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^{1}H,^{13}C, ^{19}F NMR, and ESI mass spectral characterization of two geminal difluorosteroids

Elina Sievänen,[a]* Virpi Noponen,[a] Vladimír Král,[b,c] Tomáš Bříza[b] and Erkki Kolehmainen[a]

Two geminal difluorosteroids, 3,3-difluoro-5β-cholan-24-oic acid (1) and 3,3-difluoro-5α-androstan-17-one (2), have been prepared from corresponding ketosteroids with diethylaminosulphurtrifluoride (DAST) treatment in moderate yields. The structures of 1 and 2 have been characterized by ^{1}H, ^{13}C, ^{19}F NMR, and ESI mass spectral techniques. Copyright © 2008 John Wiley & Sons, Ltd.

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Introduction

Advances in fluorinated pharmaceuticals based on natural products, such as steroids, have been reviewed recently.[1] It is known for long that fluorosteroids are physiologically and pharmaceutically active substances.[2–6] There exist also some papers on fluorinated bile acids[7–12] and on fluorinated androstane derivatives.[13,14] In addition, useful articles on fluorine substituent chemical shifts (SCS) in fluorinated cyclohexanes and related compounds are available.[15] Recently, it has been shown that interfacial properties of fluorinated bile acids deviate markedly from the natural congeners.[8] However, the pharmacological utility and possible use of fluorinated bile acids as biological labeling agents are yet to be fully explored.[8] These two arguments were the principal motivating factors for our interest toward novel fluorinated bile acids. Synthesis of fluorinated steroids using elemental fluorine owing to its extreme high reactivity is not a very safe approach,[12] whereas diethylaminosulphurtrifluoride (DAST) is a more user-friendly alternative as described, for example, in synthesis of 3,3-difluoro-5α-androstane and 4,4-difluorocholestan-3β-ol.[9] In this study, we report a facile synthesis and ^{1}H, ^{13}C, ^{19}F NMR, and ESI mass spectral characterization of two geminal difluoro derivatives, which may find use, for example, in surface chemical and pharmacological applications.

Results and Discussion

The synthetic routes leading to 3,3-difluoro-5β-cholan-24-oic acid (**1**) and 3,3-difluoro-5α-androstan-17-one (**2**) are depicted in Schemes 1 and 2, respectively.

For the structural characterization of **1** and **2** ^{1}H, ^{13}C, ^{13}C DEPT-135, ^{19}F, PFG ^{1}H, ^{13}C HMQC, and ^{1}H, ^{13}C HMBC NMR spectra as well as electrospray ionization-time of flight (ESI-TOF) mass spectra were recorded.

The ^{1}H NMR spectrum of **1** reveals the characteristic signals of the three steroidal methyl protons at carbons 18, 21, and 19 at δ 0.66 (singlet), 0.93 (doublet), and 0.97 ppm (singlet), respectively. The methylene protons at carbon 23, δ = 2.27 and 2.40 ppm, form a part of an AA'BB'-spin system with the geminal protons at C-22. The ^{1}H NMR chemical shift assignments of **1** are given in Table 2.

^{19}F NMR spectrum of **1** shows only one large doublet with strongly broadened signals at δ −88.1 ppm (from external $CFCl_3$) with a $^{2}J(^{19}F,^{19}F)$ = 232.9 Hz coupling. The signal is assumed to originate from the fluorine atom in the equatorial position (or at β-site) at C-3 in the cyclohexane ring A of steroid skeleton, since the vicinal coupling constants $^{3}J_{eqeq}$ and $^{3}J_{eqax}$ in the chair conformer of cyclohexane are small or even vanishing (and thus not resolved) according to a Karplus-type relation for both H,H and H,F-pairs.[16] Fluorine in the axial position (or at α-site) resonates at δ −99.2 ppm. Again, $^{2}J(^{19}F,^{19}F)$ = 232.9 Hz reveals the coupling to the other geminal fluorine atom. This doublet is further split to a triplet of triplets showing couplings to the axial protons, 2α-H and 4α-H ($^{3}J_{axax}(^{19}F,^{1}H)$ = 35.0 Hz), and further to the equatorial protons, 2β-H and 4β-H ($^{3}J_{axeq}(^{19}F,^{1}H)$ = 12.2 Hz), respectively.

^{13}C NMR chemical shifts of **1** and **2** were assigned based on ^{13}C, ^{13}C DEPT-135, PFG ^{1}H,^{13}C HMQC, and ^{1}H,^{13}C HMBC experiments using Bruker standard pulse programs[17] and are presented in Table 1. The carbonyl C-24 in **1** resonates at a chemical shift value of δ 178.64 ppm, indicating a free carboxylic acid group. The fluorine coupling induces splitting of resonance signals of carbons in the steroidal A-ring. The largest values for *J* indicating $^{1}J(^{19}F,^{13}C)$ coupling are observed for carbon-3 (dd, *J* = 241.8 and 239.0 Hz), which resonates at the chemical shift value of δ 124.56 ppm. The signal is deshielded for over 50 ppm compared to lithocholic acid LCA (a 3α-hydroxy derivative), due to inductive effects of two fluorine atoms. The geminal fluorine atoms at position C-3 split

* *Correspondence to: Elina Sievänen, Department of Chemistry, FIN-40014, University of Jyväskylä, Finland. E-mail: elvirtan@cc.jyu.fi*

a *Department of Chemistry, FIN-40014, University of Jyväskylä, Finland*

b *Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic*

c *Zentiva Ltd., Prague, Czech Republic*

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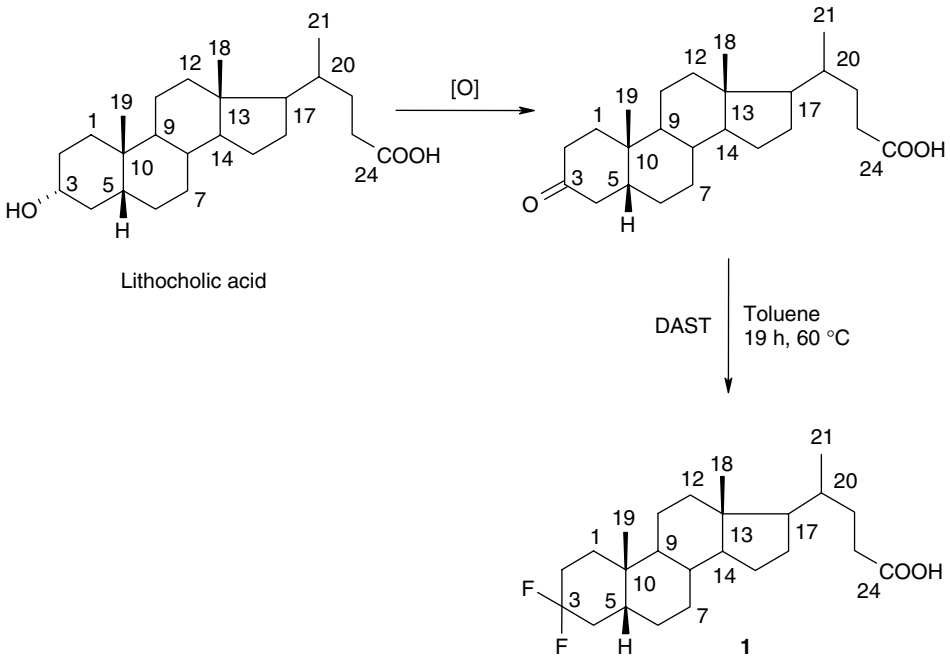
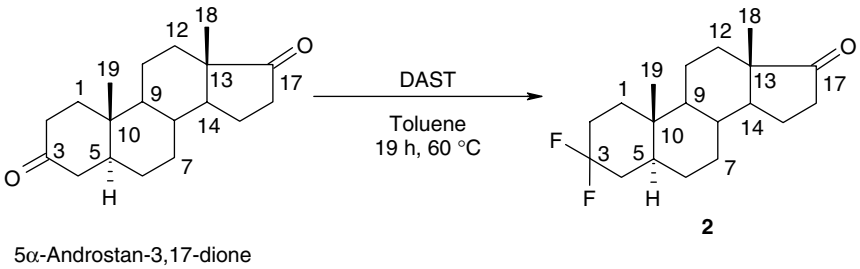


Scheme 1. Synthetic procedure leading to 3,3-difluoro-5β-cholan-24-oic acid (**1**).



Scheme 2. Preparation of 3,3-difluoro-5α-androstan-17-one (**2**).

the signals of carbons C-2 and C-4 to a doublet of doublets, with typical $^{2}J(^{19}F,^{13}C)$ coupling constant values ranging from 20.5 to 24.5 Hz. The carbons resonate at the chemical shift values of δ 34.53 (C-4) and 29.38 ppm (C-2), respectively. The signals of carbons C-1 and C-5 are split to doublets with a coupling constant value of approximately 9 Hz. The vicinal coupling between the equatorial fluorine and C-1 or C-5 is small or even negligible according to Karplus-type relation,[16] which is why only $^{3}J(^{19}F,^{13}C)$ of the axial fluorine can be observed in the spectrum. The carbons resonate at the chemical shift values of δ 40.06 (C-5) and 33.03 ppm (C-1), respectively.

The mass spectrometric measurements for the fluorinated bile acid derivative **1** resulted in MS *m/z*, ESI-TOF$^-$ found 395.41 $[M-H]^-$ in acetonitrile and 395.34 $[M-H]^-$ in methanol. $C_{24}H_{38}F_2O_2$ requires $[M-H]^- = 395.28$. The results thus ensure the molecular weight of the compound. In the spectra of **1** singly charged ions were detected. When acetonitrile was used as the solvent, $[M-H]^-$ ion was basically the only one observed in the spectrum. Methanol induced formation of $[2M-H]^-$ ion, with rather low intensity (<10%), however, compared to the main signal originating from $[M-H]^-$ ion. The observed $[M-H]^-$ and $[2M-H]^-$ ions were well in agreement with the calculated isotope patterns.

^{1}H NMR chemical shift assignments of **2** and comparisons with those of three monofluoro derivatives taken from the literature[14] are given in Table 3. As can be seen an unambiguous assignment for **2** was possible to obtain. The used DAST synthetic procedure substitutes the appropriate carbonyl groups by two fluorine atoms generating geminal difluoroderivatives. Therefore, preparation of **2** starting from a 3,17-diketosteroid could have resulted also in a 17,17-difluoro or even in a 3,3,17,17-tetrafluoro product instead of the characterized 3,3-difluoro derivative. In the ^{13}C NMR spectrum of **2**, however, a carbonyl signal at δ 220.86 ppm was observed, revealing that the investigated compound still contains one carbonyl group. Significant similarities with the investigated compound and 17-oxo-5α-androstane derivatives were observed, when compared with ^{13}C NMR chemical shifts of some selected androstane derivatives.[18] Especially the ^{13}C NMR chemical shift of C-18 seems to be affected by a carbonyl oxygen in position C-17. It is deshielded almost 3 ppm compared with hydroxyl substituted derivatives ($\delta \sim 11$ ppm). The ^{13}C NMR chemical shift value of δ 13.82 ppm of the investigated compound thus suggests that the carbonyl oxygen is attached at position C-17. The carbonyl carbon in the 17-oxo derivatives ($\delta \sim 221$ ppm) is deshielded almost 10 ppm compared to that in the 3-oxo derivatives ($\delta \sim 210$ ppm). This is another indicator suggesting that compound **2** represents a 17-oxo rather than a

Table 1. ^{13}C and ^{19}F NMR chemical shifts (in ppm from int. TMS and ext. $CFCl_3$) of **1** and **2** in $CDCl_3$ at 30 °C

Compound	1	2
C-1	33.03[a]	34.85[b]
C-2	29.38[c]	30.28[d]
C-3	124.56[e]	123.59[f]
C-4	34.53[g]	36.78[h]
C-5	40.06[i]	42.74[j]
C-6	26.12[k]	27.76
C-7	26.02[k]	30.60
C-8	35.55	35.00
C-9	39.89	53.78
C-10	34.56	35.59
C-11	21.08	20.54
C-12	40.07	31.51
C-13	42.77	47.73
C-14	56.46	51.33
C-15	24.14	21.78
C-16	28.13	35.81
C-17	55.98	220.86
C-18	12.06	13.82
C-19	22.79	11.27
C-20	35.30	
C-21	18.25	
C-22	30.77[k]	
C-23	30.73[k]	
C-24	178.64	
F-3α (or ax)	−99.2[l]	−98.8[m]
F-3β (or eq)	−88.1[n]	−89.1[o]

[a] $^3J(^{19}F,^{13}C) = 9.3$ Hz.
[b] $^3J(^{19}F,^{13}C) = 9.7$ Hz.
[c] $^2J(^{19}F,^{13}C) = 25.4$ and 22.0 Hz.
[d] $^2J(^{19}F,^{13}C) = 25.9$ and 22.2 Hz.
[e] $^1J(^{19}F,^{13}C) = 241.8$ and 239.0 Hz.
[f] $^1J(^{19}F,^{13}C) = 242.3$ and 238.6 Hz.
[g] $^2J(^{19}F,^{13}C) = 25.4$ and 20.5 Hz.
[h] $^2J(^{19}F,^{13}C) = 25.4$ and 20.8 Hz.
[i] $^3J(^{19}F,^{13}C) = 9.1$ Hz.
[j] $^3J(^{19}F,^{13}C) = 9.0$ Hz.
[k] Assignment can be *vice versa*.
[l] $^2J(^{19}F,^{19}F) = 232.9$ Hz (d), $^3J(^{19}F,^1H_{ax}) = 35.0$ Hz (t), and $^3J(^{19}F,^1H_{eq}) = 12.2$ Hz (t).
[m] $^2J(^{19}F,^{19}F) = 233.9$ Hz (d), $^3J(^{19}F,^1H_{ax}) = 34.8$ Hz (t), and $^3J(^{19}F,^1H_{eq}) = 13.0$ Hz (t).
[n] $^2J(^{19}F,^{19}F) = 232.9$ Hz,
[o] $^2J(^{19}F,^{19}F) = 233.9$ Hz.

Table 2. ^1H NMR chemical shifts (in ppm from int. TMS) of **1** in $CDCl_3$ at 30 °C

Proton	Chemical shift (ppm)
1α/β	1.78/1.26
2α/β	1.90–1.63
4α/β	2.13/1.68
5	1.99
6α/β	1.27/1.02
7α/β	1.88/1.47
8	1.27
9	1.3–1.2
11α/β	1.47–1.13
12α/β	1.7/1.2
14	1.07
15α/β	1.58/1.08
16α/β	1.88/1.29
17	1.12
18	0.66
19	0.97
20	1.42
21	0.93
22α/β	1.47–1.23
23α/β	2.40/2.27

H-4α/β $\delta = 2.13$, dtd, $J = 35.9$, 15.0 and 5.0 Hz.
H-5 $\delta = 1.99$, dt, $J = 12.5$ and ~3 Hz.
H-18 (CH_3) $\delta = 0.66$, s.
H-19 (CH_3) $\delta = 0.97$, s.
H-21 (CH_3) $\delta = 0.93$, d, $J = 6.5$ Hz.
H-23α/β $\delta = 2.40$, ddd, $J = -15.8$, 9.7 and 5.3 Hz and $\delta = 2.27$, ddd, $J = -15.8$, 9.9 and 6.6 Hz.

3-oxo derivative. The NMR chemical shifts of carbons C-9 and C-19 suggest that the investigated compound possesses α-fusion of the rings A and B. When comparing the literature values of ^{13}C NMR chemical shifts of selected androstane derivatives,[15] C-9 is deshielded almost 15 ppm and C-19 shielded approximately 10 ppm compared to the corresponding 5β-derivatives. The same trend can be seen in the chemical shifts of C-9 and C-19 of the investigated compound.

Similarly as in **1** the fluorine couplings in **2** cause splittings of resonance signals of carbons in the steroidal A-ring. The largest values for J indicating $^1J(^{19}F,^{13}C)$ coupling are again observed for C-3 (dd, $J = 242.3$ and 238.6 Hz), which resonates at the chemical shift value of δ 123.59 ppm. The signals of carbons C-2 and C-4 are split to a doublet of doublets with typical $^2J(^{19}F,^{13}C)$ coupling constant values ranging from 20.8 to 26.5 Hz by the geminal fluorine atoms at position C-3. These carbons resonate at the chemical shift values of δ 36.78 (C-4) and 30.28 ppm (C-2), respectively. Owing to small vicinal coupling in the chair conformer of cyclohexane between the equatorial fluorine and C-1 or C-5, only $^3J(^{19}F,^{13}C)$ of the axial fluorine can be observed in the spectrum ($J \sim 9$ Hz). The signals of carbons C-1 and C-5 are thus split to doublets, which resonate at the chemical shift values of δ 34.85 (C-1) and 42.74 ppm (C-5), respectively.

^{19}F NMR spectrum of **2** shows a large doublet and a doublet of triplet of triplets as in **1**. The simple doublet resonates at δ −98.1 ppm with a coupling constant J of 233.9 Hz, indicating a $^2J(^{19}F,^{19}F)$ coupling. Similarly to compound **1,** this signal is assumed to originate from the fluorine atom in the equatorial position (β-site). Fluorine in the axial position (α-site) resonates at δ −98.8 ppm. J of 233.9 Hz reveals coupling to geminal fluorine atom. This doublet is again split to a triplet of triplets showing coupling to the axial protons ($^3J_{axax}(^{19}F,^1H) = 34.8$ Hz), and further to the equatorial protons ($^3J_{axeq}(^{19}F,^1H) = 13.0$ Hz). The splitting pattern closely resembles the one obtained for 3,3-difluoro-5β-cholan-24-oic acid (**1**). Unfortunately, S/N ratio was not sufficient in the ^{19}F NMR spectra of **1** and **2** (due to many splittings in 3α-F and broadness of 3β-F) to allow observation of their ^{13}C satellite signals, which also could have been useful in ascertaining their assignments.[15b]

The most intense ion in the ESI-TOF$^+$ mass spectrum of **2** appears at a m/z value of 293.14 corresponding the ion $[M - F + 2H]^+$ ($C_{19}H_{28}F_2O$ requires $[M - F + 2H]^+ = 293.23$). Also $[M + H]^+$ ion was detected in the spectrum at an m/z value of 311.22 ($C_{19}H_{28}F_2O$

Table 3. ^{1}H NMR chemical shifts (in ppm from int. TMS) of **2** in $CDCl_3$ at 30 °C

Proton	5α-Androstan- 17-one[a]	3α-Fluoro-5α-androstan- 17-one[a]	3β-Fluoro-5α-androstan- 17-one[a]	3,3-Difluoro-5α-androstan-17-one **2**
1α	0.89	1.32	0.98	1.26
1β	1.67	1.53	1.77	1.75
2α	1.46	1.77	1.93	1.98
2β	1.46 ± 0.04	1.56	1.57	1.78
3α	1.22	–	4.46	–
3β	1.68	4.81	–	–
4α	1.29	1.63	1.74	1.8–1.6
4β	1.29 ± 0.06	1.41	1.51	1.8–1.6
5	1.07	1.57	1.11	1.46
6α	1.25 ± 0.04	1.24[g]	1.35	1.40/1.29
6β	1.25	1.16[g]	1.35	1.40/1.29
7α	0.97	1.02	0.98	1.01[b]
7β	1.78	1.81	1.81	1.81
8	1.55	1.56	1.53	1.55
9	0.72	0.82	0.68	0.79
11α	1.67	1.68	1.65	1.66
11β	1.27	1.29	1.32	1.30
12α	1.23	1.24	1.23	1.27
12β	1.79	1.80	1.78	1.82
14	1.27	1.30	1.27	1.28
15α	1.92	1.94	1.92	1.96
15β	1.50	1.51	1.48	1.52
16α	2.05	2.07	2.06	2.08[c]
16β	2.45	2.42	2.42	2.42[d]
18	0.86	0.86	0.86	0.87[e]
19	0.81	0.82	0.86	0.86[f]

[a] Taken from Ref. [14].
[b] H-7α $\delta = 1.01$, qd, $J = 12.6$ and 4.5 Hz.
[c] H-16α $\delta = 2.08$, dt, $J = -19.4$ and 9.1 Hz.
[d] H-16β $\delta = 2.42$, dd, $J = -19.4$ and 9.0 Hz.
[e] H-18 (CH_3) $\delta = 0.87$, s.
[f] H-19 (CH_3) $\delta = 0.86$, s.
[g] Interchangeable.

requires $[M + H]^+ = 311.22$). Two additional ions at *m/z* values of 273.21 and 391.28 were visible as well. All of the ions were singly charged.

In order to screen useful pharmacological and physiological properties of **1** its adduct formation experiments with common bile acids have been performed as well. In the ESI-TOF mass spectra obtained by mixing equal volumes (150 µl + 150 µl) of the methanol solution of **1** (c = 0.05 mmol/l) and that of a bile acid (3α-hydroxy-5β-cholan-24-oic acid = LCA, 3α,7β-dihydroxy-5β-cholan-24-oic acid = ursodeoxycholic acid = UDCA, 3α,7α-dihydroxy-5β-cholan-24-oic acid = chenodeoxycholic acid = CDCA, 3α,12α-dihydroxy-5β-cholan-24-oic acid = deoxycholic acid = DCA, and 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid = cholic acid = CA); c = 0.05 mmol/l; the two most intense signals were given by the singly charged ions $[\mathbf{1} - H]^-$ and $[\text{bile acid} - H]^-$. However, in all of the cases signals of $[2 \times \text{bile acid} - H]^-$, $[\mathbf{1} + \text{bile acid} - H]^-$, and $[2 \times \mathbf{1} - H]^-$ were observed in different intensity ratios.

In the case of **1** and LCA, $[\mathbf{1} + \text{LCA} - H]^-$ gave the most intense signal of those three. The intensity of $[2 \times \text{LCA} - H]^-$ was approximately 86% and that of $[2 \times \mathbf{1} - H]^-$ less than 40% of the intensity of $[\mathbf{1} + \text{LCA} - H]^-$ ion. With **1** and UDCA, $[\mathbf{1} + \text{UDCA} - H]^-$ adduct again gave the most intense signal of the three signals. The intensities induced by ions $[2 \times \text{UDCA} - H]^-$ and $[2 \times \mathbf{1} - H]^-$ were approximately 96% and 69% of the intensity induced by $[\mathbf{1} + \text{UDCA} - H]^-$ adduct, respectively. Interestingly, in the spectrum of **1** and CDCA (which is an epimer of UDCA in the respect of the hydroxyl group in position 7 of the steroid skeleton), the main signal of the three adduct signals was not the one formed by bile acid and **1**, but the ion induced by $[2 \times \text{CDCA} - H]^-$ ion. The intensity of the adduct ion $[\mathbf{1} + \text{CDCA} - H]^-$ was approximately 63% and that of $[2 \times \mathbf{1} - H]^-$ 33% of the $[2 \times \text{CDCA} - H]^-$ ion, respectively. DCA is the 3α,12α-dihydroxy isomer of UDCA and CDCA. The order of the intensities of the three adduct ion signals in the case of **1** + DCA was the same as in the case of **1** + CDCA, the intensity ratios being approximately 100%, 48%, and 36%. When CA (3α,7α,12α-trihydroxy derivative) was used in the experiment, the intensity ratio of the three adduct ions again differed from the previously observed ones, which can be due to a tendency of CA to dimerize instead of forming an adduct with **1**. In this spectrum $[2 \times \text{CA} - H]^-$ gave the most intense signal of the adduct ions. The intensity of $[2 \times \mathbf{1} - H]^-$ was approximately 42% of the signal induced by the CA-dimer and that of $[\mathbf{1} + \text{CA} - H]^-$ approximately 31%.

By these experiments, the adduct ion formation tendency of the fluorinated bile acid derivative **1** was clearly demonstrated. It

seems that the amount, position, and orientation of the hydroxyl groups of the bile acids affect the adduct formation probability. The greater amount and the α-orientation of the hydroxyl group in position 7 seem to decrease the adduct formation tendency – probably via increasing the dimerization probability of the bile acid itself. Because bile acids are endogenous compounds – they form as end products of cholesterol metabolism in the liver[19] – and have an essential role in many of the digestive processes,[20] they have a huge potential to be used in medicinal and pharmaceutical applications. Their large, rigid, and chiral steroid nucleus, amphiphilicity, a unique disposition of hydroxyl groups, availability, and inexpensivity have led to the use of bile acids in pharmacology and in supramolecular applications, as recently reviewed by us.[21,22]

In conclusion, we have shown how 3-ketosteroids can be transformed to geminal difluoro derivatives in one step by DAST treatment. Multinuclear ^{1}H, ^{13}C, and ^{19}F magnetic resonance techniques have been used to characterize the formed structures in detail. The couplings induced by fluorine have been determined to affect mainly on the ^{13}C NMR signals of the steroidal A-ring. ESI mass spectrometric results prove that **1** has a clear tendency to form adducts with bile acids. Fluorinated steroids may thus have potential in acting as agents aimed for binding and removing steroids from the human system providing new possibilities in the treatment of cardiovascular diseases, such as atherosclerosis. These studies are in progress.

Experimental

5α-Androstan-3,17-dione and bile acids (purity >95%) utilized were commercial products and used without further purification. LCA was oxidized to 3-oxo-5β-cholan-24-oic acid at ambient temperature with chromic acid.[23–25]

Synthetic procedure for 3,3-difluoro-5β-cholan-24-oic acid (1) and 3,3-difluoro-5α-androstan-17-one (2)

To a 25 ml flask equipped with a magnetic bar and starting compound (50 mg, for **1** 0.13 mmol, for **2** 0.17 mmol), toluene (5 ml), and DAST (for **1** 645.5 mg, 30 eq., for **2** 55 mg, 2 eq.) were added. The reaction mixture was heated under stirring on an oil bath for 19 h at 60 °C. The mixture was allowed to cool to ambient temperature, after which 10 ml of water was added. The formed mixture was extracted with CH_2Cl_2 (2 × 25 ml) and the extract was dried with $MgSO_4$. The yield of crude product of **1** was 49 mg. Compound **1** was purified with preparative TLC using hexane : ethyl acetate 9 : 1 as an eluent giving 21 mg (38% yield) of white crystalline material. The purity of **1** was checked by ^{1}H NMR (500 MHz, $CDCl_3$, 303 K, Table 2) and mass spectrometry. MS *m/z* ESI-TOF$^-$ found 395.34 $[M-H]^-$ (100%), $C_{24}H_{37}F_2O_2$ requires 395.28; 791.73 $[2M-H]^-$ (4.4%), $C_{48}H_{75}F_4O_4$ requires 791.56.

After isolation by preparative TLC, 35 mg of crude product of **2** was obtained. The crude product crystals were washed with hexane giving 24 mg (45% yield) of white crystalline material. The purity of **2** was checked by ^{1}H NMR (500 MHz, $CDCl_3$, 303 K, Table 3) and mass spectrometry. MS *m/z* ESI-TOF$^+$ found 273.21 (11%); 293.19 $[M-F+H]^+$ (100%), $C_{19}H_{30}FO$ requires 293.23; 311.22 $[M+H]^+$ (25%), $C_{19}H_{29}F_2O$ requires 311.22.

NMR measurements

All NMR spectra were run in dilute $CDCl_3$-solutions at 303 K using Bruker Avance DRX 500 FT NMR spectrometer equipped with a 5 mm dual inverse detection probehead (BBI) and z-gradient accessory working at 500.13 MHz for proton, 125.76 MHz for carbon-13, and 470.54 MHz for fluorine-19, respectively. In ^{1}H NMR experiments, the chemical shifts were referenced to the trace signal of $CHCl_3$ (δ = 7.26 ppm from internal TMS) and in ^{13}C NMR experiments to the center peak of $CDCl_3$ septet (δ = 77.00 ppm from internal TMS), respectively. ^{19}F NMR chemical shifts were referenced to the signal of an external neat $CFCl_3$ in a 1 mm diameter capillary inserted coaxially inside the 5 mm NMR tube.

In ^{1}H NMR experiments the spectral width was 5000 Hz (10 ppm), number of data points 65 K giving a 6.6 s acquisition time, and the relaxation delay was 1 s. The flip angle was 30° and the number of scans was 8. The FID was multiplied by a 0.1 Hz exponential window function before FT. In proton decoupled (Waltz-16) ^{13}C NMR experiments the spectral width was 30 300 Hz (230 ppm), number of data points 65 K giving a 1.1 s acquisition time, and the relaxation delay was 2 s. The flip angle was 30° and the number of scans was 2300. The FID was multiplied by a 1.0 Hz exponential window function before FT.

In ^{19}F NMR experiments the spectral width was 19 100 Hz (40 ppm), number of data points 65 K giving a 1.7 s acquisition time, and the relaxation delay was 1 s. The flip angle was 30° and the number of scans was 32. The FID was multiplied by a 0.1 Hz exponential window function before FT.

In PFG ^{1}H,^{13}C HMQC experiments (hmqcgpqf Bruker pulse program) the matrix size was 1500 Hz (3 ppm)/1024 data points for ^{1}H-axis ×7560 Hz (60 ppm)/512 data points for ^{13}C-axis. For every ^{13}C increment, 16 scans have been accumulated using garp composite pulse decoupling during acquisition. The matrix size was zerofilled to 2 K × 2 K and multiplied by a sine bell window function along both axes before FT.

In PFG ^{1}H,^{13}C HMBC experiments (hmbcgplpndqf Bruker pulse program), the matrix size was 1500 Hz (3 ppm)/1024 data points for ^{1}H-axis ×28 750 Hz (230 ppm)/1024 data points for ^{13}C-axis. For every ^{13}C increment 64 scans have been accumulated using 3.45 ms low pass filter to remove direct couplings in the beginning of the pulse program and 50 ms delay for the evolution of long-range couplings. The matrix size was zerofilled to 2 K × 2 K and multiplied by a sine bell window function along both axes before FT.

MS measurements

Electrospray mass spectrometric measurements were performed using LCT TOF mass spectrometer with ESI (Micromass LCT). Controlling the LCT as well as acquiring and processing the data were performed with a MassLynx NT software system. In each experiment, a flow rate of 40 µl/min was used for the sample solution and the sample droplets were dried with nitrogen gas. Compound **1** was best ionized with negative ion mode, whereas compound **2** gave the desired result with positive ion mode. For **1** the potentials of −65 V and −5 V for the sample and extraction cones were applied. RF lens was set at a potential of −800 V and the potential in the capillary at 5000 V. The desolvation temperature was set at 180 °C and the source temperature at 100 °C. For **2** the potentials of 35 V and 4 V for the sample and extraction cones were applied. RF lens was set at a potential of 900 V and the potential in the capillary at 4300 V. The desolvation temperature was set at 120 °C and the source temperature at 100 °C. In the adduct formation experiments, the measurement conditions were identical to those described for **1** above, except that the value of sample cone was set to −40 V.

For the MS measurements, the compounds were first dissolved in methanol and further diluted to the desired concentrations. The ionization of **2** was induced with acetonitrile : H_2O : HCOOH (50% : 50% : 0.1%) solution. For the adduct formation experiments, the sample solution of compound **1** (in p.a. methanol) was diluted to a final concentration of 0.05 mmol/l. Of ursodeoxycholic, chenodeoxycholic, deoxycholic, and cholic acids methanol solutions of a concentration of 0.05 mmol/l were prepared. LCA was first dissolved in chloroform, because of its poor solubility in methanol. Equal volumes of the sample solution and the bile acid solution (150 µl + 150 µl) were then mixed and stirred thoroughly.

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References

[1] Bégué J-P, Bonnet-Delpon D. *J. Fluorine Chem.* 2006; **127**: 992.
[2] Fried J. *J. Am. Chem. Soc.* 1954; **76**: 1455.
[3] DesMarteau DD, Resnati G, Seraglia R, Traldi P. *J. Fluorine Chem.* 1992; **58**: 299.
[4] Hanson JR. *Nat. Prod. Rep.* 1995; **12**: 567.
[5] Thomas MG, Suckling CJ, Pitt AR, Suckling KE. *J. Chem. Soc., Perkin Trans. 1* 1999; 3191.
[6] Kasal A, Slaviková B, Chodounska H, Kohout L, Kristofiková Z, Krsiak M, Uhlirová L. Preparation of novel neuroactive pregnane steroids, process of their preparation and use. CZ Patent 294278, 2004.
[7] Clerici C, Castellani D, Asciutti S, Pellicciari R, Setchell KDR, O'Connell NC, Sadeghpour B, Camaioni E, Fiorucci S, Renga B, Nardi E, Sabatino G, Clementi M, Giuliano V, Baldoni M, Orlandi S, Mazzocchi A, Morelli A, Morelli O. *Toxicol. Appl. Pharmacol.* 2006; **214**: 199.
[8] Kauffman JM, Pellicciari R, Carey MC. *J. Lipid Res.* 2005; **46**: 571.
[9] (a) Bird T, Geoffrey C, Felsky G, Fredericks PM, Jones ERH, Meakins GD. *J. Chem. Res., Synop.* 1979; 388; (b) Jibo X, Yili C, Liberatore KM, Selinsky BS. *Tetrahedron Lett.* 2003; **44**: 9295.
[10] Shen Y, Wen J. *J. Fluorine Chem.* 2002; **113**: 3.
[11] Pellicciari R, Roda A, Frigerio G. Preparation of 6-fluorinated bile acid derivatives for stimulation of bile secretion. EP 393493, 1990.
[12] Rozen S, Ben-Shushan G. *Tetrahedron Lett.* 1984; **25**: 1947.
[13] Da Col M, Cainelli G, Umani RA, Sandri S, Contento M, Fortunato G. Preparation of 17beta-fluorinated androstane esters from androstane 17beta-carbothioate intermediates. IT Patent 2002–2606 20021209.
[14] Schneider HJ, Buchheit U, Becker N, Schmidt G, Siehl U. *J. Am. Chem. Soc.* 1985; **107**: 7027.
[15] (a) Abraham RJ, Warne MA, Griffiths L. *J. Chem. Soc., Perkin Trans. 2* 1997; 203; (b) Abraham RJ, Edgar M, Griffiths L, Powell RL. *J. Chem. Soc. Perkin Trans. 2* 1995; 561.
[16] (a) Karplus M. *J. Chem. Phys.* 1959; **30**: 11; (b) Berger S, Braun S, Kalinowski H-O. *NMR Spectroscopy of the Non-metallic Elements*. John Wiley & Sons: Chichester, 1997; 594.
[17] Berger S, Braun S. *200 and More NMR Experiments*. Wiley-VCH: Weinheim, 2004.
[18] Blunt JW, Stothers JB. *Org. Magn. Reson.* 1977; **9**: 439.
[19] Kritchevsky D, Nair PP. In *The Bile Acids: Chemistry, Physiology, and Metabolism*, vol. 1, Nair PP, Kritchevsky D (eds). Plenum: New York, 1971; 3.
[20] Enhsen A, Kramer W, Wess G. *Drug Discovery Today* 1998; **3**: 409.
[21] Tamminen J, Kolehmainen E. *Molecules* 2001; **6**: 21.
[22] Virtanen E, Kolehmainen E. *Eur. J. Org. Chem.* 2004; 3385.
[23] Fieser LF, Rajagopalan S. *J. Am. Chem. Soc.* 1950; **72**: 5530.
[24] Chang FC. *J. Org. Chem.* 1979; **44**: 4567.
[25] Kolehmainen E, Kaartinen M, Kauppinen R, Kotoneva J, Lappalainen K, Lewis PT, Seppälä R, Sundelin J-P, Vatanen V. *Magn. Reson. Chem.* 1994; **32**: 441.

