PHARMACEUTICALLY ACCEPTABLE SALTS OF 5ALPHA-PREGNAN-3BETA, 16ALP HA-DIOL-20-ONE 3-SULFATE USEFUL AS PROGESTINS AND CNS DISORDERS

BACKGROUND OF THE INVENTION

The use of naturally occurring estrogenic compositions of substantial purity and low toxicity such as PREMARIN (conjugated equine estrogens) has become a preferred medical treatment for alleviating the symptoms of menopausal syndrome, osteoporosis/osteopenia in estrogen deficient women and in other hormone related disorders. The estrogenic components of the naturally occurring estrogenic compositions have been generally identified as sulfate esters of estrone, equilin, equilenin, 17-β-estradiol, dihydroequilenin and 17-β-dihydroequilenin (U.S. Patent 2,834,712). The estrogenic compositions are usually buffered or stabilized with alkali metal salts of organic or inorganic acids at a substantially neutral pH of about 6.5 to 7.5. Urea has also been used as a stabilizer (U.S. 3,608,077). The incorporation of antioxidants to stabilize synthetic conjugated estrogens and the failure of pH control with tris(hydroxymethyl)aminomethane (TRIS) to prevent hydrolysis is discussed in U.S. 4,154,820.

One of the compounds described herein, 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester sodium salt is a minor component of PREMARIN (conjugated equine estrogens).

DESCRIPTION OF THE INVENTION

In accordance with this invention, there is provided a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3 sulfate ester which is useful as a progestational agent. The structure of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester sodium salt is shown as compound (5) in Scheme I.

Pharmaceutically acceptable salts of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester include, but are not limited to, the alkali metal salts, alkaline earth metal salts, ammonium salts, alkylammonium salts containing 1-6 carbon atoms or dialkylammonium salts containing 1-6 carbon atoms in each alkyl group, trialkylammonium salts containing 1-6 carbon atoms in each alkyl group and tetraalkylammonium salts containing 1-6 carbon atoms in each alkyl group.

Alkali metal salts include sodium and potassium salts, particularly preferred are sodium salts. Alkaline earth metal salts include calcium and magnesium salts. Suitable alkyl groups include methyl, ethyl, propyl, butyl, pentyl and hexyl, preferred alkyl groups being methyl and ethyl. Where more than one alkyl group is present the groups may be the same or different. Preferred trialkylammonium salts are trimethylammonium salts and triethylammonium salts.

The salts of the invention are preferably in greater than 1 percent purity.

As 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester sodium salt is a minor component of PREMARIN (conjugated equine estrogens), this invention also provides 5α -pregnan-3β, 16α–diol-20-one 3 sulfate ester sodium salt, preferably in greater than 1 percent purity.

This invention also provides a compound consisting essentially of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester sodium salt, and a compound consisting essentially of a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3 sulfate ester.

This invention further provides a method of using 5α-pregnan-3β, 16α–diol-20-one or a pharmaceutically acceptable salt of its 3-sulfate ester as a progestational agent.

The present invention further provides compositions comprising a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester. In particular it provides compositions comprising at least 1% of a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester. One aspect of the present invention provides compositions wherein the only progestational agent is a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester . Embodiments of the present invention include compositions wherein the only active compound is a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester. In these embodiments other excipients and carriers may be included but no further active materials are included.

The present invention also provides a process for the preparation of a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester which comprises:

a) converting 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester to a pharmaceutically acceptable salt,

b) converting a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester to a different pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester,

c) converting 5α-pregnan-16α, 17 epoxy-3β-ol-20-one to a pharmaceutically acceptable salt of 5α-pregnan-16α, 17 epoxy-3β-ol-20-one 3-sulfate ester or

d) deprotecting a pharmaceutically acceptable salt of a 16-hydroxy protected 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester.

5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester may be converted to a pharmaceutically acceptable salt by neutralising the acid with an appropriate base, e.g. with an alkali metal carbonate, an alkaline earth metal carbonate or a primary, secondary, tertiary or quaternary amine carbonate. Alkali metal or alkaline earth metal salts may be prepared by using the appropriate alkali metal hydride e.g. sodium hydride, potassium hydride or lithium hydride.

A pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester may be converted to a different pharmaceutically acceptable salt by displacement, by using an ion exchange resin or by double decomposition (metastasis). Displacement of a weak base with a stronger one may be utilised to convert, e.g. an amine salt to an alkali metal salt or an alkaline earth metal salt using an appropriate base, e.g. a hydroxide. For example a trialkylamine salt such as a triethylamine salt may be converted to an alkali metal salt such as a sodium salt by treating it with an alkali metal hydroxide such as aqueous sodium hydroxide. The displacement may be carried out using an ion exchange resin. Alternatively one salt may be converted to another by double decomposition, e.g. an alkaline earth metal salt such as the calcium salt may be replaced with an alkali metal salt. E.g. the calcium salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester may be dissolved in water followed by the addition of e.g.

sodium carbonate. Insoluble calcium carbonate would then precipitate out to provide the sodium salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester.

A pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester may be prepared by directly converting 5α-pregnan-3β, 16α–diol-20-one to a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester. This may be performed by reacting it with the appropriate aminesulfurtrioxide complex, e.g. by reacting it with a trialkylaminesulfurtrioxide (such as triethylamine-sulfurtrioxide complex) to provide the corresponding trialkylamine salt (such as the triethylamine salt). If desired the salt may then be converted to another salt of the invention as described above. Alkali metal or alkaline earth metal salts may be prepared by treating pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one with the appropriate alkali metal hydride e.g. sodium hydride, potassium hydride or lithium hydride to produce the corresponding alkoxide *in situ* and then adding a trialkylaminesulfurtrioxide (such as triethylaminesulfurtrioxide complex) to provide the corresponding trialkylamine salt (such as the triethylamine salt). If desired the salt may then be converted to another salt of the invention as described above.

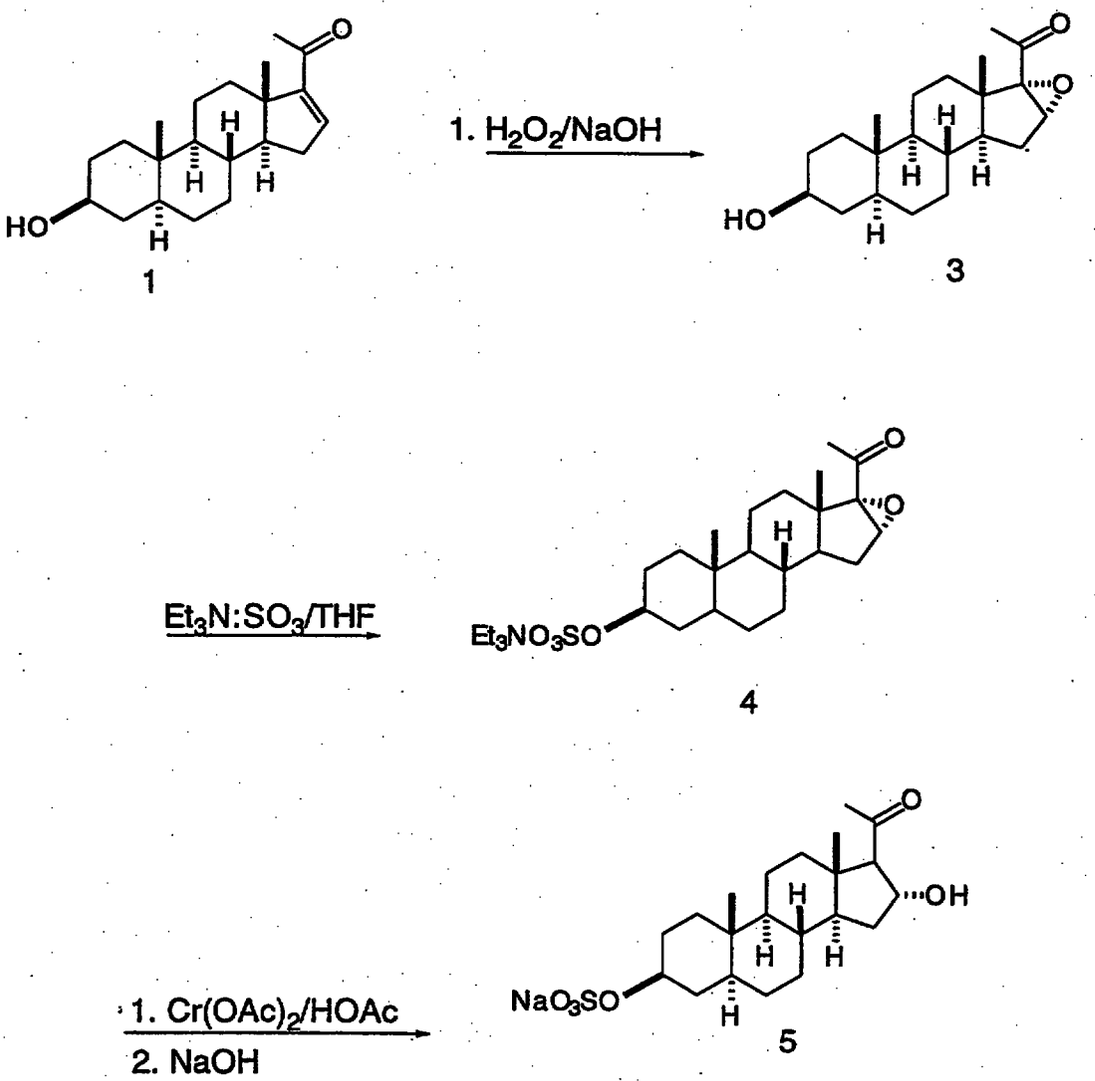
In order to prepare the desired pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester it may be necessary to protect the 16 hydroxy group. This may be achieved by conventional means using a suitable protecting group which will finally be removed to provide the product. Alternatively the hydroxyl group may be protected by forming the 16α, 17 epoxy derivative of the starting material and using it to prepare the desired pharmaceutically acceptable salt of 5α-pregnan-16α, 17 epoxy-3β-ol-20- 3-sulfate, which is then deprotected to give the desired compound of the invention.

The present invention also provides pharmaceutically acceptable salts of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester prepared by a chemical process, particularly those prepared according to the processes described above. The invention also provides pharmaceutically acceptable salts of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester obtainable by such processes.

The compounds of this invention can be prepared from readily available starting materials according to the processes in Scheme I, as shown for 5α -pregnan-3β, 16α–diol-20-one 3 sulfate ester sodium salt.

For example, according to Scheme I, 5α-pregn-16-en-3β-ol-20-one (1) is treated with hydrogen peroxide/sodium hydroxide to afford 5α-pregnane-16α, 17–epoxy-3β–ol-20-one (3) Epoxide (3) is treated with one or more equivalents of triethylamine:sulfur trioxide reagent to afford 5α-pregnan-16α, 17 epoxy-3β-ol-20-3-sulfate triethylammonium salt (4). The triethylammonium salt (4) is treated with chromous acetate in acetic acid/water then aqueous sodium hydroxide to afford 5α-pregnan-3β, 16α–diol-20-one 3 sulfate ester sodium salt (5).

Scheme I



The compounds of this invention are progestational agents, and are therefore useful as oral contraceptives (male and female), in hormone replacement therapy (particularly when combined with an estrogen), in the treatment of endometriosis luteal phase defects, benign breast and prostatic diseases and prostatic and endometrial cancers. The compounds of this invention are also useful in protecting against epileptic seizures, in cognition enhancement, in treating Alzheimer's disease, dementias, vasomotor symtpoms related to menopause, and other central nervous system disorders The compounds of this invention are further useful in stimulating erythropoises.

The compounds of this invention can be used alone as a sole therapeutic agent or can be used in combination with other agents, such as other estrogens, progestins, or and androgens.

The compounds of this invention can be formulated neat or with a pharmaceutical carrier for administration, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmacological practice. The pharmaceutical carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening

agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, lethicins, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compounds of this invention can also be administered orally either in liquid or solid composition form.

The compounds of this invention may be administered rectally or vaginally in the form of a conventional suppository. For administration by intranasal or intrabronchial inhalation or insufflation, the compounds of this invention may be formulated into an aqueous or partially aqueous solution, which can then be utilized in the form of an aerosol. The compounds of this invention may also be administered transdermally through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semipermiable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

The dosage requirements vary with the particular compositions employed, the route of administration, the severity of the symptoms presented and the particular subject being treated. Based on the results obtained in the standard pharmacological test procedures, projected daily dosages of active compound would be 0.02 µg/kg - 750 µg/kg. Treatment will generally be initiated with small dosages less than the

optimum dose of the compound. Thereafter the dosage is increased until the optimum effect under the circumstances is reached; precise dosages for oral, parenteral, nasal, or intrabronchial administration will be determined by the administering physician based on experience with the individual subject treated. Preferably, the pharmaceutical composition is in unit dosage form, e.g. as tablets or capsules. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example, packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form.

The following provides the preparation of representative compounds of this invention.

Example 1

5α-Pregnan-16α, 17-epoxy-3β-ol-20-one **(3)**

5α-pregn-16-en-3β-ol-20-one (1) (5.74g, 17.3 mmol) was dissolved in 500 ml of methanol, 25 ml of 30% hydrogen peroxide and 20 ml of 4 N sodium hydroxide. The solution was stirred at room temperature for 20 hours. The solution was poured into a mixture of 2.5l of water, 500 ml of methylene chloride and 25 ml of acetic acid. The methylene chloride layer was separated, washed with 500 ml of water, dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was dried to provide the title compound as a white solid (6g, 98%).

^{1}H NMR (300MHz, $CDCl_3$)

δ 0.82 (s, 3H), 1.00 (s, 3H), 2.03 (s, 3H), 3.58 (m, 1H), 3.67 (bs, 1H)

m/z (ES positive) 355 ($M+Na^+$), 687 ($2M+Na^+$),

5α -Pregnan-16α, 17-epoxy-3β-ol-20-one-3-sulfate, triethyl ammonium salt **(4)**

5α -Pregnan-16α, 17-epoxy-3β-ol-20-one-(3) (6.49g, 19.6 mmol) was dissolved in 100 ml of tetrahydrofuran and sulfur trioxide. In ethylamine complex (4.4g, 24.2 mmol) was added. The mixture was stirred at room temperature for 20 hours. The product was collected on a Buchner funnel, washed with tetrahydrofuran then ether and dried to provide the title compound as a white solid (8.2g, 82%).

^{1}H NMR (300MHz, DMSO-d_6)

δ 0.76 (s, 3H), 1.16 (s, 3H), 1.19 (t, 9H), 1.96 (s, 3H), 3.11 (q, 6H), 3.91 (s, 1H), 3.93 (1H)

m/z (ES negative) 411 (M-H)

5α -Pregnan-3β, 16α--diol-20-one-3-sulfate, triethyl ammonium salt.

5α-Pregnan-16α,17-epoxy-3β-ol-20-one-3-sulfate, triethylammonium salt (7.8g, 15.2 mmol) was dissolved in 200 ml of acetic acid and 60 ml of water. Chromous acetate [freshly prepared from 26g (0.5 mol) of chromium powder] was added and the mixture was stirred for 70 hours at room temperature. The solvents were removed in vacuo and the residue was slurried in water. The mixture was neutralized with sodium bicarbonate, then made alkaline with 4N sodium hydroxide. The mixture was filtered through Celite. The aqueous solution was extracted with n-butanol. The butanol extract was filtered through Celite and concentrated. The residue was purified by HPLC (converted to the triethyl ammonium salt during chromatography). (Primesphere, C18-HC, 10μ, 50x250 mm, s#171320; 10/90 to 30/70 at 4.5' to 5/45 at 7' to 60/40 at 11' to 70/30 at 18' to 90/10 at 26' (MeOH/50 mM triethylammonium acetate; pH=7) Flow=70 ml/min; UV=214 nm; 1000 PSI) to provide the title compound as a white solid (4.1g, 53%).

^{1}H NMR (300MHz, DMSO-d_6)

δ 0.52 (s, 3H), 0.75 (s, 3H), 1.14 (t, 9H), 2.08 (s, 3H), 2.42 (d, 1H), 2.97 (q, 6H), 3.93 (m, 1H), 4.49 (t, 1H);

m/z (ES negative) 413 (M-H)

5α -Pregnan-3β, 16α--diol-20-one 3 sulfate ester sodium salt (5)

5α -Pregnan-3β, 16α--diol-20-on-3 sulfate, triethyl ammonium salt (4g, 7.8 mmol) was dissolved in 150 ml of distilled water and the solution was passed through an ion exchange column (Dowex 50X8, Na^+ form). The column was rinsed with 100 ml of distilled water. The eluent was concentrated and the residue was dissolved in ethanol. The ethanol solution was concentrated in vacuo and the solid was collected with ether, filtered on a Buchner funnel and dried to provide the title compound as a white solid (3.2g, 94%).

^{1}H NMR (300MHz, DMSO-d_6)

δ 0.53 (s, 3H), 0.76 (s, 3H), 2.08 (s, 3H), 2.42 (s, 1H), 3.93 (m, 1H), 4.50 (s, 3H

m/z (ES negative) 413 (M-H)

WHAT IS CLAIMED IS:

1. A compound which is a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester.

2. The compound of claim 1 wherein the pharmaceutically acceptable salt of the 3-sulfate ester is an alkali metal salt, alkaline earth metal salt, ammonium salt, alkylammonium salts containing 1-6 carbon atoms or dialkylammonium salts containing 1-6 carbon atoms in each alkyl group, trialkylammonium salts containing 1-6 carbon atoms in each alkyl group and tetraalkylammonium salts containing 1-6 carbon atoms in each alkyl group.

3. 5α-Pregnan-3β, 16α–diol-20-one 3-sulfate ester sodium salt, which is at least 1 percent pure.

4. A compound which consists essentially of a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester.

5. A compound which consists essentially of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester sodium salt.

6. A pharmaceutical composition which comprises a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester.

7. A pharmaceutical composition which comprises at least 1% of a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester.

8. A method of providing progestational therapy to a mammal in need thereof which comprises administering a progestationally effective amount of progestationally effective amount of a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester, to said mammal.

9. A method of treating or inhibiting cancers, central nervous system disorders, dementias, or Alzheimer's disease in a mammal in need thereof, which comprises administering an effective amount of progestationally effective amount of a

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pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester, to said mammal.

10. Use of a compound as claimed in any one of claims 1 to 6 as a medicament.

11. Use of a compound as claimed in any one of claims 1 to 6 in the preparation of a medicament providing progestational therapy to a mammal in need thereof or a medicament for the treatment or prevention of cancers, central nervous system disorders, dementias, or Alzheimer's disease.

12. A process for the preparation of a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester which comprises:

a) converting 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester to a pharmaceutically acceptable salt,

b) converting a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester to a different pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester,

c) converting 5α-pregnan-16α, 17-epoxy-3β-ol-20-one to a pharmaceutically acceptable salt of 5α-pregnan-16α, 17-epoxy-3β-ol-20-one 3-sulfate ester or

d) deprotecting a pharmaceutically acceptable salt of a 16-hydroxy protected 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester.

13. A process as claimed in Claim 12 wherein a pharmaceutically acceptable salt of 5α-pregnan-16α, 17-epoxy-3β-ol-20-one 3-sulfate ester is converted to a pharmaceutically acceptable salt of 5α-pregnan-3β, 16α–diol-20-one 3-sulfate ester.

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