

PROCESS FOR THE PRODUCTION OF ESTETROL INTERMEDIATES

Field of the invention

The present invention relates to a new process for the synthesis of a key intermediate in the synthesis of Estetrol.

5 BACKGROUND OF THE INVENTION

Estrogenic substances are commonly used in methods of Hormone Replacement Therapy (HRT) and methods of female contraception. Estetrol is a biogenic estrogen that is endogenously produced by the fetal liver during human pregnancy. Recently, estetrol has been found effective as an estrogenic substance for use in HRT. Other important
10 applications of estetrol are in the fields of contraception, therapy of auto-immune diseases, prevention and therapy of breast and colon tumors, enhancement of libido, skin care, and wound healing.

The synthesis of estetrol and derivatives thereof is known in the art. Verhaar M.T; et al (WO 2004/041839) describes a process for the preparation of estetrol starting from a 3-A-oxy-estra 1,3,5(10),15-tetraen-17-one, wherein A is an C₁-C₅alkyl group, or a
15 C₇-C₁₂benzylic group. In this document, 3-A-oxy-estra 1,3,5(10),15-tetraen-17-ol is prepared in 6 steps from estrone where A is a benzyl group, the steps comprising protection of the 3-OH group by a benzyl group, then transformation of the 17-keto-group to a 17,17-ethylenedioxy derivative which is halogenated at the C₁₆ position using
20 pyridinium bromide perbromide. Dehydrohalogenation is carried out by using potassium tertbutoxyde in dimethylsulfoxide. Deprotection of the 17-keto-group is conducted using p-toluene-sulfonic acid monohydrate in aqueous acetone. Reduction of 17-keto-group affords the 17-ol derivative.

One of the disadvantages of the process described in WO 2004/041839 is the protection
25 of 3-OH function with a benzyl group which can be removed only by hydrogenation using Pd/C as catalyst in the last steps of the estetrol synthesis. Furthermore the level of this catalyst in the final drug substance must be determined and must comply with the ICH guidelines.

Another disadvantage of the synthesis described in WO 2004/041839 is the two step
30 protection/deprotection of the 17-keto function in order to generate the 15-16 double bond.

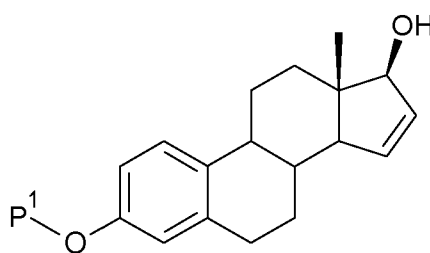
There remain a need for an improved synthesis of 3-Protected-oxy-estra-1,3,5(10),15-tetraene-17-ol.

It is therefore an object of the present invention to provide a process for the preparation of 3-Protected-oxy-estra-1,3,5(10),15-tetraene-17-ol which overcome at least one the disadvantages of the prior art.

Summary of the invention

- 5 The present inventors have now found that this object can be obtained by using a process as defined in the appended claims.

According to a first aspect of the present invention, a process for the preparation of a compound of formula (I) (3-P¹-oxy-estra-1,3,5(10),15-tetraene-17-ol) is provided:

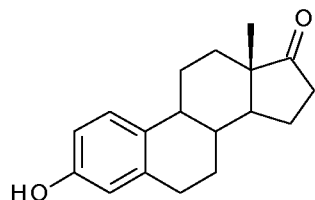


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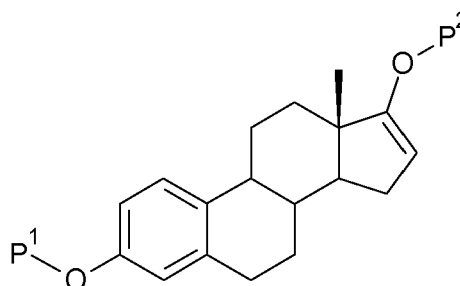
(I)

said process comprises the steps of :

- a) reacting a compound of formula (II), with an acylating or a silylating agent to produce a compound of formula (III), wherein P¹ and P² are each independently a protecting group selected from R¹CO-, or R²-Si-R³R⁴, wherein R¹ is a group selected from C₁₋₆alkyl or C₃₋₆cycloalkyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C₁₋₄alkyl; R², R³ and R⁴ are each independently a group selected from C₁₋₆alkyl or phenyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C₁₋₄alkyl;



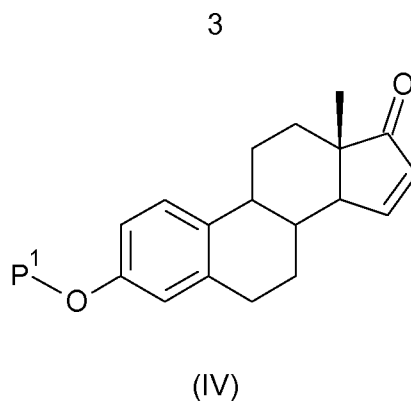
(II)



(III)

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- b) reacting the compound of formula (III) in the presence of palladium acetate or a derivative thereof to produce compound of formula (IV); and



c) reacting the compound of formula (IV) with a reducing agent to produce compound of formula (I).

5 Preferably, the present invention encompasses a process for the preparation of a compound of formula (I), said process comprising the steps of

a) reacting a compound of formula (II), with an acylating or a silylating agent to produce a compound of formula (III), wherein P^1 and P^2 are each independently a protecting group selected from $R^2-Si-R^3R^4$, or R^1CO- , wherein R^1 is a group selected from C_{1-6} alkyl or C_{3-6} cycloalkyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4} alkyl; R^2 , R^3 and R^4 are each independently a group selected from C_{1-6} alkyl or phenyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4} alkyl;

b) reacting the compound of formula (III) in the presence of palladium acetate present in catalytic or sub-stoichiometric amounts, in an oxygen atmosphere to produce compound of formula (IV); and

c) reacting the compound of formula (IV) with a reducing agent to produce compound of formula (I).

The invention provides an improved process for producing 3- P^1 -oxy-estra-1, 3, 5(10),15-tetraene-17-ol of formula (I) in significantly higher yield and/or at lower cost than possible by the previous known syntheses.

According to a second aspect, the present invention also encompasses a process for the preparation of estetrol, said process comprising preparing a compound of formula (I) by a process according to the first aspect of the invention and further reacting compound of formula (I) to produce estetrol.

According to a third aspect, the present invention also encompasses estetrol directly obtained by the process according to the second aspect of the invention, for use in a method selected from a method of hormone replacement therapy, a method of treating vaginal dryness, a method of contraception, a method of enhancing libido, of method of
5 treating skin, a method of promoting wound healing, and a method of treating or preventing a disorder selected from the group consisting of autoimmune diseases, breast tumors and colorectal tumors.

The above and other characteristics, features and advantages of the present invention will become apparent from the following detailed description, which illustrate, by way of
10 example, the principles of the invention.

Detailed description of the invention

It is also to be understood that the terminology used herein is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

As used herein, the singular forms "a", "an", and "the" include both singular and plural
15 referents unless the context clearly dictates otherwise.

The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including", "includes" or "containing", "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements or method steps. It will be appreciated that the terms "comprising", "comprises" and "comprised of" as used herein
20 comprise the terms "consisting of", "consists" and "consists of".

The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within the respective ranges, as well as the recited endpoints.

All references cited in the present specification are hereby incorporated by reference in their entirety. In particular, the teachings of all references herein specifically referred to
25 are incorporated by reference.

Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the present invention.

In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

Reference throughout this specification to “one embodiment” or “an embodiment” means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases “in one embodiment” or “in an embodiment” in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to a person skilled in the art from this disclosure, in one or more embodiments. Furthermore, while some embodiments described herein include some but not other features included in other embodiments, combinations of features of different embodiments are meant to be within the scope of the invention, and form different embodiments, as would be understood by those in the art. For example, in the appended claims, any of the claimed embodiments can be used in any combination.

The term “alkyl” by itself or as part of another substituent, refers to a straight or branched saturated hydrocarbon group joined by single carbon-carbon bonds having 1 to 6 carbon atoms, for example 1 to 5 carbon atoms, for example 1 to 4 carbon atoms, preferably 1 to 3 carbon atoms. When a subscript is used herein following a carbon atom, the subscript refers to the number of carbon atoms that the named group may contain. Thus, for example, C₁₋₆alkyl means an alkyl of one to six carbon atoms. Examples of alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *sec*-butyl, *tert*-butyl, 2-methylbutyl, pentyl iso-amyl and its isomers, hexyl and its isomers.

The term “C₃₋₆cycloalkyl”, as a group or part of a group, refers to a saturated cyclic alkyl radical containing from about 3 to about 6 carbon atoms. Examples of monocyclic C₃₋₆cycloalkyl radicals include cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

The term “C₂₋₆alkenyl” by itself or as part of another substituent, refers to an unsaturated hydrocarbyl group, which may be linear, or branched, comprising one or more carbon-carbon double bonds. Examples of C₂₋₆alkenyl groups are ethenyl, 2-propenyl, 2-butenyl, 3-butenyl, 2-pentenyl and its isomers, 2-hexenyl and its isomers, 2,4-pentadienyl and the like.

The term "C₆₋₁₀aryl", by itself or as part of another substituent, refers to a polyunsaturated, aromatic hydrocarbonyl group having a single ring (i.e. phenyl) or multiple aromatic rings fused together (e.g. naphthyl). or linked covalently, typically containing from 6 to 10 carbon atoms, wherein at least one ring is aromatic. C₆₋₁₀aryl is also intended to include the partially hydrogenated derivatives of the carbocyclic systems enumerated herein. Non-limiting examples of C₆₋₁₀aryl comprise phenyl, naphthyl, indanyl, or 1,2,3,4-tetrahydronaphthyl.

The term "C₆₋₁₀arylC₁₋₆alkyl", by itself or as part of another substituent, refers to a C₁₋₆alkyl group as defined herein, wherein one or more hydrogen atoms are replaced by one or more C₆₋₁₀aryl as defined herein. Examples of aralkyl radicals include benzyl, phenethyl, dibenzylmethyl, methylphenylmethyl, 3-(2-naphthyl)-butyl, and the like.

The term "C₁₋₆alkylcarbonyl", as a group or part of a group, represents a group of Formula -CO-R^a, wherein R^a is C₁₋₆alkyl as defined herein.

The term "C₃₋₆cycloalkylcarbonyl", as a group or part of a group, represents a group of Formula -CO-R^c, wherein R^c is C₃₋₆cycloalkyl as defined herein.

The term "C₂₋₆alkenylC₁₋₆alkanoate" refers to a compound having the Formula R^b-O-CO-R^a wherein R^a is C₁₋₆alkyl as defined herein and R^b is C₂₋₆alkenyl as defined herein.

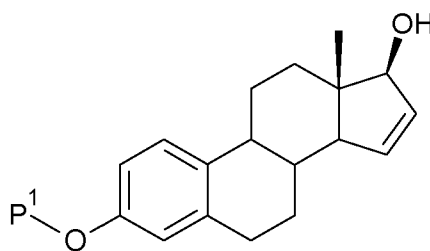
The term "C₂₋₆alkenylC₃₋₆cycloalkanoate" refers to a compound having the Formula R^b-O-CO-R^c wherein R^c is C₃₋₆cycloalkyl as defined herein and R^b is C₂₋₆alkenyl as defined herein.

The term "C₁₋₆alkylenecarbonate" refers to a compound having the Formula R^b-O-CO-O-R^a wherein R^a is C₁₋₆alkyl as defined herein and R^b is C₂₋₆alkenyl as defined herein.

The present invention relates to a process for preparing 3-P¹-oxy-estra-1,3,5(10),15-tetraene-17-ol of formula (I), wherein P¹ is a protecting group selected from R¹CO-, R²Si-R³R⁴; wherein

R^1 is a group selected from C_{1-6} alkyl or C_{3-6} cycloalkyl, each group being optionally substituted by 1, 2 or 3 substituents independently selected from fluoro or C_{1-4} alkyl; preferably R^1 is selected from the group comprising methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, each group being optionally substituted by 1, 2 or 3 substituents independently selected from fluoro or C_{1-4} alkyl; more preferably R^1 is methyl, ethyl, propyl, isopropyl, cyclopentyl, or cyclohexyl, yet more preferably R^1 is methyl, or ethyl;

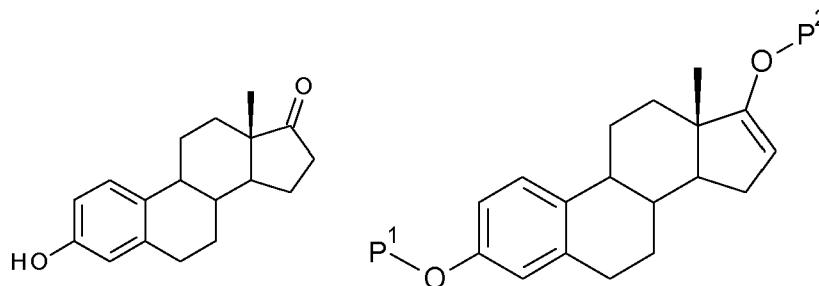
R^2 , R^3 and R^4 are each independently a group selected from C_{1-6} alkyl or phenyl, said C_{1-6} alkyl or phenyl, being optionally substituted with 1, 2 or 3 substituents independently selected from fluoro or C_{1-6} alkyl; preferably R^2 , R^3 and R^4 are each independently selected from the group comprising methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, and phenyl, each group being optionally substituted with 1, 2 or 3 substituents each independently selected from fluoro or C_{1-4} alkyl; preferably R^2 , R^3 and R^4 are each independently selected from the group comprising methyl, ethyl, propyl, isopropyl, or *tert*-butyl, and phenyl, each group being optionally substituted with 1, 2 or 3 substituents each independently selected from fluoro or C_{1-2} alkyl,



(I)

said process comprises the steps of

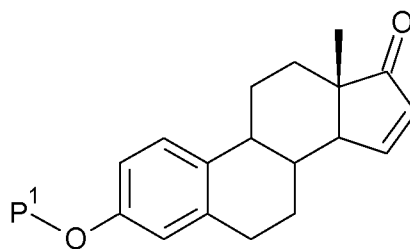
a) protecting the hydroxyl and the ketone of estrone of formula (II) to produce compound of formula (III), wherein P^1 is as defined above and P^2 is a protecting group selected from R^1CO- , $R^2-Si-R^3R^4$,



(II)

(III)

b) reacting the compound of formula (III) in the presence of palladium acetate or a derivative thereof such as palladium chloride or Tris(dibenzylideneacetone)dipalladium ($\text{Pd}_2(\text{dba})_3$) to produce a compound of formula (IV), preferably in the presence of an oxygen atmosphere; and



(IV)

c) reacting the compound of formula (IV) with a reducing agent to produce compound of formula (I);

and if necessary any protective group used in the reactions described above is cleaved concurrently or subsequently; and

if desired, compound of formula (I) is subsequently converted into another compound by routine processes applicable for conversion of functional groups,

if desired a compound of formula I thus obtained is resolved into its stereoisomers.

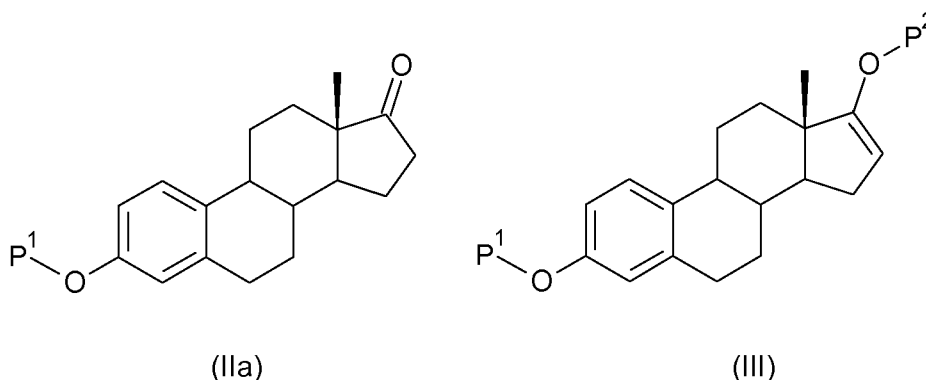
In an embodiment, P^1 is $\text{R}^1\text{CO}-$; preferably P^1 is a group selected from C_{1-4} alkylcarbonyl or C_{4-6} cycloalkylcarbonyl, each group being optionally substituted by 1, 2 or 3 substituents independently selected from fluoro or C_{1-4} alkyl; more preferably P^1 is a group selected from C_{1-2} alkylcarbonyl or C_{5-6} cycloalkylcarbonyl, each group being optionally substituted by 1, 2 or 3 substituents independently selected from fluoro or C_{1-2} alkyl; for example P^1 is selected from acetyl, or cyclohexylcarbonyl, preferably P^1 is acetyl.

In an embodiment, P^2 is $\text{R}^1\text{CO}-$; preferably P^2 is a group selected from C_{1-4} alkylcarbonyl or C_{4-6} cycloalkylcarbonyl, each group being optionally substituted by 1, 2 or 3 substituents independently selected from fluoro or C_{1-4} alkyl; more preferably P^2 is a group selected from C_{1-2} alkylcarbonyl or C_{5-6} cycloalkylcarbonyl, each group being optionally substituted by 1, 2 or 3 substituents independently selected from fluoro or C_{1-4} alkyl; for example P^2 is selected from acetyl, or cyclohexylcarbonyl, preferably P^2 is acetyl.

In an embodiment, P^1 and P^2 are each independently $\text{R}^1\text{CO}-$.

In an embodiment, P^1 is $R^2-Si-R^3R^4$. Preferably P^1 is selected from the group comprising *tert*-butyl-dimethyl-silyl, diphenyl-methyl-silyl, dimethyl-phenyl-silyl, trimethyl-silyl, triethyl-silyl and triisopropyl-silyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4} alkyl; more preferably P^1 is *tert*-butyl-dimethyl-silyl.

In an embodiment, step (a) comprises the steps of (a1) protecting the hydroxyl of compound of formula (II) with a silylating agent to produce a compound of formula (IIa), wherein P^1 is $R^2-Si-R^3R^4$; and



(a2) protecting the ketone of compound of formula (IIa) in the presence of an acylating agent to produce compound of formula (III), wherein P^2 is R^1CO- .

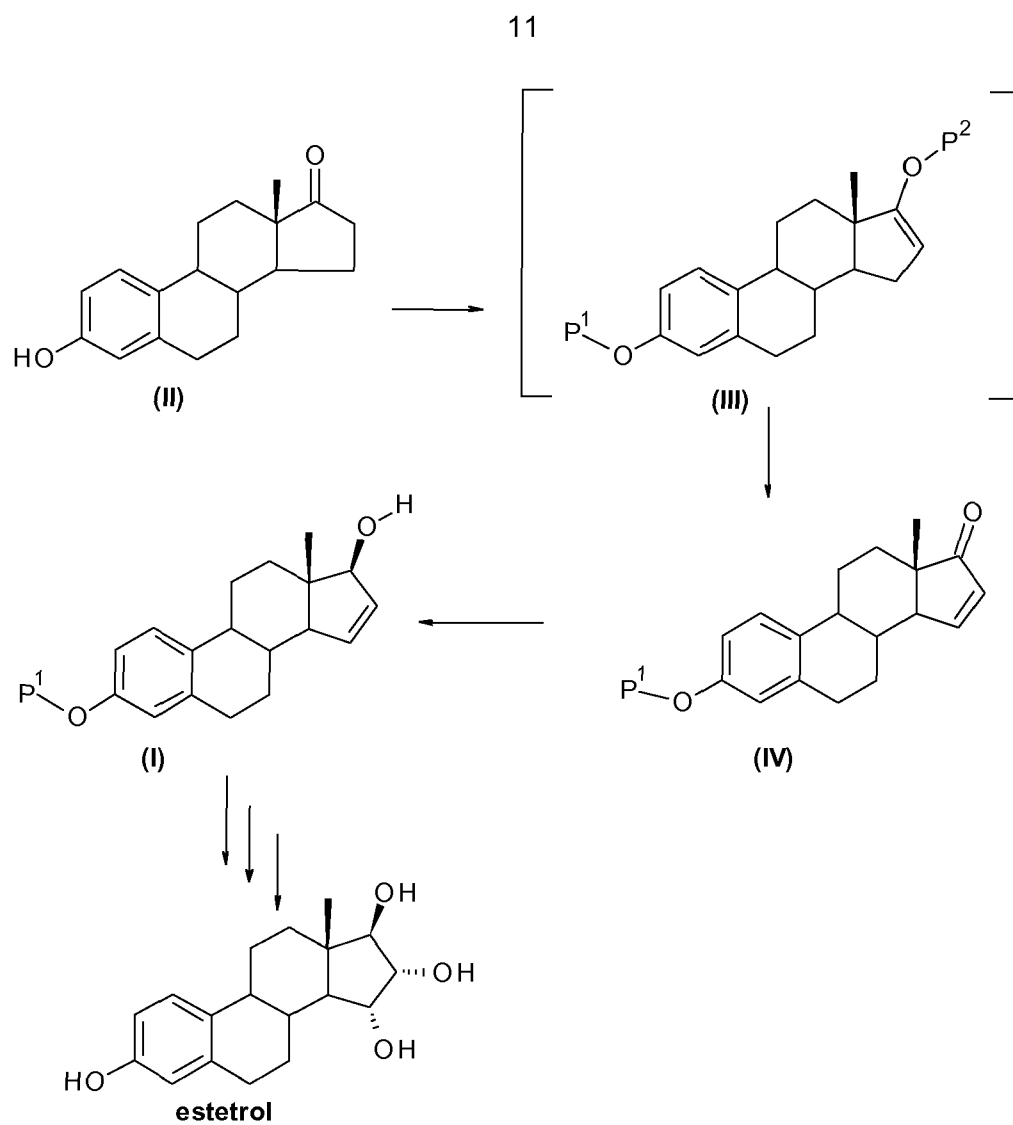
In an embodiment, P^2 is $R^2-Si-R^3R^4$; preferably P^2 is selected from the group comprising *tert*-butyl-dimethyl-silyl, diphenyl-methyl-silyl, dimethyl-phenyl-silyl, trimethyl-silyl, triethyl-silyl and triisopropyl-silyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4} alkyl, more preferably P^2 is *tert*-butyl-dimethyl-silyl.

In an embodiment, P^1 and P^2 are each independently $R^2-Si-R^3R^4$.

In an embodiment, P^1 is $R^2-Si-R^3R^4$; and P^2 is R^1CO- . Preferably P^1 is selected from the group comprising *tert*-butyl-dimethyl-silyl, diphenyl-methyl-silyl, dimethyl-phenyl-silyl, trimethyl-silyl, triethyl-silyl or triisopropyl-silyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4} alkyl; more preferably P^1 is *tert*-butyl-dimethyl-silyl; and preferably P^2 is a group selected from C_{1-6} alkylcarbonyl or C_{3-6} cycloalkylcarbonyl, each group being optionally substituted by 1, 2 or 3 substituents independently selected from fluoro or C_{1-4} alkyl; preferably P^2 is a group selected from C_{1-4} alkylcarbonyl or C_{5-6} cycloalkylcarbonyl; each group being optionally substituted by 1, 2 or 3 substituents independently selected from fluoro or C_{1-2} alkyl; more preferably P^2 is C_{1-2} alkylcarbonyl or C_{5-6} cycloalkylcarbonyl, for example P^2 is acetyl or cyclohexylcarbonyl, preferably acetyl.

In an embodiment, the silylating agent can be selected from the group comprising C_{1-6} alkylsilylchloride, C_{1-6} alkylsilyltriflate, phenylsilylchloride, phenylsilyltriflate, C_{1-6} alkylphenylsilylchloride, C_{1-6} alkylphenylsilyltriflate, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4} alkyl.

In an embodiment, the process for the preparation of 3- P^1 -estra 1, 3, 5(10),15-tetraene-17-ol of formula (I) from estrone of formula (II) can be preformed in 3 steps as shown in Scheme 1. The compound of formula (I) can then be further reacted to prepare estetrol.



Scheme 1

According to scheme 1, the hydroxyl and the ketone of estrone of formula (II) are both protected, preferably in one step, to produce compound of formula (III).

In an embodiment, wherein P^1 and P^2 are each independently R^1CO- , estrone is reacted with an acylating agent. Preferably, said acylating agent is $C_{2-6}alkenylC_{1-6}alkanoate$ or $C_{2-6}alkenylC_{3-6}cycloalkanoate$. Preferably, the acylating agent is selected from the group comprising $C_{2-6}alkenylpropanoate$, $C_{2-6}alkenylbutanoate$, $C_{2-6}alkenylpentanoate$,
5 $C_{2-6}alkenylhexanoate$, $C_{2-6}alkenylcyclopropanoate$, $C_{2-6}alkenylcyclobutanoate$, $C_{2-6}alkenylcyclopentanoate$, and $C_{2-6}alkenylcyclohexanoate$. More preferably, the acylating agent is selected from the group comprising isopropenyl acetate, isopropenyl propionate, isopropenyl butyrate, isopropenyl isobutyrate, vinyl acetate, vinyl propionate, prop-2-enyl cyclohexanecarboxylate, ethenyl cyclopentanecarboxylate, and vinyl
10 cyclohexanoate. More preferably, the acylating agent is selected from the group comprising isopropenyl acetate, isopropenyl propionate, isopropenyl butyrate, isopropenyl isobutyrate, vinyl acetate, and vinyl propionate.

The acylation can be performed in the presence of an acid, such as in the presence of sulfuric acid, or in the presence of a $C_{6-10}arylsulfonic$ acid, optionally substituted by one or
15 more chloro substituents. Non-limiting examples of a suitable acid include para-toluene sulfonic acid, and sulfuric acid.

For example, estrone of formula (II) can be was reacted with isopropenyl acetate in the presence of sulfuric acid or para-toluene sulfonic acid to give the estra-1,3,5 (10), 16-tetraene-3,17-diol, 3,17-diacetate. The reaction can be performed under reflux, optionally
20 under inert atmosphere, such as nitrogen atmosphere. The product can be used as such in the next step or further purified by known techniques in the art such as by chromatography, for example on silica with a suitable eluant such as methylene chloride/hexane or ethyl acetate/hexane.

In an embodiment, wherein P^1 and P^2 are each independently $R^2-Si-R^3R^4$, estrone of
25 formula (II) is reacted with a silylating agent. The silylating agent can be selected from the group comprising $C_{1-6}alkylsilyl$ triflate, phenylsilyltriflate, $C_{1-6}alkylphenylsilyltriflate$, $C_{1-6}alkylsilylchloride$, $C_{1-6}phenylsilylchloride$, $C_{1-6}alkylphenylsilylchloride$, each group being optionally substituted by one or more substituents independently selected from fluoro or $C_{1-4}alkyl$.

For example, formation of protected estrone silyl ether can be performed by reaction of a silylating agent such as *tert*-butyl dimethylsilyltriflate, diphenylmethylsilyltriflate, dimethylphenylsilyltriflate, trimethylsilyltriflate, triethylsilyltriflate, or triisopropylsilyltriflate. The reaction can be performed in the presence of a suitable base such as imidazole, 2,6-lutidine, collidine, triethylamine, or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The reaction can be performed at room temperature or under reflux. The reaction can be performed in the presence of a suitable solvent such as dichloromethane, toluene or dimethylformamide or a mixture thereof. The formation of protected estrone silyl ether can also be performed by reaction of a silylating agent such as *tert*-butyl dimethylsilylchloride, diphenylmethylsilylchloride, dimethylphenylsilylchloride, trimethylsilylchloride, triethylsilylchloride or triisopropylsilylchloride in the presence of a suitable base such as lithium diisopropylamide (LDA), *tert*-butyl lithium, sodium or potassium bis(trimethylsilyl)amide (NaHMDS, KHMDS) or lithium tetramethylpiperidine.

Step (b) of the present process comprises reacting the compound of formula (III) in the presence of palladium acetate or a derivative thereof such as palladium chloride or Tris(dibenzylideneacetone)dipalladium ($\text{Pd}_2(\text{dba})_3$), preferably palladium acetate or palladium chloride, more preferably palladium acetate to produce a compound of formula (IV).

In an embodiment, said palladium acetate or a derivative thereof can be present in stoichiometric amounts, or sub-stoichiometric catalytic amounts.

For example the reaction of step (b) can be performed using stoichiometric amounts of palladium acetate, palladium chloride or Tris(dibenzylideneacetone)dipalladium, preferably stoichiometric amounts of palladium acetate, preferably in a suitable solvent such as acetonitrile, benzonitrile or dimethylsulfoxide, preferably benzonitrile.

This reaction can be performed at room temperature.

In another example, said step (b) can be performed using sub-stoichiometric catalytic amounts of palladium acetate, palladium chloride, or Tris(dibenzylideneacetone)dipalladium, preferably sub-stoichiometric catalytic amounts of palladium acetate, in the presence of a C_{1-6} alkylene carbonate such as allyl carbonate and in the presence of an organotin compound as catalyst. Preferably, the organotin compound is tri-butyltin methoxide. Preferably the C_{1-6} alkylene carbonate is allyl methyl carbonate. The reaction can be performed under reflux conditions, optionally under inert atmosphere such as nitrogen or argon atmosphere.

In another example, said step (b) can be performed using sub-stoichiometric catalytic amounts of palladium acetate under an oxygen atmosphere. In another example, said step (b) can be performed using sub-stoichiometric catalytic amounts of palladium chloride, under an oxygen atmosphere. In another example, said step (b) can be performed using sub-stoichiometric catalytic amounts of Tris(dibenzylideneacetone)dipalladium, under an oxygen atmosphere.

Preferably, said oxygen atmosphere is pure molecular oxygen or atmospheric oxygen (air or circulating air, or renewable air).

Preferably, in step (b) the amount of palladium acetate, palladium chloride or Tris(dibenzylideneacetone)dipalladium is at most 0.50 equivalents, preferably at most 0.40 equivalents, more preferably at most 0.30 equivalents, yet more preferably at most 0.2 equivalents, yet more preferably at most 0.10 equivalents, yet more preferably at most 0.05 equivalents, yet more preferably at most 0.03 equivalents per equivalent of compound of formula (III).

In a preferred embodiment, step (b) is performed with at most 0.10 equivalents of palladium acetate, preferably at most 0.05 equivalents, preferably at most 0.03 equivalents per equivalent of compound of formula (III), in the presence of pure molecular oxygen or atmospheric oxygen.

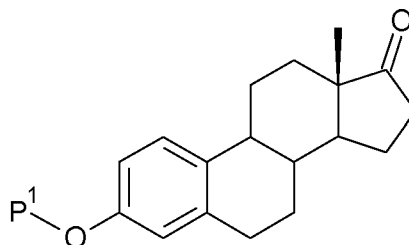
The next step in the process comprises the reduction of the compound of formula (IV) with a reducing agent to produce compound of formula (I). Preferably, said reducing agent is a metal hydride compound. For example, the metal hydride compound can be selected from the group comprising LiAlH_4 , NaBH_4 , $\text{NaBH}(\text{OAc})_3$, ZnBH_4 , and $\text{NaBH}_4/\text{CeCl}_3$. preferably, said reducing agent is $\text{NaBH}_4/\text{CeCl}_3$.

For example said reduction can be performed in a suitable solvent or a mixture thereof, such as in tetrahydrofuran, or a mixture of methanol and tetrahydrofuran. The reaction can be performed at low temperatures such as below 15°C , for example below 10°C .

In an embodiment, compound of formula (IV) is not isolated but directly reduced to the alcohol using said reducing agent. In this embodiment, step (b) and (c) are performed in one pot. This one-pot/two-step procedure is the shortest chemical pathway described to obtain compound of formula (I).

This process offers the advantages that the 17-hydroxy function of the compound of formula (I) could be also protected by a protecting group such as an acyl group, more preferably an acetyl group which could be removed in the same time that the 3-protecting group such as 3-acetyl, preferably 3-acetoxy group offering a never described synthesis of
5 estetrol in 6 steps. The 17-hydroxy function of the compound of formula (I) could be also protected by a silyl group, which could be removed in the same time that the 3-silyl protecting group offering a never described synthesis of estetrol in 6 steps.

According to another embodiment, step (a) can be performed in two steps and comprises the steps of (a1) protecting the hydroxyl of compound of formula (II) using a silylating
10 agent to produce a compound of formula (IIa), wherein $P^1 R^2-Si-R^3R^4$; and

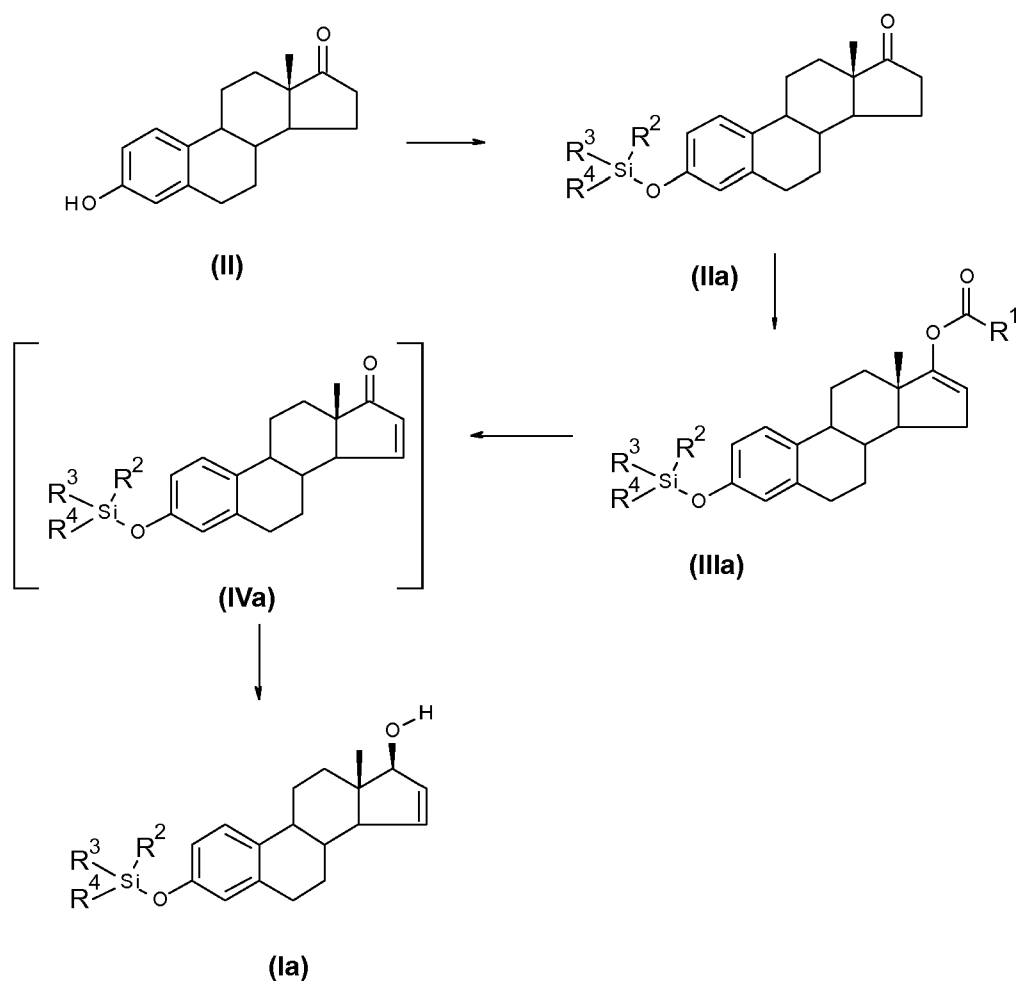


(IIa)

(a2) converting the ketone of compound of formula (IIa) to its enol ether in the presence of an acylating agent to produce a compound of formula (III).

15 According to this embodiment, the process for the preparation of 3- P^1 -estra 1, 3, 5(10),15-tetraene-17-ol of formula (I) from estrone of formula (II) can be preformed as shown in Scheme 2.

16



Scheme 2

In this embodiment, illustrated in Scheme 2, wherein P^1 independently $R^2-Si-R^3R^4$, and P^2 is $CO-R^1$, estrone of formula (II) is reacted with a silylating agent to produce compound of formula (IIa). The silylating agent can be selected from the group comprising C_{1-6} alkylsilyl chloride, phenylsilyl chloride, C_{1-6} alkylphenylsilyl chloride; each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4} alkyl.

For example, formation of protected estrone silyl ether can be performed by reaction of a silylating agent such as *tert*-butyl dimethylsilylchloride, diphenylmethylsilylchloride, dimethylphenylsilylchloride, trimethylsilylchloride, triethylsilylchloride, or triisopropylsilylchloride. The reaction can be performed in the presence of a base such as imidazole, 2,6-lutidine, collidine, triethylamine, or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

The next step comprises, converting the ketone of compound of formula (IIa) in the presence of an acylating agent to produce a compound of formula (II) wherein P^2 is acyl (compound of formula (IIIa)). Suitable acylating agents and conditions are as described herein above.

- 5 The next step in the process of scheme 2 comprises reacting the compound of formula (IIIa) in the presence of palladium acetate or a derivative thereof such as palladium chloride or Tris(dibenzylideneacetone)dipalladium ($Pd_2(dba)_3$) to produce compound of formula (IV) wherein P^1 is $R^2-Si-R^3R^4$ (compound of formula (IVa)). This reaction can be performed as described herein above.
- 10 The next step in the process comprises the reduction of the compound of formula (IVa) with a reducing agent to produce compound of formula (I) wherein P^1 is $R^2-Si-R^3R^4$ (compound of formula (Ia)). This reaction can be performed as described herein above.

The processes according to the present invention have the advantage that the protective group can be removed in situ at the end of the synthesis by conventional methods such as

15 removal of silyl protecting group with fluoride ions, such as tetra-n-butylammonium fluoride; as described in Coppola, G.M. Org Prep Proced, 2007, 39 (2), 199-292 hereby incorporated by reference; or removal of silyl protecting groups using 2,3-dichloro-5,6-dicyano-p-benzoquinone as described in Tanemura, K. J Chem Soc, Perkin Trans 1 1992, (22), 2997-2998; hereby incorporated by reference.

- 20 The present process has the advantage that 3- P^1 -oxy-estra-1,3,5(10),15-tetraen-17-ol of formula (I), and subsequently estetrol, can be obtained from estrone in a reduced number of steps compared to prior art processes, which is more convenient for an economical and industrial synthesis.

The present invention also encompasses a process for the preparation of estetrol, said

25 process comprising preparing a compound of formula (I) using the process of the invention and further reacting compound of formula (I) to produce estetrol.

The present invention also encompasses the use of estetrol directly obtained by the process the invention for the manufacture of a pharmaceutical composition, preferably for use in a method selected from a method of hormone replacement therapy, a method of

30 treating vaginal dryness, a method of contraception, a method of enhancing libido, of method of treating skin, a method of promoting wound healing, and a method of treating or preventing a disorder selected from the group consisting of autoimmune diseases, breast tumors and colorectal tumors.

The invention is illustrated but not limited by the following examples.

EXAMPLES

Example 1: Preparation of a compound of formula (I) wherein P¹ is acetyl according to an embodiment of the invention.

Step 1: Estra-1, 3, 5 (10), 16-tetraene-3, 17-diol, 3,17-diacetate

- 5 100g of 3-hydroxy-estra-1, 3, 5(10)-trien-17-one (0.370 mole) was poured in 500ml of isopropenyl acetate and 10g of para-toluene-sulfonic acid. The mixture was refluxed. Acetone and isopropenyl acetate was continuously distilled off until the temperature reached 98°C. Then the mixture was cooled to 0°C and K₂CO₃ was added.

- 10 After one hour at 0°C the mixture was filtered, the resulting solution was concentrated and diisopropyl ether added. The precipitate was collected by filtration and dried. It weighted 111.5g (yield: 85%)

¹HNMR (CDCl₃) δ 0.90 (s, 3H, CH₃ at C-18), 1.30-1.50 (m, 11H), 2.20 (s, 3H, CH₃ acetate), 2.30 (s, 3H, CH₃ acetate), 2.30-2.50 (m, 2H), 5.54 (broad s, 1H), 6.80 (broad s, 1H, H₄), 6.82 (dd, 1H, H₂), 7.27 (d, 1H, H₁) mp = 148.3°C

15 **Step 2: 3-acetoxy-estra-1, 3, 5 (10), 15-tetraen-17-one**

- To a solution of 115.5g (0.315 mole) of estra-1,3,5 (10)-tetraene-3,17-diol, 3,17, diacetate in 1500 ml of acetonitrile were added 30.4g (0.095mole) of tri-n-butyltin methoxyde and 11.2g (0.05 mole) of palladium (II) acetate and allyl methyl carbonate 20 ml. The mixture was refluxed for 2 hours then cooled to room temperature and filtered through a pad of silica gel. The reaction was then diluted with water and extracted with ethyl acetate. After concentration to one third of the initial volume diisopropyl ether 1000ml was slowly added. The precipitate was collected by filtration, washed with diisopropyl ether and used in the next step without further purification.

- 25 ¹HNMR (CDCl₃) δ 1.10 (s, 3H, CH₃ at C-18), 1.30-2.60 (m, 9H), 2.30 (s, 3H, CH₃ 3-acetate), 2.90-3.00 (m, 2H), 6.00-6.15 (m, 1H, H₁₅), 6.80 (broad s, 1H, H₄), 6.85 (dd, 1H, H₂), 7.29 (d, 1H, H₁), 7.60 (d, 1H, H₁₆), mp: 177.7°C

Step 3: 3-acetoxy-estra-1, 3, 5 (10), 15-tetraene-17-ol

The collected material was dissolved in tetrahydrofuran (THF) 300ml and a solution of cerium chloride heptahydrate (123g, 0.33mole) in methanol (300ml) was added. The mixture was cooled to 0°C and sodium borohydride (17.8g, 0.47 mole, 1.5q) was added portion wise keeping the temperature below 5°C. At this end of the addition, the mixture was stirred for one hour then quenched by addition of a 2N HCl solution (100ml). The solution was partly evaporated in situ and water (4L) was added. The precipitate was collected by filtration and dried. After crystallization from a mixture of ethanol /diisopropyl ether 3-acetoxy-estra-1, 3, 5(10),15-tetraene-17-ol was isolated in 75 % yield.

¹HNMR (CDCl₃) δ 0.85 (s, 3H, CH₃ at C-18), 1.20-2.50 (m, 8H), 2.30 (s, 3H, CH₃ 3-acetate), 2.80-3.05 (m, 2H), 4.40 (broad s, 1H, H17), 5.75 (broad s, 1H), 6.04 (broad s, 1H), 6.80 (broad s, 1H, H4), 6.84 (broad s, 1H, H2), 7.29 (d, 1H, H1), mp: 120.7°C

Example 2: Preparation of a compound of formula (I) wherein P¹ is t-butyldimethylsilyl according to an embodiment of the invention.

Step 1: 3,17-di-t-butyldimethylsiloxy-estra-1, 3, 5(10)-16-tetraene-17-ol

To a solution of estrone (50g, 0.185 mole) and 2,6-lutidine (62g, 0.58 mole) in dichloromethane 400ml was added drop wise t-butyl-dimethylsilyl-triflate (102.6g, 0.39 mole). The solution was stirred at room temperature for 6 hours. Water (300ml) was added and the organic layer was washed with a diluted solution of sodium carbonate. The dichloromethane solution was partially evaporated and ethyl acetate was added. Diisopropyl ether was added to this solution. The mixture was stirred for 2 hours at 0°C. The precipitate was collected by filtration and dried. 83 g of the title compound were obtained (90% yield).

¹HNMR (CDCl₃) δ 0.20 (s, 12H, (CH₃)₂-Si-), 0.90 (s, 3H, CH₃ at C-18), 0.95 (s, 9H, (CH₃)₃-C-Si-), 1.00 (s, 9H, (CH₃)₃-C-Si-), 1.20-2.40 (m, 11H), 2.75-2.95 (m, 2H), 4.48 (m, 1H, H16), 6.58 (broad s, 1H, H4), 6.62 (dd, 1H, H2), 7.12 (d, 1H, H1), mp: 97.6°C

Step 2: 3-t-butyldimethylsiloxy-estra-1, 3, 5 (10)-15-tetraene-17-one

To a solution of 3, 17-di-t-butyldimethylsiloxy-estra-1, 3, 5(10)-16-tetraene-17-ol 83 g (0.166 mole) in 400ml of acetonitrile was added Pd(OAc)₂ 3.8 g (0.017 mole) in an oxygen atmosphere. The mixture was stirred at 40°C for 12 hours then filtered through a pad of celite. A diluted solution of sodium carbonate was added and the mixture was extracted with ethyl acetate.

After concentration, diisopropyl ether was added and the mixture was stirred at 0°C for one hour. The product (54.7g, 86% yield) was collected by filtration and used in the next step without further purification.

¹HNMR (CDCl₃) δ 0.20 (s, 6H, (CH₃)₂-Si-), 1.00 (s, 9H, (CH₃)₃-C-Si-), 1.13 (s, 3H, CH₃ at C-18), 1.20-2.70 (m, 11H), 2.80-3.00 (m, 2H), 6.10 (dd, 1H, H15), 6.58 (broad s, 1H, H4), 6.62 (dd, 1H, H2), 7.11 (d, 1H, H1), 7.63 (dd, 1H, H16), mp: 165°C

Step 3: 3 -t-butyltrimethylsiloxy-estra-1, 3, 5 (10)-15-tetraene-17-ol

The collected material (54.7g, 0.143 mole) was dissolved in THF 300ml and a solution of cerium chloride heptahydrate (53.3g, 0.143 mole) in methanol (300ml) was added. The mixture was cooled to 0°C sodium borohydride (8.12g, 0.213 mole, 1.5eq) was added portion wise keeping the temperature below 9°C. At this end of the addition the mixture was stored for one hour then quenched by addition of a 2N HCl solution (100ml). The solution was partly evaporated in situ and water (4L) was added. The precipitate was collected by filtration and dried. After crystallization from a mixture of ethanol /diisopropyl ether the product was collected by filtration and dried. It weighted 46.6g (85% yield).

¹HNMR (CDCl₃) δ 0.20 (s, 6H, (CH₃)₂-Si-), 0.89 (s, 3H, CH₃ at C-18), 1.00 (s, 9H, (CH₃)₃-C-Si-), 1.20-2.40 (m, 10H), 2.75-2.95 (m, 2H), 4.40 (broad s, 1H, H17), 5.65-5.75 (m, 1H), 5.95-6.10 (m, 1H), 6.57 (broad s, 1H, H4), 6.60 (dd, 1H, H2), 7.13 (d, 1H, H1) mp: 107.5°C

Example 3: Preparation of a compound of formula (I) wherein P¹ is t-butyltrimethylsilyl according to an embodiment of the invention.

Step 1: 3 -t-butyltrimethylsiloxy-estra-1, 3, 5(10) -triene-17-one

To a solution of estrone (100g, 0.37 mole) in 400ml of dichloromethane, imidazole (50.36g, 0.74 mole) and t-butyl-dimethylsilyl chloride (61.3g, 0.41 mole) were added. The solution was stirred at room temperature for 24 hours. Then water (200ml) was added. The organic layer was partially evaporated and diisopropyl ether added. The white solid formed was collected by filtration and dried. It weighted 135.2g, yield 95%, mp 172°C.

¹HNMR (CDCl₃) δ 0.20 (s, 6H, (CH₃)₂-Si-), 0.90 (s, 3H, CH₃ at C-18), 1.00 (s, 9H, (CH₃)₃-C-Si-), 1.20-2.60 (m, 13H), 2.75-2.95 (m, 2H), 5.65-5.75 (m, 1H), 6.58 (broad s, 1H, H4), 6.63 (dd, 1H, H2), 7.12 (d, 1H, H1) mp: 171.6°C

Step 2: 3 -t-butyldimethylsiloxy-estra-1, 3, 5(10) -16-tetraene-17-acetate

3 -t-butyldimethylsiloxy-estra-1, 3, 5(10) -triene-17-one 135g (0.351 mole) were poured in 600ml of isopropenyl acetate and 12 g of para-toluene-sulfonic acid. The mixture was refluxed. Acetone and isopropenyl acetate were continuously distilled off until the internal
5 temperature reached 98°C. Then the mixture was cooled to 0°C and potassium carbonate added. After one hour at 0°C the mixture was filtered. The resulting solution was partially concentrated and diisopropyl ether added. The precipitate was collected by filtration and crystallized from a mixture of ethyl acetate and heptane. The product was collected by filtration and dried. It weighted 119.5g (yield 80%).

10 Step 3: 3 -t-butyldimethylsiloxy-estra-1, 3, 5 (10)-15-tetraene-17-one

To a solution of 3 -t-butyldimethylsiloxy-estra-1, 3, 5(10) -16-tetraene-17-acetate 119.5g (0.280 mole) in acetonitrile (1500ml) were added 27.2g (0.085 mole of tributyltin methoxide, 11.2 g (0.05 mole) of palladium acetate and 64 ml(0.560 mole) of allyl methyl carbonate. The mixture was refluxed for 2 hours then cooled to room temperature and
15 filtered through a pad of silica gel. The mixture was diluted with water and extracted with ethyl acetate. After concentration to one third of the initial volume diisopropyl ether was added and the solution cooled at 0°C for one hour.

The product was collected by filtration. It weighted 91g (85% yield) and was used in the next step without further purification.

20 ¹HNMR (CDCl₃) δ 0.20 (s, 6H, (CH₃)₂-Si-), 1.00 (s, 9H, (CH₃)₃-C-Si-), 1.13 (s, 3H, CH₃ at C-18), 1.20-2.70 (m, 11H), 2.80-3.00 (m, 2H), 6.10 (dd, 1H, H15), 6.58 (broad s, 1H, H4), 6.62 (dd, 1H, H2), 7.11 (d, 1H, H1), 7.63 (dd, 1H, H16), mp: 165°C

Step 4: 3 -t-butyldimethylsiloxy-estra-1, 3, 5 (10)-15-tetraene-17-ol

The reduction step was performed as described in step 3 of example 2: the collected
25 material was dissolved in THF and a solution of cerium chloride heptahydrate (1 eq) in methanol was added. The mixture was cooled to 0°C sodium borohydride (1.5eq) was added portion wise keeping the temperature below 9°C. At this end of the addition the mixture was stored for one hour then quenched by addition of a 2N HCl solution. The solution was partly evaporated in situ and water was added. The precipitate was collected
30 by filtration and dried. After crystallization from a mixture of ethanol /diisopropyl ether the product was collected by filtration and dried.

¹HNMR (CDCl₃) δ 0.20 (s, 6H, (CH₃)₂-Si-), 0.89 (s, 3H, CH₃ at C-18), 1.00 (s, 9H, (CH₃)₃-C-Si-), 1.20-2.40 (m, 10H), 2.75-2.95 (m, 2H), 4.40 (broad s, 1H, H17), 5.65-5.75 (m, 1H), 5.95-6.10 (m, 1H), 6.57 (broad s, 1H, H4), 6.60 (dd, 1H, H2), 7.13 (d, 1H, H1) mp: 107.5°C

5 Example 4:

Step 2 of Example 1 was repeated using different reagent and reactions conditions as listed in Table 1. 3-acetoxy-estra-1, 3, 5 (10), 15-tetraen-17-one was obtained. The yields and conversion rates are given in Table 1.

Table 1

Pd(OAc) ₂	Other reagents	Reaction conditions	Conversion rate (%)	Isolated Yield (%)
1.36 eq		THF, ACN, CH ₂ Cl ₂ , RT	≈ 90	18
0.08 eq	Allylmethyl carbonate (1,8 eq) tributyltin methoxide (0,3 eq)	ACN, 70°C	≈ 70	24
0.3 eq	Cu(OAc) ₂ (1 eq); O ₂	ACN, THF, 50°C	30	ND
0.1 eq	O ₂	DMSO, 80°C	≈ 70	ND
0.15 eq	O ₂	DMSO, CH ₂ Cl ₂ , 35°C	80	ND

- 10 THF: tetrahydrofuran; ACN acetonitrile; RT: room temperature; DMSO: dimethylsulfoxide; ND not determined.

Example 5:

- 15 Step 2 of Example 2 was repeated using different reagent and reactions conditions as listed in Table 2. 3-t-butyldimethylsiloxy-estra-1, 3, 5 (10)-15-tetraene-17-one was obtained. The yields and conversion rates are given in Table 2.

Table 2

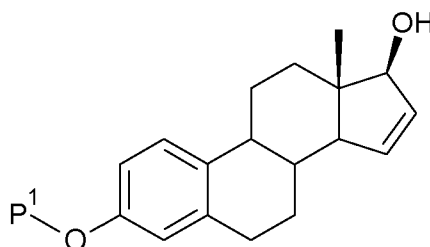
Pd(OAc) ₂	Other reagents	Reaction conditions	Conversion rate (%)	Isolated Yield (%)
1.4 eq		THF, RT	≈ 90	ND
0.1 eq	O ₂	DMSO, CH ₂ Cl ₂ , 35°C	≈ 100	71
0.1 eq	Cu(OAc) ₂ ; O ₂	DMSO, CH ₂ Cl ₂ , 35°C	≈ 100	64
0.1 eq	Air	DMSO, CH ₂ Cl ₂ , 35°C	≈ 95	65
0.1 eq	O ₂	DMSO, CH ₂ Cl ₂ , 35°C	≈ 100	93

THF: tetrahydrofuran; ACN acetonitrile; RT: room temperature; DMSO: dimethylsulfoxide; ND not determined.

It is to be understood that although preferred embodiments and/or materials have been discussed for providing embodiments according to the present invention, various modifications or changes may be made without departing from the scope and spirit of this invention.

CLAIMS

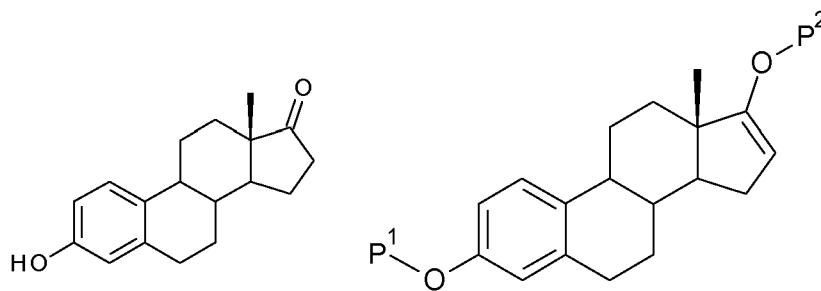
1. A process for the preparation of a compound of formula (I)



(I)

5 said process comprising the steps of

a) reacting a compound of formula (II), with an acylating or a silylating agent to produce a compound of formula (III), wherein P¹ and P² are each independently a protecting group selected from R²-Si-R³R⁴, or R¹CO-, wherein R¹ is a group selected from C₁₋₆alkyl or C₃₋₆cycloalkyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C₁₋₄alkyl; R², R³ and R⁴ are each independently a group selected from C₁₋₆alkyl or phenyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C₁₋₄alkyl;

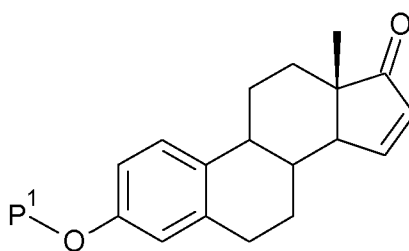


(II)

(III)

b) reacting the compound of formula (III) in the presence of palladium acetate or a derivative thereof to produce compound of formula (IV); and

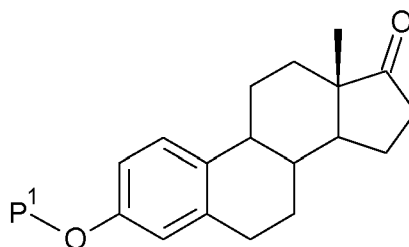
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(IV)

c) reacting the compound of formula (IV) with a reducing agent to produce compound of formula (I).

- 5 2. The process according to claim 1, wherein P¹ is R¹CO-.
3. The process according to claim 1, wherein P¹ is R²-Si-R³R⁴.
4. The process according to claim 3, wherein P² is R²-Si-R³R⁴.
5. The process according to any of claims 1 to 3, wherein P² is R¹CO-.
6. The process according to claim 5, wherein step (a) comprises the steps of (a1)
- 10 protecting the hydroxyl of compound of formula (II) with a silylating agent to produce a compound of formula (IIa), wherein P¹ has the same meaning as that defined in claim 3; and



(IIa)

- 15 (a2) protecting the ketone of compound of formula (IIa) in the presence of an acylating agent to produce compound of formula (III).
7. Process according to any of claims 1 to 3, 5, and 6 wherein the acylating agent is C₂₋₆alkenylC₁₋₆alkanoate or C₂₋₆alkenylC₃₋₆cycloalkanoate.

8. Process according to any of claims 1, 3 to 7, wherein the silylating agent is selected from the group comprising C₁₋₆alkylsilylchloride, C₁₋₆alkylsilyltriflate, phenylsilylchloride, phenylsilyltriflate, C₁₋₆alkylphenylsilylchloride, C₁₋₆alkylphenylsilyltriflate, each group being optionally substituted by one or more substituents independently selected from fluoro or C₁₋₄alkyl.
9. The process according to any of claims 1 to 8, wherein step (b) is performed in the presence of a C₁₋₆alkylene carbonate and an organotin compound.
10. The process according to any of claims 1 to 9, wherein said palladium acetate is present in stoichiometric amounts.
11. The process according to any of claims 1 to 9, wherein said reaction is performed with palladium acetate present in catalytic or sub-stoichiometric amounts, preferably the reaction is performed in an oxygen atmosphere.
12. The process according to any of claims 1 to 11, wherein the reducing agent in step (c) is selected from the group of metal hydride compounds.
13. The process according to claim 12, wherein the metal hydride compound is selected from the group comprising NaBH₄/CeCl₃, LiAlH₄, NaBH₄, NaBH(OAc)₃, and ZnBH₄.
14. Process for the preparation of estetrol, said process comprising preparing a compound of formula (I) by a process according to any of claims 1 to 13 and further reacting compound of formula (I) to produce estetrol.
15. Estetrol directly obtained by the process of claim 14 for use in a method selected from a method of hormone replacement therapy, a method of treating vaginal dryness, a method of contraception, a method of enhancing libido, of method of treating skin, a method of promoting wound healing, and a method of treating or preventing a disorder selected from the group consisting of autoimmune diseases, breast tumors and colorectal tumors.