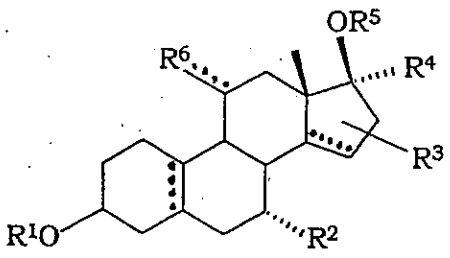
NON-AROMATIC ESTROGENIC STEROIDS WITH A HYDROCARBON SUBSTITUENT IN POSITION 11

The invention is in the field of estrogenic compounds with a steroid skeleton having a non-aromatic A-ring and a free or capped hydroxyl group at carbon atom No. 3. Estrogenic compounds have a generally recognised utility in contraception and in the treatment of estrogen-deficiency related disorders, such as menopausal complaints, and osteoporosis.

Many estrogenic compounds are known. For example, an instructive publication on estrogenic compounds with a non-aromatic A-ring and a free or capped hydroxyl group at carbon atom 3 is US patent 3,413,287. Other documents describing estrogenic or hormonal effects of non-aromatic streroids with 3-hydroxyl substitution and a 4-5 double bond are WO 94 18224, US 3,465,010, FR 2099385, US 3,652,606 and EP 145 493. A document in which non-aromatic steroids with 3-keto substitution and a 5-10 double bond are disclosed is Baran et al (US 3,377,366. Such compounds are described in general terms as agents with, among other, estrogenic or anti-estrogenic effects. Recently, in the field of drugs for estrogen receptors (ER) attention is focussed on the discovery of two distinct types of estrogen receptors, denoted ERα and ERβ (Mosselman et al., FEBS Letters 392 (1996) 49-53 as well as EP -A-0 798 378). Since these receptors have a different distribution in human tissue, the finding of compounds which possess a selective affinity for either of the two is an important technical progress, making it possible to provide a more selective treatment of estrogen-deficiency related disorders, with a lower burden of estrogen-related side-effects.

This invention provides estrogens satisfying the general formula



in which

R^1 is H, $(C_1\text{-}C_3)$alkyl or $(C_2\text{-}C_3)$acyl;

R^2 is H, α-$(C_1\text{-}C_4)$alkyl, α-$(C_2\text{-}C_4)$alkenyl or α-$(C_2\text{-}C_4)$alkynyl;

R^3 is H or $(C_1\text{-}C_4)$alkyl, $(C_2\text{-}C_4)$alkenyl or $(C_2\text{-}C_4)$alkynyl, each at location 15 or 16 of the steroid skeleton;

R^4 is H or $(C_1\text{-}C_5)$alkyl, $(C_2\text{-}C_5)$alkenyl or $(C_2\text{-}C_5)$alkynyl, each optionally substituted with halogen; preferred is ethynyl;

R^5 is H, $(C_1\text{-}C_3)$alkyl or $(C_2\text{-}C_3)$acyl;

R^6 is (C_1-C_5)alkyl, (C_2-C_5)alkenyl, (C_2-C_5)alkynyl or (C_1-C_5)alkylidene, each optionally substituted with halogen or (C_1-C_3)alkyloxy; allyl is preferred; preferred halogens in R^6 are fluorine and chlorine.

Dotted bonds represent optional double bonds. When R^6 is alkylidene, the dotted line to R^6 represents the additional bond present in an alkylidene moiety, and when R^6 is alkyl or alkenyl, the bond from atom 11 to R^6 is a single bond.

It has been found that these non-aromatic estradiol derivatives with a substituent at the 11 position of the steroid skeleton possess selective affinity for the ERα-receptor.

The compounds according to the present invention are suitable as improved estrogens, in the sense that they can be used in estrogen-related disorders, such as menopausal complaints and osteoporosis. Utility they also find in contraception, and they further may be suitable in the treatment or prevention of Alzheimer's desease, breast tumor, benign prostate hypertrophy, and cardiovascular disorders. The compounds of the invention are particularly suitable in the treatment and prevention of estrogen-deficiency related disorders under diminished estrogen-related side-effects.

In this description terms have the following meaning:

(C_1-C_5)alkyl is a branched, unbranched or cyclized alkyl group having 1-5 carbon atoms, for example methyl, ethyl, isopropyl, 2-methylcyclopropyl, butyl, sec-butyl, tert-butyl etc.;

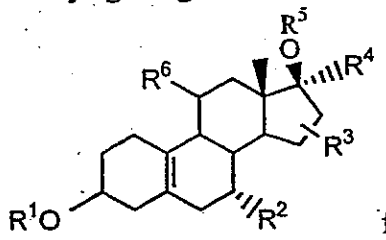
(C_2-C_5)alkenyl is a branched, unbranched or cyclized alkenyl group having 2 to 5 carbon atoms, such as ethenyl, 2-butenyl, etc.

(C_2-C_5)alkynyl is a branched or unbranched alkynyl group having 2-5 carbon atoms, such as ethynyl and propynyl.

(C_2-C_3)acyl is a group having 2-3 carbon atoms and derived from an alkylcarboxylic acid, the alkyl moiety having the meaning as defined previously.

(C_1-C_5)alkylidene is a branched, unbranched or cyclized alkylidene group having 1-5 carbon atoms, such as methylene and ethylidene.

Within the general formula given above, the compounds of the invention preferably are those satisfying the general formula II,



formula II

in which

R^1 is H, (C_1-C_3)alkyl, (C_2-C_3)acyl;
R^2 is H, α-(C_1-C_4)alkyl, α-(C_2-C_4)alkenyl, α-(C_2-C_4)alkynyl;
R^3 is H or (C_1-C_4)alkyl at location 16 of the steroid skeleton;
R^4 is ethynyl
R^5 is H or (C_1-C_3)alkyl, (C_2-C_3)acyl;
R^6 is (C_1-C_5)alkyl, (C_2-C_5)alkenyl, (C_2-C_5)alkynyl; each can be substituted with chlorine or fluorine. When R^6 is (C_1-C_2)alkyl, ethenyl or ethynyl, each optionally substituted with chlorine or fluorine, it is preferred that R^3 is methyl at location 16 of the steroid skeleton.

More preferred are the steroids of the invention in which, in the above general formula II,
R^1 is H;
R^2 is H;
R^3 is H or 16 α-methyl;
R^4 is ethynyl;
R^5 is H;
R^6 is propenyl, allyl or butenyl.

The compounds of the invention may be produced by various methods known in the art of organic chemistry in general, and especially in the art of the chemistry of steroids. See for example: Fried, J. and Edwards, J.A., *"Organic Reactions in Steroid Chemistry"*, Volumes I and II, Van Nostrand Reinhold Company, New York, 1972; and C. Djerassi, " Steroid Reactions ", Holden-Day, Inc., San Francisco, 1963.
Synthesis of steroids with particular substituents at the C7 position are e.g. available via conjugate additions of organometallic species to appropriate 4,6-diene-3-one steroids, generally producing the 7α derived steroids (along with minor amounts of 7β steroids which can easily removed via crystallisation or chromatography. Many examples of which are known from literature. Introduction of substituents at the C11 position of the steroid skeleton can be performed in several ways. Conjugate addition of organometallic species to a suitably protected 5α,10α-epoxy, 9(11)-olefin as described by Teutsch et al, Steroids 37, 361 (1981) is such an approach, but other methods, using the 11-oxo functionality of an adequately protected 19-norandrost-5-ene as a reactive functionality for functional group interconversion to e.g. a C11-aldehyde according to well known chemical methodology (see a.o. E.Ottow et al., Tetr. Lett., 5253 (1993)) may be used as well to end up with claimed compounds. Of course a combination of both approaches outlined serves similarly well to achieve the goals.
Introduction of a double bond at the 5(10) position is accomplished either by application of the so-called Birch reduction of the A ring of aromatic counterparts of suitably functionalized steroids, by dissolving metal reduction of Δ-4,5-9,11-dienones, or by ketalization of 3-keto-Δ-4,5 -steroids. This latter procedure leads either directly to the desired selective Δ-5(10)-

isomers or leads to mixtures of ketals which can be separated via chromatography or crystallization at appropriate stages of the synthesis. Careful hydrolysis of the ketal at C3 generally affords the desired 3-oxo-Δ-5(10)-isomers, which can be converted into the 3-OH compounds by hydride reductions. Saturated steroids (i.e. 5αH - derivatives) are easily available under reductive conditions like dissolving alkali metals in amines or ammonia. Introduction of double bonds at C14,15 is generally performed by firstly introducing a double bond at the C15,C16 position, followed by isomerization of this bond to the C14,C15 position according to well known procedures. The double bond introduced at C15,C16 may alternatively be used to perform a conjugate addition with e.g. cyanide, to allow the further construction of substituents at C15. The introduction of C16 substitution is easily performed by alkylation with appropriate bases and electrophiles.

The present invention also relates to a pharmaceutical composition comprising the steroid compound according to the invention mixed with a pharmaceutically acceptable auxiliary, such as described in the standard reference Gennaro et al., Remington's Pharmaceutical Sciences, (18th ed., Mack publishing Company, 1990, see especially Part 8: Pharmaceutical Preparations and Their Manufacture.). The mixture of the steroid compounds according to the invention and the pharmaceutically acceptable auxiliary may be compressed into solid dosage units, such as pills, tablets, or be processed into capsules or suppositories. By means of pharmaceutically suitable liquids the compounds can also be applied as an injection preparation in the form of a solution, suspension, emulsion, or as a spray, e.g. nasal spray. For making dosage units, e.g. tablets, the use of conventional additives such as fillers, colorants. polymeric binders and the like is contemplated. In general any pharmaceutically acceptable additive which does not interfere with the function of the active compounds can be used. The steroid compounds of the invention may also be included in an implant, a vaginal ring, a patch, a gel, and any other preparation for sustained release.

Suitable carriers with which the compositions can be administered include lactose, starch, cellulose derivatives and the like, or mixtures thereof used in suitable amounts.

Furthermore, the invention relates to the use of the steroid compound according to the invention for the manufacture of a medicament in the treatment of estrogen-deficiency related disorders such as peri- and/or post-menopausal complaints. Thus the invention also pertains to the medical indications of peri- and/or post-menopausal (climacteric) complaints and osteoporosis, i.e. a method of treatment in the field of HRT (hormone replacement therapy), comprising the administration to a patient, being a woman, of a compound as described hereinbefore (in a suitable pharmaceutical dosage form).

Further, the invention relates to the use of the steroid compound according to the invention in the manufacture of a medicament having contraceptive activity. Thus the invention also pertains to the medical indication of contraception, i.e. a method of contraception comprising the administration to a subject, being a woman or a female animal, of a progestogen and an estrogen as is customary in the field, wherein the estrogen is a compound as described hereinbefore (in a suitable pharmaceutical dosage form).

Finally the invention relates to the use of the steroid compound for the manufacture of a medicament having selective estrogenic activity, such a medicament being generally suitable in the area of HRT (hormone replacement therapy).

The dosage amounts of the present steroids will be of the normal range for estradiol derivatives, e.g. of the order of 0.01 to 10 mg per administration.

The invention is further illustrated hereinafter with reference to some unlimitative Examples and the corresponding formula schemes referred to.

EXAMPLE 1

The synthesis of compounds (3α,11β,17β)-11-(2-propenyl)-19-norpregn-5(10)-en-20-yne-3,17-diol (compound 11) and (3α,11β,16α,17β)-16-methyl-11-(2-propenyl)-19-norpregn-5(10)-en-20-yne-3,17-diol (compound 16) is described with reference to scheme 1 (next page). Compounds are referred to by numbers. The numbers refer to corresponding structural formulas in the schemes 1-7

Compound 2

To a solution of 17.3 g of CuI and 3.84 g of LiCl in 250 ml of dry THF was added at -70° C 90.6 ml of a 1M solution of allylmagnesium bromide in diethyl ether. After stirring for an additional 20 min. 11.4 ml of trimethylchlorosilane was added followed by a solution of 7.5 g of steroid 1 in 100 ml of THF. The reaction mixture was kept all the time below -60°C. After stirring for 1 h the reaction was quenched by pouring into sat. aquous NH_4Cl solution. The product was extracted with ethyl acetate and subsequently purified by column chromatography to provide 6.25 g of 2 as a colorles oil. NMR 5.20 (m, CH allyl); 5.0 (CH_2, allyl); 3.04 (m, H11).

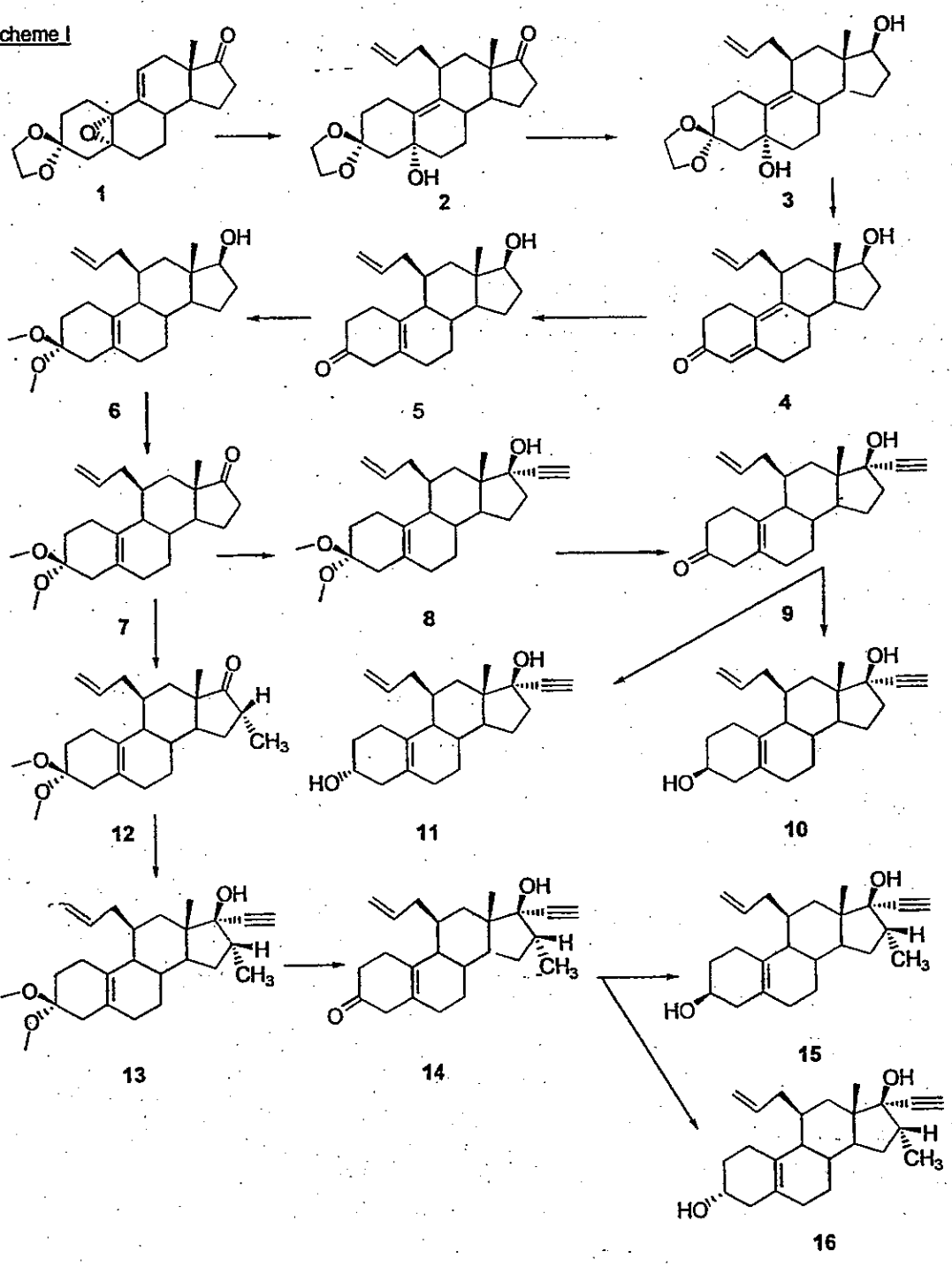
3

To a solution of 9.6 g of 2 in a mixture of 100 ml of methanol and 30 ml of methylene chloride, containing 800 mg of NaOH, was added 0.4 g of sodium borohydride at 0-

5^0C. After stirring for 1.5 h the reaction was complete and the mixture was treated with 20 ml of acetone for 0.5 h.

The reaction was then poured into water and extracted with ethyl acetate, to provide 9.5 g of 3. NMR 3.59 (t, CHOH); 2.98 (m, H11), 0.92 (s, CH_3).

Scheme I



4

To a solution of 9.5 g of 3 in 100 ml of acetone was added 8 ml of 6 N HCl. After stirring for 2 h. The mixture was neutralized with NaHCO3 and concentrated to a small volume diluted with water and extracted with ethyl acetate. This provided 8.2 g of 4 as a colorless amorphous material. NMR 5.68 (m, H4); 3.10 (m,H11); 3.65 (m, CHOH).

5

A solution of 8.2 g of 4 in 100 ml of dry THF was added to 500 ml of liq. NH3 at -70^{0}C. This mixture was treated with an amount of lithium metal (about 500 mg) until the blue color of the reaction mixture persisted for at least 15 min. The reaction was quenched by addition of a portion of NH_4Cl.

The residue which remained after evaporation of the NH_3 was diluted with water and extracted with ethyl acetate.

Chromatographic purification provided 4.0 g of 5 as a colorless oil. ; R_f 0.55 (hept./ethylacetate 1/1 v/v).

NMR 2.80 (ab, CH2 at C4); 0.93 (s, CH_3).

6

To a solution of 4.0 g of 5 in 80 ml of methanol was added 6 ml of trimethylorthoformate, followed by 0.8 g of toluenesulfonic acid. After stirring for 2 hr the ketalization was completed. The mixture was treated with 6 ml of pyridine and concentrated to a small volume. The remainders were diluted with water and extracted with ethyl acetate. The residue 4.7 g, consisted of almost pure 6 ; tlc, R_f 0.78 (hept./ethylacetate 1/1, v/v).

NMR 3.22, 3.25 (2x s, OCH_3).

7

To a solution of 33 g of 6 in 50 ml of acetone was added 6 gr of mol sieves (4A) followed by 3.2 g of N-methylmorpholine-N-oxide and 150 mg of tetrapropylammonium perruthenate. The mixture was stirred for 1 h. To the reaction mixture was added 5 g of silica gel followed by 50 ml of heptane and was stirred for an additional 5 min. The mixture was filtered over hy-flow, and after concentration in part it was taken up in ethylacetate, washed with water, and concentrated. The residue was passed over a short silica column and provided 2.9 g of 7. R_f 0.52 (heptane / ethylacetate 7/3). NMR 1.02 (s, CH_3).

8

For the ethinylation lithiumacetylide was prepared from dibromoethene and butyllithium.

To a solution of 0.74 ml of 1,2-dibromoethene in 20 ml of THF was added at -70°C 11 ml of a 1.6 M solution of BuLi in hexane. After stirring for 15 min. a solution of 800 mg of 7 in 2 ml of THF was added. The mixture was allowed to warm to room temperature in 15 min, and after an additional 15 min. at room temperature the reaction was quenched with water and the product extracted with ethyl acetate. Concentration followed by passing through a short silica gel column gave 810 mg of 8 as a white amorphous material. R_f 0.48 (heptane-ethyl acetate 7/3), R_f starting material 0.52. NMR 2.61 (s, acetylene).

9

To a suspension of 3.2 g of 8 in 60 ml of ethanol was added 0.16 g of oxalic acid in 16 ml of water. The mixture was stirred for 2.5 hr and became gradually homogeneous. The reaction mixture was treated with $NaHCO_3$ and concentrated to a small volume. Then water was added And the product was extracted with ethyl acetate. The crude product thus isolated was passed through a short silica gel column and crystallized from diisopropylether, to provide 2.3 g of 9, Mp 136°C. R_f 0.66 (heptane -ethyl acetate 1/1). NMR 2.78 (ab,2, H4); 2.61 (s, acetylene).

10, 11

To a solution of 1 g of 9 in 12 ml of THF was added 1.6 g of lithium tri-*t*-butoxy-aluminumhydride. After stirring for 1 h at room temperature the mixture was treated with water and neutralized by addition of 2N HCl. The product was extracted with ethyl acetate and chromatographed over silicagel (heptane/ ethylacetate 8/2 as eluent). This provided 0.56 g of 3β alcohol 10 (Mp 121-123 °C) and 0.24 g of 3α alcohol 11 (Mp 84-87°C).

R_f 0.53 (10) and 0.45 (11), heptane / ethylacetate 1/1. NMR (3αOH) 3.82 (m, CHOH); (3βOH) 4.08 (m, CHOH)

12

To a solution of lithium hexamethyldisilazide (prepared from 1.9 ml of 1.6M BuLi-hexane solution and 0.71 ml of hexamethyldisilazane in 4 ml of dry THF) was added at -40°C 1 g of 7 and 0.7 ml of DMPU in 5 ml of THF. The mixture was stirred for 0.5 hr at -40°C and then 225 ul of CH_3I was added by syringe. After stirring for an additional 0.5 h at -40°C the reaction was completed. The mixture was diluted with

water and extracted with ethylacetate. Chromatography of the crude product thus isolated gave 1.3 gr of 12, R_f 0.43 (heptane/ethylacetate 8/2).

13

According to the procedure described for the preparation of 8, 1.3 g of 12 were converted into the required 13, to provide 1.2 g, R_f 0.46 (heptane / ethylacetate 7/3) R_f(12) 0.55.

14

To a solution of 800 mg of 13 in 20 ml of ethanol was added a solution of 80 mg of oxalic acid in 5 ml of water. The mixture was stirred for 1 h and then neutralized by addition of $NaHCO_3$. After dilution with water and extraction with ethylacetate 0.7 g of 14 remained as crystalline material. R_f 0.47
(heptane / ethylacetate 7/3).

15,16

To a solution of 725 mg of **14** in 20 ml of a 1/1 mixture of ethanol and THF was added 130 mg of sodiumborohydride. After stirring for 1 h 2 ml of acetone were added to destroy sonme excess reagent. After 15 min the mixture was poured into water and the product was extracted with ethylacetate. The material thus obtained was purified by chromatography at silicagel, using either methylenechloride - acetone or hexane- ethylacetate as eluent. This gave 300 mg of **16** (3α-OH) and 75 mg of **15** (3β-OH). R_f **(15)** 0.47 (methylenechloride/acetone 95/5). R_f **(16)** 0.54 (methylenechloride/acetone 95/5)

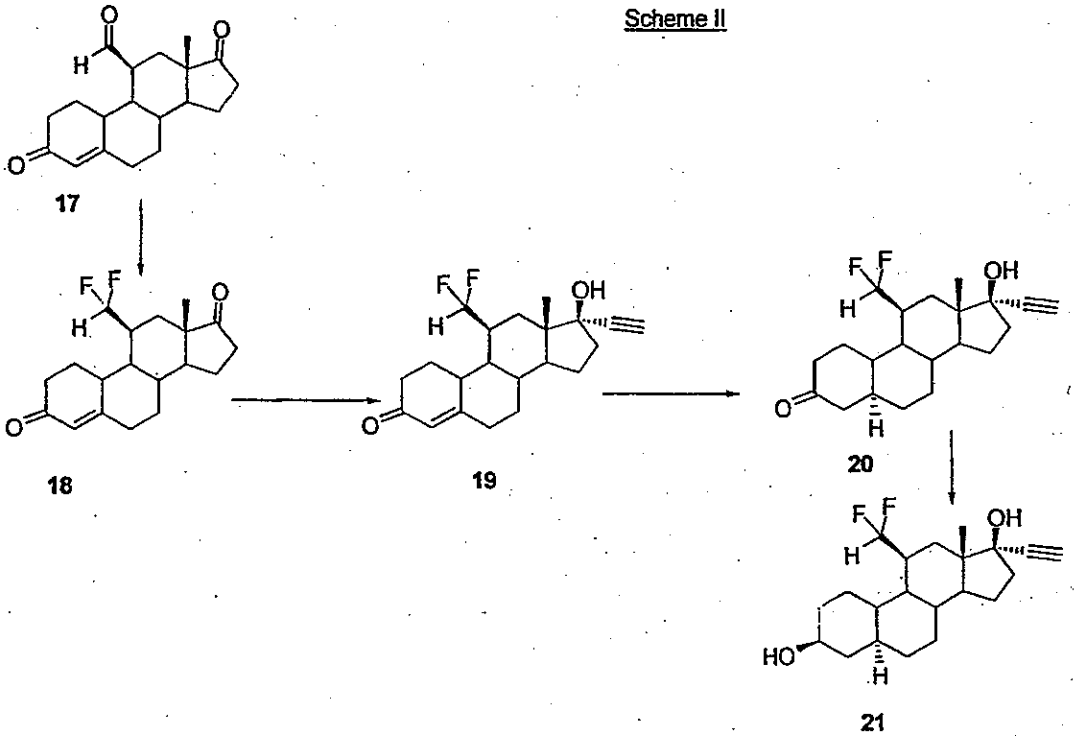
EXAMPLE 2

The synthesis of the compound (3β,5α,11β,17β)-11-difluoromethyl-19-norpregn-20-yne-3,17-diol (compound 21) is described with reference to scheme 2 (next page).

18

To a solution of 4 g of known aldehyde 17 in 200 ml of methylenechloride was added 10 ml of dimethylsulphurtrifluoride. The mixture was stirred for 48 hr at ambient temperature and then poured into ice-water and extracted with methylenechloride, followed by washing with $NaHCO_3$. After concentration and chromatography (SiO_2-heptane-ethyl acetate 2/1) 2.5 g of **18** was obtained, Mp 138-139. NMR ($CDCl_3$) 6.05 ppm double triplet of CHF_2 ;, 5.9 s, H4; 0.95 (s,CH_3).

Scheme II



19

Acetylene gas was passed for 45 min into a degassed mixture of 1.3 g of potassium-tert.butoxide and 1 g of **18** in 7 ml of THF and 2 ml of tert.butanol at 0°C. The mixture was then poured into water and extracted with ethyl acetate. After washing and concentration, the residual material wwas treated with diisopropyl ether, to yield 0.85 g of **19** as white solid. Mp 178-180. R_f 0.43 (heptane-ethylacetate 1/1).

20

To a solution of 20 mg of Li in 10 ml of liquid NH_3 was added at -60°C a solution of 300 mg of **19** in 6 ml of THF. After stirring for 1 min the mixture was treated with 0.5 g of NH_4Cl. The NH_3 was evaporated and the residue treated with water and extracted with ethylacetate. The material thus obtained was purified by chromatography, to give 140 mg of **20**, Mp 224-225, R_f 0.64 (heptane-ethylacetate 7/3).

21

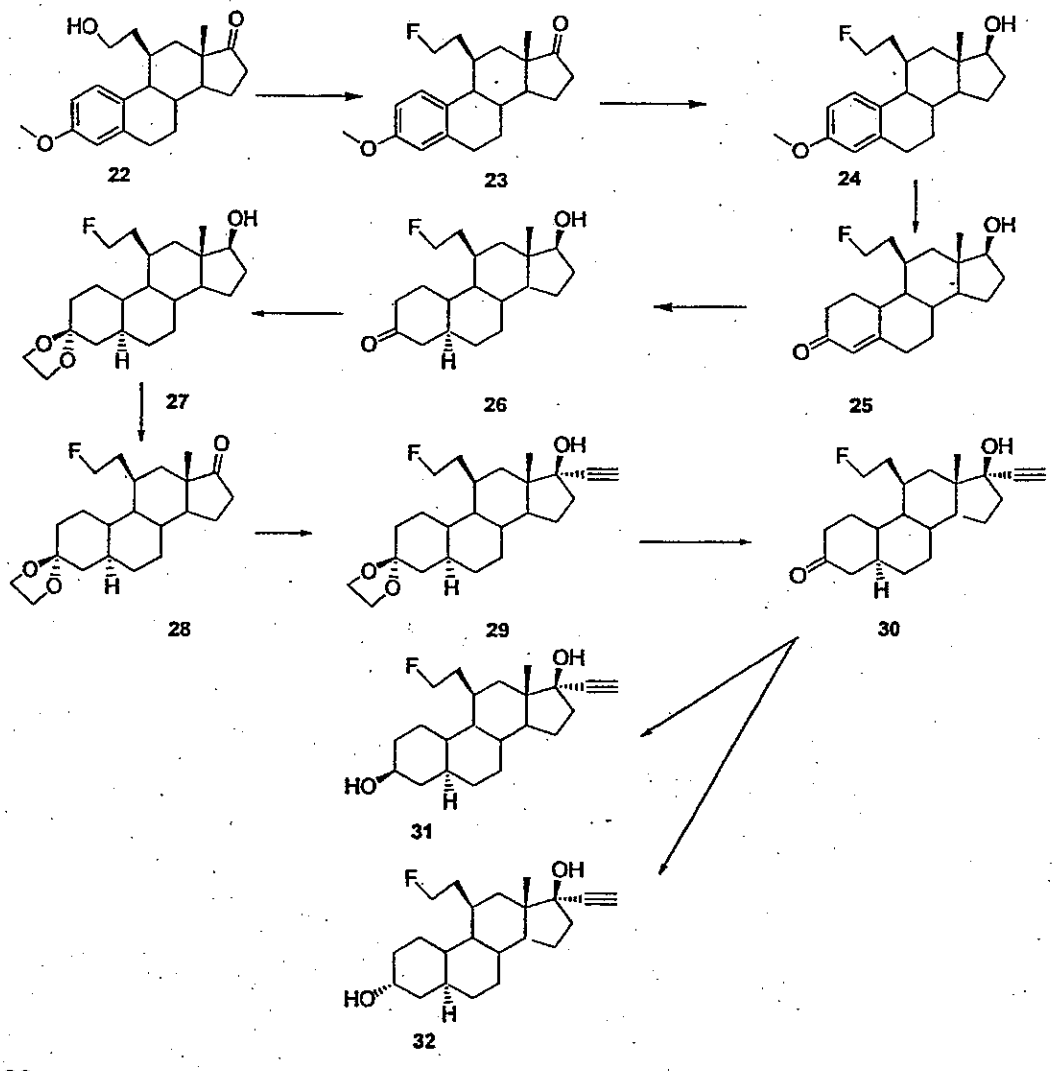
To a solution of 25 mg of $LiAlH_4$ in 4 ml of THF was added at -60°C 85 mg of **20** in 1 ml of THF. After stirring for 5 min the mixture was quickly warmed to RT and worked up by addition of 45 µl of water, 45µl of 3N NaOH solution and 140 µl of water. The precipitates were filtered and the filtrate taken up in ethylacetate and washed with 2N HCl and water. The residue which remained after drying and concentration was triturated with diisopropyl ether and gave 50 mg of **21**. Mp 168-

169°C, R_f 0.30 (toluene-ethylacetate 7/3) NMR 3.6 ppm CHOH, 2.65 CH acetylene, 6.0 double triplet CHF_2).

EXAMPLE 3

The synthesis of the compounds (3β,5α,11β,17β)-11-(2-fluoroethyl)-19-norpregn-20-yne-3,17-diol (compound 31) and (3α,5α,11β,17β)-11-(2-fluoroethyl)-19-norpregn-20-yne-3,17-diol (compound 32) is described with reference to scheme 3.

Scheme III



23

To a solution of 4.95 g of 11β-hydroxyethylestrone-3-methylether (22) in 115 ml of THF was added 66 g of mol sieves 4A, followed by 45 ml of 1M TBAF in THF (dry) and then 5.3 g of tosylfluoride. The mixture was stirred and refluxed for two hour. Then the reaction was cooled and poured onto 700 ml of 10% aq $NaHCO_3$ solution

and extracted with ethyl acetate. After concentration of the solvent and chromatography 3.4 g of 23 were isolated. R_f 0.39 (heptane/ethylacetate 7/3).

24

A solution of 4 g of 23 in a mixture of 30 ml of THF and 30 ml of methanol was treated with 0.1 ml of 4 N NaOH and then with 0.23 g of $NaBH_4$. After stirring for 1h the reaction was poured into water and extracted with ethylacetate. The crude material was filtered through a short silacolumn to provide 3.8 g of 24. R_f 0.26 (heptane/ethyl acetate 7/3) R_f 23 : 0.33.

25

To a solution of 3.8 g of 24 in a mixture of 40 ml of THF and 230 ml of liq NH_3 was added at -33°C 5 g of Li. The mixture was stirred for 5 h. Then excess lithium was destroyed by treatment with 45 ml of ethanol. Ammonia was allowed to evaporate and the residue was diluted with water and extracted with ethylacetate. Upon drying and concentration 3.7 g of crude 1,2,5(10)dienolether were obtained. This was dissolved in 30 ml of acetone, to which 3 ml of 6N HCl were added. After stirring for 3 h the mixture was neutralized with $NaHCO_3$, followed by dilution with water and extraction with ethyl acetate, to provide 2.8 g of 25. R_f 0.10 (heptane / ethyl acetate 4/6).

26

A solution of 0.84 g of 25 in a mixture of 30 ml of liq NH_3 and 6 ml of THF was treated at -60°C with small pieces of lithium until a blue color of the reaction mixture persisted for at least 5 min. Then excess reagent was destroyed by addition of a small amount of NH_4Cl and NH_3 was evaporated. The residual material was diluted with water and extracted into ethyl acetate. Concentration of the solvent left 0.80 g of essentially pure matyerial; R_f (0.39 (heptane / ethyl acetate 1/1), R_f 25, 0.24.

27

To a solution of 0.8 g of 26 in 8 ml of dichloromethane was added 2.8 ml of ethyleneglycol, 2,5 ml of triethylorthoformate and 5 mg of toluenesulfonic acid. The mixture was stirred overnight and then poured onto sat.$NaHCO_3$ solution and extracted with ethylacetate. The crude material thus obtained was purified by passing through a short silica gel column, to provide 0.72 g of 27. R_f 0.46 (heptane / ethyl acetate 1/1), R_f 26, 0.38.

28

To a solution of 0.72 g of 27 in 15 ml of acetone was added 1 gr of mol sieves (4A) followed by 0.70 g of N-methylmorpholine-N-oxide and 30 mg of tetrapropylammonium perruthenate. The mixture was stirred for 1 h. To the reaction mixture was added 1 g of silica gel followed by 15 ml of heptane and was stirred for an additional 5 min. The mixture was filtered over hy-flow, and after concentration in part it was taken up in ethylacetate, washed with water, and concentrated. The residue was passed over a short silica column and provided 0.59 g of 28. R_f 0.62 (heptane / ethylacetate 1/1).

29

For the ethinylation lithiumacetylide was prepared from dibromoethene and butyllithium.

To a solution of 0.74 ml of 1,2-dibromoethene in 20 ml of THF was added at -70°C 11 ml of a 1.6 M solution of BuLi in hexane. After stirring for 15 min. a solution of 590 mg of 28 in 2 ml of THF was added. The mixture was allowed to warm to room temperature in 15 min, and after an additional 15 min. at room temperature the reaction was quenched with water and the product extracted with ethyl acetate. Concentration followed by passing through a short silica gel column gave 430 mg of 29 as a white amorphous material. R_f 0.11 (heptane-acetone 9/1), R_f starting material 0.21.

30

To a solution of 0.43 g of 29 in 15 ml of acetone was added 1 ml of 2N HCl. The mixture was stirred for 2 h and subsequently treated with sat aq $NaHCO_3$ solution, followed by extraction with ethylacetate., to provide 0.40 g of essentially pure 30 ; R_f 0.18 (heptane/ethyl acetate 7/3) R_f 29 : 0.23.

31 / 32

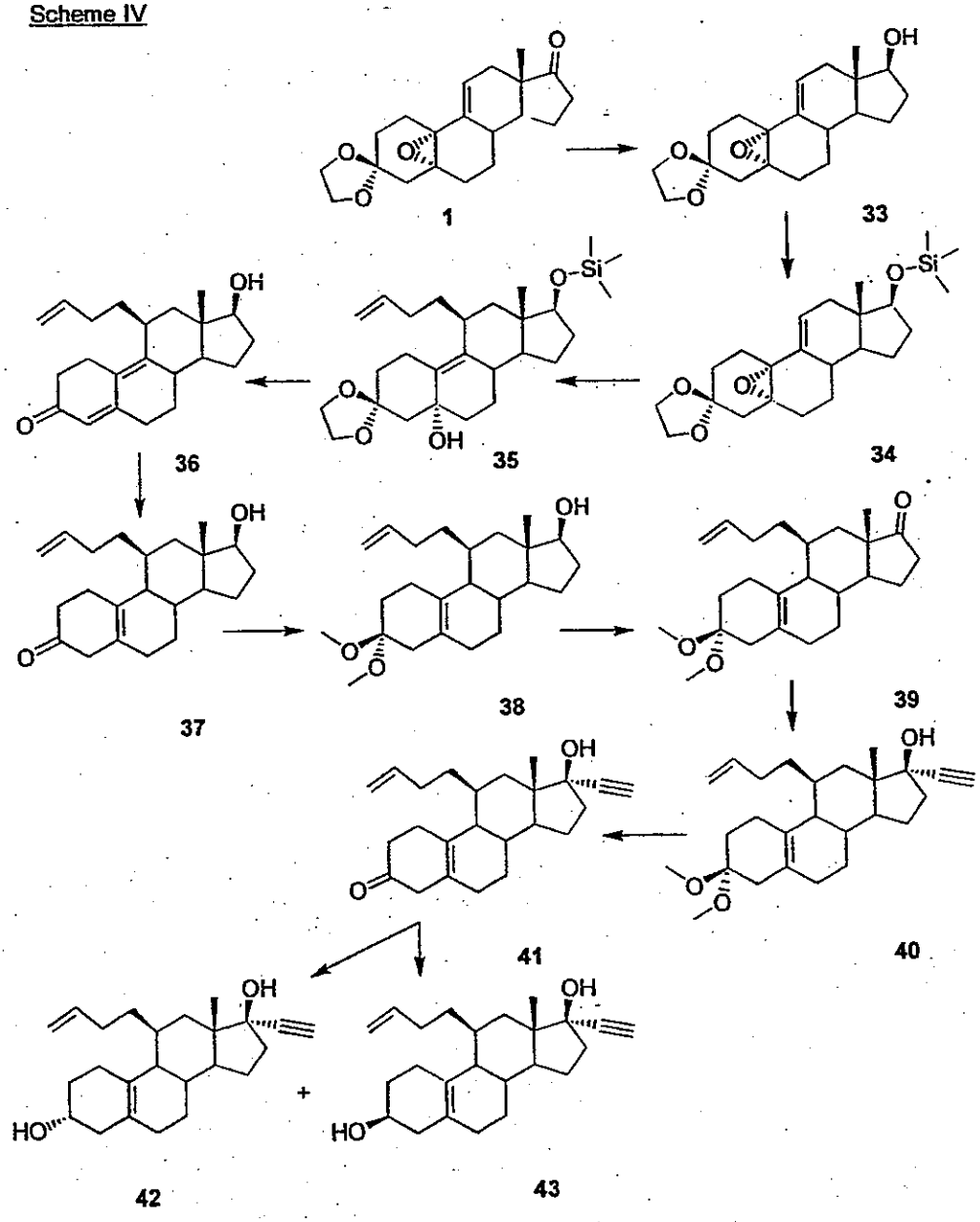
To a solution of 040 g of 30 in 4 ml of THF and 4 ml of ethanol was added 30 mg of sodium borohydride. After stirring for 0.5 h a few drops of acetone were added to decompose excess reagent. After stirring for an additional 15 min the reaction was poured onto water and extracted with ethyl acetate and the crude material thus obtained was submitted to column chromatography to provide 80 mg of 3αOH isomer 32 and 160 mg of

3βOH derivative 31. R_f 31 : 0.37, R_f 32 : 0.42, R_f starting mat. 0.48 (heptane / acetone 6/4).

EXAMPLE 4

The synthesis of the compounds (3α,11β,17β)-11-(3-butenyl)-19-norpregn-5(10)-en-20-yne-3,17-diol (compound 42) and (3β,11β,17β)-11-(3-butenyl)-19-norpregn-5(10)-en-20-yne-3,17-diol (compound 43) is described with reference to scheme 4.

Scheme IV



33

A solution of 55 g of steroid 1 in a mixture of 300 ml of THF and 300 ml of methanol was treated with a solution of 2.7 g of sodium borohydride in 30 ml of water (containing 3 mg of NaOH). After stirring for 1 h the reduction was completed and

excess hydride was destroyed by addition of 75ml of acetone. After stirring for an additional 1 h the reaction mixture was poured onto water and extracted with ethyl actetate. Concentration of the organic phase provided 54g of **33** R_f 0.31 (heptane/ethylacetate 4/6), R_f starting material 0.42.

34

To a solution of 54g of **33** in 350ml of DMF was added 35g of imidazole, followed by 37ml of trimethylsilyl chloride at -10°C. After stirring for an additional 0.5h the reaction was complete. The mixture was poured into 1.5 1 of water and extracted with ether. The residue obtained after concentration of the organic material was triturated in 80% aq. ethanol to provide 50g of pure **34** Mp 90-91°C, R_f 0.79 (heptane/ethylacetate 4/6), R_f starting material 0.36.

35

To a solution of butenylmagnesium bromide, prepared from 0.51ml of 4-bromo-1-butene and 119mg of Mg in 20ml of THF was added at –10°C 100 Mg of CuI. After stirring for 0.5h the mixture was cooled to –30°C and 1g of **34** in 5ml of THF was added. The reaction mixture was allowed to reach room temperature in about 1h. Then 100ml of sat. aq NH_4Cl solution was added followed by extraction with ethylacetate. Chromatography provided 0.9g of **35** R_f 0.54 (heptane/ethylacetate 6/4), R_f starting material 0.60.

36

To a solution of 8.65g of 35 in 80ml of acetone was added 2ml of 2N HCl. The mixture was stirred for 2h and subsequently neutralised with sat aq $NaHCO_3$ solution, concentrated to a small volume, diluted with water followed by extraction with ethylacetate. The crude material thus obtained was passed through a short silica gel column, to provide 5.3g of **36**; R_f 0.18 (heptane/ethyl acetate 7/3) R_f starting material 0.51.

37

Small pieces of lithium metal were added to a solution of 5.3g of **36** in a mixture of 340ml of liq. NH_3, 110ml of THF and 9ml of t-butanol at –70°C until the blue colour persisted (± 350mg) for 45min. Excess reagent was destroyed by addition of NH_4Cl. Ammonia gas was evaporated and the residue diluted with water and extracted with ethyl acetate. The crude material thus obtained was passed through a short silica gel column to provide 3.8g of **37**. R_f 0.46 (heptane/ethyl acetate 6/4) R_f starting material 0.22.

38

To a solution of 3.3 g of 37 in 60 ml of methanol was added 5 ml of trimethylorthoformate and 0.3 g of p-toluenesulfonic acid. After stirring at roomtemperature for 1 h the mixture was neutralized by addition of 1 ml of pyridine. The mixture was concentrated to one third of the original volume and poured into water and extracted with ethyl acetate, providing 3.7 g of 38 after chromatographic purification. R_f 0.60 (heptane/ethyl acetate 1/1) R_f starting material 0.48.

39

To a solution of 3.7 g of 38 in 45 ml of acetone was added 5 gr of mol sieves (4A) followed by 3.6 g of N-methylmorpholine-N-oxide and 100 mg of tetrapropylammonium perruthenate. The mixture was stirred for 1 h. To the reaction mixture was added 5 g of silica gel followed by 100 ml of heptane and was stirred for an additional 5 min. The mixture was filtered over hy-flow, and after concentration in part it was taken up in ethylacetate, washed with water, and concentrated. The residue was passed over a short silica column and provided 3.3 g of 39. R_f 0.51 (heptane / ethylacetate 7/3); starting material R_f 0.38.

40

For the ethinylation lithiumacetylide was prepared from dibromoethene and butyllithium.

To a solution of 0.74 ml of 1,2-dibromoethene in 20 ml of THF was added at -70^{0}C 11 ml of a 1.6 M solution of BuLi in hexane. After stirring for 15 min. a solution of 0.8 g of 39 in 2 ml of THF was added. The mixture was allowed to warm to room temperature in 15 min, and after an additional 15 min. at room temperature the reaction was quenched with water and the product extracted with ethyl acetate. Concentration followed by passing through a short silica gel column gave 0.96 g of 40 as a white amorphous material. R_f 0.15(heptane-acetone 95/5), R_f starting material 0.30

41

To a solution of 0.95 g of 40 in 20 ml of ethanol was added 0.6 g of oxalic acid in 3 ml of water. The mixture was stirred for 1.5 hr. The reaction mixture was treated with $NaHCO_3$ and concentrated to a small volume. Then water was added, and the product was extracted with ethyl acetate. The crude product thus isolated was passed through a short silica gel column, to provide 0.55 g of 41. R_f 0.33 (heptane -ethyl acetate 7/3); R_f starting material 0.40.

42/43

To a solution of 0,55g of **41** in 4ml of THF and 4ml of ethanol was added 30mg of sodium borohydride. After stirring for 0.5h a few drops of acetone were added to decompose excess reagent. After stirring for an additional 15min the reaction was poured onto water and extracted with ethyl acetate and the crude material thus obtained was submitted to column chromatography to provide 90mg of 3αOH isomer **42** Mp 159°C, and 150mg of 3βOH derivative **43**, Mp 90°C. R_f **42**: 0.31, R_f **43**: 0.23, R_f starting material 0.41 (heptane/ethyl acetate 6/4).

EXAMPLE 5

The synthesis of the compounds (3β, 7α, 11β, 17β)-11-(2-fluoroethyl)-7-methyl-19-norpregn-5(10)-en-20-yne-3,17-diol (compound 54) and (3α, 7α, 11β, 17β)-11-(2-fluoroethyl)-7-methyl-19-norpregn-5(10)-en-20-yne-3,17-diol (compound 55) is described with reference to scheme 5 (next page).

45

To a solution of 4g of **44** in 35ml of methanol was added 12ml of trimethyl orthoformate, followed by 0.3g of toluenesulfonic acid. After stirring for 1h starting material had been consumed, and the reaction mixture was neutralised by adding 1g of $NaHCO_3$ and was concentrated. The residue was diluted with water and extracted with ethylacetate. Chromatography of the crude material gave 3.4g of **45**. R_f 0.61 (heptane/ethylacetate 7/3). R_f starting material 0.35.

46

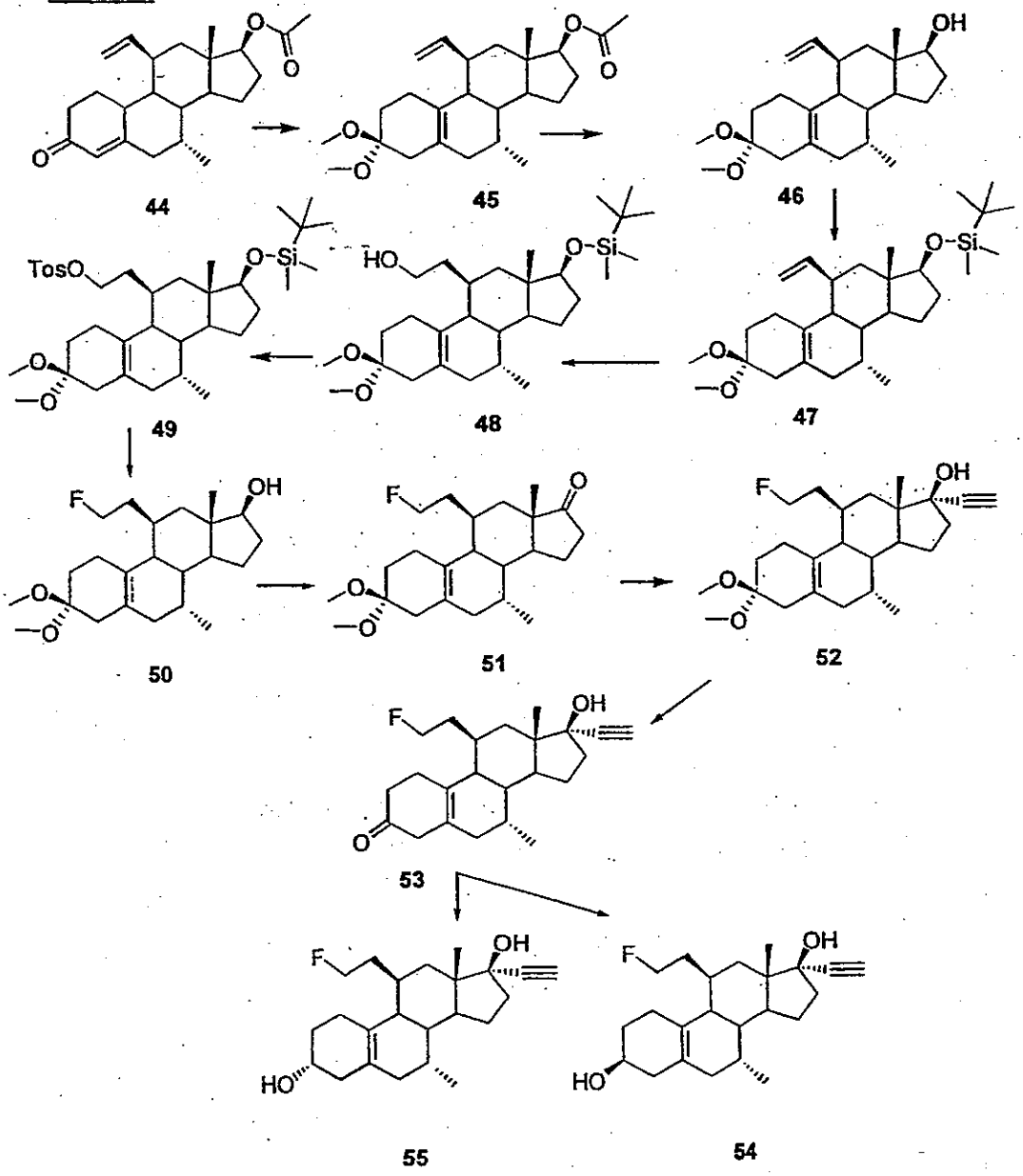
To a solution of 3.4g of **45** in 50ml of methanol was added 0.5g of NaOH. After stirring for 2h the saponification was complete. The mixture was concentrated, diluted with water and extracted with ethyl acetate, providing 3g of 46 as a colourless oil. R_f 0.31 (heptane/ethyl acetate 7/3). R_f starting material 0.65.

47

To a solution of 3.6g of **46** in 30ml of DMF was added 2.8g of imidazole, followed by 2g of tert.butyldimethylsilyl chloride. After stirring for 2h the mixture was poured into water and extracted with ethyl acetate. The crude material was passed through a short silica gel column to provide 3.8g of **47** as an oil.

R_f 0.70 (heptane/ethylacetate 7/3). R_f starting material 0.61.

Scheme V



48

A solution of 9-BBN was prepared from 1.75 ml of 1,5-cyclooctadiene and 1.4 ml of borane-dimethylsulfide complex in 40 ml of THF. To this was added 3.8 g of 47 in 5 ml of THF. The mixture was stirred for 2 h and then quenched by careful addition of water (5 ml) followed by 10 ml of 2N NaOH and 8 ml of 30% H_2O_2. After vigorous stirring for 2 h the reaction mixture was further diluted with water, extracted with ethyl acetate and washed with 10% aq. Na_2SO_3 solution. Upon concentration and chromatography 2.8 g of 48 were isolated. R_f 0.27 (heptane / ethylacetate 7/3). R_f starting material 0.70.

49

A mixture of 1g of **48** and 0.5g of tosyl chloride in 5ml of pyridine was stirred for 5h at 0-5°C. Then 1ml of water was added and stirring prolonged for 15min. The reaction mixture was further diluted with water and extracted with ethyl acetate and the crude product purified by chromatography, to provide 1.1g of **49** Mp 120°C, R_f 0.57 (heptane/ethylacetate 7/3). R_f starting material 0.30.

50

A solution of 400mg of **49** in 5ml of dry 1M TBAF in THF was stirred for 5h, leading to formation of the fluoride and concomitant cleavage of the silyl function. The mixture was poured into water and extracted with ethyl acetate. Purification by chromatography provided 185mg of **51** Mp 161-162°C, R_f 0.35(heptane/ethylacetate 7/3). R_f starting material 0.53.

51

To a solution of 180mg of **50** in 5ml of acetone was added 0.5gr of mol sieves (4A) followed by 200mg of N-methylmorpholine-N-oxide and 10mg of tetrapropylammonium perruthenate. The mixture was stirred for 1h. To the reaction mixture was added 0.5g of silica gel followed by 10ml of heptane and was stirred for an additional 5min. The mixture was filtered over hy-flow, and after concentration in part it was taken up in ethylacetate, washed with water, and concentrated to give 150mg of **51**, Mp 166°C. R_f 0.45 (heptane/ethylacetate 7/3); starting material R_f 0.35.

52

For the ethinylation lithiumacetylide was prepared from dibromoethene and butyllithium. To a solution of 0.30ml of 1,2-dibromoethene in 6ml of THF was added at –70°C 4.5ml of a 1.6M solution of BuLi in hexane. After stirring for 15min. a solution of 150mg of **51** in 1ml of THF was added. The mixture was allowed to warm to room temperature in 15min, and after an additional 15min. at room temperature the reaction was quenched with water and the product extracted with ethyl acetate. Concentration and treatment with some heptane provided 140mg of **52** as a white solid, Mp 168°C. R_f 0.38(heptane-acetone 95/5), R_f starting material 0.40.

53

To a solution of 145mg of **52** in 3ml of ethanol and 1.5ml of THF was added 0.2g of oxalic acid in 3ml of water. The mixture was stirred for 1.5hr. The reaction mixture was treated with $NaHCO_3$ and concentrated to a small volume. Then water

was added, and the product was extracted with ethyl acetate. The crude product thus isolated was passed through a short silica gel column, to provide 125 mg of **53**. R_f 0.23 (heptane -ethyl acetate 7/3); R_f starting material 0.38.

54/55

To a solution of 125 mg of **53** in 2 ml of THF and 1 ml of ethanol was added 20 mg of sodium borohydride. The reduction was complete after 0.5 h. The mixture was diluted with water and extracted with ethyl acetate. The crude product thus isolated was purified by passing through a reversed phase C-18 column, using acetonitrile-water as eluent, to provide 40 mg of 3αOH **55** and 20 mg of 3βOH **54**, both as amorphous materials with identical R_f values on silicagel ; R_f 0.43 (CH_2Cl_2/acetone 9/1), R_f starting material 0.70.

EXAMPLE 6

The compounds in this example was prepared according to scheme VI (next page)

57

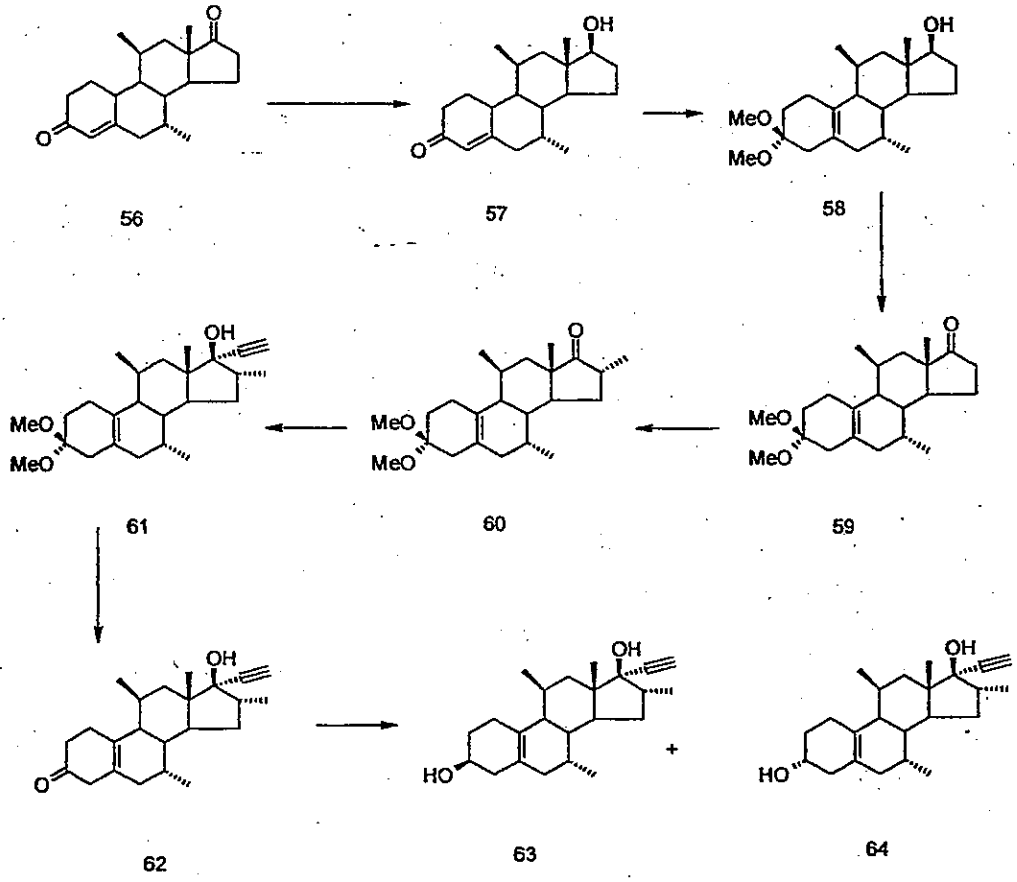
A solution of 80 mg of sodium borohydride and 112 mg of sodium hydroxide in 12 ml of methanol was added dropwise at 0°C to a solution of 7α,11β-dimethylestr-4-ene-3,17-dione (**56**) in a mixture of 18 ml of methanol and 4 ml of methylenedichloride

After stirring for one hour excess borohydride was destroyed by addition of 12 ml of acetone and stirring for an additional 15 min. The mixture was poured into water and extracted with ethyl acetate. The combined organic phases were once washed with sat NaCl solution and dried, concentrated and purified over a silica column, to provide 0.74 g of **57**, contaminated with some 3,17-diol. (The latter one was removed in the next step); R_f 0.40 (hept./ethyl ac. 6/4).NMR (CDCl3) δ 3.60 (t, 1, 17αH), 0.77 (d, 3, 7αCH3), 1.08 (d, 3, 11βCH_3).

58

To a solution of 0.74 g of **57** in 15 ml of methanol and 1.1 ml of trimethylorthoformate was added 0.2 g of p-toluenesulphonic acid. The mixture was stirred for 2 h. Then 0.1 ml of pyridine was added and poured onto water. The product was extracted with ethyl acetate and purified by chromatography over silica, using heptane /ethyl acetate as eluent, to provide 0.59 g of 3,3-dimethylketal **58**, R_f 0.47 (hept / ethylac. 6/4); NMR δ 3.20 (2 s, 6, OCH_3), 3.65 (t, 1, 17αH),0.86 (s, 3, 18CH_3), 0.77 (d, 3, 7αCH_3), 0.90 (d, 3, 11βCH_3).

Scheme VI



59

To a solution of 0.59 g of steroid **58** in 10 ml of acetone were added successively 0.6 g of N-methylmorpholine-N-oxide and 40 mg of tetrapropylammonium perruthenate. After stirring for 1 h 10 ml of heptane and 1 g of silica were added. The mixture was stirred for an additional 15 min and then filtered over Celite, concentrated and the remainders passed over a short silica column, to provide 0.56 g of **59**; R_f 0.55 (hept./ ethyl ac. 6/4); NMR δ 0.98 (s, 3, 18 CH_3), 0.82 (d, 3, 7αCH_3), 0.92 (d, 3, 11βCH_3), 3.20 (2xs, 6, OCH_3).

60

Lithium hexamethyldisilazide was prepared by addition of 1.24 ml of 1.55 M BuLi-hexane solution at –40°C to a solution of 0.44 ml of bistrimethylsilylamine in 4 ml of THF. After stirring for ½ h a solution of 0.56 g of **59** and 0.46 ml of DMPU in 8 ml of THF was added. Stirring was continued for 45 min at –40 °C followed by addition of 140 µl of methyliodide. Upon stirring for an additional 30 min the alkylation was complete. The mixture was poured into 40 ml of sat NH_4Cl solution and extracted with ethyl acetate. Chromatographic purification provided 0.55 g of 16α-methylated product **60.** R_f 0.73 (hept/ethyl ac. 1/1)starting material R_f 0.69; NMR δ 1.11 (d, 3, 16αCH_3), 1.02 (s, 3, 18CH_3), 0.82 (d, 3, 7αCH_3), 0.91 (d, 3, 11βCH_3),

61

Lithium acetyilde was prepared by addition of a solution of 9.5ml of 1.55M BuLi-hexane to 0.60ml of 1,2-dibromoethane in 20ml of THF at –60°C. After stirring for ½h, a solution of 0.55g of ketone **60** in 2ml of THF was added and the cooling device was removed so as to allow the mixture to gradually warm to room temperature. Then water was added and the product was extracted with ethylacetate. After chromatographic purification 0.50g of **61** was obtained; R_f 0.22 (hept/ethylac. 9/1) starting material R_f 0.42; NMR δ 1.18 (d, 3, 16αCH_3), 1.02 (s, 3, 18CH_3), 0.78 (d, 3, 7αCH_3), 0.94 (d, 3, 11βCH_3), 2.66 (s,1, acetylene).

62

To a solution of 0.49g of **61** in 20ml of ethanol was added 50mg of oxalic acid in 3ml of water. The mixture was stirred for 4hr. The reaction mixture was treated with 5% aq. sodium bicarbonate solution and the product extracted with ethyl acetate and passed through a silica column, to remove some impurities, yielding 250mg of **62** ; R_f 0.40 (hept/ethyl ac. 6/4), starting mat. R_f 0.49; NMR δ 2.80 (m, 2, H4), 1.19 (d, 3, 16αCH_3), 1.04 (s, 3, 18CH_3), 0.82 (d, 3, 7αCH_3), 0.93(d, 3, 11βCH_3), 2.67 (s, 1, acetylene).

63/64

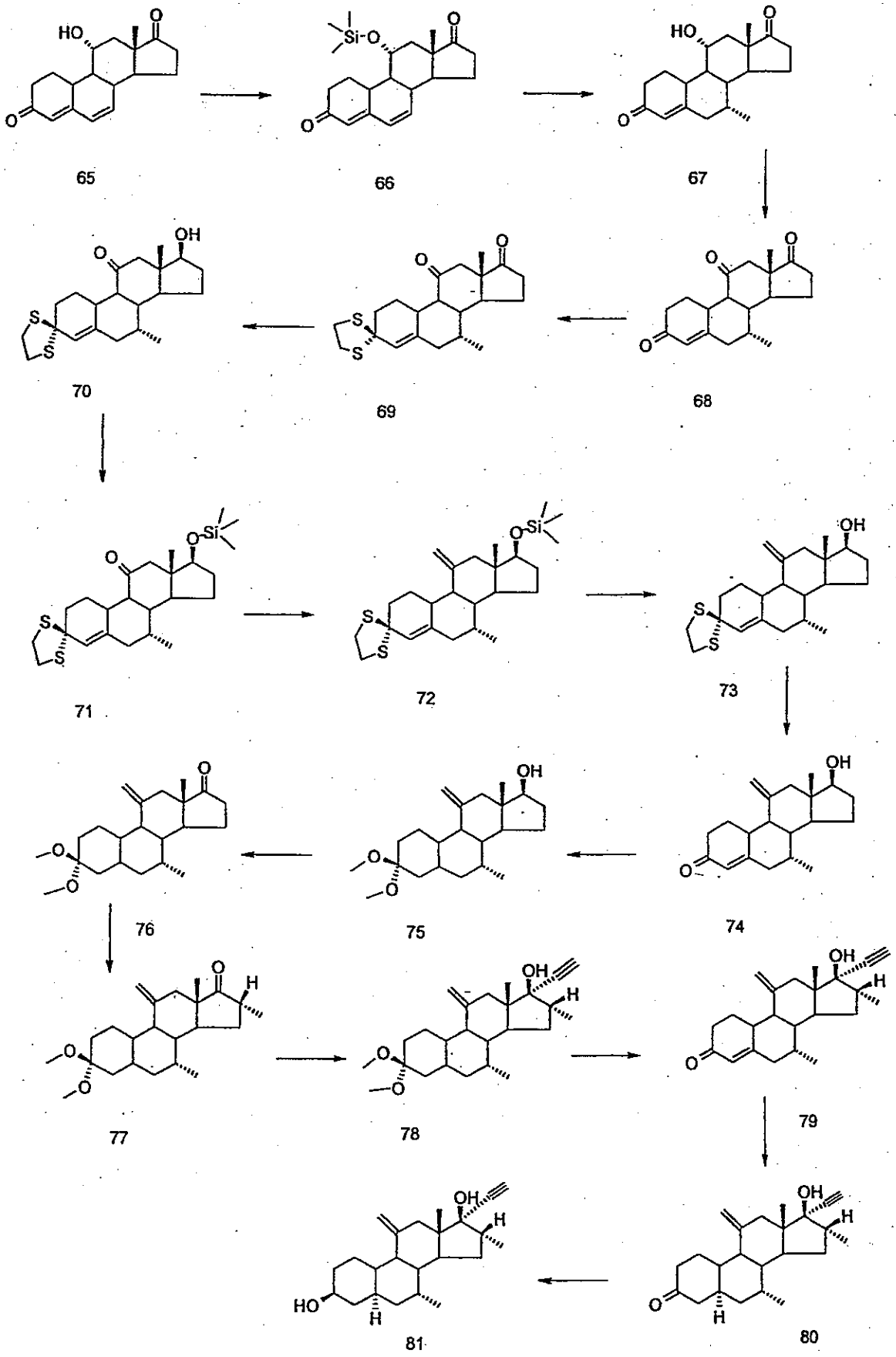
To a solution of 240mg of **62** in 5ml of THF was added 360mg of lithium(tert-butoxy)$_3$AlH. The mixture was stirred for 1h, and then poured onto water and the product extracted with ethyl acetate. The isomeric alcohols thus obtained were separated by means of preparative hplc (reversed phase C18) using a gradient of acetonitrile/water. The products obtained after concentration of the eluent were crystallised from water-ethanol. This gave 68mg of the 3α-hydroxy derivative **64**, and 70mg of the 3β-isomer **63**, R_f (63/64) 0.36 (hept/ethyl acetate 6/4), starting material R_f 0.54. Mp **(63)** 165-167°C, Mp **(64)** 171-172°C. NMR **(64)** δ 3.80 (m,1,H13), 2.67 (s,1,acetylene),), 1.17 (d, 3, 16αCH_3), 1.02 (s, 3, 18 CH_3), 0.77 (d, 3, 7αCH_3), 0.90(d, 3, 11βCH_3).

NMR **(63)** δ 4.05 (m,1,H3), 2.67 (s,1,acetylene),), 1.18 (d, 3, 16α-CH_3), 1.02 (s, 3, 18 CH_3), 0.77 (d, 3, 7αCH_3), 0.93(d, 3, 11βCH_3).

EXAMPLE 7

The compound in this example was prepared according to scheme VII (Next page):

Scheme VII



66

To a solution of 10g of 11α-hydroxynordienedione **(65)** in 45ml of DMF was added 10g of imidazole, followed at 0°C by 8.6ml of trimethylsilyl chloride. After stirring for 1hr the mixture was poured into ice water and the product was extracted with ethyl acetate. The crude material was chromatographed and then triturated with heptane, to provide 7.8g of 11-trimethylsilyloxy derivative **66**, Mp 89-90°C, NMR δ 4.08 (m, 1, 11αH), 5.80 (s,1H, H4), 6.25 (m, 2, H6,7)., 0.15 (s, 9, trimethylsilyl).

67

To a solution of 5.3g of **66** and 500mg of $Cu(Oac)_2$ in 20ml of THF was added at –30°C dropwise 9.8ml of 1M methylinagnesiuin chloride in THF. The mixture was stirred for 1h and then poured into a solution of 6ml of conc. sulphuric acid in 300ml of water and stirred overnight. The product was extracted with ethyl acetate, dried and concentrated, providing 4.8g of solid material, pure enough for further reaction. NMR δ 5.85 (s, 1, H4), 3.92 (m, 1, H11α), 0.83 (d, 3, 7αCH_3), 0.95 (s, 3, 18CH_3); R_f 0.22 (hept/ethyl ac. 7/3) starting material R_f 0.65.

68

To a solution of 4.8g of **67** and 6.6g of NMO in 100ml of acetone was added 130mg of tetrapropylaminonium perruthenate. After stirring for 2h 2g of silica gel, followed by 100ml heptane were added. The mixture was stirred for an additional ½h and filtered over Celite. The filtrate was concentrated and the residue treated with diisopropyl ether, to provide 4g of essentially pure trione **68**. NMR δ 5.89 (s,1,H4), 0.89 (s,3, 18CH_3), 0.92 (d, 3, 7αCH_3) ; R_f 0.46 (hept./ethyl ac. 7/3) starting material R_f 0.24.

69

A mixture of 3.5g of steroid **68**, 1.3ml of ethanedithiol, 300mg of p-toluenesulfonic acid and 35ml of ethanol was refluxed for 1h. The reaction was cooled to room temperature and 80ml of cold ½N NaOH was added. After stirring for 1h the product was filtered and washed with cold water. After drying in vacuo at 50°C 4.6g of **69** were obtained ; NMR δ 5.66 (s, 1, H4), 3.22 and 3.38 (m, 4, thioketal) 0.85 (s, 3, 18CH_3), 0.88 (d, 3, 7αCH_3).; R_f 0.88 (toluene/ac 7/3) starting mat. R_f 0.59.

70

To a solution of 4.0g of **69** in a mixture of 100m of methanol and 50ml of methylene chloride was added at –20°C 450mg of $NaBH_4$ in small portions. After stirring for an

additional 1 h the reduction was complete and the reaction was treated with 5 ml of acetone and then concentrated to a small volume and diluted with 100 ml of water and extracted with methylene chloride. After drying and concentration 3.9 g of essentially pure **70** was obtained; NMR δ 3.90 (m, 1, 17αH), 0.82 (d, 3, 7αCH_3), 0.73 (s, 3, 18CH_3); Rf 0.39 (hept./ethyl ac. 1/1) starting material R_f 0.65.

71

A solution of 2.5 ml of trimethylsilyl chloride in 20 ml of ether was added dropwise to a mixture of 4.7 g of **70** and 2.6 g of imidazole in 50 ml of DMF at 0°C. After stirring for 1 h the reaction was diluted with ice water and the product extracted with ethyl acetate. After drying and concentration of the organic phase the residue was triturated with 80%aq. ethanol at an ice bath and filtered,and dried at 50°C in vacuo, to provide 5.2 g of **71**; NMR δ 3.76 (t, 1, 17αH), 0.65 (s, 3, 18CH_3), 0.80 (d, 3, 7αCH_3), 5.60 (s, 1, H4); R_f 0.86 (hept./ethyl ac. 1/1) starting material R_f 0.37.

73

A suspension of 4.4 g of potassium tert-butoxide and 16.2 g of methyltriphenylphosphonium bromide in 130 ml of toluene was heated at 100 °C for ½ hr under nitrogen atmosphere. The yellow mixture was cooled to 50 °C and a solution of 4.6 g of steroid **71** in 20 ml of toluene was added. The reaction was stirred for another 1 h at 100°C, cooled and poured into 500 ml of ice water. The product was extracted with toluene and washed, dried and concentrated. The residue was chromatographed over toluene/silica gel to remove most of the reagent contamination. The crude **72** thus obtained (6.3 g) was dissolved in 20 ml of THF and a solution of 18 ml of 1M TBAF in THF was added. After stirring ~~for~~ ½ h the mixture was diluted with water and extracted with ethyl acetate. The product thus obtained was purified by chromatography, providing 4.7 g of **73**, Mp 177-180 ; NMR δ 4.77 and 4.86 (AB, 2, methylene H's) 5.60 (s, 1, H4), 3.72 (m, 1, 17αH)0.70 (s, 3, 18CH_3), 0.77 (d, 3, 7α-CH_3); R_f 0.10 (hept./ethyl ac. 9/1) starting material R_f 0.73.

74

A solution of 2.45 g of periodic acid in a mixture of 12 ml of water and 12 ml of methanol was added to a solution of 4.7 g of **73** in 40 ml of methylene chloride. The mixture was stirred for 45 min. and then diluted with water. The product was extracted with methylene chloride. The organic layer was washed several times with 5%aq.sodium thiosulphate solution and water and then dried and concentrated. The residue was purified by column chromatography over silica gel to provide 2.6 g of **74** ; NMR δ 0.73 (s, 3, 18CH3), 0.79 (d, 3, 7αCH3), 5.88

(s, 1, H4), 4.83, 4.94 (AB, 2, methylene H's), 3.78 (m, 1, 17αH); R_f 0.25 (hept./ ethyl ac. 6/4) starting mat. R_f 0.48.

75

A mixture of 2.6 g of 74, 7 ml of trimethylorthoformate, 480 mg of p-toluenesulphonic acid and 50 ml of methanol was stirred at room temperature while monitoring the reaction by tlc. After 2.5 h the reaction was poured onto sat. aq. $NaHCO_3$ and extracted with ethyl acetate. After drying and concentration in vacuo 3.1 g of a 1/1 mixture of Δ5,6 and Δ5(10) ketal was obtained; R_f 0.46 (hept./ ethyl ac. 6/4) starting material R_f 0.25 ; NMR(1/1 mixture of ketals) δ 3.15, 3.22, 3.24 (singulets, 6, signals of OCH_3), 0.77, 0.87 (2x d, 3, 7αCH_3), 0.65, 0.71 (2x s, 3, 18CH_3).

76

The crude product 75 was dissolved in 60 ml of acetone. To this were added 3.7 g of N-methylmorpholine-N-oxide and 75 mg of tetrapropylammonium perruthenate. The mixture was stirred for 2 h. then 1 g of silicagel and 60 ml of heptane were added. Upon stirring for 15 min. the reaction was filtered over Celite and the filtrate concentrated to dryness. The residue was pased through a short silica column and provided 1.9 g of the ketone 76 as a mixture of Δ5(10) and Δ5,6 ketals; NMR typical signals at δ 0.82, 0.93 (2xd, 3, 7αCH_3), 0.79, 0.85 (2xs, 3, 18CH_3), 3.15, 3.22, 3.25 (s, 6, signals of OCH_3), 4.70,4.85 and 4.87 and 4.93 (2x AB, 2, methylene H's); R_f 0.58 (hept./ ethyl ac. 6/4), starting material R_f 0.45.

77

A solution of 500 mg of 76 in 10 ml of THF and 0.4 ml of DMPU was added dropwise at –40°C to a solution of 1.6 ml of 1M Li-hexamethyldisilazide in 10 ml of THF. After stirring for an additional 45 min, 120 µl of methyliodide was added. Stirring was continued for 1 h at –20°C and then the reaction was poured into water and the product extracted with ethyl acetate. The material obtained after washing, drying and concentration of the organic phase was passed through a silica column, and provided 510 mg of 16α-methyl derivative 77, as a mixture of double bond isomers; R_f 0.56 (hept./ ethyl ac. 7/3) starting material R_f 0.48. NMR δ 1.15, 1.17 (2x d, 16αCH_3).

78

Li-acetylide was generated by dropwise addition of 7.1 ml of 1.6M BuLi-hexane to 0.46 ml of 1,2-dibromoethene in 10 ml of THF at –60°C. After stirring for ½ h a solution of 500 mg of steroid 77 in 10 ml of THF was added and the reaction mixture was stirred for ½ h while

allowing the temperature rise to room temperature during this period. Water was then added and the product extracted with ethyl acetate. The material thus obtained was passed through a short silica column, and provided 540mg of **78** as double bond isomer mixture. NMR δ 2.74 and 2.76 (2x s, 1, acetylene), 1.17, 1.19 (2xd, 3, 16αCH_3), 0.78, 0.88 (2x d, 3, 7αCH_3); R_f 0.50 (hept./ethyl ac. 7/3), starting material R_f 0.60.

79

To a solution of 440mg of **78** in 20ml of acetone was added 4ml of 4N HCl. After stirring for 1h the reaction was complete, and the mixture was poured into sat. aq. $NaHCO_3$ and extracted with ethyl acetate. Upon washing, drying and concentration, 380mg of essentially pure **79** were obtained, directly used in the next step; NMR δ 5.88 (s, 1, H-4), 5.90 (AB, 2, methylene), 2.76 (s, 1, acetylene), 1.18 (d. 3, 16αCH_3), 0.88(s, 3, 18-CH_3), 0.79 (d, 3, 7αCH_3).

80

To a solution of 280mg of **79** in a mixture of 30ml of liq. NH_3 and 10ml of THF at –40°C were added small pieces of Li foil until the blue colour persisted for 15min. Then the small excess of Li was quickly quenched by addition of solid NH_4Cl and ammonia was allowed to evaporate. To the residual material was added 100ml of water and the mixture was extracted with ethyl acetate. The organic material isolated after washing, drying and concentration contained almost pure **80**; R_f 0.57 (hept./ethyl ac. 6/4), NMR δ 4.78, 4.88 (AB, 2, methylene), 2.66 (s,1, acetylene), 1.19 (d, 3, l6αCH_3), 0.86 (s, 3, 18CH_3), 0.90 (d, 3, 7αCH_3).

81

To a solution of 160mg of **80** in 10ml of THF were added at 0°C small portions of $LiAlH_4$ until the reduction was completed. Then 0.1ml of sat. aq. Na_2SO_4 was added followed by some solid Na_2SO_4 The mixture was stirred for 15min and then filtered over Celite. The filtrate was concentrated and the residue purified by passing through a preparative HPLC column, packed with reversed phase C- 18 silica, using acetonitrile-water as eluent, to provide 55mg of **81**, Mp 198-199°C; R_f 0.33 (hept./ethyl ac. 6/4) starting material 0.57. NMR δ 3.67 (m, 1, 3αH), 4.70, 4.82 (AB, 2, methylene) 2.65 (s, 1, acetylene),), 1.18 d, 3, 16αCH_3), 0.83 (s, 3, 18CH_3), 0.89 (d, 3, 7αCH_3).

EXAMPLE 8

Compounds are tested for their estrogen receptor activity in a binding assay and in a transactivation assay.

Determination of competitive binding to cytoplasmic human estrogen receptor from rec.CHO cells is used to estimate the relative affinity (potency ratio) of a test compound for estrogen receptors present in the cytosol of recombinant Chinese hamster ovary (CHO) cells, stably transfected with the human estrogen receptor, as compared with estradiol (E_2). Cytosol prepared from recombinant CHO cells, stably transfected with the human estrogen receptor. The cell line has been made within the Department of Biotechnology and Biochemistry (BBC) (N.V. Organon) and is known under the name CHO-ER (2B1). Reference compounds are ethinylestradiol and estriol.

The antiestrogenic activity of compounds is determined in an in vitro bioassay with recombinant Chinese hamster ovary (CHO) cells stably co-transfected with the human estrogen receptor α (hERα) or β receptor (hERβ), the rat oxytocin promoter (RO) and the luciferase reporter gene (LUC). The antiestrogenic activity (potency ratio) of a test compound to inhibit the transactivation of the enzyme luciferase mediated via the estrogen receptor by the estrogen Org 2317 (estradiol, 1, 3, 5 (10)-estratriene-3, 17ß-diol) is compared with the standard Org 34790 (ICI 164.384; (7α,17ß)-N-butyl-3,17-dihydroxy-N-methylestra-1,3,5(10)-triene-7-undecanamide).

Test medium: Intact recombinant CHO cells stably co-transfected with the human estrogen receptor, the rat oxytocin promoter and the luciferase reporter gene. The cell line has been produced within the Department of Biotechnology and Biochemistry (BBC) (N.V. Organon) and is known under the name CHO-ERRO 2B1-1E9.

The results are presented in the Table below.

The data are expressed in percentage of the action of the reference compound in the assay

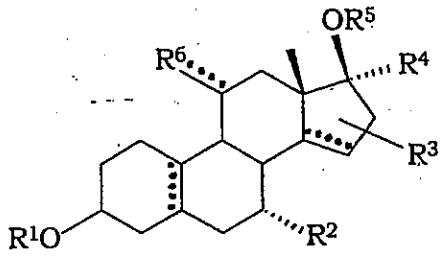
TABLE

Compound	α-binding.	α-transactivation	β-binding	β-transactivation
11	57	58	1.1	0.8
15	35	27	0.3	0.3
21	8.6	7.7	0.2	0.1
31	37	9.6	0.1	0.2
32	2,6	2.4	<0.1	0.1
42	29	2	0.1	<0.04
43	11.9	1.3	0.1	<0.1
54	27,5	29.7	0.1	1.0
55	36	61.5	0.1	1.9
63	25	22	0.4	0.1
64	35	29	0.2	0.2
81	n.t.r.	27	n.t.r.	0.1

n.t.r.: No test results available

Claims:

1. A non-aromatic estrogenic steroid satisfying the general formula I,



Formula 1

in which

R^1 is H, (C_1-C_3)alkyl or (C_2-C_3)acyl;

R^2 is H, α-(C_1-C_4)alkyl, α-(C_2-C_4)alkenyl or α-(C_2-C_4)alkynyl;

R^3 is H or (C_1-C_4)alkyl, (C_2-C_4)alkenyl or (C_2-C_4)alkynyl, each at location 15 or 16 of the steroid skeleton;

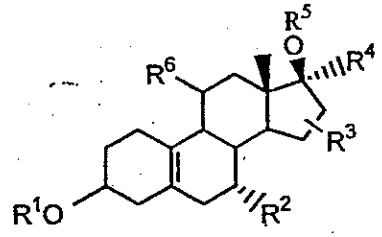
R^4 is H or (C_1-C_5)alkyl, (C_2-C_5)alkenyl or (C_2-C_5)alkynyl, each optionally substituted with halogen; preferred is ethynyl;

R^5 is H, (C_1-C_3)alkyl or (C_2-C_3)acyl;

R^6 is (C_1-C_5)alkyl, (C_2-C_5)alkenyl, (C_2-C_5)alkynyl or (C_1-C_5)alkylidene, each optionally substituted with halogen or (C_1-C_3)alkyloxy;

dotted bonds represent optional double bonds.

2. A non-aromatic estrogenic steroid according to claim 1, characterised in that it satisfies the general formula II,



formula II

in which

R^1 is H, (C_1-C_3)alkyl, (C_2-C_3)acyl;

R^2 is H, α-(C_1-C_4)alkyl, α-(C_2-C_4)alkenyl, α-(C_2-C_4)alkynyl;

R^3 is H or (C_1-C_4)alkyl at location 16 of the steroid skeleton;

R^4 is ethynyl

R^5 is H or (C_1-C_3)alkyl, (C_2-C_3)acyl;

R^6 is (C_1-C_5)alkyl, (C_2-C_5)alkenyl, (C_2-C_5)alkynyl; each can be substituted with chlorine or fluorine.

3. A non-aromatic estrogenic steroid according to claim 2, characterised in that
R^1 is H;
R^2 is H;
R^3 is H, 16 α-methyl, or 16 α-ethyl;
R^4 is ethynyl;
R^5 is H;
R^6 is propenyl, allyl or butenyl.

4. A pharmaceutical composition comprising a steroid compound according to any one of the preceding claims, and pharmaceutically acceptable auxiliaries.

5. The use of a steroid compound according to any one of claims 1-4 for the manufacture of a medicine in the treatment of estrogen-deficiency dependent disorders.