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Efficient preparation of secondary aminoalcohols through a Ti(IV) reductive amination procedure. Application to the synthesis and antibacterial evaluation of new 3β-*N*-[hydroxyalkyl]aminosteroid derivatives

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Abstract

An efficient method for the synthesis of various secondary aminoalcohols through a titanium(IV) isopropoxide-mediated reductive amination reaction of ketones is reported. Thus, different ketones gave the expected products in moderate to excellent yields up to 89% in numerous cases. A series of 3β-*N*-[hydroxyalkyl]aminosteroid derivatives were prepared according to this methodology and evaluated for their in vitro antimicrobial properties against human pathogens. All the compounds showed moderate to excellent activities against Gram-positive bacteria exhibiting similar results against *Staphylococcus aureus* and *Streptococcus faecalis* with Minimum Inhibitory Concentrations (MICs) varying from 3.12 to 25 μg/mL. No significant antibacterial activities are encountered against Gram-negative bacteria.

1. Introduction

Reductive amination of carbonyl compounds is a very important methodology for chemists to target the synthesis of primary, secondary or tertiary amines.[1] Some reducing agents are useful for this tandem reaction. Particularly, the utility of sodium cyanoborohydride is well recognized due to its versatility and compatibility.[2–4] Nevertheless, this reducing agent is highly toxic and the reaction requires up to a 5-fold excess of amine. Recently, we reported an efficient reductive amination reaction of various amines and ketones using $Ti(O\textit{i}\text{-}Pr)_4$–$NaBH_4$ reagent system.[5,6] In this case, this chemoselective reaction allows the synthesis of various primary and symmetrical secondary amines in good yields up to 98%. Aminoalcohols are important synthetic targets because of their versatile utility as building blocks in organic synthesis.[7] More precisely, chiral 1,2-aminoalcohols obtained by chiral aminoacids reduction have been used as chiral auxiliaries and as source of stereogenic center. Such chiral auxiliaries represent the vast majority of examples for the usage of aminoalcohols with many as part of a cyclic system, especially five-membered rings. However, there is still a need for new safe and efficient methodologies for direct synthesis of new classes of aminoalcohols. Herein, we wish to report an extension of our work for the synthesis of various secondary aminoalcohols through a titanium(IV) isopropoxide-mediated reductive amination reaction of ketones and its subsequent application to the synthesis of new antibacterial 3β-*N*-[hydroxyalkyl]aminosteroid derivatives.

2. Results and discussion

Initially, experiments for the titanium(IV) reductive amination of ketones were performed using acetophenone **1** and 2-aminoethanol **2** as test substrates under various experimental conditions.

Best results were obtained using polar solvents such as MeOH or *i*-PrOH leading to the formation of the expected aminoalcohol compound **3** in 85 and 68% yields, respectively (Table 1, entries 1 and 2). Performing the reaction in CH_2Cl_2,

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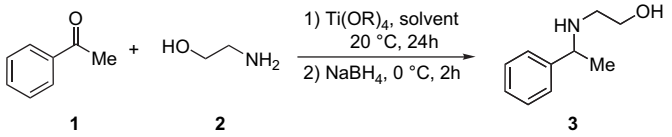
Table 1
Titanium(IV) reductive amination reaction of acetophenone with 2-aminoethanol under various experimental conditions



1 2 3

Entry[a]	Titanium source	Aminoalcohol (equiv)	Solvent	Yield[b] (%)
1	Ti(O*i*-Pr)$_4$	3	MeOH	85
2	Ti(O*i*-Pr)$_4$	3	*i*-PrOH	68
3	Ti(O*i*-Pr)$_4$	3	CH_2Cl_2	64
4	Ti(O*i*-Pr)$_4$	3	Toluene	33
5	Ti(O*i*-Pr)$_4$	3	THF	42
6	Ti(OEt)$_4$	3	MeOH	42
7	Ti(O*t*-Bu)$_4$	3	MeOH	61
8	Ti(O*i*-Pr)$_4$	1	MeOH	30
9	Ti(O*i*-Pr)$_4$	2	MeOH	39

[a] Reaction performed at 20 °C for 5 h in the desired solvent (5 mL) on a 2 mmol scale of acetophenone in the presence of Ti(OR)$_4$ (2.6 mmol) and 2-aminoethanol (6 mmol).
[b] Isolated yield.

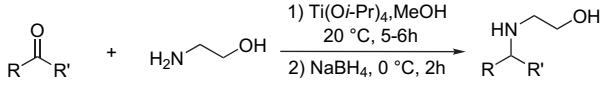
$CHCl_3$, toluene, and THF led to poor results (yields varying from 33 to 64%) (Table 1, entries 3–5). Low yields were realized using Ti(OEt)$_4$ and Ti(O*t*-Bu)$_4$ (Table 1, entries 6 and 7) compared to Ti(O*i*-Pr)$_4$. Increasing the amount of amine from 1 to 3 equiv improved the yield from 30 to 85% (Table 1, entries 1, 8, and 9).

This study has been extended to a series of different ketones using 2-aminoethanol as test substrate. The results are summarized in Table 2.

Aromatic ketones led to the formation of the desired compounds in excellent yields varying from 56 to 85% depending on the nature of the ketone (Table 2, entries 1–5). In the case of aliphatic ketone, the expected product was obtained up to 52% chemical yields (Table 2, entries 9–11). Conjugated ketones such as cyclohexenone did not afford the expected derivative and only formation of by-products such as 2-cyclohexen-1-ol were observed (Table 2, entry 7).

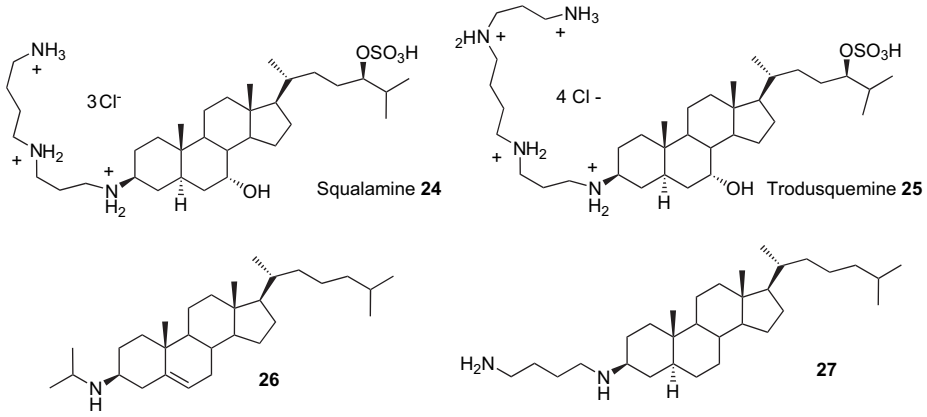
Recently, we and others reported that water soluble cationic steroids, squalamine **24** and trodusquemine **25** isolated from the dogfish shark *Squalus acanthias* exhibit potent antimicrobial and antiangiogenic activities.[8–12] To date, obtaining large quantities of **24** and **25** is difficult since the large scale production requires expensive starting materials and numerous steps with low chemical yields. Recently, we have synthesized and evaluated 3β-amino and polyamino steroid analogues **26** and **27** exhibiting interesting antimicrobial properties even toward resistant strains (Scheme 1).[13,14]

Table 2
Titanium(IV) reductive amination reaction of various ketones with 2-aminoethanol



Entry[a]	Ketone	Product	Yield[b] (%)	Entry[a]	Ketone	Product	Yield[b] (%)
1	**1**	**3**	85	7	**14**	**15**	0[c]
2	**4**	**5**	56	8	**16**	**17**	51
3	**6**	**7**	63	9	**18**	**19**	40
4	**8**	**9**	89	10	**20**	**21**	52
5	**10**	**11**	24	11	**22**	**23**	11
6	**12**	**13**	86				

[a] Reaction performed at 20 °C for 5–6 h in MeOH (5 mL) on a 2 mmol scale of ketone in the presence of Ti(O*i*-Pr)$_4$ (2.6 mmol) and 2-aminoethanol (6 mmol).
[b] Isolated yield.
[c] Only formation of by-products was noticed and major formation (60%) of 2-cyclohexen-1-ol.



Scheme 1.

As part of our studies, we became interested in the synthesis of new 3β-*N*-[hydroxyalkyl]aminosteroid derivatives **29**–**33** and **35**–**39**, their parent aminoalcohol analogues, in one pot synthesis from 4-cholesten-3-one **28** and 5α-cholestan-3-one **34** in order to evaluate the importance of an amino or a hydroxy group on the biological activities of these compounds. All these derivatives were obtained in moderate to good yields varying from 6 to 95% yield and more than 95% diastereomeric excess in all cases (Scheme 2).

All the synthesized derivatives were screened for their antimicrobial activities against several yeast strains as well as Gram-positive and Gram-negative bacterial strains (Table 3).

All these novel compounds tested in the present study were found to have little activity against yeasts such as *Saccharomyces cerevisiae* and *Candida albicans* with antifungal activities varying from 3.125 to 12.5 μg/mL in the best cases. However, these compounds showed important antibacterial activities against Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* with an average MIC of 3.125–12.5 μg/mL in numerous cases (Table 3, compounds **29**–**33**) whereas no antibacterial activity has been detected against Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes* bacteria. On the other hand, it clearly appears that saturated sterol derivatives are more active than their unsaturated parents suggesting important structure–activity relationships.

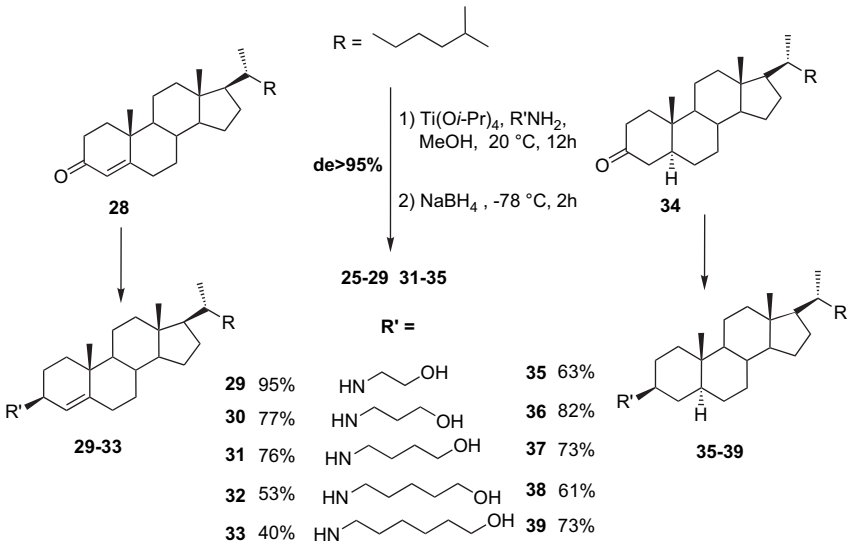
3. Conclusion

An efficient method for the synthesis of various secondary aminoalcohols through a titanium(IV) isopropoxide-mediated reductive amination reaction of ketones in moderate to excellent yields up to 89% is developed. Thus, aromatic ketones are well suited for this methodology whereas aliphatic ketones give moderate yields. Moreover, the successful synthesis of new 3β-*N*-[hydroxyalkyl]aminosteroid derivatives presenting significant antimicrobial activities has been realized.

4. Experimental section

4.1. General

All solvents were purified according to reported procedures, and reagents were used as commercially available.



Scheme 2.

Table 3
Antimicrobial activities of 3β-*N*-[hydroxyalkyl]aminosteroid derivatives **29**–**33** and **35**–**39**

MIC (μg/mL)					
Strains	Products				
	29 (**35**)	**30** (**36**)	**31** (**37**)	**32** (**38**)	**33** (**39**)
Yeasts					
S. cerevisiae	50 (>100)	>50 (>100)	**12.5** (>100)	**12.5** (>100)	**3.12** (>100)
C. albicans	>50 (>100)	>50 (>100)	>50 (>100)	>50 (>100)	>50 (>100)
Gram-positive bacteria					
S. aureus	50 (>100)	50 (>100)	**25** (>100)	**25** (>100)	**25** (>100)
E. faecalis	**12.5** (>100)	**12.5** (50)	**6.25** (50)	**12.5** (>100)	**3.12** (>100)
Gram-negative bacteria					
E. coli	>50	>50	>50	>50	>50
P. aeruginosa	>50	>50	>50	>50	>50
E. aerogenes	>50	>50	>50	>50	>50

Toluene, tetrahydrofuran (THF) were distilled from sodium/benzophenone ketyl immediately prior to use. CH_2Cl_2 was distilled from P_2O_5 prior to use. Ethylacetate, methanol, and petroleum ether (35–60 °C) were purchased from SDS and used without any further purification. Column chromatography was performed on SDS silica gel (70–230 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer working at 300 and 75 MHz, respectively (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. MS spectra were recorded using a triple Quadripole API III Plus Sciex spectrometer.

4.2. General procedure for the titanium-mediated reductive amination reaction of ketones

A mixture of the ketone (2 mmol), titanium(IV) isopropoxide (738 mg, 2.6 mmol), and 2-aminoethanol (6 mmol) in absolute methanol (5 mL) was stirred under argon at room temperature for 5–6 h. Sodium borohydride (75 mg, 2 mmol) was then added at 0 °C and the resulting mixture was stirred for an additional 2 h. The reaction was then quenched by adding water (1 mL). Stirring was continued at room temperature for 20 min then the reaction mixture was acidified with hydrochloric acid (1 M, 5 mL). After filtration over a pad of Celite washing with water and ethylacetate. The organic layer was separated and dried over Na_2SO_4, filtered, and concentrated in vacuo to afford the expected crude aminoalcohol derivative, which was purified by flash chromatography on silica gel.

*4.3. 2-(1-Phenyl-ethylamino)-éthanol **3***

Purification by column chromatography on silica gel using ethylacetate as eluent; clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.35 (d, J=9 Hz, 3H), 2.51–2.68 (m, 2H), 3.31–3.80 (m, 3H), 7.23–7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=23.75, 49.15, 58.03, 60.74, 126.45, 128.37, 144.85. $C_{10}H_{15}NO$ calcd C, 72.69; H, 9.15; N, 8.48. Found C, 72.10; H, 9.28; N, 8.41.

*4.4. 2-(1-Phenyl-propylamino)-éthanol **5***

Purification by column chromatography on silica gel using ethylacetate/méthanol 50:50 as eluent; clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.79 (t, J=9 Hz, 3H), 1.20 (t, J=9 Hz, 1H), 1.61–1.98 (m, 2H), 2.55–2,63 (m, 1H), 3.17 (s, 1H), 3.41 (s, 1H), 3.48–3.65 (m, 2H), 7.20–7.34 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=11.16, 30.96, 49.49, 61.29, 65.29, 127.52, 127.70, 128.80, 143.63. $C_{11}H_{17}NO$ calcd C, 73.70; H, 9.56; N, 7.81. Found C, 73.70; H, 9.64; N, 7.88.

*4.5. 2-(1-Methyl-2-phenyl-ethylamino)-éthanol **7***

Purification by column chromatography on silica gel using ethylacetate as eluent; clear oil. ^{13}C NMR (75 MHz, $CDCl_3$): δ=20.48, 43.86, 49.02, 54.95, 61.38, 126.65, 128.82, 129.66, 139.57. $C_{11}H_{17}NO$ calcd C, 73.70; H, 9.56; N, 7.81. Found C, 73.68; H, 9.50; N, 7.81.

*4.6. 2-(1-Furan-2-yl-ethylamino)-éthanol **9***

Purification by column chromatography on silica gel using ethylacetate/méthanol 50:50 as eluent; clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.32 (d, J=9 Hz, 3H), 1.88 (s, 1H), 2.57 (t, J=6 Hz, 2H), 3.46–3.59 (m, 2H), 3.78 (q, J=6 Hz, 1H), 6.06 (d, J=3 Hz, 1H), 6.21 (d, J=3 Hz, 1H), 7.08 (s, 1H), 7.25 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=20.27, 49.04, 51.31, 61.14, 105.87, 110.28, 141.79, 157.51. $C_8H_{13}NO_2$ calcd C, 61.91; H, 8.44; N, 9.03. Found C, 61.85; H, 8.35; N, 9.01.

*4.7. 2-[(Cyclopropyl-phényl-méthyl)-amino]-éthanol **11***

Purification by column chromatography on silica gel using methanol as eluent; clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.36–0.69 (m, 5H), 1.04–1.30 (m, 4H), 2.06–2.19 (m, 2H), 4.03 (d, J=9 Hz, 1H), 7.28–7.59 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=3.22, 4.01, 12.11, 17.57, 19.60, 78.94, 126.44, 127.92, 128.76, 128.91, 133.15, 144.27. $C_{12}H_{17}NO$

calcd C, 75.35; H, 8.96; N, 7.32. Found C, 75.32; H, 8.94; N, 7.31.

4.8. 2-(1-Cyclopropyl-ethylamino)-éthanol **13**

Purification by column chromatography on silica gel using methanol as eluent; clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.08–0.91 (m, 5H), 1.17 (d, J=6 Hz, 3H), 2.90–2.97 (m, 1H), 3.71 (t, J=6 Hz, 2H), 4.19 (t, J=6 Hz, 2H), 4.8 (s, 1H), 5.31 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=2.78, 15.99, 18.87, 19.21, 44.04, 56.89, 62.62. $C_7H_{15}NO$ calcd C, 65.07; H, 11.70; N, 10.84. Found C, 65.10; H, 12.02; N, 10.65.

4.9. 2-(1-Methyl-3-phenyl-allylamino)-éthanol **17**

Purification by column chromatography on silica gel using methanol as eluent; clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.27 (d, J=6 Hz, 3H), 2.40 (m, 2H), 2.74–2.86 (m, 1H), 3.39 (t, J=6 Hz, 2H), 3.66–3.70 (m, 2H), 6.03–6.09 (dd, J=6, 15 Hz, 1H), 6.51 (d, J=15 Hz, 1H), 7.21–7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=22.05, 48.73, 55.98, 61.29, 126.29; 127.41, 128.56, 130.14, 133.93, 136.96. $C_{12}H_{17}NO$ calcd C, 75.35; H, 8.96; N, 7.32. Found C, 75.25; H, 8.96; N, 7.14.

4.10. 2-Cyclohexylamino-éthanol **19**

Purification by column chromatography on silica gel using methanol as eluent; clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.99–1.91 (m, 10H), 2.73 (t, J=6 Hz, 2H), 3.40 (m, 2H), 3.62 (t, J=6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=25.05, 26.02, 33.26, 48.31, 56.77, 60.76. $C_{10}H_{21}NO$ calcd C, 70.12; H, 12.36; N, 8.18. Found C, 70.05; H, 12.34; N, 8.25.

4.11. 2-(1,3-Dimethyl-butylamino)-éthanol **21**

Purification by column chromatography on silica gel using methanol as eluent; clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.82 (t, J=6 Hz, 6H), 0.89 (d, J=6 Hz, 3H), 1.05–1,29 (m, 1H), 1.55–1.64 (m, 1H), 2.59–2.74 (m, 4H), 3.27 (m, 2H), 3.57 (t, J=6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=20.61, 22.76, 23.52, 25.26, 46.88, 48.96, 51.33, 60.99. $C_8H_{19}NO$ calcd C, 66.16; H, 13.19; N, 9.64. Found C, 66.05; H, 12.95; N, 9.59.

4.12. 2-(1,2,2-Trimethyl-propylamino)-éthanol **23**

Purification by column chromatography on silica gel using methanol as eluent; clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.91 (s, 9H), 1.03 (d, J=6 Hz, 3H), 1.75–2.17 (m, 2H), 2.24 (q, J=6 Hz, 1H), 2.64–2.96 (m, 2H), 3.55–3.63 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=15.45, 26.88, 34.83, 49.85, 61.37, 62.20. $C_8H_{19}NO$ calcd C, 66.16; H, 13.19; N, 9.64. Found C, 66.54; H, 13.45; N, 9.78.

4.13. 3β-N-[Hydroxyethyl]amino-4-cholestene **29**

Purification by column chromatography on silica gel using CH_2Cl_2/MeOH/NH_4OH(32%) 7:3:1 as eluent; viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.25 (s, 1H), 3.66–3.63 (m, 2H), 3.11–2.78 (m, 5H), 2.20–0.65 (m, 43H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=147.37, 122.37, 61.53, 56.57, 55.02, 54.78, 48.26, 52.87, 40.30, 39.90, 37.90, 36.78, 36.35, 36.17, 33.57, 32.87, 28.60, 28.40, 27.50, 24.62, 24.24, 23.21, 22.95, 21.55, 19.53, 19.05, 12.37. HRMS (FAB) m/z found 430.4006 $[M+H]^+$, calcd for $C_{29}H_{51}NO$ 430.4004.

4.14. 3β-N-[Hydroxypropyl]amino-4-cholestene **30**

Purification by column chromatography on silica gel using CH_2Cl_2/MeOH/NH_4OH(32%) 7:3:1 as eluent; viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.19 (s, 1H), 3.77–2.86 (m, 7H), 2.14–0.63 (m, 47H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=147.13, 121.31, 63.80, 56.10, 54.52, 54.47, 46.13, 42.40, 39.82, 39.43, 37.42, 36.23, 36.08, 35.70, 33.08, 32.38, 30.99, 28.13, 27.92, 26.65, 24.15, 23.77, 22.75, 22.49, 21.08, 19.05, 18.59, 11.90. HRMS (FAB) m/z found 444.4152 $[M+H]^+$, calcd for $C_{30}H_{53}NO$ 444.4161.

4.15. 3β-N-[Hydroxybutyl]amino-4-cholestene **31**

Purification by column chromatography on silica gel using CH_2Cl_2/MeOH/NH_4OH(32%) 7:3:1 as eluent; viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.23 (s, 1H), 3.56–2.63 (m, 8H), 2.16–0.62 (m, 46H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=147.19, 121.37, 62.40, 56.21, 54.58, 54.31, 46.20, 42.47, 39.90, 39.59, 37.50, 36.30, 36.16, 35.83, 35.77, 33.16, 32.52, 32.43, 29.03, 28.20, 27.99, 26.75, 24.23, 23.85, 22.81, 22.56, 21.15, 19.15, 18.66, 11.97. HRMS (FAB) m/z found 458.4320 $[M+H]^+$, calcd for $C_{31}H_{55}NO$ 458.4317.

4.16. 3β-N-[Hydroxypentyl]amino-4-cholestene **32**

Purification by column chromatography on silica gel using CH_2Cl_2/MeOH/NH_4OH(32%) 7:3:1 as eluent; viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.30 (s, 1H), 4.64 (s, 2H), 3.64–3.46 (m, 10H), 2.76–0.68 (m, 44H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=70.85, 56.07, 55.63, 50.48, 46.98, 46.06, 42.70, 41.19, 39.43, 39.27, 38.81, 37.78, 36.84, 36.73, 36.09, 35.77, 35.73, 31.65, 31.29, 31.24, 28.02, 27.95, 23.83, 23.64, 22.75, 22.47, 21.06, 18.55, 11.70, 11.59. HRMS (FAB) m/z found 472.4479 $[M+H]^+$, calcd for $C_{32}H_{57}NO$ 472.4474.

4.17. 3β-N-[Hydroxyhexyl]amino-4-cholestene **33**

Purification by column chromatography on silica gel using CH_2Cl_2/MeOH/NH_4OH(32%) 7:3:1 as eluent; viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.23 (s, 1H), 3.60–3.56 (m, 2H), 3.12–0.63 (m, 56H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=146.56, 122.07, 62.28, 56.23, 54.67, 46.56, 42.48, 39.93, 39.51, 37.54, 36.45, 36.17, 35.97, 35.78, 33.20, 32.69, 32.49, 30.25, 28.21,

27.59, 27.19, 26.98, 25.72, 24.23, 22.82, 22.56, 21.17, 19.10, 18.66, 11.98. HRMS (FAB) *m/z* found 486.4632 $[M+H]^+$, calcd for $C_{33}H_{59}NO$ 486.4630.

4.18. 3β-N-[Hydroxyethyl]aminocholestane **35**

Purification by column chromatography on silica gel using CH_2Cl_2/MeOH/NH_4OH(32%) 7:3:1 as eluent; viscous oil. ^1H NMR (300 MHz, $CDCl_3$): δ=3.67−3.60 (m,2H), 3.29 (s,3H), 2.84−2.50 (m, 3H), 1.96−0.62 (m, 55H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=61.19, 60.88, 57.70, 56.89, 56.69, 54.79, 52.84, 49.04, 48.39, 45.73, 42.99, 40.42, 40.11, 39.91, 37.73, 36.57, 36.28, 36.19, 35.88, 35.42, 33.51, 32.94, 32.46, 32.40, 29.20, 28.64, 28.40, 26.14, 24.59, 24.24, 23.21, 22.96, 21.55, 21.17, 19.07, 12.71, 12.47, 11.92. HRMS (FAB) *m/z* found 432.4160 $[M+H]^+$, calcd for $C_{29}H_{53}NO$ 432.4161.

4.19. 3β-N-[Hydroxypropyl]aminocholestane **36**

Purification by column chromatography on silica gel using CH_2Cl_2/MeOH/NH_4OH(32%) 7:3:1 as eluent; viscous oil. ^1H NMR (300 MHz, $CDCl_3$): δ=3.67−3.60 (m,2H), 3.29 (s,3H), 2.84−2.50 (m, 3H), 1.96−0.62 (m, 55H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=61.19, 60.88, 57.70, 56.89, 56.69, 54.79, 52.84, 49.04, 48.39, 45.73, 42.99, 40.42, 40.11, 39.91, 37.73, 36.57, 36.28, 36.19, 35.88, 35.42, 33.51, 32.94, 32.46, 32.40, 29.20, 28.64, 28.40, 26.14, 24.59, 24.24, 23.21, 22.96, 21.55, 21.17, 19.07, 12.71, 12.47, 11.92. HRMS (FAB) *m/z* found 446.4314 $[M+H]^+$, calcd for $C_{30}H_{55}NO$ 446.4317.

4.20. 3β-N-[Hydroxybutyl]aminocholestane **37**

Purification by column chromatography on silica gel using CH_2Cl_2/MeOH/NH_4OH(32%) 7:3:1 as eluent; viscous oil. ^1H NMR (300 MHz, $CDCl_3$): δ=3.80 (s, 2H), 3.57−3.51 (m, 2H), 2.67−2.45 (m, 3H), 1.94−0.60 (m, 50H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=62.73, 57.53, 56.89, 56.68, 54.82, 46.80, 45.77, 42.97, 40.43, 39.90, 37.78, 36.56, 36.30, 36.18, 35.87, 35.68, 32.91, 32.46, 29.44, 29.17, 28.54, 28.38, 24.58, 24.23, 23.20, 22.95, 21.52, 19.06, 12.70, 12.45. HRMS (FAB) *m/z* found 460.4469 $[M+H]^+$, calcd for $C_{31}H_{57}NO$ 460.4474.

4.21. 3β-N-[Hydroxypentyl]aminocholestane **38**

Purification by column chromatography on silica gel using CH_2Cl_2/MeOH/NH_4OH(32%) 7:3:1 as eluent; viscous oil. ^1H NMR (300 MHz, $CDCl_3$): δ=3.60 (s, 2H), 2.87−0.61 (m, 57H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=62.51, 57.88, 56.92, 56.69, 54.87, 49.98, 47.07, 45.77, 42.98, 40.45, 39.90, 37.83, 36.57, 36.27, 36.18, 35.89, 32.76, 32.50, 29.99, 29.57, 29.36, 28.63, 28.38, 24.59, 24.23, 23.88, 23.20, 22.95, 21.53, 19.06, 12.75, 12.46. HRMS (FAB) *m/z* found 474.4652 $[M+H]^+$, calcd for $C_{32}H_{59}NO$ 474.4630.

4.22. 3β-N-[Hydroxyhexyl]aminocholestane **39**

Purification by column chromatography on silica gel using CH_2Cl_2/MeOH/NH_4OH(32%) 7:3:1 as eluent; viscous oil. ^1H NMR (300 MHz, $CDCl_3$): δ=3.60−3.57 (m, 2H), 2.66−0.61 (m, 59H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=62.79, 57.88, 56.93, 56.69, 54.88, 47.15, 45.77, 42.98, 40.46, 39.90, 37.84, 36.57, 36.39, 36.19, 35.90, 33.10, 33.04, 32.51, 30.36, 29.12, 28.64, 28.39, 27.50, 26.99, 26.03, 24.59, 24.24, 23.20, 22.95, 21.53, 19.06, 12.75, 12.46. HRMS (FAB) *m/z* found 488.4752 $[M+H]^+$, calcd for $C_{33}H_{61}NO$ 488.4787.

4.23. Determination of minimal inhibitory concentrations

Antimicrobial activity of the compounds was studied by determination of minimal inhibitory concentrations (MIC) according to the NCCLS guidelines using the microbroth dilution methods.[15] The cells were grown overnight at 28 °C (*S. cerevisiae* ATCC 28383) or 37 °C (*E. coli* ATCC 10536, *P. aeruginosa* ATCC 15442, *E. aerogenes* ATCC 15038, *S. aureus* ATCC 6538, *E. faecalis* CIP 103015, *C. albicans* ATCC 90029) in YPD broth for *S. cerevisiae* and Sabouraud broth for *C. albicans*, LB broth for *E. coli*, *P. aeruginosa*, *E. aerogenes* and *S. aureus* or BHI broth for *S. faecalis*. Eight microlitres of a compound solution of 5 mg/mL was serially diluted by factor of two with the corresponding broth. Econazole and streptomycin were used as substrate references for all fungi and bacteria, respectively. The bacterial 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 8.5%, wt/vol) by adjusting the turbidity at 623 nm to obtain $1-3\times10^5$ CFU/mL.

Broth microdilution method was used to determine the MIC and was performed in sterile 96-well microplates. Each compound (5 mg/mL in methanol) was transferred to each microplate well, 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 EL_x 808IU (Biotek Instruments). MIC was defined for each agent from duplicate observations as the lowest concentration of compound allowing no visible growth.

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