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Synthesis and antimicrobial activity of 7-fluoro-3-aminosteroids

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Abstract—A series of 7-fluoro-3-aminosteroids were synthesized and their in vitro antimicrobial activities were evaluated against Gram-positive and Gram-negative bacteria. The nucleophilic fluorination of several 7β-hydroxysteroids by diethylaminosulfur trifluoride in *n*-pentane, followed by reductive amination of the resulting 7-fluoro-3-ketosteroids with spermidine in the presence of $NaBH_3CN$, afforded 7-fluoro-3-aminosteroids in high yield. Compound **25** showed the highest antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Escherichia coli*.

The rapid emergence of superbugs[1] poses a constant threat to humans, and although newly developed antibiotics are constantly being tested the pace at which bacteria develop resistance is much faster than the development of new antibiotics. Superbugs like the methicillin-resistant *Staphylococcus aureus*,[2] vancomycin-resistant enterococci,[3] and quinolone-resistant *Streptococcus pneumoniae*[4] are classic examples. An antibiotic can exert its effect in one of the three ways: interference with the cell wall, interference with nucleic acid synthesis, and interference with protein synthesis.[5] One such antibiotic is squalamine, which is isolated from the tissue of *Squalus acanthias*. It is a polyamine-steroid conjugate which ruptures the cell membrane of bacteria leaving it susceptible to osmotic lysis. Squalamine has antimicrobial activity against Gram-positive and Gram-negative bacteria, possessing antiangiogenic activity and exhibiting low hemolytic activity (Fig. 1).[6] Recently, seven new aminosterols related to squalamine were isolated from *S. acanthias* and some of them were more potent against bacteria than squalamine.[7] Since this discovery, other analogues of squalamine have been pursued because many of them possess potent antimicrobial, and anti-trypanosomal activity, as well as DNA binding affinity.[8,9] The synthesis of various polyamine conjugates to cholesterol, cholenic acid, and bile has been reported by us[10] and others,[11] and the compounds were evaluated for biological potency. These substances exhibited comparable antimicrobial activity to that of squalamine against Gram-positive and Gram-negative bacteria. Squalamine and analogues **1** and **2** exhibited comparable antimicrobial activity,[10b] so we were interested in evaluating the fluoro analogues of **1**. Since squalamine has a 7α-hydroxyl group at C7, we synthesized the 7α-fluoro analogues. Replacing the hydroxyl group with fluorine in aliphatic, cyclic, and heterocyclic compounds exhibited increased activity however it is difficult to substitute the hydroxyl group with fluorine in a steroid ring due to elimination and other side reactions, resulting in lower yields.[12–14] We have reported that the synthesis and antimicrobial activity of 3α-hydroxy-7α-fluoro-23,24-bisnorcholane polyamine carbamate also suffer from the aforementioned bottleneck problem.[10a]

A number of methodologies have been applied in order to substitute the hydroxyl group with fluorine in steroids. The fluorination of steroids led by elemental fluorine is usually associated with a lower yield and it is not easy to perform in the laboratory.[12] Diethylaminosulfur trifluoride (DAST), a highly effective nucleophilic fluorinating agent, is a reagent of choice in that it is reactive, easy to use, and commercially available. The fluorination of 3β-acetoxy-7α- and 7β-hydroxyandrost-5-en-17-one with DAST, in *n*-pentane, provided complex and inseparable mixtures of 7-fluoro derivatives in a 64% yield, however, the resulting fluorinated steroid, when mixed with chloroform, underwent decomposition at 5–10 °C and was susceptible to heat and acid.[13] Epimerization was found to be higher in polar solvents such as dichloromethane and lower in nonpolar solvents such as

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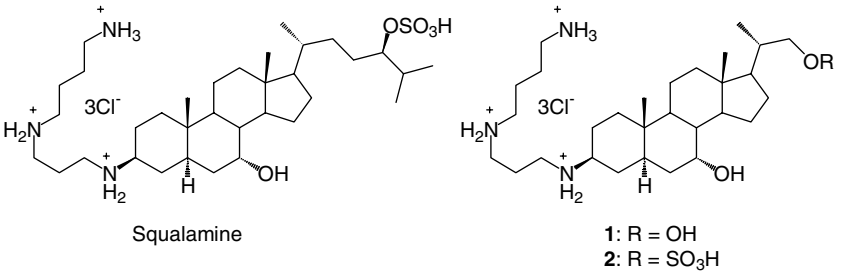
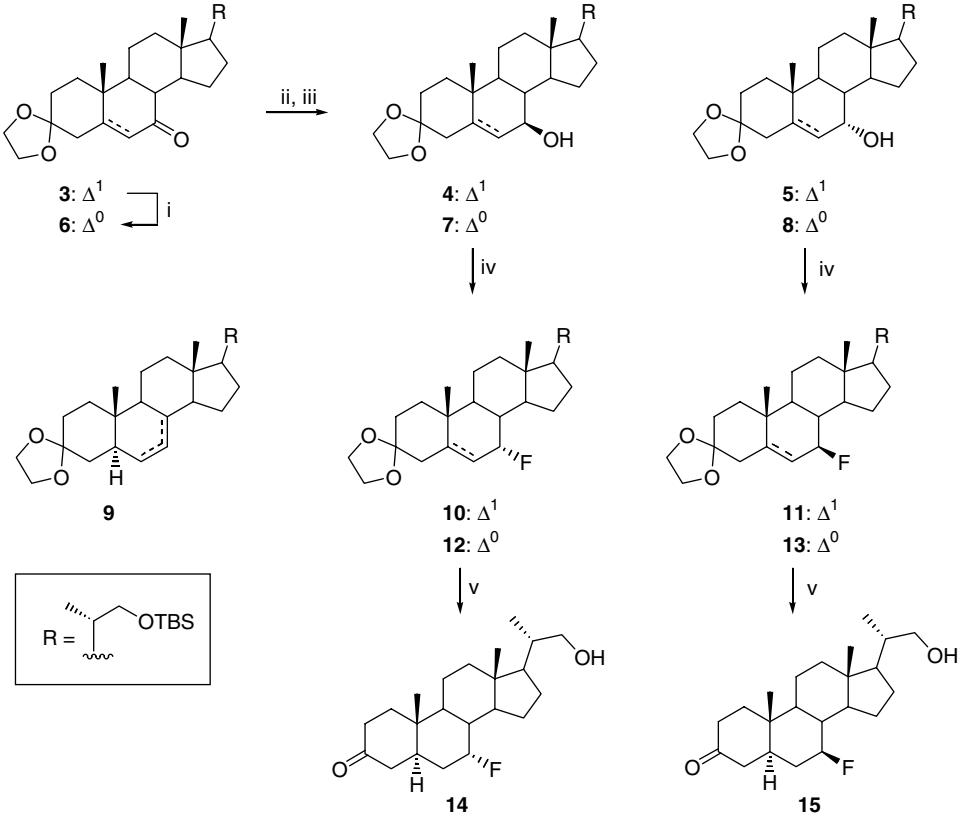


Figure 1. Squalamine and analogues.

n-pentane. 7α- and 7β-fluoro-5α-cholestan-22-al were synthesized from a stigmasterol derivative, however the yield was low.[14]

For the synthesis of 7-fluorinated squalamine analogues, we investigated a more efficient method that could produce the 7α-fluoro-3-ketosteroid. The requisite starting material, 3-dioxolane-22-*tert*-butyldimethylsilyloxy-23,24-bisnorchola-5-en-7-one (**3**), was synthesized from commercially available 23,24-bisnorchola-4-en-3-one by the literature procedure.[10b] The catalytic hydrogenation of **3** with 5% Pt/C gave **6**. The reduction of **3** with $LiAlH_4$ in dry THF afforded 7β-OH (**4**) and 7α-OH (**5**) quantitatively in a 1:1 ratio. The reduction of Δ^5 and 7-keto groups for **3** was achieved simultaneously by dissolving metal reduction. Under Li/liq. NH_3 conditions, **3** primarily furnished 7β-OH (**7**, 94%) along with the 7α-OH isomer (**8**, 5%).

The nucleophilic fluorination of the 7β-OH isomer (**7**) was accomplished by DAST in *n*-pentane, at room temperature. The simultaneous deprotection of C-3 ketal and 22-TBS with *p*TSA in acetone provided a mixture of the 7α-F isomer (**14**) and 7β-F isomer (**15**) in a ratio of 4:3 in an 83% yield and eliminated product **9** in a 14% yield.[15] The similar fluorination of allylic alcohol **4** and **5** gave **10** and **11** in 62% yields, however, it was observed to be unstable and rapidly decomposing at room temperature when mixed with deuterated chloroform. We examined other methods (e.g., the mesylate of **7** was treated with a phase transfer catalyst (KF-Ph_3SnF/sulfolane),[16] KF-18C6,[17] and ionic liquid (bmim-BF_4)[18]) but these provided **12** and **13** only in traces. On the other hand, when the 7α-OH isomer (**8**) reacted with DAST, an eliminated product (**9**) resulted. It seemed that axial-equatorial stereochemistry at C-7 played an important role, while the substitution of hydroxyl group at C-7 with fluorine (Scheme 1).



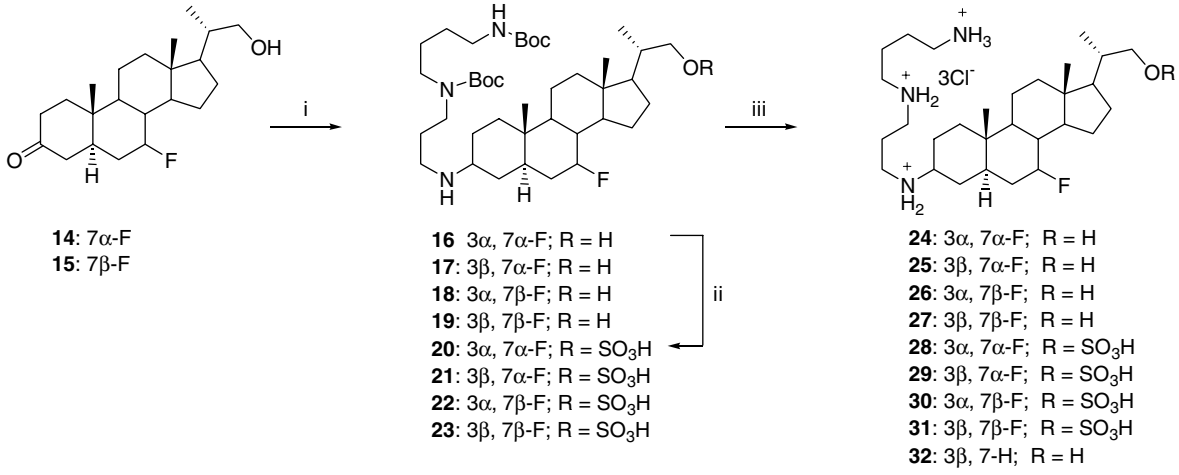
Scheme 1. The fluorination of 7-hydroxysteroids with DAST. Reagents: (i) H_2, PtO_2, EtOAc; (ii) Li/liq. NH_3, THF; (iii) $LiAlH_4$, THF; (iv) DAST, *n*-pentane; (v) *p*TSA, acetone.

The ^{1}H NMR chemical shift of H-7 in **14** was 4.57 ppm with J_{HF} = 49.1 Hz and the ^{13}C NMR chemical shift of C-7 was 90.3 ppm with J_{CF} = 171.0 Hz, which showed the axial configuration of fluorine. The ^{1}H NMR chemical shift of H-7 in **15** was 4.13 ppm with J_{HF} = 49.0 Hz, while the ^{13}C NMR chemical shift of C-7 was 96.4 ppm with J_{CF} = 174.0 Hz[15] which was consistent with an equatorial fluorine configuration. The chemical shifts and coupling constants observed were similar to those found previously.[13,14]

The reductive amination of 7-fluoro-3-ketosteroids **14** and **15** with Boc-spermidine, in the presence of $NaBH_3CN$,[19] afforded 7-fluoro-3-aminosteroids **16**–**19** in 93–94% yields, as shown in Scheme 2. The treatment of 22-hydroxyl compounds **16**–**19** with a SO_3-pyridine complex in pyridine at room temperature provided **20**–**23** in a quantitative yield. Consequently hydrochloride salts were obtained from **16** to **23** with thionyl chloride and methanol in dichloromethane at room temperature. Recrystallization of the resulting products in acetone yielded **24**–**31**. Compounds **1**, **2**, and **32** were obtained by a previously described method.[10]

Compounds **1**, **2**, **32**, and 7-fluoro-3-aminosteroids **24**–**31** were assessed against the strains of Gram-positive and Gram-negative bacteria, respectively, and the MIC (minimum inhibitory concentration) values are summarized in Table 1. Compounds **1**, **25**, and **32** share the same stereochemistry having spermidine at 3β except for a substituent at C7. The effect of the substituent at C7 is clearly apparent as the fluoro analogue **25** has a stronger antimicrobial activity than that of **1** and **32**. Compound **25** displayed the highest potency against *Pseudomonas aeruginosa* 1771M having a MIC value of 3.1 μg/mL. Compounds **25**–**27**, **30**, and **32** are the most active against *S. aureus* 503 having a MIC value of 6.3 μg/mL. *Streptococcus pyogenes* 77A is adversely affected by **24**–**28** and **30**, and in particular, **25** and **26** were the most potent with a low MIC of 6.3 μg/mL. *Escherichia coli* was the most vulnerable to **25**–**27** and **32**. Gram-positive *S. aureus* 503 was the most affected by the 22-hydroxyl analogue (**25**–**27**) having a MIC value of 6.3 μg/mL rather than the 22-sulfated counterpart **2**, **29**, and **31**. Compound **24** has an axial orientation at C-3 and C-7 was more active against *S. pyogenes* 77A and *S. aureus* 503, but it exhibited less potency, especially against Gram-negative strains. Compound **28**, a 22-sulfate analogue of **24**, showed a twofold improvement in activity against *S. pyogenes* 308A, *E. coli* DC2, and *P. aeruginosa* 9027. In general, all compounds were less potent against *Salmonella typhimurium* and *Enterobacter cloacae*.

In conclusion, we have synthesized 7-fluoro-3-aminosteroids by the fluorination of 7β-hydroxysteroids in a high yield. Our results suggest that the nature and stereochemistry of functional groups exert a great influence



Scheme 2. The synthesis of 7-fluoro-3-aminosteroids. Reagents: (i) Boc-spermidine, $NaBH_3CN$, THF–MeOH; (ii) SO_3-py complex, pyridine; (iii) $SOCl_2$, MeOH, CH_2Cl_2.

Table 1. The antimicrobial activity (MIC) of 3-aminosteroids **1**, **2**, and **24**–**32**

Strain	1	2	24	25	26	27	28	29	30	31	32
S. pyogenes 308A	25.0	NA	25.0	6.3	12.5	12.5	12.5	50.0	25.0	50.0	100.0
S. pyogenes 77A	25.0	50.0	12.5	6.3	6.3	12.5	12.5	50.0	12.5	50.0	100.0
S. aureus 503	12.5	50.0	12.5	6.3	6.3	6.3	12.5	25.0	6.3	50.0	6.3
E. coli DC2	25.0	50.0	50.0	6.3	12.5	12.5	25.0	50.0	25.0	25.0	12.5
P. aeruginosa 9027	12.5	50.0	50.0	6.3	12.5	12.5	25.0	12.5	50.0	50.0	12.5
P. aeruginosa 1771M	25.0	50.0	100.0	3.1	25.0	6.3	50.0	25.0	50.0	50.0	6.3
S. typhimurium	50.0	50.0	100.0	100.0	50.0	100.0	100.0	100.0	50.0	50.0	50.0
E. cloacae 1321E	50.0	50.0	100.0	100.0	100.0	100.0	100.0	100.0	50.0	50.0	100.0

Antimicrobial activity; IC_{50}, μg/mL; NA, non applicable.

on antimicrobial activity. We can conclude that **25**, **26**, and **27** were found to exhibit high potency, and compound **25** in particular was the most potent among the tested 7-fluoro-3-aminosteroids.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.001.

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