Synthesis of Squalamine Utilizing a Readily Accessible Spermidine Equivalent

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Introduction

Squalamine (**3**, Scheme 1) is a natural aminosterol found in the tissues of the dogfish shark, which was initially identified based on its antimicrobial activity.[1] Its structure was determined by spectroscopic[2] and chemical[3] means. More recently, squalamine was found to be antiangiogenic in that it inhibits endothelial cell function and effects the growth of solid tumors.[4−7] The need for new chemotherapeutic approaches in the treatment of cancer[8,9] and the importance of angiogenesis in tumor growth and in the development of metastatic disease[10,11] have both propelled clinical development of antiangiogenic agents such as squalamine. Although the majority of the squalamine used in preclinical studies was obtained by extraction and purification of dogfish livers, this source was projected to be too costly and unreliable to provide adequate supplies for clinical trials. Therefore, we directed our efforts to a cost-effective chemical synthesis.

The published syntheses of squalamine were not suitable for this purpose. The chemical synthesis of squalamine has been demonstrated in 17 steps from both 3β-acetoxy-5-cholenic acid[12] and 3β-hydroxy-5-cholenic acid.[13] The published methods, however, afforded a mixture of diastereomers at C-24 and required an expensive starting material. A 20-step formal stereoselective synthesis of squalamine (**3**) from the inexpensive starting material stigmasterol (**1**) was also described.[3] Stigmasterol is also the starting point for the fifteen step synthesis of squalamine described in this report (Scheme 1). The first 10 steps of this route have recently been reported (**1** to **2**).[14,15]

The final five steps involve the incorporation of the polyamine and sulfate moieties. Steroid **2** is suitably protected to allow the stepwise elaboration of the target molecule. There remained some significant challenges in completing a practical synthesis of squalamine. We expected to couple a protected polyamine to a C-3 ketone by a reductive process to afford the β-polyamine. Frye has shown that there is no stereoselectivity in the reductive amination in the presence of a C-7 benzyl[13] or benzoate.[16] Additionally, the yield of sulfation of the C-24 hydroxyl group was low with a spermidine-functionalized molecule. Therefore, our plan was to first introduce the sulfate and then cleave the benzoate group, once its function had been served; the stability of the sulfate to conditions that cleave the benzoate has been demonstrated.[16] The unprotected C-7 hydroxyl group was predicted to provide less steric hindrance to the approach of the reducing agent from the α-face during reductive amination. Supportive evidence that a small group at C-7 is advantageous was provided by the good stereocontrol noted by Moriarty in the presence of a C-7 acetate group[3]. Finally, we had to utilize a protecting group on the polyamine that would be labile under conditions that would not cleave the sulfate group. The BOC-protected spermidine derivative used by Moriarty[3] and Frye[13] is removed in neat trifluoroacetic acid, which would be expected to cleave the sulfate group. The selection of an appropriate protecting group is further complicated by the fact that the group must be stable to the reductive amination conditions. For instance, a trifluoroacetyl-group would be unsatisfactory. Ideally the polyamine should not require many steps or chromatography to prepare in analytically pure form. The BOC-protected spermidine, which is typical of described monoprotected spermidines, is prepared in multiple steps and requires chromatography.[17]

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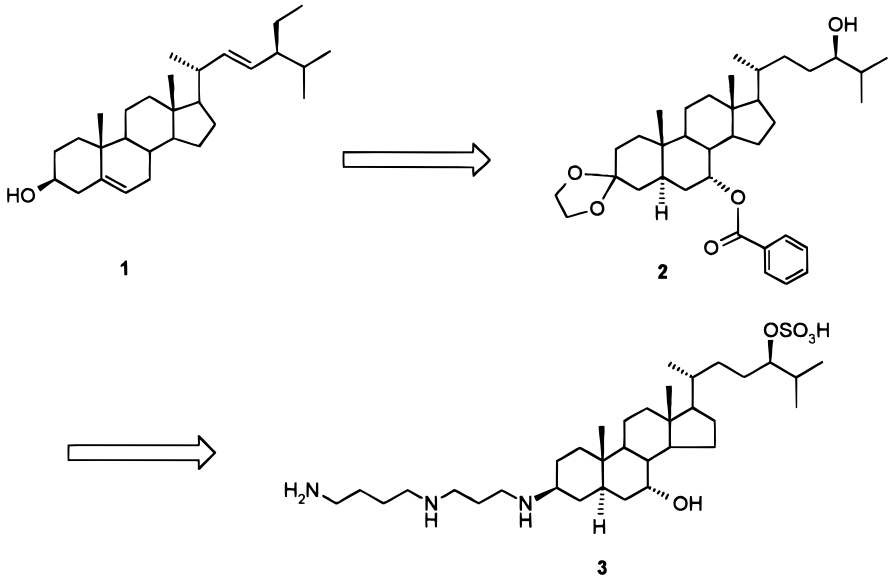
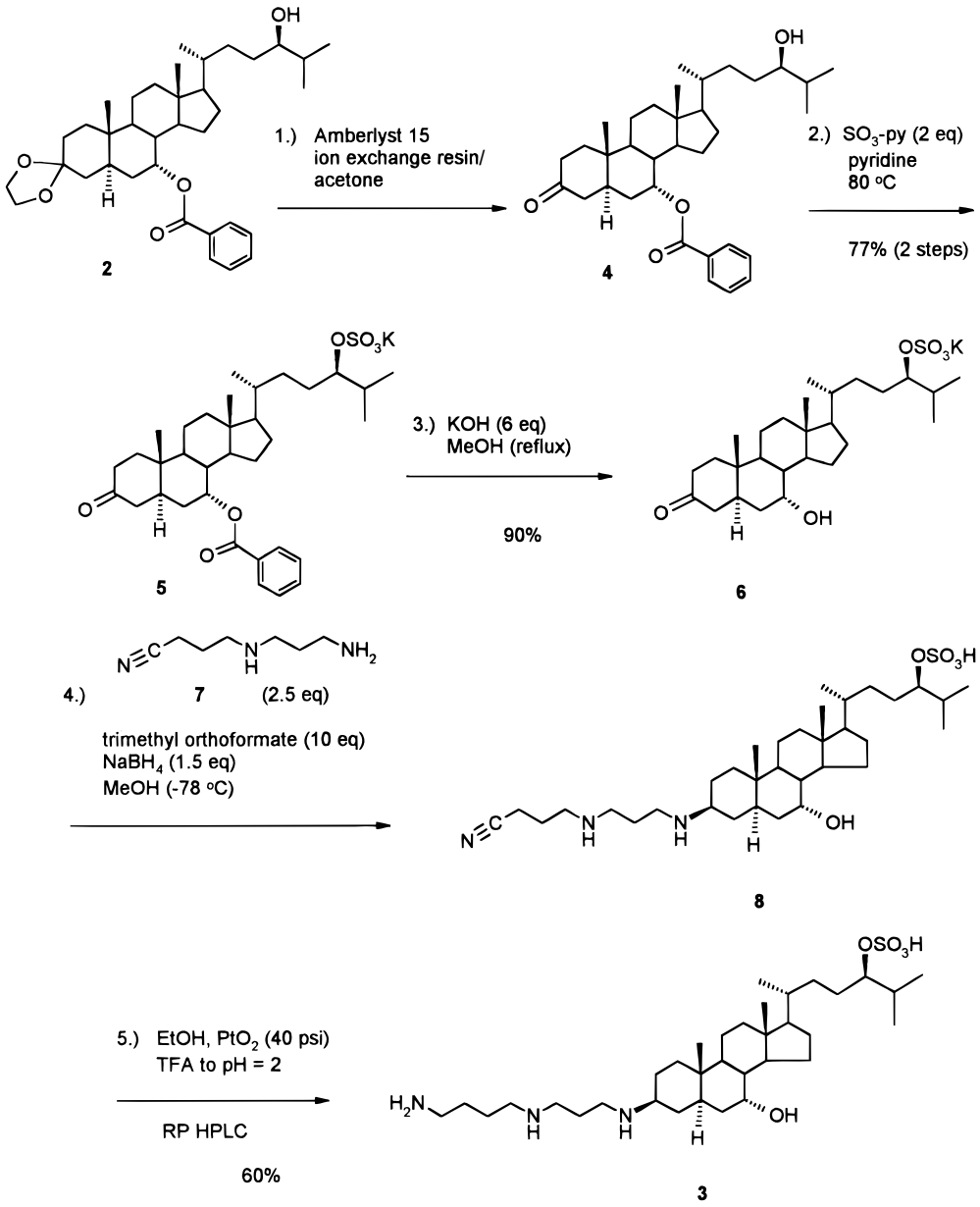
Scheme 1



1 2 3

Scheme 2



2 4 5 6 8 3

Chart 1

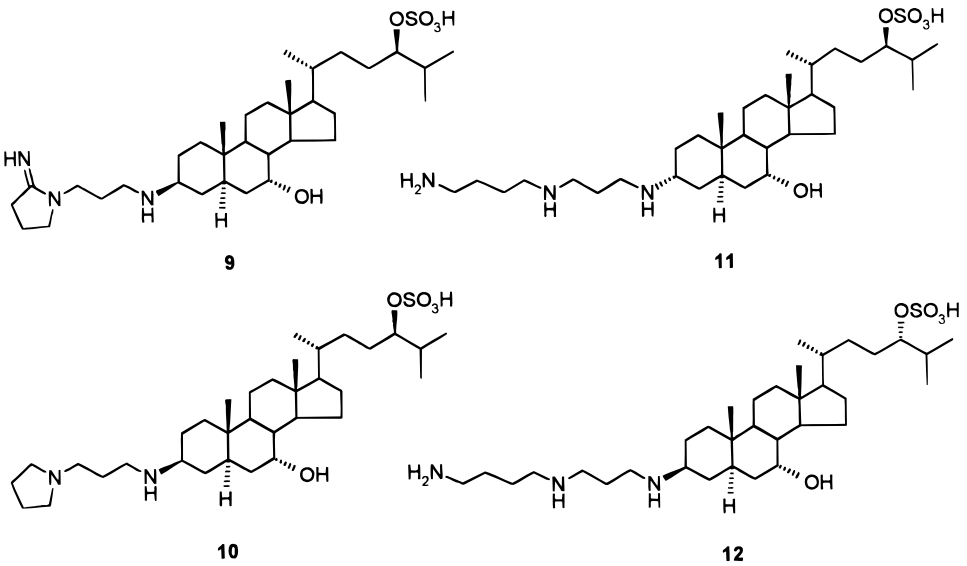


Table 1

entry	**2**, % de	**6**, % de (after purification)	**3**, de (after HPLC)
1	93	94	97

Results and Discussion

The completion of this 15-step synthesis of squalamine began (Scheme 2) with the liberation of the protected ketone functionality of intermediate **2**. Acid hydrolysis of **2** was achieved utilizing Amberlyst 15 ion-exchange resin as catalyst, which could be easily removed by filtration. Pyridine was added, and the acetone removed by distillation. The intermediate ketone **4** was treated with sulfur trioxide−pyridine complex and the reaction mixture was worked up with saturated potassium chloride solution to afford **5** in excellent yield. Cleavage of the benzoate at C-7 with potassium hydroxide afforded steroid **6**. The starting material **2** contained a small amount of the C-24 epimer and its quantity was not significantly altered during the transformation to **6** (Table 1).

To continue the synthesis, we chose the masked spermidine equivalent **7**, because the nitrile is stable to reductive amination conditions, yet is easily converted to an amino function (spermidine) by catalytic hydrogenation under conditions that do not affect the sulfate. Reagent **7** has been utilized previously as a spermidine equivalent.[18] It was now prepared in one step by adding 4-bromobutyronitrile over 1.5 h to 1,3-diaminopropane (5 equiv) at −6 °C. The crude reaction mixture was converted to the free base using a Dowex 1 × 8−100 ($^-$OH form) column and then distilled to afford **7** in good yield (73% versus 42% literature). The conditions of workup and distillation are crucial, as compound **7** readily cyclizes to 1,5-diazabicyclo[4.3.0]non-5-ene (DBN).

Squalamine (**3**) was produced by coupling steroid **6** to the masked spermidine reagent **7** using trimethyl orthoformate and sodium borohydride. The primary amine reacts preferentially to afford the imine with the elimination of water by trimethyl orthoformate. The reaction mixture was cooled to −78 °C and treated with sodium borohydride. These conditions maximize production of the desired β-orientation at the C-3 position (ratio of 6:1 of **3** to **11**, Chart 1). The nitrile of **8** was hydrogenated at low pH (1−2) using platinum oxide as catalyst to yield **3**. When the hydrogenation reaction was attempted at higher pH, significant amounts of products were obtained from cyclization of the secondary nitrogen upon the nitrile function as in compounds **9** and **10** (Chart 1). After removal of catalyst, **3** was purified by both propyl sulfonic acid ion exchange and reverse phase HPLC columns. Synthetic **3**, isolated as the trifluoroacetate salt, was compared to natural **3** by ^{1}H NMR, ^{13}C NMR, and HPLC and was found to be identical. Early fractions from the HPLC purification were enriched in the C-24 epimer of **3**. By discarding those fractions containing **12** (Chart 1), it was possible to increase the diastereomeric excess (de) of the final compound (Table 1).

Conclusion

Squalamine was efficiently prepared in five steps (37% yield overall) from **2** utilizing the latent spermidine reagent **7**. This reagent was shown to be most appropriate, as it is stable to reductive amination conditions, is converted to spermidine under weakly acidic conditions, and is easily prepared. The investigation of alternate spermidine equivalents will be reported in due course. The soybean-based sterol, stigmasterol, which is used as the starting material to prepare key intermediate **2**, is both inexpensive and readily available. This stereoselective synthesis of squalamine from steroid **2** has provided supplies for phase I clinical trials, which are currently in progress.

Experimental Section

General. The ^{13}C NMR spectra were generated at 100 MHz, utilizing 49.15 (CD_3OD) and 77.23 ($CDCl_3$) ppm as the reference. Elemental analyses were performed at Oneida Research Services, Inc., Whitesboro, NY. Fast atom bombardment mass spectral analysis was carried out at M-Scan Inc., West Chester, PA.

Potassium (5α, 7α, 24*R*)-7-Benzyloxy-3-keto-cholestan-24-yl Sulfate (5). A three-necked 3 L flask equipped with a mechanical stirrer was charged with a mixture of **2** (54 g, 95 mmol),[14] acetone (2.7 L), and Amberlyst 15 ion-exchange resin

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(H^+ form, 22 g) and stirred at room temperature for 4 h. After filtration, pyridine (6 mL) was added to the filtrate. After evaporation of the solvent in vacuo, pyridine (1.5 L) was added to the residue at room temperature under nitrogen. Sulfur trioxide−pyridine complex (30.0 g, 188 mmol) was added in one portion, and the mixture was warmed to 80 °C for 30 min, after which TLC showed completion of the reaction. The solvent was removed in vacuo, and ethyl acetate (610 mL) was added to the residue. The resulting suspension was filtered, and the filter cake was washed with ethyl acetate (2 × 85 mL). A solution of potassium chloride (22 g) in water (150 mL) was added to the vigorously stirred organic solution. After a few minutes, a thick suspension was obtained. *tert*-Butyl methyl ether (1500 mL) was added, and the suspension was cooled to 0 °C, filtered, and washed with cold water (2 × 100 mL) and *tert*-butyl methyl ether (2 × 100 mL). The solid was dried (50 °C, 2 mmHg) to yield **5** as the potassium salt (48.5 g, 77% from **2**); ^{1}H NMR (400 MHz, CD_3OD): δ 8.02 (d, J = 8 Hz, 2H), 7.62 (t, J = 7 Hz, 1H), 7.51, (t, J = 7.5 Hz, 2H), 5.14 (br s, 1H), 4.08 (q, J = 5 Hz, 1H), 2.04−1.08 (m, 27H), 0.95−0.86 (m, 12H), 0.72 (s, 3H). Anal. Calcd for $C_{34}H_{49}O_7SK\cdot1.2H_2O$ (FW 662.54): C, 61.64; H, 7.82; H_2O, 3.26. Found: C, 61.59; H, 7.85; H_2O (KF), 2.94.

Potassium (5α, 7α, 24*R*)-7-Hydroxy-3-ketocholestan-24-yl Sulfate (6). A 1 L three-necked flask equipped a mechanical stirrer, thermometer, and reflux condenser was charged with methanol (350 mL), potassium hydroxide (23.3 g of 85%, 353 mmol), and **5** (40 g, 60 mmol) under nitrogen. The mixture was heated to 60 °C and stirred overnight. The suspension was concentrated under reduced pressure, and water (275 mL) and dichloromethane (275 mL) were added to the residue with vigorous stirring. The suspension was cooled to 0 °C and Celite (11 g) was added to facilitate the filtration. The mixture was filtered, and the solid was washed with cold water (2 × 100 mL) and dichloromethane (2 × 75 mL). The solid was vacuum-dried at 50 °C to afford a solid (42.2 g). The solid was suspended in methanol (400 mL) and triethylamine (7 mL), warmed to 60 °C for 45 min, and filtered. The filter cake was washed with warm methanol (110 mL), and the combined filtrates were concentrated at reduced pressure until a thick suspension was obtained. *tert*-Butyl methyl ether (325 mL) was added to the suspension, and the mixture was cooled to 0 °C, filtered, and washed with *tert*-butyl methyl ether (100 mL). The solid was vacuum-dried at 50 °C to afford **6** as a white solid (29.4 g, 90%, mp 153−158 °C): ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.16 (m, 1H), 3.78 (br q, J = 5 Hz, 1H), 3.61 (m, 1H), 2.5−1.0 (m, 27H), 0.94 (s, 3H), 0.86 (d, J = 6 Hz, 3H), 0.81 (d, J = 7 Hz, 3H), 0.79 (d, J = 7 Hz, 3H), 0.63 (s, 3H). Anal. Calcd for $C_{27}H_{45}O_6SK\cdot0.04Na\cdot0.38H_2O$ (FW 544.58): C, 59.55; H, 8.47; H_2O, 1.26; K, 7.18; Na, 0.17. Found: C, 59.64; H, 8.39; H_2O, 1.28; K, 7.20; Na, 0.17; IR (KBr, cm^{-1}): 3436, 2936, 1708, 1470, 1390, 1208, 1056, 1038, 950, 812; HPLC 94% de.

3-[(3-Cyanopropyl)amino]propylamine (7). The following is a modification of the published procedure.[18] A flask containing 1,3-diaminopropane (2.50 kg, 33.7 mol) was stirred with a mechanical stirrer, cooled (−6 °C), and treated with 4-bromobutyronitrile (1.00 kg, 6.76 mol) over 1.5 h, maintaining the internal temperature below 0 °C. The cold bath was removed, and the reaction was allowed to stir without auxiliary temperature control for 1 h. 2-Propanol (IPA, 11 L) was added in one portion to the reaction. The mixture was stirred for 15 min after the appearance of a precipitate and then stored at 0−10 °C overnight. The solids were collected by filtration in a Buchner funnel lined with a polypropylene felt filter pad. The solids were washed with IPA (2 × 1.1 L). The combined filtrate was purified using a Dowex 1 × 8−100 ($^-$OH form) ion exhange column prepared as described below.

A 2.2 kg sample of Dowex 1 × 8−100 ($^-$Cl form) was combined with aqueous 5 N sodium hydroxide (6 L). After standing for 1 h, the mixture was poured into a suitably sized chromatography column with a coarse glass frit. The resin was washed with 5 N sodium hydroxide (44 L). A small aliquot of the eluent, neutralized to pH 7 with acetic acid, appeared hazy when aqueous 0.1 M silver nitrate was added. An additional wash with 5 N sodium hydroxide (9 L) did not visibly improve clarity. The resin was washed with deionized water (6.6 L), at which time the pH of the eluent was 7. An aliquot of the eluent was clear after the addition of aqueous 0.1 M silver nitrate. The resin was washed with IPA (11 L) and the column was ready for use.

The above filtrate, containing crude **7**, was added to the top of the ion exhange column. After the entire filtrate had passed through the column, the column was washed with IPA (14 L). A small aliquot of the combined eluent, when neutralized to pH 7 with acetic acid, was clear after the addition of aqueous 0.1 M silver nitrate. The combined eluent was concentrated to a weight of 1.39 kg using a water aspirator and a bath at 45−50 °C. Molecular sieves (3 Å, 100 g) were added to the residue, which was stored at 0−10 °C overnight. The sieves were removed by filtration, and the filtrate was distilled under reduced pressure in a 2 L flask equipped with overhead stirring, a thermometer, and a distillation head with a short Vigreaux column. Fractions distilling at less than 114 °C (0.6 mmHg) were collected and discarded (627 g). Two fractions of pure **7** were collected (total = 699 g, 73%): fraction 1 (108 g) distilled at 114−115 °C (0.6 mmHg) and fraction 2 (591 g) distilled at 110−112 (0.5 mmHg): ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.71 (t, J = 6.5 Hz, 2H), 2.68 (t, J = 6.5 Hz, 2H), 2.61 (t, J = 7 Hz, 2H), 2.39 (t, J = 7 Hz, 2H), 1.75 (p, J = 7 Hz, 2H), 1.56 (p, J = 7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 119.9, 48.1, 47.7, 40.5, 33.8, 25.8, 15.0; MS ($^+$FAB): 142 ([M + H]$^+$, 100); IR (neat, cm^{-1}): 3280, 2930, 2244, 1592, 1470, 1128, 830. Anal. Calcd for $C_7H_{15}N_3\cdot0.15H_2O$: C, 58.42; H, 10.72; N, 29.20. Found: C, 58.60, H, 10.52, N, 28.86.

(3β, 5α, 7α, 24*R*)-3-[[3-[(4-aminobutyl)amino]propyl]-amino]-7-hydroxycholestan-24-yl Hydrogen Sulfate Bis-(trifluoroacetate) Salt (Squalamine, 3). The polyamine **7** (8.00 g, 56.7 mmol) was dissolved in anhydrous methanol (650 mL) at room temperature, and trimethyl orthoformate (50 mL, 457 mmol) was added. Steroid **6** (10.0 g, 18.4 mmol) was added, and the reaction mixture was stirred for 18 h. The reaction mixture was cooled to −78 °C, treated with sodium borohydride (1.06 g, 28.0 mmol) over one min, and stirred for 3.5 h at −78 °C. The reaction was allowed to warm to room temperature and was concentrated at 31 °C under a water aspirator vacuum. The crude product was dissolved in 100% ethanol (290 mL), purged with nitrogen, and acidified to pH 1−2 with neat trifluoroacetic acid. Platinum oxide (1.00 g) was added and the mixture was shaken on a Parr apparatus (40 psi) for 18 h. The reaction mixture was filtered through paper, and the solids were washed with methanol (620 mL). The filtrate was evaporated and then dissolved in 50% ethanol in water.

A propyl sulfonic acid (PSA) ion exchange column was prepared by suspending 80 g of resin (J. T. Baker, Phillipsburg, NJ) in 10% IPA in water to form a slurry and by adding 200 mL of 10% IPA to the column, followed by the slurry. At least five column volumes of 10% IPA were eluted through the column at a flow rate of 40 mL/min. The column was washed with 0.05% trifluoroacetic acid (TFA) in 50% ethanol in water (150 mL) at 20 mL per min. The steroid from above was loaded on the column in two portions, and eluent was collected. For each portion the column was washed with two column volumes of 0.05% TFA in 50% ethanol in water and two column volumes of 0.05% TFA in 10% IPA. The column was then eluted with 4.5 M KOAc/10% IPA (pH 5), and fractions were collected (150 mL each). Fractions that contained squalamine by TLC (silica gel, eluent 6:3:1, dichloromethane:methanol:ammonium hydroxide; R_f = 0.35−0.40) were combined.

The two batches of crude **3** from the ion exchange were purified on a 25 × 5 cm plus 5 × 5 cm YMC ODS-AQ C18 reversed phase columns (YMC, Inc. Wilmington, NC). The eluent from PSA was diluted with four volumes of deionized water. The column was loaded and eluted with four column volumes of buffer A (0.05% TFA in 1% acetonitrile in water). Then the column was eluted (100 mL/min) with the following gradient of increasing percentages of buffer B (B = 0.05% TFA in 1% water in acetonitrile) (Detector: UV λ = 220 nm): 0−25% (10 min), 25−40% (50 min), and 40−80% (20 min). Between 28 and 60 min (30−40% B) fractions were collected every 30 s (50 mL). All fractions were examined by TLC, and the early and late fractions that contained **3** were analyzed by analytical HPLC with *o*-phthaldialdehyde (OPA) derivitization (see below). Fractions that were >95% pure were combined and lyophilized to afford 97% pure **3** (10.3 g, 62% yield) as the trifluoroacetate salt.

This synthetic material was identical to natural **3** by analytical HPLC (OPA method); ^{1}H NMR (400 MHz, CD_3OD): δ 4.12 (br q, 1H), 3.76 (br s, 1H), 3.2−2.9 (m, 9H), 2.1−1.0 (m, 33H), 0.94−0.90 (m, 9H), 0.84 (s, 3H), 0.67 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 86.7, 68.4, 59.2, 57.7, 51.8, 46.8, 46.0, 43.9, 43.0, 41.2, 40.1, 38.7, 38.0, 37.8, 37.5, 37.0, 32.7, 32.2, 32.1, 29.5, 28.3, 26.1, 25.7, 24.7, 24.6, 24.3, 22.3, 19.6, 18.6, 18.3, 12.6, 11.7. Anal. Calcd for $C_{34}H_{65}N_3O_5S\cdot 2TFA\cdot 2.5H_2O$ (FW 901): C, 50.65; H, 8.05; N, 4.66; S, 3.56; F, 12.65. Found: C, 50.71; H, 8.06; N, 4.70; S, 3.75; F, 12.13; HPLC 96% de.

The 3-α-isomer of **3** (**11**) eluted after squalamine on reversed phase HPLC (ratio of **3** to **11** = 6/1). **11**: ^{1}H NMR (400 MHz, CD_3OD): δ 4.14 (q, J = 8 Hz, 1H), 3.81 (br s, 1H), 3.44 (br s, 1H), 3.18−2.97 (m, 8H), 2.20−2.16 (m, 2 H), 2.04−1.14 (m, 31H), 0.96−0.93 (m, 9H), 0.87 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz): δ 86.5, 68.4, 57.7, 56.9, 51.8, 48.4, 46.8, 46.1, 44.5, 43.8, 41.1, 40.2, 37.34, 37.29, 37.2, 33.4, 32.9, 32.5, 32.1, 30.2, 29.4, 28.3, 25.6, 24.6, 24.2, 24.1, 23.9, 21.8, 19.5, 18.5, 12.5, 11.0. Anal. Calcd for $C_{34}H_{65}N_3O_5S\cdot 2TFA\cdot 2H_2O$: C, 51.17; H, 8.02; N, 4.71; S, 3.59; F, 12.78. Found: C, 51.12; H, 7.48; N, 4.66; S, 3.51; F, 12.72.

The fractions enriched in the 24*S*-isomer of **3** (**12**) were combined, concentrated in vacuo to remove the organic modifier, and purified on the same YMC column. The column was eluted (100 mL/min) with the following gradient of buffers A and B (see above): 0−25% (10 min), 25−35% (50 min), 35−60% (10 min), and 60−80 (10 min) (detector: UV λ = 220 nm). Fractions were collected every 30 s (50 mL) and analyzed by TLC and HPLC (OPA method). Fractions which contained pure **12** were combined and lyophilized: ^{1}H NMR (400 MHz, CD_3OD): δ 4.13 (br q, J = 5 Hz, 1H), 3.80 (br s, 1H), 3.19−2.97 (m, 9H), 2.16−1.06 (m, 33H), 0.99−0.93 (m, 9H), 0.88 (s, 3H), 0.72 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 86.5, 68.3, 59.2, 57.7, 51.8, 48.4, 46.7, 46.0, 43.9, 43.0, 41.2, 41.1, 40.1, 38.7, 37.85, 37.77, 37.5, 37.0, 32.6, 32.0, 31.8, 29.6, 28.6, 26.1, 25.6, 24.7, 24.5, 24.2, 22.2, 19.6, 18.9, 18.0, 12.6, 11.7. Anal. Calcd for $C_{34}H_{65}N_3O_5S\cdot 2TFA\cdot 2H_2O$: C, 51.17; H, 8.02; N, 4.71; S, 3.59. Found: C, 51.31; H, 7.99; N, 4.76; S, 3.66.

Preparation of 9 and 10. Compounds **9** and **10** are two of the major impurities identified, which elute after **3** and **11** on reversed phase HPLC. Late HPLC fractions from several syntheses of **3** that contained **9** and **10** were combined and lyophilized. Crude solid (2 g) was purified by flash chromatography on a silica gel column (5 cm diameter, gradient elution with 12/3/1 to 3/3/1 of chloroform/methanol/isopropylamine) affording first **10** and then **9** in their free base forms. Compounds **9** and **10** were converted to their trifluoroacetic acid (TFA) salts on the polystyrene resin, amberchrom (CG-161, TosoHaas, Montgomeryville, PA). The free base was dissolved in methanol, treated with amberchrom (2 mL), and concentrated in vacuo. This solid was loaded onto a column (20 × 1 cm) of the same absorbent prepared in water. The column was eluted with 0.1% TFA in water (200 mL), 0.1% TFA and 20% acetonitrile in water (200 mL), 0.1% TFA and 40% acetonitrile in water (200 mL), 0.1% TFA and 60% acetonitrile in water (200 mL), and 0.1% TFA and 80% acetonitrile in water (200 mL). Compound **9** eluted with 40% acetonitrile (550 mg), and **10** eluted with 60% acetonitrile (815 mg).

9: ^{1}H NMR (400 MHz, CD_3OD): δ 4.08 (br q, J = 3 Hz, 1H), 3.77−3.73 (m, 3H), 3.53 (t, J = 7 Hz, 2H), 3.28−3.05 (m, 3H), 2.91 (t, J = 8 Hz, 2H), 2.14 (p, J = 7.5 Hz, 2H), 2.04−1.08 (m, 29H), 0.93−0.88 (m, 9H), 0.83 (s, 3H), 0.67 (s, 3H); MS ($^+$FAB): 624.2 (M + 1, 30), 544.3 (27), 526.4 (33), 125.1 (100). Anal. Calcd for $C_{34}H_{61}N_3O_5S\cdot TFA\cdot 3H_2O$: C, 54.59; H, 8.65; N, 5.31; S, 4.05. Found: C, 54.96; H, 8.32; N, 5.31; S, 4.15.

10: ^{1}H NMR (400 MHz, CD_3OD): δ 4.10 (br q, 1H), 3.77 (br s, 1H), 3.67 (m, 2H), 3.32−3.28 (m, 2H), 3.13−3.09 (m, 5H), 2.13−1.09 (m, 33H), 0.94−0.90 (m, 9H), 0.85 (s, 3H), 0.68 (s, 3H); MS ($^+$FAB): 611.3 (M + 1, 92%), 531.4 (32), 513.4 (100). Anal. Calcd for $C_{34}H_{62}N_2O_5S\cdot TFA\cdot 2.5H_2O$: C, 56.16; H, 8.90; N, 3.64; S, 4.16. Found: C, 55.93; H, 8.69; N, 3.73; S, 4.26.

HPLC Analysis of 2 and (24*S*)-2. The diastereomeric excess for compound **2**, compared to the 24*S*-isomer of **2**, was determined by HPLC. A sample of **2** (2 mg/mL) in acetonitrile was prepared and injected (5 μL) on a Waters Nova-Pak Phenyl (3.9 × 150 mm) column at ambient temperature and eluted with 45% water in acetonitrile (1 mL/min). Detection was at 230 nm. Typical retention times for compound **2** and the 24*S*-isomer of compound **2** are 21 and 19 min, respectively.

HPLC Analysis of 6 and 24*S*-6. This HPLC analysis provides the relative quantity of **6** and the 24*S*-isomer of **6**. The analysis was performed at a flow rate of 1 mL/min and temperature of 35 °C on a Kromasil C4 RP (4.6 × 250 mm), 100 Å, 5 μm column (Phenomenex) using the following mobile phases: A: 0.1% TFA in 90/10 water/acetonitrile and B: 0.1% TFA in 10/90 water/acetonitrile and a gradient of 35−55% B (15 min), 55−85% B (10 min). The compound was observed by ultraviolet spectroscopy at 200 nm. The sample (10 μL) was prepared at a concentration of 4 mg/mL in 0.01% triethylamine in methanol. Typical retention times: 24*S*-isomer of **6**, 18 min, and **6**, 19 min.

OPA HPLC Analysis of 3. This HPLC analysis provides the relative quantities of squalamine (**3**), **12**, and **11**. Because of the lack of a strong UV chromophore, **3** was converted to its OPA derivative using *o*-phthaldialdehyde reagent (Sigma) prior to analysis. The analysis was performed at a flow rate of 1 mL/min and column temperature of 45 °C on a Kromasil C18 RP 250 × 4.6 mm, 100 Å, 5 μm column using the following mobile phases: A: 0.1% TFA in 90/10 water/acetonitrile and B: 0.1% TFA in 10/90 water/acetonitrile and a gradient of 30−50% B (12 min), 50−60% B (5 min), 60−85% B (2 min). The compound was observed by fluorescence detection (excitation = 230 nm, emission = 455 nm). The sample was prepared at a concentration of 100 μg/mL in 20 mM acetate buffer (pH = 4.0). The derivitization reaction was accomplished in the autosampler by removing 5 μL of OPA reagent, removing 10 μL of sample, removing 5 μL of OPA, and mixing in the injector. Average retention times: **12**, 11.5 min; **3**, 12.2 min; and **11**, 15.5 min.

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