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Meiosis Activating Sterols Derived from Diosgenin

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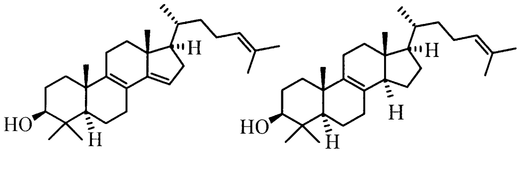
Abstract—Continuing research based on the meiosis activation properties of the endogenous sterol FF-MAS is reported. The synthesis and SAR of 16- and 26-substituted sterols are described, utilising diosgenin as starting material. Selected sterols were tested for their ability to induce oocyte maturation in hypoxanthine arrested mouse oocytes in vitro. © 2002 Elsevier Science Ltd. All rights reserved.

4,4-Dimethyl-cholest-8,14,24-trien-3β-ol (FF-MAS) is a naturally occurring sterol isolated from human follicular fluid. It is an upstream intermediate in the cholesterol biosynthetic pathway, initially formed via enzymatic 14α-demethylation of lanosterol, and has been reported to have a positive meiosis inducing effect on immature mouse oocytes cultured in vitro.[1,2] More recently, FF-MAS has also been shown to promote meiosis in other species such as rat[3] and human.[4]



FF-MAS **T-MAS**

It has been shown in vitro that FF-MAS can induce meiotic maturation in hypoxanthine arrested denuded mouse oocytes and restart the meiotic process, which results in germinal vesicle breakdown (GVB)/polar body formation (PB) of the oocyte as the initial steps.

The mode of action of FF-MAS exposure to immature oocytes and their effect on GVB induction is not yet known, however, in the original article by Byskov et al.,[1] a number of sterols were isolated and their effect on meiosis determined. It was demonstrated that whilst FF-MAS was active, both lanosterol and cholesterol were inactive in this assay. In addition, a close analogue of FF-MAS, which lacks Δ^{14} unsaturation was isolated from bull testicular tissue and given the acronym T-MAS, was also found to be a potent meiosis inducer.

Based on follow up work on these original findings we recently reported a novel synthesis of FF-MAS,[5] and this letter describes further research relating to other sterols which have an influence on the regulation of meiosis.

The synthetic route reported here (Scheme 1) utilised diosgenin as the starting material, allowing access to sterols containing substituents at the 16 and 26 position. Diosgenin was converted to 26-hydroxy cholesterol (**1**) using the established literature method,[6] and this diol could be selectively protected in the side chain with TBSCl. Introduction of the gem dimethyl group at the 4 position was done via Oppenauer oxidation of the 3-hydroxy group, dimethylation at C4 with potassium *tert*-butoxide and methyl iodide and finally lithium aluminium hydride reduction of the 3-keto group to give alcohol **2**, which underwent silyl deprotection to give the corresponding diol **3**.

Alternatively, silyl protection of the 3β-alcohol of **2** afforded compound **4**, which underwent subsequent allylic bromination and elimination[7] to provide the $\Delta^{5,7}$ intermediate **5**. The two TBS groups in **5** could be cleaved using HF to provide the $\Delta^{5,7}$ diol **6**. Similarly, reaction of **5** with HCl in ethanol at reflux isomerised the $\Delta^{5,7}$ diene system to the desired $\Delta^{8,14}$ diene with concominant deprotection of both TBS groups to generate the 26-hydroxylated compound **8**. In general for $\Delta^{5,7}$ to $\Delta^{8,14}$ isomerisation reactions, traces of unwanted diene isomers were conveniently removed by recrystallisation.

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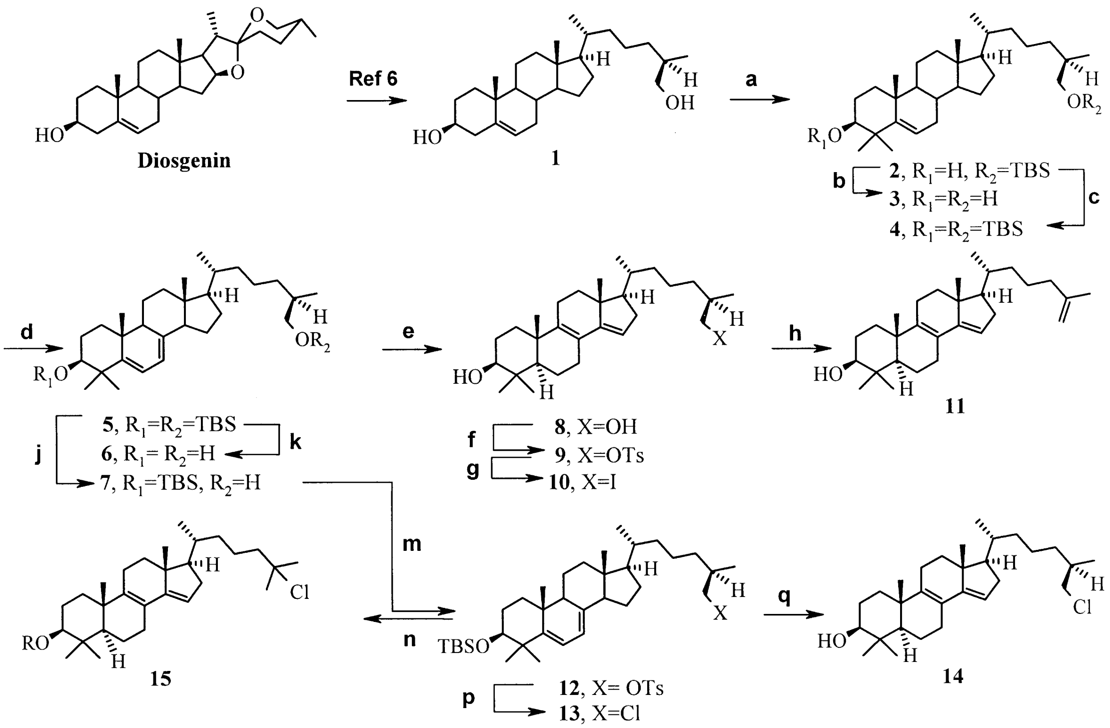


Scheme 1. Reagents and conditions: (a) (i) TBSCl, imidazole, DMF (65%); (ii) $Al(O\textit{i}Pr)_3$, *N*-methyl morpholine (91%); (iii) KO*t*Bu, MeI, *t*BuOH (76%); (iv) $LiAlH_4$, THF (93%); (b) TBAF, THF (64%); (c) TBSCl, imidazole, DMF (98%); (d) (i) dimethyl bromohydantoin, hexane/benzene; (ii) quinaldine/*o*-xylene (90% over two steps); (e) HCl, EtOH, benzene (49%); (f) TsCl, py (71%); (g) NaI, DMF (80%); (h) DBU, $CHCl_3$ (44%); (j) TBAF, THF (75%); (k) HF (72%); (m) TsCl, py (89%); (n) (i) as for (f), (g), (h) (38% over three steps); (ii) HCl, EtOH, benzene (70%); (p) LiCl, DMF (89%); (q) HCl, EtOH, benzene (36%).

It was also possible to prepare the $\Delta^{8,14,25}$ regioisomer of FF-MAS by regioselective modification of the 26-hydroxy group in **8**. Initially, since the 26-tosyloxy intermediate **9** did not react in the base induced elimination step, it was necessary to prepare the corresponding 26-iodo derivative **10**, which on treatment with DBU converted smoothly to compound **11**. Compound **5** could also be regioselectively deprotected in the 26-position using TBAF at room temperature to afford compound **7**, and in our hands it was observed that for sterols bearing the 4,4-dimethyl functionality, the 3-OTBS group was very robust to this deprotection protocol. This allowed for conversion of the 26-hydroxy group in **7** to tosylate **12**, which in turn was treated with lithium chloride in DMF to give the corresponding chloro compound **13**. Exposure of this compound to the acid isomerisation conditions reported above afforded the $\Delta^{8,14}$ compound **14**.

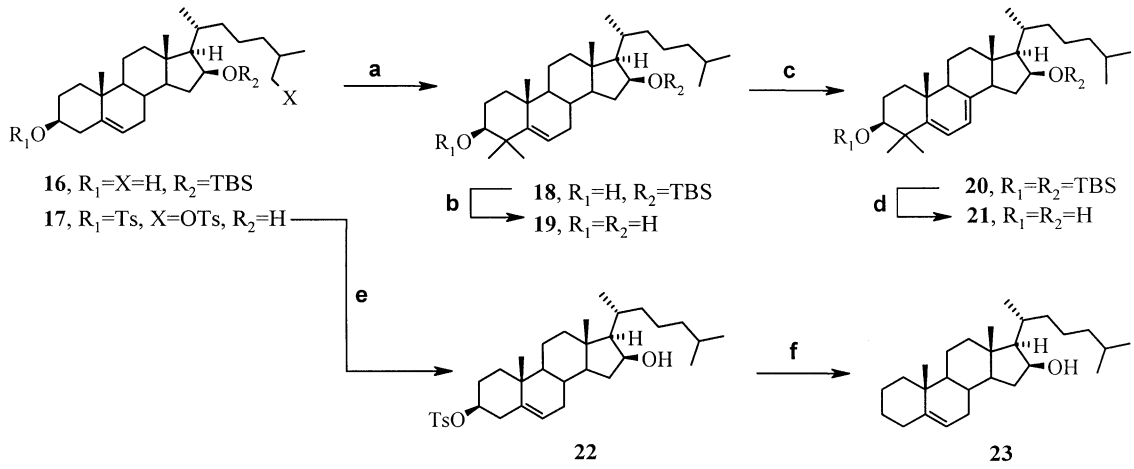
Tosylate **12** could also be transformed to the corresponding Δ^{25} alkene in a similar manner to that described for compound **11**. In this case, HCl induced isomerisation also gave addition across the Δ^{25} double bond to afford the 25-chloro compound **15** which was isolated as the major product ($\sim$70% yield, traces of diene impurities could not be completely removed).

16-Hydroxy containing sterols were prepared from sterols **16** and **17** (Scheme 2), which were again conveniently derived from diosgenin.[8] The 4,4-dimethyl group was introduced in a similar fashion to that described in Scheme 1, providing compound **18**, which under acidic conditions underwent silyl deprotection to diol **19**. Silyl protection of compound **18** at the 3-hydroxy group followed by introduction of the $\Delta^{5,7}$ diene system afforded the protected diol **20**. In this case complete deprotection of the two silyl ethers, and in particular the 16-OTBS group was not possible using either TBAF or hydrogen fluoride, however exposure to DIBAL in refluxing hexane afforded the desired diol **21**. It should be noted that it was not possible to access the corresponding 16-hydroxy-$\Delta^{8,14}$ diene, most likely due to the instability of the allylic alcohol moiety in this compound. Based on the initial biological activity of sterols containing the 16-hydroxy group (see Table 1), compound **23** was also prepared from **17** via the tosylate

Table 1. Effect of selected sterols on Germinal Vesicle Breakdown[9]

Compd	GVB relative (%)[a]
FF-MAS	100
19	113 (±4)
11	106 (±11)
21	90 (±35)
6	89 (±4)
23	87 (±9)
14	87 (±3)
10	85 (±8)
3	71 (±26)
15	62 (±9)
16	43 (±15)
1	na
8	na

[a] Values are means of 2–3 experiments, standard deviation is given in parentheses. na, not active.



Scheme 2. Reagents and conditions: (a) (i) Al(O*i*Pr)$_3$, *N*-methyl morpholine (90%); (ii) KO*t*Bu, MeI, *t*BuOH (75%); (iii) LiAlH$_4$, THF (99%); (b) HCl, EtOH (60%); (c) (i) TBSCl, imidazole, DMF (93%); (ii) dimethyl bromohydantoin, hexane/benzene; (iii) quinaldine/*o*-xylene (62% over two steps); (d) DIBAL-H, THF (46%); (e) LiAlH$_4$ (83%); (f) Super-H, THF (55%).

22, which could be reduced to the desired alcohol on treatment with lithium triethylborohydride.

Selected sterols were tested in a cell based assay for their ability to induce meiosis in immature mouse oocytes by measuring the rate of Germinal Vesicle Breakdown (GVB) compared to FF-MAS. From Table 1 it can be seen that for the $\Delta^{8,14}$ diene compounds **10** and **14**, added iodo or chloro functionality can be tolerated at the 26 position, the 26-hydroxy compound **8**, however, showed no meiosis inducing effect. The 25-chloro compound **15** also shows moderate activity, and it appears that lipophilic groups are tolerated at these positions. Compound **11**, the $\Delta^{8,14,25}$ regioisomer of FF-MAS, also showed a similar activity to FF-MAS, and it should be noted that the saturated side chain analogue of FF-MAS (not shown) also has a relative GVB value of around 70%.

The 26-hydroxy cholesterol (**1**) has poor meiosis promoting activity, however it is interesting to note that the activity in this series increases with the introduction of the gem dimethyl group at position 4 (**3**) and again after further unsaturation at Δ^7 (**6**). In the case of the 16-hydroxylated sterols we found that moderate activity could be seen even when only the Δ^5 double bond was present (**16**). In a similar fashion to the 26-hydroxy series, increased activity was observed by introduction of methyl groups at 4 and to a slightly lesser extent further unsaturation at Δ^7, and in fact compound **19** in this assay had a comparable activity to that of FF-MAS. It appears that the 16-hydroxy group is essential for this series of compounds as verified by retention of activity of 16-hydroxy-cholest-5-ene (**23**). There are still many open questions relating to the mechanism of FF-MAS and related sterols as meiosis regulators, however, the in vitro activity of these compounds gives valuable information towards improving our understanding of the structural constraints required for activity within this family of compounds. A recent article by Boer et al. has also discussed the connection between electrostatic potential and activity of a series of sterols related to FF-MAS.[10]

In conclusion, we have described the synthesis and preliminary SAR of a series of new sterols which have a stimulating effect on mouse oocyte maturation in vitro. Lipophilic substituents on the 25 and 26 positions of the sterol side chain are generally tolerated and it appears that in the 16-hydroxy series the Δ^8 or $\Delta^{8,14}$ double bond moiety is not essential for activity. Compounds of this type provide further knowledge and understanding to an area which has potential for the treatment of infertility, and further research in this area will be reported in due course.

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9. Oocytes were obtained from immature female mice weighing 13–16 g, placed in culture medium and cultured with 10 μM sterol for 24 h at 37 °C according to the procedure described in ref 2. At the end of the culture period the oocytes which had undergone germinal vesicle breakdown (GVB) and polar body formation (PB) were counted using a stereomicroscope. % GVB relative was defined as [(number of GVB + number of PB/total number of oocytes) × 100] indexed to FF-MAS which was defined as 100.
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