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Synthesis and Antimicrobial Activity of Squalamine Analogue

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Abstract—Synthesis and antimicrobial activity of squalamine analogue **2** are reported. The synthesis of **2** was accomplished from bisnoralcohol **3**. The spermidine moiety was introduced via reductive amination of an appropriately functionalized 3β-aminosterol with spermidinyl aldehyde **17** utilizing sodium triacetoxyborohydride as the reducing agent. Compound **2** shows weaker antimicrobial activity than squalamine.

Introduction

The sterol-polyamine conjugates as new classes of antibiotics have attracted much interest in recent years, due to the emergence of penicillin-resistant *Staphylococci*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococus pneumoniae* in hospitalized patients.[1–3] Squalamine (**1**) is a novel sterol-spermidine conjugate that has been isolated from the stomach extracts of the dogfish shark *Squalus acanthias*.[4] It has been shown to exhibit potent antimicrobial activity against Gram-negative bacteria, Gram-positive bacteria, and fungi.[4–6] This compound has an unusual chemical structure, consisting of cholestane ring system with 5α-hydrido, 7α-hydroxy, 3β-spermidinyl, and 24*R*-sulfate groups.[7,8] (Fig. 1) Due to the difficulty of obtaining large quantities of squalamine from natural sources, two chemical syntheses of squalamine have been accomplished from the inexpensive stigmasterol as a starting material.[9,10] Also two 17-step, low-yielding formal syntheses of squalamine both from the expensive starting materials, 3β-acetoxy-5-cholenic acid[11] and 3β-hydroxy-5-cholenic acid[12] are known. For these reasons, a wide variety of squalamine mimics which have a spermidine moiety in the sterol skeleton have been prepared from 5-cholenic acid[13] and bile acids,[14,15] and tested antimicrobial activity against bacteria and fungi.

We now wish to report the synthesis of squalamine analogue (**2**) which contains sulfate group at C-22 from the inexpensive 22-hydroxy-23,24-bisnorchola-4-en-3-one (**3**) and in vitro examination of the antimicrobial activity to define the influence of structural feature on the side chain upon antimicrobial potency.

Results and Discussion

Synthesis

The preparative method leading to the formation of **2** requires initially the synthesis followed by the coupling of 7α-hydroxy-3β-aminosterol **14** and spermidine moiety **17**. The intermediate **14** was readily synthesized in nine steps from the commercially available 22-hydroxy-23,24-bisnorchola-4-en-3-one (**3**, bisnoralcohol) in 39% yield as shown in Scheme 1. Bisnoralcohol **3** was reacted with ethylene glycol in the presence of *p*-toluenesulfonic acid in benzene to give the 3-dioxolane Δ^5-derivative **4** (85%), which was then protected with *tert*-butydimethylsilyl chloride to give **5** (95%). Ketalized compound **3** was isomerized to **4**. The structure of **5** can be confirmed by ^{1}H and ^{13}C NMR spectra which show H-6 proton at δ 5.32 and C-3,-5,-6, and -19 carbons at δ 109.4, 140.1, 122.1, and 18.8. Allylic oxidation of **5** with ruthenium chloride and 70% *tert*-butylhydroperoxide[16] gave enone **6** (65%). Hydrogenation (5% Pt/C) of enone **6** resulted in the formation of 5α-cholestan-7-one **7** (80%) along with 5α-cholestane-7β-ol **8** (16%). The latter could be oxidized to **7** with pyridinium chlorochromate (75% yield). Stereoselective reduction of **7** with K-Selectride provided exclusively the 7α-ol **9** (98%). The 7-OH configurations of compounds **8** and **9** were determined based on ^{13}C NMR data: i.e. the chemical shift of C-7 for **8**

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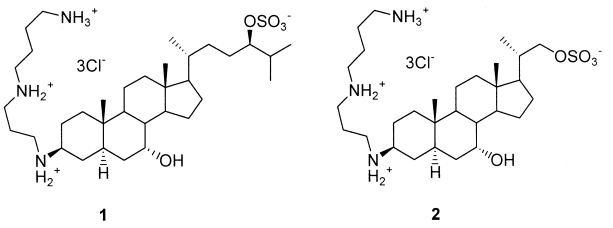
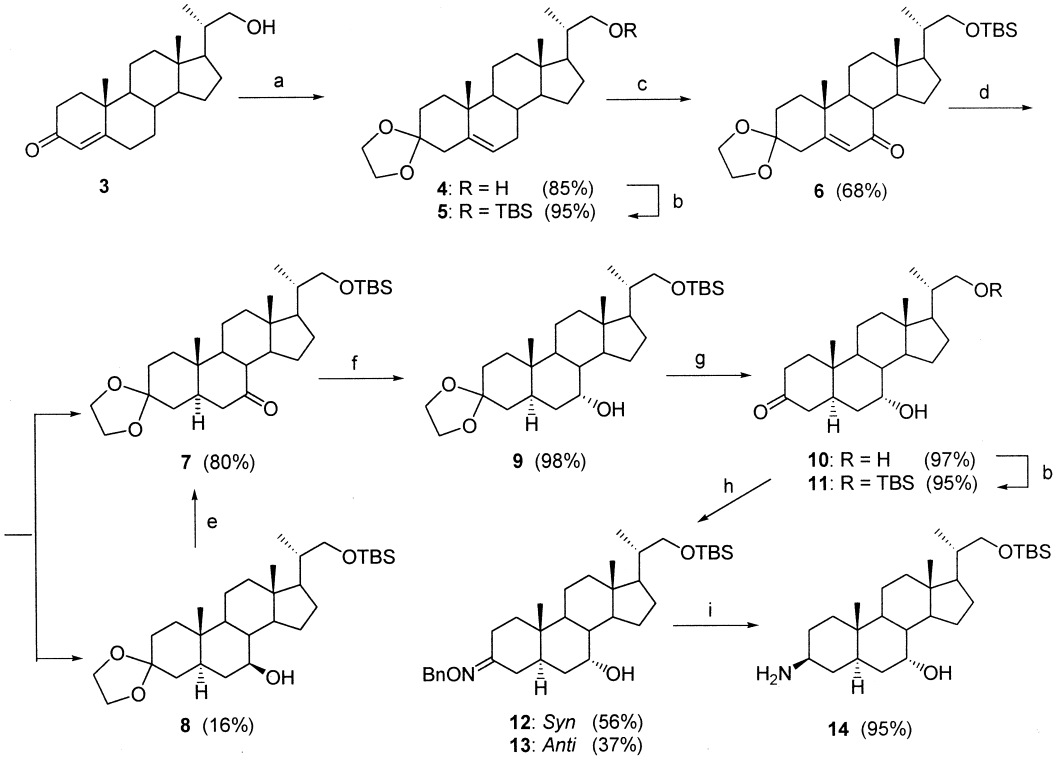


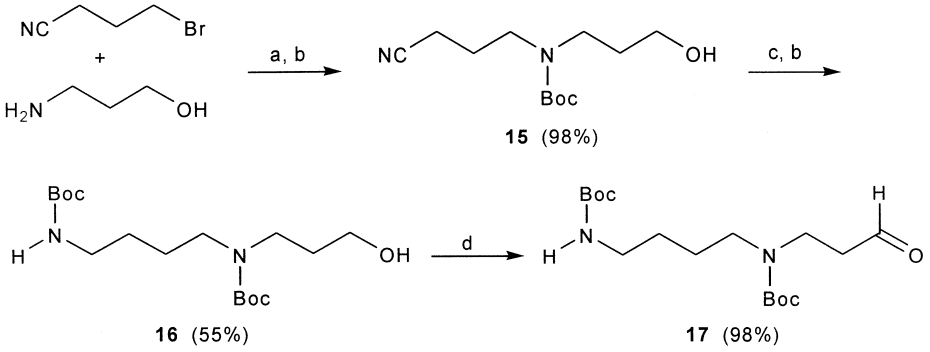
Figure 1. The structures of squalamine (**1**) and analogue (**2**).

was at 75.0, and that for **9** was at 67.9. These values are in good agreement with those reported for the corresponding compound.[17,18] Deprotection of **9** by treatment with 1N HCl afforded **10** (97%). The 3β-amino functionality was introduced onto **10** to give **14** by reduction of oximes **12** and **13**. Reaction of **10** with benzyl hydroxylamine hydrochloride in pyridine resulted in the formation of *syn*-oxime **12** (56% yield) and *anti*-isomer **13** (37% yield) after separation by flash chromatography. The ^{1}H NMR chemical shift of α-CH_2 next to oxime has been used for the assignment of configuration in the case of the *syn* and *anti*-oxime.[11] The chemical shift of α-CH_2 next to oxime in **12**, 2.96 ppm, in $CDCl_3$ is like that in *syn*-oxime (2.98 ppm), unlike that in *anti*-oxime (3.27 ppm).[11] Reduction of oximes **12** and **13** with lithium aluminum hydride gave 3β-aminosterol **14** (95%).

Spermidinyl aldehyde **17** was prepared according to the literature method[11] with slight modification as shown in Scheme 2. Namely, monoalkylation of 3-amino-1-propanol by treatment with 4-bromobutyronitrile in the presence of potassium carbonate and a catalytic amount of sodium iodide followed by protection with di-*tert*-butyl dicarbonate afforded **15** (98%). Reduction of **15** with lithium aluminum hydride and protection with di-*tert*-



Scheme 1. Synthesis of compound **14**. (a) $HOCH_2CH_2OH$, pTSA/benzene; (b) TBSCl, imidazole, DMAP/CH_2Cl_2; (c) $RuCl_3$, TBHP/cyclohexane; (d) H_2, 5% Pt/C/EtOAc; (e) PCC/CH_2Cl_2; (f) K-Selectride/THF; (g) 1N HCl/THF; (h) $BnONH_2$·HCl, pyridine/EtOH; (i) $LiAlH_4/Et_2O$.



Scheme 2. Synthesis of compound **17**. (a) NaI, K_2CO_3/CH_3CN; (b) $(Boc)_2O$/MeOH; (c) $LiAlH_4/Et_2O$; (d) PCC, CH_2Cl_2.

butyl dicarbonate gave **16** (55%). Oxidation of the latter with pyridinium chlorochromate afforded **17** (98%).

For the introduction of spermidine moiety to the steroid backbone, reductive amination was carried out as shown in Scheme 3. Reductive amination of aldehyde **17** and **14** in the presence of sodium triacetoxyborohydride[19] provided **18** (56%). The 3β-configuration of **18** was confirmed by comparing the characteristic ^{1}H NMR peak at δ 3.10 with that of the squalamine (δ 3.05).[7]

Removal of the BOC and TBS protecting groups was accomplished by treatment with 10% hydrochloric acid in methanol to give trihydrochloride salt **19** (90%). Treatment of the latter with sulfur trioxide-pyridine complex in methanol gave 22-sulfate trihydrochloride **2** (15%).

Antimicrobial activity

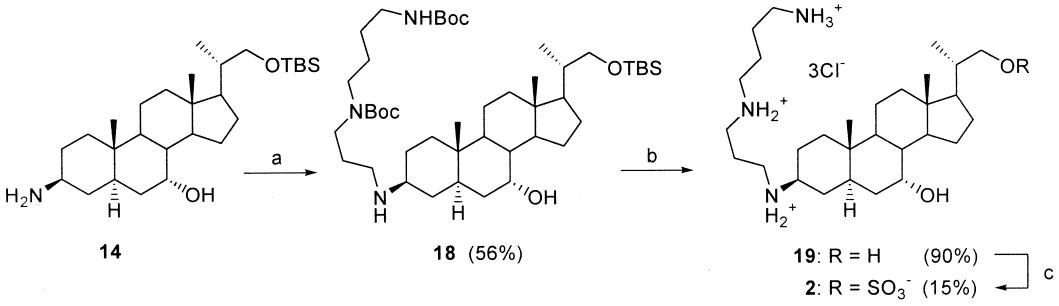
Minimum inhibitory concentrations (MIC) of compound **2** and squalamine were tested against four strains of Gram-positive bacteria (*S. aureus* 6538P, *S. equisimilis* 6580C, *M. luteus* ATCC 9341, and *B. subtilis* ATCC 6633) and six strains of Gram-negative bacteria (*E. coli* 25922, *P. aeruginosa* 27853, *P. mirabilis* 25933, *S. marcescens* 27117, *S. typhimurium* 14028, and *K. pneumoniae* 10031) to evaluate its antimicrobial activity. Compound **2** exhibited a noticeable activity against *M. luteus* 9341, *S. aureus* 6538P, *K. pneumoniae* 10031, *S. equi* 6580C, and *B. subtilis* 6633 but no activity against *E. coli* 25922, *P. aeruginosa* 27853, *P. mirabilis* 25933, *S. marcescens* 27117, and *S. typhimurium* 14028. The activity of **2** is shown in comparison with squalamine in Table 1. The antimicrobial activities of compound **2** were weaker than those of squalamine.

From these results we can conclude that squalamine analogue **2** having a shorter side chain, is capable of exhibiting comparable antimicrobial activities. These results may be used to design new sterol-spermidine conjugate of enhanced antimicrobial activities. The synthetic methodology developed for **2** is being utilized in the preparation of squalamine.

Experimental

Synthesis

General methods. Melting points were measured using a Thomas–Hoover melting point apparatus and are uncorrected. IR spectra were recorded on a Galaxy FT-IR 7000 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker AM-300 or Varian unity plus 300 instruments; unless otherwise stated, all NMR were performed in $CDCl_3$ solution. The chemical shifts of ^{1}H NMR spectra are given in ppm downfield from tetramethylsilane, and ^{13}C NMR spectra were referenced to $CDCl_3$ at 77.0 ppm. ^{1}H and ^{13}C NMR assignments were made from DEPT, COSY, HETCOR, and by comparison to spectra of similar sterols.[7–15] Low-resolution mass spectra (MS) were recorded on a Shimadzu QP-1000 spectrometer. High-resolution MS were measured on a JEOL KMS-DX 303 spectrometer. Elemental analyses were performed by CSI at Kyungpook National University. TLC analyses were carried out on precoated 0.2 mm HPTLC silica gel 60 plates (E. Merck, Darmstadt); substances were visualized by spraying with 5% ammonium molybdate in 10% H_2SO_4 followed by heating. For column chromatography, E. Merck silica gel (70–230 mesh) was used as an adsorbent. Solutions were



14 **18** (56%)

Scheme 3. Synthesis of compound **2**: (a) compound **17** then $NaBH(OAc)_3/CH_2Cl_2$; (b) 10% HCl/MeOH; (c) SO_3-pyridine/MeOH.

Table 1. In vitro antimicrobial activities of **2**[a]

	(Minimal inhibitory concentration, μg/mL)				
Strains	*E. coli*	*P. aeruginosa*	*P. mirabilis*	*M. luteus*	*S. aureus*
ATCC #	(25922)	(27853)	(25933)	(9341)	(6538P)
2	> 100.00	> 100.00	> 100.00	12.50	6.25
Squalamine	> 100.00	> 100.00	> 100.00	3.13	6.25
Strains	*S. marcescens*	*S. typhimurium*	*K. pneumoniae*	*S. equisimilis*	*B. subtilis*
ATCC #	(27117)	(14028)	(10031)	(6580C)	(6633)
2	> 100.00	> 100.00	25.00	12.50	3.13
Squalamine	> 100.00	> 100.00	12.50	3.13	1.56

[a]Activities against ten strains compared to those of the squalamine determined in identical conditions.

dried over anhydrous sodium sulfate. Squalamine was synthesized by literature procedure.[9,10] 22-Hydroxy-23, 24-bisnorchola-4-en-3-one (**3**, bisnoralcohol) was obtained from Pharmacia & Upjohn Pharmaceutical Co (Kalamazoo, MI). Dichloromethane, pyridine, diethyl ether and acetonitrile were dried over calcium hydride, and THF was dried over sodium-benzophenone.

3-Dioxolane-23,24-bisnorchola-5-en-22-ol (4). A mixture of bisnoralcohol **3** (5.00 g, 15.15 mmol), ethylene glycol (16 mL, 227.25 mmol) and *p*TSA (30 mg) in benzene (300 mL) was refluxed with Dean-Stark column for 22 h. The reaction mixture was treated with saturated $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified by column chromatography (EtOAc: hexane 1:3) to give **4** (4.82 g, 12.89 mmol, 85%) and **3** (650 mg, 1.97 mmol, 13%) as white solids: mp 173–174 °C (CH_2Cl_2–MeOH); TLC R_f 0.44 (1:2 EtOAc:hexane); IR (KBr) 3443, 2943, 2881, 1098 cm^{-1}; ^1H NMR δ 0.70 (s, 3H, 18-CH_3), 1.03 (s, 3H, 19-CH_3), 1.05 (d, J = 6.6 Hz, 3H, 21-CH_3), 3.34 (dd, J = 10.2, 6.9 Hz, 1H, 22-H_a), 3.62 (dd, J = 10.2, 3.0 Hz, 1H, 22-H_b), 3.95 (m, 4H,-OCH_2CH_2O-), 5.34 (dd, J = 5.4, 2.7 Hz, 1H, 6-H); ^{13}C NMR δ 11.8, 16.7, 18.7, 20.9, 24.3, 27.6, 30.9, 31.6, 31.8, 36.2, 36.5, 38.7, 39.5, 41.7, 42.3, 49.5, 52.3, 56.3, 64.1, 64.3, 67.7, 109.4, 122.0, 140.0; MS m/z 374 (M^+, 7), 99 (100), 55 (19). Anal. calcd for $C_{24}H_{38}O_3$: C, 76.96; H, 10.27. Found C, 76.90; H, 10.40.

3-Dioxolane-22-*tert*-butyldimethylsilyloxy-23,24-bisnorchola-5-ene (5). A solution of *tert*-butyldimethylsilyl chloride (TBSCl, 963 mg, 6.42 mmol) in CH_2Cl_2 (5 mL) was added to the mixture of **4** (2.00 g, 5.35 mmol), imidazole (1.10 g, 16.05 mmol) and 4-dimethylaminopyridine (DMAP, 10 mg) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 4 h. After the reaction was completed, 10% HCl was added to the mixture and extracted with CH_2Cl_2. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified by column chromatography (EtOAc:hexane 1:5) to give **5** (2.46 g, 5.08 mmol, 95%) as a white solid: mp 127–128 °C (CH_2Cl_2–MeOH); TLC R_f 0.75 (1:4 EtOAc: hexane); IR (KBr) 2960, 2888, 1253, 1088, 863, 838, 771 cm^{-1}; ^1H NMR δ 0.03 (s, 6H, $Si(CH_3)_2C(CH_3)_3$), 0.66 (s, 3H, 18-CH_3), 0.86 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.96 (d, J = 6.0 Hz, 3H, 21-CH_3), 1.00 (s, 3H, 19-CH_3), 3.20 (dd, J = 9.6, 7.5 Hz, 1H, 22-H_a), 3.56 (dd, J = 9.6, 3.0 Hz, 1H, 22-H_b), 3.91 (m, 4H,-OCH_2CH_2O-), 5.32 (dd, J = 5.1, 2.7 Hz, 1H, 6-H); ^{13}C NMR δ −5.4, 12.0, 16.9, 18.4, 18.8, 21.0, 24.4, 26.0, 27.7, 31.0, 31.7, 31.9, 36.3, 36.6, 39.1, 39.6, 41.8, 42.4, 49.6, 52.6, 56.4, 64.2, 64.4, 67.9, 109.4, 122.1, 140.1; MS m/z 489 (M + 1, 6), 432 (M-C_4H_8, 3), 355 (8), 99 (100), 75 (10), 55 (7). Anal. calcd for $C_{30}H_{52}O_2Si$: C, 73.71; H, 10.72. Found C, 73.62; H, 10.51.

3-Dioxolane-22-*tert*-butyldimethylsilyloxy-23,24-bisnorchola-5-en-7-one (6). To a solution of **5** (2.00 g, 4.10 mmol) and $RuCl_3 \cdot xH_2O$ (12 mg) in cyclohexane (20 mL) was added 70% *tert*-butylhydroperoxide (TBHP, 12.0 mL) via syringe pump at 17–19 °C for 6 h. The resulting mixture was stirred at same temperature for 16 h. The mixture was treated with saturated $NaHCO_3$ solution and then extracted with EtOAc. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified by column chromatograph (EtOAc:hexane 1:4) to give **6** (1.34 g, 2.67 mmol, 65%) as a white solid: mp 148–150 °C (CH_2Cl_2–MeOH); TLC R_f 0.49 (1:4 EtOAc:hexane); IR (KBr) 2948, 2888, 1666, 1460, 1252, 1082, 836 cm^{-1}; ^1H NMR δ 0.03 (s, 3H, $Si(CH_3)_2C(CH_3)_3$), 0.67 (s, 3H, 18-CH_3), 0.86 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.97 (d, J = 6.0 Hz, 3H, 21-CH_3), 1.18 (s, 3H, 19-CH_3), 3.22 (dd, J = 9.6, 7.5 Hz, 1H, 22-H_a), 3.57 (dd, J = 9.6, 3.0 Hz, 1H, 22-H_b), 3.92 (m, 4H,-OCH_2CH_2O-), 5.63 (d, J = 1.5 Hz, 1H, 6-H); ^{13}C NMR δ −5.4, 12.1, 16.9, 17.0, 18.3, 21.1, 25.9, 26.5, 28.0, 31.0, 35.6, 38.2, 38.6, 39.0, 41.7, 43.2, 45.4, 49.5, 49.7, 51.3, 64.5, 67.8, 108.9, 126.6, 164.5, 201.7; MS m/z 502 (M^+, 1), 445 (M-C_4H_9, 32), 368 (7), 326 (7), 99 (100). Anal. calcd for $C_{30}H_{50}O_4Si$: C, 71.66; H, 10.02. Found C, 71.95; H, 10.21.

3-Dioxolane-22-*tert*-butyldimethylsilyloxy-23,24-bisnor-5α-chola-7-one (7). A solution of **6** (500 mg, 1.00 mmol) in EtOAc (20 mL) was hydrogenated with 5% Pt/C (30 mg) under an atmosphere of hydrogen for 10 h. After the catalyst was removed by filtration through the Celite pad, the filtrate was concentrated to dryness. The residue was purified by column chromatograph (EtOAc: hexane 1:4) to give **7** (400 mg, 0.79 mmol, 79%) and 3-dioxolane-22-*tert*-butyldimethylsilyloxy - 23,24 - bisnor - 5α-chola-7β-ol (**8**, 76 mg, 0.15 mmol, 15%). Compound **8** was oxidized with pyridinium chlorochromate (PCC, 38 mg) in CH_2Cl_2 (3 mL) for 2 h to give **7** (57 mg, 11%) as a white solid: mp 157–158 °C (CH_2Cl_2-MeOH); TLC R_f 0.52 (1:4 EtOAc:hexane); IR (KBr) 2948, 2857, 1709, 1254, 1105, 837, 773 cm^{-1}; ^1H NMR δ 0.03 (s, 6H, $Si(CH_3)_2C(CH_3)_3$), 0.64 (s, 3H, 18-CH_3), 0.86 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.96 (d, J = 6.9 Hz, 3H, 21-CH_3), 1.05 (s, 3H, 19-CH_3), 3.23 (dd, J = 9.6, 7.5 Hz, 1H, 22-H_a), 3.55 (dd, J = 9.6, 3.0 Hz, 1H, 22-H_b), 3.89 (m, 4H,-OCH_2CH_2O-); ^{13}C NMR δ −5.4, 11.0, 12.1, 17.0, 18.3, 21.8, 25.1, 25.9, 27.9, 31.1, 35.1, 35.9, 37.8, 38.6, 38.9, 42.6, 45.6, 45.8, 48.6, 49.9, 51.5, 54.9, 64.2, 64.3, 67.8, 108.7, 211.7; MS m/z 504 (M^+, 1), 447 (M-C_4H_9, 46), 446 (M-$HOCH_2CH_2OH$, 61), 444 (49), 370 (46), 315 (20), 100 (98), 99 (100). Anal. calcd for $C_{30}H_{52}O_4Si$: C, 71.38; H, 10.38. Found C, 70.99; H, 10.46.

3-Dioxolane-22-*tert*-butyldimethylsilyloxy-23,24-bisnor-5α-chola-7β-ol (8). Mp 120–121 °C (CH_2Cl_2–MeOH); TLC R_f 0.45 (1:4 EtOAc:hexane); IR (KBr) 3529, 2928, 2857, 1470, 1254, 1096, 837 cm^{-1}; ^1H NMR δ 0.03 (s, 6H, $Si(CH_3)_2C(CH_3)_3$), 0.69 (s, 3H, 18-CH_3), 0.83 (s, 3H, 19-CH_3), 0.89 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.99 (d, J = 6.9 Hz, 3H, 21-CH_3), 3.26 (dd, J = 9.6, 7.8 Hz, 1H, 22-H_a), 3.38 (m, 1H, 7α-H), 3.48 (bs, 1H, 7β-OH), 3.60 (dd, J = 9.6, 3.2 Hz, 1H, 22-H_b), 3.93 (m, 4H,-OCH_2CH_2O-); ^{13}C NMR δ −5.4, 11.5, 12.2, 17.0, 18.3, 21.3, 25.9, 27.0, 28.1, 31.1, 34.9, 35.9, 37.5, 37.9, 38.9, 39.8, 40.9, 43.4, 43.7, 51.7, 52.1, 55.4, 64.1, 64.2, 67.9, 75.0, 109.1; MS m/z 450 (7), 449 (M-C_4H_9, 4), 432 (M-C_4H_9-OH, 44), 356 (29), 331 (20), 315 (38), 140 (30), 99 (100). Anal. calcd for $C_{30}H_{54}O_4Si$: C, 71.09; H, 10.74. Found C, 71.17; H, 10.78.

3-Dioxolane-22-*tert*-butyldimethylsilyloxy-23,24-bisnor-5α-chola-7α-ol (9). To a solution of **7** (400 mg, 0.79 mmol) in THF (10 mL) at −60 °C was added 1 M solution

of K-Selectride (3.9 mL) and stirred for 5 h. The mixture was diluted with 30% H_2O_2 (3 mL) and saturated $NaHCO_3$ solution (20 mL) and extracted with EtOAc. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified by column chromatograph (EtOAc:hexane 1:3) to give **9** (380 mg, 0.75 mmol, 95%) as a white solid: mp 170–172 °C (CH_2Cl_2–MeOH); TLC R_f 0.64 (1:2 EtOAc:hexane); IR (KBr) 3537, 2948, 2860, 1470, 1254, 1105, 836, 774 cm^{-1}; ^1H NMR δ 0.03 (s, 6H, $Si(CH_3)_2C(CH_3)_3$), 0.66 (s, 3H, 18-CH_3), 0.80 (s, 3H, 19-CH_3), 0.88 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.97 (d, J = 6.9 Hz, 3H, 21-CH_3), 3.24 (dd, J = 9.6, 7.8 Hz, 1H, 22-H_a), 3.56 (dd, J = 9.6, 3.2 Hz, 1H, 22-H_b), 3.80 (m, 1H, 7β-H), 3.91 (m, 4H,-OCH_2CH_2O-); ^{13}C NMR δ −5.4, 10.4, 11.9, 16.9, 18.3, 20.9, 23.8, 25.9, 27.6, 31.2, 35.6, 35.7, 36.1, 36.3, 37.5, 39.0, 39.3, 39.6, 42.7, 45.6, 50.3, 52.6, 64.1, 67.8, 67.9, 109.2; MS m/z 450 (M-C_4H_8, 11), 432 (M-C_4H_9-OH, 91), 431 (M-C_4H_9-H_2O, 78), 315 (27), 141 (35), 99 (100). Anal. calcd for $C_{30}H_{54}O_4Si$: C, 71.09; H, 10.74. Found C, 71.26; H, 10.93.

7α,22-Dihydroxy-23,24-bisnor-5α-chola-3-one (10). To a solution of **9** (506 mg, 1.00 mmol) in THF (20 mL) was added one drop of 1N HCl at room temperature and stirred for 8 h. The resulting mixture was treated with 10% NaOH solution and extracted with EtOAc. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified on silica gel column chromatograph (EtOAc:hexane 1:1) to give **10** (338 mg, 0.97 mmol, 97%) as a white solid: mp 204–206 °C (CH_2Cl_2–MeOH); TLC R_f 0.45 (100% EtOAc); IR (KBr) 3481, 2936, 2894, 2870, 1714, 1438, 1232, 1031 cm^{-1}; ^1H NMR δ 0.71 (s, 3H, 18-CH_3), 1.01 (s, 3H, 19-CH_3), 1.05 (d, J = 6.7 Hz, 3H, 21-CH_3), 3.37 (dd, J = 10.5, 7.0 Hz, 1H, 22-H_a), 3.49 (s, 1H, 7α-OH), 3.64 (dd, J = 10.5, 3.2 Hz, 1H, 22-H_b), 3.87 (m, 1H, 7β-H); ^{13}C NMR δ 10.4, 11.9, 16.7, 21.1, 23.7, 27.5, 35.6, 36.5, 38.1, 38.1, 38.7, 39.0, 39.2, 39.5, 42.7, 44.1, 45.1, 50.2, 52.4, 67.5, 67.9, 211.8; MS m/z 348 (M^+, 3), 331 (M-OH, 100), 315 (16), 300 (4). Anal. calcd for $C_{22}H_{36}O_3$: C, 75.82; H, 10.41. Found C, 76.15; H, 10.55.

22-*tert*-Butyldimethylsilyloxy-7α-hydroxy-23,24-bisnor-5α-chola-3-one (11). A solution of TBSCl (660 mg, 4.40 mmol) in CH_2Cl_2 (5 mL) was added to the mixture of **10** (1.39 g, 4.00 mmol), imidazole (825 mg, 12.00 mmol) and DMAP (8 mg) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature for 4 h. After the reaction was completed, 10% HCl was added to the mixture and extracted with CH_2Cl_2. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified by column chromatography (EtOAc:hexane 1:3) to give **11** (2.46 g, 5.08 mmol, 95%) as a white solid: mp 211–213 °C (CH_2Cl_2–MeOH); TLC R_f 0.63 (1:2 EtOAc:hexane); IR (KBr) 3467, 2950, 2860, 1716, 1471, 1255, 1100, 835, 773 cm^{-1}; ^1H NMR δ 0.03 (s, 6H, Si $(CH_3)_2C(CH_3)_3$), 0.69 (s, 3H, 18-CH_3), 0.88 (s, 9H, Si $(CH_3)_2C(CH_3)_3$), 0.98 (d, J = 6.9 Hz, 3H, 21-CH_3), 0.99 (s, 3H, 19-CH_3), 3.26 (dd, J = 9.6, 7.8 Hz, 1H, 22-H_a), 3.56 (dd, J = 9.6, 3.0 Hz, 1H, 22-H_b), 3.85 (m, 1H, 7β-H); ^{13}C NMR δ −5.4, 10.4, 11.9, 16.9, 18.3, 21.2, 23.8, 25.9, 27.6, 35.6, 36.5, 38.1, 38.1, 39.0, 39.0, 39.2, 39.5, 42.7, 44.1, 45.2, 50.2, 52.6, 67.5, 67.8, 211.6; MS m/z 463 (M + 1, 6), 430 (1), 406 (30), 405 (M-C_4H_9, 16), 388 (M-C_4H_9-OH, 100), 311 (63), 271 (62). Anal. calcd for $C_{28}H_{50}O_3Si$: C, 72.67; H, 10.89. Found C, 73.05; H, 11.10.

***syn*-3-Benzyloxyimino-22-*tert*-butyldimethylsilyloxy-23,24-bisnor-5α-chola-7α-ol (12) and *anti*-isomer (13).** A mixture of **11** (430 mg, 0.93 mmol), pyridine (1.13 mL) and *o*-benzyl hydroxylamine hydrochloride ($BnONH_2$·HCl, 178 mg, 1.12 mmol) in EtOH (10 mL) was refluxed for 1 h. After the solvent was removed under reduced pressure, 10% HCl was added to the mixture and extracted with EtOAc. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified by column chromatography (EtOAc:hexane 1:4) to give **12** (293 mg, 0.52 mmol, 56%) and **13** (195 mg, 0.35 mmol, 37%) as white solids: **12**: mp 124–125 °C (CH_2Cl_2–MeOH); TLC R_f 0.67 (1:4 EtOAc:hexane); IR (KBr) 3566, 3061, 3029, 2927, 2853, 1739, 1632, 1454, 1361, 1256, 1081, 1018, 841, 774 cm^{-1}; ^1H NMR δ 0.03 (s, 6H, $Si(CH_3)_2C(CH_3)_3$), 0.68 (s, 3H, 18-CH_3), 0.88 (s, 3H, 19-CH_3), 0.89 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.99 (d, J = 6.0 Hz, 3H, 21-CH_3), 2.96 (d, J = 11.7 Hz, 1H, 2-H), 3.26 (dd, J = 9.6, 6.7 Hz, 1H, 22-H_a), 3.57 (dd, J = 9.6, 3.3 Hz, 1H, 22-H_b), 3.83 (m, 1H, 7β-H), 5.04 (s, 2H, $OCH_2C_6H_5$), 7.31 (m, 5H, Ph); ^{13}C NMR δ −5.4, 10.5, 11.9, 16.9, 18.4, 20.9, 23.8, 26.0, 27.6, 36.1, 36.4, 37.7, 38.0, 39.0, 39.3, 39.4, 42.7, 45.4, 45.4, 50.2, 52.6, 67.8, 67.8, 75.2, 127.6, 128.0, 128.3, 138.1, 160.0; MS m/z 565 (M^+, 3), 491 (M-C_4H_9-OH, 100), 490 (M-C_4H_9-H_2O, 98), 401 (7), 374 (38), 90 (86). Anal. calcd for $C_{35}H_{57}NO_3Si$: C, 74.02; H, 10.12; N 2.47. Found C, 73.98; H, 10.07; N 2.12.

13: mp 136–137 °C (CH_2Cl_2–MeOH); TLC R_f 0.46 (1:4 EtOAc:hexane); IR (KBr) 3476, 3104, 3049, 2937, 2868, 1721, 1656, 1474, 1365, 1251, 1080, 1015, 853, 777 cm^{-1}; ^1H NMR δ 0.03 (s, 6H, $Si(CH_3)_2C(CH_3)_3$), 0.67 (s, 3H, 18-CH_3), 0.89 (s, 3H, 19-CH_3), 0.89 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.98 (d, J = 6.0 Hz, 3H, 21-CH_3), 3.25 (dd, J = 9.6, 6.9 Hz, 1H, 22-H_a), 3.26 (m, 1H, 2-H), 3.56 (dd, J = 9.6, 3.0 Hz, 1H, 22-H_b), 3.83 (m, 1H, 7β-H), 5.05 (s, 2H, $OCH_2C_6H_5$), 7.31 (m, 5H, Ph); ^{13}C NMR δ −5.4, 10.3, 11.9, 16.9, 18.4, 20.9, 21.4, 23.8, 26.0, 27.6, 36.2, 36.4, 37.0, 39.0, 39.0, 39.3, 39.5, 42.7, 45.4, 45.4, 50.3, 52.6, 67.7, 67.8, 75.2, 127.5, 127.8, 138.3, 160.1; MS m/z 565 (M^+, 5), 491 (M-C_4H_9-OH, 97), 490 (M-C_4H_9-H_2O, 100), 401 (5), 90 (74). Anal. calcd for $C_{35}H_{57}NO_3Si$: C, 74.02; H, 10.12; N 2.47. Found C, 74.27; H, 10.17; N 2.06.

3β-Amino-22-*tert*-butyldimethylsilyloxy-23,24-bisnor-5α-chola-7α-ol (14). A mixture of **12** and **13** (320 mg, 0.57 mmol) and $LiAlH_4$ (35 mg, 0.93 mmol) in Et_2O (15 mL) was refluxed for 16 h. After the reaction was completed, 10% NaOH solution was added to the mixture and stirred for 30 min and extracted with Et_2O. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified by column chromatography (EtOAc:hexane 1:1) to give **14** (250 mg, 0.54 mmol, 95%) as a white solid: mp 178–180 °C; TLC R_f 0.76 (7:2.5:0.5 CH_2Cl_2:MeOH:NH_4OH); IR (KBr) 3396, 2932, 2859, 1566, 1471, 1387, 1254, 1094, 1034, 8305, 774 cm^{-1}; ^1H NMR δ 0.025 (s, 6H, $Si(CH_3)_2C(CH_3)_3$), 0.67 (s, 3H, 18-CH_3), 0.79 (s, 3H, 19-CH_3), 0.89 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.98 (d, J = 6.3 Hz, 3H, 21-CH_3), 2.73

(m, 1H, 3α-H), 3.25 (dd, $J=9.6$, 8.7 Hz, 1H, 22-H_a), 3.57 (dd, $J=9.6$, 3.3 Hz, 1H, 22-H_b), 3.83 (m, 1H, 7β-H); MS *m/z* 463 (M^+, 2), 446 (M-NH_3, 1), 431 (M-NH_3-CH_3, 1), 407 (12), 389 (M-C_4H_9-NH_3, 100), 372 (6), 331 (3), 313 (11), 296 (9), 273 (13), 255 (13). Anal. calcd for $C_{28}H_{53}NO_2Si{\cdot}H_2O$: C, 69.80; H, 11.51; N, 2.91. Found C, 70.11; H, 11.41; N, 2.94.

***tert*-Butyl *N*-(4-cyanobutyl)-*N*-(3-hydroxypropyl)carbamate (15).** A mixture of 3-aminopropanol (3.00 g, 39.94 mmol), K_2CO_3 (6.10 g, 44.16 mmol) and NaI (0.60 g, 4.00 mmol) in CH_3CN (150 mL) was refluxed for 1 h. To the resulting mixture was added a solution of 4-bromobutyronitrile (5.91 g, 39.94 mmol) in CH_3CN (50 mL) for 6 h, and refluxed for 20 h. After the reaction was completed, the solvent was removed under reduced pressure, diluted with water (20 mL) and extracted with CH_2Cl_2. The organic layer was washed with brine, dried and concentrated to dryness. To a solution of the crude product (6.70 g) in MeOH (50 mL) was added a solution of di-*tert*-butyl dicarbonate (10.28 g, 47.12 mmol) in MeOH (25 mL) and stirred for 5 h at room temperature. After the reaction was completed, the solvent was removed under reduced pressure, diluted with water (20 mL) and extracted with CH_2Cl_2. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified by column chromatography (100% ethyl acetate) to give **15** (9.48 g, 39.14 mmol, 98%) as a colorless oil: TLC R_f 0.48 (100% EtOAc); IR (neat) 3446, 2984, 2262, 1667, 1482, 1419, 1370, 1296, 1250, 748 cm^{-1}; ^1H NMR δ 1.43, 1.64, 1.85, 2.32, 3.25, 3.32, 3.51, 3.59; ^{13}C NMR δ 14.4, 24.2, 28.0, 30.3, 42.7, 45.4, 58.1, 80.3, 118.9, 156.0; MS *m/z* 242 (M + 1, 2), 143 (59), 97 (49), 84 (46), 58 (100); high resolution MS, calcd for $C_{12}H_{22}N_2O_3$ (M^+) 242.1630, found 242.1632.

***tert*-Butyl *N*-(4-aminobutyl)-*N*-(3-hydroxypropyl)dicarbamate (16).** Compound **15** (1.12 g, 4.62 mmol) was stirred with $LiAlH_4$ (0.62 g, 11.63 mmol) in Et_2O (15 mL) at 0 °C for 30 min. After the reaction was completed, 1N solution of NaOH (5.0 mL) was added at the same temperature, and stirred for additional 30 min. Inorganic material was removed by filtration of the resulting mixture through the Celite pad. The filtrate was concentrated to dryness. Without further purification the residue was treated with di-*tert*-butyl dicarbonate (1.21 g, 5.55 mmol) in MeOH (15 mL). After the reaction was completed, the solvent was removed under reduced pressure, diluted with water (20 mL) and extracted with CH_2Cl_2. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified by column chromatography (100% EtOAc) to give **16** (916 mg, 2.54 mmol, 55%) as a colorless oil: TLC R_f 0.51 (100% EtOAc); IR (neat) 3357, 2977, 2872, 1693, 1527, 1367, 1172 cm^{-1}; ^1H NMR δ 1.43, 1.53, 1.64, 3.11, 3.15, 3.35, 3.54, 3.79, 4.51; ^{13}C NMR δ 25.7, 27.5, 28.4, 30.6, 40.1, 42.5, 46.7, 58.3, 79.2, 80.1, 156.0; MS *m/z* 347 (M + 1, 2), 88 (50), 58 (100); high resolution MS, calcd for $C_{17}H_{34}N_2O_5$ (M^+) 346.2468, found 346.2466.

***tert*-Butyl *N*-(4-aminobutyl)-*N*-(3-oxopropyl)dicarbamate (17).** To a mixture of **16** (1.00 g, 2.89 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added PCC (1.80 g, 8.32 mmol) and stirred for 3 h. After the reaction was completed, Et_2O (60 mL) was added to the resulting reaction mixture. The resulting mixture was filtered through the Celite pad and the filtrate was concentrated to dryness. The residue was purified by column chromatography (100% EtOAc) to give **17** (980 mg, 2.85 mmol, 98%) as a colorless oil: TLC R_f 0.62 (100% EtOAc); IR (neat) 3362, 2977, 2935, 2870, 2728, 1695, 1523, 1480, 1367, 1172 cm^{-1}; ^1H NMR δ 1.20, 1.40, 1.49, 2.65, 3.19, 3.15, 3.46, 4.57, 9.75; ^{13}C NMR δ 25.5, 27.3, 28.4, 40.1, 41.0, 43.3, 47.4, 79.1, 79.8, 155.4, 156.0, 201.0; MS *m/z* 345 (M + 1, 3), 288 (4), 245 (11), 226 (11), 215 (21), 187 (21), 171 (5), 170 (25), 126 (64), 114 (56), 82 (97), 69 (94), 57 (100); high resolution MS, calcd for $C_{17}H_{32}N_2O_5$ (M^+) 344.2311, found 344.2308.

3β-*N*-[*tert*-Butyl *N*(3-[4-aminobutyl])-1,3-diaminopropyldicarbamate]-22-*tert*-butyldimethylsilyloxy-23,24-bisnor-5α-chola-7α-ol (18). To a stirred solution of $NaBH(OAc)_3$ which was prepared from $NaBH_4$ (190 mg) and HOAc (1.0 mL) in CH_2Cl_2 (9 mL) at 0 °C was added **17** (80 mg, 0.23 mmol) and **14** (131 mg, 0.22 mmol). After the reaction was completed, saturated solution of $NaHCO_3$ was added to the resulting mixture and extracted with CH_2Cl_2. The organic layer was washed with brine, dried and concentrated to dryness. The residue was purified by column chromatography (CH_2Cl_2:MeOH:NH_4OH 16:3:1) to give **18** (98 mg, 0.12 mmol, 56%) as a white solid: mp 88–90 °C (CH_2Cl_2–MeOH); TLC R_f 0.61 (16:3:1 CH_2Cl_2:MeOH:NH_4OH); IR (KBr) 3366, 2931, 2860, 1694, 1516, 1472, 1420, 1365, 1252, 1173, 1090, 835, 775 cm^{-1}; ^1H NMR (500 MHz) δ 0.03 (s, 6H, $Si(CH_3)_2C(CH_3)_3$), 0.64 (s, 3H, 18-CH_3), 0.76 (s, 3H, 19-CH_3), 0.86 (s, 9H, $Si(CH_3)_2C(CH_3)_3$), 0.96 (d, $J=6.5$ Hz, 3H, 21-CH_3), 1.42 (s, 18H, 2×$CO_2C_4H_9$), 2.59 (m, 2H, CH_2N), 3.10 (m, 1H, 3α-H), 3.13 (m, 4H, 2×CH_2N), 3.23 (dd, $J=9.6$, 7.5 Hz, 1H, 22-H_a), 3.55 (dd, $J=9.6$, 3.1 Hz, 1H, 22-H_b), 3.80 (m, 1H, 7β-H), 4.65 (bs, 1H, N-H); ^{13}C NMR δ −5.4, 11.2, 11.9, 16.8, 18.3, 20.8, 23.7, 25.9, 27.3, 27.6, 28.4, 28.4, 36.1, 36.5, 37.2, 37.5, 39.0, 39.3, 39.3, 39.5, 40.1, 42.7, 45.9, 46.5, 50.3, 52.5, 57.2, 67.8, 67.9, 79.3, 155.9; MS *m/z* 791 (M^+, 2), 776 (M-CH_3, 1), 678 (M-OTBS, 1), 618 (M-SC, 1), 462 (M-spermidine, 30), 388 (3), 41 (100). Anal. calcd for $C_{45}H_{85}N_3O_6Si{\cdot}H_2O$: C, 66.70; H, 10.82; N, 5.19. Found C, 66.83; H, 10.70; N, 5.03.

3β-*N*-1-[*N*(3-[4-Aminobutyl])-1,3-diaminopropane]-7α,22-dihydroxy-23,24-bisnor-5α-cholane trihydrochloride (19). A solution of **18** (700 mg, 0.88 mmol) in 10% HCl in MeOH (10 mL) was stirred at room temperature for 2 h. After the solvent was removed the residue was purified by column chromatography (CH_2Cl_2:MeOH:NH_4OH 6:3:1) to give **16** (464 mg, 0.79 mmol, 90%) as a white solid: mp 255–260 °C (CH_2Cl_2–MeOH); TLC R_f 0.50 (6:3:1 CH_2Cl_2:MeOH:NH_4OH); IR (KBr) 3406, 2942, 2856, 1613, 1470, 1402, 1155, 1034, 949, 795, 553 cm^{-1}; ^1H NMR (CD_3OD) δ 0.71 (s, 3H, 18-CH_3), 0.88 (s, 3H, 19-CH_3), 1.03 (d, $J=6.8$ Hz, 3H, 21-CH_3), 2.98–3.02 (m, 9H), 3.24 (dd, 1H, $J=10.8$, 7.2 Hz, 1H, 22-H_a), 3.57 (dd, 1H, $J=10.8$, 3.0 Hz, 1H, 22-H_b), 3.80, (m, 1H, 7β-H); ^{13}C NMR (CD_3OD) δ 11.5, 12.3, 17.4, 22.0, 24.2, 24.3, 24.6, 25.5, 25.8, 28.8, 31.9, 36.7, 37.6, 38.5, 40.0, 40.2, 40.7, 40.9, 42.8, 43.7, 45.9, 46.7, 48.2, 48.3, 51.4, 53.9,

58.8, 67.9, 68.3; MS *m/z* 404 (M-$C_4H_8NH_3$, 2), 390 (6), 375 (5), 361 (8), 349 (27), 315 (10), 300 (3), 207 (4), 162 (5), 152 (20), 130 (10), 111 (86), 98 (100). Anal. calcd for $C_{29}H_{55}N_3O_2 \cdot 3HCl \cdot 3H_2O$: C, 54.32; H, 10.06; N, 6.55. Found C, 54.39; H, 9.73; N, 7.25.

3β-*N*-1-[*N*(3-[4-Aminobutyl])-1,3-diaminopropane]-7α,22-dihydroxy-23,24-bisnor-5α-cholane, 22-sulfate trihydrochloride (2). A mixture of **19** (60 mg, 0.102 mmol) and concentrated HCl (3 mL) in MeOH (30 mL) was stirred at room temperature for 15 min. After the solvent was removed, the residue was reacted with SO_3-pyridine complex in pyridine (3 mL) under argon at 50 °C. After the reaction was completed, MeOH (6 mL) was added to the reaction mixture and filtered through Celite pad. The filtrate was concentrated to dryness. The residue was purified by column chromatography (CH_2Cl_2:MeOH:NH_4OH 6:3:1) to give **2** (13 mg, 0.019 mmol, 15%) as a white solid: mp 196–198 °C (CH_2Cl_2–MeOH); TLC R_f 0.4 (6:3:1 CH_2Cl_2:MeOH:NH_4OH); IR (KBr) 3435, 2940, 2868, 1628, 1469, 1240, 1209, 1059, 1013, 978, 583 cm^{-1}; ^1H NMR (CD_3OD) δ 0.72 (s, 3H, 18-CH_3), 0.84 (s, 3H, 19-CH_3), 1.07 (d, J = 6.4 Hz, 3H, 21-CH_3), 2.92–2.63 (m, 5H), 3.79(m, 1H), 3.98, (dd, J = 6.8, 3.2 Hz, 1H); MS *m/z* 494 (1), 446 (5), 386 (8), 371 (19), 333 (23), 255 (24), 236 (25), 121 (35), 81 (100). Anal. calcd for $C_{29}H_{54}N_3O_4S \cdot 3HCl \cdot 3H_2O$: C, 48.36; H, 8.82; N, 5.83; S, 4.45. Found C, 48.32; H, 8.78; N, 5.72; S, 3.96.

Antimicrobial screens

Compound **2** and squalamine were assayed in vitro against ten bacterial strains obtained from the American Type Culture Collection (ATCC: Rockville, MD, USA). The strains were four Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538P), *Streptococcus equisimilis* (ATCC 6580C), *Micrococcus luteus* (ATCC 9341), and *Bacilius subtilis* (ATCC 6633), and the following six Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 25933), *Serratia marcescens* (ATCC 27117), *Salmonella typhimurium* (ATCC 14028), and *Klebsiella pneumoniae* (ATCC 10031). Minimal inhibitory concentrations for the bacteria were determined by an agar dilution method using Muller-Hinton medium. The culture grown overnight at 37 °C for 20 h was diluted to 3×10^6 colony-forming units (CFU)/mL and about 10^4 CFU/mL was spotted on to the agar plates containing serial two-fold dilutions of antibiotics with replacing device (Microplanter). The plates were incubated at 37 °C for 20 h. The MIC was defined as the lowest concentration of antibiotics, at which visible growth was inhibited. Control incubation in the absence of bacteria served to set a baseline value.

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