Synthesis of 24ξ-Squalamine, an Anti-Infective Steroidal Polyamine

Anthony D. Pechulis,† Frank H. Bellevue III,† Christopher L. Cioffi,† Sean G. Trapp,† John P. Fojtik,† Anthony A. McKitty,† William A. Kinney,‡ and Leah L. Frye*,†

Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York 12180 and Magainin Pharmaceuticals, Inc., Plymouth Meeting, Pennsylvania 19462

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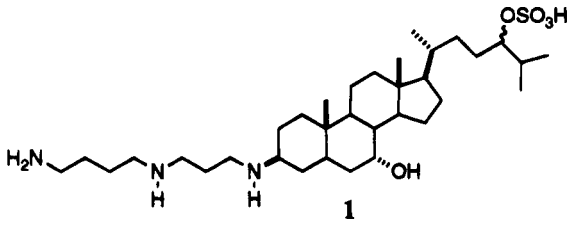
Squalamine (**1**) is a novel steroidal polyamine which exhibits broad spectrum anti-infective activity. It inhibits the growth of bacteria, both Gram positive and Gram negative, and fungi. The synthesis of 24ξ-squalamine was accomplished in 17 steps from 3β-hydroxy-5-cholenic acid. The stereospecific introduction of the 7α-hydroxyl group was achieved by allylic oxidation followed by hydrogenation of the Δ^5 olefin and reduction of the 7-keto group with K-selectride. The polyamine side chain was introduced *via* reductive amination of an appropriately functionalized 3-keto steroid with a suitably protected spermidine utilizing sodium cyanoborohydride as the reducing agent. The required 24-sulfate was introduced by selective sulfation of the 7α,24ξ-diol with sulfur trioxide–pyridine complex.

Squalamine (**1**) is a novel steroidal polyamine which has recently been isolated from the dogfish shark, *Squalus acanthias*.[1,2] This water soluble steroidal polyamine exhibits potent antimicrobial activity against Gram negative bacteria *(Escherichia coli, Pseudomonas aeruginosa)*, Gram positive bacteria *(Staphylococcus aureus, Enterococcus faecalis)*, and fungi *(Candida albicans, Candida tropicalis, Candida parapsilosis, and Candida kefyr)*. In addition, it induces the osmotic lysis of protozoa. Squalamine represents a new class of naturally occurring antibiotics of animal origin and is the first example of a natural product which is an adduct of a polyamine and a steroid. The fact that insufficient amounts of squalamine were available for mechanistic studies, coupled with the clear need for the preparation of analogs, prompted us to undertake the synthesis of this interesting molecule.



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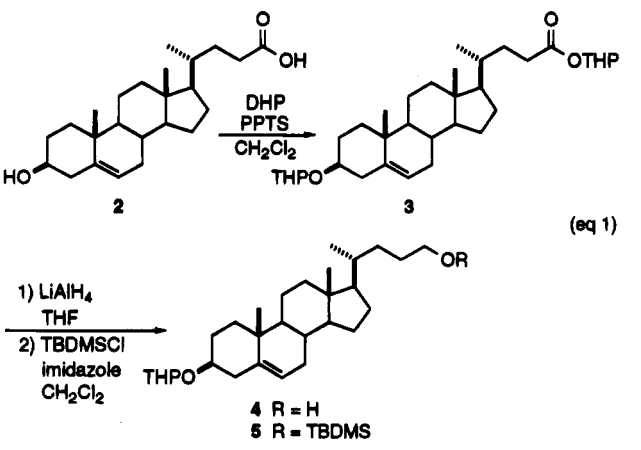
Squalamine (**1**) is a 5α-cholestanol with a 7α-hydroxyl, a C-24 sulfate (stereochemistry undetermined), and a polyamine (spermidine) attached to C-3 in the β position. We envisioned introducing the polyamino side chain *via* a reductive amination of a suitably protected spermidine analog with an appropriately functionalized 3-ketosteroid. The 5-cholenic acid **2** is an obvious starting material for the synthesis of the steroidal portion of squalamine since it contains the following: (1) functionality at C-24 for the introduction of the sulfate and the isopropyl terminus of the side chain, (2) functionality at C-3 for the introduction of the spermidine side chain, and (3) a Δ^5 double bond which allows for the introduction of oxygen functionality at C-7 and easy access to the A-B *trans* ring junction. During the preparation of this manuscript, Moriarty and co-workers reported the synthesis of squalamine (**1**) from cholenic acid.[3]

The 5-cholenic acid **2** was treated with dihydropyran and pyridinium *p*-toluenesulfonate (PPTS) in methylene chloride to provide tetrahydropyranyl (THP)-ether THP-ester **3** in 92% yield (eq 1).[4,5] This relatively unstable



molecule was reduced to 24-alcohol **4** with lithium aluminum hydride in 92% yield. The alcohol was then protected as the *tert*-butyldimethylsilyl (TBDMS) ether by treatment with *tert*-butyldimethylsilyl chloride and imidazole in methylene chloride to give compound **5** in 98% yield.

The requisite oxygen functionality at C-7 was introduced by allylic oxidation of Δ^5 sterol **5** (eq 2). This was accomplished by treatment of sterol **5** with the 1:1 complex of 3,5-dimethylpyrazole (DMP) and chromium

† Rensselaer Polytechnic Institute.
‡ Magainin Pharmaceuticals, Inc.
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(2) Wehrli, S. L.; Moore, K. S.; Roder, H.; Durell, S.; Zasloff, M. *Steroids* **1993**, *58*, 370–378.
(3) Moriarty, R. M.; Tuladhar, S. M.; Guo, L.; Wehrli, S. *Tetrahedron Lett.* **1994**, *35*, 8103–8106.
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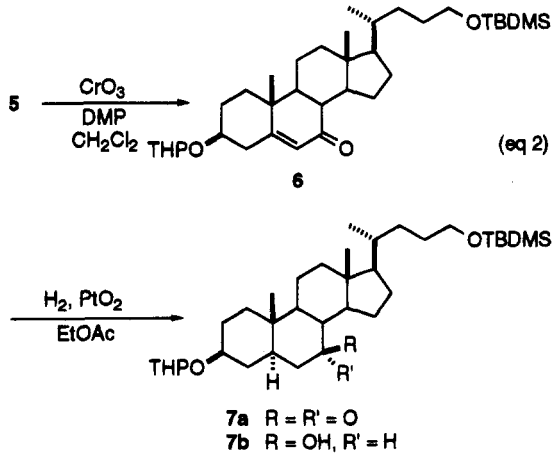
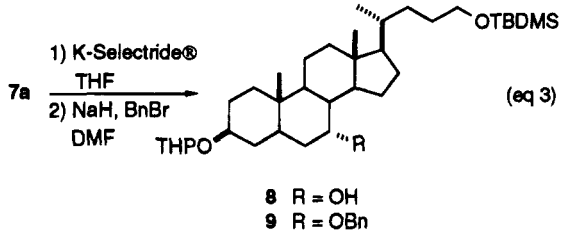
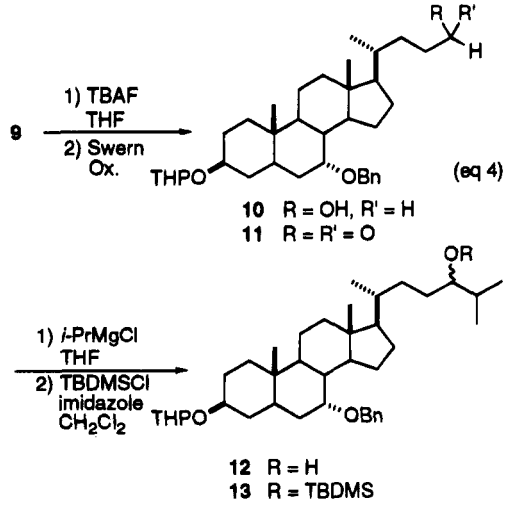


trioxide[6] to give enone **6** in 59% yield. Hydrogenation of enone using Adams catalyst at atmospheric pressure resulted in the formation of 7-ketone **7a** in 71% yield along with 7β-alcohol **7b** (21%).[7] It should be noted that 7β-alcohol **7b** could be oxidized to desired ketone **7a** with Collin's reagent in 64% yield. Stereoselective reduction of 7-ketone **7a** with K-Selectride resulted in formation of required 7α-alcohol **8** in 94% yield (eq 3).[7,8] This



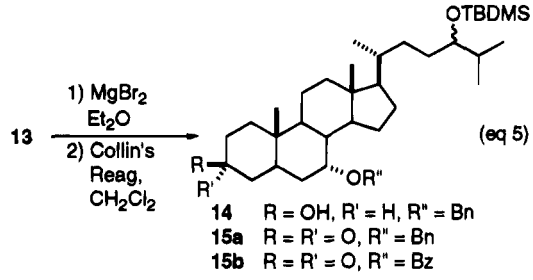
alcohol was then protected as the benzyl ether by treatment with sodium hydride followed by benzyl bromide in DMF giving compound **9** in 87% yield.

The side chain of the steroid was then completed in the following manner (eq 4). The TBDMS protecting



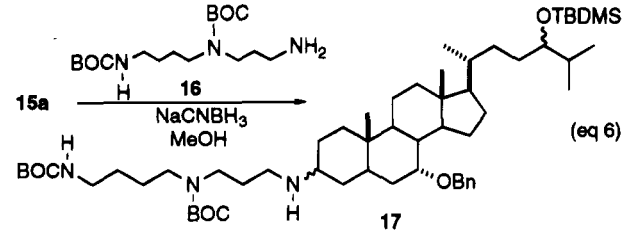
group of compound **9** was removed by treatment with tetra-*n*-butylammonium fluoride (TBAF) to give 24-alcohol **10** in 90% yield. Swern oxidation of this alcohol gave aldehyde **11** in 97% yield. The isopropyl terminus of the side chain was introduced by treatment of aldehyde **11** with isopropylmagnesium chloride giving cholestanol **12** in 77% yield as a mixture of epimers at C-24. The 24-alcohol was then protected as the TBDMS ether as described previously (96% yield). It should be noted that since the absolute stereochemistry at C-24 of squalamine (**1**) is not known, no attempts were made to separate the diastereomers.

As mentioned previously, we envisioned the introduction of the polyamine side chain *via* a reductive amination. The requisite 3-keto steroid was prepared by selective removal of the THP protecting group of compound **13**, in the presence of the TBDMS and benzyl ethers, by treatment with magnesium bromide in diethyl ether[9] affording C-3 alcohol **14** (quantitative yield) followed by oxidation with Collin's reagent to give 3-ketone **15a** (87% yield, eq 5). 7α-(Benzoyloxy)cholestanone **15b**

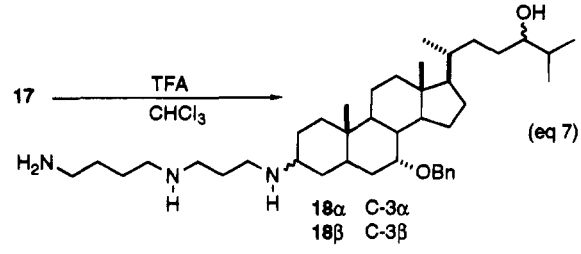


was formed as a byproduct (6.4% yield) during the oxidation *via* benzylic oxidation of the 7α-benzyloxy group. If the oxidation was run at a higher concentration or for longer periods of time, higher yields of the benzoate were obtained.

The polyamino side chain of squalamine was introduced *via* a reductive amination of 3-ketone **15a** with known suitably protected spermidine **16**[10] utilizing sodium cyanoborohydride as the reducing agent giving compound **17** in 66% yield (eq 6). Removal of the BOC



and TBDMS protecting groups was accomplished by treatment with trifluoroacetic acid in chloroform to give the 3β-polyamino sterol **18β** (48% yield) and 3α-polyamino sterol **18α** (43% yield) after separation by flash chromatography (eq 7). The mixture of α and β isomers



was convenient since we were also interested in prepar-

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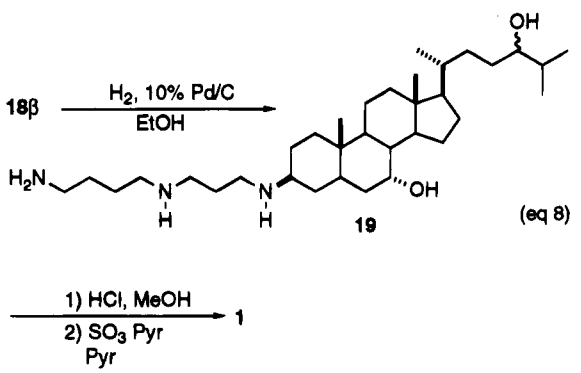
ing 3-*epi*-squalamine. The stereochemistry at C-3 for compounds **18β** and **18α** was initially assigned using ^{1}H NMR spectroscopy. As observed with squalamine **1**,[2] the resonance for the axial C-3 proton of compound **18β** is not resolved from the overlapping triplets associated with the downfield polyamino side chain methylene protons (2.9–2.6 ppm). In contrast, the resonance for the equatorial C-3 proton of compound **18α** is observed downfield from that of compound **18β** (3.43 ppm) and is resolved from the methylene protons. This is consistent with the data reported for the C-3 proton of 3α-aminocholestane (3.2 ppm) and 3β-aminocholestane (2.6 ppm).[11] In addition, the resonance for the C-3 proton of compound **18α** is relatively sharp as expected for an equatorial proton. These stereochemical assignments were confirmed by the conversion of compounds **18β** and **18α** to 24ξ-squalamine (**1**) and 3-*epi*-24ξ-squalamine, respectively. It should be noted that the byproduct obtained in the preparation of compound **17** could be treated with TFA under the same conditions to give compounds **18α** and **18β**.

Hydrogenation (Pd/C, 55 psi) of 3β-polyamino sterol **18β** resulted in removal of the benzyl protecting group from the 7α-alcohol to provide squalamine dessulfate (**19**) in 68% yield (eq 8). To reduce the nucleophilicity of the



amines of the polyamino side chain, they were protonated by treatment with concd hydrochloric acid in methanol. Subsequent treatment with sulfur trioxide–pyridine resulted in sulfation of the substantially less hindered 24-alcohol giving 24ξ-squalamine (**1**) in 56% yield which was purified by flash chromatography (SiO_2, 12:4:1 CH_2Cl_2:MeOH:NH_4OH).

Prior to biological evaluation, a sample of the synthetic squalamine free base was further purified by reverse phase HPLC (acetonitrile/water/TFA) to remove any residual silica gel. This afforded squalamine as the trifluoroacetic acid salt. The minimum inhibitory concentrations exhibited by this synthetic squalamine against a variety of microorganisms were virtually identical to those obtained for natural squalamine: *S. aureus*, 1 μg/mL; *E. coli*, 2–4 μg/mL; *P. aeruginosa*, 32 μg/mL; *C. albicans*, 8 μg/mL.

In summary, 24ξ-squalamine (**1**) has been prepared from 3β-hydroxy-5-cholenic acid in 17 steps. The methodology developed for the synthesis of this interesting steroidal polyamine is being utilized in the preparation of analogs. We are currently undertaking studies to determine the absolute configuration of natural squalamine at C-24. In addition, we are involved in studying the mechanism of action and biosynthesis of squalamine.

Experimental Section

Analytical instrumentation and spectral data formats are the same as previously described.[12] Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. FAB mass spectral data (low and high resolution) were obtained from M-Scan Inc., West Chester, PA.

An authentic sample of natural squalamine (**1**) was obtained from Magainin Pharmaceuticals, Inc., Plymouth Meeting, PA. THF and Et_2O were distilled from sodium/benzophenone ketyl. Pyridine was distilled from KOH. Methylene chloride and pentane were distilled from CaH_2. DMF was distilled from BaO under reduced pressure. Methanol was dried over 3 Å molecular sieves prior to use. PPTS was prepared *via* the method of Grieco.[5] Molecular sieves were dried in an oven (170 °C) overnight prior to use. Silica gel (EM Science Silica Gel 60, 230–400 Mesh) was used for all flash chromatography.

3β-(Tetrahydropyranyloxy)chol-5-en-24-oic Acid 24-Tetrahydropyranyl Ester (3). 3β-Hydroxy-5-cholenic acid **2** (7.58 g, 20 mmol) was suspended in a solution of dry CH_2Cl_2 (300 mL). Distilled dihydropyran (19.0 mL, 200 mmol) was added, followed by a catalytic amount of pyridinium *p*-toluenesulfonate (1.1 g, 4.0 mmol). The suspension was stirred at room temperature overnight under argon. During this period of time, the steroid went into solution. The resultant solution was washed with aqueous saturated NH_4Cl solution (2×), aqueous saturated $NaHCO_3$ solution (2×), and aqueous saturated NaCl solution. The organic layer was dried over anhyd $MgSO_4$ and filtered, and the solvent was removed *in vacuo*. The crude solid was purified by flash chromatography (SiO_2, hexanes/EtOAc 10:1), giving compound **3** as a white solid (9.8 g, 18.5 mmol, 92%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.96 (brs, 1H), 5.37–5.32 (m, 1H), 4.72 (brs, 1H), 3.95–3.87 (m, 2H), 3.71–3.64 (m, 1H), 3.58–3.44 (m, 2H), 1.01 (s, 3H), 0.94 (d, J = 6.3 Hz, 3H), 0.68 (s, 3H); IR ($CHCl_3$) 2930, 1730 cm^{-1}.

3β-(Tetrahydropyranyloxy)chol-5-en-24-ol (4). Compound **3** (16.1 g, 30 mmol) in dry tetrahydrofuran (THF, 150 mL) was added to a suspension of $LiAlH_4$ (5.5 g, 145 mmol) in dry THF (200 mL). The suspension was stirred at 0 °C with a mechanical stirrer under argon overnight. The resultant gray slurry was quenched with EtOAc, followed by aqueous saturated Na_2SO_4 solution. During the addition of the Na_2SO_4 solution, a white precipitate formed and the solution became clear. Anhydrous Na_2SO_4 was added, and the mixture was stirred for 15 min and then filtered. The filter cake was washed well with ethyl acetate, and the filtrate was concentrated *in vacuo*. The resulting solid was purified by flash chromatography (SiO_2, hexanes/EtOAc 5:1), giving compound **4** as a white solid (12.3 g, 27.7 mmol, 92%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37–5.32 (m, 1H), 4.72 (brs, 1H), 3.95–3.88 (m, 1H), 3.62–3.47 (m, 4H), 1.01 (s, 3H), 0.93 (d, J = 6.6 Hz, 3H), 0.68 (s, 3H); IR ($CHCl_3$) 3610, 2900 cm^{-1}; MS (CI/isobutane) m/z 445 (M + 1, 2%), 343 (M + 1 − THPOH, 100%); mp 130–131 °C. Anal. Calcd for $C_{29}H_{48}O_3$: C 78.33, H 10.88. Found: C 78.00, H 10.92.

24-[(*tert*-Butyldimethylsilyl)oxy]-3β-(tetrahydropyranyloxy)chol-5-ene (5). Compound **4** (7.6 g, 17 mmol) in dry CH_2Cl_2 (300 mL) was treated with a solution of *tert*-butyldimethylsilyl chloride (TBDMSCl, 1.0 M) and imidazole (0.5 M) in dry CH_2Cl_2 (38.0 mL, 38.0 mmol TBDMSCl). The solution was stirred at room temperature under argon overnight. The resultant solution was poured into an aqueous saturated $NaHCO_3$ solution and the mixture extracted with CH_2Cl_2 (3×). The combined organic layers were washed with saturated sodium chloride, dried over anhyd $MgSO_4$ and filtered, and the solvent was removed *in vacuo*. The resultant solid was purified by flash chromatography (SiO_2, hexanes/EtOAc gradient from 20:1 to 5:1) giving compound **5** (9.4 g, 17 mmol, 98%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38–5.32 (m, 1H),

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(11) Kim, J. C.; Choi, S.-K.; Cho, I.-S.; Yu, D.-S.; Ryu, S. H.; Moon, K. H. *Yakhak Hoeji* **1985**, *29*, 62–69.
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4.72 (brs, 1H), 3.95–3.88 (m, 1H), 3.60–3.46 (m, 4H), 1.01 (s, 3H), 0.93 (d, J = 6.6 Hz), 0.89 (s, 9H), 0.67 (s, 3H), 0.05 (s, 6H); IR ($CHCl_3$) 2900 cm^{-1}; MS (CI/isobutane) m/z 559 (M + 1, 1%), 474 (M + 1 − THP, 12%), 457 (M + 1 − THPOH, 18%), 343 (M + 1 − THP − TBDMSOH, 6%), 325 (M + 1 − THPOH − TBDMSOH, 100%); mp 116–118 °C. Anal. Calcd for $C_{35}H_{62}O_3Si$: C 75.21, H 11.18. Found: C 75.37, H 11.24.

24-[(*tert*-Butyldimethylsilyl)oxy]-3β-(tetrahydropyranyloxy)chol-5-en-7-one (6). Chromium trioxide (6.43 g, 64.4 mmol) was suspended in dry CH_2Cl_2 (100 mL). The mechanically stirred suspension under argon was cooled to −78 °C *via* a dry-ice/acetone bath. 3,5-Dimethylpyrazole (6.18 g, 64.4 mmol) was added to the suspension as a solid, and the mixture was allowed to stir for 25 min at −78 °C to ensure complete formation of the complex. Compound **5** (3.10 g, 5.37 mmol) was then added to the mixture as a solid, and the reaction mixture was allowed to slowly warm to room temperature and stirred overnight. The mixture was then transferred to a one-neck 500 mL round bottom flask, and silica gel (flash grade) was introduced. The slurry was concentrated to a free flowing solid which was introduced onto the top of a wet packed flash column (SiO_2), and the product was eluted with hexanes/ethyl acetate (gradient 30:1 to 15:1 to 6:1 to 3:1). The desired product, compound **6** (1.80 g, 59%), was obtained as a white solid: ^1H NMR (500 MHz, $CDCl_3$) δ 5.65 and 5.63 (2s, 1H), 4.70–4.64 (m, 1H), 3.90–3.81 (m, 1H), 3.70–3.62 (m, 1H), 3.57 (t, J = 6.6 Hz, 2H), 3.52- 3.46 (m, 1H), 1.19 (s, 3H, C-19 H), 0.93 (d, J = 6.3 Hz), 0.90 (s, 9H), 0.68 (s, 3H), 0.05 (s, 6H); IR ($CHCl_3$) 2900, 1650 cm^{-1}; MS (CI/isobutane) m/z 573 (M + 1, 11%), 489 (M + 1 − THP, 100%); mp 118–120 °C. Anal. Calcd for $C_{35}H_{60}O_4Si$: C 73.37, H 10.56. Found: C 73.41, H 10.66.

24-[(*tert*-Butyldimethylsilyl)oxy]-3β-(tetrahydropyranyloxy)-5α-cholan-7-one (7a). Compound **6** (1.0 g, 1.75 mmol) was dissolved in EtOAc (75 mL), and platinum(IV) oxide (0.012 g, 0.049 mmol) was added. The mixture was placed on a hydrogenation apparatus (atmospheric). The setup was evacuated to remove the dissolved oxygen, and then hydrogen was introduced. The evacuation and introduction of hydrogen process was repeated two times. The reaction was stirred under hydrogen at atmospheric pressure for 2.5 h. The reaction mixture was filtered through Celite and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, hexanes/EtOAc gradient starting with 20:1) giving compound **7a** as a white solid (0.70 g, 71%). 24-[(*tert*-Butyldimethylsilyl)oxy]-3β-(tetrahydropyranyloxy)-5α-cholan-7β-ol (**7b**) was obtained as a byproduct (21%). (Note: This biproduct could be converted to the desired ketone **7a** with Collin's reagent in 64% yield.) Compound **7a**: ^1H NMR (500 MHz, $CDCl_3$) δ 4.73–4.66 (m, 1H), 3.95–3.85 (m, 1H), 3.66–3.52 (m, 3H), 3.50–3.45 (m, 1H, C-3 H), 1.08 (s, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.89 (s, 9H), 0.64 (s, 3H), 0.04 (s, 6H); IR ($CHCl_3$) 2900, 1685 cm^{-1}; MS (CI/isobutane) m/z 575 (M + 1, 85%), 491 (M + 1 − THP, 100%); mp 166–170 °C. Anal. Calcd for $C_{35}H_{62}O_4Si$: C 73.12, H 10.87. Found: C 72.88, H 10.78.

24-[(*tert*-Butyldimethylsilyl)oxy]-3β-(tetrahydropyranyloxy)-5α-cholan-7α-ol (8). K selectride (potassium tri-*sec*-butylborohydride) (8.9 mL, 1 M in THF, 8.9 mmol) was added dropwise *via* syringe to a solution of ketone **7a** (1.7 g, 3.0 mmol) in dry THF (50 mL) at rt under argon. The reaction mixture was heated to 50 °C in an oil bath and stirred for 5 h. The mixture was allowed to cool to room temperature and then quenched by adding 30% H_2O_2 dropwise until the evolution of gas ceased. Saturated aqueous NH_4Cl solution was added, and the aqueous solution was extracted (3×) with Et_2O. The combined organic extracts were washed with aqueous saturated $NaHCO_3$ solution (2×), distilled H_2O (2×), and aqueous saturated NaCl solution, dried over anhyd $MgSO_4$, and filtered, and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (SiO_2, hexanes/EtOAc 10:1) giving alcohol **8** as a white solid (1.6 g, 94%): ^1H NMR (500 MHz, $CDCl_3$) δ 4.73–4.66 (m, 1H), 3.95–3.85 (m, 1H), 3.82 (s, 1H), 3.66–3.52 (m, 3H), 3.50–3.45 (m, 1H), 1.08 (s, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.89 (s, 9H), 0.64 (s, 3H,), 0.04 (s, 6H); IR ($CHCl_3$) 3430, 2860 cm^{-1}; MS (CI/isobutane) m/z 577 (M + 1, 5%), 493 (M + 1 − THP, 22%), 475 (M + 1 − THPOH, 26%), 458 (M + 1 − THPOH − H_2O, 38%), 343 (M + 1 − THPOH − TBDMSOH, 80%), 325 (M + 1 − THPOH − TBDMSOH − H_2O, 100%); mp 130–133 °C. Anal. Calcd for $C_{35}H_{64}O_4Si$: C 72.86, H 11.18. Found: C 72.69, H 11.32.

7α-(Benzyloxy)-24-[(*tert*-butyldimethylsilyl)oxy]-3β-(tetrahydropyranyloxy)-5α-cholane (9). A flame-dried round bottom flask with stirring bar was charged with sodium hydride (60% in mineral oil, 28 mg, 0.69 mmol), equipped with a septum and a gas-needle inlet and flushed with argon. The mineral oil was removed by washing (3×) with dry pentane, and the pentane was removed to provide the sodium hydride as a powder. Dry DMF (2.0 mL) was added. A solution of alcohol **8** (40 mg, 0.069 mmol) in dry THF (2 mL) was added dropwise *via* syringe. The reaction mixture was stirred overnight and then heated to 40 °C in an oil bath over a 20 min period. Freshly distilled benzyl bromide (0.165 mL, 1.38 mmol) was added dropwise, and the reaction mixture was stirred at 40 °C for 10 h. The reaction was allowed to cool to rt, and the solvent was removed under reduced pressure. The flask was placed under vacuum overnight to remove any residual DMF. The crude material was purified by flash chromatography (SiO_2, hexanes/EtOAc 50:1) giving compound **9** as a white solid (40 mg, 0.060 mmol, 87%). A gradient of increasing EtOAc concentration provided other components, including the 7α-formate (1 mg, 1%) as well as recovered starting material (3 mg, 8%). Compound **9**: ^1H NMR (500 MHz, $CDCl_3$) δ 7.35–7.20 (m, 5H), 4.73–4.66 (m, 1H), 4.535 (d, J = 12.0 Hz, $^1/_2$H), 4.53 (d, J = 12.0 Hz, $^1/_2$H), 4.26 (d, J = 12.2 Hz), 4.245 (d, J = 11.8 Hz, $^1/_2$H), 3.95–3.85 (m, 1H), 3.66–3.52 (m, 3H), 3.50–3.45 (m, 2H), 1.08 (s, 3H), 0.91 (d, J = 6.6 Hz), 0.89 (s, 9H), 0.64 (s, 3H), 0.04 (s, 6H); MS (CI/isobutane) m/z 668 (M + 1, 6%), 584 (M + 1 − THP, 18%), 475 (M + 1 − THPOH, 30%), 457 (M + 1 − THPOH − HOBn, 58%), 343 (M + 1 − THP − HOBn − TBDMSOH, 100%), 325 (M + 1 − THPOH − TBDMSOH − HOBn, 83%); IR ($CHCl_3$) 2920 cm^{-1}. Anal. Calcd for $C_{42}H_{70}O_4Si$: C 75.62, H 10.58. Found: C 75.71, H 10.40.

7α-(Benzyloxy)-3β-(tetrahydropyranyloxy)-5α-cholan-24-ol (10). Compound **9** (0.0527 g, 0.079 mmol) in anhyd THF (4 mL) under Ar was treated with tetrabutylammonium fluoride (TBAF) (0.237 mL, 1 M in THF, 0.237 mmol). The solution was stirred until no starting material remained by TLC. The solvent was removed *in vacuo*, the residue was taken up in 5 mL of CH_2Cl_2, washed with 5 mL of aqueous saturated $NaHCO_3$ solution, and the aqueous layer was extracted 2× with 5 mL of CH_2Cl_2. The combined organic layers were dried over anhyd $MgSO_4$, filtered, and the solvent removed *in vacuo*. Flash chromatography (SiO_2, 8:1 hexanes: EtOAc) gave compound **10** (0.0395 g, 90%) as a white solid foam: ^1H NMR (500 MHz, $CDCl_3$) δ 7.35–7.34 (m, 5H), 4.71–4.69 (m, 1H), 4.585 (d, J = 11.8 Hz), 4.58 (d, J = 11.8 Hz, $^1/_2$H), 4.315 (d, J = 12.0 Hz, $^1/_2$H), 4.29 (d, J = 12.0 Hz, $^1/_2$H), 3.94–3.90 (m, 1H), 3.62–3.58 (m, 3H), 3.50–3.48 (m, 1H, C-3 H), 3.45 (s, 1H), 0.92 (d, J = 6.6 Hz, 3H), 0.81 (s, 3H), 0.63 (s, 3H) (Note: This product is a mixture of diastereomers); IR ($CHCl_3$) 3600, 2900 cm^{-1}; MS (CI/isobutane) m/z 554 (M + 1, 2%), 361 (M + 1 − THP − HOBn, 42%), 343 (M + 1 − THP − HOBn, H_2O, 100%); mp 52–56 °C. Anal. Calcd for $C_{36}H_{56}O_4$: C 78.21, H 10.21. Found: C 77.93, H 10.39.

7α-(Benzyloxy)-3β-(tetrahydropyranyloxy)-5α-cholan-24-al (11). DMSO (0.01 mL, 0.14 mmol) in CH_2Cl_2 (0.1 mL) was added dropwise to a stirred solution of oxalyl chloride (0.008 mL, 0.0917 mmol) in anhyd CH_2Cl_2 (2 mL) at −78 °C under anhydrous conditions (drying tube). This solution was stirred at −78 °C for 15 min. Steroid **10** (0.0234 g, 0.0423 mmol) in dry CH_2Cl_2 (0.5 mL) was then added dropwise and the solution stirred for 40 min at −78 °C. Diisopropylethylamine (DIPEA) (0.08 mL, 0.458 mmol) was added and the solution allowed to warm to 0 °C with stirring over a 30 min period. Aqueous saturated $NaHCO_3$ solution (5 mL) was added and the solution extracted 3× with 5 mL of CH_2Cl_2. The combined organic extracts were washed 2× with 5 mL of aqueous saturated NaCl solution, dried over anhyd $MgSO_4$, and filtered, and the solvent was removed *in vacuo*. Flash chromatograpy (SiO_2, hexanes: EtOAc 10:1) gave the compound **11** (0.0226 g, 97%) as a white solid foam. ^1H NMR (500

MHz, $CDCl_3$) δ 9.76 (s, 1H), 7.35–7.34 (m, 5H), 4.71–4.69 (m, 1H), 4.59 (d, J = 11.8 Hz, ½H), 4.585 (d, J = 11.8 Hz), 4.30 (d, J = 12.0 Hz, ½H), 4.29 (d, J = 12.0 Hz, ½H), 3.95–3.89 (m, 1H), 3.63–3.58 (m, 3H), 3.50–3.47 (m, 1H), 3.45 (s, 1H), 2.49-2.42 (m, 1H), 2.37–2.31 (m, 1H), 0.958 (d, J = 6.5 Hz, 3H), 0.81 (s, 3H), 0.63 (s, 3H) (Note: This product is a mixture of diasteromers); IR ($CHCl_3$) 2900, 1700 cm^{-1}; MS (CI/isobutane) m/z 552 (M + 1, 0.4%), 465 (M + 1 − THP, 3%), 449 (M + 1 − THPO, 14%), 375 (M + 1 − THP − Bn, 7%), 359 (M + 1 − THP − HOBn, 68%), 341 (M + 1 − THP − HOBn − H_2O, 100%); mp 50–54 °C. Anal. Calcd for $C_{36}H_{54}O_4$: C 78.50, H 9.88. Found: C 78.11, H 10.04.

7α-(Benzyloxy)-3β-(tetrahydropyranyloxy)cholestan-24ξ-ol (12). A solution of compound **11** (0.374 g, 0.679 mmol) in anhyd THF (10 mL) under argon was treated with isopropylmagnesium chloride (2 mL, 2 M in THF, 5.43 mmol) at rt. The reaction was stirred until no starting material remained by TLC. Aqueous NH_4Cl solution (10%, 15 mL) was added to quench the reaction, and the THF was removed *in vacuo*. Distilled H_2O (5 mL) was added and the solution extracted 3× with 15 mL of CH_2Cl_2. The combined organic layers were washed with aqueous saturated NaCl solution (15 mL), dried over anhyd $MgSO_4$, and filtered, and the solvent was removed *in vacuo*. Flash chromatography (SiO_2, hexanes:EtOAc 12:1) gave compound **12** (0.3117 g, 77%) as a white foam: ^1H NMR (500 MHz, $CDCl_3$) δ 7.35–7.34 (m, 5H), 4.71–4.69 (m, 1H), 4.585 (d, J = 11.9 Hz, 1H), 4.31 (d, J = 12.0 Hz, ½H), 4.295 (d, J = 12.0 Hz, ½H), 3.94–3.91 (m, 1H), 3.62–3.58 (m, 1H), 3.50–3.48 (m, 1H), 3.45 (s, 1H), 3.32–3.31 (m, 1H), 0.81 (s, 3H), 0.61 (s, 1.5H), 0.65 (s, 1.5H) (Note: This product is a mixture of diastereomers); IR ($CHCl_3$) 3605, 2900 cm^{-1}; MS (CI/isobutane) m/z 595 (M + 1, 10%), 401 (M + 1 − THP − Bn − H_2O, 25%), 385 (M + 1 − THP − HOBn − H_2O, 100%); mp 55–59 °C. Anal. Calcd for $C_{39}H_{62}O_4$: C 78.74, H 10.50. Found: C 78.65, H 10.54.

7α-(Benzyloxy)-24ξ-[(*tert*-butyldimethylsilyl)oxy]-3β-(tetrahydropyranyloxy)cholestane (13). Compound **12** (0.050 g, 0.084 mmol) in dry CH_2Cl_2 (1 mL) was treated with a solution of *tert*-butyldimethylsilyl chloride (TBDMSCl, 0.5 M) and imidazole (1.0 M) in dry CH_2Cl_2 (0.80 mL, 0.40 mmol TBDMSCl). The reaction was stirred at rt under argon for 24 h. Aqueous saturated $NaHCO_3$ solution (5 mL) was added and the solution extracted 3× with 10 mL of CH_2Cl_2. The combined organic layers were washed with 10 mL of aqueous saturated NaCl solution and dried over anhyd Na_2SO_4. Filtration and removal of solvent *in vacuo* followed by flash chromatography (SiO_2, hexanes:EtOAc 20:1) gave the desired product **13** (0.057 g, 96%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$) δ 7.35–7.34 (m, 5H) 4.70–4.69 (m, 1H), 4.59 (d, J = 12.0 Hz, 1H), 4.315 (d, J = 12.0 Hz, ½H), 4.31 (d, J = 12.0 Hz, ½H), 3.94–3.91 (m, 1H), 3.62–3.58 (m, 1H), 3.50–3.48 (m, 1H), 3.45 (s, 1H), 3.37–3.35 (m, 1H), 0.89 (d, J = 1.0 Hz, 9H), 0.81 (s, 3H), 0.62 (s, 3H), 0.04 and 0.03 (2s, 6H)(Note: This product is a mixture of diastereomers); IR ($CHCl_3$) 2900 cm^{-1}; MS (CI/isobutane) m/z 709 (M + 1, 20%), 367 (M + 1 − THPOH − HOBn − TBDMSOH, 100%); mp 52–58 °C. Anal. Calcd for $C_{45}H_{76}O_4Si$: C 76.21, H 10.80. Found C 76.11, H 10.81.

7α-(Benzyloxy)-24ξ-[(*tert*-butyldimethylsilyl)oxy]-cholestan-3β-ol (14). Compound **13** (0.057 g, 0.0803 mmol) was dissolved in dry Et_2O (3 mL) under argon. $MgBr_2$ (0.142 g, 0.771 mmol) was added quickly as a solid and the reaction was stirred until no starting material remained by TLC. H_2O (10 mL) was added and the mixture was extracted 3x with 10 mL Et_2O. The combined organic layers were dried over anhyd $MgSO_4$, filtered and the solvent removed *in vacuo*. Flash chromatography (SiO_2, hexanes:EtOAc 7:1) gave compound **14** (0.0493 g, 98%) as a white foam: ^1H NMR (500 MHz, $CDCl_3$) δ 7.36–7.35 (m, 5H), 4.59 (d, J = 12.0 Hz, 1H), 4.34 (d, J = 12.0 Hz, 1H), 3.65–3.60 (m, 1H), 3.475 (d, J = 2.4 Hz, 1H), 3.40–3.36 (m, 1H), 0.91 (d, J = 0.9 Hz, 9H), 0.82 (s, 3H), 0.65-(s, 3H), 0.05 & 0.04 (s, 6H)(Note: This product is a mixture of diastereomers); IR ($CHCl_3$) 3600, 2900 cm^{-1}; MS (CI/isobutane) m/z 624 (M + 1, 3%), 501 (M + 1 − OTHP, 6%), 385 (M + 1 − OTHP − TBDMS, 68%), 367 (M + 1 − THPOH − TBDMSOH, 100%); mp 55–58 °C. Anal. Calcd for $C_{40}H_{68}O_3Si$: C 76.86, H 10.97. Found: C 76.69, H 10.87.

7α-(Benzyloxy)-24ξ-[(*tert*-butyldimethylsilyl)oxy]-cholest-3-one (15a) and 7α-(Benzoyloxy)-24ξ-[(*tert*-butyldimethylsilyl)oxy]cholestan-3-one (15b). A solution of compound **14** (0.229 g, 0.3664 mmol) in dry CH_2Cl_2 (30 mL) was treated with Collin's reagent (0.385 g, 1.49 mmol). The mixture was stirred at rt overnight under argon. At this time no starting material remained by TLC. Celite was added, and the mixture was stirred for 20 min and then filtered through a pad of Celite. The cake was rinsed well with CH_2Cl_2. The solvent was removed *in vacuo*. Flash chromatography (SiO_2, hexanes:EtOAc 20:1) gave the desired product **15a** (0.198 g, 87%) as a white solid along with the 7α-benzoate **15b** (0.015 g, 6.4%) as a white foam. Note: When the reaction was run a higher concentration, a higher yield of the benzoate was obtained. Compound **15a**: ^1H NMR (500 MHz, $CDCl_3$) δ 7.35–7.27 (m, 5H), 4.55 (d, J = 11.7 Hz, 1H), 4.32 (d, J = 11.7 Hz, 1H), 3.495 (d, J = 2.0 Hz, 1H), 3.38–3.35 (m, 1H), 1.02 (s, 3H), 0.90 (d, J = 0.8 Hz, 9H), 0.67 (s, 3H, C-18 H), 0.04 and 0.03 (s, 6H) (Note: This product is a mixture of diastereomers); IR ($CHCl_3$) 2900, 1690 cm^{-1}; MS (CI/isobutane) m/z 624 (M + 1, 50%), 534 (M + 1 − Bn, 7%), 518 (M + 1 − OBn, 36%), 492 (M + 1 − HOSi(Me)$_2$$t$-Bu, 28%), 383 (M + 1 − $C_{14}H_{30}OSi$, 100%). Compound **15b**: ^1H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, J = 7.3 Hz, 2H), 7.59 (t, J = 7.4 Hz, 1H), 7.48 (t, J = 7.7 Hz, 2H), 5.20 (br s, 1H), 3.35–3.31 (m, 1H), 1.08 (s, 3H), 0.86 (d, J = 3.7, 9H), 0.71 (s, 3H) (Note: This product is a mixture of diastereomers); IR ($CHCl_3$) 2900, 1690 cm^{-1}; MS (CI/isobutane) m/z 637 (M + 1, 3%), 516 (M + 1 − OBz, 16%), 382 (M + 1 − OBz − TBDMSOH, 100%); mp 62–65 °C.

7α-(Benzyloxy)-3ξ-[5,10-bis(*tert*-butoxycarbonyl)-1,5,-10-triazadecyl]-24ξ-[(*tert*-butyldimethylsilyl)oxy]-cholestane (17). A mixture of compound **15a** (0.07 g, 0.11 mmol), approximately 2 equiv of amino compound **16**,[10] and 3 Å molecular seives (0.5 g) in MeOH (6 mL, dried over 3 Å seives) was stirred for 12 h at rt under argon. $NaCNBH_3$ (0.33 mL, 1 M in THF, 0.33 mmol) was added and the solution stirred for 24 h at rt under argon. The mixture was filtered through Celite, the cake was washed well with MeOH and CH_2Cl_2, and the solvents were removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (10 mL), washed 2× with 5 mL of H_2O made basic with aqueous NaOH solution (5%), and washed 2× with of 5 mL aqueous saturated NaCl solution. The combined aqueous layers were back extracted with CH_2Cl_2, and the combined organic layers were dried over anhyd $MgSO_4$. Filtration, removal of the solvent *in vacuo*, and flash chromatography (SiO_2, gradient of increasing polarity from 2% MeOH in CH_2Cl_2 to 10% MeOH in CH_2Cl_2) gave the desired product **17** (0.07 g, 66%) and a more polar product which is missing one *t*-BOC group and is contaminated with excess amine. Compound **17**: ^1H NMR (500 MHz, $CDCl_3$) δ 7.36–7.28 (m, H), 4.63 (d, J = 12.0 Hz, ½H), 4.58 (d, J = 12.0 Hz, ½H), 4.33 (t, J = 1.25 Hz, 1H), 3.49 (s, 1H), 3.46–3.14 (m, 8H), 2.91–2.86 (m, 2H), 1.47-1.41 (m, 18H), 0.90 (s, 9H), 0.84 (s, 3H), 0.64 (s, 3H), 0.05 and 0.04 (s, 6H) (Note: This product is a mixture of diastereomers).

7α-(Benzyloxy)-3β-(1,5,10-triazadecyl)cholestan-24ξ-ol (18β) and 7α-(Benzyloxy)-3α-(1,5,10-triazadecyl)-cholestan-24ξ-ol (18α). TFA (1.8 mL, 24 mmol) was added to a solution of compound **17** (0.386 g, 0.4 mmol) in $CHCl_3$ (15 mL) at rt. The reaction was stirred until no starting material remained by TLC. The solvent was removed *in vacuo* and the residue purified by preparative TLC (SiO_2, 2000 μm, 6:3:1 CH_2Cl_2:MeOH:NH_4OH, R_f = 0.46) to give the desired 3β product **18β** (0.122 g, 48%) and the 3α isomer **18α** (0.109 g, 43%). It should be noted that the byproduct obtained in the previous reaction could be treated with TFA under the same conditions to give compounds **18α** and **18β**. Compound **18β**: ^1H NMR (500 MHz, CD_3OD) δ 7.32–7.35 (m, 5H), 4.57 (d, J = 11.7 Hz, 1H), 4.31 (d, J = 11.7 Hz, 1H), 3.52 (s, 1H), 3.22–3.21 (m, 2H), 2.86 (t, J = 7.1 Hz, 2H), 2.81 (t, J = 6.6 Hz, 2H), 2.74 (t, J = 7.0 Hz, 2H), 2.67 (t, J = 6.3, 2H), 0.85 (s, 3H), 0.683 (s, 1.5H, C-18 H), 0.678 (s, 1.5H, C-18 H); MS (pos. FAB) m/z 638.6 (M + 1, 100%). Compound **18α**: ^1H NMR (500 MHz, CD_3OD) δ 7.35–7.22 (m, 5H), 4.61 (d, J = 11.4 Hz, 1H), 4.28 (d, J = 11.4 Hz, 1H), 3.53 (s, 1H), 3.43 (s, 1H), 3.24–3.20 (m, 2H), 3.11 (t, J = 7.1 Hz, 2H), 3.08–3.02 (m, 2H), 2.96 (t,

J = 6.9 Hz, 2H), 0.85 (s, 3H), 0.691 (s, 1.5H), 0.686 (s, 1.5H); MS (pos. FAB) m/z 638.6 (M + 1, 100%).

3β-(1,5,10-Triazadecyl)cholesta-7α,24ξ-diol (19). To a solution of compound **18β** (0.0128 g, 0.02 mmol) in absolute EtOH (8 mL) were added a catalytic amount of 10% Pd/C and 2 drops of concd hydrochloric acid. The mixture was placed on a Parr hydrogenation apparatus and shaken under 55 psi (H_2) for 24 h. The solution was filtered through a pad of Celite, the cake was washed well with EtOH and MeOH, and the solvents were removed *in vacuo*. The desired product **19** (0.0074 g, 68%) was obtained. If the product was pure by TLC, it was used without further purification. If impurities were observed by TLC, the material was purified by flash chromatography (SiO_2, CH_2Cl_2:MeOH:NH_4OH 15:4:1): ^1H NMR (500 MHz, CD_3OD) δ 3.79 (s, 1H) 3.22–3.13 (m, 6H), 3.09 (t, J = 7.4, 2H, CH_2N), 2.99 (t, J = 7.3 Hz), 0.87 (s, 3H), 0.694 (s, 1.5H), 0.691 (s, 1.5H, C-18 H).

24ξ-Squalamine (1). Compound **19** (0.0176 g, 0.032 mmol) was dissolved in a solution of concd hydrochloric acid in MeOH (1 mL of concd hydrochloric acid in 10 mL of MeOH). The solution was stirred for 15 min and the solvent removed *in vacuo*. To the crude, dried product was added SO_3–pyridine complex (0.010 g, 0.064 mmol), and the flask was flushed with argon. Dry pyridine (1 mL) was added, and the solution was warmed to 40 °C in an oil bath and stirred for 2 h. MeOH (2 mL) was added. The flask was removed from the oil bath, and the mixture was stirred for 15 min. The solvent was removed *in vacuo*, and the residue was resuspended in MeOH and filtered through a pad of Celite. The cake was washed well with MeOH. Flash chromatography (SiO_2, CH_2Cl_2:MeOH:NH_4OH 12:4:1) gave the desired product **1** (0.0113 g, 56%, white solid) as the free base. The product was one spot by TLC (6:3:1 CH_2Cl_2/MeOH/NH_4OH, R_f = 0.46) with an R_f identical to natural squalamine: ^1H NMR (500 MHz, CD_3OD) δ 4.13–4.10 (m, 1H), 3.79 (s, 1H), 3.22–3.10 (m, 5H), 3.08 (t, J = 6.7 Hz, 2H), 2.98 (t, J = 6.8 Hz, 2H), 0.87 (s, 3H), 0.70 (s, 3H); IR ($CHCl_3$) 3447, 2943, 1690, 1471, 1381, 1200, 1136, 1051, 910, 837, 802, 723, 634, 591, 472 cm^{-1}; MS (pos. FAB) m/z 628.4 (M + 1, 57%), 548.5 (M + 1 − SO_3, 23%), 530.5 (M + 1 − H_2SO_4, 100%); high resolution MS (pos. FAB) m/z 628.4669 (calcd: 628.4723). Notes: The ^1H NMR spectrum of the synthetic squalamine is identical to that of natural squalamine (Magainin Pharmaceuticals) except for the methyl group region which is more complex in the synthetic case since it is a mixture of two diastereomers. The mass spectra of the synthetic and natural squalamine are identical.

HPLC Purification of Squalamine (1). A sample of synthetic squalamine free base was purified on a Rainin Microsorb C-18 column (8 μm, 100 Å pore size, 21.4 mm i.d., 25 cm) to remove any residual silica from the flash chromatography. Linear gradient elutions (0–20% acetonitrile in water with 0.1% trifluoroacetic acid (TFA) over 10 min followed by 20–90% acetonitrile in water with 0.1% TFA over 60 min) afforded squalmine as the TFA salt. The flow rate was 15 mL/min. One minute fractions were collected, and the fractions containing squalamine (fractions 30–32) were identified by TLC (6:3:1 CH_2Cl_2/MeOH/NH_4OH).

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Supporting Information Available: NMR peak assignments for all compounds (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from ACS; see any current masthead page for ordering information.

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