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Synthesis of [19-$^2H_3$]-analogs of dehydroepiandrosterone and pregnenolone and their sulfates

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Abstract

Deuterated analogs of pregnenolone and pregnenolone sulfate with three atoms of deuterium in position 19 were prepared. The synthetic approach was developed on derivatives of dehydroepiandrosterone, where initial intermediates were well characterized, and then applied to the pregnenolone series. Starting 19-hydroxy compounds were transformed into 3α,5-cyclo derivatives to simplify the Jones oxidation into the corresponding 19-oic acids. After oxidation, rearrangement to 3-hydroxy-5-enes, and suitable protection, two deuterium atoms were introduced by lithium aluminum deuteride reduction. Mesylate exchange by iodide in the presence of zinc and deuterium oxide added third deuterium atom. Deprotection gave title analogs with about 93–95% content of d_3-derivative, the rest was mainly not fully deuterated d_2-analogue as followed from the mass spectra analysis. Thus, 3β-hydroxy[19-$^2H_3$]androst-5-en-17-one was prepared in 14 steps from 19-hydroxy-17-oxoandrost-5-en-3β-yl acetate in 8.9% yield, the analogous sequence in the pregnenolone series gave 3β-hydroxy[19-$^2H_3$]pregn-5-en-20-one in 7.3% yield. Corresponding sulfates were prepared via pyridinium salts in 53 and 57% yields, respectively. Fully assigned NMR data of selected pregnenolone derivatives were given.

Keywords: Steroid; Synthesis; Deuterium; NMR spectra

1. Introduction

Recent interest in neuroactive steroids stimulated the need for compounds that can be traced in biological tissues together with their metabolites. Our study focused on dehydroepiandrosterone (DHEA), pregnenolone (PREG), and their sulfates. These derivatives are included among neurosteroids, i.e., they are both synthesized and accumulated in the nervous system to levels, at least in part, independent of peripheral steroidogenesis. Neurosteroids interact with various brain cell receptors, mainly through γ-aminobutyric acid (GABA) receptors or *N*-methyl-D-aspartate (NMDA) glutamatergic receptors. They have been demonstrated to exhibit a broad spectrum of biological actions, influencing memory and mental activity (e.g., learning, perception, cognition, and spatial orientation) [1–5].

Among the analytical methods for tracing neurosteroids, a combination of gas chromatography–mass spectroscopy combination is the frequently used one [6]. The principal requirement when using deuterium labeling is the presence of at least two deuterium atoms substituting protium. This allows for sufficient sensitivity, which is influenced by the interference of the first isotopic satellite caused by the natural abundance of ^{13}C isotope. Moreover, it is necessary to use such positions on the steroid skeleton where deuterium is sufficiently stable for protium exchange and for possible metabolic transformations of the steroid skeleton under study. Taking this together, labeling with three deuterium atoms at the angular methyl in position 19 fulfills all prerequisites for successful tracing.

With respect to the development of a synthetic approach for the above-mentioned deuterated derivatives of DHEA and PREG (Scheme 1), substantial background information exists in the literature. The functionalization of position 19 was feasible through lead(IV) acetate cyclization of the corresponding 6β-hydroxy derivatives [7–9]. Transformations into 19-oic acid were performed mostly on 3-oxo-4-ene derivatives [8], and the use of 6β-methoxy-3α,5-cyclo derivative was previously described in patent literature [10]. Introduction

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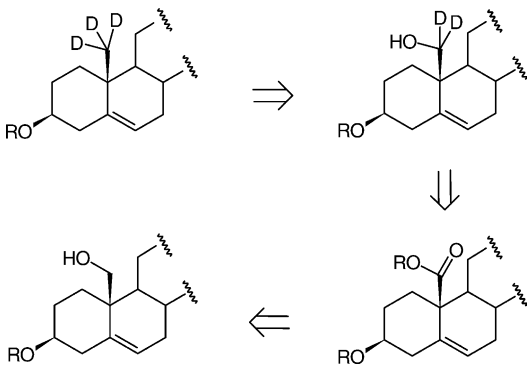


Scheme 1.

of two deuterium atoms using lithium aluminum deuteride reduction of methyl ester is a standard method; in the androstane series, it was performed on methyl 17,17-ethylenedioxy-3β-methoxyandrost-5-en-19-oate [11]. For the third deuterium atom, a general method using an exchange of the mesyloxy group could be applied [12]. However, some of the existing methods are specific for a particular type of steroid (mostly for 3-oxo-4-enes of the androstane series in connection with aromatization studies), and especially in the PREG series, even initial intermediates are not known or sufficiently documented. We, therefore, decided to develop our own approach using the androstane series for preliminary experiments and applied the sequence to the PREG series.

2. Experimental

2.1. General

Melting points were determined on a Boetius micro-melting point apparatus (Germany). Optical rotations were measured at 25 °C on a Perkin-Elmer 141 MC polarimeter, and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Infrared spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer. ^{1}H NMR spectra were taken on Varian UNITY-200 and Bruker AVANCE-400 instruments (200 and 400 MHz, FT mode) at 23 °C in $CDCl_3$ and referenced to TMS as the internal standard. Chemical shifts are given in ppm (δ-scale); coupling constants (J) and width of multiplets (W) are given in Hz. NMR spectra for selected pregnane derivatives (**13**, **15**, **16**, **18**, **23**, **29**–**32**, PREG, and PREG-Ac) were measured on Varian UNITY-500 and Bruker AVANCE-500 instruments (^{1}H at 500 MHz; ^{13}C at 125.7 MHz) under the above conditions, except for **32** that was measured in D_2O. For ^{13}C, secondary referencing was performed using the solvent signal at position $\delta(CDCl_3) = 77.0$. In addition to 1D proton and carbon NMR spectra, homonuclear 2D-spectra (H,H-PFG-COSY, ROESY and J-resolved) together with heteronuclear 2D-spectra (H,C-PFG-HSQC) were used for complete structural assignment of signals (Tables 1 and 2). Mass spectra were recorded by means of a VG Analytical ZAB-EQ spectrometer.

Thin-layer chromatography (TLC) was performed on silica gel G (ICN Biochemicals, detection by spraying with concentrated sulfuric acid followed by heating). For column chromatography, Silica gel 60 (Merck, 63–100 μm) or Aluminum oxide 90 neutral (Merck, 63–200 μm, activity III) were used. Prior to evaporation on a rotary evaporator in vacuo (0.25 kPa, bath temperature 40 °C), solutions in organic solvents were dried over anhydrous $MgSO_4$.

19-Hydroxy-3β-methoxy-3α,5-cyclo-5α-androstan-17-one (**1**) was prepared both according to Ref. [13] and by a procedure described below for PREG derivative **18** (yield from 19-hydroxy-17-oxoandrost-5-en-3β-yl acetate was 36%), m.p. 102–103 °C, $[\alpha]_D + 128$ (c 1.2, $CHCl_3$), literature [13] gave m.p. 104–107 °C, $[\alpha]_D + 145.2$ ($CHCl_3$); 19-hydroxy-20-oxopregn-5-en-3β-yl acetate (**13**) was prepared according to Ref. [9], m.p. 171–173 °C (acetone/hexane), $[\alpha]_D + 22$ (ca. 0.6, $CHCl_3$), ^{1}H and ^{13}C NMR, see Tables 1 and 2, literature gives m.p. 165–176 °C, $[\alpha]_D + 19$ [7] and m.p. 168–171 °C (diethyl ether/pentane) [9]; pregnenolone and pregnenolone acetate (PREG-Ac) were obtained from Steraloids. Jones reagent was prepared by dissolving CrO_3 (13.35 g) in concentrated H_2SO_4 (11.5 ml) and water (20 ml) and subsequently diluting to 50 ml with water. Lithium aluminum deuteride (Aldrich) had 98% $LiAlD_4$. For deuterium exchange experiments (**9** and **28**), all solid components were predried in vacuo: powdered mesylate at 50 °C for 5 h, zinc and sodium iodide at 105 °C for 10 h. 1,2-Dimethoxyethane was left for several days over 4A molecular sieves, and it had a residual water content of 30 μg/ml. Deuterium oxide (CDN isotopes, Quebec, Canada) had a 99.9% content of D_2O.

2.2. Derivatives of DHEA

2.2.1. Methyl 3β-hydroxy-17-oxoandrost-5-en-19-oate (4)

Freshly prepared Jones reagent (2.67 M, 7 ml) was added dropwise to a stirred solution of cycloderivative **1** (1.4 g, 4.40 mmol) in acetone (90 ml) under argon and in an ice bath. The reaction mixture was stirred and cooled for 3.5 h (TLC: chloroform/acetone (10:1)). 2-Propanol (1 ml) was then added, the mixture was stirred while cooling for 5 min, and then poured into saturated aqueous $KHCO_3$ (100 ml). Part of the acetone was removed on a rotary evaporator, additional $KHCO_3$ (100 ml) was added, and the product was extracted with ethyl acetate (2× 150 ml). The organic layers were collected, dried, and the solvent was evaporated. The crude acid **2** (1.2 g) was dissolved in acetone (25 ml), 35% perchloric acid (2.5 ml) was added, and the mixture was stirred at room temperature for 1 h. After pouring into ice-water (100 ml), the crystalline product was filtered off, washed with water, and dried under air. Crude **3** (1.0 g) was

Table 1
^{1}H NMR chemical shifts in $CDCl_3$ (except for **32** which was measured in D_2O at 50 °C)

Proton	**13** (a)	**15** (b)	**16** (c)	**18** (d)	**23** (e)	**29**	**30**	**31** (f)	**32**	PREG	PREG-Ac (g)
1α (ax)	1.16	1.11	1.11	0.71	1.03	1.10	1.10	1.13	1.15	1.10	1.16
1β (eq)	1.97	2.08	1.95	1.34	2.61	1.86	1.85	1.87	1.96	1.86	1.87
2α (eq)	1.88	1.87	1.86	1.76	1.95	1.86	1.85	2.18	2.04	1.85	1.87
2β (ax)	1.50	1.41	1.40	1.57	1.51	1.50	1.50	1.70	1.70	1.50	1.60
3	4.65	3.56	3.58	1.13	3.55	3.53	3.53	4.36	4.20	3.53	4.61
4α (eq)	2.43	2.37	2.39	0.64	2.48	2.29	2.31	2.64	2.53	2.31	2.34
4β (ax)	2.27	2.26	2.20	0.48	1.79	2.24	2.23	2.42	2.42	2.24	2.31
6	5.78	5.60	5.75	2.80	5.66	5.36	5.36	5.39	5.52	5.35	5.38
7α (ax)	1.58	1.55	1.57	1.07	1.59	1.58	1.48	1.55	1.61	1.59	1.58
7β (eq)	2.06	2.04	2.06	1.97	2.13	2.00	2.00	1.99	2.03	2.00	2.00
8 (ax)	1.88	1.74	1.90	2.11	1.79	1.48	1.48	1.48	1.51	1.48	1.48
9 (ax)	0.98	1.00	0.96	0.87	1.10	0.99	0.99	0.99	1.04	0.99	1.01
11α (eq)	∼1.66	1.69	∼1.67	1.55	1.78	1.63	∼1.62	1.61	1.68	1.63	1.62
11β (ax)	∼1.66	1.51	∼1.67	1.76	1.09	1.48	∼1.62	1.45	1.52	1.48	1.48
12α (ax)	1.45	1.43	1.43	1.43	1.43	1.46	1.46	1.46	1.52	1.46	1.47
12β (eq)	2.09	2.08	2.09	2.08	2.04	2.05	2.05	2.04	2.05	2.05	2.05
14 (ax)	1.07	1.08	1.06	1.12	1.11	1.16	1.15	1.15	1.27	1.15	1.18
15α	1.66	1.68	∼1.65	1.67	1.69	1.68	∼1.65	1.67	1.73	1.68	1.68
15β	1.24	1.24	1.24	1.24	1.25	1.24	1.24	1.23	1.26	1.24	1.23
16α	2.18	2.18	2.18	2.21	2.18	2.18	2.18	2.17	2.04	2.17	2.18
16β	∼1.65	1.66	∼1.65	1.64	1.66	∼1.65	∼1.65	1.66	1.73	1.65	1.65
17	2.53	2.52	2.51	2.54	2.51	2.53	2.53	2.53	2.74	2.53	2.54
18	0.684	0.651	0.688	0.723	0.567	0.634	0.634	0.625	0.63	0.634	0.632
19	3.87	4.47	3.84	3.53	–	–	0.976	–	–	1.011	1.022
19′	3.61	3.96	3.60								
21	2.125	2.118	2.118	2.126	2.103	2.127	2.127	2.125	2.21	2.127	2.127

Other protons—(a) OAc: 2.036 s; (b) OAc: 2.027 s; (c) OH: 1.05 b; (d) OH: 4.40 dd; (e) OMe: 3.702 s; (f) C_5H_5N: 8.98 m (H-2,6), 8.00 m (H-3,5), 8.49 m (H-4); (g) OAc: 2.037 s.

Table 2
^{13}C NMR chemical shifts in $CDCl_3$ (except for **32** which was measured in D_2O at 50 °C)

Carbon	**13** (a)	**15** (b)	**16**	**18** (c)	**23** (d)	**29**	**30**	**31** (e)	**32**	PREG	PREG-Ac (f)
1	33.14	33.77	33.40	29.80	33.83	37.17	37.20	37.07	36.89	37.28	37.01
2	28.04	31.77	31.09	24.08	33.04	31.60	31.61	28.83	28.61	31.63	27.73
3	73.28	71.37	71.30	23.30	70.95	71.70	71.70	78.80	80.73	71.70	73.83
4	38.13	42.36	42.24	12.53	44.17	42.24	42.24	39.17	38.86	42.27	38.07
5	134.56	135.64	135.57	35.58	134.82	140.74	140.73	140.14	141.60	140.79	139.68
6	127.95	125.51	127.04	82.01	124.39	121.38	121.40	122.04	122.17	121.37	122.31
7	31.08	31.22	31.90	32.94	30.85	31.75	31.76	31.77	31.65	31.78	31.76
8	33.25	32.92	33.31	32.06	32.03	31.86	31.86	31.82	31.86	31.87	31.83
9	50.14	50.09	50.23	47.39	48.64	49.93	49.94	49.83	49.92	50.02	49.92
10	41.57	39.64	41.50	48.44	50.73	36.30	36.37	36.29	36.68	36.53	36.61
11	21.67	21.69	21.72	22.54	23.32	21.08	21.08	21.03	21.01	21.09	21.03
12	39.03	38.99	39.09	39.50	38.64	38.63	38.83	38.79	38.40	38.86	38.80
13	44.15	44.01	44.19	44.76	43.92	43.99	44.00	43.98	44.65	43.99	43.96
14	57.68	57.46	57.78	57.44	56.54	56.90	56.90	56.84	56.60	56.93	56.85
15	24.27	24.40	24.28	24.24	24.26	24.47	24.48	24.46	24.30	24.48	24.47
16	22.79	22.84	22.79	22.81	22.81	22.80	22.80	22.79	22.87	22.85	22.85
17	63.60	63.60	63.63	63.78	63.54	63.70	63.70	63.70	63.92	63.72	63.68
18	13.55	13.28	13.57	13.86	13.16	13.21	13.22	13.20	12.82	13.21	13.20
19	62.69	64.53	62.72	66.50	174.10	18.51 [g]	18.80 [h]	18.42 [g]	17.19 [g]	19.37	19.28
20	209.50	209.28	209.56	209.52	209.28	209.58	209.58	209.59	217.93	209.46	209.42
21	31.49	31.43	31.47	31.46	31.46	31.54	31.54	31.54	31.35	31.49	31.50

(a) OAc: 170.50, 21.36; (b) OAc: 170.63, 21.03; (c) OMe: 56.56; (d) OMe: 51.80; (e) C_6D_5NH: 142.28 (C-2,6), 127.12 (C-3,5), 145.69 (C-4); (f) OAc: 170.49, 21.38; (g) heptet (^1J(C, D) = 18.7 Hz); (h) pentet (^1J(C, D) = 19.3 Hz).

dissolved in methanol (30 ml), cooled with an ice bath, and methylated with ethereal diazomethane solution (to yellow color). After 5 min (TLC: chloroform/acetone (10:1)), several drops of acetic acid were added, and the solution was evaporated to dryness (1.2 g). Column chromatography on silica gel (150 ml) in a mixture of chloroform/acetone (20:1) gave 999 mg of ester **4** (68%), m.p. 188–190 °C, $[\alpha]_D$ − 61 (c 0.6, $CHCl_3$). Literature [14] gave m.p. 188–190 °C. IR ($CHCl_3$), ν (cm^{-1}): 3607, 3480 (OH); 1733, 1723 (C=O, ester, ketone); 1677 (C=C); 1438 (CH_3, ester); 1206, 1167 (C–O). ^{1}H NMR (200 MHz), δ (ppm): 5.69 (1H, m, W = 12, H-6); 3.73 (3H, s, $COOCH_3$); 3.57 (1H, m, W = 30, H-3α); 0.82 (3H, s, 3 × H-18). Analysis calculated for $C_{20}H_{28}O_4$ (332.4): C, 72.26; H, 8.49. Found: C, 72.37; H, 8.53.

2.2.2. Methyl 17,17-ethylenedioxy-3β-(methoxymethoxy) androst-5-en-19-oate (6)

Ethylene glycol (1.5 ml, 27 mmol), triethyl orthoformate (4.5 ml, 27 mmol), and 4-toluenesulfonic acid monohydrate (10 mg) were added to a suspension of ester **4** (900 mg, 2.71 mmol) in benzene (20 ml). The mixture was stirred at room temperature for 5 h (TLC: benzene/ether (1:1)), and during this time, the suspension dissolved. The reaction mixture was poured into ice cooled, saturated aqueous $KHCO_3$ (100 ml) and extracted with ethyl acetate (100 ml). The extract was washed successively with $KHCO_3$ (2×), and with water, dried, and the solvent was evaporated. Crude ketal **5** (960 mg) was dissolved in benzene (20 ml), cooled in an ice bath, and *N*,*N*-diisopropylethylamine (5 ml, 28.7 mmol) and bromomethyl methyl ether (0.6 ml, 7.4 mmol) were added. The mixture was stirred at room temperature for 2 h (TLC: benzene/acetone (10:1)). Then, the product was partitioned between ethyl acetate (100 ml) and cold 5% aqueous citric acid (100 ml). The organic layer was washed sequentially with 5% aqueous citric acid, water, saturated aqueous $KHCO_3$ (2×), and water, dried, and the solvent was evaporated. The residue (800 mg) in minimum benzene was applied onto a short column of alumina (30 ml) in benzene, and the product was eluted with a mixture of benzene/acetone (20:1). The yield of protected ester **6** was 590 mg (52%), m.p. 134–136 °C (methanol), $[\alpha]_D$ − 118 (c 0.5, $CHCl_3$). IR ($CHCl_3$), ν (cm^{-1}): 1721 (C=O, ester); 1675 (C=C); 1437 (CH_3, ester); 1169, 1031 (C–O, ester); 1150, 1106, 1041 (COCOC, methoxymethoxy); 1106, 959, 952 (ring, dioxolane). ^{1}H NMR (200 MHz), δ (ppm): 5.67 (1H, bd, J = 5.8, H-6); 4.67 (2H, s, O–CH_2–O); 3.88 (4H, m, OCH_2CH_2O); 3.71 (3H, s, $COOCH_3$); 3.45 (1H, m, W = 30, H-3α); 3.36 (3H, s, C**H**$_3$–O–CH_2); 0.79 (3H, s, 3 × H-18). Analysis calculated for $C_{24}H_{36}O_6$ (420.6): C, 68.55; H, 8.63. Found: C, 68.67; H, 8.63.

2.2.3. 17,17-Ethylenedioxy-3β-(methoxymethoxy)[19-$^2H_2$] androst-5-en-19-ol (7)

Protected ester **6** (500 mg, 1.19 mmol) in THF (15 ml) was refluxed with lithium aluminum deuteride (140 mg, 3.33 mmol) under argon for 4 h. The reaction mixture was then chilled in an ice bath, the remaining deuteride was destroyed with saturated, aqueous Na_2SO_4, and the mixture was diluted with ether (15 ml). The solids were filtered off on a celite column (10 ml), which was then washed with ether (50 ml). The resulting solution was washed sequentially with cold 5% aqueous citric acid, water, saturated aqueous $KHCO_3$ (2×), and water. After drying, the solvents were evaporated, leaving 460 mg (98%) of hydroxy derivative **7**, m.p. 140–141 °C (methanol), $[\alpha]_D$ − 64 (c 0.2, $CHCl_3$). IR ($CHCl_3$), ν (cm^{-1}): 3624, 3577 (OH); 2217, 2116 (C^2H_2); 1667 (C=C); 1149, 1104, 1042 (COCOC, methoxymethoxy); 1104, 959, 950 (ring, dioxolane). ^{1}H NMR (200 MHz), δ (ppm): 5.76 (1H, m, W = 12, H-6); 4.69 (2H, s, O–CH_2–O); 3.87 (4H, m, OCH_2CH_2O); 3.48 (1H, m, W = 32, H-3α); 3.37 (3H, s, C**H**$_3$–O–CH_2); 0.92 (3H, s, 3 × H-18). Analysis calculated for $C_{23}H_{34}{}^2H_2O_5$ (394.6): C, 70.02; H, 8.69; ^{2}H, 1.02. Found: C, 69.94; H, 9.44 (distorted by ^{2}H).

2.2.4. 17,17-Ethylenedioxy-3β-(methoxymethoxy)[19-$^2H_3$] androst-5-ene (9)

Hydroxy derivative **7** (450 mg, 1.14 mmol) was dissolved in pyridine (5 ml), chilled in an ice bath, and methanesulfonyl chloride (0.4 ml, 5.17 mmol) was added. The reaction mixture was left aside in an ice bath for 1 h. Then it was poured onto ice, and the solid product formed was filtered off. The solids were dissolved in ether (50 ml), and the solution was washed sequentially with ice cold, 5% aqueous citric acid (2×), water, saturated aqueous $KHCO_3$ (2×), and water. After drying, the solvent was evaporated, the residue was coevaporated with benzene (3× 10 ml), and thoroughly dried in vacuo. Crude mesylate **8** (430 mg) was refluxed under an argon atmosphere in 1,2-dimethoxyethane (4 ml) with sodium iodide (430 mg, 2.87 mmol), zinc (430 mg, 6.58 mmol), and deuterium oxide (0.4 ml, 22.11 mmol) for 6 h. The reaction mixture was cooled, diluted with ether, and filtered through a celite column (10 ml), which was washed sequentially with ether (ca. 40 ml). The ethereal solution was washed with water, ice cold 5% aqueous citric acid (2×), water, saturated aqueous $KHCO_3$, 5% aqueous sodium thiosulfate pentahydrate, and water. After drying, the solvent was evaporated. The residue was dissolved in a minimum of benzene, introduced onto a short column of aluminum oxide (15 ml), and the product was eluted with a mixture of benzene/ether (10:1). The yield of protected d_3-DHEA (**9**) was 340 mg (78%), m.p. 90–92 °C (methanol), $[\alpha]_D$ − 67 (c 0.2, $CHCl_3$). IR ($CHCl_3$), ν (cm^{-1}): 2225 (C^2H_3); 1668 (C=C); 1148, 1105, 1042 (COCOC, methoxymethoxy); 1105, 959, 951 (ring, dioxolane). ^{1}H NMR (200 MHz), δ (ppm): 5.36 (1H, m, W = 12, H-6); 4.69 (2H, s, O–CH_2–O); 3.86 (4H, m, W = 30, OCH_2CH_2O); 3.42 (1H, m, W = 31, H-3α); 3.37 (3H, s, C**H**$_3$–O–CH_2); 0.86 (3H, s, 3 × H-18). Analysis calculated for $C_{23}H_{33}{}^2H_3O_4$ (379.6): C, 72.78; H, 8.76; ^{2}H, 1.52. Found: C, 72.72; H, 9.74 (distorted by ^{2}H).

*2.2.5. 3β-Hydroxy[19-$^2H_3$]androst-5-en-17-one (**10**)*

Protected d_3-DHEA (**9**) (300 mg, 0.79 mmol) was stirred in benzene (5 ml) and a methanol (5 ml) solution with 35% aqueous $HClO_4$ at 50 °C for 4 h. The reaction mixture was cooled, poured into cold, saturated aqueous $NaHCO_3$, and extracted with ethyl acetate (20 ml). The extract was washed with saturated aqueous $NaHCO_3$ and water, dried, and the solvents were evaporated. The residue was chromatographed on silica gel (20 ml) in a mixture of benzene/acetone (50:1). The yield of d_3-DHEA (**10**) was 210 mg (91%), m.p. 149–151 °C (methanol), $[\alpha]_D$ +12 (c 0.2, ethanol). Ref. [15] gives m.p. 142–143 and 152–153 °C, $[\alpha]_D$ + 10.9 (c 0.4, ethanol) for the non-deuterated analog. IR ($CHCl_3$), ν (cm^{-1}): 3608, 3458 (OH); 2225 (C^2H_3); 1732 (C=O); 1668 (C=C); 1048 (C–OH). ^{1}H NMR (200 MHz), δ (ppm): 5.39 (1H, m, $W = 12$, H-6); 3.55 (1H, m, $W = 32$, H-3α); 3.36 (1H, d, $J = 5.6$, OH); 0.89 (3H, s, 3 × H-18). EI-MS, *m/z* (%): 206 (45), 216 (15), 228 (7), 234 (12), 237 (9), 245 (9), 255 (53), 273 (46), 291 (100, M^+); 0.5 ($M-3$), 0.7 ($M-2$), 4.1 ($M-1$), 100.0 (M), 21.0 ($M+1$), 2.2 ($M+2$); DHEA for comparison: 203 (46), 213 (20), 228 (18), 231 (12), 237 (9), 242 (6), 255 (59), 270 (37), 288 (100, M^+); 0.5 ($M-2$), 0.7 ($M-1$), 100.0 (M), 21.2 ($M+1$), 2.2 ($M+2$). Analysis calculated for $C_{19}H_{25}{}^2H_3O_2$ (291.5): C, 78.30; H, 8.65; ^{2}H, 2.07. Found: C, 78.52; H, 9.86 (distorted by ^{2}H).

*2.2.6. Pyridinium 17-oxo[19-$^2H_3$]androst-5-en-3β-yl sulfate (**11**)*

A solution of d_3-DHEA (**10**) (300 mg, 1.03 mmol) and sulfur(IV) oxide–pyridine complex (300 mg, 1.88 mmol) in pyridine (5 ml) was stirred and heated to 70 °C for 1 h. The reaction mixture was then evaporated nearly to dryness, and hot water (4 ml) was added. The solution was left to attain room temperature and then applied onto a column of Lichroprep RP-18 (40–63 μm, 40 g) prewashed with water. The column was washed with water, and the product was eluted with methanol. The fractions containing the sulfate were collected, and the solvent was evaporated. Crystallization from a methanol/ether mixture gave 360 mg (78%) of sulfate **11**, m.p. 188–192 °C, $[\alpha]_D$ + 6 (c 0.4, $CHCl_3$). Ref. [16] gave m.p. 194–195 °C for non-deuterated analog. ^{1}H NMR (400 MHz), δ (ppm): 8.99, 8.49, and 8.01 (5H, pyridinium); 5.42 (1H, m, $W = 12$, H-6); 4.35 (1H, m, $W = 32$, H-3α); 0.88 (3H, s, 3 × H-18). FAB-MS, *m/z*: 451 ($M+1$). Analysis calculated for $C_{24}H_{30}{}^2H_3NO_5S$ (450.6): C, 63.97; H, 6.71; ^{2}H, 1.34; N, 3.11; S, 7.12. Found: C, 63.78; H, 7.63 (distorted by ^{2}H); N, 3.02; S, 7.11.

*2.2.7. Sodium 17-oxo[19-$^2H_3$]androst-5-en-3β-yl sulfate (**12**)*

Pyridinium salt **11** (335 mg, 0.74 mmol) was thoroughly shaken with water (6 ml), and a 10% aqueous NaCl solution (6 ml) was added in portions. After 1 h of standing in the refrigerator, the crystals were filtered off, washed with a small amount of ice cold water and dried. Crude sulfate (260 mg) was recrystallized by dissolving in methanol (5 ml) and adding chloroform (7.5 ml). The yield of sodium salt **12** was 210 mg (68%), m.p. 192–194 °C, $[\alpha]_D$ + 18 (c 0.2, methanol). Ref. [16] gave m.p. 192–193 °C and Ref. [17] gave m.p. 185–187 °C, $[\alpha]_D$ + 10.7 (c 4, methanol) for non-deuterated analog. IR (KBr), λ (cm^{-1}): 2220 (C^2H_3); 1733 (C=O); 1289–1218, 1065, 986, 969 (sulfate). Analysis calculated for $C_{19}H_{24}{}^2H_3NaO_5S \cdot H_2O$ (411.5): C, 55.46; H, 6.37; ^{2}H, 1.47; S, 7.79. Found: C, 55.16; H, 7.31 (distorted by ^{2}H); S, 7.75.

2.3. Derivatives of PREG

*2.3.1. 20-Oxopregn-5-ene-3β,19-diyl diacetate (**14**)*

Acetate **13** (6 g, 16.0 mmol) was dissolved in pyridine (50 ml), the solution was cooled in an ice bath, and 4-dimethylaminopyridine (650 mg, 5.2 mmol) and acetic anhydride (7 ml, 74.2 mmol) were added. The reaction mixture was left at room temperature for 3 h (TLC: benzene/acetone (10:1)). Then, the reaction mixture was poured into an ice and water mixture (500 ml), and the crystals were filtered off and washed with water. The crude product was dissolved in ether (300 ml) and washed sequentially with 5% HCl, saturated aqueous $KHCO_3$ (2×), and water. After drying, the solvent was evaporated leaving 6.1 g (91%) of diacetate **14** pure enough for further processing. An analytical sample was crystallized from methanol, m.p. 103–104 °C, $[\alpha]_D$ −17 (c 0.64, $CHCl_3$). Ref. [18] gave m.p. 104.5–105 °C, $[\alpha]_D$ − 21 (c 0.6, $CHCl_3$). IR ($CHCl_3$), ν (cm^{-1}): 1727 (C=O, acetate); 1703 (C=O, ketone); 1370 (CH_3, acetate); 1256, 1034 (C–O, acetate). ^{1}H NMR (200 MHz), δ (ppm): 5.63 (1H, m, $W = 12$, H-6); 4.64 (1H, m, $W = 32$, H-3α); 4.49 (1H, d, $J = 11.9$, H-19); 3.96 (1H, d, $J = 11.9$, H-19′); 2.12 (3H, s, 3 × H-21); 2.03 (6H, s, OAc); 0.65 (3H, s, 3 × H-18). Analysis calculated for $C_{25}H_{36}O_5$ (416.6): C, 72.08; H, 8.71. Found: C, 72.06; H, 8.86.

*2.3.2. 3β-Hydroxy-20-oxopregn-5-en-19-yl acetate (**15**)*

Diacetate **14** (5.6 g, 13.4 mmol) was dissolved in methanol (500 ml), 2% aqueous $KHCO_3$ (60 ml) was added under stirring, and the mixture was refluxed and stirred for 2 h (TLC: benzene/acetone (6:4)). Most of the methanol was then evaporated on a rotary evaporator, and the mixture was diluted with water (300 ml) and extracted with ether (500 ml). The ethereal layer was washed with water (2×), dried, and the solvent was evaporated, leaving a crude mixture (5.2 g) in which monoacetate **15** prevailed. Column chromatography on silica gel (70 g) in a mixture of benzene/acetone (from 20:1 to 10:1) yielded 4.4 g (87%) of monoacetate **15**, m.p. 143–145 °C (acetone/hexanes), $[\alpha]_D$ − 20 (c 0.6, $CHCl_3$). IR ($CHCl_3$), ν (cm^{-1}): 3608 (O–H); 1726 (C=O, acetate); 1701 (C=O, ketone); 1367 (CH_3, acetate); 1236 (C–O, acetate). For ^{1}H and ^{13}C NMR data, see Tables 1 and 2. Analysis calculated for $C_{23}H_{34}O_4$ (374.5): C, 73.76; H, 9.15. Found: C, 73.48; H, 9.25.

From the less polar fractions, diacetate **14** (360 mg, 6%) was regenerated.

*2.3.3. 3β,19-Dihydroxypregn-5-en-20-one (**16**)*

After further elution with a mixture of benzene/acetone (1:1), chromatography of a mixture from the preceding experiment gave a sample of diol **16** (120 mg, 3%), m.p. 195–196 °C (methanol), $[\alpha]_D$ + 28 (c 0.30, $CHCl_3$). Literature [13] gave m.p. 193–194 °C (benzene). IR ($CHCl_3$), ν (cm^{-1}): 3611, 3480 (O–H); 1699 (C=O, ketone); 1386, 1358 (CH_3); 1042 (C–OH). For ^1H and ^{13}C NMR data, see Tables 1 and 2. Analysis calculated for $C_{21}H_{32}O_3$ (332.5): C, 75.86; H, 9.70. Found: C, 76.01; H, 9.62.

*2.3.4. 19-Hydroxy-6β-methoxy-3α,5-cyclo-5α-pregnan-20-one (**18**)*

Monoacetate **15** (4.3 g, 11.5 mmol) was dissolved in pyridine (50 ml), the solution was cooled in an ice bath, and methanesulfonyl chloride (2.7 ml, 35 mmol) was added. After 1 h (TLC: benzene/acetone (10:1)), the reaction mixture was poured into an ice and water mixture (300 ml), and crystallization of the product was induced by friction of a glass stick against the walls of the beaker. Crystals were filtered off, and washed with water. The crude product was dissolved in benzene (350 ml) and sequentially washed with 5% aqueous citric acid (2×), saturated aqueous $KHCO_3$ (2×), and water. After drying, the solvent was evaporated, and the residue was dried in vacuo. The crude mesylate was dissolved in dioxane (25 ml) and methanol (50 ml), freshly fused potassium acetate (4.0 g, 41 mmol) was added, and the mixture was stirred and refluxed for 5 h (TLC: benzene/petroleum ether/acetone (10:10:1)). Methanol was then evaporated on a rotary evaporator, water and ice were added (200 ml), and the mixture was extracted with ether (200 ml). The extract was washed with water with a small amount of saturated aqueous NaCl solution (3×), dried, and the solvent was evaporated. A solution of sodium hydroxide (4.8 g, 120 mmol) in methanol (400 ml) was added to a crude cycloderivative **17**, and the mixture was refluxed and stirred for 1 h (TLC: benzene/petroleum ether/acetone (10:10:1)). After cooling, a lump of solid carbon dioxide was added, and the mixture was concentrated to a small volume. Then ice-water (200 ml) was added, and the product was extracted with ether (2× 100 ml). The ethereal layer was washed with water with small amount of saturated aqueous NaCl solution (3×), dried, and the solvent was evaporated to give 3.7 g of a foamy, crude product. Purification by column chromatography on silica gel (200 ml) in a mixture of benzene/acetone (from 20:1 to 10:1) gave 2.5 g of cycloderivative **18** (63% from **15**). After crystallization from methanol, 1.7 g of **18** (43% from **15**) was obtained, m.p. 139–141 °C, $[\alpha]_D$ + 130 (c 0.6, $CHCl_3$). IR ($CHCl_3$), ν (cm^{-1}): 3394 (OH); 3004 (CH_2, cyclopropane); 1698 (C=O); 1435 (CH_3, OMe); 1014 (cyclopropane). For ^1H and ^{13}C NMR data, see Tables 1 and 2. Analysis calculated for $C_{22}H_{34}O_3$ (346.5): C, 76.26; H, 9.89. Found: C, 76.23; H, 10.10.

*2.3.5. 19-Hydroxy-6β-methoxy-3α,5-cyclo-5α,17α-pregnan-20-one (**19**)*

This compound was a side product in the preparation of cycloderivative **18**, from less polar fractions in the final chromatography. The yield of **19** was 350 mg (9%, referred to **15**), m.p. 148–149 °C. ^1H NMR (400 MHz), δ (ppm): 4.42 (1H, dd, J = 0.9, 11.8, OH); 3.58 (1H, dd, J = 11.4, 11.8, H-19); 3.38 (3H, s, OCH_3); 3.25 (1H, d, J = 11.4, H-19′); 2.83 (1H, dd, J = 2.7, 8.5, H-17α); 2.79 (1H, t, J = 2.8, H-6α); 2.14 (3H, s, 3 × H-21); 1.02 (3H, s, 3 × H-18); 0.61 (1H, dd, J = 4.0, 5.2, H-4α); 0.46 (1H, dd, J = 8.2, 5.2, H-4β).

*2.3.6. 19-Hydroxy-3β-methoxypregn-5-en-20-one (**20**)*

This compound was a side product in the preparation of cycloderivative **18**, from more polar fractions in the final chromatography. The yield of **20** was 400 mg (10%, referred to **15**), m.p. 161–163 °C, $[\alpha]_D$ +24 (c 0.6, $CHCl_3$). ^1H NMR (200 MHz), δ (ppm): 5.75 (1H, m, W = 12, H-6); 3.84 (1H, dd, J = 2.1, 11.5, H-19); 3.59 (1H, dd, J = 9.1, 11.5, H-19′); 3.36 (3H, s, OCH_3); 3.12 (1H, m, W = 32, H-3α); 2.12 (3H, s, 3 × H-21); 0.67 (3H, s, 3 × H-18).

*2.3.7. Methyl 3β-hydroxy-20-oxopregn-5-en-19-oate (**23**)*

Freshly prepared Jones reagent (2.67 M, 9 ml) was added dropwise to a stirred solution of cycloderivative **18** (1.5 g, 4.33 mmol) in acetone (90 ml) under argon and in an ice bath. The reaction mixture was stirred and cooled for 2.5 h (TLC: chloroform/acetone (10:1)). 2-Propanol (2 ml) was then added, and the mixture was stirred while cooling for 5 min and then, poured into saturated, aqueous $KHCO_3$ (100 ml). Part of the acetone was removed on a rotary evaporator, additional $KHCO_3$ (100 ml) was added, and the product was extracted with ethyl acetate (2× 150 ml). The organic layers were collected, dried, and the solvent was evaporated. The crude product **21** (1.2 g) was dissolved in acetone (25 ml), 35% perchloric acid (2.5 ml) was added, and the mixture was stirred at room temperature for 1 h. After pouring into ice-water (100 ml), crystalline product was filtered off, washed with water, and dried under air. Crude **22** (950 mg) was dissolved in methanol (30 ml), cooled in an ice bath, and methylated with ethereal diazomethane solution (to yellow color). After 5 min (TLC: chloroform/acetone (10:1)), several drops of acetic acid were added, and the solution was evaporated to dryness (1.14 g). Column chromatography on silica gel (150 ml) in a mixture of chloroform/acetone (20:1) gave 950 mg of ester **23** (61%). An analytical sample was crystallized from a mixture of acetone/petroleum ether, m.p. 175–176 °C, $[\alpha]_D$ − 39 (c 0.6, $CHCl_3$). IR ($CHCl_3$), ν (cm^{-1}): 3608 (OH); 1720 (C=O, ester); 1701 (C=O, ketone); 1438 (CH_3, ester); 1199, 1168 (C–O); 1048 (C–OH). For ^1H and ^{13}C NMR data, see Tables 1 and 2. Analysis calculated for $C_{22}H_{32}O_4$ (360.5): C, 73.30; H, 8.95. Found: C, 73.14; H, 9.18.

*2.3.8. Methyl 20,20-ethylenedioxy-3β-(methoxymethoxy) pregn-5-en-19-oate (**25**)*

Ethylene glycol (1.4 ml, 25 mmol), triethyl orthoformate (4.1 ml, 24.6 mmol), and 4-toluenesulfonic acid monohydrate (10 mg) were added to a suspension of ester **23** (900 mg, 2.50 mmol) in benzene (20 ml). The mixture was stirred at room temperature for 2 h (TLC: petroleum ether/acetone (3:1)), and during this time the suspension dissolved. The reaction mixture was poured into ice cooled, saturated aqueous $KHCO_3$ and extracted with ethyl acetate (100 ml). The extract was washed sequentially with $KHCO_3$ (2×), water, dried, and the solvent was evaporated. Crude ketal **24** (1 g) was dissolved in benzene (20 ml), cooled in an ice bath, and *N*,*N*-diisopropylethylamine (5 ml, 28.7 mmol) and bromomethyl methyl ether (0.6 ml, 7.4 mmol) were added. The mixture was stirred at room temperature for 2 h (TLC: benzene/acetone (10:1)). Then, the product was partitioned between ethyl acetate (100 ml) and cold 5% aqueous citric acid (100 ml). The organic layer was washed sequentially with citric acid, water, saturated aqueous $KHCO_3$ (2×), and water, dried, and the solvent was evaporated. Crude product (800 mg) in benzene was applied onto a short column of alumina (30 ml) in benzene and eluted with a mixture of benzene/acetone (20:1). The yield of protected ester **25** was 660 mg (59%), m.p. 137–138 °C (methanol), $[\alpha]_D$ − 73 (c 0.5, $CHCl_3$). IR ($CHCl_3$), ν (cm^{-1}): 1720 (C=O, ester); 1675 (C=C); 1440 (CH_3, ester); 1148, 1103, 1037 (COCOC, methoxymethoxy); 1103, 1055, 950 (ring, dioxolane). ^{1}H NMR (200 MHz), δ (ppm): 5.67 (1H, m, W = 12, H-6); 4.67 (2H, s, O–CH_2–O); 3.92 (4H, m, OCH_2CH_2O); 3.70 (3H, s, $COOCH_3$); 3.44 (1H, m, W = 30, H-3α); 3.36 (3H, s, C**H**$_3$–O–CH_2); 1.28 (3H, s, 3 × H-21); 0.71 (3H, s, 3 × H-18). Analysis calculated for $C_{26}H_{40}O_6$ (448.6): C, 69.61; H, 8.99. Found: C, 69.40; H, 9.24.

*2.3.9. 20,20-Ethylenedioxy-3β-(methoxymethoxy) [19-^{2}H$_2$]pregn-5-en-19-ol (**26**)*

Protected ester **25** (419 mg, 0.93 mmol) in THF (15 ml) was refluxed with lithium aluminum deuteride (110 mg, 2.62 mmol) under argon for 4 h. The reaction mixture was then chilled in an ice bath, the remaining deuteride was destroyed with saturated aqueous Na_2SO_4, and the mixture was diluted with ether (15 ml). The solids were filtered off on a celite column, which was then washed with ether (50 ml). The resulting solution was sequentially washed with cold, 5% aqueous citric acid, water, saturated aqueous $KHCO_3$ (2×), and water. After drying, the solvents were evaporated, leaving 380 mg (96%) of hydroxy derivative **26**, m.p. 132–133 °C (methanol), $[\alpha]_D$ − 26 (c 0.6, $CHCl_3$). IR ($CHCl_3$), ν (cm^{-1}): 3624, 3574 (OH); 2218, 2115 (C^2H_2); 1666 (C=C); 1147, 1103, 1036 (COCOC, methoxymethoxy); 1103, 1051, 950 (ring, dioxolane). ^{1}H NMR (200 MHz), δ (ppm): 5.75 (1H, m, W = 12, H-6); 4.68 (2H, s, O–CH_2–O); 3.91 (4H, m, OCH_2CH_2O); 3.47 (1H, m, W = 32, H-3α); 3.36 (3H, s, CH_3O); 1.29 (3H, s, 3 × H-21); 0.83 (3H, s, 3 × H-18). Analysis calculated for $C_{25}H_{38}{}^2H_2O_5$ (422.6): C, 71.05; H, 9.06; ^{2}H, 0.95. Found: C, 71.14; H, 9.74 (distorted by ^{2}H).

*2.3.10. 20,20-Ethylenedioxy-3β-(methoxymethoxy) [19-^{2}H$_3$]pregn-5-ene (**28**)*

Hydroxy derivative **26** (350 mg, 0.83 mmol) was dissolved in pyridine (3.5 ml), chilled in an ice bath, and methanesulfonyl chloride (0.35 ml, 4.52 mmol) was added. The reaction mixture was left in an ice bath for 1 h. Then, it was poured onto ice, and the solid product formed was filtered off. Solids were dissolved in ether (30 ml), the solution was sequentially washed with ice cold 5% aqueous citric acid (2×), water, saturated aqueous $KHCO_3$ (2×), and water. After drying, the solvent was evaporated, and the residue was coevaporated with benzene (3× 5 ml) and thoroughly dried in vacuo. Crude mesylate **27** (380 mg) was refluxed under an argon atmosphere in 1,2-dimethoxyethane (4 ml) with sodium iodide (400 mg, 2.67 mmol), zinc (400 mg, 6.12 mmol), and deuterium oxide (0.4 ml, 22.11 mmol) for 6 h. The reaction mixture was cooled, diluted with ether, and filtered through a celite column, which was washed with ether (ca. 30 ml). The ethereal solution was washed sequentially with water, ice cold 5% aqueous citric acid (2×), water, saturated aqueous $KHCO_3$, 5% aqueous sodium thiosulfate pentahydrate, and water. After drying, the solvent was evaporated. The residue (330 mg) was dissolved in a minimum of benzene and introduced onto a short column of aluminum oxide (20 ml). The product was eluted with a mixture of benzene/ether (100:1). The yield of protected d_3-pregnenolone **28** was 280 mg (83%), m.p. 110–112 °C (methanol), $[\alpha]_D$ −32 (c 0.3, $CHCl_3$). IR ($CHCl_3$), ν (cm^{-1}): 2224 (C^2H_3); 1669 (C=C); 1147, 1102, 1036 (COCOC, methoxymethoxy); 1102, 1048, 950 (ring, dioxolane). ^{1}H NMR (400 MHz), δ (ppm): 5.35 (1H, m, W = 12, H-6); 4.69 (2H, s, O–CH_2–O); 3.93 (4H, m, OCH_2CH_2O); 3.42 (1H, m, W = 32, H-3α); 3.37 (3H, s, C**H**$_3$–O–CH_2); 1.30 (3H, s, 3 × H-21); 0.78 (3H, s, 3 × H-18). Analysis calculated for $C_{25}H_{39}{}^2H_3O_4$ (409.6): C, 73.71; H, 9.60; ^{2}H, 1.47. Found: C, 73.75; H, 10.37 (distorted by ^{2}H).

*2.3.11. 3β-Hydroxy[19-^{2}H$_3$]pregn-5-en-20-one (**29**)*

Protected d_3-pregnenolone **28** (170 mg, 0.42 mmol) was stirred in benzene (3 ml) and methanol (3 ml) solution with 35% aqueous $HClO_4$ (0.3 ml) at 50 °C for 2 h. The reaction mixture was cooled, poured into cold saturated aqueous $NaHCO_3$, and extracted with ethyl acetate (30 ml). The extract was washed sequentially with saturated aqueous $NaHCO_3$ and water, dried, and the solvents were evaporated. The residue was chromatographed on silica gel (20 ml) in a mixture of benzene/acetone (100:1 to 50:1). The yield of d_3-pregnenolone (**29**) was 100 mg (75%), m.p. 185–187 °C (methanol), $[\alpha]_D$ + 25 (c 0.2, ethanol). Ref. [15] gives m.p. 193 °C, $[\alpha]_D$ + 28 (ethanol) for the non-deuterated analog. For ^{1}H and ^{13}C NMR data, see Tables 1 and 2. IR ($CHCl_3$), ν (cm^{-1}): 3609, 3458 (OH); 2225 (C^2H_3); 1699

(C=O); 1668 (C=C); 1368 (CH_3); 1358 (CH_3CO); 1048 (C–OH). EI-MS, *m/z* (%): 43 (100), 208 (15), 216 (18), 234 (32), 258 (9), 283 (17), 301 (30), 319 (59, M^+); 1.2 ($M-3$), 0.8 ($M-2$), 5.4 ($M-1$), 100.0 (M), 23.1 ($M+1$), 3.0 ($M+2$); PREG for comparison: 43 (100), 205 (15), 213 (14), 231 (23), 255 (7), 283 (18), 298 (20), 316 (37, M^+); 0.2 ($M-2$), 0.8 ($M-1$), 100.0 (M), 23.2 ($M+1$), 2.6 ($M+2$). Analysis calculated for $C_{21}H_{29}{}^2H_3O_2$ (319.5): C, 78.95; H, 9.15; ^{2}H, 1.89. Found: C, 78.98; H, 10.35 (distorted by ^{2}H).

2.3.12. 3β-Hydroxy[19-$^2H_2$]pregn-5-en-20-one (30)

A sample from crude mesylate **27** (25 mg) was processed as described for the preparation of **28** with the use of H_2O instead of deuterium oxide and then deprotected as described for **29**. After chromatography, the resulting product (**30**, 7 mg) was used to obtain NMR spectra for comparison with the fully deuterated product. For ^{1}H and ^{13}C NMR data, see Tables 1 and 2. EI-MS, *m/z* (%): 43 (100), 207 (7), 215 (7), 233 (12), 257 (4), 283 (8), 300 (12), 318 (27, M^+).

2.3.13. Pyridinium 20-oxo[19-$^2H_3$]pregn-5-en-3β-yl sulfate (31)

A solution of d_3-pregnenolone (**29**) (250 mg, 0.78 mmol) and sulfur(IV) oxide–pyridine complex (250 mg, 1.57 mmol) in pyridine (4 ml) was stirred and heated to 70 °C for 1 h. The reaction mixture was then evaporated nearly to dryness and hot water (4 ml) was added. The solution was left to attain room temperature and then, applied onto a column of Lichroprep RP-18 (40–63 μm, 40 g) prewashed with water. The column was washed with water, and the product was eluted with methanol. The fractions containing the sulfate were collected, and the solvent was evaporated. Crystallization from a methanol-ether mixture gave 260 mg (69%) of sulfate **31**, m.p. 155–160 °C, $[\alpha]_D$ + 20 (c 0.4, $CHCl_3$). Ref. [19] gave m.p. 173–174 °C for the non-deuterated analog. IR ($CHCl_3$), ν (cm^{-1}): 3070, 3020, 1638, 1549, 1490 (pyridinium); 2459, 2135 (NH^+); 2224 (C^2H_3); 1699 (C=O); 1271–1175, 1050, 980, 959 (sulfate). For ^{1}H and ^{13}C NMR data, see Tables 1 and 2. Analysis calculated for $C_{26}H_{34}{}^2H_3NO_5S$ (478.7): C, 65.24; H, 7.16; ^{2}H, 1.26; N, 2.93; S, 6.70. Found: C, 65.25; H, 7.97 (distorted by ^{2}H); N, 2.87; S, 6.69.

2.3.14. Sodium 20-oxo[19-$^2H_3$]pregn-5-en-3β-yl sulfate (32)

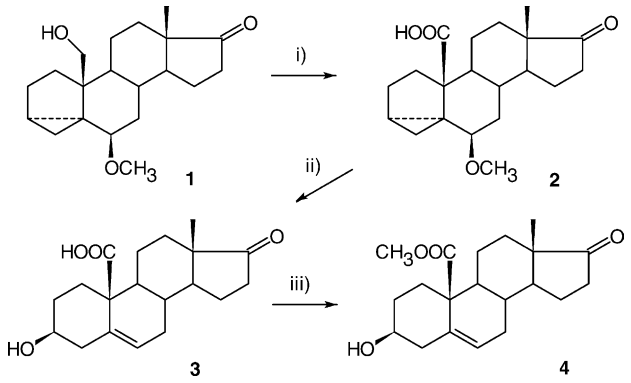
Pyridinium salt **31** (160 mg, 0.33 mmol) was thoroughly shaken with water (3 ml) and a 10% aqueous solution of NaCl was added in portions. During the addition, the gel-like solution was changed into a crystalline suspension. After being kept in refrigerator overnight, the crystals were filtered off, washed with a small amount of ice cold water, and dried. The yield of sodium salt **32** was 118 mg (83%), m.p. 201–204 °C, $[\alpha]_D$ + 19 (c 0.2, ethanol). Steraloids gave m.p. 180–182 °C, $[\alpha]_D$ + 21 (ethanol) for the non-deuterated analog. IR (KBr), ν (cm^{-1}): 2220 (C^2H_3); 1706 (C=O); 1289–1218, 1065, 986, 969 (sulfate). For ^{1}H and ^{13}C NMR data, see Tables 1 and 2. Analysis calculated for $C_{21}H_{28}{}^2H_3NaO_5S$ (418.5): C, 59.83; H, 6.70; ^{2}H, 1.43; S, 7.61. Found: C, 59.57; H, 7.49 (distorted by ^{2}H); S, 7.54.

3. Results and discussion

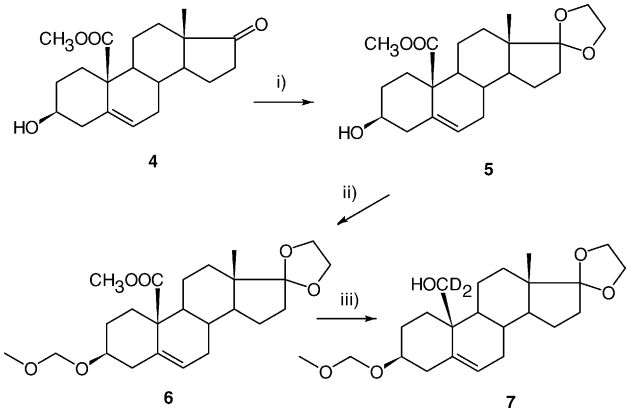
At the initial stage of our synthetic strategy (Scheme 1), we needed a 19-oate derivative. We reproduced oxidation experiments of 19-hydroxyandrost-5-ene derivatives with Jones reagent on 3β-acetoxy-19-hydroxyandrost-5-en-17-one, but we obtained only low yields of the corresponding acid in a mixture with the aldehyde. These results are comparable to findings in the literature [11]. More promising were experiments on the 3α,5-cyclo-5α-androstane skeleton (Scheme 2). We used an idea from the patent literature [10], and we were able to prepare ester **4** from 19-hydroxy-3α,5-cyclo-5α-androstan-17-one (**1**), Ref. [13], in a three-step procedure (***1*** → ***2*** → ***3*** → ***4***) in 68% yield.

For the second stage, introduction of two deuterium atoms through lithium aluminum deuteride reduction of the ester function in position 19, we needed a suitably protected derivative. For the ketone in position 17, we chose an ethylenedioxo group, and the hydroxyl in position 3 was protected as a methoxymethyl ether. We tried both possible sequences, i.e. first introducing ether and then ketal and vice versa, and we found that better yields were obtained by using ketalization before ether formation (Scheme 3). The reason for this was that acid catalysis during ketalization partially cleaved the methoxymethyl group. The protected derivative **6** was treated with lithium aluminum deuteride in boiling tetrahydrofuran to give the dideuterated alcohol **7**.

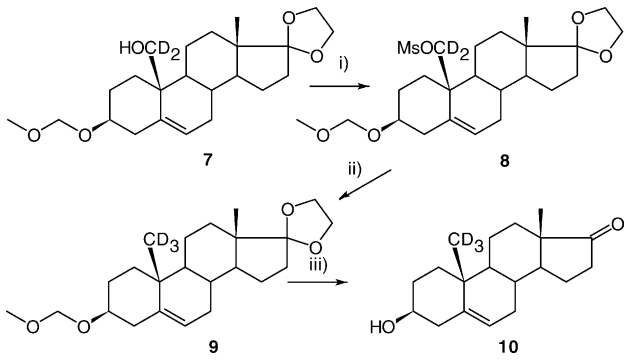
The last steps of the synthesis involved preparation of mesylate **8**, its exchange to deuterium, and final deprotection of trideutero derivative **9** (Scheme 4). We kept the conditions for the exchange reaction as anhydrous as possible. All solids, solvents, and reaction apparatus were dried before the reaction (see Section 2). After the removal of both protecting groups in acidic medium, we obtained d_3-DHEA (**10**), for the deuterium content see discussion below.



Scheme 2. (i) CrO_3, H_2SO_4, acetone; (ii) $HClO_4$, acetone; (iii) CH_2N_2, Et_2O, MeOH.



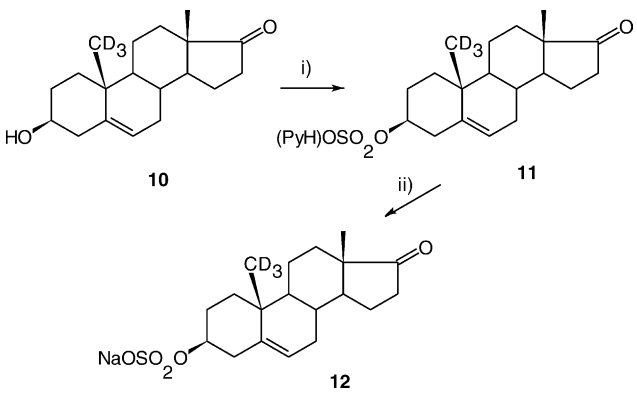
Scheme 3. (i) ethylene glycol $(EtO)_3CH$, TsOH, benzene; (ii) $BrCH_2OCH_3$, $(I\text{-}Pr)_2EtN$, benzene; (iii) $LrAlD_4$, THF.



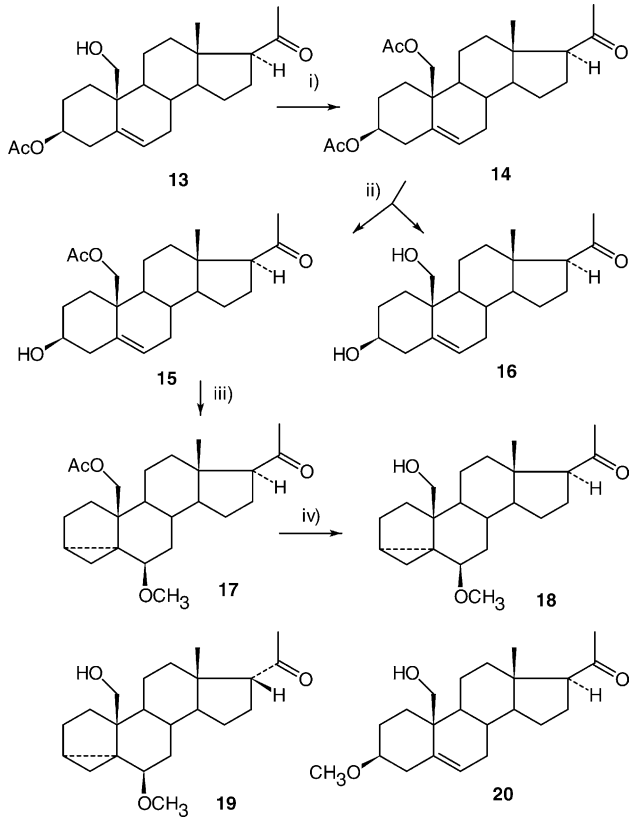
Scheme 4. (i) MsCl, Py; (ii) NaI, Zn, D_2O, DME; (iii) $HClO_4$, benzene, MeCH.

A sulfate was prepared (Scheme 5) by the reaction of **10** with sulfur(VI) oxide–pyridine complex in pyridine [19], and the resulting pyridinium salt **11** was concentrated on a reversed phase column and eluted with methanol [20]. Transformation into sodium salt **12** was achieved by treatment with aqueous sodium chloride [16].

Fewer 19-hydroxy derivatives are known in the pregnane series. We started (Scheme 6) from 19-hydroxy-20-oxopregn-5-en-3β-yl acetate (**13**) [7,9], which was acetylated to diacetate **14** and transformed into 19-acetate **15**,



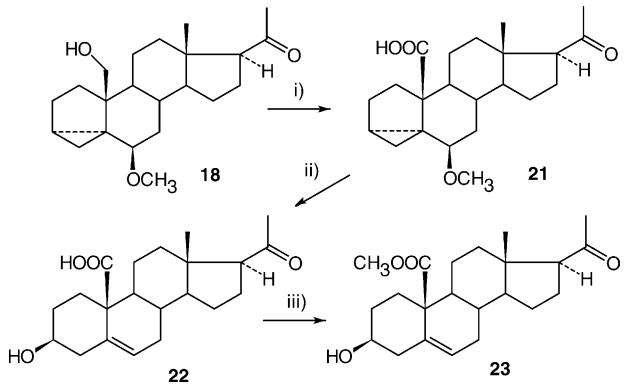
Scheme 5. (i) P_4ASO_3, P_4; (ii) NaCl, H_2O.



Scheme 6. (i) Ac_2O, Py; (ii) K_2CO_3, MeOH; (iii) 1. MsCl, Py, 2. MeOH, KOAc, dioxane; (iv) NaOH, MeOH.

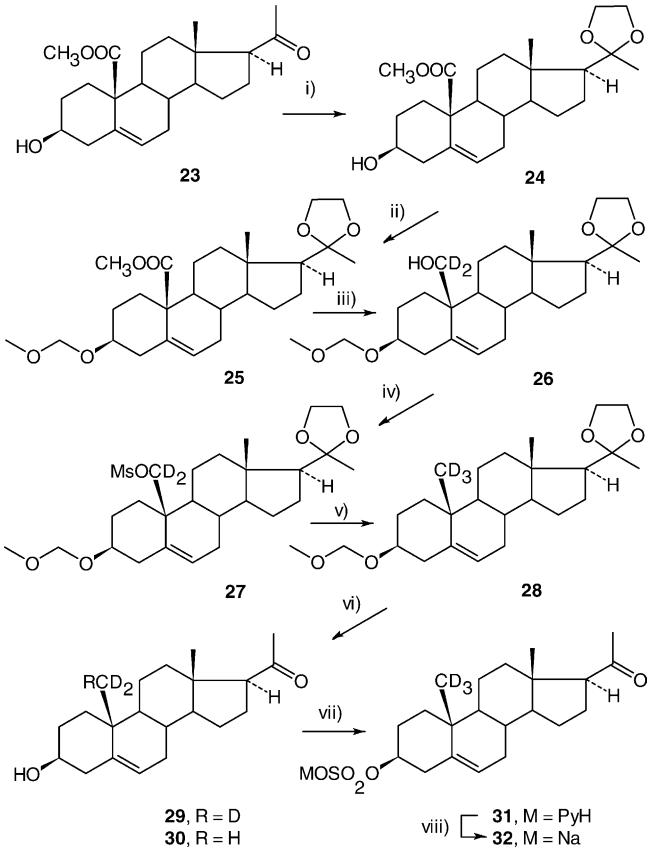
employing preferential hydrolysis of the 3-*O*-acetyl group under mild alkaline conditions [21]. We obtained diol **16** as a side product. Standard procedure then gave 3α,5-cycloderivative **17**. The next step was the alkaline hydrolysis of 19-acetate. This step was the most critical in the whole synthesis because an acetate in this position is relatively stable, and the pregnane side chain in position 17 partially isomerizes under the conditions used from 17β to 17α. We carefully monitored the composition of the reaction mixture and stopped the reaction when all of starting compound had reacted. Product **18** was purified by column chromatography and crystallization, and the structure was checked by NMR spectra. In addition, we isolated two side products of this two-step procedure (***15*** → ***17*** → ***18***), originating from different steps. 17α-Isomer **19** came from the second step and the methoxy derivative **20** from the first step. In spite of this complication, we obtained the 19-hydroxy derivative **18** in a 43% yield (from 19-acetate **15**).

The rest of the synthesis was done after the sequence corroborated in the androstane series (Scheme 7). The oxidation of cycloderivative **18** with Jones reagent gave 19-acid **21**, which was transformed without purification into 3-hydroxy-5-ene derivative **22** and methylated to ester **23**. The protection in position 20 (Scheme 8) gave the ethylenedioxo derivative **24**, and the introduction of the methoxymethyl group into position 3 gave the fully protected derivative **25**.



Scheme 7. (i) CrO_3, H_2SO_4, acetone; (ii) $HClO_4$, acetone; (iii) CH_2N_2, Et_2O, MeOH.

Lithium aluminum deuteride reduction yielded the hydroxy derivative **26**, and third deuterium embodiment via mesylate **27** gave **28**. The removal of the protecting groups resulted in 3β-hydroxy[19-$^2H_3$]pregn-5-en-20-one (**29**), for the deuterium content see discussion below. As a sample for comparison, we prepared dideuterated PREG **30** using water in mesylate **27** exchange and the same deprotection reaction as in the preparation of d_3-PREG (**29**).



Scheme 8. (i) ethylene glycol, $(ETO)_3CH$, TsOH, benzene; (ii) $BrCH_2OCH_3$, $(i\text{-}Pr)_2EtN$, benzene; (iii) $LiAlD_4$, THF; (iv) MsCl, Py; (v) NaI, Zn, D_2O, DME; (vi) $HClO_4$, benzene, MeOH; (vii) $PyASO_3$, Py; (viii) NaCl, H_2O.

The sulfate was also prepared by the same method as for the androstane series. Firstly, we obtained pyridinium salt **29**, which was then transformed into sodium salt **30**.

The structure of key compounds in the PREG series was confirmed by ^1H and ^{13}C NMR data. A general strategy based on a combination of 1D and 2D NMR methods was used for complete structural assignment of all proton and carbon signals. NMR data are summarized in Tables 1 and 2.

Compounds **13**, **15**, and **16** were selected as representatives of 19-hydroxypregnenolone derivatives. In comparison with pregnenolone and pregnenolone acetate, characteristic signals of H-19 protons in the ^1H NMR spectra were apparent in the region of $\delta = 3.6$–3.9 ($\delta = 4.0$–4.5 for acetates). In the ^{13}C NMR spectra, the carbons mostly influenced were in positions 1, 5, 6, 10, and 19 (differences in shifts >1 ppm).

Compounds **29**–**32**, where two and/or three protons in position 19 were replaced with deuterium, showed characteristic changes in the appearance and position of the 19-methyl group NMR signal. The ^1H NMR spectrum of the 19-CHD_2 group in **30** gave a characteristic pentet at $\delta = 0.976$ with $^2J(H, D) = 1.8$ Hz and the upfield isotopic shift (−0.035 ppm in comparison with nondeuterated PREG). Similarly, its ^{13}C NMR spectrum showed a pentet of the 19-CHD_2 group at $\delta = 18.80$ with $^1J(C, D) = 19.3$ Hz and an upfield isotopic shift of −0.57 ppm. The presence of the 19-CD_3 group in compounds **29**, **31**, and **32** led to the absence of a corresponding signal in the ^1H NMR spectra, while the characteristic heptets at $\delta = 18.51$, 18.42, and 17.19 with $^1J(C, D) = 18.7$ Hz and upfield isotopic shifts (−0.86 ppm for **29**) were observed in their ^{13}C NMR spectra. The complete absence of proton as well as carbon signals corresponding to less/deuterated isotopomers in position 19 proved the isotopic purity of the samples at the level higher than 95%.

In the whole series (**13**, **15**, **16**, **18**, **23**, **29**–**32**), the conservation of the 17β configuration of the pregnane side chain followed from both ^1H and ^{13}C NMR spectra (cf. Ref. [22]).

The isotopic abundance estimation was performed for title compounds d_3-DHEA (**10**) and d_3-PREG (**29**), which gave suitable EI mass spectra with sufficiently populated molecular ion. The relative abundances of partially deuterated species were calculated from normalized values of abundances in the molecular ion cluster (see Section 2). The values were sequentially corrected to the occurrence of natural isotopes ^{13}C, ^{17}O, and ^{18}O (correction factors for $M + 1$, $M + 2$; DHEA: 0.211, 0.025; PREG: 0.231, 0.029). After normalization, the abundances d_0, d_1, d_2, d_3, for deuterated DHEA amounted 0.5, 0.6, 3.8, 95.1, and for PREG 1.1, 0.5, 5.0, 93.4, respectively. The used method, however, could give only orienting results in our case because standard DHEA and PREG had some residual $M - 2$ and $M - 1$ ions (see Section 2). It could be guessed, that nondeuterated and mono-deuterated species were present up to 1%, dideuterated up to 5%, leaving d_3-steroid content about 93–95%. This corresponds to the reported results for the used labeling methods: the deuteride reduction introduced

first two deuterium atoms nearly quantitatively, for the second method, the label content varies depending on water present during the reaction [12]. The spectra of preceding deuterated intermediates in both series were not useful for estimating of deuterium content due to instability of the molecular ion caused probably by the presence of dioxolane ring: all protected derivatives displayed this behavior.

In conclusion, we developed a synthesis of trideutero derivatives of DHEA and PREG from 19-hydroxy-17-oxoandrost-5-en-3β-yl acetate and 19-hydroxy-20-oxopregn-5-en-3β-yl acetate, respectively. The approach we used tried to avoid Jones oxidations of 19-hydroxy group on a 5-ene skeleton, which gave low yields of mixtures of acids and aldehydes that were difficult to separate. The oxidation of the 3α,5-cycloderivatives proceeded much more smoothly, and although the whole synthesis was longer, the main reaction steps produced sufficiently pure intermediates to be used for preparation of labeled compounds. The synthesis gave deuterated derivatives in 14 steps (yields 8.9 and 7.3%, respectively) with d_3-DHEA and d_3-PREG content about 93–95%, sufficient for simple tracing experiments. In connection with the necessary structural check, data from fully assigned NMR spectra of selected derivatives in the PREG series were presented.

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