Rapid Construction of a Squalamine Mimic

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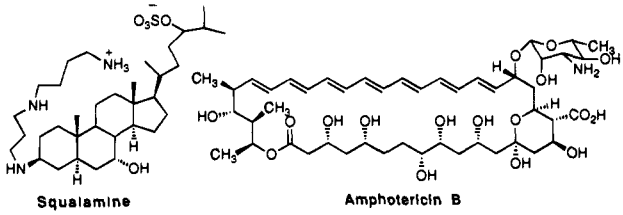
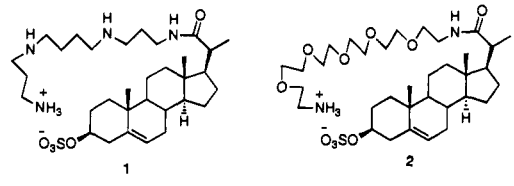
Squalamine is a novel sterol−spermidine conjugate that has recently been isolated from tissues of the dogfish shark, *Squalus acanthias*.[1] This unusual natural product has attracted considerable interest because of its potent antimicrobial activity against a broad spectrum of microorganisms.[2] At present, the feasibility of obtaining large quantities of this steroidal antibiotic from natural sources appears questionable; only trace amounts are present in the liver and gallbladder of the shark. While a recent synthesis has confirmed the structure of squalamine, the 17 steps that are needed, together with a low overall yield (0.36%) and expensive starting material (3β-acetoxy-5-cholenic acid), make such a route impractical for large-scale preparations.[3]

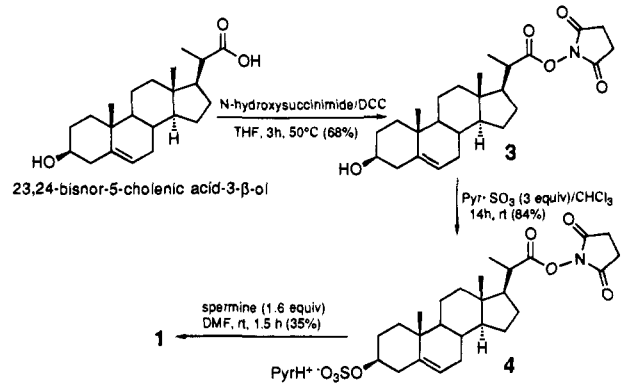
In this paper we describe the design and *three-step* synthesis of a molecule that mimics not only the structure of squalamine but also its extraordinary antimicrobial properties; i.e., a sterol−spermine conjugate, **1**. Our results *demonstrate that the placement of a pendant spermidine and sulfate group on the A and D rings of a closely related sterol can be reversed with retention of antimicrobial activity, and that much more accessible mimics are possible.*





Our design of **1** was guided by analogy to squalamine and by our hypothesis that this natural product bears a structural and functional resemblance to amphotericin B (AmB), an amphiphilic antibiotic that is known to form pores in lipid bilayers.[4,5] In particular, squalamine and **1** share three common elements that are characteristic of AmB: (i) a long and rigid hydrophobic unit; (ii) a flexible hydrophilic chain that is linked to the hydrophobic unit, which can extend across its "face";

Scheme 1



and (iii) a pendant polar head group; i.e., analogs of the heptaene, polyol, and carboxyl/mycosamine components of AmB, respectively.[5,6] Although the placement of the pendant sulfate and spermidine groups in the A and D rings of **1** are opposite to that of squalamine, we hypothesized that both compounds would show similar biological activity, since both are capable of adopting a macrocyclic conformation that resembles AmB. As has been noted previously, such a conformation may be favored via intramolecular salt bridge formation.[3] Our choice of 3β-hydroxy-23,24-bisnor-5-cholenic acid as starting material was based on its ready availability and our belief that the hydrophobic−hydrophilic balance of **1** would be similar to that of squalamine; i.e., the presence of an olefinic and an amide group was expected to compensate for the absence of a hydroxyl group at the C-7 position. For purposes of comparison, a polyether analog (**2**) was also chosen as a synthetic target.

Activation of the carboxylic acid group of 3β-hydroxy-23,24-bisnor-5-cholenic acid by reaction with *N*-hydroxysuccinimide to give **3**,[6] followed by sulfation, afforded **4**; subsequent condensation with spermine produced **1** in 20% overall yield in gram scale. Similar condensation of **4** with 1,17-diamino-3,6,9,12,15-pentaoxaheptadecane [$NH_2(CH_2CH_2O)_5CH_2CH_2NH_2$] afforded **2** in 12% overall yield.[7]

A summary of the biological activity of **1** and **2** against representative bacteria and fungi is presented in Table 1, along with data that has previously been reported for squalamine.[2] Of particular significance is the fact that **1** *mimics the antibiotic properties of squalamine by exhibiting potent activity against a broad spectrum of microorganisms*.[8] The fact that **2** exhibits negligible antimicrobial activity suggests that the pendant polyamine chain plays an important role with respect to the biological activity of **1**. In parallel studies, we have found that **1** does not lyse sheep red blood cells at concentrations as high as 100 μg/mL; it does, however, promote their aggregation. The absence of hemolytic activity, together with its antibiotic properties, indicates that **1** has potential as a chemotherapeutic agent.

In preliminary studies, we have examined the ability of **1** and **2** to promote the transport of Na^+ across egg PC bilayers by means of ^{23}Na NMR spectroscopy. Specific procedures that were used were similar to those previously described.[4] Inclusion of 1 mol % of **2** in 1000 Å diameter unilamellar vesicles promoted the entry of Na^+ into their aqueous interior, which was characterized by a first-order half-life of 3.9 h. This level of activity compares favorably with that which has been

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Table 1. Antimicrobial Properties of Sterol Conjugates

	antimicrobial activity (MIC), μg/mL[a]					
conjugate	*Escherichia coli* (25922)[c]	*Pseudomonas aeruginosa* (27853)[c]	*Staphylococcus aureus* (29123)[c]	*Proteus vulgaris* (13315)[c]	*Serratia marcescens* (8100)[c]	*Candida albicans*[b] (14053)[c]
squalamine[d]	1−2	4−8	1−2	4−8	>125	4−8
1	6.25	3.13	>100 (12.5)[e]	>100	>100[f]	12.5[g]
2	>100	>100	>100	>100	>100[f]	>100[g]

[a] Minimum concentration required for complete inhibition of growth. Micro-broth dilution methods were used. Inocula of 10^6/mL bacteria and 10^4/mL fungi were incubated in 0.5×, trypticase soy broth at 35 °C for 24 h. [b] The squalamine mimic, **1**, tested against clinical isolates of *Cryptococcus neoformans* and *Aspergillus fumigatus* gave MIC values of 3.13 and 12.5 μg/mL, respectively. [c] American Type Culture Collection (ATCC) numbers that define a given strain are in parentheses. [d] Data taken from ref 2. [e] ATCC No. 25923. [f] ATCC No. 13880. [g] ATCC No. 90028.

observed for a related sterol−bis[oligo(ethylene glycol)] conjugate.[4] In sharp contrast, no ion transport activity could be detected for **1** under similar conditions over the course of 48 h.[9] Whether or not **1** is capable of generating pores or ion channels in microbial membranes, however, remains to be established. Although we cannot presently rule out the possibility that **1** operates at the nuclear level, where DNA binding is the primary event, we consider this as unlikely on the basis of the conjugate's amphiphilic structure.[10]

The similarity between squalamine and **1**, in terms of their structure, composition, and antimicrobial properties, strongly suggests that they function by the same mechanism. While this similarity also suggests that we have correctly identified the key elements of squalamine that are necessary for biological activity, it should be noted that very small alterations in the structure of **1** can significantly affect its activity; e.g., a saturated analog of **1** (prepared from 3β-hydroxy-23,24-bisnor-5α-cholanic acid) showed substantially lower antimicrobial activity. Thus, there would appear to be considerable room for fine-tuning the biological properties of such compounds. The ready availability of **1** makes it particularly attractive as a lead compound for further exploration. In this regard, the synthetic strategy that is outlined herein should permit the rapid construction of a rich variety of analogs. Such studies are now under intensive investigation in our laboratories.

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(7) **3**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (m, 1 H), 3.53 (m, 1 H), 2.82 (s, 4 H), 2.73 (dq, J = 10.9, 6.9 Hz, 1 H), 2.27 (m, 2 H), 1.38 (d, J = 6.9 Hz, 3 H), 1.02 (s, 3 H), 0.74 (s, 3 H), 2.0−0.95 (m, 18 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 170.2, 141.1, 120.2, 70.0, 55.5, 51.9, 49.3, 47.4, 42.1, 37.1, 36.8, 35.2, 31.3, 31.1, 26.7, 25.3, 25.2, 24.3, 23.8, 20.8, 19.0, 17.0, 11.5 ppm; IR (KBr) 3440, 3330, 2931, 2850, 1812, 1781, 1737, 1626, 1575, 1366, 1207, 1065, 1049, 1019 cm^{-1}; HRMS-FAB (M + Na$^+$) calcd for $C_{26}H_{36}O_5N + Na^+$ 466.2569, found 466.2564. **4**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.92 (m, 2 H), 8.57 (m, 1 H), 8.05 (m, 2 H), 5.28 (m, 1 H), 3.84 (m, 1 H), 2.80 (s, 4 H), 2.68 (dq, J = 10.5, 6.8 Hz, 1 H), 2.38 (m, 2 H), 1.28 (d, J = 6.8 Hz, 3 H), 1.10 (s, 3 H), 0.72 (s, 3 H), 2.14−0.95 (m, 19 H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.2, 170.1, 145.3, 142.8, 140.6, 126.8, 120.8, 75.1, 55.4, 51.8, 49.3, 47.4, 42.1, 36.8, 36.0, 34.8, 31.3, 31.2, 28.7, 26.7, 25.4, 25.2, 24.4, 23.8, 20.8, 18.9, 16.9, 11.5 ppm; IR (KBr) 3030, 2938, 2850, 1808, 1780, 1739, 1251, 1208, 1064, 975 cm^{-1}; HRMS-FAB (MH$^+$) calcd for $C_{31}H_{42}O_8N_2S$ 603.2740, found 603.2741. **1**: ^{1}H NMR (500 MHz, CD_3OD) δ 5.39 (m, 1 H), 4.14 (m, 1 H), 3.19−3.24 (m, 2 H), 2.85 (t, J = 7.0 Hz, 2 H), 2.78 (t, J = 7.2 Hz, 2 H), 2.68−2.72 (m, 6 H), 2.54 (m, 1 H), 2.31 (m, 2 H), 1.75 (m, 4 H), 1.60 (m, 4 H), 1.15 (d, J = 6.7 Hz, 3 H), 1.04 (s, 3 H), 0.75 (s, 3 H), 2.10−0.96 (m, 19 H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 180.0, 141.7, 123.2, 79.8, 57.9, 54.1, 51.7, 49.8, 47.9, 47.2, 45.1, 43.5, 41.0, 40.5, 40.2, 38.5, 37.7, 37.5, 33.3, 33.0, 30.1, 30.0, 29.5, 28.6, 27.6, 27.5, 25.4, 22.2, 19.9, 18.8, 12.6 ppm; IR (KBr) 3310, 2939, 2885, 2851, 1647, 1551, 1471, 1246, 1221, 994, 981 cm^{-1}; HRMS-FAB (MH$^+$) calcd for $C_{32}H_{58}O_5N_4S$ 611.4206, found 611.4211. Anal. Calcd for $C_{32}H_{58}O_5N_4S \cdot 1.5H_2O$: C, 60.25; H, 9.64; N, 8.78; S, 5.03. Found: C, 60.12; H, 9.34; N, 8.46; S, 5.01. 1,17-Diamino-3,6,9,12,15-pentaoxaheptadecane was synthesized from hexaethylene glycol by conversion to its ditosylate, followed by standard Gabriel synthesis.

(8) Unfortunately, we were unable to obtain an authentic sample of squalamine from industrial sources for "head to head" comparisons without undue constraints.

(9) Similar results were observed under ambient pH (5.6) and at pH 7.4 [86 mM tris(hydroxymethyl)aminomethane/HCl buffer].

(10) For steroidal polyamine−DNA binding interactions, see: Hsieh, H. P.; Muller, J. G.; Burrows, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 12077.