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Articles

Synthesis of Trimegestone: The First Industrial Application of Bakers' Yeast Mediated Reduction of a Ketone

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Abstract:

Trimegestone (17α-methyl-17β-(2(*S*)-hydroxy-1-oxopropyl)-estra-4,9-dien-3-one) is a new progestomimetic molecule developed by Roussel Uclaf for the treatment of postmenopausal diseases. It is produced on an industrial scale from a related 3-ketal, 17-keto norsteroid intermediate in a nine-step sequence involving hydrocyanation of the 17-keto group, alkylation of the protected cyanohydrin with ethyl magnesium bromide to give a 17α-hydroxy 20-ketone, stereospecific 17α-methylation of the corresponding 17,20-enolate, and oxidation of the 20,-21-enolate with air and ketal deprotection to give 3,20,21-triketone. The key step of the synthesis is the chemo-, regio-, and almost stereospecific bioreduction of this triketone to the desired 21(*S*) alcohol, Trimegestone (de = 99%). This bioreduction is performed with bakers' yeast in water, thus showing the efficiency of this method at an industrial level.

Background

Trimegestone (**1**) (RU 27987) is a norsteroidal progestomimetic compound devoid of androgenic activity. It binds with high affinity to the receptors of progesterone and can directly stimulate human osteoblastic functions alone or, more efficiently, in association with estradiol.[1] Its low toxicity and its high activity at very low dosage make it a promising drug for the treatment of postmenopausal diseases.

Therefore, it has been selected for international clinical development, and its industrial synthesis has been studied (Scheme 1).

The strategy was to start from "ethylene deltenone" **2**, an intermediate of the Roussel Uclaf industrial synthesis of norsteroids, and to perform the necessary elaborations at the 17-position to obtain the ketone **3**. The major problem of this route was the stereospecific introduction of the 21(*S*) hydroxyl group (**3** → **1**).

Industrial Synthesis of Ketone 3

The industrial transformation of **2** into ketone **3** is depicted in Scheme 2. Though all these steps involve well-known chemistry on the laboratory scale, we had to solve all the problems related to industrial production, i.e., cost, selectivity and yield, but also taking into account aspects of safety, pollution, and the ease of purifications carried out. This was not straightforward, considering in particular the first step (release of hydrogen cyanide) and the last one (alkylation at −75 °C in liquid ammonia).

The hydrocyanation of **2** was performed with potassium cyanide (2 equiv) in methanol at 20 °C in the presence of acetic acid (1.5 equiv).[2] Potassium cyanide was used in excess with respect to this weak acid, so that hydrogen cyanide (pK = 9.1) can be generated *in situ* in limited quantities, and consumed progressively by the reaction. A stronger acid (e.g., H_2SO_4) causes too rapid a formation of hydrogen cyanide (bp = 25.7 °C), which is then partially eliminated in the gaseous effluent.

The very efficient formation of the β cyanohydrin results from the equilibrium between the kinetic α isomer and the thermodynamic β isomer, and also from the difference in solubility between both isomers. As the β cyanohydrin is the most insoluble compound in the reaction mixture, its crystallization causes a total shift of the overall equilibrium to the formation of β cyanohydrin (Scheme 3).[2b]

The initial ketone **2** itself is not totally soluble in the methanolic medium, so that the reaction mixture is always heterogeneous. At first glance this might have led to problems as the ketone tends to cocrystallize with the cyanohydrin. However, when conditions in which **2** is more soluble were studied (e.g., in the presence of a cosolvent, with larger amounts of acetic acid or with acetone cyanohydrin[2b,3]), side products appeared. So, degradation of **2** can be minimized by working in a constantly heterogeneous medium.

The cyanohydrin was isolated in 96% yield by simple filtration after addition of water to the reaction mixture. The alkaline medium ensured maximal safety by avoiding any release of hydrogen cyanide during the filtration.

(1) Bouali, Y.; Da Ponte, F.; Moura, A. M.; Philibert, D. Presented at the American Society for Bone and Mineral Research Meeting, Baltimore, September 1995.

(2) (a) Gasc, J. C.; Nédélec, L. *Tetrahedron Lett.* **1971**, 2005. (b) Nitta, I.; Fujimori, S.; Ueno, H. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 978 and references therein.

(3) Ercoli, A.; de Ruggieri, P. *J. Am. Chem. Soc.* **1953**, *75*, 650.

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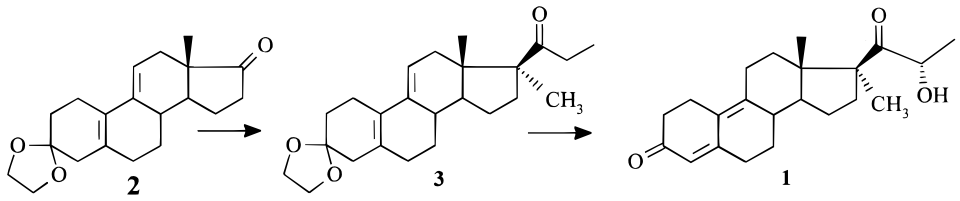
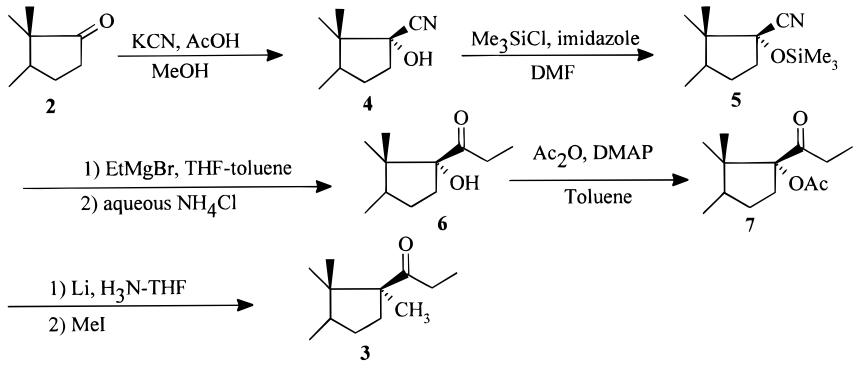
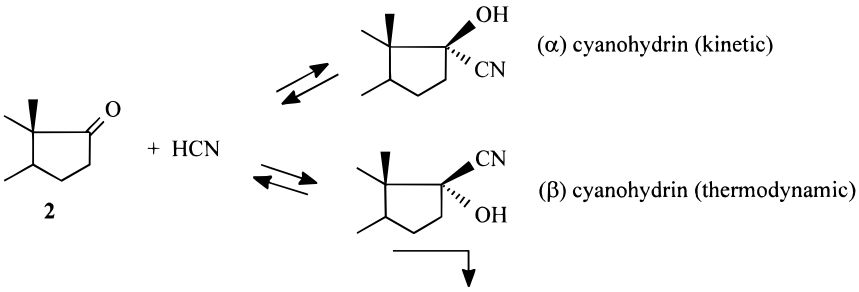
Scheme 1. **Key industrial intermediates**



Scheme 2. **Synthesis of ketone 3**



Scheme 3. **Hydrocyanation of 2**



No α cyanohydrin could be detected in the isolated product. This is of no strategic interest, since the stereochemistry is lost by the formation of the 17,20 enolate and reintroduced definitively at the α methylation step of the latter (*vide infra)*. However, the stereochemical purity of the intermediates is helpful with regard to their purification: the crystallization of a pure compound is easier than that of an isomeric mixture.

Clearly the Grignard reaction on the nitrile group cannot be performed on the unprotected cyanohydrin, because the basic Grignard reagent would first promote its degradation into ketone **2**. Thus, the hydroxyl function was protected as a trimethylsilyl ether, a group which is introduced and cleaved under mild conditions.[4]

Silylation of **4** was performed under neutral conditions with trimethylchlorosilane (1.3 equiv) and imidazole (1.5 equiv) in DMF at 25 °C, giving **5** as the sole product, which was not isolated and was used as a toluene solution for the next step.

Usually, the Grignard reactions of simpler cyanohydrin silyl ethers are performed at lower temperatures (below 37 °C) and with only a slight excess of Grignard reagent, with the hydrolysis of the intermediate silylated iminium salt demanding highly acidic conditions.[5]

On the contrary, the alkylation of **5** was sluggish and required forcing conditions: high temperature (65 °C), a long reaction time (over 18 h), and a high concentration of Grignard reagent (above 1.4 M). Large amounts of ethylmagnesium bromide were also necessary (6−7 equiv).

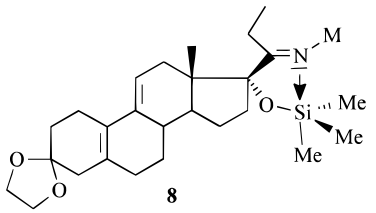
On the other hand, the hydrolysis was instantaneous in aqueous ammonium chloride at 10 °C.

The course of the reaction is noteworthy. TLC monitoring after quenching in aqueous NH_4Cl showed the following: at the end of introduction of the Grignard reagent, no **6** was present, but a mixture of starting material **5** and another product which was slightly more polar than **6**; after 5 h at 65 °C, no **5** was left, but a mixture of ketol **6** and the unknown compound from above; finally, after 18 h at 65 °C, **6** was by far the main product and was isolated in 80% yield after crystallization from isopropyl ether.

These results are consistent with the formation of an intermediate alkylated in the reaction medium before being hydrolyzed into **6**. Though we were unable to isolate it because of its instability, we propose the structure to be a silylated iminium salt[5a] **8**, which is probably a pentacoordinated silicon complex.

Thus, the first step is probably a rapid alkylation of **5** to **8a**, which under our conditions of smooth hydrolysis (aqueous NH_4Cl) could be transformed into **8b**, the unstable compound we could detect but not isolate.

(4) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Chemistry*, 2nd ed.; J. Wiley and Sons, Inc.: New York, 1991.
(5) (a) Gill, M.; Kiefel, M. J.; Lally, D. A. *Tetrahedron Lett*. **1986**, *27*, 1933. (b) Still, I. W. J.; Drewery, M. J. *J. Org. Chem*. **1989**, *54*, 290.



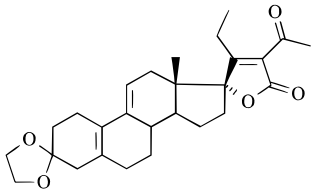
8

8a : M = MgBr

8b : M = H

This is also consistent with earlier studies in which the hydrolysis of the silylated iminium salt led to the ketol only under very acidic conditions.[5] Due to its pentacoordinated structure,[6] **8a** then could be alkylated at the silicon to the salt **9**, which on hydrolysis would give ketol **6** under very mild conditions (Scheme 4).

Because of steric hindrance around the 17-hydroxyl group, acetylation of **6** also required harsh conditions: 2.5 equiv of acetic anhydride, 0.3 equiv of DMAP, 23 h at 110 °C in toluene. Acetate **7** was isolated in 78% yield after crystallization from methanol. The main byproduct was lactone **10** (ca. 10%) resulting from an intramolecular aldolisation of the acetate **7** and subsequent acylation.



10

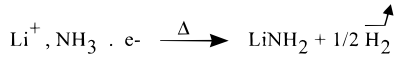
Acetylation with acid activation was ineffective: even the DCC−DMAP system, the most efficient reagent for sterically hindered alcohols,[7] gave only 20% acetylation after 3 days at 20 °C in methylene chloride (1.1 equiv of acetic acid, 1.2 equiv of DCC, 0.1 equiv of DMAP).

The 17α methyl group was then introduced according to the Weiss procedure[8] by generation of the 17,20 lithium enolate with lithium in a liquid ammonia−THF mixture at −75 °C and quenching of the enolate with methyl iodide at the same temperature (Scheme 5).

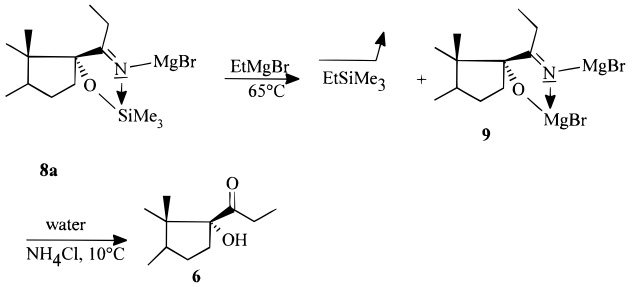
This alkylation occurs stereospecifically from the α face. No trace of the β methyl isomer could be detected, either in the crystallized product or in its mother liquors.

The reaction was relatively clean, yielding ca. 80% pure **3** after crystallization from methanol (multikilogram scale). The main byproduct was lactol **11** (ca. 10%), the formation of which was increased by higher temperatures (above −70 °C) or traces of water. This lactol results from an enolization process of the 17α acetate (Scheme 6).

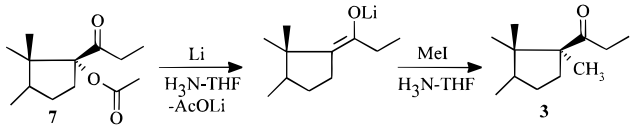
Under strictly anhydrous conditions, the base responsible for this enolization is lithium amide, which could result from a thermal decomposition of the solvated electron in ammonia:



Scheme 4. **Proposed alkylation of intermediate 8**



Scheme 5. **Alkylation at C-17**



Therefore, the temperature had to be kept as low as possible during the reaction (−75 °C in the industrial process). Higher temperatures or uncontrolled exotherms led to higher amounts of **11**.

Preparative Synthesis of Trimegestone

In order to produce the first amounts of Trimegestone needed for development, the following preparative route was first checked at the laboratory scale and then used in the pilot plant without further process study[9] (Scheme 7).

The potassium enolate of **3** was oxidized by air in the presence of triethylphosphite in DMF,[10] so that the intermediate hydroperoxide could be reduced *in situ* into ketol **12** (diastereoisomeric ratio *S*/*R* = 6/4) (Scheme 8). As the α ketol moiety is sensitive to acidic conditions, **12** is protected as acetate **13** before the ketal cleavage is performed. Methanolysis of **14** followed by separation of the isomers by preparative HPLC yielded after crystallization from diethyl ether 28% pure **1** and 19% pure **15** (overall yields from **3**; multikilogram scale).

The 21(*R*) isomer **15** was converted to Trimegestone as depicted in Scheme 9.

The mesylate derived from **15** was substituted by the acetate ion with inversion of configuration. Then methanolysis of **14a** and purification by chromatography followed by crystallization from diethyl ether yielded 58% pure Trimegestone.

However, although we were able to produce the required amounts of Trimegestone in the pilot plant by this route, we thought the scheme oulined above was not efficient enough for production purposes, for three main reasons: (1) the overall yield is low (39% from **3**, including inversion of the *R* isomer to give the *S* isomer), (2) the separation of both isomers by HPLC is tedious and expensive, and (3) the overall scheme is long (seven steps from **3** including the transformation of the *R* isomer).

Alternative Routes

To avoid these drawbacks other solutions were sought. The first one was to hydroxylate **3** in a diastereoselective way (Scheme 10).

(6) (a) Carré, F.; Cerveau, G.; Chuit, C.; Corriu, R.; Reyé, C. *New J. Chem.* **1992**, *16*, 63. (b) Boudin, A.; Cerveau, G.; Chuit, C.; Corriu, R.; Reyé, C. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 101.

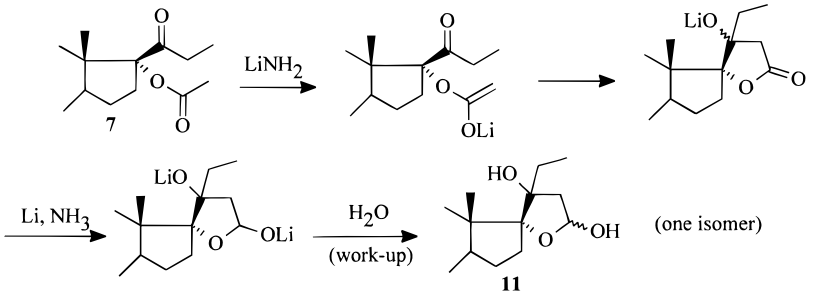
(7) (a) Haslam, E. *Tetrahedron* **1980**, *36*, 2409. (b) Neises, B.; Steglich, W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 522.

(8) Weiss, M. J.; Schaub, R. E.; Allen, G. R., Jr.; Poletto, J. F.; Pidacks, C.; Conrow, R. B.; Coscia, C. J. *Tetrahedron*, **1964**, *20*, 357.

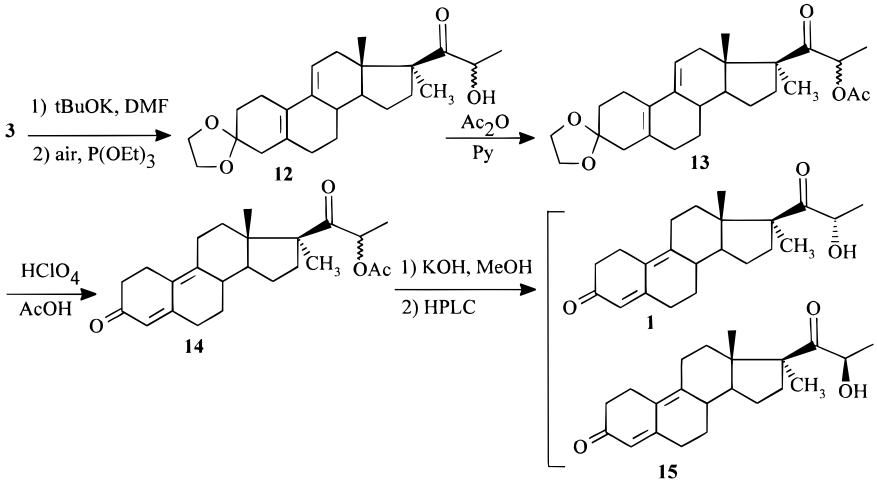
(9) (a) Coussedière, D. EP 7,823 (Roussel Uclaf). (b) Coussedière, D. EP 46,-001 (Roussel Uclaf).

(10) Gardner, J. N.; Carlon, F. E.; Gnoj, O. *J. Org. Chem.* **1968**, *33*, 3294.

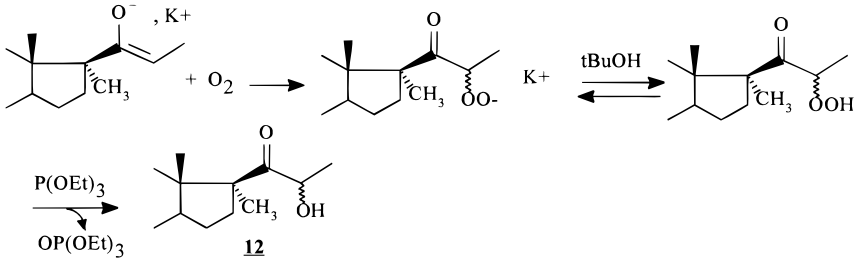
Scheme 6. **Proposed pathway to 11**



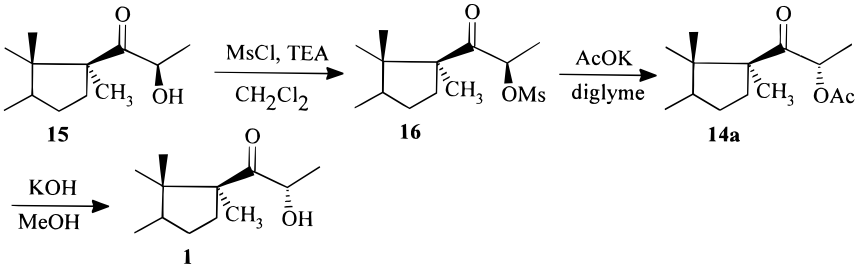
Scheme 7. **Preparative route to Trimegestone**



Scheme 8. **α-Hydroxylation of 3**



Scheme 9. **Inversion of isomer 15 to Trimegestone**



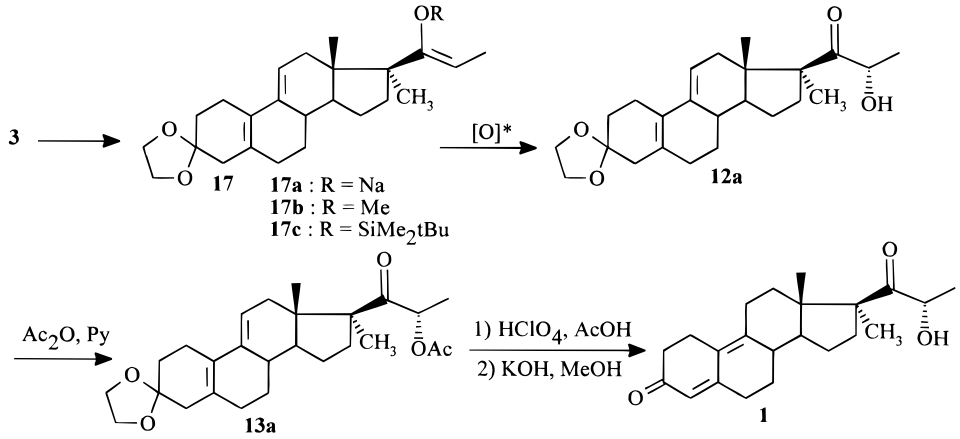
Among the various possible catalytic methods, the simplest and the most selective solutions to this problem would have been, for example, (1) to oxidize the sodium enolate **17a** with molecular oxygen in the presence of triethylphosphite and a chiral phase transfer catalyst,[11] and (2) to dihydroxylate the methyl enol ether **17b** or the silyl enol ether **17c** with Sharpless' "AD mix α".[12] However, these methods have also some drawbacks with regard to scaling up: the chiral inducers are expensive, so they must be recovered and recycled; the enantioselectivity is seldom total, which implies the need for purification steps; the overall route is long (four to five steps from **3** to **1**); in the case of the asymmetric dihydroxylation, any trace of osmium must be removed totally from the product, for safety reasons. Therefore, another route[13] was then tested on a laboratory scale, based on the diastereoselective biohydrolysis of acetate **14** (Scheme 11).

(11) Masui, M.; Ando, A.; Shioiri, T. *Tetrahedron Lett.* **1988**, *29*, 2835.

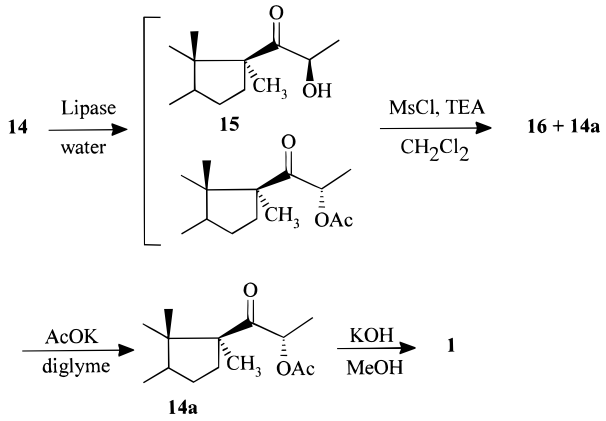
(12) (a) Hashiyama, T.; Morikawa, K.; Sharpless, K. B. *J. Org. Chem.* **1992**, *57*, 5067. (b) Kolb, H. C.; Van Nieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.

(13) Buendia, J.; Godard, J. Y.; Mackiewicz, P.; Richard, C. EP 581,649 (Roussel Uclaf).

Scheme 10. **Diastereoselective hydroxylation**



Scheme 11. **Enzymatic hydrolysis**



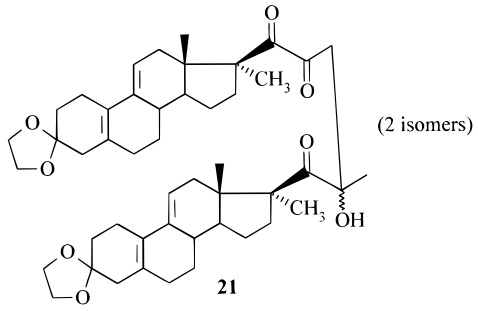
Among the various esterases and lipases tested in a water−*n*-butanol mixture at 37 °C, lipase PS Amano (*Pseudomonas cepacia)* gave the best results: at 49% conversion, 21*(R)* acetate was almost exclusively hydrolyzed (de ≥99%), so that the remaining 21*(S)* acetate **14a** was also optically very pure (de ca. 96%). The crude mixture was then treated with first methanesulfonyl chloride,[14] and then potassium acetate in diglyme in order to transform the 21*(R)* hydroxyl group into 21*(R)* mesylate and then into 21*(S)* acetate, whereas the unreacted 21*(S)* acetate **14a** remained unchanged.

Methanolysis gave finally Trimegestone in a 67% overall yield from **14** (gram scale). Unfortunately, the optical purity of the ketol so obtained was only 95%: some epimerization of the 21(*S*) acetate occurred under the conditions of the nucleophilic substitution of the mesylate. This is the main drawback of this route, the second one being the cost of the lipase, used here in large quantities.

Industrial Synthesis of Trimegestone

We then focused our efforts on a shorter route, based on the selective reduction of the 20,21 diketone moiety to the 21(*S*) ketol[15] (Scheme 12). Thus, the potassium enolate **3** was submitted to oxidation by molecular oxygen in DMF or preferentially in dimethylacetamide[16] (DMAC) at −15 °C, in the absence of reducing agent.[17] Under these conditions the intermediate hydroperoxide **20** was decomposed *in situ* by the base, giving diketone **18** in a 84% yield (Scheme 13).

The main byproduct of this step was aldol **21**, formed in the presence of a large excess of potassium *tert*-butylate (over 2 equiv). Therefore, its formation could easily be controlled and minimized.



The deketalization of diketone **18** was best performed in acetic acid solution, in the presence of small quantities of water and a very strong acid. Hydrochloric acid was as efficient as perchloric acid, which had been used in the preparative synthesis (Scheme 7), without the safety problems of the latter.

In acetic acid, ethylene diacetate was detected in the medium, thus proving that the reaction is not a hydrolysis but a solvolysis (Scheme 14).

For the selective reduction of triketone **19** into Trimegestone, only those systems that have the potential to avoid the side reduction of the 4 and 9 double bonds were selected. The reduction products from such reagents are shown in Scheme 15.

First, some achiral metal hydrides were examined. DIBAL-H[18] (1 equiv) reacted very slowly at −65 °C in methylene chloride (26% conversion after 2.5 h), giving a complex mixture of reduction products with almost no stereoselectivity at C-21. The results obtained with other hydrides in THF are depicted in Table 1.

(14) Danda, H.; Nagatomi, T.; Maehara, A.; Umemura, T. *Tetrahedron* **1991**, *47*, 8701.

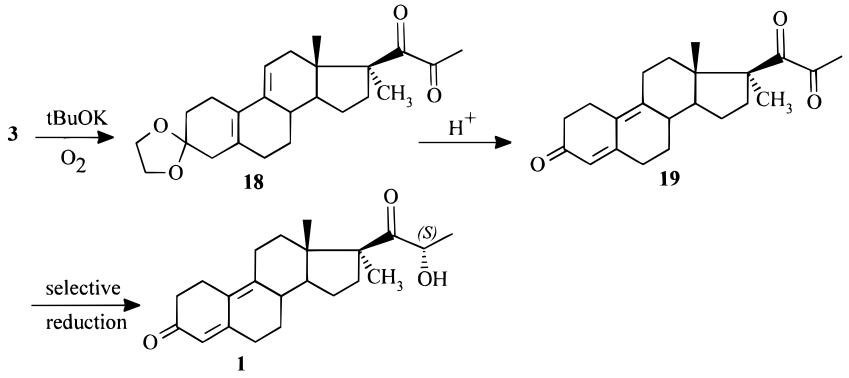
(15) Buendia, J.; Crocq, V.; Masson, C.; Prat, D.; Vivat, M. EP 574,317 (Roussel Uclaf).

(16) Differential thermic analysis of potassium *tert*-butylate solutions in the presence of air at 21 °C showed no oxidation process in DMAC and a slightly exothermic phenomenon in DMF. On the other hand, *N*-methylpyrrolidone gave a rapid and exothermic reaction even at 0 °C.

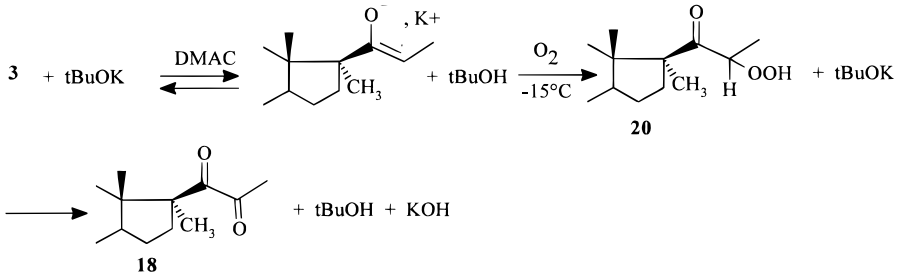
(17) (a) Bailey, E. J.; Barton, D. H. R.; Elks, J.; Templeton, J. F. *J. Chem. Soc.* **1962**, 1578 and references cited. (b) Hanna, R.; Ourisson, G. *Bull. Soc. Chim. Fr.* **1961**, 1945.

(18) (a) Wilson, K. E.; Seidner, R. T.; Masamune, S. *J. Chem. Soc., Chem. Commun.* **1970**, 213. (b) Winterfeldt, E. *Synthesis* **1975**, 617. (c) Nicolaou, K. C.; Kubota, S.; Li, W. S. *J. Chem. Soc., Chem. Commun.* **1989**, 512.

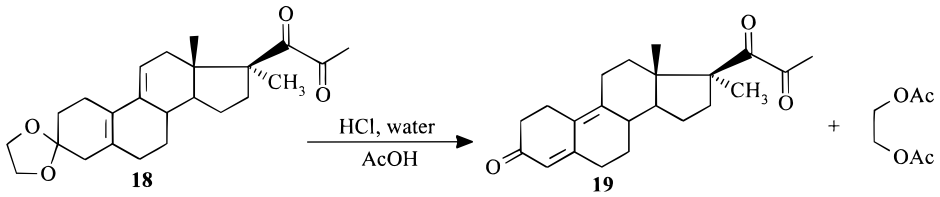
Scheme 12. **Industrial transformation of 3 to Trimegestone**



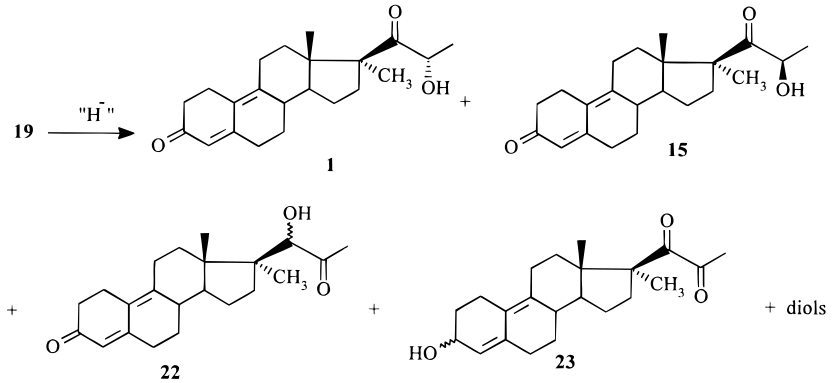
Scheme 13. **Oxidation of 3**



Scheme 14. **Deketalization of 18**



Scheme 15. **Products of the chemical reduction of 19**



L-Selectride[19] and K-Selectride[20] reacted similarly, with a high conversion but a poor overall selectivity (less than 20% **1** formed). Nevertheless, the main reduction product at C-21 is the *S* isomer (*S*/*R* = 9/1) (entries 1 and 2).

Lithium tris-*tert*-butoxyaluminium hydride[21] gave the most promising results: an almost complete conversion and a high chemo-, regio-, and stereoselectivity of reduction at C-21 (*S*/*R* = 95/5) (entry 3). Under these conditions,

Table 1. **Chemical reduction (%) of triketone 19**[a]

entry	hydride	21(*S*) OH **1**	21(*R*) OH **15**	20-OH **22**	triketone **19**
1	L-Selectride	19	2	17	8
2	K-Selectride	17	1.6	10.5	2.6
3	$LiAlH(OtBu)_3$	77	3.7	8	0.5

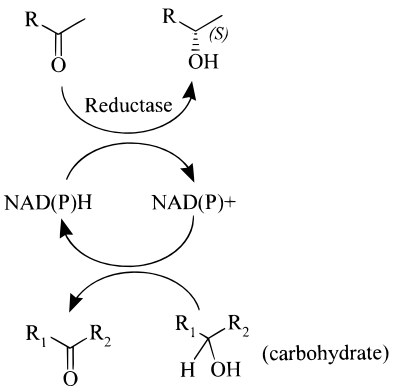
[a] Conditions: 1 equiv of hydride; 2.5 h at −65 °C; yields determined by quantitative HPLC (see Experimental Section).

Trimegestone was isolated in 68% yield and 93% purity after crystallization from isopropyl ether.

In order to improve the *Si*-selectivity at C-21, the CBS[22] system was then tested with the (*R*)-diphenyloxazaborolidine

(19) Bonnert, R. V.; Howarth, J.; Jenkins, P. R.; Lawrence, N. J. *J. Chem. Soc., Perkin Trans. 1*, **1991**, 1225.
(20) (a) Ganem, B. *J. Org. Chem.* **1975**, *40*, 146. (b) Fortunato, J. M.; Ganem, B. *J. Org. Chem.*, **1976**, *41*, 2194.
(21) (a) Burgstahler, A. W.; Nordin, I. C. *J. Am. Chem. Soc.* **1961**, *83*, 198. (b) Haubenstock, H. *J. Org. Chem.* **1972,** *37*, 656. (c) Paryzek, Z.; Martynow, J.; Strasko, M. *Synth. Commun.* **1989,** *19*, 439.

Scheme 16. **Bioreduction of ketone RCOMe by bakers' yeast**



(0.1 equiv). The reaction was slow in methylene chloride at 0 °C in the presence of BH_3·THF (1 equiv) and gave mainly the C-3 reduction product **23** (α/β ca. 1/1).[23]

Under these conditions the C-3 protected diketone **18** remained largely unchanged, and thus the CBS system does not react on the 20,21 diketone moiety.

In the same way, we tested biocatalysts, which are known for their very high selectivity.

Bakers' yeast (*Saccharomyces cerevisiae*) was selected because it seemed to be particularly applicable to our problem, giving very high "*Si*" enantioselectivity in the reduction of prochiral ketones bearing a carbonyl function in α or β position.[24,25]

Another advantage of resting cells such as bakers' yeast is that they do not need extra cofactor, as they possess all the necessary enzymes for cofactor regeneration. Only a hydrocarbonated substrate such as glucose is needed, and the overall reaction can be considered as a reduction of the ketone by this substrate[26] (Scheme 16).

The energy necessary for this reaction is also produced by the metabolism of the carbohydrate, i.e., glycolysis and, under aerobic conditions, respiration.[27]

Bakers' yeast has been used extensively to perform reductions at the laboratory scale,[24] and it has several advantages with regard to industrial applications: no toxicity or ecotoxicity; a constant quality, as a result of its use in the baking industry; and a very low price. The main drawback of bakers' yeast as a chemical tool is its low productivity, thus requiring the use of large amounts of yeast in highly diluted media. Moreover, the recovery of the product from the enzymatic medium is tedious, particularly the filtration stage. Therefore, to our knowledge it has not been hitherto used on an industrial scale.

(22) For recent reviews, see: (a) Singh, V. K. *Synthesis*, **1992**, 605. (b) Wallbaum, S.; Martens, J. *Tetrahedron: Asymmetry* **1992**, *3*, 1475.
(23) The (*S*)-diphenyloxazaborolidine gave also only C-3 reduction (α/β ca. 1/3).
(24) Seebach, D.; Sutter, M. A.; Weber, R. H.; Züger, M. F. *Org. Synth.* **1984**, *63*, 1.
(25) For recent reviews see: (a) Servi, S. *Synthesis* **1990**, 1. (b) Csuk, R.; Glänzer, B. I. *Chem. Rev.* **1991**, *91*, 49. (c) Faber K. *Biotransformations in Organic Chemistry—A Textbook*, 2nd ed.; Springer Verlag: New York, 1992. (d) Halgas J. *Biocatalysts in Organic Synthesis*; Elsevier: Amsterdam, 1992.
(26) Kometani, T.; Morita , Y.; Furui, H.; Yoshii, H.; Matsuno, R. *J. Fermen. Bioeng.* **1994**, *77*, 13.
(27) Curtis H. *Biology* 4th ed.; Worth Publishers, Inc.: New York, 1983; Chapter 9.

Scheme 17. **Analogous reduction of a 20,21 diketone**

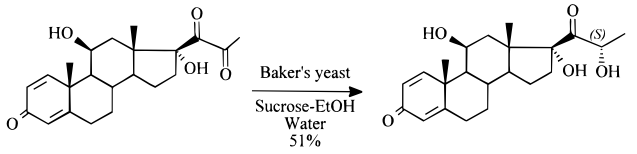


Table 2. **Reduction of 19 by bakers' yeast**[a]

entry	yeast (g/g)	substrate (g/g)	conversion (%)
1	120	saccharose (12)	64
2	120	glucose (12)	71
3	120	glycerol (12)	76
4	240	glucose (8)	74
5	240	glucose (24)	93
6	240	glycerol (24)	97
7	240	glycerol (24)	57

[a] Conditions: 2 g of **19**; total volume = 1.61; pH = 5.0; T = 40 °C; entries 1−6, air flow rate = 50 L/h; entry 7, N_2 atmosphere.

Nevertheless, this bioreduction has been described in cortisonic series at the laboratory scale[28] (Scheme 17).

In a preliminary test, triketone **19** was transformed very selectively into Trimegestone (yield = 49%; ee = 99.5%; 100 mg scale).[15] Hence we decided to set up our industrial synthesis of Trimegestone using this bakers' yeast mediated reduction, and to begin an optimization study of this reaction.

At the start the main difficulties were an incomplete reaction, a high dilution of the substrate in the medium, a tedious filtration of the yeast, and the necessary purification of crude Trimegestone by a column chromatography.

Optimization of the Bakers' Yeast Mediated Reduction

pH. This parameter was not critical; the efficiency of the yeast was the same at pH 4.0 as at pH 7.0. However, too acidic conditions (under pH 3) stopped the reduction definitively, probably because of the lysis of the yeast.

Temperature. The ideal temperature for the reduction was 40 ± 2 °C; at temperatures greater than 42 °C the productivity of the yeast decreased, probably because of thermic lysis. At less than 38 °C the reduction required longer times (e.g., 16 h at 30 °C).

Carbohydrated Substrate. Some carbohydrates were compared as feeding substrates in the presence of a limited amount of yeast: the conversion into Trimegestone was higher with glycerol than with saccharose or glucose (entries 1−3, Table 2). The other drawback of these two latter substrates is that they were metabolized rapidly by yeast before the reduction, causing an important release of carbon dioxide with the formation of foams and a rapid acidification of the medium. Large amounts of ammonium hydroxide were then needed to maintain the pH.

On the other hand, glycerol was steadily metabolized along with the bioreduction, without foam, and the pH could be regulated very easily. So, glycerol is particularly well adapted to the process because its rate of metabolism is similar to that of the bioreduction.

About 10% (weight) of carbohydrate substrate with respect to the yeast was needed to ensure an almost complete

(28) Agnello, E. J.; et al. *J. Org. Chem.* **1963**, *28*, 1531.

Scheme 18. **Proposed scheme for the bioreduction of 19 in the presence of glycerol**

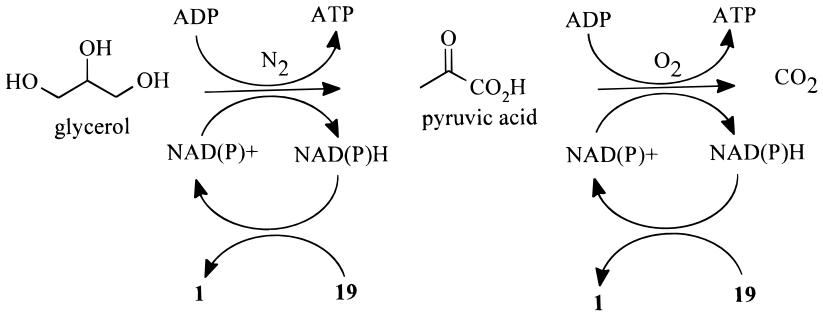


Table 3. **Optimization of the amount of yeast**[a]

entry	mass of yeast/ mass of **19**	maximum conversion
1	120	76%
2	170	90%
3	180	94%
4	240	98%

[a] Conditions: substrate, glycerol (10% weight/yeast); pH = 5.0; $T = 40 \pm 1$ °C; 2 g of **19**; total volume = 2 L; maximal time = 5 h.

conversion of the triketone (entries 4−6); however, some reduction also took place with no substrate added (about 40% under the same conditions), due to the metabolization of the sugar reserves of the yeast.

Oxygenation. Though the bioreduction was far more efficient under aerobic conditions, some reaction was observed also under anaerobic conditions, with glycerol as a substrate (entries 6 and 7). Thus, the cofactor NAD(P)H is regenerated during both the proposed glycolytic process of changing glycerol into pyruvic acid and the respiratory chain producing carbon dioxide (Scheme 18).

The reaction medium must be aerated, but the efficiency of the oxygen transfer is more important than the flow rate of air itself. So, the flow rate must be adapted to the apparatus used for the bioreduction.

Yeast. The main problem with the bioreduction using *S. cerevisiae* is the large amount of yeast needed for a high conversion (≥97%) of triketone **19** (Table 3). Hence, in order to increase the productivity of this process, screening of yeasts was carried out. The most promising ones are listed in Table 4. Though most of the samples tested displayed a better productivity than *S. cerevisiae*, the difference was not large enough to be of interest economically, due to the cost of the culture and the control of the yeast. In fact, bakers' yeast is very cheap and can be used directly in the commercial pressed form (33% dry content). Though the large amounts of yeast required a highly diluted medium (over 500 L/kg of triketone), the reaction solvent was water, an ideal solvent for industrial purposes. Moreover, no special apparatus such as a fermentor was needed for the process; for example, a classical 1000 L stainless steel vessel was used routinely at the pilot plant.

Morphology of the Triketone. The triketone is poorly soluble in the reaction solvent, water (0.01%). In order to improve its consumption by the yeast, it must thus be introduced in the most finely divided form possible or, more accurately, in the form having the largest specific area. The addition of a cosolvent such as 2-propanol, acetone, or DMF

Table 4. **Comparison of the productivity of the yeasts tested**

yeast[a]	MUCL no.	time (h)	specific production[b]
Saccharomyces chevalieri	27815	24	97
Saccharomyces hienipiensis	27820	24	82
Saccharomyces italicus	27822	24	48
Saccharomyces uvarum	27835	144	45
Octosporomyces japonicus	27840	24	45
Octosporomyces octosporus	27842	24	120
Saccharomyces carlsbergensis	28756	48	67
Saccharomyces pastorianus	29299	23	40
Kluyveromyces thermotolerans	28822	23	72
Kluyveromyces marxianus	27725	23	51
Schizosaccharomyces pombe	28824	23	95
Saccharomyces cerevisiae		23	45

[a] Yeasts from the Mycotheque de l'Université Catholique de Louvain. [b] Trimegestone produced (mg)/biomass (g).

was not possible as the solvents have a lethal effect on the yeast.

Workup. The workup is the other difficulty encountered in the bakers' yeast mediated reductions, because the filtration of the yeast cake is particularly tedious. In the case of a water-soluble compound, the reaction mixture can be centrifuged and the product collected in the supernatant. But Trimegestone crystallizes in the aqueous medium and is lost in the solid residue after centrifugation.

On the other hand, as it is soluble in ethanol−water or acetone−water mixtures, a simple addition of acetone to the reaction mixture totally solubilized Trimegestone in the liquid phase. Moreover, acetone caused an immediate lysis of the yeast and a rapid sedimentation of the fragments. Then, an addition of toluene followed by gentle stirring gave two easily separable phases: an upper organic phase (toluene−acetone) containing the crude Trimegestone and a lower aqueous phase (water−acetone) containing the solid yeast fragments, which were thus removed by simple decantation. These decantations can probably be performed continuously in pulsed columns. The solvents can be recycled, thus limiting the process cost and the environmental problems.

Washing of the organic phase with water and concentration *in vacuo* gave a 1/1 oily mixture of steroids and fatty acids from the yeast. Crude Trimegestone was crystallized by trituration in *n*-heptane, and the fatty acids were removed in the mother liquors.

Finally, crystallization in isopropyl ether yielded 75% pure Trimegestone (purity: 99%; multikilogram scale).

Selectivity. A mass balance study indicated a deficit in steroid: 5−10% was missing. This is probably the result

of a partial metabolism of the triketone or Trimegestone by the yeast.

The selectivity of the bioreduction was checked by HPLC analysis of the combined organic solution after decantations: they contained *ca.* 0.5% of 21(*R*) isomer and less than 0.2% of any other reduction impurity.

Thus, the reaction is chemo- and regiospecific and very diastereoselective (de = 99%).

Conclusion

In conclusion, these results show that bakers' yeast mediated reduction of ketones not only is a very useful method at the laboratory scale but also can be scaled up to an industrial level. As it requires high dilutions, its field of applications is limited to high-value substrates. In these cases, the selectivity of the reaction makes up for the dilution cost, since it avoids the chromatographic purifications. Moreover, no particular investment is necessary: the bioreduction can be performed in a classical stainless steel vessel. Finally, the separation of the product from the yeast can be performed by simple decantations.

Experimental Section

All the NMR, IR, mass spectra and quantitative HPLC were recorded and analyzed by our Analytical Department.

The ^{1}H NMR spectra were obtained using a 300 MHz instrument. Purity of the products was determined by HPLC analysis, by comparison with a standard. The preparative HPLC were performed at the pilot scale in a stainless steel column (diameter 45 cm) on silica gel supplied by Merck, ref 11763.

Bakers' yeast was purchased from Fould Springer. The usual bakery quality was used (pressed; dry content ca. 33%). All the reactions were performed under a nitrogen atmosphere, except for the enolization with lithium in liquid ammonia, which required an argon atmosphere. The gaseous effluents were washed in a tower charged with an appropriate solution, e.g., a bleach and soda solution for the cyanation reaction.

The yields and equivalents of reagents are not corrected for their purity.

TLC was carried out on 60F254 Silica gel coated plates (Merck) (UV 254 nm detection). The melting points were determined by differential scanning calorimetry (heating rate: 5 °C/min).

17β Cyanohydrin 4 (3,3-Ethylenedioxy-17α-hydroxy-17β-cyanoestra-5(10),9(11)-diene). To a stirred suspension of ketone **2** (100 g; MW = 314.4; 0.318 mol) and potassium cyanide (Prolabo, 96%; 41.4 g; 2 equiv) in methanol (800 mL) at 20 °C was added acetic acid (27.3 mL; 1.5 equiv) over 15 min at 20 °C. The white suspension was stirred for 18 h at 20 °C (TLC monitoring: $CHCl_3$−acetone, 97/3 (v/v)). Filtration, washing with water, and drying *in vacuo* gave cyanohydrin **4** (104.1 g; yield = 96%; purity = 95%): $C_{21}H_{27}NO_3$; MW = 341.4; mp = 175 °C; IR ($CHCl_3$, cm^{-1}) ν 3598, 2240; ^{1}H NMR ($CDCl_3$, ppm) δ 0.94 (s, 3H), 3.99 (s, 4H), 5.61 (bs, 1H), 6.84 (s, 1H).

Ketol 6 (3,3-Ethylenedioxy-17α-Hydroxy-17β-(1-oxopropyl)estra-5(10),9(11)-diene). To a stirred suspension of cyanohydrin **4** (100 g; 0.293 mol; purity = 95%) and imidazole (30 g; 1.5 equiv) in DMF (150 mL) at 20 °C was added trimethylchlorosilane (48 mL; 1.3 equiv) over 15 min. The mixture was stirred for 2 h at 25 °C; a total solubilization was observed, followed by the crystallization of imidazole hydrochloride (TLC monitoring: $CHCl_3$−acetone, 97/3 (v/v)). Toluene (400 mL) and water (200 mL) were then added. After decantation, the toluene solution of silylated cyanohydrin **5** was washed with water and dried over magnesium sulfate. This solution was added over 45 min to a 2 M solution of ethylmagnesium bromide in THF (950 mL; 6.5 equiv) at 65 °C. The mixture was stirred for 24 h at 65 °C (TLC monitoring: $CHCl_3$−acetone, 97/3 (v/v)) and poured carefully during 15 min into a 25% aqueous ammonium chloride solution at 0 °C (**CAUTION**: Release of ethane!). After decantation, the toluene phase was washed with water, dried ($MgSO_4$), filtrated on a silica gel plug, and concentrated. Crystallization from isopropyl ether gave ketol **6** (87.3 g; yield = 80%; purity = 94%) as white crystals: $C_{23}H_{32}O_4$; MW = 372.5; mp = 165 °C; IR ($CHCl_3$, cm^{-1}) ν 3618, 3513, 1706, 1691, 1640, 1617; ^{1}H NMR ($CDCl_3$, ppm) δ 0.66 (s, 3H), 1.07 (t, J = 7 Hz, 3H), 2.50 (dq, J = 18 and 7 Hz, 1H), 2.79 (bs, 1H), 2.80 (dq, J = 18 and 7 Hz, 1H), 3.99 (bs, 4H), 5.56 (m, 1H); MS (*m*/*z*) 372 (62, M^+), 315 (5), 297 (8), 99 (48), 86 (49), 57 (100).

Acetate 7 (3,3-Ethylenedioxy-17α-acetyl-17β-(1-oxopropyl)estra-5(10),9(11)-diene). To a stirred solution of ketol **6** (80 g; 0.215 mol; purity = 94%) and DMAP (8 g; 0.3 equiv) in toluene (240 mL) was added acetic anhydride (51.2 mL; 2.5 equiv) during 5 min at 110 °C. The brown solution was stirred for 23 h at 110 °C (TLC monitoring: $CHCl_3$−acetone, 97/3 (v/v)), cooled to 20 °C, and poured into a 20% aqueous solution of ammonium chloride (320 mL) at 20 °C. After stirring for 15 min, the aqueous phase was decanted and the organic phase was washed with saturated aqueous sodium hydrogen carbonate and water, then dried ($MgSO_4$), and concentrated. Recrystallization from methanol gave acetate **7** as white crystals (69.4 g; yield = 78%; purity = 97%): $C_{25}H_{34}O_5$; MW = 414.5; mp = 184 °C; IR ($CHCl_3$, cm^{-1}) ν 1729, 1715; ^{1}H NMR ($CDCl_3$, ppm) δ 0.59 (s, 3H), 1.05 (t, J = 7 Hz, 3H), 2.07 (s, 3H), 3.99 (bs, 4H), 5.58 (m, 1H); MS (FAB) *m*/*z* 415 (MH^+).

Lactone 10 was isolated by a chromatographic purification of the methanolic mother liquors (eluent: heptane−ethyl acetate, 8/2 (v/v)), as an oil: $C_{27}H_{34}O_5$; MW = 438.6; IR ($CHCl_3$, cm^{-1}) ν 1747, 1688, 1602; ^{1}H NMR ($CDCl_3$, ppm) δ 0.96 (s, 3H), 1.16 (t, J = 7 Hz, 3H), 2.58 (s, 3H), 3.98 (bs, 4H), 5.49 (m, 1H); MS (*m*/*z*) 438 (84, M^+), 366 (10), 257 (24), 167 (53), 99 (33), 86 (66), 43 (100).

Ketone 3 (3,3-Ethylenedioxy-17α-methyl-17β-(1-oxopropyl)estra-5(10),9(11)-diene). Ammonia (640 mL) was condensed in a strictly anhydrous 2 L glass vessel under an argon atmosphere (**CAUTION**: Lithium may react violently with nitrogen!). Lithium (2.68 g, 4.0 equiv) was added in small portions at −75 °C over 30 min, and then THF (800 mL) and a solution of acetate **7** (40 g; 96.5 mmol; purity = 97%) in THF (240 mL) were added over 10 min at −75 °C to the dark blue solution. Stirring was continued for 1 h at −75 °C, and methyl iodide (30 mL; 5 equiv) was added over 10 min at −75 °C: the mixture turned to pale yellow. After stirring for 1 h at −75 °C, the medium was allowed to warm

up slowly to 20 °C (**CAUTION**: Release of ammonia around −33 °C!). Most of the THF was then evaporated *in vacuo* and replaced by methylene chloride (400 mL) and water (400 mL). Decantation, washing with water, drying ($MgSO_4$), filtration on a silica gel pad, concentration, and crystallization from methanol gave ketone **3** as white crystals (25.1 g; yield = 70%; purity = 95%): $C_{24}H_{34}O_3$; MW = 370.5; mp = 105 °C; IR ($CHCl_3$, cm^{-1}) ν 1695; ^1H NMR ($CDCl_3$, ppm) δ 0.60 (s, 3H), 1.04 (t, J = 7 Hz, 3H), 1.12 (s, 3H), 3.98 (bs, 4H), 5.58 (m, 1H); MS (*m*/*z*) 370 (100, M^+).

Lactol 11 was obtained by a chromatographic purification of the methanolic mother liquors (eluent: hexane−ethyl acetate, 4/6 (v/v)) as a white powder: $C_{25}H_{36}O_5$; MW = 416.5; IR ($CHCl_3$, cm^{-1}) ν 3609, 3515; ^1H NMR ($CDCl_3$, ppm) δ 0.84 (s, 3H), 1.04 (t, J = 7 Hz, 3H), 3.98 (bs, 4H), 3.18 (bs, 1H), 4.96 (bs, 1H), 5.39 (dd, J = 5 and 8.5 Hz, 1H), 5.53 (m, 1H); MS (*m*/*z*) 416 (5, M^+), 415 (15), 414 (45), 372 (48), 315 (15), 298 (80), 297 (100), 253 (20) 225 (23), 211 (44).

Trimegestone via Ketol 12 (Preparative Synthesis). To a solution of potassium *tert*-butylate (8.37 g; 1.1 equiv) in DMF (350 mL) was added a solution of ketone **3** (25 g; 67.5 mmol; purity = 95%) in DMF (250 mL) during 5 min at 20 °C. The brown solution was cooled to −50 °C, and triethyl phosphite (21 mL; 1.8 equiv) was added. Dry air (flow rate: 13 L/h) was introduced at −50 °C for 3.5 h until completion (TLC monitoring: toluene−ethyl acetate, 8/2 (v/v)). Then, air was replaced by nitrogen and the mixture was poured at 0 °C into a 10% aqueous solution of acetic acid (200 mL). Extractions with isopropyl ether (8 × 125 mL), washing with water, drying over sodium sulfate, and concentration gave crude ketol **12** as an oily residue (30.2 g; theory, 26.1 g; $C_{24}H_{34}O_4$; MW = 386.5).

This residue was dissolved in pyridine (50 mL), and acetic anhydride (25 mL) was added. The solution was stirred for 7 h at 20 °C (TLC monitoring: toluene−ethyl acetate, 8/2 (v/v)) and then poured into water (625 mL) and methylene chloride (100 mL). The mixture was stirred for 1 h at 20 °C and decanted. Washing with 5 N hydrochloric acid, water, saturated aqueous sodium hydrogen carbonate, and again water, drying over sodium sulfate, and concentration gave a methylene chloride solution (50 mL) of acetate **13** (theory, 28.9 g; $C_{26}H_{36}O_5$; MW = 428.6).

To this solution were added acetic acid (250 mL) and then 65% aqueous perchloric acid (25 mL) at 20 °C. The mixture was stirred for 2 h at 20 °C (TLC monitoring: toluene−ethyl acetate, 5/5 (v/v)) and then poured over 5 min into water (600 mL) at 15 °C. The biphasic mixture was stirred for 15 min and decanted. The organic phase was washed successively with water, saturated aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), and concentrated to a final volume of 50 mL. Methylene chloride was then replaced by methanol by continuous distillation *in vacuo* at constant volume. Acetate **4** was obtained as a methanolic solution (theory, 25.9 g; $C_{24}H_{32}O_4$; MW = 384.5).

This solution was diluted with methanol (200 mL), and potassium hydroxide (0.3 g; 0.08 equiv) was added at 20 °C. The brown solution was stirred for 3 h at 20 °C (TLC monitoring: toluene−ethyl acetate, 5/5) and then neutralized with aqueous 0.1 N hydrogen chloride (50 mL). The mixture was concentrated *in vacuo* to 75 mL final volume. Methylene chloride (125 mL) and water (100 mL) were added. After decantation, the organic phase was washed with water, dried (Na_2SO_4), and concentrated. The crude mixture of **1** and **15** was purified by medium-pressure chromatography (10−15 atm) in a stainless steel column (diameter 8 cm) charged with silica gel (1 kg) (eluent: toluene−ethyl acetate, 9/1 (v/v)). Concentration of the homogeneous fractions and crystallization from diethyl ether gave **19**, **15**, **1**, and a mixture of **1** and **15** as detailed below.

*Triketone **19***: 1.77 g; yield = 8%.

*21(R) ketol **15** (17α-methyl-17β-(2(R)-hydroxy-1-oxopropyl)estra-4,9-dien-3-one):* (4.17 g; yield = 18%; $C_{22}H_{30}O_3$; MW = 342.5; mp = 190 °C; $[\alpha]^{20}_D$ = −340 (*c* = 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) ν 3465, 1686, 1652, 1606, 1588 (sh); ^1H NMR ($CDCl_3$, ppm) δ 0.81 (s, 3H), 1.16 (s, 3H), 1.32 (d, J = 7 Hz, 3H), 3.69 (d, J = 6.5 Hz, 1H), 4.56 (dq, J = 6.5 and 7 Hz, 1H), 5.68 (s, 1H); MS (*m*/*z*) 342 (34, M^+), 297 (10), 269 (100), 213 (15), 161 (30), 45 (62),

*Pure Trimegestone **1** (17α-methyl-17β-(2(S)-hydroxy-1-oxopropyl)estra-4,9-dien-3-one):* 5.43 g; yield = 23.5%; purity ≥99%; $C_{22}H_{30}O_3$; MW = 342.5; mp = 119 °C; $[\alpha]^{20}_D$ = −200 (*c* = 1, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) ν 3540, 1697, 1653, 1607, 1588 (sh); ^1H NMR ($CDCl_3$, ppm) δ 0.83 (s, 3H), 1.18 (s, 3H), 1.32 (d, J = 6.5 Hz, 3H), 2.93 (d, J = 10 Hz, 1H), 4.43 (dq, J = 10 and 6.5 Hz, 1H), 5.69 (s, 1H); MS (*m*/*z*) 342 (22, M^+), 297 (7), 269 (100), 213 (16), 161 (24), 45 (20).

*Mixture (75/25) of **1** and **15**:* 2.77 g; yield = 12%. This mixture was not crystallized. However, at the pilot plant, it was separated by an extra chromatography.

Mesylate 16 (17α-Methyl-17β-(2(*R*)-(methylsulfonyloxy)-1-oxopropyl)estra-4,9-dien-3-one). To a solution of ketol **15** (15 g; 43.8 mmol) in methylene chloride (150 mL) was added triethylamine (11 mL; 1.8 equiv) at 20 °C. The solution was cooled to 0 °C and methanesulfonyl chloride (5.1 mL; 1.5 equiv) was added. The mixture was stirred for 1 h at 20 °C (TLC monitoring: toluene−ethyl acetate, 6/4 (v/v)), and water (30 mL) was added. The biphasic mixture was stirred for 10 min at 20 °C and decanted. The organic phase was washed with water, dried (Na_2SO_4), and concentrated to give mesylate **16** (18.5 g; 100%; purity = 99%): $C_{23}H_{32}O_5S$; MW = 420.6; IR ($CHCl_3$, cm^{-1}) ν 1713, 1652, 1607, 1350, 1176; ^1H NMR ($CDCl_3$, ppm) δ 0.90 (s, 3H), 1.18 (s, 3H), 1.57 (d, J = 6.5 Hz, 3H), 5.52 (q, J = 6.5 Hz, 1H), 3.6 (s, 3H), 5.68 (s, 3H).

Acetate 14a (17α-Methyl-17β-(2(*S*)-(acetyloxy)-1-oxopropyl)estra-4,9-dien-3-one). To a solution of mesylate **16** (18.5 g; 43.8 mmol) in diglyme (113 mL) was added potassium acetate (12.9 g; 3 equiv). The mixture was stirred for 20 h at 90 °C (TLC monitoring: toluene−ethyl acetate, 5/5 (v/v), then cooled to 20 °C, and poured into water (300 mL). After stirring for 4 h at 20 °C, filtration and washing with water furnished acetate **14a** (15.9 g; yield = 94%): $C_{24}H_{32}O_4$; MW = 384.5; IR ($CHCl_3$, cm^{-1}) ν 1739, 1710, 1653, 1606; ^1H NMR ($CDCl_3$, ppm) δ 0.82 (s, 3H), 1.20 (s, 3H), 1.39 (d, J = 6.5 Hz, 3H), 2.11 (s, 3H), 5.42 (q, J = 6.5 Hz, 1H), 5.68 (s, 3H) (5−10% of the 21*(S)* isomer was detected by NMR).

Trimegestone from Acetate 14a. To a solution of acetate **14a** (15 g; 39 mmol) in methanol (150 mL) was added potassium hydroxide (0.174 g; 0.08 equiv) at 20 °C. The brown solution was stirred for 3 h at 20 °C (TLC monitoring: toluene−ethyl acetate, 5/5 (v/v)) and then neutralized by addition of 0.1 N aqueous hydrochloric acid (24 mL). Most of the methanol was evaporated, and methylene chloride (75 mL) and water (60 mL) were added. After decantation, the organic phase was washed with water, dried (Na_2SO_4), and concentrated. Crude Trimegestone was purified by medium-pressure column chromatography on silica gel (750 g; eluent, methylene chloride−acetone, 95/5 (v/v)), followed by a crystallization from diethyl ether. Pure Trimegestone (5.6 g; yield = 42%; purity >99%) was obtained as white crystals.

Diketone 18 (3,3-Ethylenedioxy-17α-methyl-17*β*-(1,2-dioxopropyl)estra-5(10),9(11)-diene). A 2 L glass reactor equipped with a mechanical stirrer and a gas input with efficient dispersion was charged with ketone **3** (100 g; 0.27 mol; purity = 95%) and DMAC (750 mL) under nitrogen at 20 °C. After cooling to −15 °C, a solution of potassium *tert*-butylate (60.6 g; 2 equiv) in DMAC (250 mL) was added over 5 min. Dry air was introduced to the brown solution (flow rate: 25 L/h) for 1.5 h at −15 °C (TLC monitoring: toluene−ethyl acetate, 92/8 (v/v)). The mixture was then poured into a 10% aqueous solution of sodium bisulfite (2 L) at 10 °C. Toluene (1 L) was added, and after stirring and decantation, the organic phase was washed with water, dried ($MgSO_4$), filtrated on a silica gel pad, and concentrated to give crude diketone **18** as a yellow solid (87.2 g; yield = 84%; purity = 88%): $C_{24}H_{32}O_4$; MW = 384.5; mp = 126 °C; IR ($CHCl_3$, cm^{-1}) ν 1715, 1694; ^1H NMR ($CDCl_3$, ppm) δ 0.66 (s, 3H), 1.26 (s, 3H), 2.32 (s, 3H), 3.98 (bs, 4H), 5.59 (m, 1H).

Aldol 21 was obtained by chromatography of crude **18** on a silica gel column (eluent: toluene−ethyl acetate, 92/8 (v/v) as a yellow powder: $C_{48}H_{64}O_8$, MW = 768.9; IR ($CHCl_3$, cm^{-1}) ν 3530, 1693; ^1H NMR ($CDCl_3$, ppm) δ 0.66 (s, 3H) 0.67 (s, 3H), 1.23 (s, 3H), 1.24 (s, 3H), 1.39 (s, 3H), 2.80 (d, J = 18 Hz, 1H), 3.53 (d, J = 18 Hz, 1H), 3.98 (bs, 8H), 4.18 (bs, 1H), 5.59 (m, 2H); MS (FAB), m/z 769 (MH^+).

Triketone 19 (17α-Methyl-17*β*-(1,2-dioxopropyl)estra-4,9-dien-3-one). A solution of crude diketone **18** (40 g; 0.104 mol; purity = 88%) in acetic acid (200 mL) was treated with water (4 mL) and aqueous 36% hydrochloric acid (4 mL) for 2 h at 20 °C (TLC monitoring: toluene−dioxane, 85/15 (v/v)). Water (800 mL) was then added over 15 min at 20 °C, together with seeding crystals of pure triketone **19**. After stirring for 2 h at 20 °C, filtration and washings with water gave crude triketone **19** (34.6 g; purity = 85%) as a yellow amorphous solid. Crystallization from 2-propanol furnished in two crops pure triketone **19** (26 g; yield = 73%; purity = 96%) as yellow crystals: $C_{22}H_{28}O_3$; MW = 340.5; mp = 101 °C; IR ($CHCl_3$, cm^{-1}) ν 1716, 1694, 1653, 1607; ^1H NMR ($CDCl_3$, ppm) δ 0.86 (s, 3H), 1.26 (s, 3H), 2.33 (s, 3H), 5.68 (s, 1H).

Reduction of Triketone 19 by Metal Hydrides. Commercial (Aldrich) 1 M solutions of hydrides in THF (CH_2Cl_2 for DIBALH) were used.

To a solution of triketone **19** (2 g; 5.87 mmol; purity = 96%) in THF (20 mL) (CH_2Cl_2 for DIBALH) was added 5.9 mL of the metal hydride solution (1 equiv) over 5 min at −65 °C. The mixture was stirred for 2.5 h at −65 °C (TLC monitoring: toluene−dioxane, 85/15 (v/v)) and allowed to warm to 0 °C. Then it was poured into a 15% aqueous solution of ammonium chloride. The products were extracted with methylene chloride (2 × 20 mL), and the organic solution was washed with water, dried over magnesium sulfate, and evaporated. The crude mixture was analyzed by quantitative HPLC with respect to a standard of each component. HPLC conditions: symmetry C18, 5 μm, length = 25 cm, diameter = 4.6 mm; eluent, acetonitrile−water−AcOH, gradient 40/59/1 to 95/4/1 (v/v); flow rate, 1.3 mL/min; detection UV 280 nm; injection = 20 μL of a 0.05% solution.

Reduction of Triketone 19 by Cultured Yeasts. *Medium.* Each yeast strain was grown in the following medium (amounts per liter): KH_2PO_4. (6 g), $Na_2HPO_4 \cdot 12H_2O$ (6 g), $(NH_4)_2SO_4$ (10 g). After autoclaving 20 min at 121 °C, the following solutions were added: 1 mL/L of autoclaved 25% $CaCl_2 \cdot 2H_2O$ and 1 mL/L of autoclaved 25% $MgSO_4 \cdot 7H_2O$ plus $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (150mg), $ZnSO_4 \cdot 7H_2O$ (500 mg), citric acid (100 mg), and 2 mL/L sterilized vitamin solution (biotin, 5 mg; *meso*-inositol, 10 g; sodium pantothenate, 1 g; nicotinic acid, 1 g; pyridoxine hydrochloride, 700 mg; distilled water, 100 mL).

Culture Conditions. Cultures were carried out in 500 mL conical flasks containing 100 mL of medium inoculated with 0.5 mL of 16% glycerol. Flasks were incubated for 24 h at 30 °C and at 150 rpm in a 2.5 cm excentric orbital shaker. The optical density at 600 nm was read on a KONTRON 930 spectrophotometer after 1/20 dilution.

Bioconversion Conditions. Bioconversions were assayed in 100 mL magnetically stirred (400 rpm) conical flasks equipped with an aeration system. Bioconversion was started as follows: 25 mL of fresh medium, 25 mL of the culture described above, and 50 mg of triketone **19**. Flasks were incubated at 30 °C with microaeration in a water bath. Samples were taken for analysis of total dry weight, substrate, and product. The proportion of solubilized product was estimated to be 33% of the total Trimegestone formed. On the basis of these data, final biomass concentration was estimated and Trimegestone/biomass ratio was calculated.

Trimegestone 1 by Reduction of 19 with Bakers' Yeast. A 10 L glass reactor equipped with a "sparger" air input and an efficient stirrer was charged with water (4 L), potassium dihydrogen phosphate (12 g), disodium hydrogen phosphate (12 g), glycerol (240 g), and structol J673 (2 mL; antifoaming agent). Air (100 L/h) was introduced into the medium with vigorous stirring, and bakers' yeast (2.4 kg) was introduced during 10 min at 20 °C. The acidity was maintained at pH = 5.0 ± 0.2 by addition of ammonium hydroxide (1 M aqueous solution) or phosphoric acid (1 M aqueous solution). An aqueous suspension (1.4 L) of triketone **18** (10 g; 29.4 mmol; purity = 96%; particle size <200 μm) was added over 15 min, while the mixture was warmed to 40 °C. Air introduction and stirring at 40 °C and pH = 5.0 were continued for 6 h. Then, the input of air was stopped and replaced by a nitrogen atmosphere.

Acetone (8 L) was added, and the mixture was stirred for 1 h at 20 °C. Toluene (2 L) was added, and the biphasic medium was decanted. The aqueous phase containing the residues of yeast was extracted with toluene (2 × 2 L) with gentle stirring (emulsions may occur; addition of acetone is helpful in this case). The combined toluene phases were washed with water, dried ($MgSO_4$), and concentrated. The brown residue was taken up with heptane (50 mL) and stirred for 2 h at 20 °C. Filtration and washing with heptane gave crude Trimegestone (8.2 g; purity = 94%) as beige crystals. After dissolution in methylene chloride (80 mL), decolourization with carbon black, and filtration on silica gel, a final crystallization from isopropyl ether gave pure Trimegestone (7.4 g; yield = 74%; purity ≥99%) as white crystals.

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