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Synthesis of new 7-aminosterol squalamine analogues with high antimicrobial activities through a stereoselective titanium reductive amination reaction

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Abstract—A series of 7-amino- and polyaminosterol analogues of squalamine and trodusquemine were synthesized involving a new stereoselective titanium reductive amination reaction in high chemical yields of up to 95% in numerous cases. These derivatives were evaluated for their in vitro antimicrobial properties against human pathogens. All the compounds present excellent activities against Gram-positive bacteria exhibiting similar results against *Staphylococcus aureus* and *Streptococcus faecalis* with minimum inhibitory concentrations (MICs) varying from 2.5 to 10 μg/mL. Numerous derivatives possess also MICs against Gram-negative *Escherichia coli* bacteria (MICs varying from 2.5 to 10 μg/mL) suggesting that nature of the amino group attached to the sterol moiety plays an important role on the activities of such products.

1. Introduction

Chemical antibiotics were one of the great health successes of the 20th century. Antibiotics both naturally-derived and synthetic have resulted in huge decreases in both morbidity and mortality from bacterial infections. However, this has led to high levels of inappropriate prescribing, where antibiotics may be given to fulfill patient expectations rather than for clinical benefit. Along with unwise uses in agriculture and elsewhere, this has contributed to recent rises in numbers of antibiotic-resistant bacteria. As a result, many commentators have described the end of the antibiotic age,[1] and the term 'superbug' has entered the common vocabulary for multidrug-resistant bacteria such as vancomycin-resistant *Enterococcus* (VRE), multidrug-resistant *Staphylococcus aureus* (MRSA), and multidrug-resistant *Pseudomonas aeruginosa* (MRPA).[2] In this context, the growing resistance of bacteria against conventional antibiotics has led to an intense research for new types of antibiotics such as antibiotics peptides, lipids, and alkaloids isolated as host defense agents from diverse animal species.[3–7] Among these substances, two water soluble cationic steroids, squalamine **1** and trodusquemine **2** have been isolated from the dogfish shark *Squalus acanthias* exhibiting potent antimicrobial and antiangiogenic activities (Fig. 1).[8–15]

To date, obtaining large quantities of **1** and **2** is questionable from natural sources or synthetic process since only traces of these compounds are present in the liver and gallbladder of the shark and that the chemical production requires expensive starting materials and numerous steps with low chemical yields. To date few studies have been devoted to the synthesis of molecules based on cholestane or bis-nor cholenic acid skeleton mimicking not only the structure of squalamine but also its remarkable biological activities.[16–19] Recently, we synthesized and evaluated 7α- and 7β-aminocholesterol derivatives **3a** and**3b** exhibiting interesting antimicrobial properties even toward resistant strains.[20–22] These derivatives hydroxylated at C-3 with an amino function at C-7 are in inverse position compared with squalamine. Nevertheless, this methodology is not applicable to the synthesis of various parent amino compounds. In this context, even if **1** and **2** possess three different functional groups, we have focused our studies on a systematic structure–activity relationships of derivatives possessing a C-7β-polyamine moiety and a C-3β-hydroxyl group but devoid of a sulfate moiety in position C-24. Recently, we demonstrated that reductive amination of carbonyl compounds is a very important and powerful tool for chemists to target the synthesis of primary, secondary or tertiary amines.[23,24] In the frame of our work, we report herein the development of a totally stereoselective titanium reductive amination procedure for the synthesis of various

Abbreviations: MIC, minimum inhibitory concentration; VRE, vancomycin-resistant *Enterococcus*; MRSA, multidrug-resistant *Staphylococcus aureus*; MRPA, multidrug-resistant *Pseudomonas aeruginosa*.

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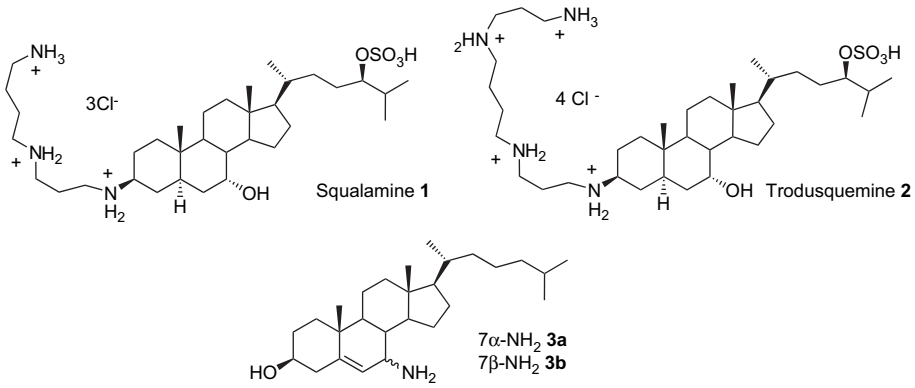


Figure 1. Structures of squalamine **1**, trodusquemine **2**, 7α-NH_2 **3a** and 7β-NH_2 **3b**.

new 7-amino or polyaminocholestanol derivatives in a few step procedure according to the following synthetic pathway and the evaluation of their biological properties (Scheme 1).

2. Results and discussion

Initial experiments for the titanium(IV) reductive amination reaction were performed using 3β-acetoxy-7-keto-5α-cholestane **5** easily prepared in 49% overall yield in a three-step synthesis from cholesterol and 1,2-diaminoethane as test substrates under various experimental conditions.

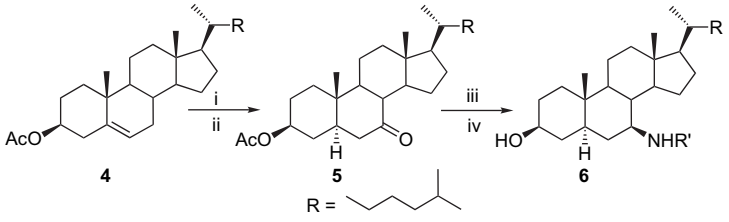
First of all, it clearly appears that isolated yields of compound **6a** are highly solvent dependent. Thus, the expected amino derivative **6a** was obtained in 61% yield performing the reaction in MeOH (Table 1, entry 1) whereas only moderate yields varying from 30, 19, and 33% yields were encountered performing the reaction in CH_2Cl_2, toluene, and THF, respectively (Table 1, entries 5–7). Influence of nature of the titanium source involved was also investigated by performing the reaction in MeOH and chemical yield variations from 30 to 61% were obtained (Table 1, entries 1, 8–10), best result having been observed using Ti(O*i*-Pr)$_4$ as titanium source. Furthermore, under these best experimental conditions, increasing the reaction temperature from −78 to 0 °C led to a significant decrease of the diastereoselectivity from 95 to 60% de, respectively (Table 1, entries 1 and 4). Moreover, variation of the amount of amine from 1 to 3 equiv was also taken into consideration, increasing the yield from 5 to 61% (Table 1, entries 1–3).

A mechanistic rationale can be proposed including a nucleophilic attack of the amino group to a carbonyl compound activated by a Lewis acid. Thus, as we have already demonstrated in the case of simple aliphatic or aromatic ketones, the reaction may proceed through the formation of a titanium complex undergoing a transient imine species, which can be subsequently reduced. A transition state model (Scheme 2) is proposed to account for the stereoinduction in the reaction leading exclusively to the formation of the β-amino or polyamino derivative due to steric hydrogen hindrance suggesting that the hydride attack occurs at the C-7 carbon in an α position generating principally the 7β-aminocholestanol derivative **6**. Thus, this transition state allows us to justify that the formation of the 7α-parent derivative is unfavored at low temperature (Scheme 3).[25–28]

Under the best experimental conditions (Table 1, entry 1), the use of numerous different diamines has been envisioned. Whatever be the nature of the considered diamine, the expected product was obtained in chemical overall yields varying from 6 to 77% and with excellent diastereoselectivity of up to 95% de in all cases (Table 2).

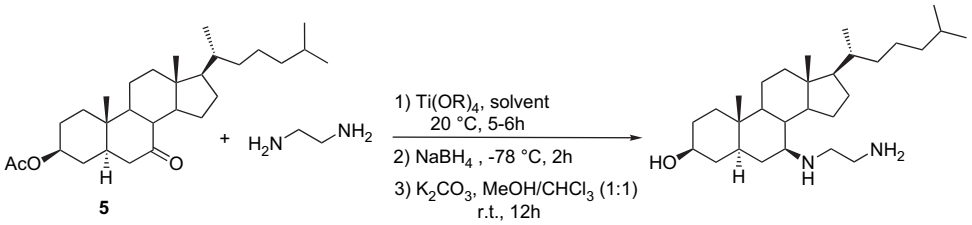
All the synthesized compounds were screened for antimicrobial activity against several yeast strains as well as Gram-positive and Gram-negative bacterial strains and found to possess activities against the microorganisms listed in Table 3. Thus, almost all of the compounds tested in the present study were found to possess good activity against *Saccharomyces cerevisiae* whereas few of them are efficient against this *Candida albicans* strain. The presence of an amino group possessing a well defined chain length, typically 3–5 carbons between the two amino groups (Table 3, compounds **6d–6f**) seems to be a crucial parameter since in all the other cases no activities have been detected against *C. albicans*.

On the other hand, except for derivative **6b**, all the compounds present excellent activities against Gram-positive bacteria exhibiting similar results against *S. aureus* and *Streptococcus*



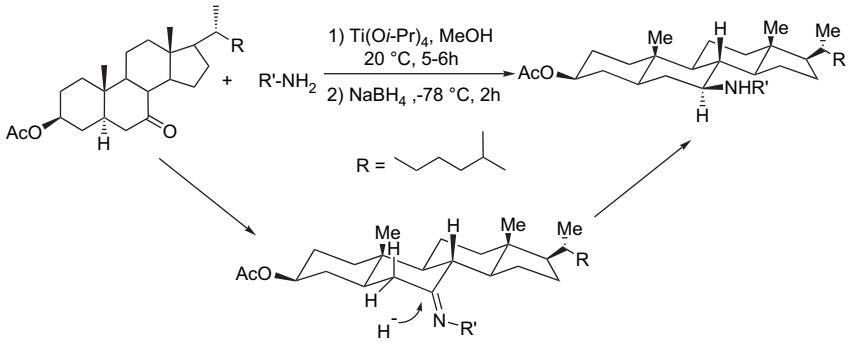
Scheme 1. Conditions: (i) (a) O_2, *N*-hydroxyphthalimide, acetone/ethyl acetate (1:1), benzoyl peroxide, 50 °C, 48 h (b) $CuCl_2$, pyridine, 0 °C, 24 h (72%); (ii) PtO_2 (5 mol %), H_2 (3 bar), 12 h; (iii) (a) R′NH_2, Ti(O*i*-Pr)$_4$, MeOH 20 °C, 5–6 h (b) $NaBH_4$, 2 h; (iv) K_2CO_3 (4 equiv), $CHCl_3$/MeOH (50:50).

Table 1. Titanium(IV) reductive amination reaction of 3β-acetyl-7-ketocholestane **5** with 1,2-diaminoethane under various experimental conditions

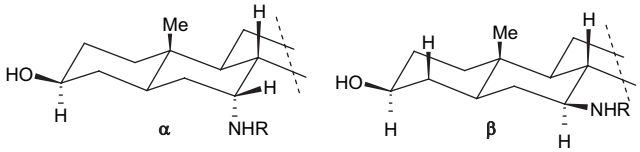


Entry	Titanium source	Solvent	Yield[e] (%)	de[f] (%)
1[a]	Ti(O*i*-Pr)$_4$	MeOH	61	>95
2[b]	Ti(O*i*-Pr)$_4$	MeOH	5	n.d.[g]
3[c]	Ti(O*i*-Pr)$_4$	MeOH	<5	n.d.[g]
4[d]	Ti(O*i*-Pr)$_4$	MeOH	90	60
5[a]	Ti(O*i*-Pr)$_4$	CH_2Cl_2	30	>95
6[a]	Ti(O*i*-Pr)$_4$	Toluene	19	>95
7[a]	Ti(O*i*-Pr)$_4$	THF	33	>95
8[a]	Ti(OEt)$_4$	MeOH	41	>95
9[a]	Ti(OBu)$_4$	MeOH	30	>95
10[a]	Ti(O*t*-Bu)$_4$	MeOH	30	>95

[a] Reaction performed at −78 °C using 3 equiv of amine.
[b] Reaction performed at −78 °C using 2 equiv of amine.
[c] Reaction performed at −78 °C using 1 equiv of amine.
[d] Reaction performed at 0 °C using 3 equiv of amine.
[e] Isolated overall yield.
[f] Diastereomeric excesses were evaluated by ^{1}H and ^{13}C NMR analysis. In all cases, the 7β-diastereomer has been obtained as the major compound.
[g] Not determined.



Scheme 2. Postulated titanium reductive amination mechanism.



δ 7β-H = 4.11 ppm $J_{7\beta\text{-}8}$ = 9 Hz $J_{7\beta\text{-}6}$ = 4 Hz

δ 7α-H = 4.22 ppm $J_{7\alpha\text{-}8}$ = 12 Hz $J_{7\alpha\text{-}6}$ = 6 Hz

Scheme 3. ^{1}H NMR data revealing α- and β-amino derivatives differences.

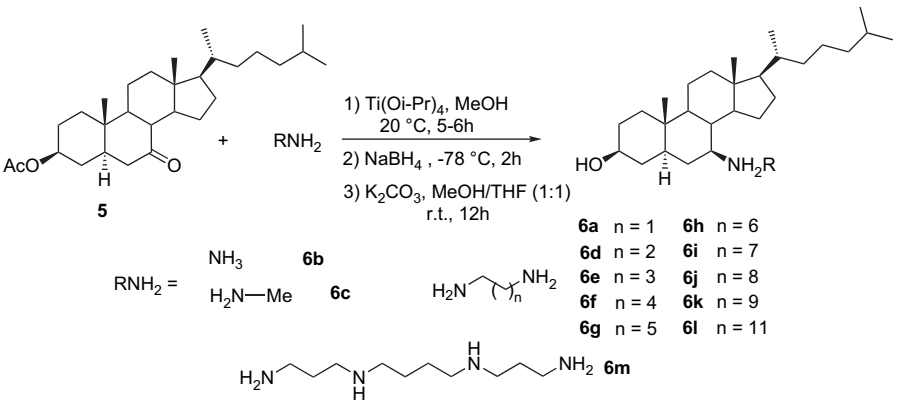
faecalis with MICs varying from 2.5 to 10 μg/mL. Moreover, except for compounds **6b** and **6c**, all these derivatives possess MICs against Gram-negative *Escherichia coli* bacteria (MIC varying from 2.5 to 10 μg/mL)suggesting that nature of the amino group attached to the sterol moiety plays an important role on the potential activities of our products.

Finally, mimic derivative of trodusquemine **6m** appears to be interesting antimicrobial candidate since it leads to the same results, in terms of biological activities, than squalamine **1**.

3. Conclusion

Current studies are underway to evaluate the potentiality of such derivatives in vivo, by determining the cytotoxicity of these compounds and establishing the mechanism of action of this new class of antimicrobial agents.

Table 2. Titanium(IV) reductive amination reaction of 3β-acetyl-7-ketocholestane **5** with various diamines



Entry[a]	Product	Amine	Yield[b] (%)	de[c] (%)
1	**6b**	Ammonia	6	>95
2	**6c**	Methylamine	77	>95
3	**6a**	1,2-Diaminoethane	61	>95
4	**6d**	1,3-Diaminopropane	35	>95
5	**6e**	Putrescine	21	>95
6	**6f**	Cadaverine	42	>95
7	**6g**	1,6-Diaminohexane	33	>95
8	**6h**	1,7-Diaminoheptane	37	>95
9	**6i**	1,8-Diaminooctane	34	>95
10	**6j**	1,9-Diaminononane	39	>95
11	**6k**	1,10-Diaminodecane	25	>95
12	**6l**	1,12-Diaminododecane	44	>95
13	**6m**	Spermine	45	>95

[a] Reaction performed at −78 °C for 12 h in MeOH on a 0.39 mmol scale of 3β-acetoxy-7-ketocholestane **5** in the presence of Ti(O*i*-Pr)$_4$ (0.51 mmol) and the desired amine (1.17 mmol).
[b] Isolated overall yield.
[c] Diastereomeric excesses were evaluated by ^{1}H and ^{13}C NMR analysis. In all cases, the 7β-diastereomer has been obtained as the major compound.

Table 3. Antimicrobial activities of squalamine analogues **6a–6m**

Sample CIP	Antimicrobial activity (MIC), μg/mL				
	S. cerevisiae (28383)	*C. albicans* (1180-79)	*S. aureus* (4.83)	*E. faecalis* (103015)	*E. coli* (54127)
Squalamine **1**	25	8	2	12.5	2
6b	>100	>100	>100	>100	>100
6c	2.5	>100	2.5	2.5	>100
6a	2.5	>100	3.12	5	5
6d	1.25	10	2.5	2.5	2.5
6e	1.25	5	2.5	2.5	2.5
6f	2.5	6.25	5	5	5
6g	2.5	>100	5	5	5
6h	2.5	>100	10	5	5
6i	2.5	25	2.5	2.5	6.25
6j	5	>100	3.12	12.5	12.5
6k	5	>100	3.12	5	20
6l	25	>100	10	6.25	50
6m	10	>100	5	5	10

4. Experimental section

4.1. General

All solvents were purified according to reported procedures, and reagents were used as commercially available. Methanol, ethyl acetate, dichloromethane, ammonia, and petroleum ether (35–60 °C) were purchased from SDS and used without further purification. Column chromatography was performed on SDS silica gel (70–230 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AC 300 spectrometer working at 300 and 75 MHz, respectively (the usual abbreviations are used: s: singlet, d: doublet, t: triplet, q: quadruplet, m: multiplet). Tetramethylsilane was used as internal standard. All chemical shifts are given in parts per million. Cholesteryl-3β-acetate **4** has been prepared from cholesterol according to well known procedures. Purity of all the new compounds is up to 99% and has been evaluated by HPLC analysis.

4.2. Synthesis of 7-keto-5α-cholesteryl-3β-acetate

Cholesteryl-3β-acetate **4** (10 g, 0.023 mol) and *N*-hydroxyphthalamide (3.73 g, 0.025 mol) were dissolved in EtOAc/acetone (600 mL, 1:1 v/v) in a 2 L glass reactor equipped with a condenser and a mechanical stirrer. Benzoyl peroxide (1 g) was added at 50–60 °C. Air was bubbled into the reaction solution and stirring was maintained for 36 h at 50–60 °C. Additional 50:50 EtOAc/acetone was added to the reaction as needed to replenish what was lost due to air flow through the system. The reaction was followed by TLC on silica gel (EtOAc/petroleum ether, 1:6) and judged to be completed after 36 h. After evaporation of all the solvents in vacuo, dichloromethane (100 mL) was added and a yellow precipitate is filtered off. The organic layer is concentrated under vacuum and the residue dissolved in pyridine (200 mL). The pyridine solution was cooled to 0 °C and 15 mL of acetic anhydride added. The solution was stirred overnight allowing the solution to warm to room

temperature. After concentration under vacuum, the oily residue is dissolved in dichloromethane and this organic layer was washed with saturated $CuSO_4$ solution until no trace of pyridine was observed. The organic layer is dried over Na_2SO_4 and concentrated in vacuo. The oily residue was purified by chromatography on a silica gel column using EtOAc/petroleum ether as eluent (1:6) affording the expected 7-ketocholesterylacetate in 52% yield, white solid. ^{1}H NMR: δ=5.72 (s, 1H), 4.73 (m, 1H), 2.55–0.69 (m, 44H). ^{13}C: δ=202.31, 170.66, 164.22, 127.11, 72.60, 55.17, 50.35, 45.81, 43.50, 39.87, 39.24, 38.70, 38.26, 28.93, 28.38, 27.75, 26.35, 24.22, 23.20, 22.94, 21.65, 19.25, 17.65, 12.36. $C_{30}H_{50}O_3$ calcd C 78.55, H 10.99; found C 78.50, H 11.01.

4.3. Synthesis of 3β-acetoxy-7-keto-5α-cholestane 5

A solution of 7-keto-5α-cholesteryl-3β-acetate (1.2 g, 2.24×10^{-5} mol) in ethyl acetate (10 mL) was stirred at room temperature in the presence of platinum oxide (30 mg) under an atmosphere of H_2 (3 bar) for 24 h. The reaction mixture was filtered over Celite, the solvents removed under reduced pressure, and the crude residue purified by chromatography on a silica gel column (eluent: petroleum ether/ethyl acetate 9:1 to 8:2) affording the expected compound **5** as a white powder in 95% yield (1.1 g), white solid. ^{1}H NMR: δ=4.62–4.52 (m, 1H), 2.28–0.56 (m, 47H). ^{13}C: δ=211.39, 170.32, 72.65, 54.87, 49.83, 48.76, 46.36, 45.74, 42.38, 39.35, 38.58, 36.02, 35.82, 35.71, 35.54, 33.72, 28.29, 27.86, 26.98, 34.87, 23.66, 22.70, 22.46, 21.67, 21.21, 18.68, 11.94, 11.58. $C_{30}H_{52}O_3$ calcd C 78.21, H 11.38; found C 78.30, H 12.01.

4.4. General procedure for the titanium-mediated reductive amination reaction of 6a

A mixture of 3β-acetoxy-7-keto-5α-cholestane **5** (173 mg, 0.39 mmol), titanium(IV) isopropoxide (151 μL, 0.51 mmol), and 1,2-diaminoethane (70 mg, 1.17 mmol) in absolute methanol (5 mL) was stirred under argon at room temperature for 12 h. Sodium borohydride (15 mg, 0.39 mmol) was then added at −78 °C and the resulting mixture was stirred for an additional 2 h. The reaction was then quenched by adding water (1 mL) and stirring was maintained at room temperature for 20 min. The resulting inorganic precipitate was filtered off over a pad of Celite and washed with Et_2O and ethylacetate. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo to afford the expected crude amino acetate derivative, which was subsequently deprotected by treatment with 4 equiv of K_2CO_3 (215 mg, 1.56 mmol) in 10 mL of a 1:1 MeOH/$CHCl_3$ solvent mixture. The resulting mixture was stirred for 12 h, then quenched by adding water (15 mL) and stirring was maintained at room temperature for 20 min. The resulting solution was filtered over a pad of Celite and washed with Et_2O and ethylacetate. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was purified by flash chromatography affording the expected amino derivative.

4.4.1. 7β-(1,2-Diaminoethane)-3β-hydroxycholestane 6a. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 61% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=4.62 (s, 1H), 3.66–3.62 (m, 1H), 2.82–0.82 (m, 55H), 0.65 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=71.03, 56.13, 54.74, 50.63, 49.81, 46.18, 42.66, 41.80, 39.46, 39.06, 37.87, 36.88, 36.82, 36.11, 35.82, 35.72, 31.96, 31.34, 28.07, 27.98, 23.80, 23.63, 22.79, 22.52, 21.13, 18.61, 11.74, 11.66. MS (ESI$^+$) *m*/*z* 447.3 (100%, [M+H]$^+$). $C_{30}H_{58}N_2O$ calcd C 77.86, H 12.63, N 6.05; found C 77.80, H 12.59, N 6.08.

4.4.2. 7β-Amino-3β-hydroxycholestane 6b. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 6% yield. ^{13}C NMR (75 MHz, $CDCl_3$): δ=71.18, 68.03, 56.10, 50.58, 45.88, 42.65, 39.49, 37.72, 37.06, 36.73, 36.13, 35.76, 35.55, 31.92, 31.60, 31.41, 29.68, 29.64, 28.18, 27.99, 23.73, 22.79, 22.54, 20.99, 18.64, 11.83, 11.23. MS (ESI$^+$) *m*/*z* 404.3 (100%, [M+H]$^+$). $C_{28}H_{53}NO$ calcd C 80.12, H 12.73, N 3.34; found C 80.11, H 12.57, N 6.04.

4.4.3. 7β-Methylamino-3β-hydroxycholestane 6c. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 77% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.65–3.57 (m, 1H), 2.47–2.46 (m, 1H), 2.00–0.62 (m, 49H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=71.05, 56.90, 56.07, 53.37, 50.66, 46.24, 42.67, 39.47, 39.01, 38.00, 36.84, 36.08, 35.82, 35.67, 34.83, 31.37, 31.00, 28.04, 27.96, 23.66, 23.56, 22.76, 22.52, 21.13, 18.59, 11.69, 11.63. MS (ESI$^+$) *m*/*z* 418.4 (100%, [M+H]$^+$). $C_{29}H_{55}NO$ calcd C 80.30, H 12.78, N 3.23; found C 80.20, H 12.82, N 3.20.

4.4.4. 7β-(1,3-Diaminopropane)-3β-hydroxycholestane 6d. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 35% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.63–3.58 (m, 1H), 2.87–0.62 (m, 55H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=70.93, 56.10, 55.29, 50.58, 46.61, 46.12, 42.68, 41.01, 39.44, 39.35, 38.90, 37.88, 36.84, 36.80, 36.10, 35.80, 35.72, 32.58, 31.50, 31.34, 28.04, 27.96, 23.81, 23.63, 22.78, 22.50, 21.10, 18.54, 11.72, 11.64. MS (ESI$^+$) *m*/*z* 461.5 (100%, [M+H]$^+$). $C_{31}H_{60}N_2O$ calcd C 78.09, H 12.68, N 5.87; found C 78.01, H 12.59, N 5.91.

4.4.5. 7β-(1,4-Diaminobutane)- 3β-hydroxycholestane 6e. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 21% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=4.25–4.11 (m, 1H), 3.62–2.47 (m, 10H), 2.15–0.63 (m, 47H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=70.91, 68.13, 56.06, 55.81, 50.33, 48.28, 46.21, 42.82, 39.48, 39.27, 38.86, 38.69, 37.44, 36.75, 36.12, 35.80, 35.73, 31.77, 31.25, 31.33, 28.89, 27.99, 23.83, 23.77, 23.71, 22.95, 22.81, 22.54, 18.61, 11.73, 11.66. MS (ESI$^+$) *m*/*z* 475.4 (100%, [M+H]$^+$). $C_{32}H_{62}N_2O$ calcd C 78.30, H 12.73, N 5.71; found C 78.32, H 12.70, N 5.73.

4.4.6. 7β-(1,5-Diaminopentane)-3β-hydroxycholestane 6f. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 42% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.59–3.57 (m, 1H), 2.71–0.61 (m, 59H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=71.05, 56.11, 56.02, 54.74, 50.57,

50.41, 47.84, 46.06, 42.69, 41.76, 39.45, 39.03, 37.91, 36.82, 36.68, 36.10, 35.81, 35.71, 32.79, 31.79, 31.32, 29.94, 27.96, 34.60, 23.77, 23.60, 22.76, 22.49, 21.11, 18.59, 11.70, 11.65. MS (ESI$^+$) *m/z* 489.5 (100%, [M+H]$^+$). $C_{33}H_{64}N_2O$ calcd C 78.51, H 12.78, N 5.55; found C 78.51, H 12.79, N 6.10.

4.4.7. 7β-(1,6-Diaminohexane)-3β-hydroxycholestane 6g. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 33% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=4.70–4.18 (m, 1H), 3.62–3.50 (m, 1H), 2.72–0.62 (m, 60H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=71.06, 56.13, 54.85, 50.54, 47.85, 46.06, 42.71, 41.75, 39.46, 39.03, 36.70, 36.12, 35.81, 35.73, 32.74, 31.75, 31.33, 29.97, 28.87, 28.09, 27.98, 27.16, 26.60, 23.82, 23.59, 22.78, 22.52, 21.43, 21.13, 18.60, 11.72, 11.68. MS (ESI$^+$) *m/z* 503.6 (100%, [M+H]$^+$). $C_{34}H_{66}N_2O$ calcd C 78.70, H 12.82, N 5.40; found C 78.71, H 12.84, N 5.44.

4.4.8. 7β-(1,7-Diaminoheptane)-3β-hydroxycholestane 6h. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 37% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.62–3.55 (m, 1H), 2.67–0.61 (m, 63H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=70.97, 66.13, 59.66, 50.66, 47.89, 46.14, 42.67, 42.14, 39.46, 39.09, 38.12, 36.90, 36.73, 36.11, 35.83, 35.71, 33.56, 31.96, 31.39, 30.32, 29.29, 28.07, 27.87, 27.37, 26.90, 26.79, 23.76, 23.57, 22.77, 22.51, 21.13, 18.60, 11.72, 11.68. MS (ESI$^+$) *m/z* 517.5 (100%, [M+H]$^+$). $C_{35}H_{68}N_2O$ calcd C 78.86, H 12.86, N 5.26; found C 78.87, H 12.92, N 5.26.

4.4.9. 7β-(1,8-Diaminooctane)-3β-hydroxycholestane 6i. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 34% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.62–3.55 (m, 1H), 2.68–0.61 (m, 65H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=70.96, 56.12, 54.68, 50.61, 50.34, 47.76, 46.09, 42.68, 41.96, 39.46, 39.08, 38.09, 36.86, 36.69, 36.12, 35.83, 35.71, 33.32, 31.89, 31.35, 30.15, 29.45, 29.27, 28.07, 27.96, 27.27, 26.78, 23.77, 23.58, 22.76, 22.51, 21.13, 18.60, 11.71, 11.67. MS (ESI$^+$) *m/z* 531.5 (100%, [M+H]$^+$). $C_{36}H_{70}N_2O$ calcd C 79.05, H 12.90, N 5.12; found C 79.25, H 12.62, N 5.14.

4.4.10. 7β-(1,10-Diaminononane)-3β-hydroxycholestane 6j. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH(32%), 7:3:1) afforded a pale yellow viscous oil in 39% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.63–3.56 (m, 1H), 2.70–0.62 (m, 67H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=70.86, 56.15, 54.70, 50.64, 47.89, 46.09, 42.69, 41.79, 39.48, 39.11, 38.10, 36.86, 36.74, 36.14, 35.84, 35.73, 32.90, 31.98, 31.45, 30.25, 29.52, 29.33, 28.09, 27.98, 27.33, 26.76, 23.79, 23.61, 22.79, 22.52, 21.14, 18.62, 11.73, 11.68. MS (ESI$^+$) *m/z* 545.4 (100%, [M+H]$^+$). $C_{37}H_{72}N_2O$ calcd C 79.22, H 12.94, N 4.99; found C 79.25, H 12.92, N 5.02.

4.4.11. 7β-(1,10-Diaminodecane)-3β-hydroxycholestane 6k. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 25% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.69–0.63 (m, 70H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=71.06, 56.15, 54.67, 51.77, 50.65, 47.96, 46.09, 42.71, 42.10, 39.49, 39.12, 36.84, 36.74, 36.15, 35.86, 35.75, 33.46, 31.94, 31.50, 30.27, 29.80, 29.54, 29.48, 29.40, 28.11, 28.00, 27.40, 26.80, 23.80, 22.80, 22.54, 21.16, 18.63, 11.74, 11.69. MS (ESI$^+$) *m/z* 559.5 (100%, [M+H]$^+$). $C_{38}H_{74}N_2O$ calcd C 79.37, H 12.97, N 4.87; found C 79.37, H 12.92, N 4.82.

4.4.12. 7β-(1,12-Diaminododecane)-3β-hydroxycholestane 6l. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH(32%), 7:3:1) afforded a pale yellow viscous oil in 44% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.62–0.57 (m, 74H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=70.55, 56.02, 54.54, 50.54, 49.64, 47.96, 45.99, 42.58, 42.02, 39.38, 39.00, 37.96, 36.76, 36.63, 36.02, 35.75, 35.63, 33.58, 31.81, 31.40, 30.21, 29.44, 29.34, 27.99, 27.87, 27.38, 26.73, 23.67, 23.50, 22.68, 22.42, 21.03, 18.51, 11.62, 11.56. MS (ESI$^+$) *m/z* 587.5 (100%, [M+H]$^+$). $C_{40}H_{78}N_2O$ calcd C 79.67, H 13.04, N 4.65; found C 79.64, H 13.12, N 4.59.

4.4.13. 7β-(Spermino)-3β-hydroxycholestane 6m. Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 45% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.51–0.59 (m, 72H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=70.38, 56.07, 54.76, 50.72, 49.99, 49.81, 48.87, 47.73, 46.71, 46.15, 42.51, 40.36, 39.32, 38.93, 38.05, 36.90, 36.81, 35.99, 35.74, 35.57, 33.42, 31.76, 31.34, 30.17, 27.93, 27.83, 23.66, 23.45, 22.65, 22.39, 21.01, 18.48, 11.60, 11.56. MS (ESI$^+$) *m/z* 589.7 (100%, [M+H]$^+$). $C_{38}H_{76}N_4O$ calcd C 75.43, H 12.66, N 9.26; found C 75.39, H 12.59, N 9.20.

4.5. Determination of minimal inhibitory concentrations

Antimicrobial activity of the compounds was studied by determination of minimal inhibitory concentration (MIC) according to the NCCLS guidelines M7-A2 using the microbroth dilution methods. All the strains were issued from the Institut Pasteur collection (Paris). The yeast was grown overnight at 28 °C (*S. cerevisiae* CIP 28383) or 37 °C (*C. albicans* CIP 1180–79) in YPD broth. The bacterial strains were grown on trypticase soy agar (Becton Dickinson) at 37 °C for 24 h (*E. coli* CIP 54127, *S. aureus* CIP 4.83, *E. faecalis* CIP103015) in LB broth for *E. coli* and *S. aureus* or BHI broth for *S. faecalis*. Inocula were prepared in TCE (tryptone 0.1%, NaCl 8%, wt/vol) by adjusting the turbidity at 623 nm to obtain 1–3$\times10^5$ CFU/mL.

Antimicrobial activities of the compounds were determined by using a broth microdilution method performed in sterile 96-well microplates. All compounds were solubilized in methanol at a concentration of 5 mg/mL and were transferred to each microplate well (in all cases concentrations of the desired molecules in methanol do not exceed 2% of the total proportion), in order to obtain a 2-fold serial dilution in 100 μL of broth and 100 μL of inocula containing 2–6$\times10^5$ CFU of each bacteria and yeast were added to each well. A number of wells were reserved for positive controls, inoculum viability, and solvent effect. After 24 or 48 h incubation, growth was assayed by absorbance measurement at 623 nm with an IEMS Labsystem automatic plate reader.

MIC was defined for each agent from duplicate observations as the lowest concentration of compound allowing no visible growth.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.10.032.

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