ORGANIC LETTERS

2000
Vol. 2, No. 19
2921−2922

A Short Formal Synthesis of Squalamine from a Microbial Metabolite

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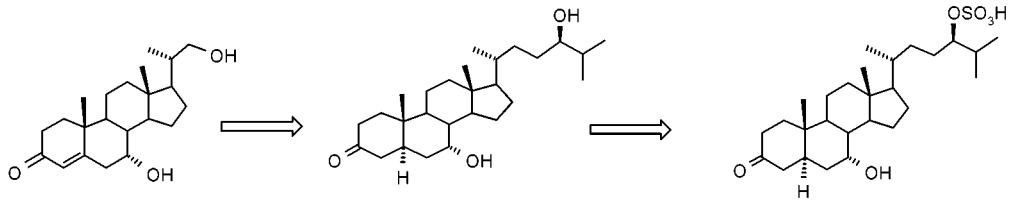
Received April 13, 2000

ABSTRACT



2 **10** **11**

A short formal synthesis of squalamine is described, utilizing the biotransformation product 2, which is available in one step from commercially available 3-keto-23,24-bisnorchol-4-en-22-ol (1). Regioselective C-22 oxidation and C-24 sulfation of the corresponding alcohols in the presence of a free C-7 alcohol make for an efficient preparation of squalamine intermediate 11.

Squalamine, by virtue of its potent antiangiogenic activity,[1] entered clinical development as an antitumor agent in the autumn of 1997. A large-scale stereoselective synthesis was developed to satisfy the requirements for rapid entry into clinical trials.[2] Anticipating greater needs for this agent as it progresses through clinical trials, a much more efficient process has been developed that reduces the number of steps from 16 to 11. The two remaining impediments to reducing the length of the synthesis were the use of protecting groups and the tedious introduction of the 7α-hydroxyl group. It was expected that a microbial hydroxylation could be utilized to dramatically shorten the route, as has been done routinely by others in steroid chemistry.[3] Despreaux has described the microbial 7α-hydroxylation of 3-keto-23,24-bisnorchol-4-en-22-ol (**1**, Scheme 1) using the species *Diplodia gossypina*.[4] The product of this hydroxylation, **2**, was obtained in up to 45% yield with the recovery of a similar amount of starting steroid. Although some optimization of this fermentation procedure would be required, we found **2** to be an appealing starting material for the synthesis of squalamine (**12**).

Starting from steroid **2**, two regioselective reactions would be necessary to deliver squalamine without the use of a protecting group at C-7. In intermediate **3**, it was expected that the primary C-22 hydroxyl group could be selectively manipulated in the presence of the hindered secondary C-7 hydroxyl group. A more troublesome selectivity issue was the selective sulfation that would be required in **10** to deliver the C-24 sulfate **11**. Both alcohols are secondary, although C-24 would be more accessible on steric grounds. Some C-24 selectivity has been shown in the sulfation reaction on a spermidinyl-steroidal diol. However, the ratio was not carefully quantified and the yield was low (10%).[5] The conversion of **2** to **11** would represent a short formal

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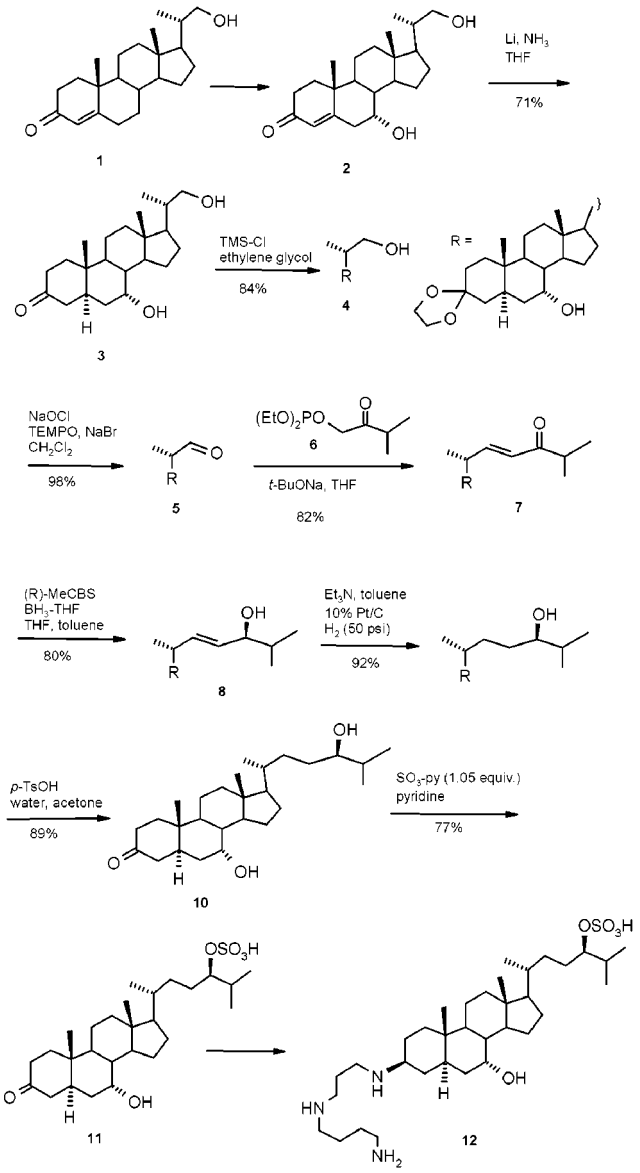
10.1021/ol0059495 CCC: $19.00
Published on Web 08/31/2000

Scheme 1. Eleven-Step Preparation of Squalamine, **12**, from **1**



synthesis of squalamine, because **11** has been previously used as an intermediate to yield the natural product in two steps.[2,6]

The best preliminary result at adopting the fungal 7α-hydroxylation of **1** with *D. gossypina* (ATCC 20576) is described in detail in the Supporting Information. The yield of **2** was estimated to be 800 mg/L at its peak concentration during fermentation (26% yield), based on HPLC analysis. This yield was obtained at a 3 g/L substrate concentration, substantially higher than that described in the literature (1.0 g/L).[4a] Therefore, the yield per liter is slightly improved relative to the literature (lit. 450 mg/L, 45% yield). After extraction and purification, 155 mg/L of **2** was recovered (lit. 110 mg/L).

The viability of the 10 chemical steps was demonstrated successfully. The reduction of **2**[4a] to **3**[12] was accomplished in 71% yield using lithium in ammonia. This method is commonly used to afford the trans AB-ring junction.[7] Ketalization was performed utilizing ethylene glycol in chlorotrimethylsilane in good yield.[8] This reaction was accomplished at 10% concentration of substrate, which allows for efficient scale-up of this procedure. Selective oxidation of the C-22 alcohol with bleach and TEMPO as catalyst[9] afforded **5** in 98% yield. Wadsworth−Emmons reagent **6**[10] was utilized to afford enone **7** efficiently (82%). Steroid **7** was reduced stereoselectively as before with borane and (*R*)-MeCBS[11] to yield **8** in good yield. The diastereomeric excess was not evaluated at this stage, but was after conversion to **11**. The product **8** was isolated by recrystallization and converted to **9** by hydrogenation. Deprotection of the ketal afforded intermediate **10**, which contains the C7,-24-diol. The key step to this short route is the selective sulfation of the C24-hydroxyl group in **10** to afford **11**. Selective sulfation was accomplished successfully with a very small excess (5%) of sulfur trioxide−pyridine complex. The diastereomeric excess in the sulfate **11** was calculated to be 95% based on the HPLC method, which is comparable to what was achieved previously (94%).[2] This suggests that the stereoselectivity of the chiral reduction is not significantly influenced by the protecting group on C-7.

The short 10-step route to squalamine **12** from the fungal metabolite **2** has been accomplished. The overall method has potential to improve the supply and cost of this promising Phase II clinical candidate. Research activity must now be directed at the biotransformation step in order to make this potential manufacturing method a reality.

Acknowledgment. This research was supported by a Small Business Innovation Research Grant (1 R43 CA 80473-01) from the National Cancer Institute. The authors thank Hong-Seok Kim, Kyungpook National University, Taegu, South Korea, for providing a reference sample of compound **3**.

Supporting Information Available: Experimental procedures and analytical data for compounds **2**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0059495

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