3α-FLUORO ANALOGUES OF "ALLOPREGNANOLONE" AND THEIR BINDING TO $GABA_A$ RECEPTORS+

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Dedicated to the memory of late Dr Václav Černý.

(Diethylamino)sulfur trifluoride (DAST) was used for the preparation of 3α-fluorides (*e.g.*, 3α-fluoro-5α-pregnane-12,20-dione, 3α-fluoro-16α-[(methoxycarbonyl)methyl]-5α-pregnan-20-one, methyl 3α-fluoro-5α-androstane-17β-carboxylate, 3α-fluoro-5β-pregnan-20-one) from the corresponding 3β-alcohols and for the preparation of 3,3-difluorides from 3-ketones (*e.g.*, 3,3-difluoro-5α-pregnan-20-one). Boron trifluoride etherate was used for the conversion of an epoxide into 3α-fluoro-2β-hydroxy-5α-pregnan-20-one. The *in vitro* binding of the 3α-fluorides and the corresponding 3α-alcohols to the $GABA_A$ receptor was established using [^{3}H]muscimol and [^{35}S]-*tert*-butylbicyclo[2.2.2]phosphorothionate as ligands.

Keywords: Allopregnanolone; Epalon; Neuroactive steroids; Fluorosteroids; Fluorination; ^{1}H NMR spectroscopy.

Compounds which positively modulate the effects of γ-aminobutyric acid (GABA) – one of the neurotransmitters affecting anxiety, pain, insomnia and drug dependance – have a great therapeutic potential. One receptor of γ-aminobutyric acid ($GABA_A$ receptor) is also modulated by a few metabolites of progesterone. The simplest neurosteroid – 3α-hydroxy-5α-pregnan-20-one (**1**) – is known as "allopregnanolone" or "epalon". It acts[2–4] at concentrations 10 times higher than benzodiazepines and 100 times higher than barbiturates but, at larger concentrations, affects the neurons even in the absence of GABA. Further, it combines both the effects of barbiturates

+ Part CDXIII in the series On Steroids; Part CDXII see ref.[1]

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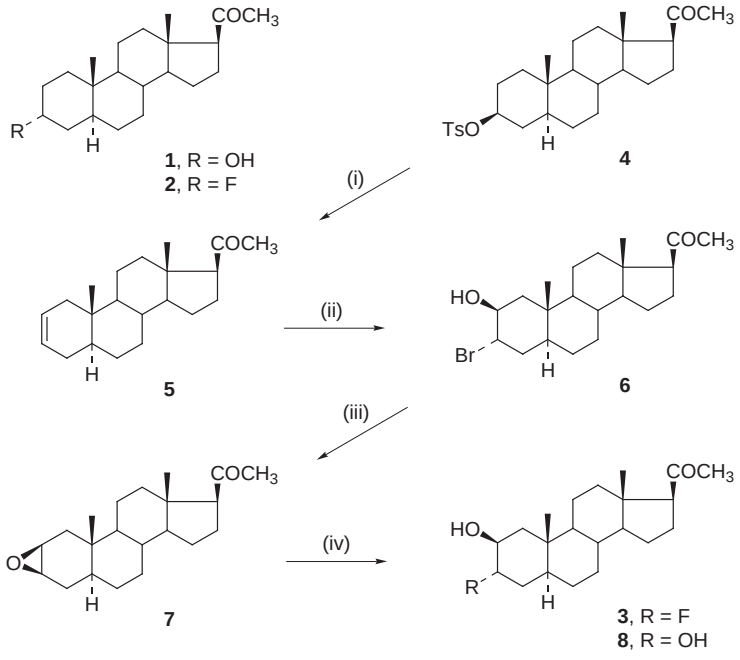
and benzodiazepines as far as the frequency and duration of the chloride ion channel opening are concerned[5]. Yet the steroidal partial agonists of $GABA_A$ receptors are being developed as anxiolytics because they show fewer and less severe side effects compared to conventional benzodiazepines and have a better selectivity for the $GABA_A$ receptor subtypes.

Practical use of compound **1** has been hampered by its very fast metabolism and low solubility in water and, consequently, various new analogues have been designed in order to overcome these drawbacks. Thus, compound **2** exerts a high GABA modulatory activity in spite of having the hitherto essential 3α-hydroxy group replaced with the 3α-fluoro substituent[6]. The well-known strength of the C–F bond yielded a product with a high metabolic stability. However, the presence of the C–F bond also increased the lipophilicity of compound **2**. Striving for an increased hydrophilicity of the products, we modified the structure of fluoride **2** in various positions (*e.g.*, 2, 12, 16 and 17), where the new substituents would not jeopardize the activities of the products.

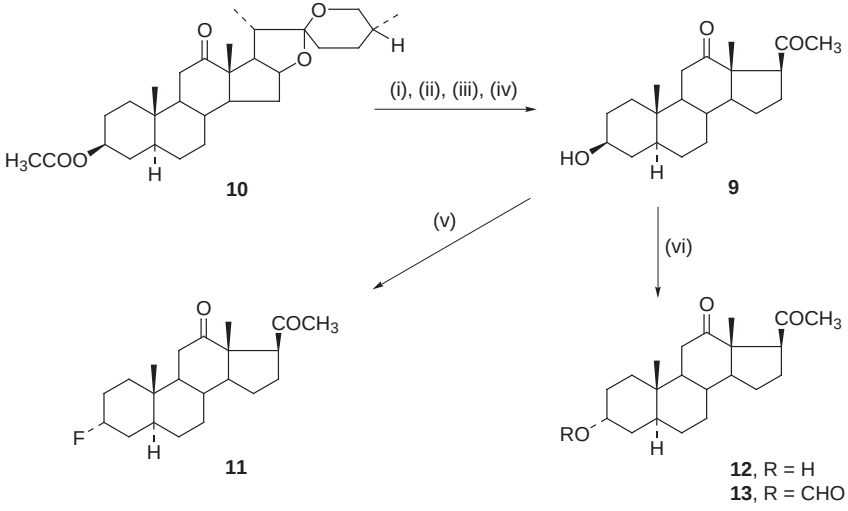


(i) Collidine, Δ; (ii) $HClO_4$, *N*-Bromoacetamide; (iii) KOH; (iv) $BF_3 \cdot Et_2O$

SCHEME 1

The 2β-hydroxy derivative **3** was prepared from 20-oxo-5α-pregnan-3β-yl *p*-tosylate[7] (**4**) *via* 5α-pregn-2-en-20-one[8] (**5**). Hypobromous acid addition yielded diaxial bromohydrin[9] **6** which was converted to epoxide[9] **7**. Compound **7** afforded fluorohydrin **3** together with 2β,3α-diol **8** upon treatment with boron trifluoride etherate (Scheme 1).

Another polar substituent introduced into structure **2** was a 12-oxo group. 3β-Hydroxy-5α-pregnane-12,20-dione[10] (**9**), available from hecogenin acetate[10,11] (**10**), was treated with (diethylamino)sulfur trifluoride (DAST) to yield the expected 3α-fluoro derivative **11**; its ^{1}H NMR spectrum (a doublet of narrow multiplets, $J(3\beta H,3\alpha F)_{gem}$ = 49 Hz) proved the presence of the fluorine atom in an axial position. For the sake of comparison, the 3α-hydroxy derivative **12** was also prepared by subjecting compound **9** to the Mitsunobu reaction and hydrolysis of the corresponding 3α-formate **13** (Scheme 2).



(i) Ac_2O; (ii) CrO_3, AcOH; (iii) KOH, *t*-BuOH; (iv) H_2/Pd; (v) DAST; (vi) PPh_3, diethyl azodicarboxylate, HCOOH

SCHEME 2

Quaternary ammonium salts of carboxylic acids are much more polar compounds. Thus, the 16α-(methoxycarbonyl)methyl derivative[12] **14**, previously used for the preparation of quaternary ammonium salt[12] **15**, was treated with DAST to produce the 3α-fluoro derivative **16**. For the $GABA_A$ receptor tests, the corresponding free acid **17** and its triethylammonium salt **18** were prepared in a standard way.

Similarly, methyl 3β-hydroxyandrost-5-ene-17β-carboxylate[13] (**19**) was hydrogenated to afford the 5α-dihydro derivative[13,14] **20** which was converted into the 3α-fluoro ester **21**, the corresponding free acid **22** and its salts **23** and **24**. The fluoro alcohol **25** was obtained by the reduction of ester **21** with lithium aluminum hydride. Oxidation of alcohol **25** with chromium trioxide yielded another potential neuroactive steroid – the fluoro aldehyde **26**.

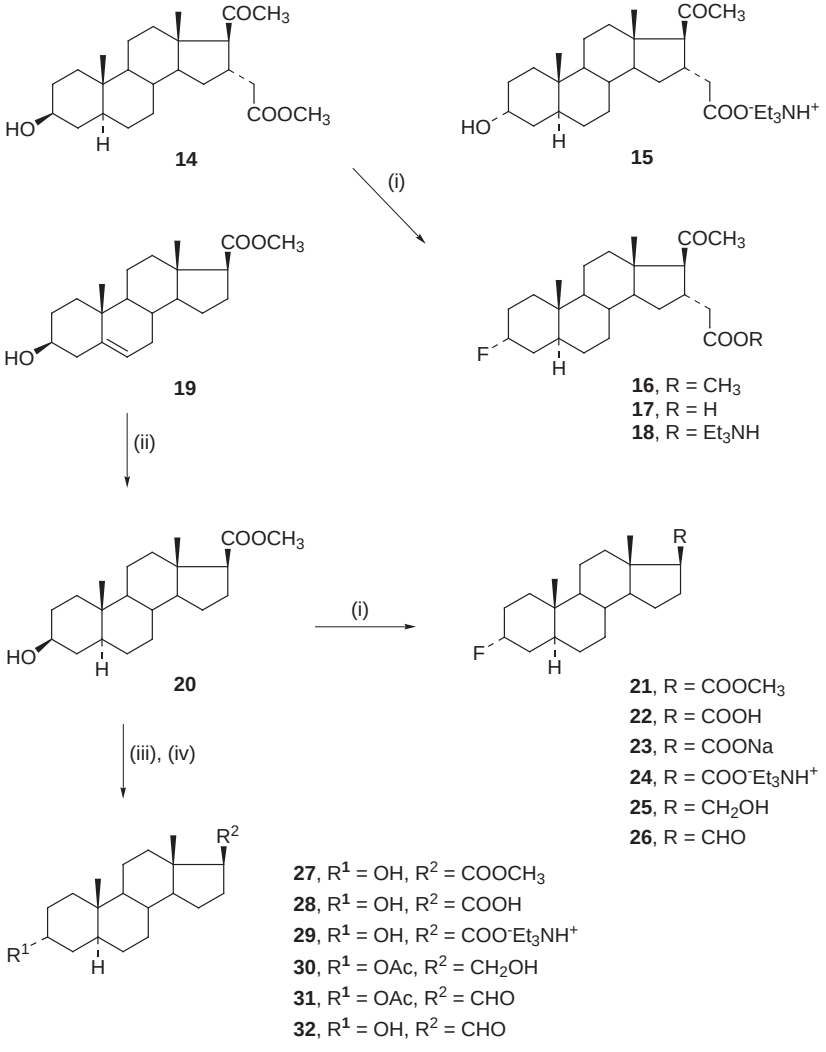
For the purpose of comparison, a similar sequence was carried out in the 3α-hydroxy series: the known[15] 3α-hydroxy ester **27** was hydrolyzed to the free acid[16] **28** and neutralized to yield salt **29**. The hydroxy acid **28** was then acetylated at the 3-hydroxyl, and acylated at the 17-carboxyl with ethyl chloroformate; the intermediate mixed anhydride was reduced with sodium borohydride to yield 3α-acetoxy-20-alcohol **30**. Chromium trioxide oxidation and hydrolysis afforded 3α-acetoxy- and 3α-hydroxy aldehydes **31** and **32** (ref.[17]), respectively (Scheme 3).

Hogenkamp and others[18] have shown that 3β-substituents, introduced into the structure of neurosteroid **1**, improve the anaesthetic properties of such products. In this context, we were interested in the preparation and testing of the 3β-fluoro derivative of compound **2**, *i.e.* the 3,3-difluoro analogue[19] **33**. The target compound was produced from 20-hydroxy-5α-pregnan-3-ones[20] **34** and **35**. The 20-hydroxy groups were protected by benzoylation, and benzoates[21] **36** and **37** were treated with DAST at 80 °C for 8 h. Difluoro benzoates **38** and **39** were hydrolyzed with potassium hydroxide to alcohols **40** and **41** which were oxidized to difluoro ketone **33**.

The anaesthetic properties of allopregnanolone (**1**) are surpassed by its 5β-epimer **42** – another natural neurosteroid. Therefore, we decided to prepare its 3α-fluoro analogue, *i.e.* 3α-fluoro-5β-pregnan-20-one (**43**). It was produced by the reaction of 3β-alcohol[22] **44** with DAST at room temperature (Scheme 4).

The binding of the above 3α-hydroxy- and 3α-fluoro compounds to $GABA_A$ receptors was tested *in vitro* using neural membranes of male rat brains. The specific steroid binding was detected by the increase of the specific [^{3}H]muscimol and decrease of the specific [^{35}S]-*tert*-butylbicyclo-[2.2.2]phosphorothionate (TBPS) binding after application of the tested compounds. The results (see Tables I and II) could be summarized as follows: (i) The impact of the substitution of fluorine for the 3α-hydroxyl does not follow a coherent line. In five cases (compounds **3**, **11**, **21**, **24**, **43**), the muscimol binding of fluoro analogues seems to be strongly reduced when compared with the 3α-alcohols. These data may be due to either the incom-

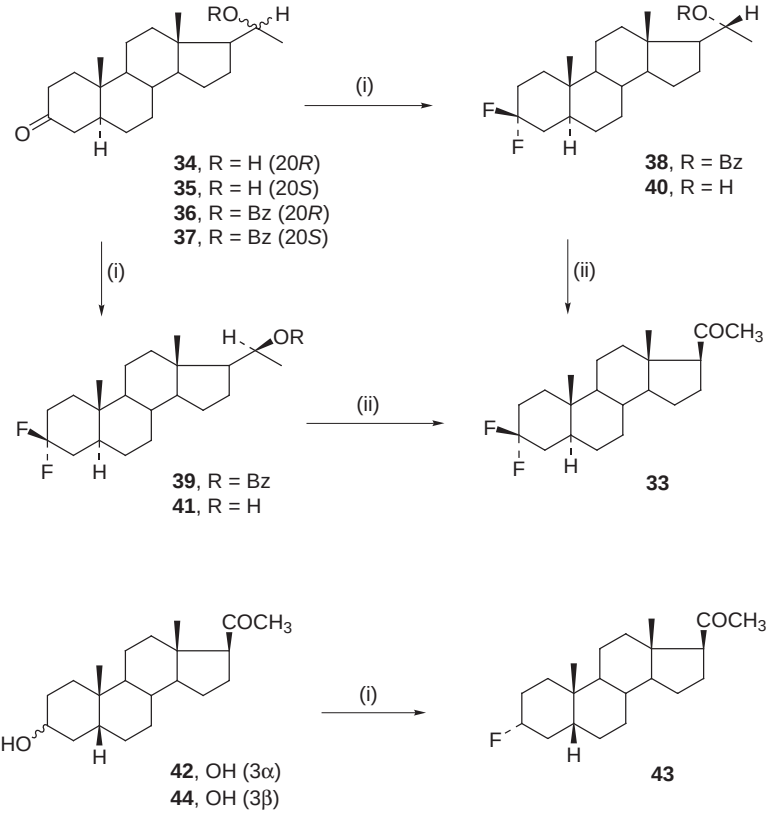
patibility of the substrates with the $GABA_A$ receptor or due to their lower solubility in the testing medium caused by a higher lipophilicity of the fluorides as compared with the corresponding alcohols. Also, fluoride **2** seems to be slightly less active than its hydroxylic counterpart, allopregnanolone (**1**), even though behavioral tests with mice show antiaggressive effects. On the other hand, two fluoro compounds (**18**, **26**) increase the muscimol



(i) DAST; (ii) H_2/Pd; (iii) DCC, CuCl, HCOOH; (iv) KOH

SCHEME 3

binding more profoundly that the corresponding 3α-alcohols **15** and **32**. (ii) Modification of the allopregnanolone structure in positions other than 3α leads to increased binding of muscimol. However, should a good positive modulator of GABA pass both the muscimol and TBPS tests used, only two compounds would be selected: 12-oxo-allopregnanolone (**12**) and a salt of the fluoro derivative (**18**). It is noteworthy that the former is a 3α-alcohol and the latter is a 3α-fluoride.



SCHEME 4

Of all the above compounds, no general rule about the 3α-fluoro analogues of allopregnanolone can be formulated. In some cases, the 3α-fluoro analogue surpasses the GABA-like activity of its 3α-alcohol, in other cases it does not.

EXPERIMENTAL

Melting points were determined on a melting point micro apparatus Boetius (Germany) and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Optical rotations were measured in chloroform ($[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}). IR spectra of chloroform solutions were recorded on a Bruker IFS 88 spectrometer, wavenumbers are given in cm^{-1}. NMR spectra were measured on a FT-NMR spectrometer Varian Unity-200 (at 200 MHz) in $CDCl_3$ with tetramethylsilane as internal reference. Chemical shifts are given in ppm (δ-scale), coupling constants and widths of multiplets in Hz. Unless otherwise stated, the data were interpreted as the first-order spectra. Thin-layer chromatography (TLC) was performed on silica gel (ICN Biochemicals). Preparative TLC (PLC) was car-

TABLE I
Modulatory effect of 3α-hydroxy (R = OH) and 3α-fluoro (R = F) products on $GABA_A$ receptors

Compound	R	[^{3}H]-Muscimol binding		
		EC_{50}[a], nM	E_{max}[b], %	E_{100nM}[c], %
1	OH	154.7	122.6	123.7
2	F	1 000.0	104.1	101.5
8	OH	166.3	186.3	115.2
3	F	[d]	[d]	43.7
12	OH	10.0	147.3	109.1
11	F	[d]	[d]	85.3
15	OH	116.2	126.2	103.8
18	F	99.4	192.8	154.6
27	OH	550	162	178.3
21	F	[d]	[d]	67.4
29	OH	500	144	126.5
24	F	[d]	[d]	71.8
32	OH	[d]	[d]	110.1
26	F	98.1	200.0	127.9
33	F	[d]	[d]	61.1
42	OH	633.3	145.8	110.5
43	F	[d]	[d]	78.9

[a] The steroid concentration producing a half-maximal response. [b] The maximal enhancement of the binding. [c] Related to the DMSO binding. [d] Not measured due to low solubility of the compound.

ried out on 200 × 200 mm plates coated with a 0.7 mm thick layer of the same material. For column chromatography, 60–120 μm silica gel was used. Whenever aqueous solutions of hydrochloric acid, potassium hydrogencarbonate and potassium carbonate were used, their concentration was always 5%. Before evaporation on a rotary evaporator in a vacuum (bath temperature 50 °C), solutions in organic solvents were dried over anhydrous sodium sulfate.

Receptor binding assay. Membranes[23] were preincubated at 37 °C for 10 min in 50 mM Tris-HCl buffer (pH 7.4) with or without steroid. The mixture was incubated with [^{3}H]muscimol (5 nM) and, after 10 min, the reaction was terminated by a rapid vacuum filtration through a Whatman GF/B glass-fiber filter. Nonspecific binding was determined in the presence of 1 mM GABA.

TABLE II
Modulatory effect of 3α-hydroxy (R = OH) and 3α-fluoro (R = F) products on $GABA_A$ receptors

Compound	R	[^{35}S]-TBPS binding		
		IC_{50}[a], nM	I_{max}[b], %	I_{100nM}[c], %
1	OH	80.0	79.0	56.2
2	F	50.0	17.0	90.2
8	OH	[d]	[d]	135.4
3	F	[d]	[d]	[d]
12	OH	50.0	43.0	53.1
11	F	[d]	[d]	[d]
15	OH	40.0	19.0	78.6
18	F	75.0	34.0	76.9
27	OH	[d]	[d]	96.6
21	F	[d]	[d]	[d]
29	OH	[d]	[d]	126.6
24	F	[d]	[d]	[d]
32	OH	[d]	[d]	[d]
26	F	[d]	[d]	111.9
33	F	[d]	[d]	[d]
42	OH	8.0	21.0	62.2
43	F	[d]	[d]	[d]

[a] The steroid concentration producing a half-maximal inhibition. [b] The maximal suppression of the binding. [c] Related to the DMSO binding. [d] Not determined.

Preliminary experiments with 5 nM [^{3}H]muscimol were carried out with 100 nM concentration of steroids (see Table I). Experiments with 2 nM [^{35}S]TBPS were performed only with the 100 nM concentration of steroids (see Table II).

General Procedure for the Conversion of Alcohols to Fluorides

An azeotropic mixture (10 ml) was distilled off from a solution of a steroidal alcohol (1.57 mmol) in dichloromethane (20 ml). (Diethylamino)sulfur trifluoride (DAST, 366 mg, 2.27 mmol) was added to the mixture at room temperature. When the reaction was complete (TLC monitoring), the mixture was diluted with dichloromethane (40 ml) and washed with the potassium hydrogencarbonate solution and water. After evaporation of the solvent, the residue was separated by flash chromatography on a column of silica gel (50 g). Yields varied from 30 to 50%.

5α-Pregn-2-ene-20-one (**5**)

A solution of tosylate[7] **4** (1.10 g, 2.33 mmol) in 2,4,6-trimethylpyridine (11 ml) was heated to reflux for 4 h. The reaction mixture was poured into dilute hydrochloric acid (10%, 35 ml) and the product was extracted with ether. The extract was washed with water, the potassium hydrogencarbonate solution and dried. After evaporation of the solvent, an admixture of 3-ene was removed by partial sulfation[24]. Yield of olefin **5**: 0.545 g (78%), m.p. 125–126 °C (methanol, ref.[8] gives 126–129 °C). ^{1}H NMR: 0.61 s, 3 H (3 × H-18); 0.76 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 5.31 m, 2 H, $W_{1/2}$ = 4 (H-2 and H-3).

3α-Bromo-2β-hydroxy-5α-pregnan-20-one (**6**)

Perchloric acid (1 N, 0.51 ml) was added to a solution of olefin **5** (690 mg, 2.3 mmol) in dioxane (45 ml) at 15 °C upon stirring. *N*-Bromoacetamide (317 mg, 2.1 mmol) was added in 6 portions over 30 min. After an additional 30 min, the mixture was quenched by the addition of a sodium hydrogensulfite solution (10%, 3.3 ml). After 18 h, a precipitate was collected and crystallized from methanol. Yield: 730 mg (80%), m.p. 207–209 °C (ref.[9] recorded 210.5–212 °C). ^{1}H NMR: 0.61 s, 3 H (3 × H-18); 1.00 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 4.35 m, 1 H, $W_{1/2}$ = 6 (H-2); 4.22 m, 1 H, $W_{1/2}$ = 8 (H-3).

2β,3β-Epoxy-5α-pregnan-20-one (**7**)

A solution of potassium hydroxide (100 mg, 1.78 mmol) in a mixture of water (0.3 ml) and methanol (1 ml) was added to a solution of bromohydrin **6** (350 mg, 0.88 mmol) in methanol (40 ml). The reaction mixture was heated to reflux for 2 h. On addition of brine, a precipitate was formed. The solid was collected, washed with water and dried. Crystallization from methanol gave 320 mg (73%) of compound **7**, m.p. 173–175 °C (ref.[9] recorded 174–175.5 °C). ^{1}H NMR: 0.56 s, 3 H (3 × H-18); 0.81 s, 3 H (3 × H-19); 2.09 s, 3 H (3 × H-21); 3.13 m, 2 H, $W_{1/2}$ = 16 (H-2, H-3); 2.49 t, 1 H, J = 9 (H-17).

3α-Fluoro-2β-hydroxy-5α-pregnan-20-one (**3**)

Boron trifluoride diethyl etherate (123 mg, 0.86 mmol) was added to a solution of epoxy compound **7** (70 mg, 0.22 mmol) in a mixture of toluene (3.5 ml) and ether (3.5 ml). After 2 h, the mixture was washed with the potassium carbonate solution and water, and dried.

After evaporation of the solvent, the residue was applied on two preparative TLC plates which were developed with light petroleum–ethyl acetate (8 : 2). The polar zones yielded 26 mg (35%) of compound **3**, m.p. 194–197 °C (acetone–heptane), $[\alpha]_D$ +98 (*c* 1.2). IR: 3 622 (OH), 1 697 (C=O), 1 030 (C–OH), 994 (C–F). ^{1}H NMR: 0.61 s, 3 H (3 × H-18); 0.99 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.53 t, 1 H, *J* = 9 (H-17); 4.10 m, 1 H, $W_{1/2}$ = 15 (H-2); 4.61 dm, 1 H, *J*(F,H-3) = 46 (H-3). For $C_{21}H_{33}FO_2$ (336.5) calculated: 74.96% C, 9.89% H; found: 74.83% C, 9.78% H.

2β,3α-Dihydroxy-5α-pregnan-20-one (**8**)

The less polar zones from the preparative plates of the above experiment gave 31 mg (42%) of compound **8**, m.p. 212–219 °C (ethanol), $[\alpha]_D$ +67 (*c* 1.0). IR: 3 616, 3 390 (OH); 1 697 (C=O); 1 021, 1 003 (C–OH). ^{1}H NMR: 0.61 s, 3 H (H-18); 1.01 s, 3 H (H-19); 2.12 s, 3 H (H-21); 4.15 m, 2 H, $W_{1/2}$ = 19 (H-2, H-3).

3β-Hydroxy-5α-pregnane-12,20-dione (**9**)

Hecogenin acetate (**10**) was converted into compound **9** according to refs[10,11], m.p. 193–196 °C (ref.[10] recorded 189–195 °C). IR: 3 610, 1 035 (OH); 1 694 (C=O); 1 385, 1 360 (CH_3). ^{1}H NMR: 0.90 s, 3 H (3 × H-18); 0.95 s, 3 H (3 × H-19); 2.26 s, 3 H (3 × H-21); 2.50 t, 1 H, *J* = 13 (H-17); 3.31 t, 1 H, *J* = 9 (H-11β); 3.61 m, 1 H, $W_{1/2}$ = 32 (H-3).

3α-Fluoro-5α-pregnane-12,20-dione (**11**)

Following the general procedure, alcohol **9** (100 mg, 0.3 mmol) was treated with DAST (97 mg, 0.60 mmol) in dichloromethane (1 ml) at room temperature for 2 h. Chromatography was carried out using two preparative TLC plates (light petroleum–ether, 7 : 3). Yield of **11**: 36 mg (36%), m.p. 146–149 °C (acetone–heptane), $[\alpha]_D$ +166 (*c* 1.1). IR: 1 702 (C=O), 992 (C–F). ^{1}H NMR: 0.87 s, 3 H (3 × H-18); 0.95 s, 3 H (3 × H-19); 2.27 s, 3 H (3 × H-21); 2.48 t, 1 H, *J* = 13 (H-17); 3.32 t, 1 H, *J* = 9 (H-11β); 4.82 dm, 1 H, *J*(F,H-3) = 49 (H-3). For $C_{21}H_{31}FO_2$ (334.5) calculated: 75.41% C, 9.34% H; found: 75.35% C, 9.26% H.

3α-Hydroxy-5α-pregnane-12,20-dione (**12**)

A solution of potassium hydrogencarbonate (50 mg, 0.50 mmol) in water (1 ml) was added to a solution of formate **13** (96 mg, 0.27 mmol) in methanol (9 ml), and the reaction mixture was heated to reflux for 10 min. The solution was concentrated to a quarter of its original volume, and compound **12** (80 mg, 90%) precipitated upon the addition of brine (5 ml). M.p. 199–201 °C and 229–230 °C (acetone–heptane, 71 mg, 80%), $[\alpha]_D$ +170 (*c* 1.0). ^{1}H NMR: 0.87 s, 3 H (3 × H-18); 0.95 s, 3 H (3 × H-19); 2.27 s, 3 H (3 × H-21); 2.46 t, 1 H, *J* = 13 (H-17); 3.32 t, 1 H, *J* = 9 (H-11β); 4.07 m, 1 H, $W_{1/2}$ = 7 (H-3). IR: 3 616, 3 482, 3 434, 3 390, 1 085 (OH); 1 702 (C=O); 1 383, 1 362 (CH_3). For $C_{21}H_{32}O_3$ (332.5) calculated: 75.86% C, 9.70% H; found: 75.86% C, 9.65% H.

12,20-Dioxo-5α-pregnan-3α-yl Formate (**13**)

From a mixture of compound **9** (120 mg, 0.36 mmol) and triphenylphosphine (300 mg, 1.14 mmol) in benzene (10 ml) and toluene (20 ml), an azeotropic mixture (10 ml) was distilled off. The flask was quickly (within 3 min) cooled in ice-cool water and 10 drops of di-

ethyl azodicarboxylate (1.44 mmol) were dripped in. The yellow solution was stirred for 8 min and then formic acid (3 drops, 1.43 mmol) was added. After 3 h, the mixture was washed successively with the solution of potassium hydrogencarbonate and water, dried and evaporated. The residue was applied on three preparative TLC plates that were developed with a mixture of benzene and ether (3 : 1). The major band (R_F 0.5) was eluted with ether, yielding 105 mg (81%) of formate **13**, m.p. 195–197 °C (acetone), $[\alpha]_D$ +178 (*c* 0.9). ^{1}H NMR: 0.89 s, 3 H (3 × H-18); 0.95 s, 3 H (3 × H-19); 2.26 s, 3 H (3 × H-21); 2.50 t, 1 H, *J* = 13 (H-17); 3.32 t, 1 H, *J* = 9 (H-11β); 5.18 m, 1 H, $W_{1/2}$ = 7 (H-3); 8.05 s, 1 H (CH=O). For $C_{22}H_{32}O_4$ (360.5) calculated: 73.30% C, 8.95% H; found: 73.04% C, 9.01% H.

Methyl (3α-Fluoro-20-oxo-5α-pregnan-16α-yl)acetate (**16**)

Following the general procedure, a solution of ester **14** (ref.[12], 1.0 g, 2.55 mmol) in dichloromethane (20 ml) was treated with DAST for 18 h. Chromatography of the product was carried out on a column of silica gel (100 ml). Light petroleum–ether (9 : 1) eluted compound **16** (0.236 g, 23%), m.p. 140–142 °C (acetone–heptane), $[\alpha]_D$ +57 (*c* 1.3). IR: 1 731, 1 438, 1 161 ($COOCH_3$); 1 701, 1 358 (CH_3CO); 991 (C–F). ^{1}H NMR: 0.63 s, 3 H (3 × H-18); 0.78 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.21 dd, 1 H, *J* = 7 and *J*′ = 14 (H-16a$_1$); 2.30 dd, 1 H, *J* = 7 and *J*′ = 14 (H-16a$_2$); 2.39 d, 1 H, *J* = 9 (H-17); 3.62 s, 3 H (OCH_3); 4.81 dm, 1 H, *J*(F,H-3) = 49 (H-3). For $C_{24}H_{37}FO_3$ (392.6) calculated: 73.43% C, 9.50% H; found: 73.27% C, 9.38% H.

(3α-Fluoro-20-oxo-5α-pregnan-16α-yl)acetic Acid (**17**)

Ester **16** (238 mg, 0.61 mmol) was hydrolyzed with a solution of hydrochloric acid (17%, 4 ml) in acetone (20 ml) at room temperature. After 48 h, the mixture was concentrated to a quarter of its original volume and diluted with dichloromethane (20 ml). The resultant solution was washed with brine and dried. Evaporation of the solvent and crystallization of the residue from acetone–heptane afforded acid **17** (141 mg, 61%), m.p. 183–185 °C, $[\alpha]_D$ +69 (*c* 1.3). IR: 3 515, 1 743 (COOH); 984 (C–F). ^{1}H NMR: 0.64 s, 3 H (3 × H-18); 0.78 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 2.30 d, 2 H, *J* = 8 (H-16a); 2.38 d, 1 H, *J* = 9 (H-17); 3.00 m, 1 H, $W_{1/2}$ = 24 (H-16); 4.81 dm, 1 H, *J*(F,H-3) = 48 (H-3). For $C_{23}H_{35}FO_3$ (378.5) calculated: 72.98% C, 9.32% H; found: 72.84% C, 9.23% H.

Triethylammonium (3α-Fluoro-20-oxo-5α-pregnan-16α-yl)acetate (**18**)

A solution of carboxylic acid **17** (200 mg, 0.53 mmol) in triethylamine (16 ml) was heated to reflux for 20 min. The solvent was evaporated and the residue crystallized from ether at –60 °C, yielding compound **18** (127 mg, 50%), m.p. >300 °C (decomp.). IR: 1 702 (C=O), 1 407 (COO^-), 984 (C–F). ^{1}H NMR: 0.64 s, 3 H (3 × H-18); 0.78 s, 3 H (3 × H-19); 1.24 t, 9 H, *J* = 7 (3 × C**H**$_3$-CH_2); 2.12 s, 3 H (3 × H-21); 2.22 d, 2 H, *J* = 8 (2 × H-16a); 2.40 d, 1 H, *J* = 9 (H-17); 2.96 m, 1 H, $W_{1/2}$ = 24 (H-16); 3.00 q, 6 H, *J* = 8 (3 × CH_2-N); 4.81 dm, 1 H, *J*(F,H-3) = 48 (H-3). For $C_{29}H_{50}FNO_3$ (479.7) calculated: 72.61% C, 10.51% H, 2.92% N; found: 72.52% C, 10.38% H, 2.84% N.

Methyl 3β-Hydroxy-5α-androstane-17β-carboxylate (**20**)

Compound **20** was prepared by a palladium-catalyzed hydrogenation of methyl 3β-hydroxy-androst-5-ene-17β-carboxylate (**19**; 10.0 g, 30.1 mmol) in methanol (400 ml) according to ref.[14], m.p. 168–173 °C (methanol, ref.[13] gives 172 °C, ref.[14] gives 168–171 °C). ^{1}H NMR: 0.64 s, 3 H (3 × H-18); 0.81 s, 3 H (3 × H-19); 2.33 t, 1 H, *J* = 9 (H-17); 3.59 m, 1 H, $W_{1/2}$ = 28 (H-3); 3.66 s, 3 H (OCH_3).

Methyl 3α-Fluoro-5α-androstane-17β-carboxylate (**21**)

Following the general procedure, alcohol **20** (1.0 g, 2.99 mmol) was treated with DAST (0.702 g, 4.36 mmol) in dichloromethane (20 ml) for 18 h. The product crystallized without chromatographic purification. Yield: 0.392 g (39%), m.p. 139–141 °C (light petroleum), $[\alpha]_D$ +50 (*c* 1.3). IR: 1 725, 1 198 (COO); 989 (C–F). ^{1}H NMR: 0.65 s, 3 H (3 × H-18); 0.78 s, 3 H (3 × H-19); 2.33 t, 1 H, *J* = 9 (H-17); 3.66 s, 3 H (OCH_3); 4.81 dm, 1 H, *J*(F,H-3) = 49 (H-3). For $C_{21}H_{33}FO_2$ (336.5) calculated: 74.96% C, 9.89% H; found: 74.81% C, 9.70% H.

3α-Fluoro-5α-androstane-17β-carboxylic Acid (**22**)

A solution of potassium hydroxide (570 mg, 10.18 mmol) in water (1.5 ml) was added to a solution of ester **21** (570 mg, 1.70 mmol) in dioxane (10.4 ml) and the mixture was heated at reflux for 8 h. The mixture was diluted with brine (20 ml), acidified with dilute hydrochloric acid and the precipitate formed was extracted with ethyl acetate. After removing the solvent, the residue crystallized from acetone–heptane yielding acid **22** (170 mg, 31%). Chromatography of mother liquor on five preparative plates afforded an additional crop (340 mg, 62%) of compound **22**, m.p. 230–231 °C, $[\alpha]_D$ +27 (*c* 1.2). IR: 3 514, 1 732, 1 428 (COOH); 990 (C–F). ^{1}H NMR: 0.72 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 2.40 t, 1 H, *J* = 9 (H-17); 4.81 dm, 1 H, *J*(F,H-3) = 49 (H-3). For $C_{20}H_{31}FO_2$ (322.5) calculated: 74.50% C, 9.69% H; found: 74.46% C, 9.60% H.

Sodium 3α-Fluoro-5α-androstane-17β-carboxylate (**23**)

A solution of acid **22** (54 mg, 0.17 mmol) in methanol (1 ml) was treated with a methanolic solution of sodium methoxide (0.87 M, 0.2 ml, 0.17 mmol). After 1 h, the solvent was evaporated and the residue was triturated with ether. The mixture was filtered, the filtrate was evaporated; the residue (**23**; 52 mg, 90%) did not melt below 300 °C, $[\alpha]_D$ +36 (*c* 1.0, CH_3OH–$CHCl_3$, 1 : 1). IR spectrum (KBr): 1 552, 1 414, 678, 655, 607 (COO^-); 1 383 (CH_3); 987 (C–F). ^{1}H NMR ($CDCl_3$ + CD_3OD): 0.68 s, 3 H (3 × H-18); 0.78 s, 3 H (3 × H-19); 2.21 t, 1 H, *J* = 8.8 (H-17); 4.81 dm, 1 H, *J*(F,H-3) = 49 (H-3). For $C_{20}H_{30}FNaO_2$ (344.4) calculated: 69.74% C, 8.78% H; found: 69.47% C, 8.51% H.

Triethylammonium 3α-Fluoro-5α-androstane-17β-carboxylate (**24**)

A solution of acid **22** (50 mg, 0.16 mmol) in triethylamine (4.0 ml, 28.7 mmol) was heated to reflux for 20 min. On cooling, salt **24** crystallized from the reaction mixture. Yield: 51 mg (78%), m.p. 243–254 °C, $[\alpha]_D$ +34 (*c* 1.2). IR: 2 476 (Et_3NH^+); 1 700, 1 594 (COO^-); 989 (C–F). ^{1}H NMR: 0.71 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 1.16 t, 9 H, *J* = 7 (3 × C**H**$_3$-CH_2); 2.34 t, 1 H, *J* = 9 (H-17); 2.85 q, 6 H, *J* = 7 (3 × CH_2-N); 4.81 dm, 1 H, *J*(F,H-3) =

49 (H-3). For $C_{26}H_{46}FNO_2$ (423.7) calculated: 73.71% C, 10.94% H, 3.31% N; found: 73.48% C, 10.70% H, 3.09% N.

3α-Fluoro-21-nor-5α-pregnan-20-ol (**25**)

Compound **21** (60 mg, 0.18 mmol) was reduced with lithium aluminum hydride (7.8 mg, 0.21 mmol) in tetrahydrofuran (0.5 ml) at room temperature. After 24 h, the excess reagent was decomposed with moist ether and with a saturated aqueous solution of sodium sulfate. The mixture was saturated with anhydrous sodium sulfate and the solution was filtered. On removal of the solvent from the filtrate, compound **25** (45 mg, 82%) was obtained, m.p. 146–148 °C (acetone–heptane), $[\alpha]_D$ +8 (*c* 1.2). IR: 3 622, 3 457 (OH); 990 (C–F). ^{1}H NMR: 0.64 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 3.54 dd, 1 H, *J* = 7 and *J*′ = 10 (H-20a); 3.72 dd, 1 H, *J* = 7 and *J*′ = 10 (H-20b); 4.81 dm, 1 H, *J*(F,H-3) = 49 (H-3). For $C_{20}H_{33}FO$ (308.5) calculated: 77.87% C, 10.78% H; found: 77.73% C, 10.71% H.

3α-Fluoro-21-nor-5α-pregnan-20-al (**26**)

Pyridine (0.1 ml) was added dropwise at 0 °C in argon atmosphere to a stirred suspension of chromium(VI) oxide (62 mg, 0.62 mmol) and anhydrous magnesium sulfate (50 mg, 0.42 mmol) in dichloromethane (1.75 ml). After stirring at 0 °C for 30 min, a solution of alcohol **25** (45 mg, 0.15 mmol) in dichloromethane (0.9 ml) was added. The mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. The mixture was diluted with light petroleum (10 ml) and filtered through a column of alumina (10 g) which was then washed with the same solvent (20 ml). The combined filtrates were concentrated in vacuum. Pyridine was removed from the residue by coevaporation with toluene. Yield of aldehyde **26**: 26 mg (58%), m.p. 136–137 °C (acetone–heptane, under argon), $[\alpha]_D$ +59 (*c* 1.1). IR: 2 820, 2 725 (C–H in CHO); 1 713 (C=O); 990 (C–F). ^{1}H NMR: 0.75 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 4.81 dm, 1 H, *J*(F,H-3) = 49 (H-3); 9.77 d, 1 H, *J* = 2.2 (CH=O). For $C_{20}H_{31}FO$ (306.5) calculated: 78.38% C, 10.20% H; found: 78.27% C,10.11% H.

3α-Hydroxy-5α-androstane-17β-carboxylic Acid (**28**)

Potassium hydroxide (1.0 g, 17.82 mmol) in water (2.5 ml) was added to a solution of methyl 3α-hydroxy-5α-androstane-17β-carboxylate[15] **27** (1.0 g, 2.99 mmol) in ethylene glycol (15 ml) and the reaction mixture was heated at reflux for 20 min. The mixture was poured into brine with hydrochloric acid and the product was extracted with ethyl acetate, washed with water, and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded carboxylic acid **28** (0.88 g, 92%), m.p. 282–284 °C (ref.[16] gives 282–285 °C). IR: 3 615 (OH); 3 512, 2 666 (COOH); 1 737, 1 700 (C=O); 1 002 (C–OH). ^{1}H NMR: 0.72 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 2.39 t, 1 H, *J* = 9.4 (H-17); 4.05 m, 1 H, $W_{1/2}$ = 6.8 (H-3).

Triethylammonium 3α-Hydroxy-5α-androstane-17β-carboxylate (**29**)

A solution of carboxylic acid **28** (120 mg, 0.37 mmol) was heated at reflux with triethylamine (10 ml) for 2 h. The solvent was removed in a vacuum and the residue was crystallized from acetone–heptane, yield 56 mg (35%), m.p. 266–268 °C. IR: 3 289 (OH); 1 740, 1 408 (COO^-); 1 004 (C–OH). ^{1}H NMR: 0.71 s, 3 H (3 × H-18); 0.78 s, 3 H (3 × H-19); 1.20 t, 9 H, *J* = 7.3 (3 × C**H**$_3$-CH_2); 2.37 t, 1 H, *J* = 9.4 (H-17); 2.92 q, 6 H, *J* = 7.3 (3 × CH_2-N); 4.04

m, 1 H, $W_{1/2}$ = 6.8 (H-3). For $C_{26}H_{47}NO_3$ (421.7) calculated: 74.06% C, 11.24% H, 3.32% N; found: 73.89% C, 11.08% H, 3.19% N.

3α-Acetoxy-21-nor-5α-pregnan-20-ol (**30**)

Acetic anhydride (0.90 g, 8.82 mmol) was added to a solution of carboxylic acid **28** (100 mg, 0.31 mmol) in pyridine (1.5 ml) and the mixture was allowed to stand at room temperature for 18 h. The mixture was then poured in ice-cool water with hydrochloric acid. The product was extracted with ethyl acetate, washed with water and dried over sodium sulfate. After evaporation to dryness, the residue (90 mg) and triethylamine (0.04 ml) were dissolved in tetrahydrofuran (1.4 ml) and cooled to 0 °C. Ethyl chloroformate (0.02 ml, 0.2 mmol) was added, followed with a solution of sodium borohydride (47 mg, 1.24 mmol) in water (1.75 ml) after 20 min. After standing at room temperature for 2 h, the mixture was poured into water acidified by hydrochloric acid. The product was extracted with ether, washed successively with dilute hydrochloric acid, water, the saturated solution of potassium hydrogencarbonate, water and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded alcohol **30** (60 mg, 54%), m.p. 100–102 °C (acetone). IR: 3 623, 3 509 (OH); 1 726, 1 714 (C=O); 1 266, 1 251, 1 241, 1 020 (C–O); 1 061 (C–OH). ^{1}H NMR: 0.64 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 2.05 s, 3 H (CH_3COO); 3.56 dd, 1 H, J = 7 and J' = 10 (H-20A); 3.72 dd, 1 H, J = 7 and J' = 10 (H-20B); 5.01 m, 1 H, $W_{1/2}$ = 7 (H-3). For $C_{22}H_{36}O_3$ (348.5) calculated: 75.82% C, 10.41% H; found: 75.70% C, 10.36% H.

3α-Acetoxy-21-nor-5α-pregnan-20-al (**31**)

Pyridine (0.2 ml) was added dropwise at 0 °C in argon atmosphere to a stirred suspension of chromium(VI) oxide (124 mg, 1.24 mmol) and anhydrous magnesium sulfate (100 mg, 0.84 mmol) in dichloromethane (3.5 ml). After stirring at 0 °C for 30 min, a solution of alcohol **30** (100 mg, 0.29 mmol) in dichloromethane (2.0 ml) was added. The mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. The mixture was diluted with hexane (20 ml) and filtered through a column of alumina (20 g) which was then washed with the same solvent (30 ml). The combined filtrates were concentrated in a vacuum. Pyridine was removed from the residue by coevaporation with toluene. Yield of aldehyde 70 mg (70%), m.p. 118–120 °C (acetone–heptane). IR: 2 820, 2 724 (CH=O); 1 724, 1 714 (C=O); 1 266, 1 251, 1 241 (C–O). ^{1}H NMR: 0.75 s, 3 H (3 × H-18); 0.80 s, 3 H (3 × H-19); 2.05 s, 3 H (CH_3COO); 5.01 m, 1 H, $W_{1/2}$ = 7 (H-3); 9.77 d, 1 H, J = 2.0 (CH=O). For $C_{22}H_{34}O_3$ (346.5) calculated: 76.26% C, 9.89% H; found: 76.29% C, 9.81% H.

3α-Hydroxy-21-nor-5α-pregnan-20-al (**32**)

Potassium carbonate (33.5 mg, 0.24 mmol) was added to a solution of aldehyde **31** (70 mg, 0.20 mmol) in methanol (3.4 ml). The reaction mixture was allowed to stand at room temperature for 18 h. The mixture was then neutralized with brine containing hydrochloric acid and the product was extracted with chloroform. The organic phase was washed with water and dried over anhydrous sodium sulfate. Evaporation of the solvent and chromatography of the residue on two preparative plates afforded the starting compound **31** (33 mg) and alcohol **32** (31 mg, 50%), m.p. 136–138 °C (acetone–heptane) (ref.[17] gives 137–138 °C). IR: 3 615 (OH), 2 725 (C–H in CHO), 1 715 (C=O). ^{1}H NMR: 0.74 s, 3 H (3 × H-18); 0.79 s, 3 H (3 × H-19); 4.05 m, 1 H, $W_{1/2}$ = 7 (H-3); 9.77 d, 1 H, J = 1.8 (CH=O).

3,3-Difluoro-5α-pregnan-20-one (**33**)

A) A solution of (20*R*)-3,3-difluoro-5α-pregnan-20-ol (**40**; 50 mg, 0.15 mmol) in acetone (1 ml) was oxidized with Jones reagent (1 ml) until purple colour of the mixture persisted for 2 min. After standing at room temperature for 5 min, the excess reagent was reduced with 2-propanol (2 ml) and the mixture was poured into water. The product was extracted with ether, washed with water, the potassium hydrogencarbonate solution, water and dried. Evaporation of the solvent gave compound **33** that crystallized from acetone–heptane, yield 25 mg (50%), m.p. 138–140 °C (ref.[19] gives 145–147 °C), $[\alpha]_D$ +87 (*c* 1.2). IR: 1 698, 1 362 ($COCH_3$); 1 092 (C–F). ^{1}H NMR: 0.61 s, 3 H (3 × H-18); 0.83 s, 3 H (3 × H-19); 2.11 s, 3 H (3 × H-21); 2.52 t, 1 H, *J* = 9 (H-17).

B) Analogously, (20*S*)-3,3-difluoro-5α-pregnan-20-ol (**41**; 50 mg, 0.15 mmol) yielded ketone **33** (32 mg, 64%), identical with the sample prepared above.

(20*R*)-3-Oxo-5α-pregnan-20-yl Benzoate (**36**)

The benzoate **36** was prepared from (20*R*)-20-hydroxy-5α-pregnan-3-one[20] (**34**) in the same way as compound **37**, m.p. 183–185 °C (ethanol) (ref.[21] gives 184-185 °C). ^{1}H NMR: 0.68 s, 3 H (3 × H-18); 0.96 s, 3 H (3 × H-19); 1.26 d, 3 H, *J* = 6 (3 × H-21); 5.13 m, 1 H, $W_{1/2}$ = 21 (H-20); 7.48 m, 3 H and 8.05 m, 2 H (benzoate).

(20*S*)-3-Oxo-5α-pregnan-20-yl Benzoate (**37**)

Benzoyl chloride (0.5 ml, 4.3 mmol) was added to a solution of (20*S*)-20-hydroxy-5α-pregnan-3-one[20] (**35**; 400 mg, 1.26 mmol) in pyridine (0.5 ml) and the reaction mixture was left standing at room temperature. After 20 h, the mixture was poured into hot water and the product was extracted with chloroform. The extract was successively washed with water, dilute hydrochloric acid, water, the potassium hydrogencarbonate solution and dried. After evaporation of the solvent, the residue crystallized from ethanol yielding benzoate **37** (420 mg, 79%), m.p. 185–187 °C. IR: 1 706, 1 284 (COO); 1 378 (CH_3); 3 026, 1 603, 1 451 (arom.). ^{1}H NMR: 0.74 s, 3 H (3 × H-18); 1.01 s, 3 H (3 × H-19); 1.35 d, 3 H, *J* = 6 (3 × H-21); 5.16 m, 1 H, $W_{1/2}$ = 21 (H-20); 7.48 m, 3 H and 8.05 m, 2 H (benzoate). For $C_{28}H_{38}O_3$ (422.6) calculated: 79.58% C, 9.06% H; found: 79.50% C, 8.94% H.

(20*R*)-3,3-Difluoro-5α-pregnan-20-yl Benzoate (**38**)

(20*R*)-Benzoate **36** (118 mg, 0.28 mmol) was treated with DAST (1.22 g, 7.57 mmol) in the same way as in the preparation of compound **39**. Yield of compound **38** was 55 mg (44%), m.p. 153–154 °C (methanol), $[\alpha]_D$ –21 (*c* 1.3). IR: 1 705, 1 282, 960 (COO); 1 376 (CH_3); 3 031, 1 603, 1 452, 1 002 (arom.); 1 093 (C–F). ^{1}H NMR: 0.66 s, 3 H (3 × H-18); 0.78 s, 3 H (3 × H-19); 1.26 d, 3 H, *J* = 6 (3 × H-21); 5.13 m, 1 H, $W_{1/2}$ = 18 (H-20); 7.48 m, 3 H and 8.05 m, 2 H (benzoate). For $C_{28}H_{38}F_2O_2$ (444.6) calculated: 75.64% C, 8.61% H; found: 75.50% C, 8.49% H.

(20*S*)-3,3-Difluoro-5α-pregnan-20-yl Benzoate (**39**)

Benzoate **37** (100 mg, 0.24 mmol) in a glass tube was dissolved in DAST (1.22 g, 7.57 mmol). The tube was sealed and heated to 80 °C. After 8 h, the tube was cooled to 0 °C and opened. The mixture was diluted with dichloromethane (8 ml), and the solution was

washed with the potassium hydrogencarbonate solution (30 ml) and with water. The solvent was evaporated in a vacuum and the residue was chromatographed on a column of silica gel (10 ml). Elution with a mixture of light petroleum–ether (98 : 2) and crystallization yielded compound **39** (50 mg, 47%), m.p. 189–191 °C (methanol), $[\alpha]_D$ +35 (*c* 1.3). IR: 1 707, 1 284, 964 (COO); 1 375 (CH_3); 3 031, 1 603, 1 451 (arom.); 1 093 (C–F). ^{1}H NMR: 0.72 s, 3 H (3 × H-18); 0.83 s, 3 H (3 × H-19); 1.35 d, 3 H, *J* = 6 (3 × H-21); 5.18 m, 1 H, $W_{1/2}$ = 16 (H-20); 7.48 m, 3 H and 8.05 m, 2 H (benzoate). For $C_{28}H_{38}F_2O_2$ (444.6) calculated: 75.64% C, 8.61% H; found: 75.48% C, 8.53% H.

(20*R*)-3,3-Difluoro-5α-pregnan-20-ol (**40**)

Benzoate **38** (157 mg, 0.35 mmol) was hydrolyzed in a solution of potassium hydroxide in ethanol (5%, 15 ml) at reflux temperature. After 3 h, the mixture was concentrated in a vacuum to a half of its original volume and poured into brine. The precipitate was extracted with chloroform, and the organic phase was washed with the solution of hydrochloric acid, water, the potassium hydrogencarbonate solution and water. The solution was dried and the solvent removed. Crystallization afforded compound **40** (57 mg, 47%), m.p. 173–174 °C (acetone–heptane), $[\alpha]_D$ +3 (*c* 1.5). IR: 3 610, 3 464, 1 102 (OH); 1 374 (CH_3); 1 092 (C–F). ^{1}H NMR: 0.75 s, 3 H (3 × H-18); 0.83 s, 3 H (3 × H-19); 1.13 d, 3 H, *J* = 6 (3 × H-21); 3.72 m, 1 H, $W_{1/2}$ = 18 (H-20). For $C_{21}H_{34}F_2O$ (340.5) calculated: 74.08% C, 10.06% H; found: 73.91% C, 9.89% H.

(20*S*)-3,3-Difluoro-5α-pregnan-20-ol (**41**)

Benzoate **39** (50 mg, 0.11 mmol) was hydrolyzed in the same way as above to yield compound **41** (22 mg, 57%), m.p. 158–159 °C (acetone–heptane), $[\alpha]_D$ +12 (*c* 1.4). IR: 3 615, 3 464, 1 072 (OH); 1 374 (CH_3); 1 093 (C–F). ^{1}H NMR: 0.66 s, 3 H (3 × H-18); 0.83 s, 3 H (3 × H-19); 1.24 d, 3 H, *J* = 6 (3 × H-21); 3.70 m, 1 H, $W_{1/2}$ = 16 (H-20). For $C_{21}H_{34}F_2O$ (340.5) calculated: 74.08% C, 10.06% H; found: 73.92% C, 9.84% H.

3α-Fluoro-5β-pregnan-20-one (**43**)

Following the general procedure, 3β-hydroxy-5β-pregnan-20-one[22] (**44**; 376 mg, 1.18 mmol) was treated with DAST (366 mg, 2.27 mmol) in dichloromethane (8 ml) at room temperature for 2 h. Chromatography was carried out on a column of silica gel (20 g). Elution with a mixture of light petroleum–ether (96 : 4) yielded 102 mg of compound **43** (27%), m.p. 107–108 °C (acetone–heptane), $[\alpha]_D$ +106 (*c* 1.2). IR: 1 698 (C=O), 1 002 (C–F). ^{1}H NMR: 0.60 s, 3 H (3 × H-18); 0.93 s, 3 H (3 × H-19); 2.12 s, 3 H (3 × H-21); 4.52 dm, 1 H, *J*(F,H-3) = 49 (H-3). For $C_{21}H_{33}FO$ (320.5) calculated: 78.70%C, 10.38% H; found: 78.63% C, 10.29% H.

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