139.4 (8-CH), 117.6 (7-CH), 70.9 (3-CH), 58.1 (3″-CH_2), 55.5 (1′-CH_2), 54.8 (CH), 54.2 (CH), 49.4 (CH), 48.7 (1″-CH_2), 45.6 (N(CH_3)$_2$), 43.5 (13-C), 40.0 (CH), 39.5 (CH_2), 38.0 (CH_2), 37.1 (CH_2), 36.8 (CH), 34.2 (10-C), 31.5 (CH_2), 29.6 (CH_2), 28.1 (2″-CH_2), 27.7 (CH_2), 23.0 (CH_2), 21.5 (CH_2), 17.8 (21-CH_3), 13.0 (19-CH_3), 11.9 (18-CH_3). HRMS Found 416.3739 (Calcd 416.3767). IR (KBr, cm^{-1}): 3389, 3174, 2944, 2849, 2827, 1672, 1469, 1374, 1100, 1057.

5.4.2.3. (3*S*,20*S*)-20-[(2-Pyrrolidin-1-yl-ethylamino)-methyl]-pregn-7-en-3-ol (5c). Ester **4c** (171 mg, 0.36 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 1:9:0.5) (144 mg, 94%). Mp 143 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (m, 1H, H-7), 3.58 (m, 1H, H-3), 2.74 (m, 1H), 2.64 (m, 2H), 2.58 (m, 2H), 2.50 (m, 4H), 2.30 (dd, 1H, H-1′b, J = 11.5 Hz, J = 8.5 Hz), 2.00 (ddd, 1H, H-12a, J = 12.1 Hz, J = 3.7 Hz, J = 2.9 Hz), 1.87–1.19 (m, 25H), 1.07 (ddd, 1H, H-1b, J = J = 13.5 Hz, J = 3.6 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.78 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 139.4 (8-CH), 117.6 (7-CH), 70.9 (3-CH), 55.9 (2″-CH_2), 55.7 (1′-CH_2), 54.8 (CH), 54.2 (CH, 2 CH_2), 49.4 (CH), 48.9 (1″-CH_2), 43.5 (13-C), 40.2 (CH), 39.4 (CH_2), 38.0 (CH_2), 37.1 (CH_2), 36.8 (CH), 34.2 (10-C), 31.4 (CH_2), 29.6 (CH_2), 27.7 (CH_2), 23.4 (2 CH_2), 23.0 (CH_2), 21.5 (CH_2), 17.9 (21-CH_3), 13.0 (19-CH_3), 11.9 (18-CH_3). HRMS Found 428.3795 (Calcd 428.3767). IR (KBr, cm^{-1}): 3383, 3224, 2949, 2924, 2877, 2846, 1678, 1463, 1443, 1362, 1101, 1057.

5.4.2.4. (3*S*,20*S*)-20-(Methylamino-methyl)-pregn-7-en-3-ol (5d). Ester **4d** (180 mg, 0.47 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 1:9:0.8) (110 mg, 68%). Mp 149 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (m, 1H, H-7), 3.58 (m, 1H, H-3), 2.57 (dd, 1H, H-1′a, J = 11.7 Hz, J = 3.2 Hz), 2.41 (s, 3H, N-CH_3), 2.31 (dd, 1H, H-1′b, J = 11.7 Hz, J = 8.0 Hz), 2.02 (ddd, 1H, H-12a, J = 11.5 Hz, J = J = 3.5 Hz), 1.91–1.20 (m, 27H), 1.07 (ddd, 1H, H-1b, J = J = 13.3 Hz, J = 3.0 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.79 (s, 3H, H-19), 0.55 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 139.4 (8-CH), 117.6 (7-CH), 71.0 (3-CH), 57.9 (1′-CH_2), 54.8 (CH), 54.0 (CH), 49.4 (CH), 43.5 (13-C), 40.2 (CH), 39.5 (CH_2), 38.0 (CH_2), 37.1 (CH_2), 36.9 (N-CH_3), 36.6 (CH), 34.2 (10-C), 31.5 (CH_2), 29.6 (CH_2), 27.7 (CH_2), 23.0 (CH_2), 21.5 (CH_2), 17.9 (21-CH_3), 13.0 (19-CH_3), 11.9 (18-CH_3). HRMS Found 345.3049 (Calcd 345.3032). IR (KBr, cm^{-1}): 3424, 2944, 2875, 1663, 1468, 1444, 1381, 1051, 1041.

5.4.2.5. (3*S*,20*S*)-20-(Ethylamino-methyl)-pregn-7-en-3-ol (5e). Ester **4e** (170 mg, 0.42 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 5:5:0.5) (128 mg, 85%). Mp 148 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.16 (m, 1H, H-7), 3.59 (m, 1H, H-3), 2.62 (m, 3H), 2.31 (dd, 1H, H-1′b, J = 11.6 Hz, J = 8.6 Hz), 2.02 (ddd, 1H, H-12a, J = 12.3 Hz, J = J = 3.4 Hz), 1.90–1.24 (m, 21H), 1.10 (t, 3H, H-2″, J = 7.3 Hz, with underneath m, 1H, H-1b), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.78 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 139.4 (8-CH), 117.5 (7-CH), 71.0 (3-CH), 55.3 (1′-CH_2), 54.8 (CH), 54.2 (CH), 49.4 (CH), 44.4 (1″-CH_2), 43.5 (13-C), 40.2 (CH), 39.4 (CH_2), 37.9 (CH_2), 37.1 (CH_2), 36.8 (CH), 34.2 (10-C), 31.4 (CH_2), 29.6 (CH_2), 27.7 (CH_2), 23.0 (CH_2), 21.4 (CH_2), 17.9 (21-CH_3), 15.3 (2″-CH_3), 13.0 (19-CH_3), 11.9 (18-CH_3). HRMS Found 359.3205 (Calcd 359.3188). Anal. Calcd: C, 80.16; H, 11.49; N, 3.90. Found: C, 79.61; H, 11.67; N, 3.72. IR (KBr, cm^{-1}): 3383, 2944, 2899, 1469, 1445, 1379, 1099, 1052, 826.

5.4.2.6. (3*S*,20*S*)-20-(Propylamino-methyl)-pregn-7-en-3-ol (5f). Ester **4f** (180 mg, 0.43 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 5:5:0.5) (140 mg, 87%). Mp 120 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.15 (m, 1H, H-7), 3.59 (m, 1H, H-3), 2.62 (dd, 1H, H-1′a, J = 11.6 Hz, J = 3.1 Hz), 2.54 (m, 2H), 2.29 (dd, 1H, H-1′b, J = 11.6 Hz, J = 8.7 Hz), 2.02 (ddd, 1H, H-12a, J = 13.0 Hz, J = J = 3.3 Hz), 1.89–1.20 (m, 23H), 1.07 (ddd, 1H, H-1b, J = J = 13.0 Hz, J = 3.3 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.91 (t, 3H, H-3″, J = 7.4 Hz), 0.79 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 139.4 (8-CH), 117.5 (7-CH), 71.0 (3-CH), 55.4 (1′-CH_2), 54.8 (CH), 54.2 (CH), 52.2 (1″-CH_2), 49.4 (CH), 43.5 (13-C), 40.2 (CH), 39.4 (CH_2), 38.0 (CH_2), 37.1 (CH_2), 36.8 (CH), 34.2 (10-C), 31.4 (CH_2), 29.6 (CH_2), 27.7 (CH_2), 23.1 (2″-CH_2), 23.0 (CH_2), 21.5 (CH_2), 17.9 (21-CH_3), 13.0 (19-CH_3), 11.9 (18-CH_3), 11.8 (3″-CH_3). HRMS Found 373.3383 (Calcd 373.3345). Anal. Calcd: C, 80.37; H, 11.60; N, 3.75. Found: C, 79.91; H, 11.94; N, 3.60. IR (KBr, cm^{-1}): 3410, 2932, 2872, 1448, 1378, 1105, 1051.

5.4.2.7. (3*S*,20*S*)-20-(Butylamino-methyl)-pregn-7-en-3-ol (5g). Ester **4g** (170 mg, 0.40 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl

ether/EDMA 7:3:0.2) (132 mg, 85%). Mp 96 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.14 (m, 1H, H-7), 3.58 (m, 1H, H-3), 2.61 (ddd, 1H, H-1′a, J = 11.6 Hz, J = 3.7 Hz), 2.54 (m, 2H), 2.29 (dd, 1H, H-1′b, J = 11.6 Hz, J = 8.8 Hz), 2.02 (dm, 2H, H-12, J = 12.6 Hz), 1.89–1.20 (m, 23H), 1.12 (ddd, 1H, H-1b, J = J = 13.0 Hz, J = 3.2 Hz), 0.99 (d, 3H, H-21, J = 6.4 Hz), 0.90 (t, 3H, H-4″, J = 7.2 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 139.4 (8-CH), 117.5 (7-CH), 71.0 (3-CH), 55.3 (1′-CH_2), 54.8 (CH), 54.2 (CH), 49.8 (1″-CH_2), 49.4 (CH), 43.5 (13-C), 40.2 (CH), 39.5 (CH_2), 38.0 (CH_2), 37.1 (CH_2), 36.6 (CH), 34.2 (10-C), 31.8 (2″-CH_2), 31.4 (CH_2), 30.0 (CH_2), 27.7 (CH_2), 23.0 (CH_2), 21.5 (CH_2), 20.5 (3″-CH_2), 18.0 (21-CH_3), 14.0 (4″-CH_3), 13.0 (19-CH_3), 12.0 (18-CH_3). HRMS Found 387.3501 (Calcd 387.3501). Anal. Calcd: C, 80.56; H, 11.70; N, 3.61. Found: C, 80.40; H, 11.63; N, 3.52. IR (KBr, cm^{-1}): 3396, 3316, 2937, 2874, 2850, 1468, 1446, 1380, 1050, 784.

5.4.2.8. (3*S*,20*S*)-20-(Pentylamino-methyl)-pregn-7-en-3-ol (5h). Ester **4h** (188 mg, 0.42 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 5:5:0.5) (132 mg, 78%). Mp 98 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.16 (m, 1H, H-7), 3.59 (m, 1H, H-3), 2.58 (m, 3H), 2.29 (dd, 1H, H-1′b, J = 11.4 Hz, J = 8.7 Hz), 2.00 (ddd, 1H, H-12a, J = 12.6 Hz, J = J = 3.3 Hz), 1.89–1.21 (m, 27H), 1.08 (ddd, 1H, H-1b, J = J = 13.3 Hz, J = 3.2 Hz), 0.99 (d, 3H, H-21, J = 6.4 Hz), 0.90 (t, 3H, H-5″, J = 6.9 Hz), 0.79 (s, 3H, H-19), 0.55 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 139.4 (8-CH), 117.5 (7-CH), 71.0 (3-CH), 55.6 (1′-CH_2), 54.9 (CH), 54.3 (CH), 50.4 (1″-CH_2), 49.4 (CH), 43.5 (13-C), 40.2 (CH), 39.5 (CH_2), 38.0 (CH_2), 37.1 (CH_2), 36.9 (CH), 34.2 (10-C), 31.5 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 27.7 (CH_2), 23.0 (CH_2), 22.6 (CH_2), 21.5 (CH_2), 17.9 (21-CH_3), 14.0 (5″-CH_3), 13.0 (19-CH_3), 11.9 (18-CH_3). HRMS Found 401.3694 (Calcd 401.3658). Anal. Calcd: C, 80.74; H, 11.79; N, 3.49. Found: C, 80.51; H, 11.87; N, 3.48. IR (KBr, cm^{-1}): 3421, 2930, 2870, 2800, 1448, 1376, 1130, 1096,1046, 726.

5.4.2.9. (3*S*,20*S*)-20-[(2-Methylpropyl)amino-methyl]-pregn-7-en-3-ol (5i). Ester **4i** (200 mg, 0.46 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 8:2:0.5) (141 mg, 79%). Mp 117 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.15 (m, 1H, H-7), 3.58 (m, 1H, H-3), 2.62 (dd, 1H, H-1′a, J = 11.6 Hz, J = 3.1 Hz), 2.41 (dd, 1H, H-1″a, J = 11.6 Hz, J = 7.0 Hz), 2.32 (dd, 1H, H-1″b, J = 11.6 Hz, J = 6.8 Hz), 2.25 (dd, 1H, H-1′b, J = 11.6 Hz, J = 2.9 Hz), 2.00 (ddd, 1H, H-12a, J = 12.5 Hz, J = J = 3.3 Hz), 1.89–1.20 (m, 22H), 1.07 (ddd, 1H, H-1b, J = J = 13.0 Hz, J = 3.3 Hz), 0.99 (d, 3H, H-21, J = 6.4 Hz), 0.89 (d, 6H, 2 CH_3, J = 6.8 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 139.4 (8-CH), 117.5 (7-CH), 71.0 (3-CH), 58.2 (1″-CH_2), 55.4 (1′-CH_2), 54.8 (CH), 54.3 (CH), 49.4 (CH), 43.5 (13-C), 40.2 (CH), 39.4 (CH_2), 37.9 (CH_2), 37.1 (CH_2), 36.7 (CH), 34.2 (10-C), 31.4 (CH_2), 29.6 (CH_2), 28.0 (2″-CH), 27.7 (CH_2), 23.0 (CH_2), 21.5 (CH_2), 20.68 and 20.63 (2 CH_3), 17.8 (21-CH_3), 13.0 (19-CH_3), 11.9 (18-CH_3). HRMS Found 387.3528 (Calcd 387.3501). IR (KBr, cm^{-1}): 3409, 2951, 2869, 1471, 1444, 1378, 1365, 1097, 1039, 749.

5.4.2.10. (3*S*,20*S*)-20-[(1*R*,*S*)-(1,2-Dimethyl-propylamino)-methyl]-pregn-7-en-3-ol (5j) (mixture of diastereomers). Ester **4j** (220 mg, 0.50 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 9:1:0.5) (164 mg, 82%). Mp 141 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (m, 2H, H-7), 3.58 (m, 2H, H-3), 2.67 (dd, 1H, H-1′a, J = 11.2 Hz, J = 2.8 Hz), 2.61 (dd, 1H, H-1′a, J = 11.8 Hz, J = 3.1 Hz), 2.37 (m, 2H, H-1″), 2.27 (dd, 1H, H-1′b, J = 11.8 Hz, J = 8.6 Hz), 2.17 (dd, 1H, H-1′b, J = 11.2 Hz, J = 8.8 Hz), 2.00 (ddd, 2H, H-12a, J = 12.5 Hz, J = J = 3.3 Hz), 1.88–1.18 (m, 44H), 1.06 (ddd, 2H, H-1b, J = J = 12.7 Hz, J = 3.1 Hz), 1.00 (d, 3H, H-21, J = 6.6 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.94 (d, 3H, H-1‴, J = 6.4 Hz), 0.93 (d, 3H, H-1‴, J = 6.4 Hz), 0.89 (d, 3H, H-3″, J = 6.7 Hz), 0.88 (d, 3H, H-3″, J = 6.7 Hz), 0.86 (d, 3H, H-3″, J = 6.6 Hz), 0.84 (d, 3H, H-3″, J = 6.6 Hz), 0.78 (s, 6H, H-19), 0.54 (s, 6H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 139.4 (8-CH), 117.5 (7-CH), 71.04 and 70.99 (3-CH), 58.9 and 58.1 (1″-CH_2), 54.9 (CH), 54.3 (CH), 53.1 and 52.7 (1′-CH_2), 49.48 and 49.43 (CH), 43.51 and 43.49 (13-C), 40.2 (CH), 39.50 and 39.48 (CH_2), 38.0 (CH_2), 37.3 (CH), 37.19 and 37.13 (CH_2), 36.7 (CH), 34.3 and 34.2 (10-C), 32.5 and 31.6 (2″-CH), 31.53 and 31.47 (CH_2), 29.68 and 29.63 (CH_2), 27.72 and 27.69 (CH_2), 23.0 (CH_2), 21.5 (CH), 19.53 and 19.49 (3″-CH_3), 18.04 and 17.91 (21-CH_3), 17.5 and 17.0 (3″-CH_3), 16.3 and 16.0 (1‴-CH_3), 13.0 (19-CH_3), 11.9 (18-CH_3). HRMS Found 358.3140 (Calcd 358.3110 for $C_{24}H_{40}NO$). Anal. Calcd: C, 80.74; H, 11.79; N, 3.49. Found: C, 80.53; H, 11.89; N, 3.51. IR (KBr, cm^{-1}): 3427, 2947, 2873, 1471, 1444, 1381, 1099, 1052.

5.4.2.11. (3*S*,20*S*)-20-(Dodecylamino-methyl)-pregn-7-en-3-ol (5k). Ester **4k** (158 mg, 0.29 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 6:4:0.5) (109 mg, 75%). Mp 76 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.16 (m, 1H, H-7), 3.58 (m, 1H, H-3), 2.63–2.47 (m, 3H, H-1″, H-1′a), 2.28 (dd, 1H, H-1′b, J = 11.7 Hz, J = 8.6 Hz), 2.01 (ddd, 1H, H-12a, J = 12.5 Hz, J = 4.0 Hz, J = 2.9 Hz), 1.89–1.19 (m, 41H), 1.07 (ddd, 1H, H-1b, J = J = 13.4 Hz, J = 3.5 Hz), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.88 (t, 3H, H-12″, J = 6.9 Hz), 0.79 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 139.4 (8-CH), 117.6 (7-CH), 71.0 (3-CH), 55.5 (1′-CH_2), 54.8 (14-CH), 54.2 (17-CH), 50.4 (1″-CH_2), 49.4 (9-CH), 43.5 (13-C), 40.2 (5-CH), 39.5 (12-CH_2), 38.0 (4-CH_2), 37.1 (1-CH_2), 36.8 (20-CH), 34.2 (10-C), 31.9 (CH_2 side chain), 31.5 (2-CH), 30.1, 29.65, 29.62, 29.61, 29.58 (CH_2 side chain and 6-CH_2), 29.3 (CH_2 side chain), 27.7 (16-CH_2), 27.4 (CH_2 side chain), 23.0 (15-CH_2), 22.7 (CH_2 side chain), 21.5 (11-CH_2), 17.9 (21-CH_3), 14.1 (12″-CH_3), 13.0 (19-CH_3), 12.0 (18-CH_3). HRMS Found 499.4768 (Calcd 499.4753). Anal. Calcd: C, 80.74; H, 11.79; N, 3.49. Found: C, 80.53; H, 11.89; N, 3.51. IR (KBr, cm^{-1}): 3418, 2918, 2849, 1635, 1472, 1372, 1051, 1040.

5.4.3. (3*S*,20*S*)-20-[(*N*-Butyl-*N*-methyl-amino)-methyl]-pregn-7-en-3-yl acetate (6)

To a stirred solution of ester **4g** (1.80 g, 4.10 mmol) in dichloromethane (10 mL) was successively added, at 0 °C, formaldehyde solution (37% in water, 4.0 mL, 49 mmol) and formic acid (566 mg, 12.3 mmol). The reaction mixture was then heated at 80 °C for 2 h. After cooling to ambient temperature aqueous NaOH [2 N] (6 mL) was slowly added and the mixture was stirred for 10 min at ambient temperature. Then it was extracted with dichloromethane (3 × 10 mL), the combined organic layers were dried over sodium sulfate, and evaporated to dryness under reduced pressure. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 9:1:0.1) to afford **6** as a white solid (1.7 g, 94%). Mp 128 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.37 (ddd, 1H, H-1″a, J = 12.0 Hz, J = 8.6 Hz, J = 6.3 Hz), 2.14 (s, 3H, CH_3N), 2.11 (m, 2H), 2.05 (ddd, 1H, H-12a, J = 12.9 Hz, J = J = 3.3 Hz), 2.02 (s, 3H, CH_3CO), 1.97 (dd, 1H, H-1′b, J = 12.0 Hz, J = 10.4 Hz), 1.91–1.11 (m, 22H), 1.12 (m, 2H), 0.99 (d, 3H, H-21, J = 6.6 Hz), 0.90 (t, 3H, H-4″, J = 7.3 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7 (CO), 139.4 (8-CH), 117.4 (7-CH), 73.4 (3-CH), 64.1 (1′-CH_2), 58.3 (1″-CH_2), 55.2 (CH), 54.7 (CH), 49.2 (CH), 43.6 (13-C), 43.2 (CH_3N), 40.0 (CH), 39.4 (CH_2), 36.8 (CH_2), 35.4 (CH), 34.2 (10-C), 33.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 27.9 (CH_2), 27.5 (CH_2), 23.1 (CH_2), 21.5 (CH_2, *CH_3*CO), 20.7 (3″-CH_2), 18.2 (21-CH_3), 14.1 (4″-CH_3), 12.9 (19-CH_3), 12.0 (18-CH_3). HRMS Found 443.3769 (Calcd 443.3763). Anal. Calcd: C, 78.50; H, 11.13; N, 3.16. Found: C,

78.38; H, 10.94; N, 3.10. IR (KBr, cm^{-1}): 2948, 2867, 2844, 2800, 1734, 1465, 1369, 1246, 1031, 975, 894.

5.4.4. (3*S*,20*S*)-20-[(*N*-Butyl-*N*-methyl-amino)-methyl]-pregn-7-en-3-ol (7)

Ester **6** (200 mg, 0.450 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 8:2:0.2) (164 mg, 91%). Mp 122 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.16 (m, 1H, H-7), 3.60 (m, 1H, H-3), 2.37 (m, 1H), 2.15 (s, 3H, CH_3N), 2.13 (m, 2H), 2.06 (ddd, 1H, H-12a, J = 13.0 Hz, $J = J$ = 3.5 Hz), 1.99 (dd, 1H, H-1′b, J = 11.8 Hz, J = 10.7 Hz), 1.90 (m, 1H), 1.84–1.19 (m, 22H), 1.08 (m, 2H), 1.00 (d, 3H, H-21, J = 6.3 Hz), 0.91 (t, 3H, H-4″, J = 7.4 Hz), 0.80 (s, 3H, H-19), 0.56 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 139.5 (8-CH), 117.6 (7-CH), 71.0 (3-CH), 64.2 (1′-CH_2), 58.3 (1″-CH_2), 55.2 (CH), 54.7 (CH), 49.5 (CH), 43.7 (13-C), 43.1 (CH_3N), 40.3 (CH), 39.5 (CH_2), 38.0 (CH_2), 37.3 (CH_2), 35.4 (CH), 34.2 (10-C), 31.5 (CH_2), 29.6 (CH_2), 29.4 (2″-CH_2), 27.9 (CH_2), 23.1 (CH_2), 21.5 (CH_2), 20.7 (3″-CH_2), 18.2 (21-CH_3), 14.1 (4″-CH_3), 13.0 (19-CH_3), 11.9 (18-CH_3). HRMS Found 401.3653 (Calcd 401.3658). Anal. Calcd: C, 80.74; H, 11.79; N, 3.49. Found: C, 80.75; H, 11.77; N, 3.40. IR (KBr, cm^{-1}): 3433, 2936, 2865, 1633, 1462, 1375, 1046.

5.4.5. (3*S*,20*S*)-20-[(*E*,*Z*)-2-Methoxyethylen-1-yl]-pregn-7-en-3-yl acetate (8a) and (3*S*,20*S*)-20-[(*E*,*Z*)-2-methoxy- ethylen-1-yl]-pregn-7-en-3-ol (8b)

To a stirred solution of methoxymethyltriphenyl-phosphonium chloride (553 mg, 1.61 mmol) in anhydrous THF (10 mL) under nitrogen atmosphere was added dropwise *n*-butyllithium (2.5 M solution in hexanes, 0.6 mL, 1.5 mmol) at 0 °C. The reaction mixture was allowed to warm up to ambient temperature and was added dropwise to a stirred solution of aldehyde **3** (300 mg, 0.810 mmol) in anhydrous THF (5 mL). The reaction mixture was stirred at room temperature for 16 h and then quenched with a saturated aqueous solution of sodium bicarbonate (15 mL). The biphasic mixture was extracted with diethyl ether (3 × 15 mL), the combined organic layers were washed with water (15 mL), dried over sodium sulfate, and evaporated to dryness under reduced pressure. The crude product was subjected to SCC (pentane/diethyl ether 9:1) to afford ether **8a**, further elution with pentane/diethyl ether (3:7) afforded ether **8b**.

Compound **8a** (110 mg, 34%). Mp 160 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 6.28 (d, 1H, H-2′(*E*), J = 12.6 Hz), 5.82 (d, 1H, H-2′(*Z*), J = 6.6 Hz), 5.11 (m, 2H, H-7), 4.57 (m, 3H), 4.12 (dd, 1H, H-1′(*Z*), J = 6.6 Hz, J = 9.5 Hz), 3.48 (s, 3H, OCH_3 (*Z*)), 3.38 (s, 3H, OCH_3 (*E*)), 2.50 (under the DMSO-peak, 1H, H-20 (*Z*)), 1.97 (s, 6H, CH_3CO with underneath m, 3H, H-12a, H-20 (*E*)), 1.79–1.20 (m, 37H), 1.10 (m, 2H), 1.00 (d, 3H, H-21 (*E*), J = 6.6 Hz), 0.98 (d, 3H, H-21 (*Z*), J = 6.6 Hz), 0.76 (s, 6H, H-19), 0.51 (s, 6H, H-18). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 170.2 (CO), 146.0 (2′-CH(*E*)), 145.0 (2′-CH(*Z*)), 139.5 (8-CH), 117.0 (7-CH), 113.5 (1′-CH (*Z*)), 110.0 (1′-CH (*E*)), 72.5 (3-CH), 58.8 (CH_3O (*Z*)), 55.9 (CH), 55.6 (CH), 55.3 (CH_3O (*E*)), 54.5 (CH), 54.3 (CH), 48.4 (CH), 42.7 (13-C), underneath the DMSO-peak (CH), 38.6 (CH_2), 36.0 (CH_2), 35.5 (20-CH (*E*)), 33.5 (CH_2, 10-C), 31.0 (20-CH (*Z*)), 28.8 (CH_2), 27.9 (CH_2), 27.0 (CH_2), 22.3 (CH_2, 21-CH_3 (*E*)), 21.0 (CH_2, 21-CH_3 (*Z*), CH_3CO), 12.6 (19-CH_3), 11.7 (18-CH_3). HRMS Found 400.3011 (Calcd 400.2978). IR (KBr, cm^{-1}): 3443, 2945, 2869, 2849, 1733, 1662, 1453, 1367, 1240, 1097, 1028.

Compound **8b** (50 mg, 17%). Mp 117 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.24 (d, 1H, H-2′ (*E*), J = 12.5 Hz), 5.73 (d, 1H, H-2′ (*Z*), J = 6.2 Hz), 5.15 (m, 2H, H-7), 4.59 (dd, 1H, H-1′ (*E*), J = 12.5 Hz, J = 9.2 Hz), 4.17 (dd, 1H, H-1′ (*Z*), J = 6.2 Hz, J = 9.7 Hz), 3.59 (m, 2H, H-3), 3.55 (s, 3H, OCH_3 (*Z*)), 3.47 (s, 3H, OCH_3 (*E*)), 2.59 (m, 1H, H-20 (*Z*)), 1.98 (m, 1H, H-20 (*E*)), 1.84–1.19 (m, 42H), 1.10 (m, 2H, H-1b), 1.03 (d, 3H, H-21 (*E*), J = 6.6 Hz), 0.98 (d, 3H, H-21 (*Z*), J = 6.6 Hz), 0.79 (s, 6H, H-19), 0.57 and 0.54 (2 s, 6H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 145.3 (2′-CH (*E*)), 143.8 (2′-CH (*Z*)), 139.6 and 139.4 (8-CH), 117.5 and 117.4 (7-CH), 114.2 (1′-CH (*Z*)), 110.3 (1′-CH (*E*)), 71.0 (3-CH), 59.4 (CH_3O (*Z*)), 56.5 (CH), 56.3 (CH), 55.8 (CH_3O (*E*)), 55.2 (CH), 55.1 (CH), 49.4 (CH), 49.3 (CH), 43.2 (13-C), 40.2 (CH), 39.4 (CH_2), 37.9 (CH_2), 37.1 (CH_2), 36.1 (20-CH (*E*)), 34.2 (10-C), 31.8 (20-CH (*Z*)), 31.4 (CH_2), 29.6 (CH_2), 28.4 (CH_2), 27.6 (CH_2), 22.9 (CH_2), 22.8 (CH_2), 22.1 (21-CH_3 (*E*)), 21.5 (CH_2), 21.2 (21-CH_3 (*Z*)), 13.0 (19-CH_3), 12.01 and 11.98 (18-CH_3). HRMS Found 358.2852 (Calcd 358.2872). IR (KBr, cm^{-1}): 3448, 2935, 2870, 2853, 1656, 1448, 1376, 1211, 1102, 1042, 934.

5.4.6. (3*S*,20*R*)-20-(Formylmethyl)-pregn-7-en-3-yl acetate (9a) and (3*S*,20*R*)-(3-Hydroxypregn-7-en-20-yl)-ethanal (9b)

To a solution of ether **8a** (287 mg, 0.720 mmol) in THF (6 mL) was added sulfuric acid [2 N] (3 mL) at ambient temperature. The reaction mixture was stirred at 70 °C for 5 h. After cooling to ambient temperature the reaction mixture was extracted with dichloromethane (2 × 10 mL), the combined extracts were washed successively with water (10 mL), brine (10 mL) and water (10 mL), dried over sodium sulfate, and evaporated to dryness under reduced pressure. The crude product was subjected to SCC (pentane/diethyl ether 85:15) to afford **9a**, further elution with diethyl ether afforded **9b**.

Compound **9a** (158 mg, 58%). Mp 85 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.75 (dd, 1H, CHO, J = 3.3 Hz, J = 1.2 Hz), 5.14 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.46 (ddd, 1H, H-1′a, J = 16.0 Hz, J = 2.7 Hz, J = 1.2 Hz), 2.17 (ddd, 1H, H-1′b, J = 16.0 Hz, J = 9.4 Hz, J = 3.3 Hz), 2.04 (m, 1H), 2.02 (s, 3H, CH_3CO), 1.99 (m, 1H), 1.89–1.22 (m, 18H), 1.13 (ddd, 1H, H-1b, $J = J$ = 13.1 Hz, J = 3.2 Hz), 1.02 (d, 3H, H-21, J = 6.6 Hz), 0.81 (s, 3H, H-19), 0.58 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 203.3 (CHO), 170.6 (CO), 139.0 (8-CH), 117.7 (7-CH), 73.4 (3-CH), 55.8 (CH), 54.9 (CH), 50.8 (1′-CH_2), 49.2 (CH), 43.5 (13-C), 40.2 (CH), 39.3 (CH_2), 36.8 (CH_2), 34.2 (10-C), 33.8 (CH_2), 31.9 (CH), 29.5 (CH_2), 28.1 (CH_2), 27.5 (CH_2), 22.8 (CH_2), 21.42 (CH_2), 21.39 (CH_3CO), 20.1 (21-CH_3), 12.9 (19-CH_3), 11.8 (18-CH_3). HRMS Found 386.2823 (Calcd 386.2821). Anal. Calcd: C, 77.68; H, 9.91. Found: C, 77.46; H, 9.98. IR (KBr, cm^{-1}): 3440, 2946, 2874, 2851, 1729, 1718, 1364, 1246, 1037, 898.

Compound **9b** (71 mg, 29%). Mp 123 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.75 (d, 1H, CHO, J = 3.4 Hz, J = 1.4 Hz), 5.16 (m, 1H, H-7′), 3.59 (m, 1H, H-3′), 2.47 (dd, 1H, H-2a, J = 16.0 Hz, J = 2.7 Hz), 2.17 (ddd, 1H, H-2b, J = 16.0 Hz, J = 9.1 Hz, J = 3.4 Hz), 2.04 (m, 1H), 1.90–1.21 (m, 18H), 1.11 (ddd, 1H, H-1′b, $J = J$ = 13.1, J = 3.3 Hz), 1.02 (d, 3H, H-21′, J = 6.6 Hz), 0.81 (s, 3H, H-19′), 0.58 (s, 3H, H-18′). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 203.4 (CHO), 139.0 (8′-CH), 117.8 (7′-CH), 71.0 (3′-CH), 55.7 (CH), 54.9 (CH), 50.8 (2-CH_2), 49.3 (CH), 43.5 (13′-C), 40.2 (CH), 39.4 (CH_2), 37.9 (CH_2), 37.1 (CH_2), 34.3 (10′-C), 32.0 (CH), 31.4 (CH_2), 29.6 (CH_2), 28.1 (CH_2), 22.8 (CH_2), 21.4 (CH_2), 20.1 (21′-CH_3), 13.1 (19′-CH_3), 11.8 (18′-CH_3). HRMS Found 344.2728 (Calcd 344.2715). IR (KBr, cm^{-1}): 3417, 2942, 2873, 1725, 1444, 1380, 1098, 1054.

5.4.7. (3*S*,20*R*)-20-(Propylamino-ethyl)-pregn-7-en-3-yl acetate (10)

Aldehyde **9a** (158 mg, 0.410 mmol) and *n*-propylamine (130 μL, 1.63 mmol) in anhydrous THF (5 mL) were treated as described in procedure A. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 9:1:0.5) (127 mg, 72%). Mp 106 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.14 (m, 1H, H-7), 4.68 (m, 1H, H-3), 2.65 (m, 1H), 2.57 (m, 2H), 2.54 (m, 1H), 2.02 (s, 3H, CH_3CO), 2.00 (m, 1H), 1.92–1.18 (m, 24H), 1.12 (ddd, 1H, H-1b, $J = J$ = 13.0 Hz, J = 3.0 Hz), 0.94 (d, 3H, H-21, J = 6.6 Hz), 0.91 (t, 3H, H-3″, J = 7.5 Hz), 0.80 (s, 3H, H-19), 0.53 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$,

125 MHz): δ 170.6 (CO), 139.4 (8-CH), 117.3 (7-CH), 73.4 (3-CH), 56.2 (CH), 54.9 (CH), 52.1 (1″-CH_2), 49.2 (CH), 47.5 (2′-CH_2), 43.3 (13-C), 40.0 (CH), 39.4 (CH_2), 36.8 (CH_2), 36.4 (1′-CH_2), 34.6 (CH), 34.1 (10-C), 33.7 (CH_2), 29.5 (CH_2), 27.9 (CH_2), 27.4 (CH_2), 23.2 (2″-CH_2), 22.8 (CH_2), 21.4 (CH_2, *C*H_3CO), 19.0 (21-CH_3), 12.9 (19-CH_3), 11.8 (18-CH_3, 3″-CH_3). HRMS Found 429.3618 (Calcd 429.3607). Anal. Calcd: C, 78.27; H, 11.03; N, 3.26. Found: C, 78.18; H, 10.73; N, 3.23. IR (KBr, cm^{-1}): 2947, 2864, 2758, 1731, 1472, 1456, 1379, 1365, 1244, 1032, 972,897.

5.4.8. (3*S*,20*R*)-20-(Propylamino-ethyl)-pregn-7-en-3-ol (11)

Ester **10** (150 mg, 0.35 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether/EDMA 7:3:0.5) (110 mg, 81%). Mp 114 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (m, 1H, H-7), 3.59 (m, 1H, H-3), 2.65 (m, 1H), 2.56 (m, 2H), 2.53 (m, 1H), 2.02 (ddd, 1H, H-12a, *J* = 13.0 Hz, *J* = 3.3 Hz), 1.90 (m, 1H), 1.83–1.18 (m, 24H), 1.08 (ddd, 1H, H-1b, *J* = *J* = 13.2 Hz, *J* = 3.3 Hz), 0.95 (d, 3H, H-21, *J* = 6.6 Hz), 0.91 (t, 3H, H-3″, *J* = 7.4 Hz), 0.80 (s, 3H, H-19), 0.54 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 139.5 (8-CH), 117.5 (7-CH), 71.0 (3-CH), 56.3 (CH), 55.0 (CH), 52.1 (1″-CH_2), 49.4 (CH), 47.5 (2′-CH_2), 43.4 (13-C), 40.2 (CH), 39.5 (CH_2), 38.0 (CH_2), 37.1 (CH_2), 36.4 (1′-CH_2), 34.7 (CH), 34.2 (10-C), 31.5 (CH_2), 29.6 (CH_2), 28.0 (CH_2), 23.2 (2″-CH_2), 22.9 (CH_2), 21.5 (CH_2), 19.1 (21-CH_3), 13.0 (19-CH_3), 11.8 (18-CH_3, 3″-CH_3). HRMS Found 387.3522 (Calcd 387.3501). Anal. Calcd: C, 80.56; H, 11.70; N, 3.61. Found: C, 80.04; H, 11.46; N, 3.53. IR (KBr, cm^{-1}): 3420, 2932, 2872, 1470, 1376, 1100, 1044.

5.4.9. (3*S*,20*S*)-20-Hydroxymethylpregn-7-en-3-yl acetate (12)

To a stirred solution of aldehyde **3** (920 mg, 2.47 mmol) in 25 mL chloroform/methanol (3:2) was added sodium borohydride (94 mg, 2.5 mmol). The reaction mixture was stirred for 10 min at ambient temperature and then quenched with saturated aqueous ammonium chloride solution (20 mL). The biphasic mixture was extracted with chloroform (3 × 20 mL), the combined organic layers were dried over sodium sulfate, and evaporated to dryness under reduced pressure. The crude product was subjected to SCC (pentane/diethyl ether gradient from 7:3 to 5:5) to afford **12** as a white solid (800 mg, 87%). Mp 168 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (m, 1H, H-7), 4.69 (m, 1H, H-3), 3.64 (dd, 1H, H-1′a, *J* = 10.5 Hz, *J* = 3.0 Hz), 3.38 (dd, 1H, H-1′b, *J* = 10.5 Hz, *J* = 6.9 Hz), 2.02 (s, 3H, CH_3CO), 2.00 (m, 1H), 1.89–1.22 (m, 20 H), 1.13 (ddd, 1H, H-1b, *J* = *J* = 13.5 Hz, *J* = 3.5 Hz), 1.06 (d, 3H, H-21, *J* = 6.6 Hz), 0.81 (s, 3H, H-19), 0.56 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.7 ($COCH_3$), 139.2 (8-CH), 117.5 (7-CH), 73.4 (3-CH), 67.9 (CH_2OH), 54.7 (CH), 52.3 (CH), 49.2 (CH), 43.4 (13-C), 40.0 (CH), 39.3 (CH_2), 39.1 (CH), 36.8 (CH_2), 34.2 (10-C), 33.7 (CH_2), 29.5 (CH_2), 27.4 (2xCH_2), 23.0 (CH_2), 21.5 (*C*H_3CO), 21.4 (CH_2), 16.9 (21-CH_3), 12.9 (19-CH_3), 11.9 (18-CH_3). HRMS Found 374.2857 (Calcd 374.2821). IR (KBr, cm^{-1}): 3324, 2947, 2897, 1734, 1469, 1446, 1381, 1367, 1250, 1034.

5.4.10. (3*S*,20*S*)-20-Iodomethylpregn-7-en-3-yl acetate (13)

To a stirred solution of methyltriphenoxy-phosphonium iodide (907 mg, 2.00 mmol) in anhydrous THF (4 mL) was added a solution of alcohol **12** (300 mg, 0.800 mmol) in anhydrous THF (3 mL) at ambient temperature. The reaction mixture was stirred for 16 h in the dark and then was quenched with water (10 mL). The biphasic mixture was extracted with diethyl ether (3 × 10 mL). The combined organic extracts were washed with water (10 mL), sodium thiosulfate solution [1 M] (10 mL), dried over sodium sulfate and evaporated to dryness under reduced pressure. The crude product was subjected to SCC (pentane/diethyl ether 9:1) to afford **13** as a white solid (343 mg, 88%). Mp 166 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.14 (m, 1H, H-7), 4.68 (m, 1H, H-3), 3.33 (dd, 1H, H-1′a, *J* = 9.6 Hz, *J* = 2.4 Hz), 3.16 (dd, 1H, H-1′b, *J* = 9.6 Hz, *J* = 5.7 Hz), 2.02 (s, 3H, CH_3CO), 1.95 (ddd, 1H, H-12a, *J* = 12.6 Hz, *J* = 3.6 Hz, *J* = 3.0 Hz), 1.98–1.08 (m, 19 H), 1.03 (d, 3H, H-21, *J* = 6.4 Hz), 0.80 (s, 3H, H-19), 0.56 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7 (CO), 139.1 (8-CH), 117.6 (7-CH), 73.4 (3-CH), 55.3 (CH), 54.6 (CH), 49.1 (CH), 43.3 (13-C), 40.0 (CH), 39.1 (CH_2), 37.4 (CH), 36.8 (CH_2), 34.1 (10-C), 33.7 (CH_2), 29.6 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 22.8 (CH_2), 21.5 (CH_2), 21.4 (*C*H_3CO), 20.90 (21-CH_3), 20.87 (CH_2I), 12.9 (19-CH_3), 12.5 (18-CH_3). HRMS Found 484.1826 (Calcd 484.1839). IR (KBr, cm^{-1}): 3442, 2948, 1734, 1247, 1031.

5.4.11. (3*S*,20*S*)-20-(Butylsulfanyl-methyl)-pregn-7-en-3-yl acetate (14)

To a stirred solution of *n*-butanethiol (0.12 mL, 1.13 mmol) in anhydrous THF (3 mL) was added dropwise *n*-butyllithium (1.6 M solution in hexanes, 0.40 mL, 0.64 mmol) at 0 °C. The resulting mixture was then slowly added to a solution of iodide **13** (317 mg, 0.65 mmol) in anhydrous THF (5 mL) at 0 °C. The reaction mixture was stirred for 16 h at ambient temperature, quenched with water (4 mL) and evaporated to dryness under reduced pressure. The crude product was subjected to SCC (pentane/diethyl ether 19:1) to afford **14** as a white solid (252 mg, 87%). Mp 81 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.14 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.66 (dd, 1H, H-1′a, *J* = 12.4 Hz, *J* = 2.6 Hz), 2.48 (t, 2H, H-1″, *J* = 7.4 Hz), 2.25 (dd, 1H, H-1′b, *J* = 12.4 Hz, *J* = 8.9 Hz), 2.02 (s, 3H, CH_3CO), 2.00 (m, 1H), 1.92 (m, 1H), 1.98–1.20 (m, 22 H), 1.12 (ddd, 1H, H-1b, *J* = *J* = 13.0 Hz, *J* = 3.5 Hz), 1.07 (d, 3H, H-21, *J* = 6.4 Hz), 0.91 (t, 3H, H-4″, *J* = 7.4 Hz), 0.80 (s, 3H, H-19), 0.56 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7 (CO), 139.2 (8-CH), 117.5 (7-CH), 73.4 (3-CH), 55.3 (CH), 54.8 (CH), 49.2 (CH), 43.5 (13-C), 40.0 (CH), 39.7 (1′-CH_2), 39.2 (CH_2), 37.2 (CH), 36.8 (CH_2), 34.2 (10-C), 33.8 (CH_2), 32.7 (1″-CH_2), 32.0 (CH_2), 29.5 (CH_2), 27.9 (CH_2), 27.4 (CH_2), 22.9 (CH_2), 22.0 (CH_2), 21.5 (*C*H_3CO), 21.4 (CH_2), 18.9 (21-CH_3), 13.7 (4″-CH_3), 12.9 (19-CH_3), 12.5 (18-CH_3). HRMS Found 446.3172 (Calcd 446.3219). IR (KBr, cm^{-1}): 3443, 2948, 2848, 1734, 1466, 1370, 1247, 1032.

5.4.12. (3*S*,20*S*)-20-(Butylsulfanyl-methyl)-pregn-7-en-3-ol (15)

Ester **14** (200 mg, 0.45 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether 1:1) (149 mg, 82%). Mp 89 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.14 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.66 (dd, 1H, H-1′a, *J* = 12.4 Hz, *J* = 2.6 Hz), 2.48 (t, 2H, H-1″, *J* = 7.4 Hz), 2.25 (dd, 1H, H-1′b, *J* = 12.4 Hz, *J* = 8.9 Hz), 2.02 (s, 3H, CH_3CO), 2.00 (m, 1H), 1.92 (m, 1H), 1.98–1.20 (m, 22 H), 1.12 (ddd, 1H, H-1b, *J* = *J* = 13.0 Hz, *J* = 3.5 Hz), 1.07 (d, 3H, H-21, *J* = 6.4 Hz), 0.91 (t, 3H, H-4″, *J* = 7.4 Hz), 0.80 (s, 3H, H-19), 0.56 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 139.3 (8-CH), 117.6 (7-CH), 71.0 (3-CH), 55.3 (CH), 54.8 (CH), 49.3 (CH), 43.5 (13-C), 40.2 (CH), 39.7 (1′-CH_2), 39.2 (CH_2), 37.4 (CH_2), 37.14 (CH), 37.01 (CH_2), 34.2 (10-C), 32.7 (1″-CH_2), 31.9 (CH_2), 31.4 (CH_2), 29.6 (CH_2), 27.9 (CH_2), 22.9 (CH_2), 22.0 (CH_2), 21.5 (CH_2), 18.9 (21-CH_3), 13.7 (4″-CH_3), 13.0 (19-CH_3), 11.9 (18-CH_3). HRMS Found 404.3119 (Calcd 404.3113). IR (KBr, cm^{-1}): 3397, 2953, 2872, 1445, 1381, 1041.

5.4.13. (*R*,*S*)-*S*-Butyl-*S*-[(3*S*,20*S*)-(3-hydroxypregn-7-en-20-yl)-methyl]-*S*-methyl-sulfonium iodide (16) (mixture of diastereomers)

A solution of thioether **15** (70 mg, 0.17 mmol) in iodomethane (CAUTION: iodomethane is cancerogenic!) (1 mL) was stirred for three days at room temperature. The resulting precipitate was filtered off, and triturated one after the other with pentane, diethyl ether, dichloromethane, and finally with methanol. The methanol solution was evaporated to dryness under reduced pressure to afford **16** as a white solid (29 mg, 31%). Mp 252 °C. ^{1}H NMR (MeOD-

d_4, 45 °C, 500 MHz): δ 5.20 (m, 2H, H-7′), 3.51 (m, 2H, H-3′), 3.46–3.33 (m, 6H), 3.15 (m, 2H), 2.94 and 2.92 (s, 6H, CH_3S^+), 2.05 (m, 2H), 1.98 (m, 4H), 1.92–1.13 (m, 44 H), 1.20 (d, 6H, H-21′, J = 6.4 Hz), 1.10 (ddd, 2H, H-1′b, $J = J$ = 13.7 Hz, J = 3.6 Hz), 1.02 (t, 6H, H-4″, J = 7.3 Hz), 0.82 (s, 6H, H-19′), 0.64 (s, 6H, H-18′). ^{13}C NMR (MeOD-d_4, 45 °C, 125 MHz): δ 140.1 (8′-CH), 119.4 (7′-CH), 71.6 (3′-CH), 56.8 (CH), 56.6 (CH), 56.02 (CH), 55.96 (CH), 51.4 (1-CH_2), 50.9 (CH), 50.8 (1-CH_2), 45.2 (13′-C), 43.7 and 42.5 (1″-CH_2), 41.7 (CH), 40.7 (CH_2), 38.7 (CH_2), 38.4 (CH_2), 35.4 (CH, 10′-C), 34.9 (CH), 32.2 (CH_2), 30.8 (CH_2), 28.8 (CH_2), 27.0 (CH_2), 24.3 (CH_3S^+), 24.0 (CH_2), 23.2 (CH_3S^+), 22.6 (CH_2) 19.4 and 19.2 (21′-CH_3), 13.6 (C-4″-CH_3), 13.4 (19′-CH_3), 12.3 (18′-CH_3). HRMS Found 419.3338 (Calcd 419.3342). IR (KBr, cm^{-1}): 3423, 2932, 1636, 1457, 1443, 1379, 1054, 1041.

5.4.14. General procedure C (Wittig reaction)

To a stirred solution of the picolyltriphenylphosphonium chloride in anhydrous THF (0.80 mL/mmol) was added dropwise *n*-butyllithium (1.6 M solution in hexanes, 2.0 equiv) at 0 °C. The reaction mixture was allowed to warm up to ambient temperature over 1 h, and a solution of aldehyde **3** in anhydrous THF (4 mL/mmol) was then added dropwise. The reaction mixture was stirred at room temperature and then quenched with saturated aqueous sodium bicarbonate solution (20 mL). The biphasic mixture was extracted with dichloromethane (3 × 30 mL), the combined organic extracts were dried over sodium sulfate and evaporated to dryness under reduced pressure. The resulting solid was subjected to SCC to afford the vinylpyridines **17a** and **17b** as white solids.

5.4.14.1. (3S,20R)-20-[(E)-2-(Pyridin-2-yl)-ethenyl]-pregn-7-en-3-yl acetate (17a). 2-Picolyltriphenylphosphonium chloride (1.00 g, 2.57 mmol) and aldehyde **3** (478 mg, 1.29 mmol) were treated as described in procedure C. The crude product was subjected to SCC (pentane/diethyl ether 7:3) (488 mg, 85%). Mp 158 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.50 (d, 1H, H-6″, J = 4.2 Hz), 7.58 (ddd, 1H, H-4″, $J = J$ = 8.7 Hz, J = 1.6 Hz), 7.22 (d, 1H, H-3″, J = 7.9 Hz), 7.06 (ddd, 1H, H-5″, J = 7.5 Hz, J = 4.9 Hz, J = 1.1 Hz), 6.59 (dd, 1H, H-1′, J = 15.4 Hz, J = 8.8 Hz), 6.41 (d, 1H, H-2′, J = 15.4 Hz), 5.14 (m, 1H, H-7), 4.68 (m, 1H, H-3), 2.30 (m, 1H), 2.02 (s, 3H, CH_3CO), 1.70–1.20 (m, 21H), 1.15 (d, 3H, H-21, J = 6.7 Hz), 0.81 (s, 3H, H-19), 0.59 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.7 (CO), 156.3 (2″-C), 149.3 (6″-CH), 141.8 (1′-CH), 139.2 (8-CH), 136.4 (4″-CH), 127.5 (2′-CH), 121.4 (5″-CH), 120.9 (3″-CH), 117.5 (7-CH), 73.4 (3-CH), 55.5 (CH), 54.9 (CH), 49.2 (CH), 43.5 (13-C), 40.6 (CH), 40.0 (CH), 39.3 (CH_2), 36.8 (CH_2), 34.2 (10-C), 33.7 (CH_2), 29.5 (CH_2), 28.0 (CH_2), 27.4 (CH_2), 22.8 (CH_2), 21.5 (CH_2), 21.4 (CH_3CO), 20.2 (21-CH_3), 12.9 (19-CH_3), 12.1 (18-CH_3). HRMS Found 447.3149 (Calcd 447.3137). IR (KBr, cm^{-1}): 2946, 2850, 1727, 1585, 1471, 1240, 1032, 970, 766.

5.4.14.2. (3S,20R)-20-[(E)-2-(Pyridin-4-yl)-ethenyl]-pregn-7-en-3-yl acetate (17b). 4-Picolyltriphenylphosphonium chloride (1.04 g, 2.57 mmol) and aldehyde **3** (500 mg, 1.34 mmol) were treated as described in procedure C. The crude product was subjected to SCC (pentane/diethyl ether 4:6) to afford 85 mg (14%) of (*Z*)-isomer. Further elution with pentane/diethyl ether 3:7) afforded 390 mg (65%) of the (*E*)-isomer **17b**. Mp 177 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.48 (d, 2H, H-2″ and H-6″, J = 6.0 Hz), 7.18 (d, 2H, H-3″ and H-5″, J = 6.0 Hz), 6.33 (dd, 1H, H-1′, J = 16.0 Hz, J = 8.5 Hz), 6.25 (d, 1H, H-2′, J = 16.0 Hz), 5.15 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.29 (m, 1H), 2.03 (s, 3H, CH_3CO, with underneath m, 1H, H-12a), 1.85–1.27 (m, 18H), 1.14 (d, 3H, H-21, J = 6.6 Hz, with underneath m, 1H, H-1b), 0.82 (s, 3H, H-19), 0.59 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7 (CO), 149.9 (2 CH), 145.4 (4″-C), 142.1 (1′-CH), 139.1 (8-CH), 125.4 (2′-CH), 120.6 (2 CH), 117.7 (7-CH), 73.4 (3-CH), 55.5 (CH), 54.9 (CH), 49.2 (CH), 43.6 (13-C), 40.8 (CH), 40.0 (CH), 39.3 (CH_2), 36.8 (CH_2), 34.2 (10-C), 33.8 (CH_2), 29.5 (CH_2), 27.9 (CH_2), 27.5 (CH_2), 22.9 (CH_2), 21.4 (CH_2, CH_3CO), 20.1 (21-CH_3), 12.9 (19-CH_3), 12.2 (18-CH_3). HRMS Found 447.3128 (Calcd 447.3137). IR (KBr, cm^{-1}): 2947, 2867, 1733, 1594, 1364, 1244, 1029, 973, 800.

5.4.15. (3S,20R)-20-[2-(Pyridin-2-yl)-(E)-ethenyl]-pregn-7-en-3-ol (18a)

Ester **17a** (250 mg, 0.560 mmol) was treated as described in procedure B. The crude product was subjected to SCC (pentane/diethyl ether gradient from 4:6 to pure diethyl ether) (200 mg, 88%). Mp 209 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.51 (d, 1H, H-6″, J = 5.0 Hz), 7.57 (ddd, 1H, H-4″, $J = J$ = 7.8 Hz, J = 1.6 Hz), 7.22 (d, 1H, H-3″, J = 7.8 Hz), 7.06 (ddd, 1H, H-5″, J = 7.8 Hz, J = 5.0 Hz, J = 1.1 Hz), 6.58 (dd, 1H, H-1′, J = 15.6 Hz, J = 8.7 Hz), 6.41 (d, 1H, H-2′, J = 15.6 Hz), 5.15 (m, 1H, H-7), 3.59 (m, 1H, H-3), 2.30 (m, 1H), 2.02 (ddd, 1H, H-12a, J = 12.1 Hz, $J = J$ = 3.3 Hz), 1.84–1.24 (m, 21H), 1.14 (d, 3H, H-21, J = 6.6 Hz), 1.08 (ddd, 1H, H-1b, $J = J$ = 13.3 Hz, J = 3.2 Hz), 0.80 (s, 3H, H-19), 0.60 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 156.4 (2″-C), 149.4 (6″-CH), 141.7 (1′-CH), 139.3 (8-CH), 136.3 (4″-CH), 127.6 (2′-CH), 121.4 (5″-CH), 120.9 (3″-CH), 117.7 (7-CH), 71.0 (3-CH), 55.6 (CH), 54.9 (CH), 49.5 (CH), 43.5 (13-C), 40.6 (CH), 40.2 (CH), 39.5 (CH_2), 38.0 (CH_2), 37.2 (CH_2), 34.2 (10-C), 31.4 (CH_2), 29.6 (CH_2), 28.0 (CH_2), 23.0 (CH_2), 21.5 (CH_2), 20.2 (21-CH_3), 13.1 (19-CH_3), 12.1 (18-CH_3). HRMS Found 405.3041 (Calcd 405.3032). Anal. Calcd: C, 82.91; H, 9.69; N, 3.45. Found: C, 82.91; H, 9.69; N, 3.45. IR (KBr, cm^{-1}): 3430, 2929, 1649, 1591, 1469, 1052, 971, 776.

5.4.16. (3S,20R)-20-[(E)-2-(Pyridin-4-yl)-ethenyl]-pregn-7-en-3-ol (18b)

Ester **17b** (226 mg, 0.510 mmol) was treated as described in procedure B. The crude product was subjected to SCC (diethyl ether) (190 mg, 92%). Mp 240 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.48 (d, 2H, H-2″ and H-6″, $J = J$ = 6.0 Hz), 7.18 (d, 2H, H-3″ and H-5″, $J = J$ = 6.0 Hz), 6.33 (dd, 1H, H-1′, J = 16.0 Hz, J = 8.5 Hz), 6.25 (d, 1H, H-2′, J = 16.0 Hz), 5.15 (m, 1H, H-7), 4.69 (m, 1H, H-3), 2.29 (m, 1H), 2.03 (s, 1H), 1.85–1.27 (m, 18H), 1.14 (d, 3H, H-21, J = 6.6 Hz, with underneath m, 1H, H-1b), 0.82 (s, 3H, H-19), 0.59 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 147.8 (2 CH), 147.5 (4″-C), 144.1 (1′-CH), 139.0 (8-CH), 125.0 (2′-CH), 121.1 (2 CH), 117.9 (7-CH), 71.0 (3-CH), 55.4 (CH), 54.9 (CH), 49.4 (CH), 43.6 (13-C), 41.0 (CH), 40.2 (CH), 39.4 (CH_2), 37.9 (CH_2), 37.1 (CH_2), 34.2 (10-C), 31.4 (CH_2), 29.6 (CH_2), 27.9 (CH_2), 22.9 (CH_2), 21.5 (CH_2), 20.0 (21-CH_3), 13.0 (19-CH_3), 12.2 (18-CH_3). HRMS Found 405.3011 (Calcd 405.3032). IR (KBr, cm^{-1}): 3282, 2962, 2922, 2848, 1599, 1058, 971.

5.4.17. 2-(3S,20R)-[(E)-2-(3-Hydroxypregn-7-en-20-yl)-ethenyl]-1-methylpyridinium iodide (19a)

To a stirred solution of **18a** (50 mg, 0.13 mmol) in anhydrous THF (2 mL) was added iodomethane (76 μL, 1.23 mmol) (CAUTION: iodomethane is cancerogenic!) at ambient temperature. After six days stirring at room temperature the residue was filtered off and washed with diethyl ether to afford the pure product **19a** as a white solid (34 mg, 48%). Mp 230 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.85 (d, 1H, H-6, J = 6.0 Hz), 8.43 (t, 1H, H-4, $J = J$ = 7.7 Hz), 8.27 (d, 1H, H-3, J = 7.7 Hz), 7.87 (dd, 1H, H-5, J = 7.5 Hz, J = 6.0 Hz), 6.88 (m, 2H, H-1′ and H-2′), 5.14 (m, 1H, H-7″), 4.46 (d, 1H, OH, J = 4.4 Hz), 4.23 (s, 3H, CH_3N^+), 3.33 (under the water peak m, 1H, H-3″), 2.01 (d, 1H, H-12″a, J = 12.0 Hz), 1.84 -1.22 (m, 19H), 1.16 (d, 3H, H-21″, J = 6.6 Hz), 1.02 (ddd, 1H, H-1″b, $J = J$ = 13.7 Hz, J = 3.3 Hz), 0.73 (s, 3H, H-19″), 0.59 (s, 1H, H-18″). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 153.0 (2′-CH), 152.5 (2-C), 145.7 (6-CH), 144.4 (4-CH), 138.7 (8″-CH), 125.0 (3-CH, 5-CH), 118.4 (1′-CH), 117.5 (7″-CH), 68.9 (3″-CH), 54.2 (2 CH), 48.7 (CH),

45.8 (CH_3N^+), 43.3 (13″-C), 40.8 (CH), 39.9 (CH), 38.6 (CH_2), 37.8 (CH_2), 36.6 (CH_2), 33.8 (10″-C), 31.2 (CH_2), 29.2 (CH_2), 27.0 (CH_2), 22.5 (CH_2), 21.0 (CH_2), 19.4 (21″-CH_3), 12.8 (19″-CH_3), 12.0 (18″-CH_3). HRMS Found 420.3260 (Calcd 420.3261). IR (KBr, cm^{-1}): 3363, 2944, 2873, 1621, 1570, 1508, 1458, 1306, 777.

5.4.18. 4-[(3*S*,20*R*)-[(*E*)-2-(3-Hydroxypregn-7-en-20-yl)-ethenyl]-1-methylpyridinium iodide (19b)

To a stirred solution of **18b** (37 mg, 0.09 mmol) in chloroform (1 mL) was added iodomethane (60 μL, 0.91 mmol) (CAUTION: iodomethane is cancerogenic!) at ambient temperature. After four days stirring at ambient temperature the reaction mixture was evaporated under reduced pressure and the crude compound was washed with pentane and diethyl ether to afford the pure product as a white solid (39 mg, 78%). Mp 227 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.80 (d, 2H, H-2 and H-6, J = 6.9 Hz), 8.01 (d, 2H, H-3 and H-5, J = 6.9 Hz), 7.03 (dd, 1H, H-2′, J = 15.6 Hz, J = 8.7 Hz), 6.63 (d, 1H, H-1′, J = 15.6 Hz), 5.12 (m, 1H, H-7″), 4.45 (d, 1H, OH, J = 4.6 Hz), 4.21 (s, 3H, CH_3N^+), 3.32 (under the water peak m, 1H, H-3″), 2.39 (m, 1H), 1.99 (d, 1H, H-12″a, J = 11.9 Hz), 1.81 (m, 1H), 1.73–1.18 (m, 17H), 1.13 (d, 3H, H-21″, J = 6.4 Hz), 1.00 (ddd, 1H, H-1″b, J = J = 13.5 Hz, J = 3.2 Hz), 0.73 (s, 3H, H-19″), 0.56 (s, 3H, H-18″). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 152.7 (4-C), 150.7 (2′-CH), 145.0 (2 CH), 138.7 (8″-CH), 123.5 (1′-CH), 123.2 (2 CH), 117.4 (7″-CH), 68.9 (3″-CH), 54.5 (CH), 54.1 (CH), 48.7 (CH), 46.7 (CH_3N^+), 43.2 (13″-C), 40.5 (CH), 39.4 (CH), 38.6 (CH_2), 37.8 (CH_2), 36.6 (CH_2), 33.7 (10″-C), 31.2 (CH_2), 29.1 (CH_2), 27.0 (CH_2), 22.4 (CH_2), 20.9 (CH_2), 19.4 (21″-CH_3), 12.7 (19″-CH_3), 11.9 (18″-CH_3). HRMS Found 420.3253 (Calcd 420.3261). IR (KBr, cm^{-1}): 3388, 2927, 2870, 1632, 1471, 1193, 1040.

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