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Original article

New stereoselective titanium reductive amination synthesis of 3-amino and polyaminosterol derivatives possessing antimicrobial activities

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Abstract

A series of 3-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. Activity was highly dependent on the different compounds' structures involved and best results have been obtained with aminosterol derivatives **4b**, **4e** and **6i** exhibiting activities against yeasts, Gram positive and Gram negative bacteria at average concentrations of 6.25–12.5 μg/mL.

Keywords: Squalamine; Trodusquemine; Aminosterol derivatives; Antifungal activity; Antibacterial activity; Reductive amination

1. Introduction

With the extensive use of antibiotics, there is a growing need for a novel class of antibacterial agents due to infections by increasingly common multi-drug resistant bacterial strains. In recent years, a wide variety of low molecular weight antibiotics including peptides, lipids and alkaloids have been synthesized or isolated from diverse animal species [1–5]. Among these substances, two water soluble cationic sterols, squalamine **1** and trodusquemine **2** have been isolated from the dogfish shark *Squalus acanthias* exhibiting potent antimicrobial and antiangiogenic activities (Fig. 1) [6–13]. These natural amphiphilic steroids are 7-24-dihydroxylated-24-sulfated cholestane conjugated to spermine or spermidine at C-3.

At present, feasibility of obtaining large quantities of steroidal antibiotic from natural sources appears questionable since only trace amounts of **1** and **2** are present in the shark liver and gallbladder. 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 antimicrobial properties. Recently, we [14] and others [15–18] synthesized and evaluated some aminosterol derivatives exhibiting interesting antimicrobial activities [19–21]. Nevertheless, all these syntheses were performed in numerous steps and overall chemical yield was low. Reductive amination of carbonyl compounds is a very important and powerful tool for chemists to target the synthesis of primary, secondary or tertiary amines. Recently, we described an efficient method for the synthesis of various secondary amines through a titanium(IV)isopropoxide-mediated reductive amination reaction of ketones and its extension to the diastereoselective synthesis of chiral amines from various prochiral aliphatic and aromatic ketones leading to the expected products with moderate to excellent yields and diastereoselectivities up to 100% in some cases [22,23]. In the frame of our work on biologically active aminosterol derivatives [21], we report herein the development of a stereoselective titanium

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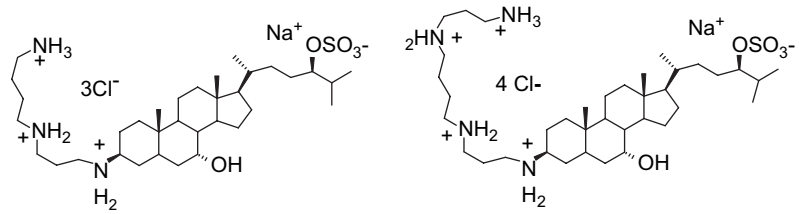


Fig. 1. Structure of squalamine **1** and trodusquemine **2**.

reductive amination procedure for the synthesis of various new 3-amino or polyaminosterol derivatives in one step synthesis and the evaluation of their antimicrobial activities. In this context, even if squalamine possesses three different functional groups, we have exclusively focused our studies on a systematic structure–activity relationships of the C-3-polyamine moiety in the absence of hydroxy and sulfate groups.

2. Results and discussion

Classical reductive amination procedures including for example activation of the keto derivative by using molecular sieves have felt in a first approach and led to the use of a Lewis acid such as titanium isopropoxide as an activation agent. Initial experiments for the titanium(IV) reductive amination reaction were performed using 4-cholesten-3-one **1** and methylamine as test substrates under various experimental conditions.

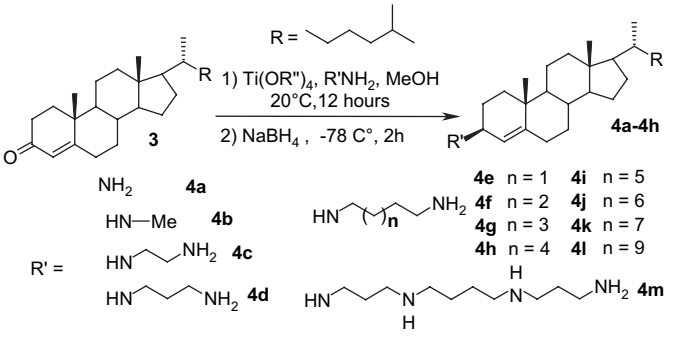
As demonstrated in our previous studies [22,23], polar solvent such as methanol has been chosen as the best solvent for the reaction leading, in this case, to the formation of the expected amine derivative **4b** in 75% yield (Table 1, entry 1). Influence of the nature of titanium source involved was also taken into account and chemical yields' variations from 50 to 84% were encountered (Table 1, entries 3–5). Moreover, lowering temperature from 20 °C to −78 °C led to a significant increase of the isolated yield and diastereoselectivity of the reaction from 60 to up to 95% de (Table 1, entries 1–2). Due to it's low price and easiness to handle, Ti(O*i*-Pr)$_4$ was used in our following investigations. Under the best experimental conditions (Table 1, entry 1), we have investigated the use of different amines or polyamines. Whatever be the considered amine, the expected product was obtained in chemical yields varying from 50 to 93% and a diastereoselectivity of up to 95% de was measured. Thus, 1,2-diaminoethane or spermine, for example, afforded the desired product in moderate to excellent yield with excellent purity, demonstrating the generality of this reaction since mono, diamino or polyamino compounds are involved (Table 1, entries 6, 7, 17) even if ammonia does not lead to the expected derivative (**4a**).

Due to the nucleophilic property of amines, a plausible mechanism has been proposed including a nucleophilic attack of the amino group to a Lewis acid activated carbonyl compound [24,25] (Scheme 1).

In this case, the reaction may proceed through the titanium complex **A** which could be either reduced directly or via a transient imine species **B** [26]. A transition state model is proposed to account for the reaction stereoinduction leading to the formation of the 3β-amino or polyamino derivative (Scheme 2).

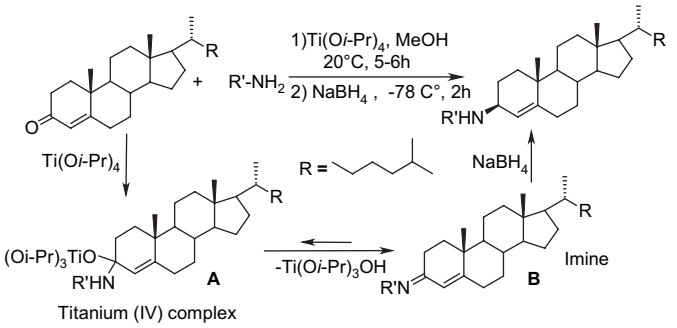
In this favorable transition state assembly and due to steric hindrance, the hydrogen hydride attack occurs from the α-side at the C-3 carbon generating principally the 3β-amino allylic cholesteryl derivative **4**. Thus, this transition state allows us to justify that the formation of the 3α-parent derivative is unfavoured at low temperature. In the case of total synthesis of squalamine [27] or numerous spermine analogues [28] of this natural product, introduction of the polyamino spermine

Table 1
Titanium(IV) reductive amination reaction of 4-cholesten-3-one **3** under various experimental conditions



Entry[a]	Product	Titanium source	Amine	Yield (%)[b]
1	**4b**	Ti(O*i*-Pr)$_4$	Methylamine	75
2	**4b**	Ti(O*i*-Pr)$_4$	Methylamine	50[c]
3	**4b**	Ti(OEt)$_4$	Methylamine	84
4	**4b**	Ti(OBu)$_4$	Methylamine	50
5	**4b**	Ti(O*t*-Bu)$_4$	Methylamine	64
6	**4a**	Ti(O*i*-Pr)$_4$	Ammonia	–
7	**4c**	Ti(O*i*-Pr)$_4$	1,2-Diaminoethane	60
8	**4d**	Ti(O*i*-Pr)$_4$	1,3-Diaminopropane	50
9	**4e**	Ti(O*i*-Pr)$_4$	Putrescine	50
10	**4f**	Ti(O*i*-Pr)$_4$	Cadaverine	74
11	**4g**	Ti(O*i*-Pr)$_4$	1,6-Diaminohexane	86
12	**4h**	Ti(O*i*-Pr)$_4$	1,7-Diaminoheptane	90
13	**4i**	Ti(O*i*-Pr)$_4$	1,8-Diaminooctane	78
14	**4j**	Ti(O*i*-Pr)$_4$	1,9-Diaminononane	93
15	**4k**	Ti(O*i*-Pr)$_4$	1,10-Diaminodecane	89
16	**4l**	Ti(O*i*-Pr)$_4$	1,12-Diaminododecane	94
17	**4m**	Ti(O*i*-Pr)$_4$	Spermine	52

[a] Reaction performed at 20 °C for 12 h in MeOH on a 0.78 mmol scale of 4-cholesten-3-one **3** in the presence of Ti(O*i*-Pr)$_4$ (1.03 mmol) and the desired amine (2.34 mmol).
[b] Isolated yield.
[c] Diastereomeric excesses was evaluated in this case to be 63% de.



Scheme 1. Mechanistic rationale for the titanium(IV) reductive amination reaction.

moiety has always been performed involving a stereoselective reduction of an imine derivative intermediate with sodium borohydride at −78 °C and in all cases the major diastereomer encountered possesses a 3β configuration (diastereomeric ratio α/β 14:86, respectively) correlating our own experimental results [29,30].

This methodology has been successfully applied to the synthesis of two other classes of aminosterol derivatives from cholestan-3-one **5** and 5-cholesten-3-one **7** in a one step synthesis. All compounds were obtained in moderate to excellent yields varying from 45 to 96% and always with a diastereoselectivity affording the major β-amino derivatives (Scheme 3).

All the synthesized compounds were screened for antimicrobial activity against several yeast strains as well as Gram positive and Gram negative bacteria strains (Table 2) [31].

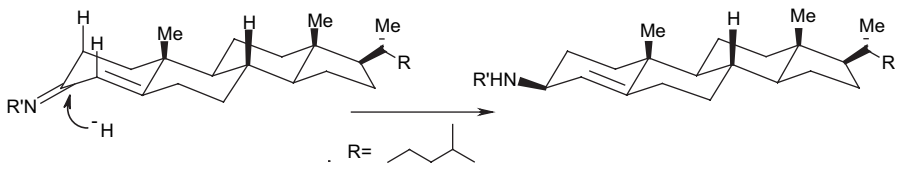
Nine out of 27 novel compounds tested in the present study were found to have no activity against the microorganisms and are listed in Table 2. Results of the remaining 18 compounds showed that they have important antibacterial activities against Gram positive bacteria such as *Staphylococcus aureus* and *Enterococcus feacalis* with an average MIC of 3.125–6.25 μg/mL in numerous cases (Table 2, compounds **4e**, **6b**, **6f**, **6i**, **8d**, **8e**). On the other hand, only five compounds (**4e**, **4m**, **8c**–**8e**) possess MICs against Gram negative *Escherichia coli* bacteria (MIC varying from 6.25 to 50 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. Nevertheless, to date, no pertinent explanation can be rationalized in order to explain these results, but some researches are underway and will be reported in due course. Moreover, five products (**4b**, **4e**, **6b**, **6i**, **8b**) possess pertinent antifungal activities against *Saccharomyces cerevisiae* and *Candida albicans* with antimicrobial activities varying from 3.125 to 50 μg/mL in the best cases. Thus, compound **4e**, bearing a putrescine moiety, presents the best antimicrobial activities on all the tested strains and at present, we are investigating the possibility for this product to be an inhibitor of spermidine synthase. Furthermore, results obtained from trodusquemine analogues **4m**, **6i**, **8i** clearly suggest that the antimicrobial activities measured are very dependent on the sterol derivative structure. Thus, presence of a double bond in the derivative structure at Δ4–5 or Δ5–6 position led to inactive products towards yeasts and bacteria (Table 2, compounds **4m** and **8i**). On the other hand, the saturated derivative **6i** differing only from trodusquemine **2** by the absence of hydroxy and sulfate groups led to similar antimicrobial activities against yeasts and Gram positive bacteria, whereas no antibacterial activity against *E. coli* was noticed, suggesting the importance of these two functional groups in the trodusquemine mechanism of action. Current studies are underway to demonstrate importance of the presence of such functional groups and their involvements in the activity of the considered derivatives.

3. Conclusion

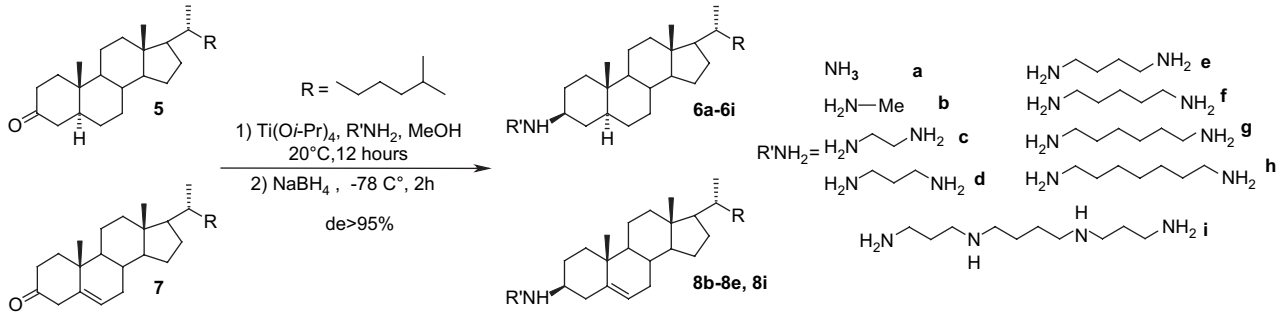
In conclusion, an efficient stereoselective titanium reductive amination reaction was involved for the synthesis of a series of 3-amino and polyaminosterol analogues of squalamine and trodusquemine in high chemical yields of up to 95% in numerous cases. Several derivatives presenting interesting antimicrobial activities on various different strains will be the subject of a thorough examination and the results will be reported in due course.

4. Experimental section

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 300.00 MHz 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 ppm. All the products were prepared from well known ketones prepared according to classical methodology. Cholestan-3-one **5** is purchased from Sigma and 5-cholesten-3-one **7** has been prepared according to the previously reported method [32].



Scheme 2. Transition state model accounting for the stereoinduction in the titanium(IV) reductive amination reaction.



Compound[a]	6a	6b	6c	6d	6e	6f	6g	6h	6i	8b	8c	8d	8e	8i
Yield (%)[b]	45	70	95	77	91	54	60	48	95	58	63	96	58	80

[a]Reactions performed at 20ºC for 12 h in MeOH on a 0.78 mmol scale of ketone **5** or **7** in the presence of Ti(O*i*-Pr)$_4$ (1.03 mmol) and the desired amine (2.34 mmol). [b]Isolated yields.

Scheme 3. Titanium(IV) Reductive Amination Reaction of Cholestan-3-one **5** and 5-Cholesten-3-one **7** using Various amines.

*4.1. General Procedure for the titanium-mediated reductive amination reaction of **4b***

A mixture of 4-cholesten-3-one **3** (300 mg, 0.78 mmol), titanium(IV)isopropoxide (302 μL, 1.03 mmol) and methylamine (854 μL, 2 M methanol, 2.34 mmol) in absolute methanol (5 mL) was stirred under argon at room temperature for 12 h. Sodium borohydride (29 mg, 0.78 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 amine **4b** which was purified by flash chromatography on silicagel.

Table 2
Antimicrobial activities of aminosterol derivatives **4b–4m**, **6a–6i**, **8b–8e**, **8i**

Sample CIP	Antimicrobial activity (MIC), μg/mL				
	S. cerevisiae (28383)	*C. albicans* (1180-79)	*S. aureus* (4.83)	*E. feacalis* (103015)	*E. coli* (54127)
Squalamine **1**[a]	50	>100	<3.125	12.5	1.56
Trodusquemine **2**[b]	–	4	1	–	4
4b	12.5	12.5	1.56	25	>50
4c	>50	>50	12.5	>50	>50
4d	>50	25	12.5	>50	>50
4e	12.5	6.25	6.25	6.25	25
4f	>50	>50	6.25	6.25	>50
4g	>50	>50	>50	>50	>50
4h	>50	>50	>50	>50	>50
4i	>50	>50	>50	>50	>50
4j	>50	>50	>50	>50	>50
4k	>50	>50	>50	>50	>50
4l	>50	>50	>50	>50	>50
4m	>50	50	6.25	>50	50
6a	>50	50	>50	50	>50
6b	12.5	12.5	6.25	6.25	>50
6c	50	>50	12.5	>50	>50
6d	>50	>50	>50	>50	>50
6e	50	25	12.5	>50	>50
6f	25	50	6.25	50	>50
6g	>50	>50	>50	>50	>50
6h	>50	>50	>50	>50	>50
6i	6.25	50	3.125	12.5	>50
8b	3.125	>50	6.25	6.25	>50
8c	>50	>50	12.5	25	12.5
8d	>50	>50	6.25	6.25	6.25
8e	>50	>50	1.56	6.25	6.25
8i	>50	>50	6.25	>50	>50

[a] Antimicrobial activities measured in our laboratory.
[b] Antimicrobial activities obtained from literature (Ref. [13]).

*4.1.1. 3β-Methylamino-4-cholestene **4b***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 75% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.22$ (s, 1H), 2.96 (s, 1H), 0.64–2.41 (m, 47H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.61$, 121.68, 56.15, 56.06, 54.61, 42.44, 39.88, 39.46, 37.51, 36.32, 36.12, 35.93, 35.73, 33.14, 33.07, 32.44, 28.16, 27.95, 26.52, 24.19, 23.80, 22.76, 22.51, 21.13, 19.08, 18.61, 11.93. MS (ESI$^+$) *m/z* 400.1 (100%, $[M+H]^+$).

*4.1.2. 3β-(1,2-Diaminoethane)-4-cholestene **4c***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 60% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.14$ (s, 1H), 3.29–2.62 (m, 5H), 2.32–0.55 (m, 46H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.32$, 122.05, 56.03, 54.45, 49.37, 48.82, 42.27, 41.80, 39.72, 39.30, 37.30, 36.24, 35.97, 35.76, 35.59, 33.00, 32.28, 28.01, 27.78, 26.92, 24.04, 23.67, 22.63, 22.37, 20.97, 18.92, 18.47, 11.78. MS (ESI$^+$) *m/z* 429.0 (100%, $[M+H]^+$).

*4.1.3. 3β-(1,3-Diaminopropane)-4-cholestene **4d***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 50% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.84$–0.60 (m, 55H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 147.30$, 123.63, 56.07, 54.48, 53.72, 42.37, 39.79, 39.39, 38.49, 37.40, 36.04, 35.84, 35.67, 35.59, 33.86, 33.03, 32.38,

28.09, 27.89, 24.13, 24.07, 23.72, 22.71, 22.46, 21.05, 20.92, 19.00, 18.55, 15.17, 11.86. MS (ESI^+) *m/z* 443.5 (100%, $[M+H]^+$).

4.1.4. 3β-(1,4-Diaminobutane)-4-cholestene ***4e***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 50% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.46-5.20$ (m, 1H), 3.57–2.45 (m, 11H), 2.13–0.64 (m, 45H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.63$, 121.67, 56.09, 54.53, 54.48, 54.18, 46.16, 42.43, 41.55, 39.78, 39.37, 37.40, 36.78, 36.28, 36.03, 35.83, 35.65, 33.05, 32.36, 30.81, 28.08, 27.87, 27.49, 24.10, 23.72, 22.69, 22.44, 21.03, 18.97, 18.54, 11.85. MS (ESI^+) *m/z* 457.4 (100%, $[M+H]^+$).

4.1.5. 3β-(1,5-Diaminopentane)-4-cholestene ***4f***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 74% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.50$ (s, 1H), 4.13–2.63 (m, 10H), 2.19–0.63 (m, 47H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.73$, 121.48, 56.07, 54.51, 42.42, 42.34, 39.77, 39.36, 37.38, 36.74, 36.02, 35.64, 33.03, 32.35, 30.12, 29.71, 28.07, 27.85, 26.55, 24.49, 24.09, 23.71, 23.07, 22.68, 22.43, 21.32, 21.02, 18.95, 18.52, 11.84. MS (ESI^+) *m/z* 471.5 (100%, $[M+H]^+$).

4.1.6. 3β-(1,6-Diaminohexane)-4-cholestene ***4g***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 86% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.20$ (s, 1H), 3.66–2.61 (m, 9H), 2.11–0.62 (m, 50H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 147.05$, 120.89, 56.03, 54.49, 45.97, 42.28, 41.28, 39.72, 39.32, 37.32, 36.13, 35.98, 35.76, 35.59, 32.95, 32.31, 32.06, 29.51, 29.16, 28.02, 27.80, 26.91, 26.47, 26.31, 26.12, 24.04, 23.67, 22.63, 22.38, 20.97, 18.89, 18.48, 11.80. MS (ESI^+) *m/z* 485.6 (100%, $[M+H]^+$).

4.1.7. 3β-(1,7-Diaminoheptane)-4-cholestene ***4h***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 90% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.21$ (s, 1H), 3.05–2.53 (m, 5H), 2.13–0.64 (m, 56H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.33$, 121.99, 56.07, 54.54, 46.57, 42.34, 41.91, 39.79, 39.37, 37.40, 36.32, 36.02, 35.83, 35.64, 33.32, 33.06, 32.35, 30.14, 29.23, 28.07, 27.85, 27.27, 26.89, 26.65, 24.09, 23.71, 22.68, 22.42, 21.03, 18.96, 18.52, 11.84. MS (ESI^+) *m/z* 499.6 (100%, $[M+H]^+$).

4.1.8. 3β-(1,8-Diaminooctane)-4-cholestene ***4i***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 78% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.17$ (s, 1H), 3.32 (s, 1H), 2.60 (m, 6H), 1.93–0.61 (m, 56H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 145.97$, 122.32, 56.03, 55.98, 54.54, 54.51, 54.29, 46.72, 42.29, 42.02, 39.75, 39.31, 37.36, 36.32, 35.97, 35.79, 35.58, 33.62, 33.03, 32.30, 30.33, 29.27, 28.01, 27.80, 27.24, 26.65, 24.04, 23.65, 22.62, 22.36, 20.98, 18.90, 18.47, 11.78. MS (ESI^+) *m/z* 513.6 (100%, $[M+H]^+$).

4.1.9. 3β-(1,9-Diaminononane)-4-cholestene ***4j***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 93% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.25$ (s, 1H), 3.36 (m, 1H), 2.65–2.60 (m, 5H), 2.38–0.62 (m, 59H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.87$, 122.16, 56.10, 54.58, 54.27, 46.71, 42.46, 42.38, 42.07, 39.40, 37.45, 36.84, 36.05, 35.67, 33.61, 32.39, 30.17, 29.75, 29.47, 29.32, 27.90, 27.27, 26.76, 24.13, 23.73, 22.71, 22.45, 21.59, 20.97, 19.00, 18.55, 11.93.

4.1.10. 3β-(1,10-Diaminodecane)-4-cholestene ***4k***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 89% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.14$ (s, 1H), 3.37–3.30 (m, 1H), 2.67–2.45 (m, 6H), 1.81–0.53 (m, 60H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 145.82$, 122.25, 55.97, 55.92, 54.40, 54.21, 46.66, 42.27, 41.91, 39.64, 39.21, 37.24, 36.70, 36.22, 35.87, 35.68, 35.48, 33.52, 32.94, 32.19, 30.18, 30.01, 29.28, 27.91, 27.69, 27.16, 26.97, 26.59, 23.94, 23.55, 22.52, 22.26, 21.37, 20.87, 18.80, 18.37, 14.97, 11.67. MS (ESI^+) *m/z* 541.5 (100%, $[M+H]^+$).

4.1.11. 3β-(1,12-Diaminododecane)-4-cholestene ***4l***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 94% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 2.63-2.57$ (m, 6H), 2.11–0.61 (m, 66H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.18$, 122.21, 56.07, 54.58, 46.82, 42.35, 42.05, 39.79, 39.37, 37.42, 36.83, 36.36, 36.03, 35.84, 35.64, 33.63, 33.08, 32.36, 30.15, 29.49, 29.37, 28.07, 27.96, 27.31, 26.76, 24.10, 23.75, 22.68, 22.43, 21.56, 21.04, 18.97, 11.84. MS (ESI^+) *m/z* 569.5 (100%, $[M+H]^+$).

4.1.12. 3β-(Spermino)-4-cholestene ***4m***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 52% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.27$ (s, 1H), 4.39 (s, 3H), 3.21 (s, 1H), 2.79–0.65 (m, 65H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 148.33$, 119.81, 56.17, 56.10, 54.57, 54.49, 53.38, 48.68, 47.98, 44.53, 42.44, 39.88, 39.46, 37.45, 36.12, 35.88, 35.74, 33.03, 32.46, 28.15, 27.96, 26.65, 25.60, 24.19, 23.81, 22.76, 22.51, 22.11, 19.07, 18.61, 11.93. MS (ESI^+) *m/z* 571.6 (100%, $[M+H]^+$).

4.1.13. 3β-Amino-cholestane ***6a***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 45% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 4.12-0.63$ (m, 49H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 56.24$, 53.79, 46.69, 46.25, 42.69, 42.57, 41.25, 40.23, 39.89,

39.49, 36.14, 35.77, 35.38, 34.11, 31.71, 29.99, 29.69, 28.22, 27.99, 25.46, 24.21, 23.81, 22.79, 22.54, 22.32, 18.64, 14.05, 12.05.

4.1.14. 3β-Methylamino-cholestane ***6b***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 70% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.22$ (s, 1H), 2.51 (s, 2H), 2.30–0.53 (m, 48H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 58.95$, 56.46, 56.24, 54.41, 45.23, 42.52, 39.99, 39.45, 37.30, 36.11, 35.91, 35.73, 35.43, 35.00, 33.14, 32.04, 28.80, 28.40, 28.17, 27.93, 24.13, 23.79, 22.74, 22.49, 21.08, 18.60, 12.25, 12.00. MS (ESI$^+$) *m/z* 402.4 (100%, $[M+H]^+$).

4.1.15. 3β-(1,2-Diaminoethane)-cholestane ***6c***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 95% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.63$–3.37 (m, 6H), 2.89–2.46 (m, 4H), 1.97–0.57 (m, 44H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 58.95$, 56.46, 56.24, 54.41, 45.23, 42.52, 39.99, 39.45, 37.30, 36.11, 35.91, 35.73, 35.43, 35.00, 33.14, 32.04, 28.80, 28.45, 28.17, 27.93, 24.13, 23.79, 22.74, 22.49, 21.08, 18.60, 12.25, 12.00. MS (ESI$^+$) *m/z* 431.4 (100%, $[M+H]^+$).

4.1.16. 3β-(1,3-Diaminopropane)-cholestane ***6d***

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, $CDCl_3$): $\delta = 3.37$–3.30 (m, 1H), 2.81–2.43 (m, 5H), 1.98–0.59 (m, 50H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 57.45$, 56.38, 56.15, 54.36, 45.25, 44.79, 42.43, 40.44, 39.91, 39.35, 38.88, 37.35, 36.03, 35.87, 35.64, 35.36, 33.99, 31.98, 29.20, 28.75, 28.09, 27.83, 24.05, 23.69, 22.66, 22.41, 21.00, 18.52, 12.21, 11.91. MS (ESI$^+$) *m/z* 445.5 (100%, $[M+H]^+$).

4.1.17. 3β-(1,4-Diaminobutane)-cholestane ***6e***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 91% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.72$–0.57 (m, 58H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 57.37$, 56.46, 56.23, 54.42, 52.23, 51.70, 47.22, 46.66, 45.31, 42.52, 41.88, 39.99, 39.67, 39.43, 37.39, 36.11, 35.94, 35.72, 35.43, 32.62, 32.05, 31.39, 29.03, 28.81, 28.17, 27.92, 27.75, 24.12, 23.77, 22.74, 22.49, 21.07, 20.71, 18.60, 12.28, 12.00, 11.48. MS (ESI$^+$) *m/z* 459.4 (100%, $[M+H]^+$).

4.1.18. 3β-(1,5-Diaminopentane)-cholestane ***6f***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 54% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.54$ (s, 5H), 2.84 (m, 3H), 1.98–0.65 (m, 52H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 57.15$, 56.21, 54.14, 47.08, 44.91, 42.51, 39.95, 39.43, 36.96, 36.10, 35.96, 35.70, 35.36, 31.88, 28.63, 28.16, 27.93, 26.03, 24.76, 24.11, 23.76, 22.74, 22.48, 21.05, 20.78, 18.60, 12.15, 12.00, 11.47. MS (ESI$^+$) *m/z* 473.4 (100%, $[M+H]^+$).

4.1.19. 3β-(1,6-Diaminohexane)-cholestane ***6g***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 60% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.67$–0.60 (m, 62H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 57.45$, 56.51, 56.28, 54.49, 51.25, 47.44, 46.91, 45.37, 42.56, 42.06, 40.04, 39.68, 39.46, 37.45, 36.14, 35.98, 35.74, 35.48, 33.61, 32.66, 32.09, 30.21, 29.13, 28.86, 28.19, 27.94, 27.25, 26.74, 24.15, 23.80, 22.75, 22.49, 21.10, 20.74, 18.62, 12.31, 12.02, 11.50. MS (ESI$^+$) *m/z* 487.6 (100%, $[M+H]^+$).

4.1.20. 3β-(1,7-Diaminoheptane)-cholestane ***6h***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 48% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.70$–0.58 (m, 64H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 57.36$, 56.43, 56.19, 54.41, 54.28, 52.10, 47.35, 46.90, 45.29, 42.47, 41.91, 39.96, 39.52, 39.40, 37.38, 36.06, 35.91, 35.67, 35.40, 33.45, 32.58, 32.03, 30.16, 29.25, 29.11, 28.79, 28.13, 27.87, 27.29, 26.68, 25.73, 24.09, 23.73, 22.70, 22.44, 21.03, 20.66, 18.56, 12.25, 11.96, 11.43. MS (ESI$^+$) *m/z* 499.6 (100%, $[M+H]^+$).

4.1.21. 3β-Spermino-cholestane ***6i***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 95% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.14$ (s, 6H), 3.52–3.45 (m, 2H), 2.96–0.65 (m, 64H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 64.13$, 62.23, 57.31, 56.47, 56.25, 54.37, 52.40, 45.67, 45.12, 42.55, 42.26, 39.99, 39.47, 37.56, 36.13, 35.73, 35.43, 33.03, 32.03, 30.48, 28.77, 28.20, 27.96, 26.98, 25.13, 24.15, 23.79, 22.77, 22.52, 21.10, 20.82, 18.63, 17.19, 12.28, 12.04. MS (ESI$^+$) *m/z* 573.6 (100%, $[M+H]^+$).

4.1.22. 3β-(Methylamino)-5-cholestene ***8b***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 58% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.25$ (s, 1H), 2.61 (d, 2H), 2.02–0.65 (m, 46H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.24$, 122.14, 56.08, 54.56, 46.69, 42.36, 42.05, 39.80, 39.38, 37.43, 36.03, 35.65, 33.58, 33.08, 32.36, 29.44, 29.30, 28.08, 27.87, 26.73, 24.11, 23.71, 22.68, 22.43, 21.04, 18.97, 18.53, 11.85. MS (ESI$^+$) *m/z* 400.6 (100%, $[M+H]^+$).

4.1.23. 3β-(1,2-Diaminoethane)-5-cholestene ***8c***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 63% yield; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.21$ (s, 1H), 4.15–2.69 (m, 6H), 2.08–0.64 (m, 45H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.58$, 122.17, 56.14, 54.61, 54.58, 49.17, 42.42, 42.07, 39.86, 39.45, 37.48, 36.38, 36.10,

35.91, 35.72, 33.14, 32.44, 28.15, 27.94, 27.11, 24.17, 23.78, 22.76, 22.50, 21.11, 19.06, 18.60, 11.91. MS (ESI^+) *m/z* 429.4 (100%, $[M+H]^+$).

4.1.24. 3β-(1,3-Diaminopropane)-5-cholestene ***8d***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 96% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.20$ (s, 1H), 4.31–2.72 (m, 12H), 2.13–0.63 (m, 41H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 147.04$, 121.35, 56.15, 56.12, 54.66, 54.55, 50.07, 44.34, 42.42, 40.24, 39.84, 39.43, 37.46, 36.26, 36.10, 35.88, 35.72, 33.10, 32.61, 32.42, 28.15, 27.93, 26.52, 24.17, 23.79, 22.75, 22.49, 21.09, 19.04, 18.60, 11.91. MS (ESI^+) *m/z* 443.5 (100%, $[M+H]^+$).

4.1.25. 3β-(1,4-Diaminobutane)-5-cholestene ***8e***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 58% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.20$ (s, 1H), 4.14–2.67 (m, 11H), 1.92–0.63 (m, 45H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.63$, 121.84, 56.13, 54.58, 46.32, 42.49, 41.79, 41.34, 39.83, 39.42, 37.47, 36.85, 36.34, 36.08, 35.70, 33.11, 32.42, 31.20, 30.48, 30.18, 28.12, 27.92, 27.64, 24.15, 23.77, 22.73, 22.48, 21.08, 19.03, 18.58, 11.90. MS (ESI^+) *m/z* 457.5 (100%, $[M+H]^+$).

4.1.26. 3β-(Spermino)-5-cholestene ***8i***

Purification by column chromatography (silica gel; CH_2Cl_2/MeOH/NH_4OH (32%), 7:3:1) afforded a pale yellow solid in 80% yield; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.15$ (s, 1H), 3.31–2.56 (m, 12H), 2.10–0.59 (m, 57H). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 146.20$, 122.18, 56.05, 54.58, 54.50, 49.75, 49.67, 48.32, 47.67, 45.11, 42.31, 40.35, 39.76, 39.34, 37.38, 36.29, 36.00, 35.80, 35.62, 33.51, 33.06, 32.33, 30.51, 28.04, 27.83, 27.69, 27.00, 24.07, 23.68, 22.66, 22.41, 21.01, 18.97, 18.51, 11.82. MS (ESI^+) *m/z* 571.6 (100%, $[M+H]^+$).

5. Determination of minimal inhibitory concentrations

Antimicrobial activity of the compounds was studied by determination of minimal inhibitory concentrations (MIC) according to the NCCLS guidelines M7-A2 using the microbroth dilution methods. All the strains were issued from the collection of the institut Pasteur (Paris). The cells were grown overnight at 28 °C (*S. cerevisiae* CIP 28383) or 37 °C (*E. coli* CIP 54127, *S. aureus* CIP 4.83, *E. faecalis* CIP103015, *C. albicans* CIP 1180-79) in YPD broth for *S. cerevisiae* and *C. albicans*, LB broth for *E. coli* and *S. aureus* or BHI broth for *S. faecalis.* The bacteria strains were grown on trypticase soy agar (Becton Dickinson) at 37 °C for 24 h and the yeast on Sabouraud agar for 48 h. Inocula were prepared in TCE (tryptone 0.1%, NaCl 8%, wt/vol) by adjusting the turbidity at 623 nm to obtain $1-3 \times 10^5$ 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 total proportion), in order to obtain a two-fold serial dilution in 100 μL of broth and 100 μL of inocula containing $2-6 \times 10^5$ 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 obsrvations as the lowest concentration of compound allowing no visible growth.

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