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Chemical synthesis of 15β-hydroxytestosterone and its derivatives using a (4-methoxyphenyl)methyl protecting group

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Reaction of 3β-hydroxyandrosta-5,15-dien-17-one with 4-methoxybenzyl alcohol followed by acetylation gave mainly 15β-[(4-methoxyphenyl)methoxy]-17-oxoandrost-5-en-3β-yl acetate. This product was transformed by borohydride reduction and organosilyl derivatization into the orthogonally protected 17β-(dimethylthexylsiloxy)-15β-[(4-methoxyphenyl)methoxy]androst-5-en-3β-yl acetate and 17β-(dimethylisopropylsiloxy)-15β-[4-methoxyphenyl)methoxy]androst-5-en-3β-yl acetate. After deacetylation, these intermediates were submitted to Oppenauer oxidation and both yielded testosterone derivatives 17β-(dimethylthexylsiloxy)-15β-[(4-methoxyphenyl)methoxy]adrost-4-en-3-one and 17β-(dimethylisopropylsiloxy)-15β-[(4-methoxyphenyl)-methoxy]androst-4-en-3-one. Removal of the (4-methoxyphenyl)methyl group from position 15 by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone treatment gave the partially protected derivatives 17β-(dimethylthexylsiloxy)-15β-hydroxyandrost-4-en-3-one and 17β-(dimethylisopropylsiloxy)-15β-hydroxyandrost-4-en-3-one. After acidic deprotection, the dimethylthexylsilyl derivative yielded 15β-hydroxytestosterone (15β,17β-dihydroxyandrost-4-en-3-one). Dimethylisopropylsilyl derivative was converted to the corresponding 15-hemisuccinate and 15-hemiglutarate (17β-hydroxy-3-oxoandrost-4-en-15β-yl 15-hemisuccinate and 15-hemiglutarate, respectively), which were designed as model haptens for immunoassay studies. (Steroids **61**:58–64, 1996)

Keywords: synthesis; 15β-hydroxytestosterone; protective groups

Introduction

Derivatives of testosterone carrying hydroxy groups in various positions are important for biological studies, especially as haptens for immunoassays. In the present communication, we describe derivatives functionalized in position 15β, which have not been available by chemical synthesis so far.

Intermediates with 15β-hydroxy groups have been reported as products of microbial hydroxylations.[1–3] These products, which have several hydroxy groups of similar reactivity, are not generally suitable as starting compounds for the preparation of partially protected derivatives.

Two synthetic methods for introducing of the 15β-hydroxy group into suitable androstane derivatives have been described in the literature[4,5]; both make use of 3β-hydroxyandrosta-5,15-dien-17-one (**1**) as the starting compound. In the first case, the functionalization is affected by addition of benzyl alcohol in the presence of sodium hydroxide. The synthesis ends, however, with androst-5-ene-3β,15β,17β-triol as the final product. The partial protection of this compound may cause problems. In the second case, the 15-double bond of ketone **1** is selectively transformed into the epoxide. Following chromium(II) acetate reduction, the 15-hydroxy derivative is produced, but only limited experimental data have been reported with respect to this.

In the present paper, we describe a novel method for introducing of a hydroxyl group into position 15, which

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involves addition of 4-methoxybenzyl alcohol to the 15-en-17-one system. This approach is analogous to the former concept,[4] but removal of the resulting (4-methoxyphenyl) methyl protective group (MPM = *p*-methoxybenzyl = PMB) is achieved under mild oxidative conditions (see below). Therefore, this group can be combined with a broad variety of other protecting groups, which are removable independently either by acid or by base (orthogonal protection[6]) and consequently the synthetic utility of the addition is enhanced.

Experimental

Melting points (m.p.) were determined on a Boetius micro melting point apparatus (Germany). Optical rotations were measured 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 taken in chloroform on a Bruker IFS 88 spectrometer. NMR spectra were obtained on Varian XL-200 (^{1}H at 200 MHz) or on Varian UNITY-500 (^{1}H at 500 MHz, ^{13}C at 125.7 MHz) instruments at 23°C, in deuteriochloroform, and with tetramethylsilane as the internal standard. Chemical shifts are given in ppm (δ-scale); coupling constants (*J*) and widths of multiplets (W) are given in Hz. Mass spectra were recorded on a VG Analytical ZAB-EQ spectrometer (energy of ionizing electrons 70 eV; ion source temperature 170–200°C). Column chromatography was performed on silica gel (60–120 μm) or on neutral alumina (Reanal, activity II); for thin-layer chromatography, the silica gel G (ICN Biochemicals) was used. Solutions in organic solvents were dried over anhydrous sodium sulfate, and the solvents were evaporated on a rotary evaporator in vacuo (about 2 kPa). 2-(Trimethylsilyl)ethyl hydrogen glutarate was prepared similarly to the corresponding hemisuccinate (see reference 7); ^{1}H NMR (200 MHz, ref. $CDCl_3$: 7.26 ppm) 4.17 (2 H, m, W 16, OCH_2CH_2Si), 2.43 (2 H, t, *J* = 7.3, CH_2CH_2CO), 2.37 (2 H, t, *J* = 7.3, CH_2CH_2CO), 1.95 (2 H, p, *J* = 7.2, $OCCH_2CH_2CH_2CO$), 0.94 (2 H, m, W = 16, OCH_2CH_2Si), 0.04 (9 H, s, $(CH_3)_3Si$).

15β-[(4-Methoxyphenyl)methoxy]-17-oxoandrost-5-en-3β-yl acetate (2)

A 50% oil suspension of sodium hydride (40 mg, 0.8 mmol) was added to a suspension of the dienone **1** (2.0 g, 7 mmol)[4] in 4-methoxybenzyl alcohol (4.0 mL, 32 mmol) and under argon. The mixture was stirred for 16 h. Pyridine (10 mL) and acetic anhydride (20 mL) were added, and the mixture was further stirred for 4 h. The mixture was poured into saturated aqueous NaCl (300 mL) and extracted with ether (400 mL). The extract was then washed twice with 10% HCl, once with saturated aqueous NaCl, and twice with saturated aqueous $KHCO_3$. After drying and evaporation, the product was chromatographed on silica gel with 2% acetone in a petroleum ether-benzene mixture (1:1, v/v). After elution of 4-methoxybenzyl acetate the crude main product (1.7 g) was eluted. Crystallization from minimum volume of ether gave **2** (1.33 g, 41%), m.p. 136–138°C, $[\alpha]_D^{25}$ –42 (c = 1.3, $CHCl_3$). IR: 2839 and 1181 (OCH_3), 1731 (C=O$_{ketone}$ and C=O$_{acetate}$), 1613, 1587, 1515, 1441 (arom), 1252 and 1032 (C–O). ^{1}H NMR (200 MHz): 7.22 (2 H, br d, *J* = 8, 2′-H and 6′-H), 6.88 (2 H, br d, *J* = 8.5, 3′-H and 5′-H), 5.39 (1 H, br d, *J* = 4, 6-H), 4.60 (1 H, m, W = 32, 3α-H), 4.29 and 4.49 (2 H, AB system, *J* = 11.3, CH_2O), 4.13 (1 H, t, *J* = 5.2, 15α-H), 3.81 (3 H, s, OCH_3), 2.68 (1 H, br d, *J* = 18, 16β-H), 2.04 (3 H, s, CH_3CO), 1.16 (3 H, s, 18-H_3), 1.07 (3 H, s, 19-H_3). ^{13}C NMR see Table 1. Analysis calculated for $C_{29}H_{38}O_5$ (466.6): C, 74.65; H, 8.21. Found: C, 74.69; H, 8.37.

Further elution resulted in a the fraction containing a mixture of polar compounds (0.7 g). This mixture did not contain any aromatic protons according to NMR data and was not further analyzed.

Table 1 ^{13}C NMR chemical shifts in $CDCl_3$

	δ$_C$					
Carbon	**2**	**3**	**4**	**5**	**12**	**14**
1	37.00	36.93	36.96	36.93	35.78	35.61
2	27.70	27.65	27.68	27.65	33.94	33.79
3	73.74	73.69	73.71	73.64	199.53	199.97
4	38.07	38.04	37.96	37.93	123.99	123.86
5	139.83	140.20	138.96	139.42	170.94	171.66
6	121.92	121.42	122.76	122.02	32.64	32.55
7	30.18	30.36	31.25	31.32	31.04	30.75
8	27.71	27.51	31.78	31.68	31.46	31.54
9	50.54	50.45	49.78	49.64	54.27	54.10
10	36.79	36.82	36.64	36.56	38.76	38.71
11	20.30	20.25	20.03	20.04	20.54	20.48
12	32.61	32.69	32.01	32.11	37.85	37.64
13	46.89	46.63	49.71	50.52	42.22	42.57
14	55.96	55.83	55.42	57.72	55.16	53.57
15	73.93	67.24	77.08	71.06	69.13	72.12
16	43.09	46.77	43.27	46.07	43.42	40.77
17	219.00	219.76	216.58	216.28	81.12	80.54
18	17.29	17.42	15.15	15.02	13.68	13.07
19	19.18	19.19	19.35	19.33	17.30	17.29

Other signals: **2** 21.40 (CH_3CO), 55.26 (OCH_3), 71.19 (OCH_2Ph), 113.75, 129.10, 130.30, 159.16 (Ph), 170.52 ($COCH_3$), **3** 21.39 (CH_3CO), 170.56 ($COCH_3$), **4** 21.39 (CH_3CO), 55.27 (OCH_3), 71.79 (OCH_2Ph), 113.88, 129.27, 130.10, 159.28 (Ph), 170.54 ($COCH_3$), **5** 21.37 (CH_3CO), 170.56 ($COCH_3$), **14** 28.92, 29.35 (2 × CH_2 succinate), 171.54, 176.36 (2 × C=O succinate).

15β-Hydroxy-17-oxoandrost-5-en-3β-yl acetate (3)

A solution of **2** (195 mg, 0.42 mmol) in dichloromethane (8 mL) was stirred with water (0.2 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (120 mg, 0.53 mmol) for 20 min. After dilution with chloroform (50 mL), the mixture was washed with saturated aqueous $KHCO_3$ (3×) and water, dried, and the solvents were evaporated. The product was chromatographed on silica gel with benzene until the side products were washed out. Further chromatography with a benzene-ether (8:2, v/v) mixture eluted **3** (133 mg, 92%), m.p. 152–154°C (ether-hexane), $[\alpha]_D^{25}$ +33 (c = 0.9, $CHCl_3$); CD spectrum (ethanol) $\Delta\epsilon_{298.0}$ +2.54. IR: 3619 and 3502 (OH), 1731 (C=O$_{ketone}$ and C=O$_{acetate}$), 1670 (C=C), 1254 and 1031 (C–O). ^{1}H NMR (500 MHz): 5.42 (1 H, dt, *J* = 5.3 and 2 × 1.8, 6-H), 4.61 (1 H, ddt, *J* = 2 × 11.4, 5.6, and 4.3, 3α-H), 4.56 (1 H, dd, *J* = 6.0 and 4.5, 15α-H), 2.59 (1 H, br d, *J* = 19.6, 16β-H), 2.51 (1 H, dd, *J* = 19.6 and 6.0, 16α-H), 2.04 (3 H, s, CH_3CO), 1.31 (1 H, dd, *J* = 11.5 and 4.5, 14α-H), 1.19 (3 H, s, 18-H_3), 1.09 (3 H, s, 19-H_3). ^{13}C NMR see Table 1. Analysis calculated for $C_{21}H_{30}O_4$ (346.5): C, 72.80; H, 8.73. Found: C, 72.72; H, 8.46.

15α-[(4-Methoxyphenyl)methoxy]-17-oxoandrost-5-en-3β-yl acetate (4)

After crystallization, mother liquors of **2** were rechromatographed in the same solvent mixture as for **2** and 150 mg of isomer **4** was isolated from more polar fractions, m.p. 131–132°C (ethanol), $[\alpha]_D^{25}$ +33 (c = 0.9, $CHCl_3$). IR: 2839 and 1181 (OCH_3), 1730 (C=O$_{ketone}$ and C=O$_{acetate}$), 1613, 1587, 1515, 1442 (arom), 1252 and 1034 (C–O). ^{1}H NMR (200 MHz): 7.24 (2 H, br d, *J* = 9, 2′-H and 6′-H), 6.88 (2 H, br d, *J* = 8.5, 3′-H and 5′-H), 5.43 (1 H, br d, *J* = 4, 6-H), 4.61 (1 H, m, W = 32, 3α-H), 4.41 and 4.46 (2 H, AB system, *J* = 11.4, CH_2O), 4.04 (1 H, ddd, *J* = 9.7, 7.6, and 6.5,

15β-H), 3.82 (3 H, s, OCH_3), 2.91 (1 H, dd, J = 18 and 7.6, 16β-H), 2.10 (1 H, dd, J = 18 and 6.5, 16α-H), 2.04 (3 H, s, CH_3CO), 1.05 (3 H, s, 19-H_3), 0.90 (3 H, s, 18-H_3). ^{13}C NMR see Table 1. Analysis calculated for $C_{29}H_{38}O_5$ (466.6): C, 74.65; H, 8.21. Found: C, 74.59; H, 8.39.

15α-Hydroxy-17-oxoandrost-5-en-3β-yl acetate (5)

Derivative **5** (65 mg, 87%) was prepared from derivative **4** (100 mg, 0.21 mmol), as described for acetate **3,** m.p. 211–212°C (ethanol-ether), $[\alpha]_D^{25}$ +8 (c = 0.7, $CHCl_3$), CD spectrum (ethanol) $\Delta\epsilon_{298.0}$ +2.67. IR: 3624 and 3497 (OH), 1732 ($C{=}O_{ketone}$ and $C{=}O_{acetate}$) 1255, 1032 (C–O). ^1H NMR (500 MHz): 5.41 (1 H, dt, J = 5.3, and 2 × 1.8, 6-H), 4.61 (1 H, ddt, J = 2 × 11.5, 5.3, and 4.2, 3α-H), 4.41 (1 H, dddd, J = 10, 8, 6.7, and 5, 15β-H), 2.99 (1 H, dd, J = 19.1 and 8, 16β-H), 2.12 (1 H, dd, J = 19.1 and 6.7, 16α-H), 2.04 (3 H, s, CH_3CO), 1.06 (3 H, s, 19-H_3), 0.92 (3 H, s, 18-H_3). ^{13}C NMR see Table 1. Analysis calculated for $C_{21}H_{30}O_4$ (346.5): C, 72.80; H, 8.73. Found: C, 72.75; H, 8.89.

Diacetate **6,** prepared by acetic anhydride in pyridine, had m.p. 188–190°C (ethanol), $[\alpha]_D^{25}$ +33 (c = 0.9, $CHCl_3$), lit.[8] m.p. 187–188°C (methanol), $[\alpha]_D^{23}$ +32.6 (c = 0.11).

17β-Hydroxy-15β-[(4-methoxyphenyl)methoxy]-androst-5-ene-3β-yl acetate (7)

Sodium borohydride (315 mg, 13.1 mmol) was added gradually (5 min) to a solution of **2** (3.25 g, 6.96 mmol) in a mixture of ethyl acetate (25 mL) and methanol (50 mL), which was stirred and cooled to 10°C. After an additional 5 min of stirring at the same temperature, the excess hydride was destroyed by acetic acid (1 mL) and water (1 mL). The solution was concentrated under reduced pressure, diluted with ethyl acetate, and washed successively with saturated aqueous NaCl, 10% HCl, water, saturated aqueous $KHCO_3$, and water. After drying and evaporation, the syrupy residue was crystallized from the mixture of ether and petroleum ether and yielded 3.0 g (92%) of **7,** m.p. 141–142°C, $[\alpha]_D^{25}$ –89 (c = 1.1, $CHCl_3$). IR: 3611 and 3502 (OH), 2839 and 1181 (OCH_3), 1723 (C=O), 1671 (C=C), 1613, 1587, 1514, 1441 (arom), 1252 and 1032 (C–O). ^1H NMR (200 MHz): 7.22 (2 H, br d, J = 8, 2′-H and 6′-H), 6.86 (2 H, br d, J = 8, 3′-H and 5′-H), 5.36 (1 H, br d, J = 4, 6-H), 4.60 (1 H, m, W = 32, 3α-H), 4.17 and 4.44 (2 H, AB system, J = 12, CH_2O), 3.80 (3 H, s, OCH_3), 3.77 (1 H, m, 15α-H, overlapped with OCH_3), 3.60 (1 H, br t, J = 8, 17α-H), 2.03 (3 H, s, CH_3CO), 1.04 (3 H, s, 19-H_3), 1.00 (3 H, s, 18-H_3). Analysis calculated for $C_{29}H_{40}O_5$ (468.6): C, 74.33; H, 8.60. Found: C, 74.36; H, 8.64.

17β-(Dimethylthexylsiloxy)-15β-[(4-methoxyphenyl)-methoxy]androst-5-en-3β-yl acetate (8a)

Triethylamine (1.0 mL, 7.2 mmol), dichloromethane (3 mL), dimethylthexylsilyl chloride (0.5 mL, 2.54 mmol), and 4-dimethylaminopyridine (5 mg) were added to a derivative **7** (539 mg, 1.15 mmol). After 5 days at 45°C, the mixture was diluted with ether and washed successively with 10% aqueous citric acid, water, saturated aqueous $KHCO_3$ (2×), and water. The mixture was then dried and evaporated. After chromatography on alumina with 1% ether in a benzene-petroleum ether mixture (1:1, v/v), **8a** was obtained in the form of an oil (563 mg, 80%), and it was sufficiently pure for further use. ^1H NMR (200 MHz): 7.22 (2 H, br d, J = 8, 2′-H and 6′-H), 6.86 (2 H, br d, J = 8, 3′-H and 5′-H), 5.36 (1 H, m, W = 10, 6-H), 4.60 (1 H, m, W = 32, 3α-H), 4.15 and 4.45 (2 H, AB system, J = 12, CH_2O), 3.80 (3 H, s, OCH_3), 3.74 (1 H, m, W = 20, 15α-H), 3.50 (1 H, br t, J = 8, 17α-H), 2.03 (3 H, s, CH_3CO), 1.04 (3 H, s, 19-H_3), 0.96 (3 H, s, 18-H_3), 0.89 (6 H, d, J = 6.8, $(CH_3)_2CH$), 0.84 (6 H, s, $(CH_3)_2C$), 0.09 (6 H, s, $(CH_3)_2Si$).

17β-(Dimethylthexylsiloxy)-15β-[(4-methoxyphenyl)-methoxy]androst-5-en-3β-ol (9a)

Derivative **8a** (550 mg, 0.9 mmol) was refluxed for 10 min with benzene (5 mL), methanol (5 mL), and 2 M aqueous potassium hydroxide (0.5 mL). The solution was concentrated under reduced pressure, diluted with benzene-ether (10:1, v/v), and washed successively with 10% aqueous citric acid, water, saturated aqueous $KHCO_3$, and water. After drying and evaporation, chromatography on silica gel in 5% ether in benzene gave **9a** as a foam (335 mg, 65%). This product was directly used in the next step. ^1H NMR (200 MHz): 7.22 (2 H, br d, J = 8, 2′-H and 6′-H), 6.86 (2 H, br d, J = 8, 3′-H and 5′-H), 5.32 (1 H, m, W = 10, 6-H), 4.14 and 4.45 (2 H, AB system, J = 12, CH_2O), 3.80 (3 H, s, OCH_3), 3.74 (1 H, m, W = 20, 15α-H), 3.53 (1 H, m, 3α-H), 3.45 (1 H, br t, J = 8, 17α-H), 1.03 (3 H, s, 19-H_3), 0.96 (3 H, s, 18-H_3), 0.89 (6 H, d, J = 7, $(CH_3)_2CH$), 0.84 (6 H, s, $(CH_3)_2C$), 0.09 (6 H, s, $(CH_3)_2Si$).

17β-(Dimethylisopropylsiloxy)-15β-[(4-methoxyphenyl)methoxy]androst-5-en-3β-ol (9b)

Derivative **7** (3.0 g, 6.4 mmol) was stirred with triethylamine (3.0 mL, 21 mmol), dichloromethane (15 mL), and dimethylisopropylsilyl chloride (3 mL, 19 mmol) under argon for 1 h. The mixture was diluted with ether, washed with ice-water, and filtered through a column of alumina layered with Na_2SO_4. Evaporation gave crude **8b,** which was deacetylated as was **8a.** After chromatography on alumina in acetone (2%–10%) in a benzene-petroleum ether mixture (1:1, v/v), **9b** was obtained as an oil (2.8 g, 83%), and was directly used for further experiments. IR: 3610 and 3468 (OH), 2895, 1249, 832 ($Si(CH_3)_2$), 2841 (OCH_3), 1673 (C=C), 1613, 1587, 1514, 1441 (arom), 1088 (C–OSi). ^1H NMR (200 MHz): 7.22 (2 H, br d, J = 8, 2′-H and 6′-H), 6.86 (2 H, br d, J = 8, 3′-H and 5′-H), 5.34 (1 H, m, W = 10, 6-H), 4.14 and 4.45 (2 H, AB system, J = 12, CH_2O), 3.80 (3 H, s, OCH_3), 3.74 (1 H, dt, J = 2 × 7.5 and 2.5, 15α-H), 3.52 (1 H, m, 3α-H), 3.50 (1 H, t, J = 8.4, 17α-H), 1.03 (3 H, s, 19-H_3), 0.97 (3 H, s, 18-H_3), 0.96 (6 H, d, J = 5, $(CH_3)_2CH$), 0.03 (6 H, s, $(CH_3)_2Si$). Mass spectrum (EI mode): 526 (M^+, 2%), 508 (3), 483 (2), 419 (2), 405 (86), 269 (65), 121 (100).

17β-(Dimethylthexylsiloxy)-15β-[(4-methoxyphenyl)-methoxy]androst-4-en-3-one (10a)

Toluene (3 mL) was distilled off under argon from a refluxing solution of **9a** (335 mg, 0.59 mmol) in toluene (15 mL) with 1-methyl-4-piperidone (0.4 mL, 3.2 mmol). A solution of aluminum isopropoxide (123 mg, 0.6 mmol) in toluene (3 mL) was subsequently added. After 4 h of refluxing, the reaction mixture was cooled, diluted with ether, and processed with citric acid as in the preparation of **9a.** Chromatography on silica gel using 1% acetone in benzene yielded **10a** as a foam (290 mg, 87%), which was further used. ^1H NMR (200 MHz): 7.23 (2 H, br d, J = 9, 2′-H and 6′-H), 6.86 (2 H, br d, J = 9, 3′-H and 5′-H), 5.72 (1 H, br s, 4-H), 4.15 and 4.49 (2 H, AB system, J = 13, CH_2O), 3.81 (3 H, s, OCH_3), 3.76 (1 H, m, W = 20, 15α-H), 3.48 (1 H, br t, J = 8, 17α-H), 1.20 (3 H, s, 19-H_3), 0.99 (3 H, s, 18-H_3), 0.89 (6 H, d, J = 7, $(CH_3)_2CH$), 0.84 (6 H, s, $(CH_3)_2C$), 0.09 (6 H, s, $(CH_3)_2Si$).

17β-(Dimethylisopropylsiloxy)-15β-[(4-methoxyphenyl)methoxy]androst-4-en-3-one (10b)

Derivative **10b** was prepared from derivative **9b** (2.8 g, 5.3 mmol) by the above mentioned procedure and after chromatography on

alumina with 2% acetone in a benzene-petroleum ether mixture (1:1, v/v). Yield 2.49 g (89%). ^{1}H NMR (200 MHz): 7.22 (2 H, br d, J = 9, 2′-H and 6′-H), 6.87 (2 H, br d, J = 9, 3′-H and 5′-H), 5.72 (1 H, br s, 4-H), 4.14 and 4.49 (2 H, AB system, J = 13, CH_2O), 3.80 (3 H, s, OCH_3), 3.75 (1 H, dt, J = 2 × 6.6 and 2.5, 15α-H), 3.50 (1 H, br t, J = 8.4, 17α-H), 1.20 (3 H, s, 19-H_3), 1.00 (3 H, s, 18-H_3), 0.96 (6 H, br d, J = 6.5, $(CH_3)_2CH$), 0.02 (6 H, s, $(CH_3)_2Si$). Mass spectrum (EI mode): 524 (M^{+}, 7%), 481 (4), 403 (21), 388 (60), 270 (65), 137 (80), 129 (73), 121 (100).

*17β-(Dimethylthexylsiloxy)-15β-hydroxyandrost-4-en-3-one (**11a**)*

Compound **10a** (280 mg, 0.49 mmol) was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone as in the preparation of **3**. Chromatography on silica gel in benzene-petroleum ether (1:1, v/v) yielded 198 mg (89%) of **11a** in the form of a glassy solid. IR: 3619 and 3467 (OH), 1662 (C=O), 1616 (C=C), 1252, 884, 825 ($Si(CH_3)_2$), 1084 (C–OSi). ^{1}H NMR (200 MHz): 5.73 (1 H, br s, 4-H), 4.17 (1 H, m, W = 20, 15α-H), 3.48 (1 H, br t, J = 9, 17α-H), 1.23 (3 H, s, 19-H_3), 1.01 (3 H, s, 18-H_3), 0.89 (6 H, d, J = 7, $(CH_3)_2CH$), 0.84 (6 H, s, $(CH_3)_2C$), 0.09 (6 H, s, $(CH_3)_2Si$). Analysis calculated for $C_{27}H_{46}O_3Si$ (446.8): C, 79.59; H, 10.38. Found: C, 79.43; H, 10.17.

*17β-(Dimethylisopropylsiloxy)-15β-hydroxyandrost-4-en-3-one (**11b**)*

The procedure described in the preparation of **3** was applied to **10b** (1.7 g, 3.24 mmol), and after chromatography on alumina in acetone (2%–10%) in a benzene-petroleum ether mixture (1:1, v/v), it yielded **11b** in the form of a foam (1.24 g, 95%). Crystallization from ether gave crystals (860 mg, 66%), m.p. 129–132°C, $[\alpha]_D^{25}$ +58 (c = 1.1, $CHCl_3$). IR: 3620 and 3478 (OH), 2895, 1251, 886, 829 ($Si(CH_3)_2$), 1662 (C=O), 1615 (C=C), 1085 (C–OSi). ^{1}H NMR (200 MHz) 5.73 (1 H, br s, 4-H), 4.17 (1 H, dt, J = 2 × 6.5 and 2.5, 15α-H), 3.48 (1 H, t, J = 8.4, 17α-H), 1.22 (3 H, s, 19-H_3), 1.02 (3 H, s, 18-H_3), 0.95 (6 H, d, J = 6, $(CH_3)_2CH$), 0.02 (6 H, s, $(CH_3)_2Si$). Analysis calculated for $C_{24}H_{40}O_3Si$ (404.7): C, 71.24; H, 9.96. Found: C, 71.13; H, 9.99.

*15β,17β-Dihydroxyandrost-4-en-3-one (**12**)*

Derivative **11a** (150 mg, 0.34 mmol) was stirred in a mixture of benzene (5 mL), methanol (5 mL), and concentrated HCl (0.5 mL) for 7 h. After addition of chloroform, the solution was washed with saturated aqueous $KHCO_3$ and water. After drying and evaporation, the product was chromatographed on silica gel in 1% methanol in chloroform, giving **12** (98 mg, 96%), m.p. 223–225°C (ethanol). $[\alpha]_D^{25}$ +60 (c = 0.6, ethanol; lit.[1] gives m.p. 220–222°C, $[\alpha]_D^{25}$ +57 (ethanol)). IR: 3451 and 3315 (OH), 1655 (C=O), 1612 (C=C) 1062 and 1047 (C–O). ^{13}C NMR see Tables 1 and 2. ^{1}H NMR see Table 3. Mass spectrum (EI mode) 304 (M^{+}, 100%), 286 (21), 268 (12), 262 (63), 124 (100).

*17β-Hydroxy-3-oxoandrost-4-en-15β-yl 15-hemisuccinate (**14**)*

2-(Trimethylsilyl)ethyl hydrogen succinate (430 mg, 2 mmol),[7] 1 M *N,N′*-dicyclohexylcarbodiimide in benzene (1.2 mL, 1.2 mmol), and 4-dimethylaminopyridine (8 mg) were added to a solution of **11b** (450 mg, 1.1 mmol) in benzene (8 mL). After 3 h of stirring, one drop of water was added, the mixture was diluted with petroleum ether (5 mL), and it was filtered through celite, which was eluted with a benzene-petroleum ether (1:1, v/v) mixture. The filtrate was concentrated, and the product was chromatographed on alumina in 2% acetone in a benzene-petroleum ether mixture (1:1, v/v), giving **13** (660 mg, 98%). ^{1}H NMR (200 MHz): 5.73 (1 H, br s, 4-H), 5.02 (1 H, dt, J = 2 × 6.5 and 2.5, 15α-H), 4.18 (2 H, m, W = 17, OCH_2CH_2Si), 3.51 (1 H, t, J = 8.4, 17α-H), 2.60 (4 H, m, $OCCH_2CH_2CO$), 1.23 (3 H, s, 19-H_3), 0.98 (2 H, m, W = 16, OCH_2CH_2Si), 0.96 (3 H, s, 18-H_3), 0.94 (6 H, d, J = 6.4, $(CH_3)_2CH$), 0.04 (9 H, s, $(CH_3)_2Si$), 0.01 (6 H, s, $(CH_3)_2Si$).

Succinate **13** (640 mg, 1.1 mmol) was then stirred for 5 h in THF (15 mL) with 1 M solution of tetrabutylammonium fluoride in THF (4 mL, 4 mmol). Acetic acid (0.25 mL) and benzene (15 mL) were added, and the mixture was evaporated with silica gel (5 g). The product on the silica gel was applied onto the column of silica gel (60 mL) and eluted with 0.1% of acetic acid in a chloroform-methanol (50:1, v/v) mixture. Crystallization from an absolute ethanol-ether mixture yielded **14** (225 mg, 52%), m.p. 183–185°C, $[\alpha]_D^{25}$ +17 (c = 0.8, $CHCl_3$). IR: 3611 and 3435 (OH), 3505 sh (COOH), 2664, and 2560 (COOH dimer), 1728 ($C=O_{ester}$), 1718 ($C=O_{acid}$), 1662 ($C=O_{ketone}$), 1615 (C=C), 1057 (C–O). ^{13}C NMR see Table 1. ^{1}H NMR see Table 3. Analysis calculated for $C_{23}H_{32}O_6$ (404.5): C, 68.29; H, 7.97. Found: C, 68.19, H, 8.12.

*17β-Hydroxy-3-oxoandrost-4-en-15β-yl 15-hemiglutarate (**17**)*

The above-mentioned procedure for **14** was applied to **11b** (400 mg, 2 mmol) with 2-(trimethylsilyl)ethyl hydrogen glutarate (700 mg, 3 mmol) and gave **15** (575 mg, 90%). ^{1}H NMR (200 MHz) 5.73 (1 H, br s, 4-H), 5.00 (1 H, ddd, J = 7.8, 5.7, and 2.7, 15α-H), 4.17 (2 H, m, W = 18, OCH_2CH_2Si), 3.51 (1 H, t, J = 8, 17α-H), 2.57 (1 H, ddd, J = 15, 8, and 7.8, 16α-H), 2.35 (4 H, m, W = 25, $OCCH_2CH_2CH_2CO$), 1.93 (2 H, p, J = 7, $OCCH_2CH_2CH_2CO$), 1.23 (3 H, s, 19-H_3), 0.97 (2 H, m, W = 19, OCH_2CH_2Si), 0.96 (3 H, s, 18-H_3), 0.94 (6 H, d, J = 7, $(CH_3)_2CH$), 0.04 (9 H, s, $(CH_3)_3Si$), 0.01 (6 H, s, $(CH_3)_2Si$).

Glutarate **15** (550 mg, 0.9 mmol) was processed with tetrabutylammonium fluoride as above but with a different workup. The mixture was diluted with ethyl acetate and washed with 10% aqueous H_2SO_4 and saturated aqueous NaCl. After drying and evapo-

Table 2 ^{13}C NMR TAI-induced shifts and substituent effects of a hydroxy group in position 15

	TAI-shifts[a]		Substituent shifts[b]		
Carbon	3	5	3	5	12
1	−0.04	−0.02	0.03	0.03	0.17
2	−0.08	−0.07	−0.05	−0.05	0.14
3	−0.08	−0.16	0.09	0.04	0.13
4	−0.09	−0.07	−0.06	−0.17	−0.39
5	−0.13	0.05	0.30	−0.48	−0.06
6	−0.15	−0.42	−0.38	0.22	−0.06
7	−0.18	−0.17	−1.14	−0.18	−0.46
8	0.20	−0.43	−3.99	0.18	−3.54
9	0.02	−0.12	0.35	−0.46	0.37
10	−0.09	−0.06	0.12	−0.14	0.16
11	−0.13	−0.11	−0.15	−0.36	−0.06
12	−0.07	−1.15	1.89	1.31	1.45
13	0.16	−0.61	−0.77	3.12	−0.48
14	−1.24	−3.57	4.23	6.12	4.76
15	6.26	4.61	45.34	49.16	45.93
16	−2.69	−3.26	10.97	10.27	13.32
17	−3.17	−2.90	−0.84	−4.33	0.12
18	−0.70	−0.24	3.92	1.52	2.68
19	0.01	−0.06	−0.21	−0.07	0.00

[a]Defined as δ(R-OCONHCOCCl$_3$) − δ(R-OH).
[b]Defined as δ(R-OH) − δ(RH), reference data taken from reference 9.

Table 3 500 MHz ^{1}H NMR data for testosterone derivatives

	δ_H ($CDCl_3$)			δ_H ($CDCl_3$)	
Proton	**12**	**14**	Proton	**12**	**14**
1α	1.71	1.70	11α	1.61	1.62
1β	2.05	2.04	11β	1.46	1.46
2α	2.35	2.36	12α	1.08	1.08[a]
2β	2.44	2.44	12β	1.85	1.88
4	5.75	5.76	14	0.85	1.03
6α	2.31	2.26	15α	4.22	5.05
6β	2.48	2.40[a]	16α	2.64	2.69
7α	1.12	1.08[a]	16β	1.60	1.56
7β	2.11	1.80	17α	3.59	3.63
8	2.00	1.94	18	1.07	1.01
9	0.99	0.97	19	1.24	1.23

	$J_{x,y}$			$J_{x,y}$	
x,y	**12**	**14**	x,y	**12**	**14**
1α,1β	13.4	13.4	8β,9α	11.0	11.0
1α,2α	4.6	4.6	8β,14α	11.2	11.2
1α,2β	14.5	14.4	9α,11α	4.2	4.2
1β,2α	3.1	3.2	9α,11β	12.5	12.2
1β,2β	5.0	5.0	11α,11β	13.5	13.8
2α,2β	17.0	17.0	11α,12α	4.5	4.2
2α,4	0.7[b]	0.0[b]	11α,12β	3.0	3.2
4,6β	2.0	1.7	11β,12α	13.0[b]	13.1[b]
6α,6β	14.7	14.9[b]	11β,12β	3.9	4.0
6α,7α	4.2	4.2	12α,12β	12.3	12.7[b]
6α,7β	2.4	2.4	14α,15α	5.6	5.9
6β,7α	13.9[b]	13.2[b]	15α,16α	7.6	7.6[b]
6β,7β	5.5	5.6[b]	15α,16β	2.4	2.5
7α,7β	12.7	12.6[b]	16α,16β	14.7	15.3[b]
7α,8β	11.2[b]	11.2[b]	16α,17α	8.7	8.7[b]
7β,8β	3.4	3.4	16β,17α	8.7	8.7

[a]Not fully resolved signals.
[b]Couplings estimated from the better resolved multiplet.

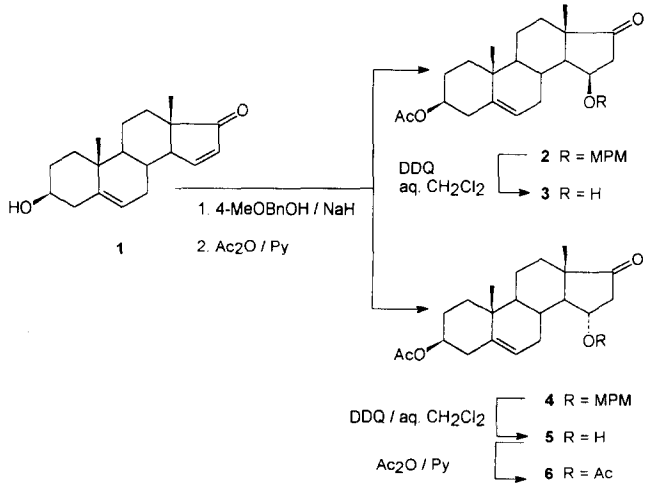
ration, the chloroform solution of the product was applied onto the column and chromatographed as in the preparation of **14.** After crystallization of the main fraction from ethanol, **17** (275 mg, 74%) was obtained, m.p. 198–200°C, $[\alpha]_D^{25}$ +12 (c = 1.0, $CHCl_3$); IR (KBr): 3521, 3080 (COOH), 3440 sh and 3400 sh (OH), 1731 ($C{=}O_{ester}$), 1714 ($C{=}O_{acid}$), 1650 ($C{=}O_{ketone}$), 1611 (C=C), 1194 (C–O). ^{1}H NMR (200 MHz) 5.68 (1 H, br s, 4-H), 4.97 (1 H, ddd, J = 7.8, 5.7, and 2.7, 15α-H), 3.54 (1 H, t, J = 8.3, 17α-H), 2.64 (1 H, ddd, J = 15, 8, and 7.8, 16α-H), 2.34 (4 H, m, W = 19, $OCCH_2CH_2CH_2CO$), 1.90 (2 H, p, J = 7, $OCCH_2CH_2CH_2CO$), 1.19 (3 H, s, 19-H_3), 0.95 (3 H, s, 18-H_3). Analysis calculated for $C_{24}H_{34}O_6$ (418.5): C, 68.88; H, 8.19. Found: C, 68.89; H, 8.23.

Results and discussion

Reaction of **1** with 4-methoxybenzyl alcohol in the presence of an oil suspension of sodium hydride (Scheme 1) gave 41% of the 15β-adduct **2** after acetylation, chromatography, and crystallization. The yield was comparable with the yield from the corresponding addition of benzyl alcohol, which is mediated by powdered sodium hydroxide.[4] We found that there is no difference between hydride or hydroxide promotion, and for practical reasons (e.g., sensitivity of the reagent to air moisture), we preferred to use the oil suspension of the hydride. We additionally isolated a minor amount of the 15α-isomer **4** from the mother liquors. ^{1}H NMR spectra of both isomers **2** and **4** gave characteristic signals of the (4-methoxyphenyl)methyl (MPM) and acetyl groups and differed mainly at 15-H and 18-H. The signal of 15α-H from **2** formed a triplet at δ 4.13, and the singlet of 18-H was shifted downfield to δ 1.16 compared with the parent 17-ketone (17-oxoandrost-5-en-3β-yl acetate: δ 0.89). However, 15β-H in **4** was split into a doublet of doublets of doublets resonating at δ 4.04, and 18-H was found at δ 0.90, i.e., it was much less influenced by the 15-hydroxy substitution. ^{13}C NMR spectra of **2** and **4** (Table 1) exhibited in comparison with the parent 17-ketone,[9] shifts caused by the α-effect on C-15 (52.0 and 55.2 ppm, respectively), β-effect on C-14 (4.4 and 3.8 ppm, respectively) and on C-16 (7.3 and 7.5 ppm, respectively), in accord with the substitution at position 15. Configurationally more dependent were γ-effects: for C-8 –3.8 and 0.3 ppm, for C-13 –0.5 and 2.3 and for C-17 –1.6 and –4.0, respectively. Effects on C-18 (3.8 and 1.7 ppm) were also noticeable.

Both derivatives **2** and **4** were smoothly deprotected: after 20 min of treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane with water,[10] we obtained corresponding hydroxy compounds **3** and **5.** The latter was transformed to the known diacetate **6** with m.p. and optical rotation in accord with published data.[8]

The structural assignments were further confirmed by NMR spectroscopy. ^{1}H NMR spectra of **3** and **5** were not fully resolved, but all the protons of D-ring were assigned (see Experimental). Coupling constants $J_{14,15}$ were 4.5 Hz for **3** and 10.0 Hz for **5,** in accord with respective 14α,15α (a,e) and 14α,15β (a,a) arrangements. Tentative assignments for 16α-H and 16β-H were done by comparing with data for androst-5-ene-17-one,[11] which gave substituent shifts for **3** and **5** for 16α-protons 0.4 and 0.0 ppm and for 16β-protons 0.1 and 0.5 ppm, respectively. For **3,** this assignment independently followed from values of coupling constants $J_{15\alpha,16\alpha}$ = 6.0 and $J_{15\alpha,16\beta} \approx 0$ Hz, and in the case of **5** NOE enhancement on 16β was observed during irradiation of 18-H. When the spectrum for **5** was measured immediately after dissolution, coupling constant $J_{15,OH}$ (5.0 Hz) could be detected. With respect to structural elucidation, ^{13}C NMR data were more convincing (Table 1). All



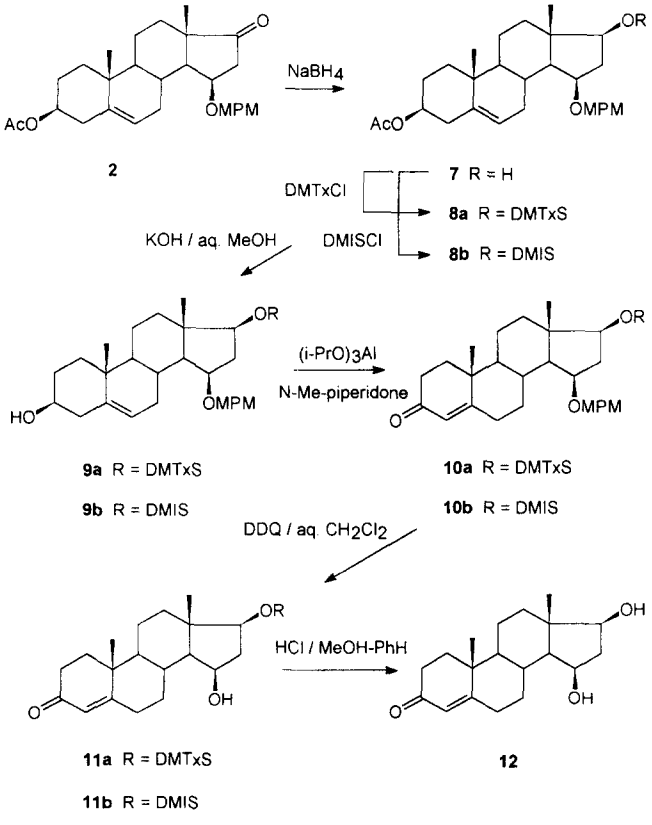
Scheme 1

substituent shifts (Table 2) are in good agreement with published data.[9] The shifts after trichloroacetyl isocyanate (TAI) derivatization were added for future comparison.

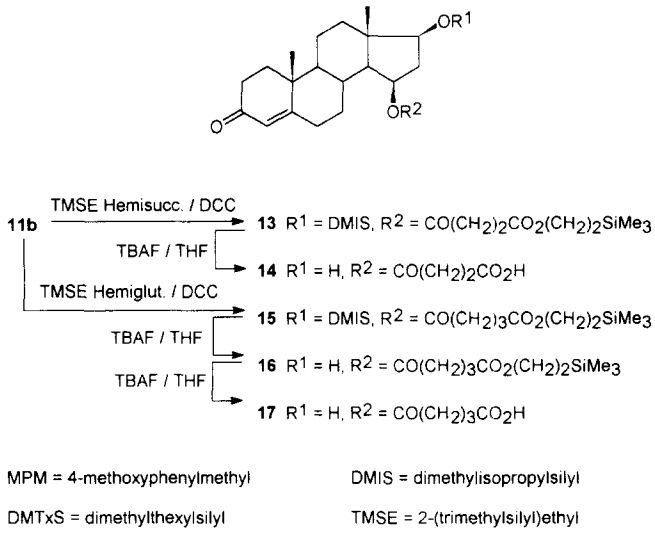
Ketone **2** was reduced by sodium borohydride in a methanol-ethyl acetate mixture (Scheme 2) giving exclusively 17β-hydroxy derivative **7.** For protection of the hydroxyl group at position 17, we chose organosilyl groups, which enabled the independent cleavage of both MPM and acetyl protecting groups at positions 3 and 15. At first we checked the use of the dimethylthexylsilyl (DMTxS) group[12] as the more stable variant for protection. The reaction of **7** with dimethylthexylsilyl chloride and triethylamine in dichloromethane with a catalytic amount of 4-dimethylaminopyridine needed prolonged time and elevated temperature for completion (5 days and 45°C). The product **8a** was deacetylated to the derivative **9a,** which has a free hydroxy group at position 3. Oppenauer oxidation with 1-methyl-4-piperidone and aluminum isopropoxide in boiling toluene[13] gave the protected testosterone derivative **10a.**

Removal of the MPM group from **10a** was accomplished by DDQ in the same manner as in the case of derivatives **2** and **4.** It is known from the literature[14] that DDQ is capable to cleaving silyl ethers. We observed, that dimethylthexylsilyl group was not cleaved under these conditions. This was caused by its relative stability and by the dichloromethane medium, in which the cleavage proceeds only slowly.[14]

The resulting dimethylthexylsilyl derivative **11a** was, however, stable under conditions usually used for organosilyl group deprotection. It could not be completely cleaved by tetrabutylammonium fluoride in THF even under elevated temperature. The use of protic acid led to 15β-



Scheme 2



Scheme 3

hydroxytestosterone **12** with m.p. and rotation in accord with published[1] data from microbial hydroxylation products. The 500 MHz ^{1}H NMR spectrum of **12** was analyzed with the aid of the general rules given in reference 15 and could be fully resolved (Table 3). ^{13}C NMR data for **12** in $CDCl_3$ (Table 1) were comparable with published data in deuteriopyridine[3] and computed substituent shifts related to testosterone (Table 2) are in agreement with general data.[9]

Because acidic medium is not compatible with the presence of labile ester functions, we searched[16] for an organosilyl protecting group, cleavable with tetrabutylammonium fluoride. Model experiments were done with *t*-butyldimethylsilyl and dimethylisopropylsilyl (DMIS) groups and showed that the latter was ideal for the 17β-hydroxy group in the androstane skeleton. This group could be smoothly introduced by reaction with chlorodimethylisopropylsilane and triethylamine in dichloromethane and could be cleaved by tetrabutylammonium fluoride in reasonable time.

The synthesis of 15-hemisuccinate **14** was therefore accomplished from the DMIS-protected derivative **8b.** After deacetylation to **9b,** Oppenauer oxidation to **10b,** and DDQ deprotection, we obtained the 15β-hydroxytestosterone derivative **11b.** Unlike in DMTxS protection, the DMIS group was partially cleaved by DDQ, as indicated by the presence of traces of **12** on TLC. Nevertheless, the yield of partially protected derivative **11b** was practically unaffected.

For the completion of the synthesis, we used an indirect method[7] for preparing hemisuccinates (Scheme 3). In the first step, we prepared succinate **13** by *N,N'*-dicyclohexylcarbodiimide-mediated condensation of (2-trimethylsilyl) ethyl hemisuccinate with **11b.** In the second step, the protecting groups from both carboxyl and hydroxyl were cleaved simultaneously by tetrabutylammonium fluoride, giving 15β-hydroxytestosterone 15-hemisuccinate **14.**

The same concept was used for the synthesis of homologous hemiglutarate **17.** However, glutarate **15** was more resistant to tetrabutylammonium fluoride, and prolonged treatment was necessary. From the initial experiments, which were interrupted before completion, partially depro-

tected derivative **16** was separated. This is evidence of preferential cleavage of DMIS over the 2-(trimethylsilyl)ethyl group.

In summary, addition of 4-methoxybenzyl alcohol onto the 15-ene-17-one grouping of androstane derivatives produced a very flexible method for 15-hydroxy substitution. As examples, model haptens of 15β-hydroxytestosterone, 15-hemisuccinate, and 15-hemiglutarate, which have spacers of variable length, were prepared. Various organosilyl protecting groups were checked, with respect to their suitable stability at position 17 of the steroid skeleton.

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