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Stereoselective synthesis of some methyl-substituted steroid hormones and their *in vitro* cytotoxic activity against human gastric cancer cell line MGC-803

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ABSTRACT

A series of 3-, 7-, 15-, and 16-methyl-substituted steroid analogs were synthesized *via* a highly stereoselective 1,6-conjugate addition. Under the catalysis of CuBr, $AlMe_3$ reacted with four steroid dienone precursors to afford either the corresponding α-epimer of C-3 and C-7 methyl-substituted steroids as the major products, and the ratio of α/β was up to 10/1. No β-epimer has been detected for methyl addition at C-16. However, under the same reaction conditions, enantioselective methyl addition at C-15 afforded the 15β-epimer as the major product. The preliminary SAR analysis showed that the methyl substituents at C-7α and C-15β positions lead to a dramatical increase in potency against human gastric cancer cell line MGC-803.

1. Introduction

Introduction of a methyl group at a certain position of steroids may significantly change their bioactivities. For example, 2α-methyl cortisol [1] exhibits a striking increase in mineralocorticoid activity over its nonmethylated analog, the methyl substitution at the 6α- [2] or 16α-positions [3] of 17-spirolactones enhances its anti-inflammatory activity, and the introduction of a methyl group at 6α-position of 17α-acetoxyprogesterone [4] enhances its progestational activity.

Furthermore, introduction of methyl group at C-7 position of steroids may also afford important biological active compounds, such as tibolone [5] (therapeutic against tumors, cardiovascular disorders), 7α-methyltestosterone (antifertility) [6], 7α,17α-dimethyl-19-nortestosterone (mibolerone) (a compound devoid of estrogenicity) [7–9], and 7α-methylnortestosterone (a potent inhibitor of spermatogenesis in mammals) [10–12].

Introducing methyl groups into the steroid core at various positions was usually achieved by the conjugate addition of either lithium dimethylcuprate [13,14] or methylmagnesium halides in the presence of copper salts to the steroid enone/dienone [15]. In general, the addition gives both α-, and β-methyl epimers with various ratios for different dienones. In the case of conjugate addition at C-15, the α-side was more sterically hindered due to the blocking effect of the 14-H, therefore 15β-methyl isomers are produced as major isomers [16]. Campbell et al. reported that 11β-hydroxy steroids reacted with methylmagnesium bromide in the presence of cuprous chloride to produce 7β-epimers as the major isomer. They suggested that the addition from the β-side can be promoted by a neighboring group which may orient the Grignard reagent to attack the front of the molecule [17].

Westermann demonstrated that highly regioselective conjugate additions can be achieved with trimethylaluminium (Me_3Al) in the presence of catalytic amounts of CuBr or CuCN [18]. Inspired by Westermann's work, in this paper, we developed a highly stereoselective 1,6-conjugate addition of methyl group to C-3, C-7, C-15 and C-16 positions on steroid compounds. Thus, the syntheses of 3α-methylandrost-5-en-7,17-dione, 11α,17β-diacetoxy-7α-methylandrost-4-en-3-one, 11α-acetoxy-7α-methylandrost-5-ene-3,17-dione cyclic 3-(1,2-ethanediyl acetal), and 7α-methylandrost-4,9(11)-diene-3,17-dione are reported herein for the first time. The structure and absolute configuration of 3α-methylandrost-5-en-7,17-dione were confirmed by single-crystal X-ray diffraction analysis. All of these compounds were assayed *in vitro* to characterize their biological profiles, and the interesting results were also reported in this paper.

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2. Experimental

Melting points were determined on a melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured on a Bruker 500 MHz/400 MHz spectrometer at 25 °C in $CDCl_3$ with TMS as the internal standard. Chemical shifts are given in ppm (δ-scale), and coupling constants and widths of multiplets are given in Hz. ^{13}C NMR spectra were measured on a spectrometer (^{13}C at 100 MHz). HR-MS were recorded on a Bruker micrOTOF II spectrometer (ESI ionization). The α/β methyl isomer ratio in the products of 1,6-conjugate addition was determined by ^{1}H NMR analyses of the crude reaction mixtures. Optical rotations were measured in dichloromethane. Thin-layer chromatography (TLC) was performed on silica gel.

2.1. Synthesis of 3-methyl steroids

2.1.1. Androst-3,5-dien-7,17-dione (**2**)

To a solution of 3β-hydroxyandrost-5-en-7,17-dione (**1**, 2.0 g, 6.6 mmol) in methanol (80 ml) was added 70% aqueous $HClO_4$ (4 ml). The reaction mixture was stirred for 12 h, then diluted with iced water and neutralized with sodium bicarbonate. Some white solid precipitated. The mixture was extracted with ethyl acetate (3× 20 ml), and the combined extracts were purified by chromatography (EtOAc/petroleum ether) to give **2** (1.5 g, 80%). m.p. 167–168 °C; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 0.93 (s, 3H, 18-CH_3), 1.15 (s, 3H, 19-CH_3), 5.65 (s, 1H, 6-H), 6.12–6.24 (m, 2H, 3-H and 4-H).

2.1.2. 3α-Methylandrost-5-en-7,17-dione (**3**) and 3β-methylandrost-5-en-7,17-dione (**4**)

To a solution of compound **2** (0.57 g, 2.0 mmol) and CuBr (6.0 mg, 0.04 mmol) in THF (10 ml) at 20 °C, under N_2, was added 2.37 mol/L Me_3Al solution (0.94 ml, 2.2 mmol). After completing the addition, trimethylsilyl chloride (TMSCl) (0.26 g, 2.4 mmol) was then added, and the resulting mixture was stirred at room temperature for 2 h. H_2O (0.2 ml) was added, and the solution was stirred for 24 h. The mixture was filtered, and solid was washed twice with THF and separated by chromatography to give both isomers **3** and **4** with ca. 6:1 ratio. The total yield was 65%. ^{1}H NMR spectrum of α-isomer (**3**), δ (ppm): 5.75 (s, 1H, 6-H), 1.24 (s, 3H, 18-H), 0.93 (s, 3H, 19-H), 0.90 (d, 3H, 3α-CH_3, J = 7.0 Hz); ^{1}H NMR spectrum of β-isomer (**4**), δ (ppm): 5.31 (s, 1H, 6-H), 1.24 (s, 3H, 18-CH_3), 0.92 (d, 3H, 3β-CH_3, J = 7.0 Hz), 0.89 (s, 3H, 19-CH_3); ^{13}C NMR spectrum of α-isomer (**3**), δ (ppm): 13.8, 17.4, 17.7, 20.3, 24.3, 27.4, 28.9, 30.8, 33.2, 35.7, 39.0, 39.3, 44.4, 45.9, 47.9, 50.4, 126.5, 168.2, 200.9, 220.5; ^{13}C NMR spectrum of β-isomer (**4**), δ (ppm): 13.7, 18.5, 20.4, 21.2, 22.7, 25.7, 29.4, 30.7, 32.6, 35.5, 37.4, 44.5, 47.3, 48.6, 50.2, 53.3, 129,2, 139.0, 208.7, 220.0; Compound **3**: $[\alpha]_D = -118.3$ (c = 1.0 in CH_2Cl_2); EI-MS m/z (%): 300 (M^+, 100), 285 (12), 267 (20), 203 (57), 177 (74), 150 (71), 135 (48), 121 (66); HR-MS for $C_{20}H_{28}O_2Na$ required 323.1987, found 323.1982 $[M+Na]^+$.

2.2. Synthesis of 7-methyl steroids

2.2.1. 17β-Acetoxyl-7α-methylandrost-4-en-3-one (**8**) and 17β-acetoxyl-7β-methylandrost-4-en-3-one (**9**)

*2.2.1.1. 17β-Hydroxylandrost-4,6-dien-3-one (**6**).* Testosterone (**5**, 1.0 g, 3.5 mmol) and tetrachlorobenzoquinone (2.4 g, 9.7 mmol) were dissolved in 70 ml of *t*-butanol, and the solution was refluxed, under N_2, for 7 h. After the solvent was removed, the residue was dissolved in dichloromethane (50 ml) and washed with 5% aqueous NaOH and water, dried over anhydrous Na_2SO_4, and evaporated to dryness to give **6** as a yellow solid (0.58 g, 59%). ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 6.08 (m, 2H, 6-H and 7-H), 5.66 (s, 1H, 5-H), 1.10 (s, 3H, 18-CH_3), 0.82 (s, 3H, 19-CH_3).

*2.2.1.2. 17β-Acetoxylandrost-4,6-dien-3-one (**7**).* To a solution of compound **6** (0.58 g, 2.0 mmol) in dichloromethane (30 ml) were added DMAP (0.25 g, 2.0 mmol) and acetic anhydride (0.4 ml, 4.0 mmol). After completing the addition, the mixture was stirred at room temperature for half an hour. The solution was washed with $NaHCO_3$ (50 ml), 1 M HCl (200 ml), and brine (200 ml), respectively, and then dried in vacuo. This gave 0.63 g product **7** as yellow solid (96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.14 (m, 2H, 6-H and 7-H), 5.69 (s, 1H, 5-H) 2.07 (s, 3H, 17-CH_3CO), 1.12 (s, 3H, 18-CH_3), 0.91 (s, 3H, 19-CH_3).

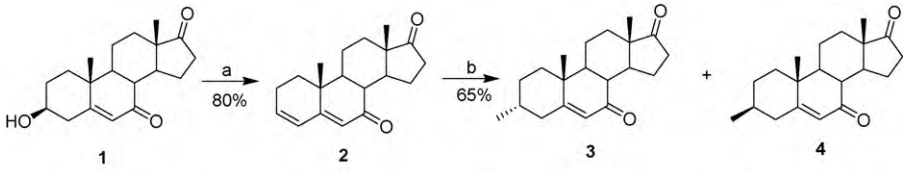
*2.2.1.3. 17β-Acetoxyl-7α-methylandrost-4-en-3-one (**8**) and 17β-acetoxyl-7β-methylandrost-4-en-3-one (**9**).* Compound **7** (0.5 g, 1.53 mmol) was methylated as described in synthesis of **3** and **4** to give 0.38 g of product (75%), and both isomers **8** and **9** was in a 5:1 ratio. Further purification with chromatography could gave 7α- and 7β-epimers, respectively. ^{1}H NMR spectrum of α-isomer (**8**), δ (ppm): 5.73 (s, 1H, 4-H), 4.61 (t, 1H, 17-H, J = 8.5 Hz), 2.05 (s, 3H, $COCH_3$), 1.20 (s, 3H, 18-CH_3), 0.85 (s, 3H, 19-CH_3), 0.77 (d, 3H, 7α-CH_3, J = 7.5 Hz); ^{1}H NMR spectrum of β-isomer (**9**), δ (ppm): 5.71 (s, 1H, 4-H), 4.56 (t, 1H, 17-H, J = 8.5 Hz), 2.05 (s, 3H, $COCH_3$), 1.16 (s, 3H, 18-CH_3), 1.05 (d, 3H, 7α-CH_3, J = 6.5 Hz), 0.85 (s, 3H, 19-CH_3); ^{13}C NMR spectrum α-isomer (**8**), δ (ppm): 11.9, 12.6, 17.8, 20.5, 21.1, 22.8, 27.3, 30.9, 33.9, 35.9, 36.4, 37.9, 38.7, 40.7, 42.4, 46.1, 46.5, 82.4. 125.8, 169.4, 171.0, 198.9; HR-MS for $C_{22}H_{33}O_3$ required 345.2430, found 345.2416.

2.2.2. 7α-Methyl-17β-acetoxyl-nortestosterone (**13**) and 7β-methyl-17β-acetoxyl-nortestosterone (**14**)

*2.2.2.1. 3,17β-Diacetoxyl-3,5-estradiene (**11**).* 19-Nortestosterone (**10**, 5.0 g, 18.2 mmol), 20 ml of acetic anhydride and 24 ml of acetyl chloride were mixed and refluxed for 3.5 h. After completion of the reaction the acetyl chloride was distilled under normal pressure, then the excess of acetic anhydride was removed. This gave a yellow solid which was dissolved in ethyl acetate (100 ml), then washed twice with brine, dried over Na_2SO_4 and evaporated to dryness to give 6.0 g of **11** with a yield of 92%. m.p. 165–169 °C; ^{1}H NMR: (500 MHz, $CDCl_3$), δ (ppm): 5.77 (d, 1H, J = 2.0 Hz), 5.48 (t, 1H, J = 2.5 Hz), 4.62 (t, 1H, J = 8.5 Hz), 2.45 (m, 1H), 2.13 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 0.82 (s, 3H, 18-Me).

*2.2.2.2. 17β-Acetoxyl-4,6-estradiene-3-one (**12**).* A solution of compound **11** (3.5 g, 9.77 mmol) in DMF (23 ml) and water (0.7 ml) was cooled to 0 °C. 1.85 g (10 mmol) of NBS was added in portions. After stirring for 45 min at room temperature LiBr (1.69 g, 20 mmol) and Li_2CO_3 (3.42 g, 46 mmol) were added, and the mixture was stirred for 20 min followed by 45 min stirring at 110 °C. The mixture was poured into a solution of water (135 ml) with acetic acid (11.3 ml), and extracted with dichloromethane (3× 100 ml). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness to give **12** as yellow solid with (2.4 g, 80%). m.p. 104–105 °C; ^{1}H NMR: (500 MHz, $CDCl_3$) δ: 6.18 (d, 2H, 6H and 7H, J = 3.9 Hz), 5.78 (s, 1H, 4-H), 4.63 (t, 1H, 17-H, J = 8.3 Hz), 2.06 (s, 3H, CH_3CO), 0.88 (s, 3H, 18-Me).

*2.2.2.3. 7α-Methyl-17β-acetoxyl-nortestosterone (**13**) and 7β-methyl-17β-acetoxyl-nortestosterone (**14**).* Compound **12** (0.5 g, 1.58 mmol) was methylated as described in synthesis of **3** and **4** to give 0.41 g product (79%), and both isomers **13** and **14** was in a 5:1 ratio. Further purification with chromatography gave 7α- and 7β-epimers, respectively. m.p. 141–142 °C; ^{1}H NMR of **13** (500 MHz, $CDCl_3$), δ (ppm): 5.86 (s, 1H, 4-H) 4.64 (bt, 1H, J = 7.4 Hz), 2.07 (s, 3H, 17-CH_3CO), 0.88 (s, 3H, 18-CH_3), 0.78 (s, 3H, 7α-CH_3); ^{1}H NMR of **14** (500 MHz, $CDCl_3$), δ (ppm): 5.78 (s, 1H), 3.61 (bt, 1H, J = 7.5 Hz), 1.04 (s, 3H, 7β-CH_3), 0.80 (s, 3H, 18-CH_3).



Reagents and conditions: a) $HClO_4$ (70%), MeOH, rt. b) Me_3Al, CuBr, TMSCl, THF, rt.

Scheme 1. Synthetic route to 3-methylandrost-5-en-7,17-dione (**3** and **4**) from 3β-hydroxyandrost-5-en-7,17-dione (**1**).

*2.2.3. 7α-Methylandrost-4-en-3,11,17-trione (**17**) and 7β-methylandrost-4-en-3,11,17-trione (**18**)*

*2.2.3.1. Androst-4,6-dien-3,11,17-trione (**16**).* A solution of androst-4-en-3,11,17-trione (**15**, 2.0 g, 8.3 mmol) and tetrachlorobenzoquinone (5.72 g, 23.0 mmol) in *t*-butanol (170 ml) was refluxed, under N_2, for 7 h. The solvent was evaporated. The residue was dissolved in dichloromethane (150 ml) and washed with 5% aqueous NaOH and water, dried over Na_2SO_4, filtered, and dried in vacuo. This gave the product **16** (1.7 g) as yellow solid (68%), and which could be used directly without further purification. m.p. 242–245 °C; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 0.91 (s, 3H, 18-CH_3), 1.31 (s, 3H, 19-CH_3), 5.69 (s, 1H, 4-H), 6.15–6.22 (m, 2H, 6-H and 7-H).

*2.2.3.2. 7α-Methylandrost-4-en-3,11,17-trione (**17**) and 7β-methylandrost-4-en-3,11,17-trione (**18**).* Compound **16** (1.0 g, 3.4 mmol) was methylated as described in synthesis of **3** and **4** to give 0.94 g white solid product (94%), and ^{1}H NMR analysis revealed that the products **17** and **18** in a ratio of 9:1. ^{1}H NMR spectrum of α-isomer (**17**), δ (ppm): 5.73 (s, 1H, 4-H), 1.43 (s, 3H, 18-CH_3), 0.91 (d, 3H, 7α-CH_3, J = 7.0 Hz), 0.88 (s, 3H, 19-CH_3); ^{13}C NMR spectrum of **17** (100 MHz, $CDCl_3$), δ (ppm): 12.3, 14.6, 17.4, 21.1, 29.8, 33.7, 35.0, 35.7, 38.5, 38.7, 39.9, 46.0, 49.7, 50.2, 56.9, 126.6, 166.3, 198.9, 208.2, 216.7; EI-MS *m*/*z* (%): 314 (M^+, 35), 300 (8), 192 (29), 165 (61), 150 (75), 122 (100), 107 (49). HR-MS for $C_{20}H_{26}O_3Na$ required 337.1780, found 337.1781[M+Na]$^+$.

*2.2.4. 11α,17β-Diacetoxyl-7α-methylandrost-4-en-3-one (**24**) and 11α,17β-diacetoxyl-7β-methylandrost-4-en-3-one (**25**)*

*2.2.4.1. 3β,11α,-Diacetoxylandrost-3,5-dien-17-one (**20**) and 3β,11α,-diacetoxylandrost-3,5-dien-17-ol (**21**).* A solution of compound 11α-hydroxyandrost-4-en-3,17-dione (**19**) (25 g, 79.1 mmol), 100 ml of acetic anhydride, and 112.5 ml of acetic chloride was refluxed for 3.5 h. After the excess of acetic chloride and acetic anhydride were distilled, the yellow solid was dissolved in ethyl acetate (300 ml). The solution was washed twice with brine and dried over Na_2SO_4, filtered, evaporated to dryness to give a yellow solid product which was dissolved in 200 ml of methanol, and the solution was cooled with iced water. $NaBH_4$ (6.25 g, 1.16 mol) was added, and then H_2O (300 ml) was added. After the reaction, the solution was extracted with ethyl acetate (200 ml × 2), and the combined organic layer was washed with brine, dried over Na_2SO_4, evaporated to dryness. This afforded 30.5 g of product **21** (99%). ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 5.68 (s, 1H, 4-H), 5.43 (t, 1H, 5-H, J = 4.0 Hz), 5.25 (m, 1H, 11-H), 3.69 (s, 1H, 17-H), 2.12 (s, 3H, 3-$COCH_3$), 2.00 (s, 3H, 11-$COCH_3$), 1.12 (s, 3H, 18-CH_3), 0.86 (s, 3H, 19-CH_3).

*2.2.4.2. 11α-Acetoxyl-17β-hydroxyandrost-4,6-dien-3-one (**22**).* Compound **21** (30.5 g, 79.0 mmol) was methylated as described in synthesis of compounds **3** and **4** to give 19.4 g of the product **22** (77%). ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 6.12 (dd, 1H, 7-H, J = 2.5, 10.0 Hz), 6.05 (dd, 1H, 6-H, J = 1.5, 10.0 Hz), 5.70 (s, 1H, 5-H), 5.29 (m, 1H, 11-H), 3.73 (m, 1H, 17-H), 2.04 (s, 3H, 11-CH_3CO), 1.13 (s, 3H, 18-CH_3), 0.91 (s, 3H, 19-CH_3).

*2.2.4.3. 11α,17β-Diacetoxylandrost-4,6-dien-3-one (**23**).* Compound **22** (2.72, 7.9 mmol) was dissolved in 50 ml of dichloromethane, DMAP (0.97 g, 7.9 mmol) and acetic anhydride (1.5 ml, 15.8 mmol) were added. The solution was stirred for half an hour. Saturated $NaHCO_3$ solution (100 ml) was added. After 0.5 h stirring, the solution was washed with 1 M HCl (100 ml), brine (100 ml), dried over Na_2SO_4, evaporated to dryness to afford 2.68 g of product **23** (99%). ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 6.13 (dd, 1H, 7-H, J = 2.3, 10.3 Hz), 6.05 (dd, 1H, 6-H, J = 2.3, 9.6 Hz), 5.70 (s, 1H, 5-H), 5.21 (m, 1H, 11-H), 4.66 (t, 1H, 17-H, J = 8.6 Hz), 2.06 (s, 3H, 11-CH_3CO), 2.00 (s, 3H, 17-CH_3CO), 1.21 (s, 3H, 18-CH_3), 0.94 (s, 3H, 19-CH_3).

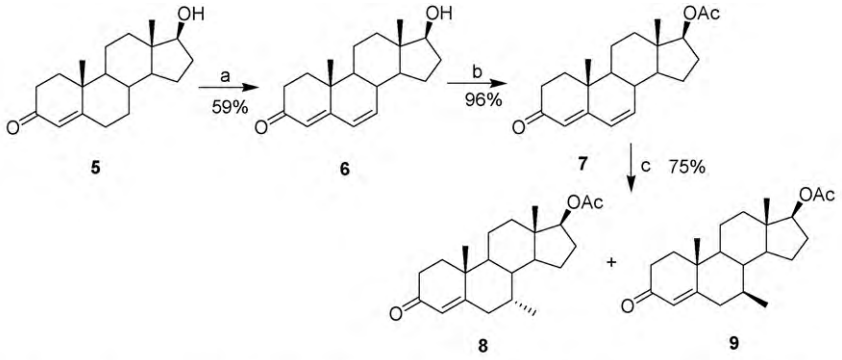
*2.2.4.4. 11α,17β-Diacetoxyl-7α-methylandrost-4-en-3-one (**24**) and 11α,17β-diacetoxyl-7β-methylandrost-4-en-3-one (**25**).* Compound **23** (0.5 g, 1.3 mmol) was methylated as described in synthesis of **3** and **4** to give 0.40 g white solid product (76%), and ^{1}H NMR analysis revealed that the products **24** and **25** in a ratio of 10:1. ^{1}H NMR (500 MHz, $CDCl_3$) of **24**, δ (ppm): 5.73 (s, 1H, 5-H), 5.24 (m, 1H, 11-H), 4.65 (m, 1H, 17-H), 2.04 (s, 3H, 17-CH_3CO), 2.01 (s, 3H, 11-CH_3CO), 1.27 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 0.79 (d, 3H, 7α-CH_3, J = 6.9 Hz); $[\alpha]_D$ = +52.3° (c = 1.0 in CH_2Cl_2).

*2.2.5. 17β-Acetoxyl-1α,7α-dimethylandrost-4-en-3-one (**37**) and 17β-acetoxyl-1α,7β-dimethylandrost-4-en-3-one (**38**)*

*2.2.5.1. 17β-Acetoxyl-1α-methylandrost-4-en-3-one (**34**).* Compound **33** (2.0 g, 6.1 mmol) was methylated as described in synthesis of **3** and **4** to give 1.8 g of white solid product (85%). ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 5.69 (s, 1H, 4-H), 4.58 (t, 1H, 17-H, J = 8.5 Hz), 2.03 (s, 3H, $COCH_3$), 1.27 (s, 3H, 18-CH_3), 0.91 (d, 3H, 1α-CH_3, J = 3.4 Hz), 0.85 (s, 3H, 19-CH_3).

*2.2.5.2. 3β,17β-Diacetoxyl-1α-methylandrost-3,5-diene (**35**).* A solution of compound **34** (5.0 g, 14 mmol), 10 ml of acetic anhydride, and 12 ml of acetic chloride was refluxed for 3.5 h. After the excess of acetic chloride and acetic anhydride were distilled, the yellow solid was dissolved in 100 ml of ethyl acetate. The solution was washed twice with brine and dried over Na_2SO_4, filtered, evaporated to dryness to give 5.5 g product **35** (98%).

*2.2.5.3. 17β-Acetoxyl-1α-methylandrost-4,6-dien-3-one (**36**).* A solution of the previous obtained product **35** in DMF (32 ml) and water (1.0 ml) was stirred at 0 °C. NBS (2.66 g, 14.9 mmol) was added in portions, then the mixture was stirred at 7 °C for 45 min. Lithium bromide (2.4 g, 28.4 mmol) and lithium carbonate (4.9 g, 66 mmol) were added. After 20 min at room temperature the mixture was stirred for about 80 min at 110 °C. The mixture was poured into a solution of water (200 ml) with acetic acid (16 ml), and then extracted with dichloromethane (100 ml × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, evaporated to dryness, and purified by column



Reagents and conditions: a) tetrachlorobenzoquinone, *t*-BuOH. b) $(CH_3CO)_2O$, DMAP, CH_2Cl_2. c) Me_3Al, CuBr, TMSCl, THF, rt.

Scheme 2. Synthetic route to 17β-acetoxyl-7-methylandrost-4-en-3-one (**8** and **9**) from 17β-hydroxyandrost-4-en-3-one (**5**).

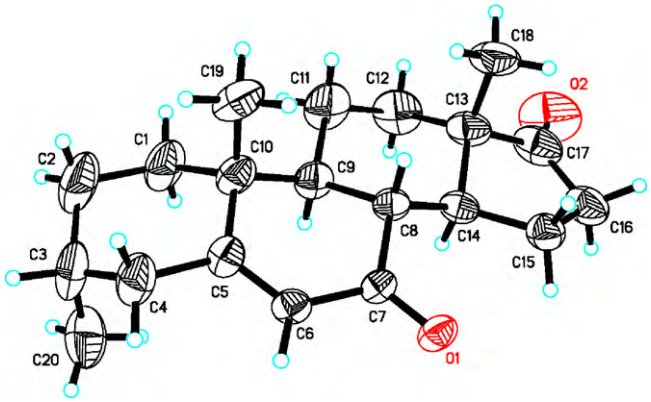


Fig. 1. ORTEP drawing of compound **3**.

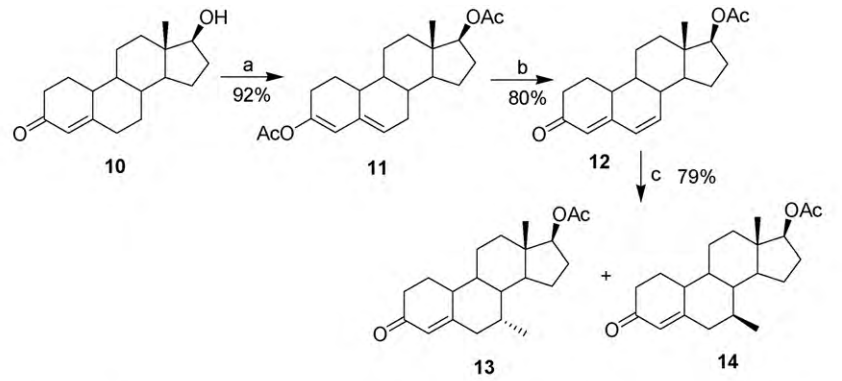
chromatography to give 3.7 g of the product **36** (76%). ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 6.14 (dd, 1H, 7-H, *J* = 2.5, 10.0 Hz), 6.05 (dd, 1H, 6-H, *J* = 2.3, 10.0 Hz,), 5.68 (s, 1H, 4-H), 4.64 (t, 1H, 17-H, *J* = 8.3 Hz), 2.05 (s, 3H, 17-CH_3CO), 1.20 (s, 3H, 18-CH_3), 0.93 (d, 3H, 1α-CH_3, *J* = 7.0 Hz), 0.87 (s, 3H, 19-CH_3).

*2.2.5.4. 17β-Acetoxyl-1α,7α-dimethylandrost-4-en-3-one (**37**) and 17β-acetoxyl-1α,7β-dimethylandrost-4-en-3-one (**38**).* Compound **36** (0.5 g, 1.45 mmol) was methylated as described in synthesis of **3** and **4** to give 0.40 g white solid product (77%), and ^{1}H NMR analysis revealed that the products **37** and **38** in a ratio of 5:1. The pure two isomers were further separated by column chromatography. ^{1}H NMR spectrum of 1α,7α-isomer (**37**), δ (ppm): 5.72 (s, 1H, 4-H), 4.63 (t, 17-H, *J* = 8.5 Hz), 2.05 (s, 3H, $COCH_3$), 1.30 (s, 3H, 18-CH_3), 0.97 (d, 3H, 1α-CH_3, *J* = 7.0 Hz), 0.85 (s, 3H, 19-CH_3), 0.77 (d, 3H, 7α-CH_3, *J* = 7.0 Hz); ^{13}C NMR spectrum of 1α.7α-isomer (**37**): 11.8, 12.8, 15.3, 19.6, 20.4, 21.0, 22.8, 27.2, 30.4, 36.4, 36.9, 37.6, 38.4, 40.8, 41.6, 42.4, 42.5, 46.6, 82.4, 125.1, 166.4, 170.9, 198.2; HR-MS for $C_{23}H_{34}O_3Na$ required 381.2406, found 381.2406.

2.3. Synthesis of 3-methoxyestra-1,3,5(10)-trien-15β-methyl-17-one

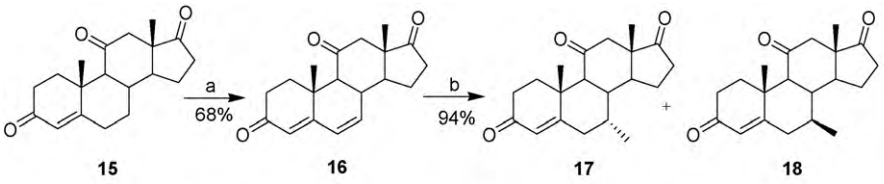
*2.3.1. 3-Methoxyestra-1,3,5(10)-trien-17-one (**40**)*

A mixture of compound **39** (5.4 g, 20 mmol), tetrabutylammonium iodide (0.4 g, 1.0 mmol), and iodomethane (10.8 g, 76 mmol) in dichloromethane (100 ml) and 10% NaOH (100 ml) was refluxed for 7 h. The two liquid phases were separated and the aqueous layer was extracted twice with dichloromethane (50 ml × 2). The combined organic extracts were washed twice with brine, dried over Na_2SO_4, filtered, and evaporated to dryness to give 5.4 g of product **40** (95%). ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 0.91 (s, 3H, 18-CH_3), 3.78 (s, 3H, $ArOCH_3$), 6.65 (d, 1H, H-4, *J* = 2.5 Hz), 6.72 (dd, 1H, H-2, *J* = 8.5, 2.5 Hz), 7.20 (d, 1H, H-1, *J* = 8.5 Hz); EI-MS *m*/*z*: 284(M)$^+$.



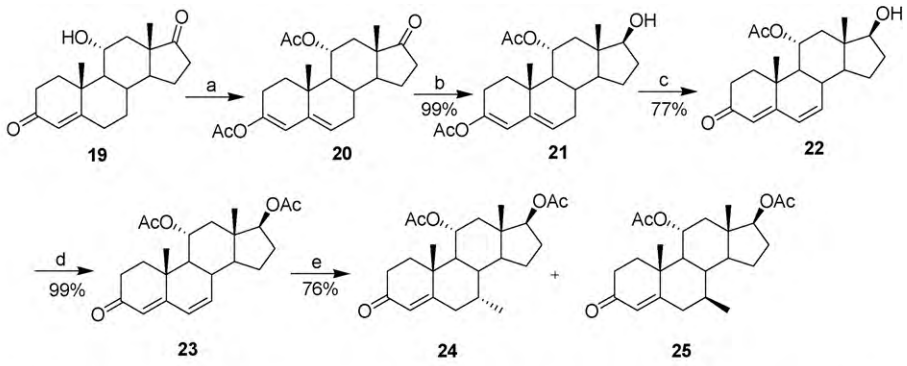
Reagents and conditions: a) $(CH_3CO)_2O$, CH_3COCl. b) NBS, DMF, LiBr, $LiCO_3$. c) Me_3Al, CuBr, TMSCl, THF, rt.

Scheme 3. Synthetic route to 17β-acetoxyl-7-methyl-nortestosterone (**13** and **14**) from 19-nortestosterone (**10**).



Reagents and conditions: a) tetrachlorobenzoquinone, *t*-BuOH. b) Me_3Al, CuBr, TMSCl, THF, rt.

Scheme 4. Synthetic route to 7-methylandrost-4-en-3,11,17-trione (**17** and **18**) from androst-4-en-3,11,17-trione (**15**).



Reagents and conditions: a)Ac_2O, CH_3COCl. b) $NaBH_4$, MeOH. c) NBS, DMF. d) DMAP, CH_2Cl_2, Ac_2O. e) Me_3Al, CuBr, TMSCl, THF, rt.

Scheme 5. Synthetic route to 11α,17β-diacetoxy-7-methylandrost-4-en-3-one (**24** and **25**) from 11α-hydroxyandrost-4-en-3,17-dione (**19**).

*2.3.2. 17-Trimethylsilyloxy-3-methoxyestra-1,3,5(10), 16-tetraene (**41**)*

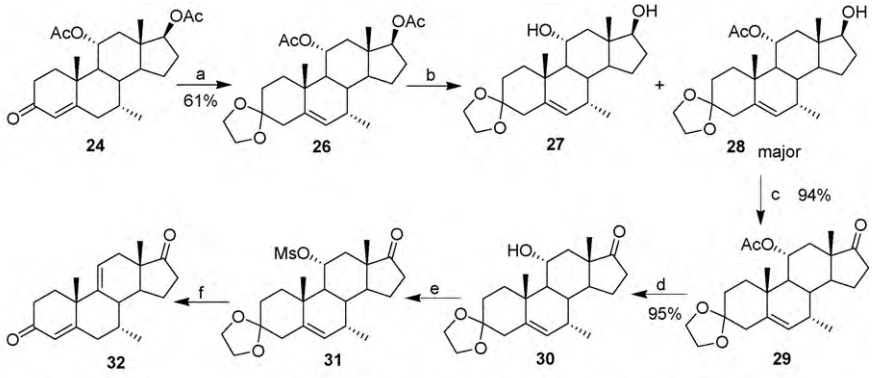
Diisopropylamine (0.92 ml, 6.4 mmol) was dissolved in 5 ml of THF under nitrogen in a three-neck flask. After the solution was cooled to 0 °C, 2.5 M butyllithium (4.1 ml, 10.2 mmol) was added dropwise, and then the mixture was stirred for another 15 min. Afterwards the reaction mixture was cooled to −78 °C. Compound **40** (0.94 g, 3.3 mmol) was dissolved in 15 ml THF in another one-neck flask, and this solution was then added to the three-neck flask. After completing the addition, the mixture was stirred for 40 min. Triethylamine and TMSCl were added, and the reaction solution was stirred at room temperature for 30 min. Saturated $NaHCO_3$ solution (50 ml) was added, and it was extracted twice with dichloromethane (50 ml × 2). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and Evaporated to dryness to give 1.0 g of **41** which was used in the next step without further purification.

*2.3.3. 3-Methoxyestra-1,3,5(10),15-tetraen-17-one (**42**)*

A solution of compound **41** (1.0 g, 2.8 mmol) and palladium acetate (0.6 g, 2.8 mmol) in acetonitrile (20 ml) and of dichloromethane (5 ml) was stirred at 40 °C for 1 h. Evaporation of solvent to give **42** (1.0 g), and total yield for the two steps was 64%. ^{1}H NMR (500Hz, $CDCl_3$), δ (ppm): 1.02 (s, 3H, 18-CH_3), 3.78 (s, 3H, $ArOCH_3$), 6.08 (s, 1H, 16-H), 6.66 (s, 1H, 4-H), 6.73 (d, 1H, 2-H, J = 8.5 Hz), 6.72 (d, 1H, J = 8.5 Hz, 1-H), 7.62 (d, 1H, 15-H, J = 6.0 Hz).

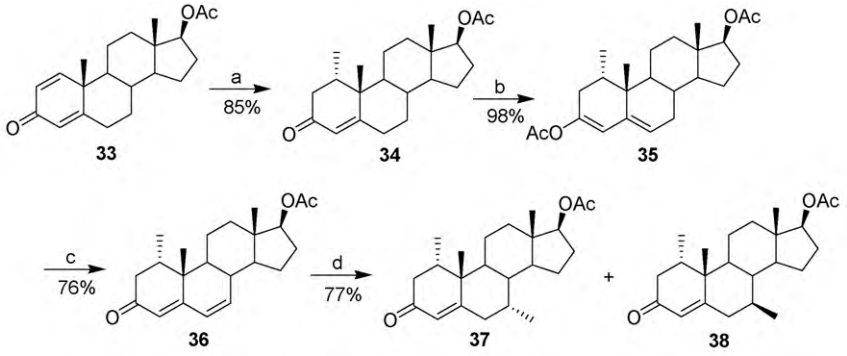
*2.3.4. 3-Methoxyestra-1,3,5(10)-trien-15β-methyl-17-one (**43**)*

Compound **42** (1.12 g, 4.0 mmol) was methylated as described in synthesis of **3** and **4** to give 0.6 g of product **43** with a yield of 51%. m.p. 124–126 °C;. ^{1}H NMR (500 Hz, $CDCl_3$): δ = 1.16 (s, 3H,



Reagents and conditions: a) *p*-TsOH, CH_2OHCH_2OH. b) 5% NaOH. c) IBX, DMSO. d) 10% LiOH. e) MsCl. f) KOAc, HOAc.

Scheme 6. Synthetic route to compounds **28** and **32**.



Scheme 7. Synthetic route to 17β-acetoxyl-1α,7-dimethylandrost-4-en-3-one (**37** and **38**) from 17β-acetoxyl-androst-1,4-dien-3-one (**33**).

18-CH_3), 1.24 (d, 3H, 15β-CH_3, J = 6.5 Hz), 3.77 (s, 3H, $ArOCH_3$), 6.65 (d, 1H, H-4, J = 2.0 Hz), 6.71 (dd, 1H, H-2, J = 8.5 Hz, 2.0 Hz), 7.18 (d, 1H, H-1, J = 8.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 221.2, 157.7, 137.8, 132.5, 125.9, 113.9, 111.4, 55.2, 52.3, 47.4, 44.7, 44.5, 36.0, 34.1, 29.5, 27.6, 26.8, 25.5, 17.9, 16.9; $[\alpha]_D$ +78° (c 1.0, CH_2Cl_2); EI-MS m/z: 298 $(M)^+$.

2.4. Synthesis of 16-methyl steroids

2.4.1. 3-Acetoxyl-16α-methylpregn-5-en-20-one (*46*)

Compound **45** (0.36 g, 1.0 mmol) was methylated as described in synthesis of **3** and **4** to give 0.28 g of product **46** with a yield of 75%. m.p. 173–176 °C; ^{1}H NMR (500 Hz, $CDCl_3$), δ (ppm): 0.66 (s, 3H, 18-CH_3), 0.95 (d, 3H, 16-CH_3, J = 6.9 Hz), 1.02 (s, 3H, 19-CH_3), 2.04 (s, 3H, CH_3CO), 2.12 (s, 3H, 20-CH_3), 4.61 (m, 1H, H-3), 5.38 (d, 1H, H-6, J = 2.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$),δ (ppm): 209.3, 170.5, 139.6, 122.3, 73.8, 73.2, 55.3, 50.0, 45.6, 39.0, 38.1, 37.0, 36.6, 33.3, 32.1, 31.7, 31.6, 31.0, 27.7, 22.2, 21.4, 20.9, 19.3, 13.9.

2.4.2. 3-Hydroxy-16α-methylpregn-5-en-20-one (*47*)

A solution of compound 46 (7.4 g, 20 mmol) in 5% NaOH (80 ml) was stirred for 1 h. H_2O (250 ml) was added. The precipitate was filtered, washed with water, and dried. This gave the product **47** (5.6 g, 83%). ^{1}H NMR (500 Hz, $CDCl_3$), δ (ppm): 0.66 (s, 3H, 18-CH_3), 0.94 (d, 3H, 16-CH_3, J = 6.9 Hz), 1.00 (s, 3H, 19-CH_3), 2.12 (s, 3H, 20-CH_3), 3.52 (m, 1H, H-3), 5.35 (t, 1H, H-6, J = 2.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 140.8, 121.4, 73.2, 71.7, 55.3, 50.1, 45.7, 42.2, 39.0, 37.2, 36.5, 33.4, 32.1, 31.7, 31.9, 31.6, 31.0, 22.2, 21.0, 19.4, 13.9.

2.4.3. 16α-Methylpregn-4-en-3,20-dione (*48*)

A solution of compound 47 (0.33 g, 1.0 mmol) and aluminum isopropoxide (0.41 g, 2.0 mmol) in toluene (8 ml) and cyclohexanone (2 ml) was refluxed for 5 h. After evaporation of toluene, dichloromethane (20 ml) was added. The solution was washed with 3 M aqueous H_2SO_4, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, and evaporated to dryness to give product **48** (0.26 g, 79%). ^{1}H NMR (500 Hz, $CDCl_3$), δ(ppm): 0.69 (s, 3H, 18-CH_3), 0.94 (d, 3H, 16-CH_3, J = 7.0 Hz), 1.18 (s, 3H, 19-CH_3), 2.12 (s, 3H, 20-CH_3), 5.72 (d, 1H, H-4, J = 12.0 Hz).

2.4.4. 16α-Methylpregn-1,4-dien-3,20-dione (*49*)

A solution of compound **48** (0.33 g, 1.0 mmol), p-TsOH (0.03 g, 0.2 mmol), and 2-iodoxybenzoic acid (IBX [19], 4.0 g, 0.01 mol) in DMSO (30 ml) and toluene (15 ml) was stirred at 70 °C for 24 h. The raw material was not completely consumed. The mixture was filtered and the solid was washed with toluene and then discarded. The filtrate was evaporated and ethyl acetate (20 ml) was added. After washing with brine, drying over Na_2SO_4, and evaporating the solvent, 0.1 g of product **49** (31%) was obtained by purification with chromatography. ^{1}H NMR (400 Hz, $CDCl_3$), δ (ppm): 0.65 (s, 3H, 18-CH_3), 0.87 (d, 3H, 16-CH_3, J = 8.0 Hz), 1.16 (s, 3H, 19-CH_3), 2.05 (s, 3H, 20-CH_3), 6.01 (s, 1H, H-4), 6.17 (dd, 1H, H-2, J = 8.0 Hz, 1 Hz), 6.98 (d, 1H, H-1, J = 2.0 Hz).

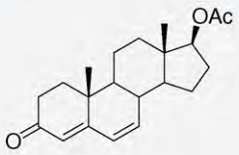
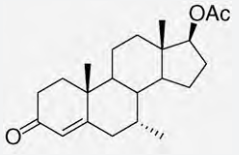
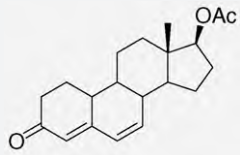
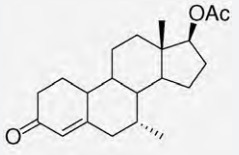
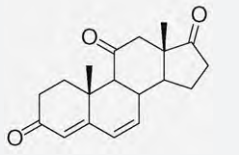
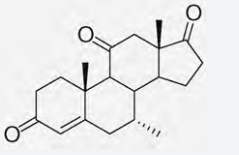
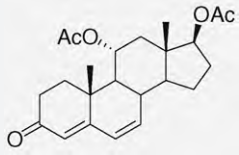
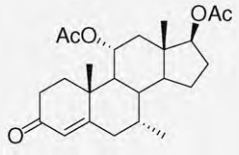
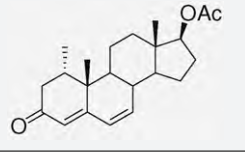
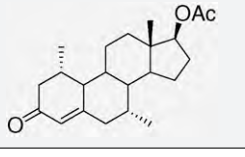
2.4.5. 3-Hydroxy-16α-methylpregn-1,3,5(10)-trien-20-one (*50*)

A mixture of metal lithium (0.12 g, 17 mmol) and biphenyl (0.76 g, 4.9 mmol) in diphenylmethane (0.4 ml) and dried THF (20 ml) was refluxed. A solution of compound **49** (0.4 g, 1.2 mmol) in dried THF (10 ml) was prepared in another flask, and then added dropwise to the former mixture. After another 1 h refluxing, the solution was quenched with water. After evaporating the solvent 1 M HCl (20 ml) was added, and then extracted with ethyl acetate. The combined organic layer was washed with saturated $NaHCO_3$ and brine, evaporated to dryness and purified by column chromatography. This afforded 0.21 g of product **50** (55%). ^{1}H NMR (400 Hz, $CDCl_3$), δ (ppm): 0.61 (s, 3H, 18-CH_3), 0.80 (d, 3H, 16-CH_3, J = 8.0 Hz,), 2.09 (s, 3H, 20-CH_3), 4.59 (s, 1H, ArOH), 6.49 (d, 1H, H-4, J = 2.0 Hz), 6.56 (dd, 1H, H-2, J = 8.0 Hz, 2.0 Hz), 7.07 (d, 1H, H-1, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 126.4, 115.2, 112.7, 106.7, 73.3, 54.1, 46.1, 43.7, 38.5, 33.0, 32.1, 31.0, 29.6, 27.5, 26.6, 22.3, 14.1; EI-MS m/z: 312$(M)^+$.

Table 1
Crystal data for compound **3**.

Empirical formula	$C_{20}H_{28}O_2$
Formula weight	300.42
Crystal_colour, habit	Colourless, prismatic
Wavelength (nm)	0.71073
Crystal system	Monoclinic
Space group	$P2(1)$
a (nm)	1.13813(18)
b (nm)	0.66429(10)
c (nm)	1.16414(18)
β (°)	103.017(3)
V (nm^3)	0.8575(2)
Z	2
D_{Calc} (Mg/m^3)	1.163
$F(000)$	328
Temperature	293(2) K
Crystal size (mm)	0.481 × 0.468 × 0.221
Residuals: R; R_w	0.066; 0.187
θ_{max} (°)	26.99
θ_{min} (°)	1.80
Goodness-of-fit indicator	0.991
μ	0.073
Maximum peak in final diff. Map (e/nm^3)	0.26
Minimum peak in final diff. Map (e/nm^3)	−0.16

Table 2
Synthesis of C-7-methyl steroids by 1,6-conjugate addition with Me_3Al reagent.

Entry	Substrate	Major product	α/β	Yield (%)[a]	Chemical shift of 7α-CH_3
1			5/1	75	0.77
2			5/1	79	0.78
3			9/1	94	0.91
4			10/1	76	0.80
5			5/1[b]	77	0.77

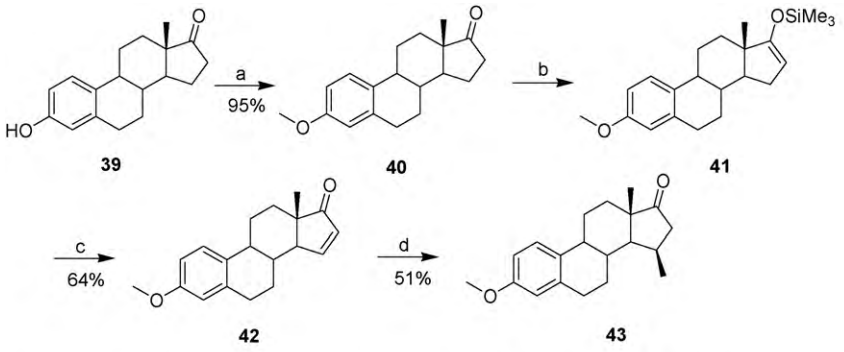
[a] Yield of isolated and purified product.
[b] The ratio of 7α/7β

*2.4.6. 3-Methoxy-16α-methylpregn-1,3,5(10)-trien-20-one (**51**)*

Compound **50** (0.03 g, 0.1 mmol), tetrabutylammonium iodide (0.002 g), and iodomethane (0.054 ml, 0.4 mmol) were dissolved while stirring in dichloromethane (5 ml) and 10% NaOH solution (5 ml). The mixture was refluxed for 7 h. The two liquid phases were separated and the aqueous layer was extracted twice with dichloromethane (5 ml × 2). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness to give 0.03 g of product **51** (97%). ^{1}H NMR (400 Hz, $CDCl_3$), δ (ppm): 0.67 (s, 3H, 18-CH_3), 0.98 (d, 3H, 16-CH_3, *J* = 8.0 Hz), 2.16 (s, 3H, 20-CH_3), 3.78 (s, 3H, $ArOCH_3$), 6.63 (s, 1H, H-4), 6.71 (d, 1H, H-2, *J* = 8.0 Hz), 7.21 (d, 1H, H-1, *J* = 8.0 Hz).

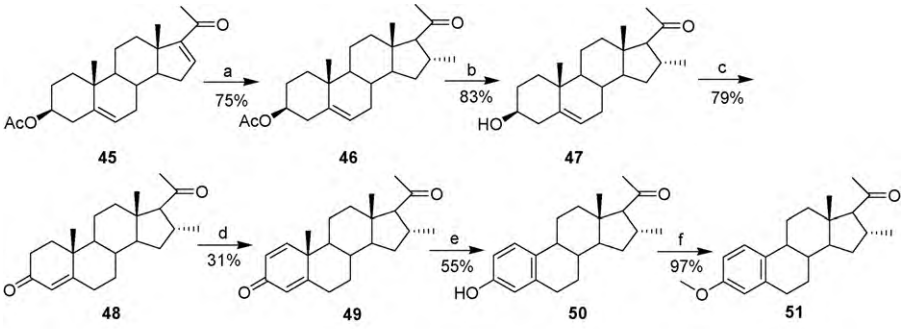
*2.5. X-ray structure determination of 3α-methylandrost-5-en-7,17-dione (**3**)*

A colourless prismatic crystal of dimensions 0.48 mm × 0.47 mm × 0.22 mm was chosen for the measurement. Diffraction data were collected on a Rigaku AFC7R diffractometer with graphite monochromated Mo Kα radiation (λ = 0.071073 nm) at 293 K.



Reagents and conditions: a) $Bu_4N^+I^-$, CH_3I, CH_2Cl_2, NaOH. b) $(i\text{-}Pr)_2NH$, THF, BuLi, Et_3N, TMSCl. c) $Pd(OAc)_2$, CH_3CN, CH_2Cl_2. d) Me_3Al, CuBr, TMSCl, THF, rt.

Scheme 8. Synthetic route to 3-methoxyestra-1,3,5(10)-trien-15β-methyl-17-one (**43**) from estrone (**39**).



Reagents and conditions: a) Me_3Al, CuBr, TMSCl, THF, rt. b) 5% NaOH. c) $Al(Oi\text{-}Pr)_3$. d) *p*-TsOH, IBX, DMSO. E) Li, biphenyl. f) $Bu_4N^+I^-$, CH_3I.

Scheme 9. Synthetic route to 3-methoxy-16α-methylpregn-1,3,5(10)-trien-20-one (**51**) from 16-DPA (**45**).

A total of 2017 reflections were collected within the range of $1.80 \leq \theta \leq 26.99^\circ$ using ω–2θ scan technique, of which 1475 reflections were observed with $I > 2\sigma(I)$. The structure was solved using direct methods, and refined by full-matrix least squares method. The final R indices were $R_1 = 0.066$, w$R_2 = 0.175$ and R indices (all data) $R_1 = 0.082$, w$R_2 = 0.187$. The goodness-of-fit on F^2 was 0.991 and the largest peak and deepest hole in the final difference Fourier map were 0.26×10^{-3} and -0.16×10^{-3} e/nm^3, respectively.

3. Results and discussion

3.1. Synthesis

3.1.1. The stereoselective synthesis of 3α-methyl-substituted steroid analog

Dehydration of 3β-hydroxyandrost-5-en-7,17-dione provided compound **2.** Under the catalysis of CuBr, Me_3Al reacted with compound **2** *via* 1,6-conjugate addition to produce the 3-methyl-substituted compound **3** (Scheme 1). TLC analysis and ^{1}H NMR spectral data showed that both 3α-methyl and 3β-methyl diastereomers were produced with the ratio of 6:1 (α:β). The chemical shifts of the 3α-methyl group and the 3β-methyl group were 0.90 (d, $J = 7.0$ Hz) and 0.92 (d, $J = 7.0$ Hz), respectively. The ^{1}H chemical shifts of 3α-CH_3 and 3β-CH_3 are nearly identical since the extent of shielding or deshielding effect, which comes of α,β-unsaturated ketone unit at C-5,6,7 position, is almost the same. The pure isomers, 3α-methylandrost-5-en-7,17-dione (**3**) and 3β-methylandrost-5-en-7,17-dione (**4**), were isolated by column chromatography with total yield of 65%.

The major product was recrystallized with a solvent mixture (petroleum/acetyl acetate) and the stereochemistry was assigned by single-crystal X-ray diffraction analysis. The crystal structure of compound **3** was shown in Fig. 1, and the crystal data were listed in Table 1.

The X-ray crystallography confirmed that the methyl group at C-3 position was equatorial stereochemistry, and which is just on opposite face of A ring when compared with the hydroxyl group at C-3 position of 17α-Oxa-D-homo-abdrost-5-en-3β,7α-diol (axial stereochemistry of 3-OH) [20].

3.1.2. The stereoselective synthesis of 7α-methyl-substituted steroid analog

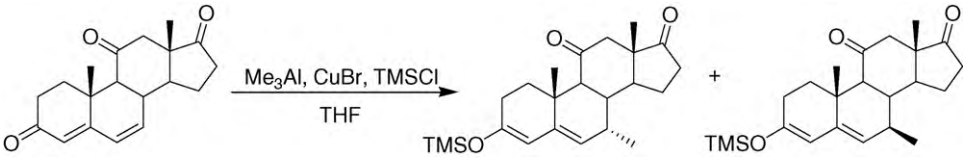
Stereoselective 1,6-conjugate addition to form C-7 methyl derivatives was also successfully achieved to produce 17β-acetoxyl-7-methylandrost-4-en-3-one in 75% yield with an α/β isomer ratio of 5:1 (Scheme 2).

To evaluate the influence of various substituents at different positions of the steroid skeleton to the stereoselectivity of this 1,6-conjugate addition, we synthesized a series of structurally diverse dienones **12**, **16**, **23**, **36** from 19-nortestosterone, androst-4-en-3,11,17-trione, 11α-hydroxyandrost-4-en-3,17-dione, and 1α-methyltestosterone-17-acetate, respectively (Schemes 3–7). The results of stereoselective 1,6-conjugate addition of **12**, **16**, **23**, **36** were listed in Table 2.

As shown in Table 2, the presence of a methyl group at C-1 and C-19 position hardly influenced the stereoselectivity of 1,6-conjugate addition on C-7 position (Table 2, entries 1, 2, and 5). However, the substituents at C-11 position strongly affected the stereoselectivity of 1,6-conjugate addition on C-7 position, and remarkably increased the proportion of α-isomer. For example, the ratio of α:β addition products with11α-acetoxy substituent was 10:1 (Table 2, entry 4).

The carbonyl group at C-11 position of 7α-methylandrost-4-en-3,11-17-trione caused a 0.14 ppm downfield shift in the ^{1}H NMR signal of the C-7α-methyl group (Table 2, entry 3) due to the carbonyl deshielding effect.

The presence of TMSCl could accelerate the reaction rate [21]. Monitoring the reaction process by ^{1}H NMR revealed that 3-trimethylsilyloxy ether was produced in the addition reaction and then desilylation happened during the work-up process (adding citric acid or quenching with water or stirring for about 24 h at room temperature after the reaction).

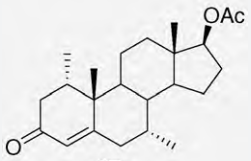
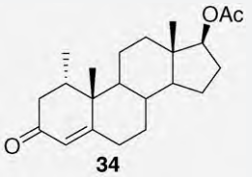
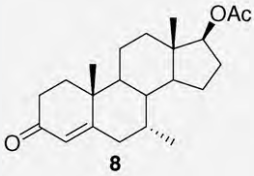
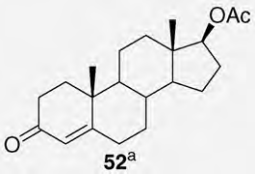
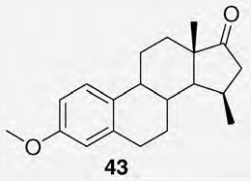
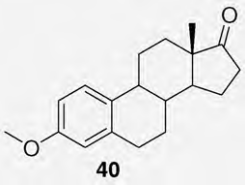
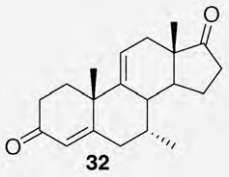
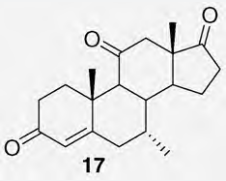
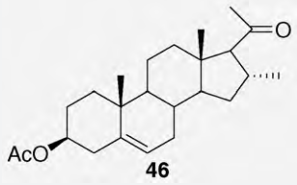
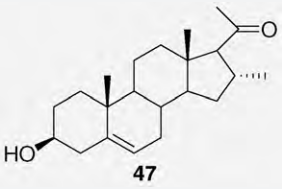
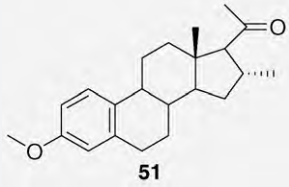
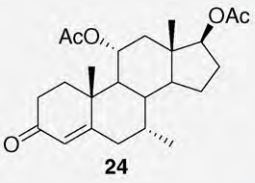
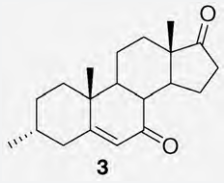
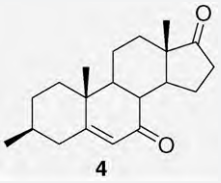


3.1.3. Methylation at C-15 position of estrogen

We selected estrone derivative **42** as the starting material to investigate the stereoselectivity of conjugate addition at C-15 position (Scheme 8).

Only one of the two possible epimers was obtained. This product has a melting point 124–126 °C, a rotation value $[\alpha]_D$ +78°, and ^{1}H NMR data of 13-CH_3 at δ 1.16.

Table 3
The *in vitro* cytotoxic activity (IC_{50}, in μM) of compounds against human gastric cancer cell line MGC-803.

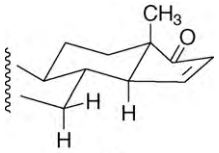
Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
 **37**	1.04 ± 0.03	 **34**	16.21 ± 0.02
 **8**	1.00 ± 0.02	 **52**[a]	3.7 ± 0.02
 **43**	0.99 ± 0.03	 **40**	33.02 ± 0.03
 **32**	4.71 ± 0.01	 **17**	>40
 **46**	1.28 ± 0.03	 **47**	1.50 ± 0.02
 **51**	>40	 **24**	>40
 **3**	>40	 **4**	>40
Doxorubicin[b]	0.98 ± 0.06		

[a] Purchased from Sinopharm Chemical Reagent Co. Ltd.
[b] Positive control.

Groen et al. have reported two criteria for deducing the configuration of C-15 substituted compounds [22]: (1) For estrone or estradiol derivatives, the 15β-epimer shows a small value in optical rotation while the corresponding 15α-epimer shows a large value in optical rotation (2) Due to the 1,3-diaxal interaction, a 15β-substituent with spherical or approximately cylindrical symmetry (halogen, OR, C≡N, CH_3) causes an appreciable downfield shift of the C-13 methyl H signal in the ^{1}H NMR, compared to that of its 15-unsubstituted analog. The 15α-substituent, which is lack of the 1,3-diaxal interaction, has much less change in the chemical shift. For instance, the H signal of C-13 methyl of C-15-unsubstituted compound **40** in ^{1}H NMR was δ 0.91 ppm, and the NMR data for the corresponding product **43** has downfield shifted 0.26 ppm. In addition, the rotation value of the product **43** was $[\alpha]_D$ +78° which was far lower than that of C-15α-methyl isomer, $[\alpha]_D$ +192° [23]. Furthermore, the melting point of this product matches the reported data of the known C-15β-methyl compound. This further confirmed that the conjugate addition gave the 15β-isomer.

The D ring in steroids has three possible conformations (two "envelope" and one "half-chair" conformations). In the case of

17-keto steroids 14α-Δ^{15}-17-one, its D ring (**44**) prefers one of the "envelope" conformations, which has less 1,3-interaction. This working model reveals that the conjugate addition favors β-side at C-15, since it was suggested that the methyl addition from the α-side could cause a marked eclipsed 1,2-interaction between the nucleophile and the C-14 α-hydrogen in the transition state. Thus β-addition at C-15 is a kinetically controlled and irreversible process [16].



44

3.1.4. Synthesis of 3-hydroxy-16α-methyl-19-norpregna-1,3,5(10)-trien-20-one

The stereoselectivity of 1,4-conjugate addition of Me_3Al reagent to 16-DPA was also investigated. 16α-isomer was exclusively obtained as the product. Such a high enantioselectivity was because the β-side is blocked by the steric hindered 13β-methyl group and 17β-acyl group. Thus, using 16-DPA as the starting material, the estranol compound was prepared *via* 16-methylation reaction, 3-hydrolysis, oxidation reaction, dehydrogenation, and aromatization reaction (Scheme 9).

3.2. Cytotoxic activity against a human gastric cancer cell line MGC-803

3.2.1. Material and method

3.2.1.1. Material. Trichloroacetic acid (TDA) was purchased from Shanghai Jingchun Reagent Co. Ltd. and dissolved in distilled H_2O at 50% (m/v). Acetic acid was purchased from Shanghai Chemical Reagent Co. Ltd. and diluted with water at 1% sulforhodamine B (SRB) was purchased from Sigma and dissolved with 1% acetic acid at 0.4% (w/v). Tris was purchased from Bio Basicinc, and dissolved in distilled H_2O at 10 mM. RPMI1640 was purchased from Gibco. Fetal bovine serum (FBS) was purchased from Front Biomedicals, USA.

3.2.1.2. Cell line and cell culture. Human gastric cancer MGC-803 cell line was cultured in RPMI 1640 medium with 10% FBS, which was obtained from the Cell Bank of the Shanghai Institute of Cell Biology.

3.2.1.3. Cytotoxicity assay. Proliferative activity was evaluated by colorimetric sulforhodamine B (SRB) assay as described by Skehan et al. [24]. Briefly, 5×10^3 cells were seeded in 96-well plate. After cell adhering, they were treated with different compounds in a dose-dependent way for 48 h. Then the cells were fixed by 10% TDA for 1 h and stained by SRB for 10 min. After washed with acetic acid to remove the excess dye, protein bounding dye were dissolved in 10 mM Tris and detected by a 96-well microplate reader (SPECTRA MAX 190) at 515 nM. Doxorubicin was used as a positive control and the vehicle (DMSO) as a negative control [25].

3.2.2. Results

Cytotoxicity effect of these steroid analogs on MGC-803 was detected *in vitro*, which is a kind of human gastric cancer cell line. According to our cytotoxicity assay, some of these steroid analogs significantly inhibited cell viability in a dose-dependent manner, while others had no effects even at doses up to 40 μM (Table 3). Compounds **37**, **8** with a 7α-methyl substituent, the norandrostene **43** with a 15β-methyl substituent and **46**, **47** with a 16α-methyl substituent their IC_{50} were about 1 μM significantly suppressed the cell viability after treating cells for 48 h. Our data also indicated that introduction of a 7α-methyl or 15β-methyl substituent resulted in a dramatic increase in inhibition potency. These substituted analogs were about 3.7- to 33-fold more potent than their corresponding parent compounds (e.g., compounds **37** vs **34**, **8** vs **52** and **43** vs **40**). However, incorporation of a methyl group at the 1α-position dramatically decreased cytotoxic activity of the resulting new compound (**34** vs **52**). And from our initial data in Table 3, we cannot draw any conclusion that introducing 3α-methyl, 3β-methyl and 16α-methyl would improve or decrease the inhibitory activity. These results indicated that modifications of the parent steroid compounds with 7α-methyl and 15β-methyl groups improved inhibitory activity on MGC-803 cell line.

4. Conclusion

In summary, we have synthesized a number of 3-, 7-, 15-, and 16-methyl-substituted steroids by using a highly stereoselective 1,6-conjugate addition. With Me_3Al in the presence of catalytic amounts of CuBr, the addition of methyl group predominantly formed α-epimer at C-3 (α/β up to 6/1), C-7 (α/β up to 10/1) and C-16 (no β-epimer). However, introduction of methyl group at C-15 position stereoselectively form β-epimer, as the major isomer, under the same reaction conditions. The absolute configuration of 3α-methylandrost-5-en-7,17-dione, which was reported herein for the first time, was determined by single-crystal X-ray diffraction analysis. The preliminary SAR analysis showed that the compounds with methyl group at 7α-, 15β- and 16α-position have significantly impact on human gastric cancer cell line MGC-803. Further research on the development of methyl-substituted steroids as promising anticancer agents is ongoing in our laboratory.

Supporting information

CCDC 767821 contains the supplementary crystallographic data of compound 3 for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 33.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.steroids.2010.05.008.

References

[1] Hogg JA, Lincoln FH, Jackson RW, Schneider WP. The adrenal hormones and related compounds. III. Synthesis of 2-alkyl analogs. J Am Chem Soc 1955;77:6401–2.
[2] Spero GB, Thompson JL, Magerlein BJ, Hanze AR, Murray HC, Sebek OK, et al. Adrenal hormones and related compounds. IV. 6-Methyl steroids. J Am Chem Soc 1956;78:6213–4.
[3] Arth GE, Johnston DBR, Fried J, Spooncer WW, Hoff DR, Sarett LH. 16-Methylated steroids. I. 16α-methylated analogs of cortisone, a new group of anti-inflammatory steroids. J Am Chem Soc 1958;80:3160–1.

[4] Babcock JC, Gutsell ES, Herr ME, Hogg JA, Stucki JC, Barnes LE, et al. 6α-Methyl-17α-hydroxyprogesterone 17-acylates; a new class of pontent progestins. J Am Chem Soc 1958;80:2904–5.
[5] Zheng YH, Li YC. Novel stereoselective synthesis of 7α-methyl-substituted 5-androstene derivatives. J Org Chem 2003;68:1603–6.
[6] Kendle KE. Further investigation of the antifertility effects in the rat of the antiprogestational steroid, RMI 12,936, and related compounds. J Reprod Fertil 1978;52:373–7.
[7] Emmens CW, Humphrey K, Martin L, Owen WH. Antifertility properties of two non-oestrogenic steroids and MRL 37. Steroids 1967;9:235–43.
[8] Jaglan PS. In vitro covalent binding of 14C-mibolerone to rat liver microsomes. Adv Exp Med Biol 1986;197:919–24.
[9] Perry JE, Tindall DJ. Androgens regulate the expression of proliferating cell nuclear antigen posttranscriptionally in the human prostate cancer cell line, LNCaP. Cancer Res 1996;56:1539–44.
[10] Shetty G, Wilson G, Hardy MP, Niu E, Huhtaniemi I, Meistrich ML. Inhibition of recovery of spermatogenesis in irradiated rats by different androgens. Endocrinology 2002;143:3385–96.
[11] Sundaram K, Kumar N. 7Alpha-methyl-19-nortestosterone (MENT): the optimal androgen for male contraception and replacement therapy. Int J Androl 2000;23:13–5.
[12] Joannou GE, Reeder AY. 15 beta-hydroxysteroids (Part V). Steroids of the human perinatal period: the synthesis of 3 beta, 15 beta, 17 alpha-trihydroxy-5-pregnen-20-one from 15 beta, 17 alpha-dihydroxy-4-pregnen-3,20-dione. Steroids 1996;61:18–21.
[13] Grunwell JF, Benson HD. Method for the control of fertility. US patent 4000273 1976; Chem Abstr 1977; 86:121629.
[14] Joannou GE, Reeder AY. Steroids 1996;61:18–21.
[15] Stoelwinder J, Ostendorf M, Buggenum VPAM. Process for the preparation of 7α-methylsteroids. WO patent 03/059931 2003; Chem Abstr 2003; 139:117580.
[16] Brutcher Jr FV, Bauer Jr W. Conformations of substituted cyclopentanes. II. Maximally puckered envelope and half-chair models for fused ring systems. J Am Chem Soc 1962;84:2233–6.
[17] Campbell JA, Babcock JC. The synthesis of some 7α-and 7β-methyl steroid hormones. J Am Chem Soc 1959;81:4069–81.
[18] Westermann J, Neh H, Nickisch K. Control of regioselectivity in the transition-metal-catalyzed conjugate additions of alkylaluminum compounds and methyltitaniumate complexes to androsta-1,4-diene-3,17-dione. Chem Ber 1996;129:963–6.
[19] Nicolaou KC, Zhong YL, Baran PS. A new method for the one-step synthesis of α,β-unsaturated carbonyl systems from saturated alcohols and carbonyl compounds. J Am Chem Soc 2000;122:7596–7.
[20] Hunter AC, Coyle E, Morse F, Dedi C, Dodd HT, Koussoroplis S-J. Transformation of 5-ene steroids by the fungus *Aspergillus tamari KITA*: mixed molecular fate in lactonization and hydroxylation pathways with identification of a putative 3β-hydroxy-steroid dehydrogenase/Δ^5–Δ^4 isomerase pathway. Biochim Biophys Acta 2009;1791:110–7.
[21] Martynow J, Krupa M, Les A, Kutner A, Szelejewski W. Optimization of copper(i)-catalyzed 1,6-conjugate addition of a methyl group to 17β-acetoxy-4,6-estradien-3-one. Org Proc Res Dev 2004;8:846–51.
[22] Groen MB, Zeelen FJ, Recueil J. Biomimetic total synthesis of steroids. IV. Stereoselective synthesis of 15α-methyl-19-norsteroids. Royal Neth Chem Soc 1979;98(4):239–42.
[23] Michiel J. Synthesis of 15,15-dialkylestradiols. J Chem Soc Perkin Trans 1996;1:1269–76.
[24] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New colorimetric antiproliferative activity assay for anticancer-drug screening. J Natl Cancer Inst 1990;82:1107–12.
[25] Huang XF, Lin YY, Kong LY. Steroids from the roots of *Asparagus officinalis* and their cytotoxic activity. J Integr Plant Biol 2008;50:717–22.