Canadian Journal of Chemistry
Revue canadienne de chimie

Oxo-analogues of 20-hydroxyecdysone in the synthesis of novel fluorinated ecdysteroid derivatives

Journal:	<i>Canadian Journal of Chemistry</i>
Manuscript ID	cjc-2017-0629.R1
Manuscript Type:	Article
Date Submitted by the Author:	01-Dec-2017
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Is the invited manuscript for consideration in a Special Issue?:	N/A
Keyword:	20-hydroxyecdysone, fluorinated ecdysteroids, DAST-fluorination, hydride reduction

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**Oxo-analogues of 20-hydroxyecdysone in the synthesis of novel
fluorinated ecdysteroid derivatives**

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Draft

Abstract

Novel side chain ω -fluorinated ecdysteroid analogues were obtained starting from ω -functionalized ecdysteroids. Thus, the reaction of diacetonides of 25*RS*-hydroxy- and 24-hydroxy 27-*nor*- and 25,26,27-*tris-nor*-20-hydroxyecdysone derivatives with diethylaminosulfur trifluoride (DAST) involves replacement of the terminal hydroxyl group and dehydration at the 14-position to give 25*RS*-fluoro- and 24-fluoro-14-anhydro analogues of ecdysteroids.

Draft

Introduction

Ecdysteroids are known as insect hormones which control molting, metamorphosis, and reproduction [1]. Some ecdysteroids act as hormone antagonists, disturbing insect molting process [2]; however, these compounds cannot efficiently affect the population of insects, which has acquired defense mechanisms against exogenous ecdysteroids during evolution. In view of this fact, a synthesis of novel ecdysteroid analogues would provide compounds for efficient regulation of vital processes in insects [3].

The non-hormonal biological activity of ecdysteroids towards mammals is intensively studied [4-6]. It has been reported that non-polar ecdysteroids possess chemo-sensitizing activity towards various cancer cell lines [7-10]. The fluorination of steroids, juvenile hormones, and pheromones can influence the biological activity of these compounds [11]. The interest in fluorinated analogues of natural compounds is caused by features of the fluorine atom, which significantly affects the biological activity and the metabolic stability of a molecule due to high electronegativity, and small size and to low polarizability of the C-F bond. Thus, the introduction of fluorine atom in natural products is the attractive strategy to obtain new leads for drug discovery [12]. The most common synthetic route to fluorinated derivatives is fluorination with various nucleophilic reagents [13]. Among them, diethylaminosulfur trifluoride (DAST) is widely used in deoxyfluorination of oxygen-containing organic compounds; moreover, this reagent is effective for geminal fluorination of acid-sensitive substrates such as aldehydes and ketones [14].

The aim of the present study was to involve new side chain analogues of 20-hydroxyecdysone in the DAST-fluorination and to extend the scope of the reaction to the synthesis of biologically active fluorinated analogues of ecdysteroids.

Results and Discussion

In this regard, we began our research from the study of DAST fluorination of the side chain-modified oxoecdysteroids **1** and **2**, which were obtained by the ozonolysis of $\Delta^{24,25}$ - and $\Delta^{25,26}$ -derivatives of 20-hydroxyecdysone [16]. It should be noted that 20-hydroxyecdysone is a polyhydroxylated molecule with three secondary and three tertiary hydroxyl groups and it reacts with the DAST reagent to give a complex reaction mixture. DAST fluorination of ecdysteroids **1** and **2** having an acid-sensitive γ -hydroxy- α,β -enone moiety gave only 14-anhydro derivatives **3** and **4**. Moreover, it was found that the oxo groups of ecdysteroid side chain are inert to the DAST-mediated geminal difluorination. That is why terminal alcohols **5** and **6** were chosen as alternative precursors for the synthesis of ecdysteroid fluorinated analogues. Such alcohols with the intact native 7-en-6-one chromophore were prepared via chemo- and stereoselective reduction of ω -oxo-substrates **1** and **2** by L-selectride (Scheme 1).

As follows from the integral intensities of the H-25 signal (δ_{H} 3.82 and 4.03 ppm) in the ^1H NMR spectrum, compound **5** is a diastereomeric mixture of 25*S*/*R* alcohols present in 85:15 ratio. In order to assign the absolute configuration of the C^{25} stereogenic center in the major and minor epimers, alcohols **5** were transformed into esters **7** through the reaction with (*R*)-2-methoxy-2-phenylacetyl chloride (*R*-MPACl). It is known that the major structure of *R*-MPA ester derivatives have the syn-periplanar (*sp*) conformation, in which H-25 atoms and methoxy and carbonyl groups are located in the same plane [17]. In the conformer of *R*-MPA ester **7**, these groups are arranged in such a way that the methyl group (CH_3^{26}) should be shielded by the phenyl group (Figure 1). The ^1H NMR spectrum of a 6 : 1 mixture of diastereomeric esters **7** shows that the signal for the C^{26} -methyl group of the major epimer is shifted to the upfield with respect to the minor epimer signals, and the diastereomeric effect is $\Delta\delta^{RS}(\text{C}^{26}\text{H}_3) = 0.13$ ppm. On the contrary, C^{24} -diastereotopic proton signals of the major diastereomer are shifted to the downfield and $\Delta\delta^{RS}(\text{C}^{24}\text{H}_2) = -0.16$ and -0.07 ppm. Taking into account all observed effects, we identify the major diastereomer **7** as 25*S*, whereas the minor one is 25*R*.

Scheme 1. Reaction of ω -oxoecdysteroids **1**, **2** with DAST and L-selectride. **Fig. 1.** Model for configurational of the major diastereomer of *R*-MPA ester **7**.

Fluorination of alcohol **5** bearing one tertiary and one secondary hydroxyl groups provides a mixture of 14-anhydro-25*RS*-fluoro-diastereomers **8**. The formation of the $\Delta^{14,15}$ -double bond is confirmed by the presence of two signals at δ_C 148.8 and 128.7 ppm and by the disappearance of the signal for the tertiary C-14 atom at δ_C 84.85 ppm. Previously, the same 14-dehydration product was observed in the fluorination of 20-hydroxyecdysone diacetone [3]. The substitution of fluorine for the 25-hydroxyl group is evidenced by characteristic downfield shift of the C-25 signal ($\Delta\delta_C$ 21.8 ppm) occurring at 91.5 ppm as a doublet ($J_{C-F}=162$ Hz). The corresponding H-25 signal is revealed as two multiplets with hyperfine structure and equal intensity at δ_H 4.64 and 4.73 ppm (HSQC). The fluorination of secondary and tertiary alcohols catalyzed by DAST follows the S_N1 mechanism with the involvement of oxonium (**A**) and carbenium-dication (**B**) as described previously [18] (Figure 2). The subsequent addition of the fluoride ion to C-25 and the removal of the H-15 proton give diastereomeric mixture **8**.

Fig. 2. Reaction of diol **5** with the DAST-reagent.

The reaction of primary alcohol **6** with DAST gives isomeric 24-fluoro-8(9),14(15)-diene **10** (apparently as a result of allylic rearrangement of H-9 to C-7 atom), in addition to the expected 24-fluoro-7(8),14(15)-diene **9** (similarly to fluorination of alcohol **5**). The ^{13}C NMR spectrum of compound **9** exhibits four signals of sp^2 -hybridized carbon atoms, whereas the ^1H NMR spectrum contains resonance lines for two olefinic protons at δ_{H} 6.00 and 6.10 ppm, which correlate with the C-15 and C-17 signals in the HSQC experiment, respectively. The assignment of the H-15 signal (δ_{H} 6.00 ppm) unambiguously follows from the HMBC spectrum, which shows cross-peaks with the C-13 (δ_{C} 47.49 ppm) and C-17 signals (δ_{C} 57.37 ppm). The correlation between the signal at H-15 and the quaternary carbon signal at δ_{C} 148.66 ppm attests that the latter belongs to the C-14 atom. The signal of quaternary sp^2 -hybridized carbon atom at δ_{C} 153.67 ppm is assigned to C-8 relying on its correlation with the H-7 signal in the HMBC spectrum. The ^{13}C NMR spectrum of **10** also exhibits four signals of sp^2 -hybridized carbon atom; however, unlike the spectrum of compound **9**, three of the signals correspond to tetrasubstituted atoms (DEPTq) and were assigned to C-8, C-9, and C-14 on the basis of HSQC and HMBC data.

The replacement of the 24-hydroxyl group in compounds **9** and **10** by fluorine atom is evidenced by significant downfield shifts of the C-24 signal ($\Delta\delta_C$ 20.5 ppm) appearing as a doublet with the coupling constant $^1J_{CF}=164$ Hz. The corresponding 1H NMR signals of the $H_2FC-(24)$ -group are located in lower field and are manifested as hyperfine-structure multiplets (δ_H 4.60 and 4.70 ppm), which attests to their diastereotopicity.

Fig. 3. Reaction of diol **6** with the DAST-reagent.

Conclusion

Thus, novel side chain ω -fluorinated ecdysteroid analogues were obtained starting from ω -functionalized ecdysteroids. It was ascertained that under the action of DAST reagent, dehydration of the tertiary 14-hydroxyl group occurs together with fluorination of side-chain primary or secondary ω -hydroxyls.

Experimental

One-dimensional (1H , ^{19}F , ^{13}C) and two-dimensional (COSY, NOESY, HSQC, and HMBC) NMR spectra were recorded on a Bruker Avance 500 spectrometer (500.17 MHz for 1H , 470.65 for ^{19}F and 125.78 MHz for ^{13}C) chemical shifts are reported to Me_4Si and CF_3Cl . Mass spectra of samples **3**, **4** and **8-10** were measured using MALDI TOF/TOF method on a Bruker Autoflex III spectrometer with registration of positive ions. Atmospheric pressure chemical ionization (APCI) mass spectra of samples **5**, **6** were obtained on a HPLC mass-

spectrometer LCMS-2010EV (Shimadzu) (direct syringe sample inlet, sample solution was in acetonitrile, mobile phase was acetonitrile/water (95:5)) in positive and negative ions mode at the ionizing electrode potential of 4.5 kV and -3.5 kV, respectively. The mobile phase flow rate was 0.1 mL min^{-1} . The temperature of the APCI interface was 250°C , the heater's and the desolvation line's temperature was 200 and 230°C , respectively. The nebulizer gas (nitrogen) flow rate was 2.5 L min^{-1} . Elemental analysis for compounds **7** was performed on *Carlo Erba EA-1108 CHNS-O* analyzer.

Specific rotations were measured on Perkin-Elmer-341 polarimeter. Melting points were determined on Boetius hot stage. Column chromatography and TLC were performed using silica gel ($<0.06 \text{ mm}$) and pre-coated silica gel (Silufol plates), respectively; spots were processed by treatment with a solution of 4-hydroxy-3-methoxybenzaldehyde in ethanol, acidified with sulfuric acid.

The stock compounds were synthesized from 20-hydroxyecdysone [(mp $239\text{--}240^\circ\text{C}$, $[\alpha]_D^{20} + 54.3^\circ$ (c 1.45, MeOH); lit.: mp 246°C (EtOAc-MeOH, 9:1), $[\alpha]_D^{17} + 65.3^\circ$ (c 1.0, MeOH) [19]. 20-Hydroxyecdysone was isolated from the juice of *Serratula coronata* L. [19]. The starting compounds were purchased from Acros Organics.

General procedure for the reaction of ω -oxo-ecdysteroids **1 and **2** with DAST.**

DAST (2 mmol) was added to the cooled (-70°C , Ar) and a stirred solution of compound **1** or **2** (1 mmol) in 5 ml of CH_2Cl_2 . The reaction mixture was kept under stirring for 1 hour, neutralized with saturated NaHCO_3 solution, extracted with CH_2Cl_2 , the extract was washed with NaCl solution, dried with MgSO_4 and evaporated. After column chromatography, compounds **3** and **4** were isolated.

(20R,22R)-2 β ,3 β :20,22-Diisopropylidenedioxy-25-oxo-27-nor-5 β -cholest-7,14-dien-6-one (3**)**

White solid, 0.04g (69%), mp $84\text{--}86^\circ\text{C}$, $[\alpha]_D^{21} - 182^\circ$ (c 0.64, CHCl_3). ^1H NMR (500.17 MHz, CDCl_3) δ : 0.98 (s, 3H, 19-Me), 1.06 (s, 3H, 18-Me), 1.16 (s,

3H, 21-Me), 1.28 and 1.95 (m, 2H, 1-H), 1.29, 1.35, 1.42 and 1.50 (s, 12H, Me_2C), 1.66 (m, 2H, 23-H), 1.73 (m, 2H, 11-H), 2.04 (m, 1H, 17-H), 2.18 (s, 3H, 26-Me), 2.23 (m, 2H, 12-H), 2.34 (m, 1H, 5-H), 2.36 and 2.62 (m, 2H, 4-H), 2.47 (m, 1H, 9-H), 2.57 and 2.53 (m, 2H, 24-H), 3.68 (m, 1H, 22-H), 4.18 (m, 1H, 2-H), 4.28 (m, 1H, 3-H), 5.99 (d, 1H, $J = 2.5$ Hz, 7-H), 6.10 (d, 1H, $J = 2$ Hz, 15-H). ^{13}C NMR (125.78 MHz, CDCl_3) δ : 19.12 (C^{18}), 20.54 (C^{11}), 21.17 (C^{21}), 22.86 (C^{23}), 23.28 (C^{19}), 26.19, 26.87, 28.51 and 28.81 ($2\text{Me}_2\text{C}$), 28.86 (C^{26}), 30.13 (C^4), 31.54 (C^{16}), 38.60 (C^9), 38.65 (C^1), 38.40 (C^{12}), 39.55 (C^{10}), 40.82 (C^{24}), 47.48 (C^{13}), 50.76 (C^5), 57.32 (C^{17}), 71.72 (C^3), 72.08 (C^2), 80.19 (C^{22}), 83.18 (C^{20}), 108.23 and 108.29 ($2\text{Me}_2\text{C}$), 120.69 (C^7), 128.22 (C^{15}), 148.65 (C^{14}), 153.87 (C^8), 202.25 (C^6), 208.29 (C^{25}). MS (MALDI TOF/TOF), m/z : 565 $[\text{M}+\text{K}]^+$, 549 $[\text{M}+\text{Na}]^+$, calcd for: $\text{C}_{32}\text{H}_{46}\text{O}_6$ 526.

(20R,22R)-2 β ,3 β :20,22-Diisopropylidenedioxy-24-oxo-25,26,27-trisnor-5 β -cholest-7,14-dien-6-one (4)

White solid, 0.035g (65%), mp 96-98°C, $[\alpha]_D^{21} - 287^\circ$ (c 1.36, CHCl_3). ^1H NMR, (500.17 MHz, CDCl_3) δ : 0.99 (s, 3H, 19-Me), 1.08 (s, 3H, 18-Me), 1.23 (s, 3H, 21-Me), 1.34, 1.36, 1.45 and 1.51 (s, 12H, Me_2C), 1.15-2.73 (m, 17H, CH, CH_2), 4.09-4.30 (m, 3H, 2-H, 3-H, 22-H), 5.99 (d, 1H, $J = 2$ Hz, 7-H), 6.10 (d, 1H, $J = 2.5$ Hz, 15-H), 9.83 (s, 1H, CHO). ^{13}C NMR (125.78 MHz, CDCl_3) δ : 19.10 (C^{18}), 20.52 (C^{11}), 21.50 (C^{21}), 23.27 (C^{19}), 26.40, 26.83, 28.51 and 28.73 ($2.3:20.22\text{Me}_2\text{C}$), 26.83 (C^4), 31.48 (C^{16}), 37.58 (C^1), 38.40 (C^{12}), 39.55 (C^{10}), 39.59 (C^9), 43.39 (C^{23}), 47.53 (C^{13}), 50.74 (C^5), 57.34 (C^{17}), 71.70 (C^3), 72.07 (C^2), 75.28 (C^{22}), 82.98 (C^{20}), 108.03 and 108.33 ($2\text{Me}_2\text{C}$), 120.83 (C^7), 127.93 (C^{15}), 148.62 (C^{14}), 153.83 (C^8), 199.82 (C^{24}), 202.18 (C^6). MS (MALDI TOF/TOF), m/z : 499 $[\text{M}+\text{H}]^+$, calcd for: $\text{C}_{30}\text{H}_{42}\text{O}_6$ 498.

General procedure for the reduction of ω -oxo-ecdysteroids 1 and 2.

A solution of the stirred starting substrate (0.1 mmol) **1** or **2** in 5 ml of dry THF was cooled to -10 °C in the argon atmosphere. To the solution 1.2 moles of a 1M L-Selectride were added. The reaction mixture was stirred for 0.5 h at -10°C,

and then the temperature was brought to room temperature and stirred for an additional 1 h, monitoring the reaction by TLC until the starting substrate disappeared. At the end of the reaction, the reaction mixture was acidified with saturated NH_4Cl solution, extracted with EtOAc. The extract was dried with Na_2SO_4 , evaporated and chromatographed on SiO_2 , eluent CHCl_3 .

(20R,22R,25R/S)-14 α ,25-Dihydroxy-2 β ,3 β :20,22-diisopropylidenedioxy-27-nor-5 β -cholest-7-en-6-one (5)

White solid, 0.05g (56%), mp 125-127°C, $[\alpha]_D^{21} + 23.8^\circ$ (c 0.71, CHCl_3). ^1H NMR (500.17 MHz, CDCl_3) δ : 0.78 (s, 3H, 18-H), 0.98 (s, 3H, 19-H), 1.15 (s, 3H, 21-H), 1.20 (d, 3H, $J = 6.0$ Hz, 26-H), 1.24 and 1.95 (m, 2H, 1-H), 1.33 and 1.49 (s, 6H, 2,3- Me_2C), 1.34 and 1.42 (s, 6H, 20,22- Me_2C), 1.52 and 2.02 (m, 2H, 4-H), 1.55 (m, 2H, 23-H), 1.24 and 1.95 (m, 2H, 24-H), 1.65 and 1.72 (m, 2H, 16-H), 1.71 (m, 2H, 11-H), 1.78 (m, 2H, 15-H), 1.82 (m, 2H, 12-H), 2.24 (m, 1H, 17-H), 2.32 (dd, 1H, $J = 5.0$ Hz and $J = 12.0$ Hz, 5-H), 2.81 (m, 1H, 9-H), 3.82 (m, 1H, minor diastereomer, 25R, 25-H), 4.03 (m, 1H, major diastereomer, 25S, 25-H), 3.70 (m, 1H, 22-H), 4.24 (m, 1H, 2-H), 4.27 (m, 1H, 3-H), 5.81 (s, 1H, 7-H). ^{13}C NMR (125.78 MHz, CDCl_3) δ : 17.02 (C^{18}), 20.52 (C^{11}), 21.19 (C^{16}), 21.78 (C^{21}), 23.59 (C^{19} and C^{26}), 25.95 (C^{23}), 26.46 and 26.87 (20,22 Me_2C), 26.66 (C^{15}), 28.54 and 28.91 (2,3 Me_2C), 30.97 (C^{12}), 31.59 (C^4), 34.50 (C^9), 37.41 (C^1), 37.58 (C^{10}), 37.78 (C^{24}), 47.45 (C^{13}), 48.99 (C^{17}), 50.79 (C^5), 68.14 (C^{25}), 71.62 (C^3), 72.14 (C^2), 81.71 (C^{22}), 84.50 (C^{20}), 84.85 (C^{14}), 107.67 and 108.29 (2 Me_2C), 121.32 (C^7), 163.42 (C^8), 202.92 (C^6). MS (APCI), m/z : 547 $[\text{M}+\text{H}]^+$, 581 $[\text{M}+\text{Cl}]^+$, calcd for: $\text{C}_{32}\text{H}_{50}\text{O}_7$ 546.

(20R,22R)-14 α ,24-Dihydroxy-2 β ,3 β :20,22-diisopropylidenedioxy-25,26,27-trisnor-5 β -cholest-7-en-6-one (6)

White solid, 0.063g (75%), mp 138-140°C, $[\alpha]_D^{21} + 28.1^\circ$ (c 0.75, CHCl_3). ^1H NMR (500.17 MHz, CDCl_3) δ : 0.76 (s, 3H, 18-Me), 0.95 (s, 3H, 19-Me), 1.19 (s, 3H, 21-Me), 1.20 and 1.94 (m, 2H, 1-H), 1.30; 1.31; 1.39 and 1.46 (s, 12H, 2,3:20,22- Me_2C), 1.74 and 1.98 (m, 2H, 4-H), 1.80 and 2.20 (m, 2H, 15-H), 1.82 and 1.98 (m, 2H, 16-H), 2.23 (m, 1H, 17-H), 2.38 (dd, 1H, $J = 5.0$ Hz and $J = 12.5$

Hz, 5-H), 2.83 (m, 1H, 9-H), 3.86 (dd, 1H, $J = 1.5$ Hz and $J = 10.5$ Hz, 22-H), 4.20 m (1H, 2-H), 4.23 m (1H, 3-H), 5.78 s (1H, 7-H). ^{13}C NMR (125.78 MHz, CDCl_3) δ : 16.96 (C^{18}), 20.52 (C^{11}), 21.16 (C^{16}), 22.02 (C^{21}), 23.56 (C^{19}), 26.45 и 26.98 (20,22 Me_2C), 26.59 (C^{15}), 28.52 and 28.93 (2,3 Me_2C), 30.95 (C^{12}), 31.31 (C^4 and C^{23}), 34.48 (C^9), 37.53 (C^1), 37.74 (C^{10}), 47.43 (C^{13}), 48.95 (C^{17}), 50.76 (C^5), 61.22 (C^{24}), 71.62 (C^3), 72.12 (C^2), 79.79 (C^{22}), 84.34 (C^{20}), 84.63 (C^{14}), 107.54 and 108.27 (2 Me_2C), 121.15 (C^7), 163.90 (C^8), 203.17 (C^6). MS (APCI), m/z : 519 $[\text{M}+\text{H}]^+$, 553 $[\text{M}+\text{Cl}]^+$, calcd for: $\text{C}_{30}\text{H}_{46}\text{O}_7$ 518.

R-MPA ester of (20R,22R,25R/S)-14 α ,25-Dihydroxy-2 β ,3 β :20,22-diisopropylidenedioxy-27-nor-5 β -cholest-7-en-6-one (7).

To a solution of alcohol **5** 0.016 g (0.03 mmol) in 2 ml of pyridine the *R*-MPA anhydride chloride 0.01 ml (0.05 mmol) and 0.003 g of DMAP were added successively,. The reaction mixture was stirred for 8 hours, evaporated and chromatographed (SiO_2 , eluent CHCl_3).

White solid, 0.012g (78%), mp 138-140°C. ^1H NMR (500.17 MHz, CDCl_3) δ : 0.79 (s, 3H, 18-Me), 1.01 (s, 3H, 19-Me), 1.07 (s, 3H, 21-Me), 1.15 (d, 3H, $J = 6.0$ Hz, major diastereomer 25*S*, 26-Me), 1.27 and 1.98 (m, 2H, 1-H), 1.28 (m, 3H, minor diastereomer, 25*R*, 26-Me), 1.32 and 1.42 (s, 6H, 2,3- Me_2C), 1.36 and 1.52 (s, 6H, 20,22- Me_2C), 1.45 (m, 2H, 23-H), 1.55 (m, 2H, 15-H), 1.52 and 1.71 (m, 2H, minor diastereomer, 25*R*, 24-H), 1.59 and 1.87 (m, 2H, major diastereomer 25*S*, 24-H), 1.66 and 1.75 (m, 2H, 16-H), 1.82 (m, 2H, 11-H), 1.85 and 2.15 (m, 2H, 4-H), 1.86 (m, 2H, 12-H), 2.11 (m, 1H, 17-H), 2.38 (m, 1H, 5-H), 2.83 (m, 1H, 9-H), 3.44 (s, 3H, OCH_3), 3.64 (d, 1H, $J = 9.5$ Hz, 22-H), 4.26 (m, 1H, 3-H), 4.30 (m, 1H, 2-H), 4.78 (s, 1H, 2'-H), 4.95 (m, 1H, minor diastereomer 25*R*, 25-H), 5.08 (m, 1H, major diastereomer 25*S*, 25-H), 5.85 (s, 1H, 7-H), 7.34-7.46 (m, 5H, Ph). ^{13}C NMR (125.78 MHz, CDCl_3) δ : 17.01 (C^{18}), 19.38 (C^{26}), 20.58 (C^{16}), 21.05(C^{11}), 21.80 (C^{21}), 23.64 (C^{19}), 25.95 (C^{23}), 26.47 and 26.89 (20,22 Me_2C), 26.70 (C^4 and C^{15}), 28.55 and 28.99 (2,3 Me_2C), 31.03 (C^{12}), 33.51 (C^{24}), 34.63 (C^9), 37.61 (C^1), 37.77 (C^{10}), 47.51 (C^{13}), 48.97 (C^{17}), 50.82 (C^5), 57.27 (OCH_3), 71.33 (C^{25} , major diastereomer 25*S*), 71.63 (C^3), 72.20 (C^2), 72.31 (C^{25} , minor

diastereomer 25*R*), 80.58 (C^{22}), 84.07 (C^{20}), 85.03 (C^{14}), 107.05 and 108.30 ($2Me_2C$), 121.43 (C^7), 127.13, 127.43, 128.26, 136.27 (Ph). 163.42 (C^8), 170.00 ($C^{1'}$), 202.73 (C^6). Anal. Calcd for $C_{41}H_{58}O_9$ (694.89): C, 70.87; H, 8.41. Found: C, 70.81; H, 8.38.

General procedure for the deoxyfluorination of alcohols **5** and **6**.

To the cooled (-70°C , Ar) and a stirred solution of compound **5** or **6** (1 mmol) in 5 ml of CH_2Cl_2 DAST (1.2 mmol) was added. The reaction mixture was kept under stirring for 1 hour, neutralized with saturated $NaHCO_3$ solution, extracted with CH_2Cl_2 , the extract was washed with NaCl solution, dried with $MgSO_4$ and evaporated. After column chromatography (eluent, compounds **8**, **9** and **10** were isolated.

(20*R*,22*R*)-2*β*,3*β*:20,22-Diisopropylidenedioxy-25-fluoro-27-nor-5*β*-cholest-7,14-dien-6-one (**8**)

White solid, 0.012g (42%), mp $100-102^\circ\text{C}$, $[\alpha]_D^{21} -16^\circ$ (c 0.82, $CHCl_3$). 1H NMR (500.17 MHz, $CDCl_3$) δ : 0.99 (s, 3H, 19-Me), 1.08 (s, 3H, 18-Me), 1.21 (s, 3H, 21-Me), 1.27 and 1.97 (m, 2H, 1-H), 1.32; 1.35; 1.44 and 1.51 (s, 12H, 2,3:20,22 Me_2C), 1.32 and 1.76 (m, 2H, 4-H), 1.39 (d, 3H, $J = 6.0$ Hz, 26-Me), 1.48 and 2.22 (m, 2H, 12-H), 1.58 and 1.62 (m, 2H, 23-H), 1.64 (m, 2H, 24-H), 1.76 (m, 2H, 11-H), 1.99 (m, 1H, 17-H), 2.36 (d, 1H, $J = 5.0$ Hz, 5-H), 2.63 (m, 2H, 16-H), 2.66 (m, 1H, 9-H), 3.73 (dd, 1H, $J = 3$ Hz; $J = 10$ Hz, 22-H), 4.18 (m, 1H, 3-H), 4.28 (m, 1H, 2-H), 4.64 and 4.73 (m, 1H, 25-H), 5.99 (s, 1H, 15-H), 6.10 (d, 1H, $J = 2.0$ Hz, 7-H). ^{13}C NMR (125.78 MHz, $CDCl_3$) δ : 19.10 (C^{18}), 20.55 (C^{11}), 21.21 (C^{21}), 21.29 (C^{26} , $^2J_{C-F} = 25.0$ Hz), 23.28 (C^{19}), 25.27 (C^{23}), 26.41 and 26.83 (20,22 Me_2C), 26.83 (C^4), 28.52 and 28.90 (2,3 Me_2C), 31.59 (C^{16}), 35.09 (C^{24} , $^3J_{C-F} = 20.6$ Hz), 37.60 (C^1), 38.41 (C^9), 38.66 (C^{10}), 39.58 (C^{12}), 47.51 (C^{13}), 50.76 (C^5), 57.50 (C^{17}), 71.73 (C^3), 72.09 (C^2), 81.17 (C^{22}), 83.12 (C^{20}), 91.51 d (C^{25} , $J_{C-F} = 162.0$ Hz), 107.08 and 108.31 ($2Me_2C$), 120.73 (C^7), 128.73 (C^{15}), 148.76 (C^{14}), 153.75 (C^8), 202.22 (C^6). ^{19}F NMR ($CDCl_3$, $CFCl_3$, MHz) δ 85.80 ($C^{25}HF$). MS (MALDI TOF/TOF), m/z 531 $[M+H]^+$, calcd for: $C_{32}H_{47}FO_5$ 530.

(20R,22R)-2 β ,3 β :20,22-Diisopropylidenedioxy-24-fluoro-25,26,27-trisnor-5 β -cholest-7,14-dien-6-one (9)

White solid, 0.015g (36%), mp 90-92°C, $[\alpha]_D^{21} -58.1^\circ$ (*c* 0.16, CHCl₃). ¹H NMR (500.17 MHz, CDCl₃) δ : 0.99 (s, 3H, 19-Me), 1.08 (s, 3H, 18-Me), 1.22 (s, 3H, 21-Me), 1.28 and 1.96 (m, 2H, 1-H), 1.34; 1.35; 1.45 and 1.51 (s, 12H, 2,3:20,22 Me₂C), 1.44 and 2.22 (m, 2H, 12-H), 1.76 (m, 2H, 11-H), 1.78 (m, 2H, 4-H), 1.81 and 1.86 (m, 2H, 23-H), 2.00 (dd, 1H, *J* = 8.0 Hz; 11.0 Hz, 17-H), 2.34 (dd, 1H, *J* = 5.0 Hz; 13.0 Hz, 5-H), 2.36 (m, 2H, 9-H), 2.67 (dd, 1H, *J*_{16,16} = 15.5 Hz; *J*_{16,17} = 11.0Hz, 16-H), 3.95 (m, 1H, 22-H), 4.17 (m, 1H, 2-H), 4.28 (m, 1H, 3-H), 4.56 and 4.65 (m, 2H, 24-H), 6.00 (t, 1H, *J* = 2.0 Hz, 15-H), 6.10 (d, 1H, *J* = 2.0 Hz, 7-H). ¹³C NMR (125.78 MHz, CDCl₃) δ : 19.12 (C¹⁸), 20.55 (C¹¹), 21.18 (C²¹), 23.28 (C¹⁹), 26.41 and 26.82 (20,22 Me₂C), 26.83 (C⁴), 28.52 and 28.84 (2,3 Me₂C), 30.35 (C²³, *J* = 19.5 Hz), 31.55 (C¹⁶), 37.60 (C¹), 38.64 (C¹⁰), 38.84 (C⁹), 39.61 (C¹²), 47.49 (C¹³), 50.76 (C⁵), 57.37 (C¹⁷), 71.72 (C³), 72.09 (C²), 76.77 (C²²), 81.62 (C²⁴, *J*_{CF} = 165.0 Hz), 82.95 (C²⁰), 107.45 and 108.32 (2Me₂C), 120.76 (C⁷), 128.13 (C¹⁵), 148.66 (C¹⁴), 153.67 (C⁸), 202.19 (C⁶). ¹⁹F NMR (CDCl₃, CFCl₃, MHz) δ : 85.81 (C²⁴H₂F). MS (MALDI TOF/TOF), *m/z* 503 [*M*+H]⁺, 525 [*M*+Na]⁺, 541 [*M*+K]⁺, calcd for: C₃₀H₄₃FO₅ 502.

(20R,22R)-2 β ,3 β :20,22-Diisopropylidenedioxy-24-fluoro-25,26,27-trisnor-5 β -cholest-8,14-dien-6-one (10)

White solid, 0.005g (12%), mp 50-52°C, $[\alpha]_D^{21} -9.6^\circ$ (*c* 0.06, CHCl₃). NMR ¹H (500.17 MHz, CDCl₃) δ : 1.17 (s, 3H, 19-Me), 1.02 (s, 3H, 18-Me), 1.23 (s, 3H, 21-Me), 1.28; 1.35; 1.45 and 1.53 (s, 12H, 2,3:20,22 Me₂C), 1.50 and 2.20 (m, 2H, 12-H), 1.60 and 1.98 (m, 2H, 1-H), 1.75 (m, 2H, 23-H), 1.87 (dd, 1H, *J* = 7.6 Hz; 10.8 Hz, 17-H), 1.98 and 2.07 (m, 2H, 4-H), 2.16 (m, 2H, 11-H), 2.35 and 2.71 (m, 2H, 16-H), 2.55 (dd, 1H, *J* = 4.8 Hz; 10.0 Hz, 5-H), 2.83 (d, 2H, ²*J*_{7,7} = 21.0 Hz, 7-H), 3.97 (m, 1H, 22-H), 4.10 (m, 1H, 2-H), 4.30 (m, 1H, 3-H), 4.55 and 4.66 (m, 2H, 24-H), 5.41 (s, 1H, 15-H). NMR ¹³C (125.78 MHz, CDCl₃) δ : 17.43 (C¹⁸), 21.24 (C²¹), 23.36 (C¹¹), 26.43 (C⁴), 28.39 (C¹⁹), 26.87 and 28.49 (20,22 Me₂C), 28.89 and 29.71 (2,3 Me₂C), 30.39 (C²³, *J* = 19.0 Hz), 31.50 (C¹⁶), 36.57 (C¹²),

37.39 (C¹), 38.85 (C⁷), 42.12 (C¹⁰), 45.90 (C¹³), 53.13 (C⁵), 55.96 (C¹⁷), 71.26 (C³), 72.80 (C²), 76.70 (C²²), 81.67 (C²⁴, $J_{C-F} = 164.0$ Hz), 83.13 (C²⁰), 121.73 (C⁸), 107.35 and 108.40 (2Me₂C), 119.15 (C¹⁵), 136.36 (C⁹), 147.91 (C¹⁴), 211.53 (C⁶). ¹⁹F NMR (CDCl₃, CFCl₃, MHz) δ : 85.79. MS (MALDI TOF/TOF), m/z 503 [M+H]⁺, calcd for C₃₀H₄₃FO₅ 502.

The study was financially supported by the Russian Foundation of Basic Research (research project No. 17-00-00603a). The structural studies of compounds were carried out at the Center for Collective Use "Agidel" at the Institute of Petrochemistry and Catalysis, Russian Academy of Sciences.

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