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Two Syntheses of FF-MAS

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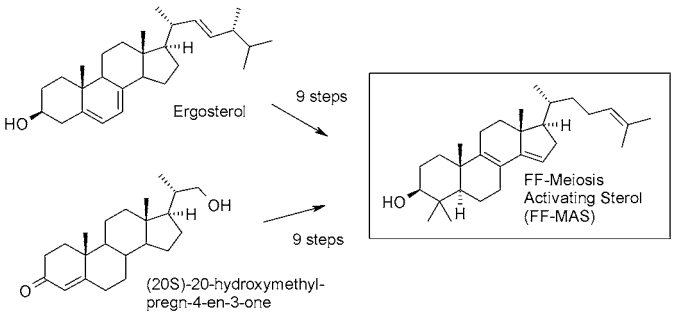
ABSTRACT



Follicular fluid-meiosis activating sterol (FF-MAS) has been shown to be an efficient inducer of meiotic maturation. It can potentially be used for improvements of in vitro fertilization techniques. Two short synthesis of FF-MAS are presented in this article. Both syntheses are based on microbiological degradations of sterol side chains. FF-MAS can be synthesized in nine steps from commercially available starting materials by both routes.

In 1995, Byskov et al. discovered FF-MAS **1** (follicular fluid-meiosis activating sterol: 4,4-dimethyl-5α-cholesta-8,14,-24-trien-3β-ol) in the follicular fluid of women.[1] It has been demonstrated that FF-MAS and analogous sterols, which can reach physiological concentrations in the gonads in the μM range,[2] can activate the nuclear maturation of oocytes in vitro. Furthermore, it has been shown that FF-MAS markedly improves the quality of the mature mouse oocyte, leading to significantly higher fertilization rates in vitro.[3] Because of its potential to be used as a fertility promoter, the compound has gained considerable attention in the recent literature.[4]

Although the exact role of MAS as a native paracrine regulator remains controversial,[5] first trials with human oocytes show clear potential toward improvements of in vitro fertilization results.[6]

FF-MAS itself can be isolated from natural sources only in submilligram quantities. Recently, a fermentation method with metabolically engineered strains of *Saccharomyces cerevisiae* has been published.[7] With this method, only milligram amounts of FF-MAS have been produced so far.

Therefore, chemical synthesis starting from readily available sterols or steroids seems to be the most attractive way to obtain reasonable amounts of pure FF-MAS for biological and clinical studies.

Several chemical syntheses of FF-MAS have been described in the organic chemical literature[8] and in patent applications.[9] All of them are partial syntheses starting from well-known steroidal precursors. The main drawbacks of the published syntheses are elaborate protection/deprotection

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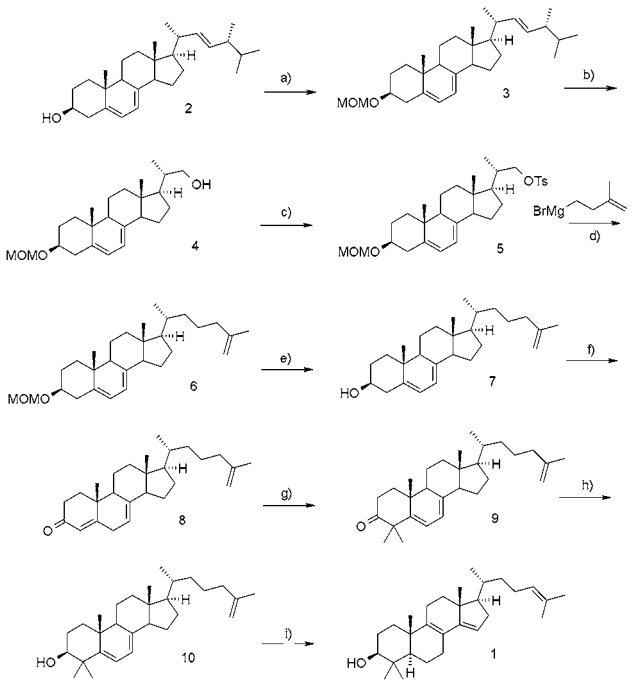
strategies for the diene system in the steroidal core and the separation of isomeric diene mixtures. The approaches described herein avoid the protection of double bonds in the steroidal core by using microbiological degradations of the sterol side chains in multigram scale during the synthesis.

Our first approach starts from ergosterol **2** (route A,[10] see Scheme 1). After protection of the 3-hydroxy group in ergosterol as a methoxymethyl (MOM) ether, which is necessary for the microbiological step, the side chain was cleaved by mycobacteria as described in the patent literature to give **4** in a yield of 75%.[11] The alcohol in the side chain was converted to the tosylate, which can be coupled with Grignard reagents in the presence of lithium chlorocuprate.[12] We chose the homoallylic side chain for coupling, because different attempts at coupling of an allylic (prenylic) side chain gave unsatisfactory results. The next steps are deprotection of the 3-hydroxy group with pyridinium *p*-toluenesulfonate (PPTS),[13] Oppenauer oxidation[14] to the ketone with migration of one double bond, dimethylation in position 4 with potassium *tert*-butoxide as the base, and subsequent reduction of the 3-keto group with lithium aluminum hydride, which gives 3β-alcohol **10** as the major diastereomer. The final step is the acid-catalyzed isomerization, which includes the fast isomerization of the Δ25 to the Δ24 double bond[15] and slower isomerization of the Δ-5,7-diene system to the thermodynamically more stable Δ-8,14-diene[16] to give FF-MAS as the major component[17] of the isomerization mixture.

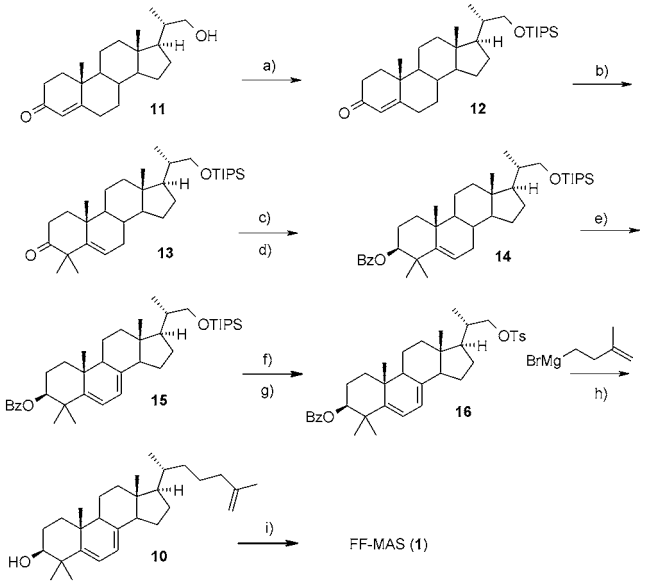
FF-MAS can be purified further by crystallization, HPLC, or chromatography on silver-coated columns.[18] We favored two consecutive recrystallizations from methanol. This procedure allowed us to isolate FF-MAS in >90% isomeric purity and with full biological activity[19] in a very convenient way.

Scheme 1. Synthesis of FF-MAS from Ergosterol: Route A[a]



[a] Conditions: (a) formaldehydedimethylacetal, P_2O_5, 100%; (b) *Mycobaterium* sp.; (c) 1.5 equiv of TsCl, pyridine, rt, 8 h, 92%; (d) Li_2CuCl_3, THF, −30 °C to rt, overnight, 71%; (e) PPTs, ^{t}BuOH, reflux, 1 h, 72%; (f) Al(O^{i}Pr)$_3$, toluene, cyclohexanone, reflux, 30 min, 73%; (g) KOtBu, MeI, ^{t}BuOH, rt; 30 min, 68%; (h) $LiAlH_4$, THF, rt, 30 min, 79%; (i) 6N H_2SO_4, dioxane, reflux, 70 h, 69%.

Scheme 2. Synthesis of FF-MAS from (20*S*)-20-Hydroxymethyl-pregn-4-en-3-one: Route B[a]



[a] Conditions: (a) TIPSCl, imidazole, CH_2Cl_2, rt, 4 h; (b) KOtBu, MeI, ^{t}BuOH, rt; 30 min; (c) $LiAlH_4$, THF, rt, 30 min; (d) BzCl, pyridine; 0 °C, 1 h; 52% over four steps; (e) 1,3-dibromo-5,5-dimethyl-imidazolidine-2,4-dione, benzene, hexane, 70 °C 30 min, then 2,4,6-trimethylpyridine, toluene, reflux, 2 h; (f) TBAF, THF, rt, 1 h, 61% over two steps; (g) *p*-TsCl, pyridine, 4-DMAP, 50 °C, 2 h, 72%; (h) Li_2CuCl_3, THF, −30 °C to rt, overnight; 100%; (i) 6 N H_2SO_4, dioxane, reflux, 70 h, 69%.

We pursued a second synthesis (route B, see Scheme 2) of FF-MAS starting from commercially available (20*S*)-20-

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(10) Strategy of this approach (route A) has been disclosed in a German patent application; see ref 9b.

(11) Weber, A.; Kenneke, M.; Neef, G. PCT Int. Appl. 92/03465.

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(15) Analogous side chain isomerization under iodine catalysis has been described: Kircher, H. W.; Rosenstein, F. U. *J. Org. Chem.* **1987**, *52*, 2586.

(16) Diene isomerizations have been described in the literature: Dolle, R. E.; Kruse L. I. *J. Org. Chem.* **1986**, *51*, 4047 and references therein.

hydroxymethyl-pregn-4-en-3-one **11**.[20] For our needs, this starting material was produced from the sterol sitosterin by side chain degradation with mycobacteria as described in the patent literature.[21]

The alcohol in the side chain of **11** was protected as a triisopropylsilyl ether. Dimethylation in position 4 and subsequent reduction of the 3-keto group was performed in analogy to route A. The resulting 3-β-OH group was protected as a benzoate. In this step, the first purification during synthesis was done. Pure **14** was isolated after a simple crystallization from methanol in a yield of 50% over four steps.

A bromination/dehydrobromination reaction[22] gave diene **15**, which was deprotected in the side chain with tetrabutylammonium fluoride (TBAF), and the resulting alcohol was tosylated under standard conditions. Coupling with the homoallyl-Grignard reagent as in route A gave the Δ-25 side chain and concomitant deprotection of the 3-hydroxy group. The final isomerization step is identical to route A. The overall yield for synthesis using route B was 10% (over nine steps).

The FF-MAS was purified as described for route A. Both route A and route B are attractive methods for the preparation of FF-MAS in gram scale. Route B is in our view especially interesting because it contains fewer purification steps and allows late introduction of side chains to produce biologically active analogues of FF-MAS for structure−activity relationship studies.[23]

In summary, we have developed two syntheses of FF-MAS that can produce the product in gram scale. Both routes are based on microbiological oxidations of sterol side chains and require only nine steps from commercially available starting materials and thus constitute attractive routes for the syntheses of FF-MAS and biologically active analogues.

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Supporting Information Available: ^{1}H NMR and mass spectra of all intermediates and the final product. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Isomeric ratio of Δ24:Δ25 in the side chain is approximately 8:1; the ratio of dienes Δ8,14:Δ6,8(14) is approximately 2.5:1, which corresponds to a content of FF-MAS (Δ8,14,24-triene) of ca. 60%.

(18) See ref 8b.

(19) Oocyte assay has been adopted from the literature (see ref 1). Naked oocytes (NO) were punctured from ovaries of mice primed with follicle stimulation hormone. After in vitro culture in the presence of 3 mM hypoxanthine and 10 μM of the corresponding test compounds for 15−24 h, germinal vesicle breakdown (GVB) and polarbody (PB) values are measured. The values for GVB and PB are comparable to the data reported in the literature.

(20) Commercially available from Research: 10 g costs ca. $300 $.

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