Aminosterols from the Dogfish Shark *Squalus acanthias*

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Seven new aminosterols related to squalamine (**8**) were isolated from the liver of the dogfish shark *Squalus acanthias*. Their structures (**1**−**7**) were determined using spectroscopic methods, including 2D NMR and HRFABMS. These aminosterols possess a relatively invariant cholestane skeleton with a trans AB ring junction, a spermidine or spermine attached equatorially at C3, and a steroidal side-chain that may be sulfated. The structure of the lone spermine conjugate, **7** (MSI-1436), was confirmed by its synthesis from (5α,7α,24*R*)-7-hydroxy-3-ketocholestan-24-yl sulfate. Some members of this family of aminosterols exhibit a broad spectrum of antimicrobial activity comparable to squalamine.

In the search for novel host defense agents, squalamine (**8**) (Figure 1) was identified as the first aminosterol from the dogfish shark, *Squalus acanthias* (Squalidae), by its antimicrobial activity.[1,2] Squalamine also exhibits antiangiogenic and antitumor properties[3] and is currently in Phase II clinical trials for the treatment of advanced nonsmall cell lung cancer. Attempts to obtain large amounts of squalamine from the dogfish shark resulted in the discovery, isolation, and characterization of a family of novel aminosterols. These aminosterols possess a relatively invariant steroid skeleton with a trans AB ring junction, a polyamine attached equatorially at C3, and a cholestane-related side chain that may be sulfated.

Results and Discussion

The original purification scheme for squalamine[1] was modified to make practical the processing of 40-kg batches of dogfish liver. The process includes (a) an extraction into water/acetic acid/ethanol/ammonium sulfate; (b) batch adsorption and extraction from XAD resin; (c) adsorption and elution from a cation exchange column (propylsulfonic acid resin); and, finally, (d) reversed-phase separation by HPLC (YMC-ODS column). Although squalamine is the most abundant aminosterol found in dogfish shark liver (400−800 mg, 0.001−0.002%), seven additional aminosterols (**1**−**7**) are found in amounts of 20−100 mg (0.00005−0.00025%) for a typical shark liver preparation (40 kg). Other minor aminosterols, found in trace quantities of <2 mg/40 kg shark liver, have not been fully characterized.

The aminosterols **1**−**7** reported here are water-soluble, elute before squalamine, and are numbered in order of their elution on preparative reversed-phase HPLC. These compounds have a broad spectrum of antimicrobial activity comparable to squalamine (**8**) (Table 1) and, in the case of aminosterols **4**−**7**, exhibit similar potency.

Full assignments of the proton and carbon signals for these aminosterols (Figure 2, Tables 2 and 3) were obtained by ^{1}H−^{1}H- and ^{1}H−^{13}C-correlated 2D spectroscopy (COSY, HMQC, HMBC). The stereochemistry of C-20 and C-24 in squalamine (**8**) and compound **7** was proven to be 20*R*,24*R* by comparison with synthetic squalamine[4,7] and synthetic **7**. The synthetic **7** reported here was made from a 2.5-fold excess of spermine and the stereochemically defined 3-keto derivative used previously in the synthesis of **8**.[7] For the other aminosterols with stereogenic centers at C-24, the stereochemistry is presumed to be 24*R*. The stereochemistry of compounds **1**−**3** at C-25 is not yet established.

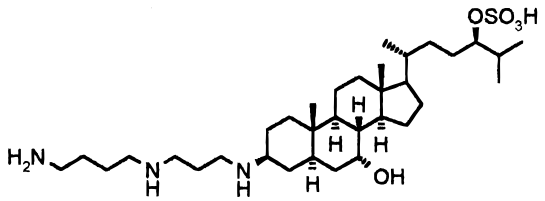

Figure 1. Structure of squalamine (**8**).

Table 1. MIC of Aminosterols[a]

	MIC (μg/mL)			
compound	*S. aureus* (29213)	*E. coli* (25922)	*P. aeruginosa* (27853)	*C. albicans* (90028)
1	8−16	256	256	128
2	4−8	128	32	16
3	8	128	128	32
4	8−16	16	16	32
5	2	8	16	32
6	2	16	16	2
7	1	1	4	4
8	1	4	16	16

[a] ATCC numbers in parentheses; squalamine (**8**) is included for comparison, and data compare well with previously reported antimicrobial data.[1]

The steroid core of these compounds is minimally substituted, with the polyamine spermidine found at C-3 in compounds **1**−**6** and **8**. Substitution at the 3-equatorial position is confirmed by large diaxial couplings to protons identified as H-2$_{ax}$ and H-4$_{ax}$. The polyamine region in **8** and **2**−**6** (3.2−2.9 ppm) in the ^{1}H NMR spectra integrates for nine protons and includes the 3-axial methine proton on the A-ring and the eight methylene protons adjacent to nitrogen atoms in the polyamine. In **1**, the polyamine region is overlapped by signals from H-28 protons next to a sulfur atom. Similarly, the polyamine spermine found in **7** integrates for 13 protons and includes the 3-axial methine proton and the 12 methylene protons adjacent to nitrogen atoms. The ^{1}H−^{1}H COSY correlations between these downfield proton signals and those for the interior methylene protons found upfield are diagnostic for the attached

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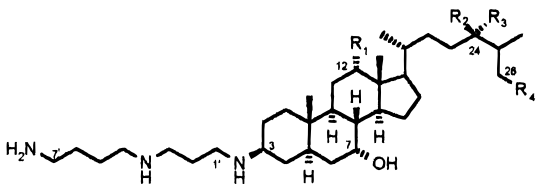
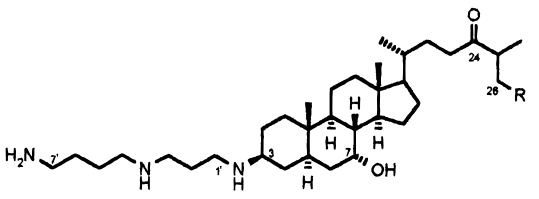
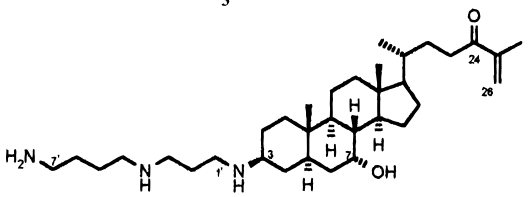


2: R_1=H, R_2=OH, R_3=H, R_4=OSO_3H
4: R_1=OH, R_2=OSO_3H, R_3=H, R_4=H
5: R_1=H, R_2=OSO_3H, R_3=CH_2OH, R_4=H
8: R_1=H, R_2=OSO_3H, R_3=H, R_4=H



1: R=$SCH_2CH(NH_2)CO_2H$
3: R=OSO_3H



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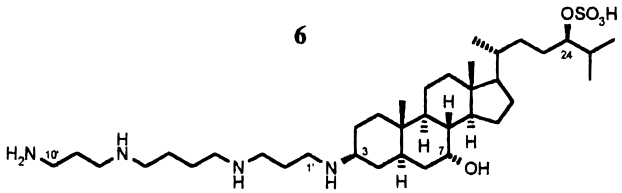

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Figure 2. Structures of major aminosterols isolated from dogfish shark liver.

polyamine. Only aminosterol **4** has an additional oxygen-bearing methine carbon signal at C-12, which correlates with a single proton observed at δ 3.95. The lack of a large diaxial coupling with adjacent protons on C-11 indicates that the hydroxyl group is attached axially [$J_{11e,12} = J_{11a,12} \approx 4$ Hz (COSY)]. Characteristic of all aminosterols reported here is the broad singlet for H-7 found at δ 3.8 correlating with a carbon methine signal at δ 68 in methanol-d_4.

The structural diversity within this group of compounds is observed primarily in the side chain. However, the side chains of **4** and **7** are identical to that of **8**, as seen by comparison of their NMR data. For example, typical HMBC experiments for these molecules gave correlations between C-24 and H-25 and the terminal methyl groups H-26/H-27, as well as between H-24 and C-23, C-25, C-26/C-27.

Compound **5** is 24-hydroxymethyl-substituted squalamine. The ^{1}H NMR spectrum displayed an AB quartet (δ 3.95) that correlated with a methylene carbon signal at δ 72.5. From HMBC data, cross-peaks were clearly seen between these diastereotopic protons (H-$28_{a,b}$) and the quaternary carbon C-24 possessing the sulfate (δ 76.3), C-25 (δ 34.3), and C-23 (δ 32.4). HRFABMS produced a fragment corresponding to the desulfated ion $[M + H - SO_3]^+$. The origin of this atypical sterol with 28 carbons may be the plant sterol, campesterol.

The ^{13}C NMR and DEPT-135 spectra of compound **2** revealed one methylene and two methine carbons substituted with an oxygen. In the HMBC spectrum both the methine carbon at δ 74.2 and the methylene carbon at δ 71.7 gave correlations with the proton signals of the methyl group (H-27) and methine (H-25) of the side chain. Additional correlations were evident between the diastereotopic protons on H-26 (δ 4.12, 3.96) and C-24, C-25 (δ 32.2), and C-27 (δ 14.4). Assignment of the sulfate to C-26 was based on the chemical shift data as well as the fragmentation pattern in the FABMS, which produced a $[M + H - SO_3]^+$ fragment from the primary aliphatic sulfate.

Initial ^{1}H$-^1$H COSY studies of compound **3** were distinguished by the absence of through-bond connectivities between positions 23 and 25, while ^{13}C NMR showed a characteristic carbonyl signal at δ 212.2. Upon interrogation by HMBC, this resonance correlated with protons at 2.82 (H-25) and 2.66, 2.46 (2H, H-$23_{a,b}$), consistent with a C-24 keto functionality. Cross-peaks in the HMBC are also seen between C-24 and δ 3.78 (m, 2H, H-$26_{a,b}$) and 0.95 (d, 3H, H-27). Evidence for the position of the sulfate on C-26 included the downfield shift of the methylene carbon (δ 67.3) correlated with the multiplet at δ 3.78 on HMQC and fragmentation of the primary sulfate on FABMS to give an $[M + H - SO_3]^+$ ion.

The ^{13}C NMR spectrum of compound **6** exhibited three downfield carbon signals at δ 205.2, 145.9, and 125.9, suggesting both a keto functionality and a double bond. The multiplicities of the carbons at δ 145.9 and 125.9 were found to be quaternary and methylene, respectively. Supporting evidence for a double bond was the observation of olefinic proton signals integrating for one proton each at δ 6.05 and 5.84. The ^{1}H$-^1$H COSY revealed that the downfield H-27 methyl (δ 1.83, dd) shared cross-peaks with the protons at δ 6.05 and 5.84. This evidence and additional HMBC data placed the double bond between C-25 and C-26. Only one sp^3 carbon bearing an oxygen atom was detected by ^{13}C NMR, and it was identified as the typical C-7 resonance on the sterol core. It is apparent that compound **6** is related to **3** by the elimination of H_2SO_4. In fact, samples of **3** were found to contain traces of **6** after storage for several years at freezer temperatures. Compound **3** is unique in containing the sulfate in a β-position relative to a carbonyl, making it more prone to elimination reactions than sulfates **2**, **4**, **5**, **7**, or **8**. These aminosterol sulfates were stable to similar isolation and storage conditions.

Compound **1** has ^{1}H and ^{13}C NMR signals consistent with a cysteine residue as well as a keto function, which would result from cysteinylation of **6**. Cysteinylation is a well-described metabolic modification in nonsteroidal xenobiotics.[8,9]

The biosynthetic origin of these compounds remains to be elucidated. The structures clearly resemble the known bile alcohols of the shark, which are frequently found as sulfuric acid esters.[10] However, the aminosterols are uniquely distinguished from bile alcohols by the polyamine modification. Although aminosterols of this family have not been identified as yet in mammals, it should be noted that closely related steroidal alcohols have been discovered, which represent logical biosynthetic precursors to the aminosterol class. Human astrocytes, for example, can metabolically convert cholesterol to 24-hydroxycholesterol, 7α,25-dihydroxycholest-4-en-3-one, and other alcohols.[11] Further conversion of 3-keto-hydroxycholestanes of this type to aminosterols of the squalamine family would

Table 2. ^{1}H NMR Spectral Data of Major Aminosterols (δ, ppm, in CD_3OD, except as noted; J in Hz, in Parentheses)

compound position	**1**	**2**	**3**[a]	**4**	**5**	**6**	**7**	**8**
1ax	1.12	1.14	0.98	1.18	1.15	1.12	1.14	1.15
1eq	1.86	1.86	1.70	1.83	1.87	1.86	1.86	1.85
2ax	1.56	1.58	1.44	1.56	1.59	1.56	1.59	1.60
2eq	1.96	2.00	1.83	2.04	2.02	1.96	2.01	2.03
3	3.13	3.14	3.04	3.16	3.13	3.12	3.12	3.11
4ax	1.43	1.43	1.31	1.42	1.43	1.42	1.44	1.44
4eq	1.61	1.65	1.49	1.65	1.64	1.63	1.65	1.64
5	1.74	1.76	1.62	1.75	1.75	1.74	1.75	1.75
6ax	1.53	1.54	1.37	1.54	1.55	1.53	1.55	1.54
6eq	1.45	1.46	1.28	1.46	1.48	1.45	1.46	1.46
7	3.80	3.80	3.61	3.81	3.80	3.80	3.80	3.79
8	1.44	1.40	1.29	1.47	1.44	1.41	1.40	1.44
9	1.23	1.25	1.17	1.59	1.28	1.23	1.27	1.29
11ax	1.35	1.34	1.20	1.58[b]	1.37	1.34	1.36	1.37
11eq	1.54	1.54	1.44	1.64[b]	1.55	1.54	1.55	1.56
12ax	1.13	1.18	1.05		1.20	1.14	1.16	1.18
12eq	1.98	2.00	1.88	3.95[c] br s	2.01	1.99	2.00	2.00
14	1.43	1.43	1.33	1.96	1.45	1.47	1.44	1.44
15a,b	1.78, 1.14	1.77, 1.12	1.67, 0.98	1.76, 1.11	1.78, 1.13	1.76, 1.11	1.76, 1.12	1.77, 1.11
16a,b	1.89, 1.35	1.93, 1.32	1.77, 1.24	1.90, 1.31	1.96, 1.32	1.89, 1.34	1.92, 1.34	1.92, 1.34
17	1.14	1.18	1.06	1.82	1.22	1.16	1.17	1.18
18	0.70 s, 3H	0.71 s, 3H	0.61 s, 3H	0.72 s, 3H	0.71 s, 3H	0.70 s, 3H	0.70 s, 3H	0.71 s, 3H
19	0.86 s, 3H	0.87 s, 3H	0.74 s, 3H	0.85 s, 3H	0.87 s, 3H	0.86 s, 3H	0.86 s, 3H	0.87 s, 3H
20	1.43	1.44	1.32	1.42	1.37	1.45	1.44	1.44
21	0.94 d, 3H (6.9)	0.97 d, 3H (6.4)	0.87 d, 3H (6.2)	1.02 s, 3H (6.4)	0.99 d, 3H (6.6)	0.96 d, 3H (6.6)	0.96 d, 3H (6.3)	0.97 d, 3H (6.5)
22a	1.75	d	1.62	1.49	1.47	1.77	1.50	1.54
22b	1.23	d	1.16	1.31	1.11	1.28	1.22	1.31
23a	2.54	1.50	2.66	1.71	1.72	2.75	1.71	1.71
23b	2.54	1.44	2.46	1.51	1.39	2.67	1.53	1.53
24		3.44		4.14			4.12	4.13
25	2.89	1.85	2.82	2.08	1.88		2.05	2.06
26	1.16 d, 3H (6.9)	4.12, 3.96	3.78	0.96 d, 3H (6.9)	0.96 d, 3H (6.9)	6.05, 5.84	0.95 d, 3H (7.7)	0.96 d, 3H (6.8)
27	2.85, 2.62	0.99 d, 3H (6.8)	0.95 d, 3H (7.1)	0.93 d, 3H (6.9)	0.95 d, 3H (6.9)	1.83 dd, 3H (1.3, 1.0)	0.94 d, 3H (7.7)	0.94 d, 3H (6.8)
28	3.14, 2.97				3.95 ABq (9.7)			
29	3.96							
30	*d*							
1′	3.15	3.16	2.99	3.19[e]	3.18	3.14	3.17	3.17
2′	2.11	2.11	1.93	2.16	2.12	2.10	2.12	2.13
3′	3.15	3.16	2.99	3.16[e]	3.18	3.14	3.17	3.17
4′	3.08	3.09	2.92	3.08	3.10	3.07	3.09	3.09
5′	1.80	1.80	1.62	1.82	1.79	1.80	1.81	1.80
6′	1.75	1.76	1.58	1.75	1.78	1.76	1.81	1.77
7′	2.98	2.99	2.81	2.99	2.99	2.98	3.09	2.98
8′			*f*				3.14	
9′							2.09	
10′							3.06	

[a] In DMSO-d_6. [b,e] Assignments within column may be interchanged. [c] $J_{11e,12} = J_{11a,12} \approx 4$ Hz (COSY). [d] Not observed. [f] $NH_{\alpha,\beta} = 8.73$ s, 1H; 8.62 br s, 1H, $NH_{2\gamma} = 7.79$ s, 2H.

require the presence of an unknown enzyme that could couple spermidine or spermine to the A ring of the steroid.

Although the aminosterols reported here possess antibiotic activity, their biological functions in the shark are not well understood in every case. Compound **7** (MSI-1436), however, has been studied extensively in vivo and has been shown to induce profound appetite suppression and weight loss in mammals, including mice, rats, dogs, and monkeys.[12] It remains to be proven whether compound **7** is responsible for the sporadic feeding behavior of the dogfish, an animal that normally eats only once every two weeks. Additional studies of biological and pharmacological activities of the other aminosterols described in this report are in progress.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded at 300 K on a Bruker AC or AMX-400 at 400 and 100 MHz, respectively, using MeOH-d_4 or DMSO-d_6 with tetramethylsilane (^{1}H NMR) and solvent peaks (^{13}C NMR, δ 49.15 and 39.51, respectively) as reference standard. ^{1}H−^{1}H DQF COSY and COSY-45, inverse-detected ^{1}H−^{13}C HMQC, and HMBC experiments were performed using Bruker software and conventional pulse sequences. Carbon multiplicities were confirmed using DEPT experiments. FABMS were measured on a VG Analytical ZAB 2SE spectrometer with 3-nitrobenzyl alcohol and PEG-600 as matrix (M-Scan, West Chester, PA 19380). Negative ion ESIMS were measured on a Micromass Autospec at the University of Iowa, Iowa City, IA. Alternatively, laser desorption mass spectra (MALDI) were obtained on a PerSeptive Biosystems Voyager instrument.

Fresh dogfish shark liver, normally a waste product, was obtained from the commercial harvest of shark for edible products at Seatrade Fishery, Portsmouth, NH.

Minimum inhibitory concentrations (MIC) for the bacteria and yeast were determined by incubating $(0.9-1.1) \times 10^5$ cfu/mL of log-phase microbes in 0.5× trypticase soy broth with increasing amounts of aminosterol in 96-well microtiter plates

Table 3. ^{13}C NMR Spectral Data of Major Aminosterols (δ, ppm, in CD_3OD, except as noted)

compound position	**1**	**2**	**3**[a]	**4**	**5**	**6**	**7**	**8**
1	37.7	37.8	35.8	37.6	37.9	37.7	37.7	37.9
2	26.0	26.1	24.1	25.9	26.1	26.1	25.9	26.1
3	59.0	59.1	55.9	59.2	59.2	59.0	59.0	59.2
4	32.2	31.9	30.1	32.1	32.1[b]	32.2[c]	32.0	32.1
5	38.7	38.7	36.4	38.7	39.7	38.6	38.5	38.7
6	37.7	37.8	36.5	37.7	37.8	37.7	37.7	37.8
7	68.4	68.4	65.3	68.4	68.4	68.4	68.3	68.4
8	41.1	41.1	39.1	41.2	41.2	41.1	41.0	41.2
9	46.9	46.9	44.7	40.4	46.8	46.9	46.7	46.8
10	36.8	36.9	35.1	36.5	37.0	37.0	36.8	37.0
11	22.1	22.2	20.4	29.8	22.3	22.1	22.1	22.3
12	41.0	41.1	39.1	73.8	41.1	41.0	41.0	45.4
13	43.8	43.9	41.9	47.5	43.9	43.8	43.7	43.0
14	51.8	51.8	49.9	43.5	51.8	51.8	51.7	51.8
15	24.6	24.7	23.0	24.3	24.8	24.6	24.4	24.7
16	29.3	29.6	27.5	28.8	29.6	29.3	29.4	29.5
17	57.6	58.0	55.5	48.3	57.7	57.6	57.5	57.7
18	12.4	12.6	11.6	13.1	12.6	12.4	12.4	12.6
19	11.5	11.7	10.6	11.4	11.7	11.5	11.6	11.7
20	36.9	37.4	34.7	37.2	38.0	36.9	37.2	37.4
21	19.1	19.5	18.3	18.3[d]	19.7	19.1	19.4	19.6
22	30.8	33.3	28.9	33.0	30.2	32.3[c]	32.5	32.7
23	40.1	32.2	37.3	28.0	32.4[b]	35.5	28.2	28.3
24	216.3	74.2	212.2	86.3	76.3	205.2	86.5	86.6
25	47.5	40.7	45.3	32.3	34.3	145.9	32.0	32.2
26	35.5	71.7	67.3	18.4[d]	17.4	125.9	18.4	18.6
27	17.1	14.4	13.1	18.2[d]	17.4	17.9	18.3	18.4
28	34.2				72.5			
29	54.5							
30	171.7							
1′	42.9[e]	43.1[f]	40.7[g]	43.0[h]	43.1[i]	43.0[j]	42.9[k]	43.9[l]
2′	24.6	24.6	22.5	24.6	24.6	24.7[m]	24.6[n]	24.6
3′	46.0[e]	46.1[f]	43.8[g]	46.0[h]	46.0[i]	46.1[j]	45.9[k]	46.0[l]
4′	48.9	48.5	46.0	48.4	48.4	48.5	48.2[o]	48.4
5′	24.4	24.4	22.5	24.2	24.3	24.5[m]	24.2[p]	24.3
6′	25.7	25.7	24.0	25.9	25.7	25.8	24.1[p]	25.7
7′	40.1	40.2	38.1	40.1	40.2	40.1	48.2[o]	40.1
8′							45.9	
9′							25.4[n]	
10′							37.9	

[a] In DMSO-d_6. [b−p] Assignments within column may be interchanged.

(Corning, Corning, NY) at 37 °C for 18−24 h.[1] Initial sample concentrations were 2 mg/mL in 250 mM of sodium acetate, pH 6.6.

Extraction and Isolation. Freshly ground dogfish shark liver (20 kg) was suspended in a solution of 12% (v/v) acetic acid (82 L) at 75 °C for 1 h. Ammonium sulfate (18 kg) and 95% ethanol (17 L) were added, followed by vigorous agitation for 5 min. The suspension was allowed to stand for 6 days, and the organic phase was discarded. After the aqueous phase was filtered through cheesecloth, the solution from two such 20-kg batches containing the crude aminosterols was slowly stirred with XAD-16 resin (5 kg, Supelco, Bellefonte, PA) for 20 h. The bulk resin was rinsed with H_2O (10 L) and resuspended in 70% EtOH (20 L) for 20 min.

The ethanolic extracts from 10 batches of XAD resin (200 L) were filtered through a 5-μm Polypure DCF filter (Gelman Sciences, Ann Arbor, MI) and applied to a cation exchange column of propylsulfonic acid resin (3 kg, J. T. Baker, Phillipsburg, NJ). The column was washed with 20% 2-propanol followed by washing with 0.4 M potassium acetate in 10% 2-propanol until absorption at 254 nm was constant. The aminosterols were then eluted with either 3.6 or 4.5 M potassium acetate in 10% 2-propanol (15−30 L). The separate eluates were diluted to 1 M salt concentrations with H_2O and further purified on a YMC-ODS (10 μm, 120 Å, 25 × 10 cm, YMC, Wilmington, NC). The YMC column was washed with 0.1% TFA in H_2O (40 L) and 0.1% TFA in 25% CH_3CN (10 L). Aminosterols were eluted with a gradient of 25−34% CH_3CN containing 0.1% TFA (470 mL/min, 0.5 L fractions) over 13 min, then 34−40% CH_3CN containing 0.1% TFA (470 mL/min, 235-mL fractions) over 22 min.

Chromatographic purification of separate YMC column fractions on a column of propylsulfonic acid resin (700 mL) as described above resulted in homogeneous aminosterol compounds. If the cation exchange eluates were not clear, the solution was ultrafiltered (3-kD cutoff, Amicon, Woburn, MA) prior to loading on a YMC-ODS (25 × 1 cm) or Dynamax (25 × 4 cm, Rainin, Woburn, MA) column. A gradient of CH_3CN/H_2O containing 0.1% TFA eluted the aminosterols.

The isolated aminosterols were analyzed as their *o*-phthalaldehyde (OPA) (precolumn-OPA-treated) derivatives using a YMC-ODS-AQ (120 Å, 250 × 4.6 mm) column with fluorescence detection. The mobile phase was a gradient (20−70%B, 30 min; 70−100%B, 5 min) of Buffer A (0.1% TFA in 90:10 H_2O/CH_3CN) and Buffer B (0.1% TFA in 10:90 H_2O/CH_3CN) at 1 mL/min. Fluorescence excitation was at 230 nm and detection at 455 nm.

The ^1H and ^{13}C NMR data for compounds **1**−**8** are displayed in Tables 2 and 3. Additional analytical and experimental information is described below.

Compound 1: white powder; HRFABMS *m/z* 665.5043 [M + H]$^+$ (calcd for $C_{37}H_{69}N_4O_4S$ 665.5039).

Compound 2: white powder; FABMS *m/z* 642 [M − H]$^-$ (calcd for $C_{34}H_{64}N_3O_6S$ 642.5), 565 [M + H − SO_3]$^+$; HRESIMS *m/z* 642.4502 [M − H]$^-$ (calcd for $C_{34}H_{64}N_3O_6S$ 642.4515).

Compound 3: white powder; HRFABMS *m/z* 562.4933 [M + H − SO_3]$^+$ (calcd for $C_{34}H_{64}N_3O_3$ fragment 562.4947); MALDI MS 642 [M + H]$^+$ (calcd for $C_{34}H_{64}N_3O_6S$ 642).

Compound 4: white powder; HRFABMS *m/z* 644.4687 [M + H]$^+$ (calcd for $C_{34}H_{66}N_3O_6S$ 644.4672); *anal.* calcd for $C_{34}H_{65}N_3O_6S/2TFA/3H_2O$; C 49.29%, H 7.95%, N 4.54%, S 3.46%; found: C 49.17%, H 7.86%, N 4.53%, S 3.29%.

Compound 5: white powder; HRFABMS *m/z* 578.5264 [M + H − SO_3]$^+$ (calcd for $C_{35}H_{68}N_3O_3$ fragment 578.5260), MALDI MS 658 [M + H]$^+$ (calcd for $C_{35}H_{68}N_3O_6S$ 658).

Compound 6: white powder; HRFABMS *m/z* 544.4823 [M + H]$^+$ (calcd for $C_{34}H_{62}N_3O_2$ 544.4842).

Compound 7: white powder; HRESIMS *m/z* 683.5130 [M − H]$^-$ (calcd for $C_{37}H_{71}N_4O_5S$ 683.5145); *anal.* calcd for $C_{37}H_{72}N_4O_5S/3TFA/H_2O$; C 49.42%, H 7.43%, N 5.36%, S 3.07%, F 16.36%; found: C 49.34%, H 7.35%, N 5.08%, S 2.76%, F 16.44%.

Synthesis of Compound 7. To a solution of spermine hydrochloride (870 mg, 2.5 mmol) in CH_3OH (15 mL) was added sodium methoxide (0.5 M, 24.5 mL, 12 mmol) in CH_3OH. Potassium (5α,7α,24*R*)-7-hydroxy-3-ketocholestan-24-yl sulfate (536 mg, 1.0 mmol)[7] was added and stirred overnight at room temperature. The reaction mixture was cooled to −78 °C, treated with sodium borohydride (70 mg, 1.8 mmol), and stirred for 3.5 h. After warming to room temperature, the solvent was removed, and the solid material was dried in vacuo. The solid was dissolved in H_2O, acidified with trifluoroacetic acid to pH 2, and filtered on Celite. The solution was applied to preparative reversed-phase HPLC and gave **7** (534 mg, 52%) as the trifluoroacetate salt. The ^1H and ^{13}C NMR were in agreement with data obtained for the natural material. A product (85 mg, 8.0%) that eluted after **7** was identified as the 3α-isomer of **7**: ^1H NMR (CD_3OD) δ 4.13 (m, 1H, H-24), 3.80 (br s, 1H, H-7), 3.44 (m, 1H, H-3), 3.15−3.04 (m, 12H), 2.10−1.00 (m, 35H), 0.95, 0.95, 0.93 (ddd, 9H, H-21, H-26, H-27), 0.86 (s, 3H, H-19), 0.70 (s, 3H, H-18); ^{13}C NMR (CD_3OD) δ 86.5 (C-24), 68.4 (C-7), 57.6, 56.8, 51.9, 48.3, 48.3, 46.8, 46.1, 46.0, 44.4, 43.8, 41.1, 41.0, 38.0, 37.3, 37.3, 37.2, 33.4, 32.9, 32.5, 32.1, 30.2, 29.4, 28.2, 25.5, 24.6, 24.3, 24.3, 24.1, 23.8, 21.8, 19.5, 18.5, 18.5, 12.5, 11.0.

Compound 8: white powder; for ^1H and ^{13}C NMR data in DMSO-d_6, see Wehrli et al.[2]

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